

# **The Humoral Immune Response to SARS-CoV-2 Vaccination and Infection**

**Alexa Keeshan**

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School of Epidemiology and Public Health | École d'épidémiologie et de santé publique  
Faculty of Medicine | Faculté de médecine  
University of Ottawa | Université d'Ottawa

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# Preface to the Thesis

**Supervisor:** Dr. Curtis Cooper

**Advisory Committee Members:** Dr. Marc-André Langlois, Dr. Ellen Freeman, Dr. Tim Ramsay, Dr. Sahar Saeed, Dr. Kumanan Wilson

The necessary approvals to conduct the research presented herein were obtained. The Stop the Spread Ottawa cohort study protocol was approved by the Ottawa Health Science Network Research Ethics Board (#2020-0481-01H). Informed consent was obtained from all study participants. No funders contributed to design of the study, data collection, analysis, interpretation, or manuscript writing.

The doctoral candidate's contributions—and those of all co-authors and colleagues involved—for each component article of this doctoral thesis are summarized below:

**Results of the Stop the Spread Ottawa (SSO) cohort study** (Chapter 2): AK completed the data analysis and manuscript preparation. YG: laboratory analysis, data analysis, manuscript preparation. AH: data analysis, manuscript preparation. EC: study design, study recruitment, manuscript preparation. PSM: laboratory analysis, data analysis. CA: laboratory analysis, data analysis. RS: study design, manuscript preparation. RB: laboratory analysis, manuscript preparation. JL: study design, data analysis, manuscript preparation. MM: study design, manuscript preparation. CAB: study design, manuscript preparation. AC: study design, laboratory analysis, data analysis, manuscript preparation. M-AL: study design, laboratory analysis, data analysis, manuscript preparation. CLC: study design, study recruitment, data analysis, manuscript preparation. CC: guarantor.

### **Immune Compromising States Impair SARS-CoV-2 Vaccine Humoral**

**Immunogenicity Despite Multiple Booster Doses** (Chapter 3): **A.K.**, S.S., C.A.G., M-A.L., C.L.C. conceptualized and designed the study and reviewed the manuscript. **A.K.**, C.L.C. jointly prepared the manuscript. **A.K.** conducted the data analysis. M-A.L. completed the laboratory testing and related data analysis. C.L.C. completed the study recruitment.

### **Obesity Does Not Influence SARS-CoV-2 Vaccine Humoral Immunogenicity**

(Chapter 4): M.D.S., **A.K.**, C.A.G., M-A.L., C.L.C. designed the study and reviewed the manuscript. M.D.S., **A.K.**, C.L.C. jointly prepared the manuscript. **A.K.** conducted the data analysis. M-A.L. completed the laboratory testing and related data analysis. C.L.C. completed the study recruitment. M.D.S. and **A.K.** contributed equally to manuscript preparation and are both designated co-first authors.

### **Alcohol Consumption Does Not Influence SARS-CoV-2 Vaccine**

**Immunogenicity: A stop the spread Ottawa cohort analysis** (Chapter 5): Alicia Vachon: conceptualization, writing – original draft / review & editing, **Alexa Keeshan**: methodology, formal analysis, Yannick Galipeau: investigation, writing – review & editing, Angela M. Crawley: resources, Marc-André Langlois: investigation, supervision, writing – review & editing, Curtis L. Cooper: conceptualization, investigation, methodology, writing - review & editing, supervision.

### **Selective Serotonin Reuptake Inhibitors Do Not Impact SARS-CoV-2 Vaccine Humoral Immunogenicity or the Risk of Post-COVID-19 Condition** (Chapter 6):

**A.K.**, S.S., M-A.L., C.L.C. conceptualized and designed the study and reviewed the manuscript. **A.K.**, C.L.C. jointly prepared the manuscript. **A.K.** conducted the data analysis. M-A.L. completed the laboratory testing and related data analysis. C.L.C. completed the study recruitment.

**Immune imprinting: The persisting influence of the first antigenic encounter with rapidly evolving viruses** (Chapter 7): M.M., **A.K.**, C.C., and M.-A.L. designed the study. M.M. and M.-A.L. designed the figures. M.M., **A.K.**, and M.-A.L. wrote the manuscript. All authors edited the final version of the manuscript.

# Abstract

**Background:** The coronavirus disease 2019 (COVID-19) pandemic resulted in over seven million deaths as of 2026 and an excess mortality of 18.2 million as of 2022. COVID-19 vaccines were instrumental in improving population immunity. However, initial phase 2/3 clinical trials excluded vulnerable clinical groups. Observational research was therefore needed to evaluate the long-term effectiveness of COVID-19 vaccines among high-risk clinical groups in a real-world context. The Stop the Spread Ottawa (SSO) cohort was utilized to accomplish this goal. **Objectives:** I. Describe the demographic and clinical characteristics of the SSO cohort; II. Identify predictors of COVID-19 vaccine humoral immunogenicity; III. Determine if immune compromised (IC) status, obesity, excessive alcohol consumption, or SSRI medication use influence the production and durability and/or neutralization activity of antibodies generated by COVID-19 vaccination; V. Determine if SSRIs impact the odds of developing post COVID-19 condition (PCC); VI. Explore the potential impact of imprinting on COVID-19 vaccine immunogenicity. **Methods:** Predictors of antibody titers after the second COVID-19 vaccine dose were identified by multivariable quantile regression. The impact of IC (after five doses), obesity (three doses), excessive alcohol consumption (four doses), and SSRI use (three doses) were assessed by multivariable multilevel robust linear regression with flexible modelling of time, segmented multilevel Bayesian robust linear regression, segmented multilevel linear regression, and multilevel robust linear regression with flexible modelling of time, respectively. **Results:** The SSO cohort was primarily White (89.0%), female (66.6%), and employed (83.2%) at baseline. A wide range of ages were represented. Older age and high-level IC (e.g., malignancy, organ/bone marrow transplant, systemic immunosuppressant, rheumatological disease, suppressed HIV) predicted impaired antibody production and neutralization activity following COVID-19 vaccination, whereas hybrid immunity and a wider interval between doses were associated with heightened humoral response. Antibody production and durability were

impaired for high-level IC participants but not for obese participants, excessive alcohol consumers, or SSRI users. SSRI use was not associated with the odds of PCC. **Conclusion:** COVID-19 vaccination generated robust humoral response among obese individuals, excessive alcohol drinkers, and SSRI users. Humoral immunogenicity was impaired for high-level IC participants and older individuals; our findings support the prioritization of these populations in public health vaccination guidelines.

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*“I have no idea what's awaiting me, or what will happen when this all ends. For the moment I know this: there are sick people and they need curing.” - Albert Camus, The Plague*



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## Glossary & Abbreviations

BAU/mL – Binding antibody units per millilitre, calibrated using the World Health Organization (WHO) International reference standard for anti-SARS-CoV-2 immunoglobulin. These units are used to report antibody quantification results. See IU/mL.

CCSA – Canadian Centre on Substance Abuse and Addiction.

CI – Confidence interval.

COVID-19 – Coronavirus disease 2019.

ELISA – Enzyme-linked immunosorbent assay.

IgA – Immunoglobulin A.

IgG – Immunoglobulin G.

IgM – Immunoglobulin M.

IQR – Interquartile range.

IU/mL – International units per millilitre. These units are used to report antibody quantification results. See BAU/mL.

MERS-CoV – Middle East respiratory syndrome coronavirus.

N – SARS-CoV-2 nucleocapsid antigen.

PBMCs – Peripheral blood mononuclear cells.

RBD – SARS-CoV-2 receptor-binding domain antigen.

RCT – Randomized controlled trial.

S – SARS-CoV-2 spike antigen.



SARS-CoV-2 – Severe acute respiratory syndrome coronavirus 2.

SD – Standard deviation. Note: in Chapter 5, SD refers to standard drinks where indicated.

Vaccine immunogenicity – The ability of a vaccine to generate an immune response.

# Chapter 1 – Introduction

## **Background**

Throughout human history pandemic viruses have emerged, spread, and caused widespread loss of life, severe morbidity, and significant harm. Their impacts have shifted population dynamics and shaped societies.<sup>1</sup> The 1918 influenza pandemic was a major public health crisis in the 20<sup>th</sup> century and caused the deaths of approximately 50 million individuals.<sup>2,3</sup> It served as a warning for later generations of the devastation that novel viruses can wreak. For decades, scientists have remained vigilant, anticipating the appearance of the next pandemic virus.<sup>2</sup> The severe acute respiratory syndrome (SARS) outbreak in 2002 and the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 raised concerns among scientists and public health experts and emphasized coronaviruses as a candidate for the next pandemic virus and a serious threat to public health.<sup>4,5</sup> Surveillance efforts highlighted the zoonotic potential of coronaviruses circulating in bat reservoirs after a live coronavirus capable of binding to angiotensin-converting enzyme 2 (ACE2) receptors and infecting human cells was isolated from Chinese horseshoe bats in China in 2016.<sup>4,5</sup> Indeed, in late 2019 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified for the first time in Wuhan, China and rapidly spread around the world.<sup>6</sup> The subsequent coronavirus disease 2019 (COVID-19) pandemic resulted in broad and devastating global consequences.

The total human cost of the COVID-19 pandemic continues to be realized. In the first two years, nearly six million official deaths were reported around the world and excess mortality due to COVID-19 was estimated to be 18.2 million (95% uncertainty interval: 17.1-19.6 million).<sup>7</sup> In Canada, the total number of confirmed COVID-19 cases and deaths were approximately 5 million (as of October 4, 2024) and 60,871 (as of as of September 21, 2024), respectively.<sup>8</sup> The healthcare burden from SARS-CoV-2 was enormous and surges in COVID-19 cases and

hospitalizations placed overwhelming pressure on already strained healthcare systems around the world.<sup>9,10</sup> Multiple waves occurred in Canada during which high rates of SARS-CoV-2 circulation was largely driven by the development of newer variants of concern possessing mutations associated with immune escape and increased transmissibility (e.g., the Delta variant in mid-2021 and the Omicron variant December 2020 onwards).<sup>11–13</sup> Prior to the development of SARS-CoV-2 vaccines, non-pharmaceutical interventions such as gathering restrictions, lockdowns and masking and social distancing mandates were implemented to “flatten the curve”, reduce strain on hospitals, and reduce the number of cases and deaths.<sup>14</sup> The public health strategy was successful at decreasing transmission and mortality.<sup>15</sup> In Canada the closure of non-essential workplaces was associated with a decrease in the mortality rate and an estimated 0.531 deaths per 10,000 people ( $P < 0.001$ ) were averted.<sup>14</sup> However, these policies disrupted economic activity and worsened mental health.<sup>16</sup> Other indirect consequences of the COVID-19 pandemic include interruptions to health services aimed at preventing and treating other diseases (e.g., cancer screening and treatment, heart disease prevention and treatment).<sup>17–19</sup>

SARS-CoV-2 is a betacoronavirus (Kingdom *Orthornavirae*, Order *Nidovirales*, Family *Coronaviridae*) that infects the respiratory tract and can produce a variety of symptoms ranging in severity from mild to life-threatening, including fever, cough, fatigue, and less commonly, headache, sore throat, nausea, vomiting, diarrhea, anosmia, ageusia, myalgia and chest pain.<sup>20,21</sup> Asymptomatic infection also occurs, particularly in children and young adults who serve as major drivers of SARS-CoV-2 circulation.<sup>22</sup> SARS-CoV-2 is highly contagious.<sup>20</sup> High viral load and viral shedding is not limited to serious cases and have been documented for mild and asymptomatic cases.<sup>20,23</sup> In addition, patients are capable of spreading the virus early in the course of infection during the incubation period and prior to developing symptoms.<sup>20,23,24</sup> The primary route of transmission is through the inhalation of droplets and aerosolized particles produced by breathing, sneezing, coughing, or talking.<sup>24</sup>

Serious outcomes of COVID-19 include hypoxia, cytokine storm, acute respiratory distress syndrome, organ damage, respiratory failure and death.<sup>25,26</sup> A meta-analysis from 2024 calculated a case fatality rate of 4.77% in North America when the Wuhan variant predominated and an overall case fatality rate of 2.48% to 2.65% over the period of 2020 to 2023.<sup>27</sup> After the resolution of acute infection, some COVID-19 convalescent patients suffer from long-term impacts of infection. Critical illness is associated with prolonged physical, psychiatric, and cognitive impairments and debility consistent with post-intensive care syndrome (PICS) up to at least one year after hospitalization.<sup>28</sup> Additionally, an estimated 10-15% of COVID-19 patients develop a complex and chronic multi-organ syndrome called post COVID-19 condition (PCC), also referred to as post-acute sequelae of SARS-CoV-2 infection (PASC) or Long COVID.<sup>29</sup>

The communicability and severity of COVID-19 is not consistent across demographic and clinical groups. Certain populations, such as older adults, males and racial minorities (e.g., Black, South Asian, East Asian, Mixed, Hispanic, Indigenous) are especially vulnerable to SARS-CoV-2 infection and experienced higher rates of hospitalization and death.<sup>20,30,31</sup> Throughout the pandemic, the observed case fatality rate was consistently higher among older adults compared to younger adults.<sup>27,30</sup> Individuals with pre-existing comorbidities, including hypertension, obesity, cardiovascular disease, asthma, chronic obstructive pulmonary disease, diabetes mellitus, and immune compromising diseases (e.g., AIDS, immunoglobulin deficiency, immunosuppressant medication use, malignancy, hematopoietic stem cell or solid organ transplantation) also have an heightened risk of experiencing serious COVID-19 illness.<sup>32-34</sup>

Nearly one year after the first official COVID-19 case was reported in Wuhan, China, vaccines targeting the SARS-CoV-2 virus were developed and administered around the world.<sup>35</sup> Vaccines were instrumental in reducing susceptibility to SARS-CoV-2 infection and improving population immunity compared to the pre-vaccine time period.<sup>22</sup> Prior to vaccine rollout in Canada, the Canadian Blood Services identified a seropositivity rate for antibodies targeting

SARS-CoV-2 of less than one percent for 10,000 blood donor samples in May and June of 2020.<sup>36</sup> By November 2021, a national vaccine coverage of 79% was achieved.<sup>35</sup> COVID-19 vaccines do not provide sterilizing immunity, especially following the emergence of the more transmissible variant Omicron; however, they significantly reduce the severity of SARS-CoV-2 infection and the risk of mortality.<sup>37–39</sup>

Ioannidis et al. estimated that COVID-19 vaccines were responsible for preventing over 2.5 million deaths (sensitivity range estimates: 1.4–4.0 million) and preserving 14.8 million life-years (sensitivity range estimates: 7.4–23.6 million life-years) globally from 2020 to 2024.<sup>40</sup> The large healthcare burden of non-fatal COVID-19 cases was also mitigated by COVID-19 vaccine rollout. Robinson et al. estimated that COVID-19 vaccination and treatment policies in the United States resulted in gains of 0.01, 0.02, and 3.15 quality-adjusted life years (QALYs) for each mild, severe and critical case prevented.<sup>41</sup>

## **Review of the Literature and Thesis Rationale**

Prior to the approval of novel COVID-19 vaccines, randomized controlled clinical trials (RCTs) were conducted in accordance with international regulatory guidelines and ethical standards to evaluate vaccine efficacy and safety.<sup>42–44</sup> BNT162b2, the lipid encased nucleoside-modified mRNA COVID-19 vaccine produced by Pfizer-BioNTech, was assessed in a phase 2/3 randomized placebo-controlled trial involving 43,548 healthy adults.<sup>42</sup> Vaccine efficacy in the trial was high and two doses of BNT162b2 provided 95% protection (95% credible interval: 90.3–97.6%) against laboratory confirmed symptomatic SARS-CoV-2 infection. However, pregnant and immune compromised individuals were excluded. The mRNA-1273 COVID-19 vaccine is another lipid encapsulated mRNA vaccine encoding the SARS-CoV-2 spike protein, manufactured by Moderna.<sup>43</sup> 30,420 adults were enrolled in the phase 3 placebo-controlled trial and vaccine efficacy was 94.1% (95% CI: 89.3–96.8%). Pregnant or breastfeeding individuals and immune compromised adults were excluded. Likewise, the phase 2/3 trial of the AstraZeneca

vaccine (ChAdOx1) involved more than 20,000 participants, all of whom were deemed healthy and/or in stable condition.<sup>44</sup>

By design, RCTs employ strict eligibility criteria and often exclude high-risk clinical populations in order to protect trial participant safety and assess vaccine efficacy in controlled and ideal conditions.<sup>45</sup> However, this prohibits the inclusion of vulnerable groups such as the immune compromised, frail and elderly, pregnant or breastfeeding, or obese.<sup>39,46,47</sup> Due to this selection bias, the results of phase 2/3 RCTs evaluating COVID-19 vaccines are less generalizable to the overall Canadian population and to key subpopulations such as the elderly, obese, and immune compromised. In other words, although the internal validity of COVID-19 vaccine RCTs is improved through the implementation of restrictive eligibility criteria, it comes at the cost of external validity or the generalizability of trial findings to other samples with differing characteristics.<sup>45</sup> This fact necessitates the conduct of high-quality observational research regarding COVID-19 vaccines in a real-world setting utilizing heterogeneous cohorts with diverse demographic and clinical characteristics represented.

Initial COVID-19 vaccine RCTs were also restricted in terms of the length of follow-up and the number of doses administered.<sup>48,49</sup> Data beyond 6 months after the second dose was often not collected or analyzed and humoral immunity dynamics were not evaluated in the long-term.<sup>42–44</sup> Therefore, the durability of COVID-19 vaccine-elicited humoral immunity in the long-term was unknown. Similarly, due to limited follow-up clinical trials do not address important issues that arise as secular changes occur over time, such as the development of new variants of concern. This information is needed to inform boosting recommendations for the general population and for vulnerable clinical groups at an increased risk of severe COVID-19 illness. We therefore rely on observational studies to characterize the durability of COVID-19 vaccine humoral immunogenicity in the long-term and elucidate the impact of multiple boosting doses given the spread of newer variants. Cohort or case-control studies on COVID-19 vaccine

humoral immunogenicity are largely restricted by the same limitations of initial COVID-19 RCTs in terms of length of follow-up and/or a low number of boosting doses.<sup>48–50</sup> The smaller number of studies analyzing the humoral response to a high number of doses (e.g., four or five doses) are face limitations by other factors, such as small sample size.<sup>51–53</sup>

The COVID-19 vaccines were constructed using established vaccine technologies (e.g., viral-vector vaccines, subunit vaccines, inactivated viruses, DNA vaccines) as well as newer technologies (mRNA vaccines).<sup>54,55</sup> mRNA vaccines are a recent innovation and although prior work has been conducted investigating potential applications in the fields of HIV, influenza, and cancer, the COVID-19 pandemic was the first time mRNA vaccines were utilised as a public health tool and administered at the population level to bolster immunity against a respiratory pathogen.<sup>56</sup> Research is needed to study the widespread use of novel COVID-19 vaccines and determine optimal strategies to maximize immunity and protection against SARS-CoV-2 in the general population and in high-risk clinical subpopulations as variants continue to emerge and circulate.<sup>54,55</sup>

Pragmatic vaccine rollout was pursued in Canada in order to maximize population coverage in light of vaccine supply constraints.<sup>39,57</sup> As a result, there was mixing of vaccine types and increased time intervals between first and second vaccine doses among recipients. The decision was made based on scientific evidence available at the time showing sufficient single dose efficacy and the aim was to improve population immunity and curb SARS-CoV-2 transmission, while prioritizing clinically vulnerable populations.<sup>39</sup> These deviations from COVID-19 vaccine clinical trial protocols provided a valuable opportunity to study on a large scale the impact of mixing vaccine types and increasing the time interval between first and second doses.

Initial research completed early in the pandemic indicated that immune compromised individuals, the obese, and excessive alcohol drinkers may be at a higher risk of acquiring a

SARS-CoV-2 infection and of experiencing severe clinical outcomes (e.g., hospitalization or mortality). From March 2020 to February 2022, unvaccinated immune compromised COVID-19 patients in the United States had an increased odds of intensive care unit admission (adjusted odds ratio [aOR]: 1.26; 95% CI: 1.08-1.49) and death following hospitalization (aOR: 1.34; 95% CI: 1.05-1.70) compared to non-immune compromised unvaccinated patients.<sup>58</sup> Elevated BMI is associated with severe COVID-19 and a higher risk of hospitalization and death.<sup>59</sup> Excessive alcohol consumption has been linked to immune activity suppression and increased susceptibility to respiratory infections such as pneumonia, raising concerns that alcohol consumption may also increase the risk of acquiring a SARS-CoV-2 infection and the risk of serious COVID-19 outcomes.<sup>60,61</sup> It was also not known how repeated vaccination would shape immunological memory in these groups. These populations are disproportionately impacted by COVID-19 and experience a high burden of disease; the need to elucidate the nuances of the humoral response to COVID-19 vaccines in these vulnerable groups is high.

Alcohol use is a prevalent public health concern. According to the 2023 Canadian Community Health Survey (CCHS), 77% and 45% of adults living in the Canadian provinces consumed at least one alcoholic drink in the previous year or in the previous week, respectively.<sup>62</sup> The magnitude of alcohol consumption—and the associated health impacts of alcohol consumption—vary across sociodemographic strata. As reported by the 2023 CCHS, very high alcohol consumption (defined by the Canadian Centre on Substance Use and Addiction as seven or more standard drinks in the past week) was more common in males (20%) than females (11%) and residents of rural communities (19%) than urban residents (14%).<sup>62</sup> Household income was also positively associated with alcohol consumption (11% of adults in the lowest income quintile drank at least seven drinks in the past week compared to 22% of adults in the highest income quintile). Conversely, younger age was correlated with lower alcohol consumption: 33% of individuals 18 to 22 years old consumed an alcoholic beverage in the past week compared to older age groups (43%-49%). The 2023 Canadian Substance Use Survey



(CSUS), which is representative of the 32.7 million Canadians 15 years of age and older living in the provinces, found that alcohol consumption also varied according to race and education level.<sup>63</sup> Of those who consumed alcohol in the past month, 27% of Indigenous respondents, 17% of Southeast Asian respondents, and 16% of Latin American respondents consumed at least five alcoholic drinks in a single occasion, whereas the proportions for other races were lower. Additionally, 22% of alcohol consumers with at most a high school diploma drank five or more standard drinks in a single occasion compared to 7.7% of those with at least a bachelor's degree. Many of these groups for which excessive alcohol consumption is more common experience heightened risk of SARS-CoV-2 infection and of severe COVID-19; the need to determine the impact of alcohol consumption on COVID-19 vaccine humoral immunogenicity is high.<sup>64</sup>

Immune imprinting is a phenomenon observed previously for influenza virus whereby initial antigenic exposure shapes the immune response upon subsequent exposure to related antigens.<sup>65</sup> Recall of cross-reactive B cells may limit the *de novo* generation of targeted B cells specific to antigenically drifted epitopes. This is of particular concern as updated vaccines targeting newer variants (e.g., Omicron) are produced and administered. Investigation into the potential role of immune imprinting in COVID-19 vaccination is warranted.

Preliminary research suggests that selective serotonin reuptake inhibitor (SSRI) use may protect against severe COVID-19 and lower the risk of post-acute sequelae (e.g., PCC). A retrospective study of 17,908 COVID-19 patients in the United States reported a lower relative risk of presumed PCC diagnosis for those taking an SSRI medication.<sup>66</sup> A scoping review of clinical trials investigating SSRIs as a treatment for COVID-19 identified that 10 of 14 studies reported a beneficial impact by SSRIs on COVID-19 morbidity and/or mortality.<sup>67</sup> Treatment with SSRI medication was associated with improved well-being and reductions in brain fog, sensory issues, chronic fatigue, and dysautonomia among 95 patients with PCC.<sup>68</sup> Conversely, a nationwide Danish study identified a higher risk of mortality following SARS-CoV-2 infection

among SSRI users (adjusted hazard ratio: 1.32; 95% CI: 1.16 -1.50; P=0.015).<sup>69</sup> A scientific consensus regarding the influence of SSRIs on COVID-19 severity and on the risk of serious clinical outcomes of SARS-CoV-2 infection is lacking. More research is needed to explore the potential use of SSRI medications as a prophylactic treatment for preventing PCC and evaluate safety in COVID-19 patients.

The 2022 Mental Health and Access to Care Survey reported that 7.6% (95% CI: 6.9-8.3%) and 5.2% (95% CI: 4.7-5.8%) of Canadians 15 years of age or older qualified for a major depressive episode or generalized anxiety disorder diagnosis within the past year.<sup>70</sup> The prevalence of mood or anxiety disorders was the highest among younger individuals, females, and White respondents. Although formal diagnoses were more common among White individuals, there is evidence suggesting that mental health symptoms are worse among racialized groups. In a 2021 Statistics Canada crowdsourcing data collection initiative, 38% of Indigenous participants reported poor to fair mental health.<sup>71</sup> A cross-sectional study of 4,220 Canadians reported a high prevalence of anxiety symptoms among Indigenous participants (48%), as well as Arab (39%), Asian (36%), and Black (30%) participants, compared to White participants (27.74 %).<sup>72</sup> Poor mental health also is associated with poverty, unemployment, and inadequate housing, all of which also increase vulnerability to SARS-CoV-2 infection.<sup>73,74</sup>

There is an urgent need to generate high-quality observational research to assess the immune response to novel COVID-19 vaccines in a real-world setting. The Stop the Spread Ottawa cohort was formed in 2020 and this thesis was conducted to meet this need and address these major knowledge gaps in the scientific literature. We conducted multiple studies in which we characterized the humoral immunogenicity of COVID-19 vaccination in the general population and in important clinical subgroups at a heightened risk of experiencing severe COVID-19 disease (the immune compromised, obese, excessive alcohol drinkers). We also generated knowledge regarding the impact of SSRI medication use on COVID-19 vaccine

humoral immunogenicity and the odds of developing PCC and explored the topic of immune imprinting as it relates to the SARS-CoV-2 and influenza viruses. The findings of this thesis will support the public health response to the COVID-19 pandemic and inform policies aimed at managing the ongoing circulation of SARS-CoV-2 variants and maximizing protection against SARS-CoV-2 infection, particularly among vulnerable clinical groups.

## **Methodological Considerations**

The Stop the Spread Ottawa cohort was formed to support analyses aimed at assessing the immune response to SARS-CoV-2 infection and vaccination.<sup>75</sup> Adult participants living in the Ottawa region were recruited starting in September 2020. Early recruitment occurred prior to COVID-19 vaccine rollout in Ontario and ensured the collection of pre-vaccination data, enabling baseline comparisons. Recruitment efforts targeted populations at high risk of SARS-CoV-2 infection due to occupation (e.g., healthcare workers, long-term care staff, homeless shelter employees, dental care workers, elementary or secondary school or daycare staff, and air travel, transit, or retail employees) or clinical risk factors (e.g., the elderly, comorbidities such as HIV infection, chronic viral hepatitis, or haematological malignancy). Relevant sample size and power calculations were conducted previously<sup>76</sup>: Based on prior research showing a clinically meaningful impact of impaired anti-S (spike) IgG (immunoglobulin G) antibody production associated with older age, we calculated a minimum sample size of 438 participants (219 participants for each comparison group) to detect a difference in anti-S IgG titers of 0.15 log(BAU/mL), given an alpha level of 0.05 and power of 80%.<sup>77–80</sup> The SSO cohort included 1,034 participants at baseline.

Serial blood draws were obtained from participants and detailed demographic and clinical data was collected in serial questionnaires. The SSO data collection protocol is outlined in Appendix Table 1. Extensive baseline data was collected, which enabled thorough adjustment for confounding. As shown in the directed acyclic graph depicted in Figure 1, there are many

important sources of confounding on the relationship between the primary exposures investigated in this thesis and the humoral immune response to COVID-19 vaccination. We identified a sufficient set for each analysis based on a priori evidence and a causal conceptual framework and included these key factors as covariates in multivariable models. Effect modification was also investigated (e.g., gender on the impact of obesity on antibody titers following vaccination).

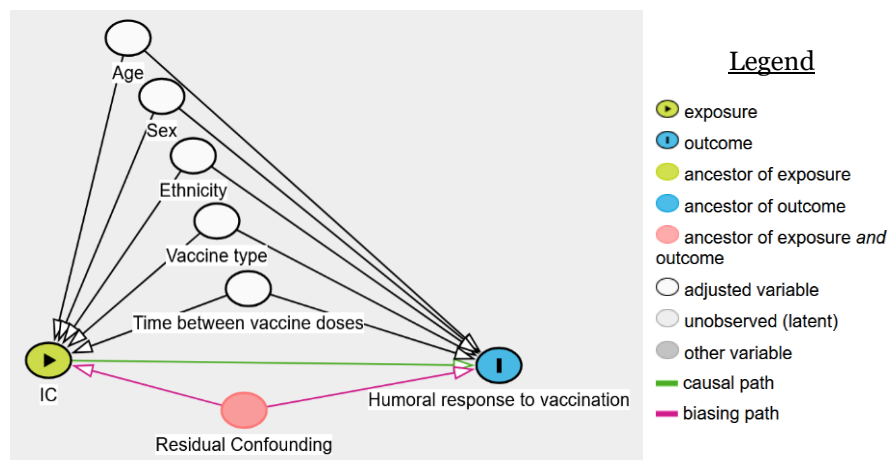


**Figure 1:** Directed acyclic graph depicting the causal framework for the relationship between immune compromised status (IC) and the humoral immune response to COVID-19 vaccination.<sup>81</sup>

## Thesis Objectives and Hypotheses

**Research Goal:** To prospectively characterize the humoral immune response to COVID-19 vaccination in key clinical groups: the immune compromised, the obese, excessive alcohol drinkers, selective serotonin reuptake inhibitor (SSRI) medication users. Immune compromising states include conditions such as tissue or organ transplant, cancer, diabetes, or HIV as well as the use of systemic immunosuppressants.

## Objectives:

- I. Describe the demographic and clinical characteristics of the SSO cohort;
- II. Identify predictors of COVID-19 vaccine humoral immunogenicity;

- III. Determine if immune compromised status, obesity, excessive alcohol consumption, or SSRI medication use influence the production and durability and/or neutralization activity of antibodies targeting SARS-CoV-2 following COVID-19 vaccination;
  - a. Longitudinally characterize antibody titers generated by COVID-19 vaccination in immune compromised individuals according to immune compromising conditions/medications and level of immunosuppression;
- IV. Identify if SSRI medication use increases or reduces the odds of developing post COVID-19 condition (PCC);
- V. Explore the impact of other factors that may influence COVID-19 in the long-term, such as immune imprinting.

### **Hypotheses:**

- I. Immune compromised status, obesity, and excessive alcohol consumption predict lower vaccine-elicited antibody titers and/or antibody neutralization activity compared to non-immune compromised status, overweight, normal weight or underweight status, and non-excessive alcohol consumption, respectively.
  - a. High-level immune compromised states (e.g., cancer, organ/tissue transplant, use of systemic immunosuppressants) are associated with a greater impairments in antibody production following vaccination compared to low-level immune compromised states (e.g., asthma/COPD, diabetes, chronic kidney disease).
- II. SSRI medication use predicts higher vaccine-elicited antibody titers compared to no SSRI medication use.
- III. SSRI medication use predicts lower odds of developing post COVID condition (PCC).

### **Overview of Chapters and Component Articles**

The component articles of this thesis are presented in **Chapter 2** to **Chapter 7**. An integrated discussion and conclusion follow in **Chapter 8**. With the exception of **Chapter 7**,

subheadings for each component chapter are organized as follows: Preface; Abstract; Introduction; Methods; Results; Discussion; Tables; Figures. The first article of this thesis (**Chapter 2**) fulfills the first and second objectives of this thesis. The entire SSO cohort is described in terms of baseline demographic and clinical data. The humoral immunogenicity and safety of the first two COVID-19 vaccine doses are reported. Data collected during the initial 10 months of follow-up was analyzed by multivariable quantile regression to identify predictors of COVID-19 vaccine humoral immunogenicity (specifically anti-S and anti-RBD IgG titers [ $\log_{10}$  BAU/mL] and neutralization activity [%] after dose two) among SSO participants with a prior SARS-CoV-2 infection at the time of vaccination and those with vaccine-elicited immunity only. The results of this study serve as the foundation of all analyses reported in the chapters that follow by providing the context for subsequent work and informing key aspects of study methodology (e.g., confounder selection). The articles presented in **Chapters 3 – 6** collectively address the third and fourth research objectives. The influence of immune status, obesity, excessive alcohol consumption and SSRI use on the humoral immune response following multiple COVID-19 vaccine doses are characterized over time. As described in the Methods section of each relevant chapter, robust statistical methods were employed which incorporated random effects, restricted cubic splines, interaction terms, robust regression, and/or a Bayesian modelling approach. Objective five is addressed in **Chapter 6**, where the adjusted odds of PCC according to SSRI medication use is reported. The final component article of this thesis is a review article (**Chapter 7**). In this review, the potential influence of immune imprinting on humoral immunity generated by boosting with updated COVID-19 vaccines containing antigens from newer variants is explored and the relevant research published to date is discussed. Together, these articles complete the research goal of this thesis to prospectively characterize the humoral immune response to COVID-19 vaccination in key clinical groups and contribute impactful new knowledge to the rapidly evolving literature on SARS-CoV-2 vaccine humoral immunogenicity.

# Chapter 2 – Results of the Stop the Spread Ottawa (SSO) cohort study: a Canadian urban-based prospective evaluation of antibody responses and neutralisation efficiency to SARS-CoV-2 infection and vaccination

## **Preface**

In this chapter, the SSO cohort is described. Baseline questionnaire data was analyzed to determine the demographic and clinical profile of the cohort. Predictors of COVID-19 vaccine humoral immunogenicity were identified by quantile regression analysis. Reactogenicity symptoms related to the first three COVID-19 vaccine doses are reported. The work presented in this chapter fulfills research objectives I and II.

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study design, manuscript preparation. CAB: study design, manuscript preparation. AC: study design, laboratory analysis, data analysis, manuscript preparation. M-AL: study design, laboratory analysis, data analysis, manuscript preparation. CLC: study design, study recruitment, data analysis, manuscript preparation. CC: guarantor.

**Ethics Approval:** All participants provided informed consent. Stop the Spread Ottawa is approved by the Ottawa Health Science Network Research Ethics Board (#2020-0481-01H).

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**Relevant Appendices:** N/A.



## **Abstract**

**Background:** Predictors of COVID-19 vaccine immunogenicity and the influence of prior severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection require elucidation.

**Methods:** Stop the Spread Ottawa is a prospective cohort of individuals at-risk for or who have been infected with SARS-CoV-2, initially enrolled for 10 months beginning October 2020. This cohort was enriched for public-facing workers. This analysis focuses on safety and immunogenicity of the initial two doses of COVID-19 vaccine. **Results:** Post-vaccination data with blood specimens were available for 930 participants. 22.8% were SARS-CoV2 infected prior to the first vaccine dose. Cohort characteristics include: median age 44 (IQR: 22–56), 66.6% women, 89.0% White, 83.2% employed. 38.1% reported two or more comorbidities and 30.8% reported immune compromising condition(s). Over 95% had detectable IgG levels against the spike and receptor binding domain (RBD) 3 months post second vaccine dose. By multivariable analysis, increasing age and high-level immune compromise predicted diminishing IgG spike and RBD titers at month 3 post second dose. IgG spike and RBD titers were higher immediately post vaccination in those with SARS-CoV-2 infection prior to first vaccination and spike titers were higher at 6 months in those with wider time intervals between dose 1 and 2. IgG spike and RBD titers and neutralisation were generally similar by sex, weight and whether receiving homogeneous or heterogeneous combinations of vaccines. Common symptoms post dose 1 vaccine included fatigue (64.7%), injection site pain (47.5%), headache (27.2%), fever/chills (26.2%) and body aches (25.3%). These symptoms were similar with subsequent doses. **Conclusion:** The initial two COVID-19 vaccine doses are safe, well-tolerated and highly immunogenic across a broad spectrum of vaccine recipients including those working in public facing environments.

## Introduction

COVID-19 vaccines have proven to be a key tool for combating the pandemic and have lowered infection rates and risk of severe disease.<sup>1,2</sup> Pragmatic vaccine roll-out in Canada resulted in extended dosing schedules beyond the time periods used in clinical trials, the mixing of vaccine types and the widespread administration of vaccines primarily based on messenger RNA (mRNA) technology.<sup>3,4</sup> As new viral variants have evolved over time, additional booster doses have been employed and bivalent vaccines have been introduced.

Clinical trials and observational population studies indicate that multiple vaccine technologies induce robust IgG levels against SARS-CoV-2.<sup>5</sup> An analysis involving 30 individuals from two randomised controlled trials (RCTs) for the ChAdOx1 vaccine in the UK (COV001 and COV002) found that longer intervals between the first and second doses (up to 44–45 weeks) resulted in higher antibody titers 28 days after the second dose. Heightened humoral response and slower antibody decay up to 6 months were observed in the Com-COV2 RCT following mixed vaccination with ChAdOx1 and then Pfizer-BioNTech or Moderna vaccines compared with homologous vaccination. However, immunogenicity varies in different populations<sup>6</sup> and the generalisability of the phase I–III randomised clinical trials for COVID-19 vaccines was limited due to restricted eligibility criteria that excluded older, younger and at-risk populations.<sup>2,7</sup> Therefore, prospective observational evaluation of COVID-19 vaccine immunogenicity in diverse populations and in real-world settings is needed to fully elucidate the role of vaccine type, time between doses, number of doses and patient characteristics (such as sex, race, age and comorbidities). The long-term durability of vaccine-mediated immunity and risk for breakthrough infections are not fully characterised. Longitudinal research is required to determine how long vaccine response persists, the level of protection provided against emerging variants of concern, and the immunogenicity of bivalent COVID-19 vaccines.

The Stop the Spread Ottawa (SSO) cohort was established in September of 2020 to address these knowledge gaps. SSO employed less restrictive eligibility criteria than clinical trials and multiple subgroups are represented in the cohort such as those working in public-facing occupations such as healthcare, childcare and the transportation sector. Additionally, older age groups, immune compromised individuals, those with multimorbidities and people with post-COVID condition (PCC) were also specifically sought.<sup>8</sup> In this paper we report on a multiparameter humoral response to COVID-19 vaccination and identify factors that influence vaccine immunogenicity in a complex, real-world population.

## **Methods**

SSO is a prospective, longitudinal, cohort study investigating immune response to SARS-CoV-2 natural infection and vaccination.<sup>8</sup> Recruitment began 14 September 2020 and was completed 28 September 2021. Enrolled participants were followed for 10 months with the option to extend for an additional 24 months. Those with a documented history of SARS-CoV-2 infection at baseline based on quantitative reverse transcription PCR (RT-PCR) or serology testing were allocated to the convalescent cohort for longitudinal sampling and participants without prior SARS-CoV-2 infection were included in the surveillance cohort. During follow-up, participants who tested positive for SARS-CoV-2 by RT-PCR or rapid antigen test (RAT) or received SARS-CoV-2 vaccine(s) were asked to cross-over from the surveillance cohort to follow the study protocol for the convalescent cohort.

Individuals 18 years or older, residing in the Ottawa-region and at-risk for SARS-CoV-2 exposure/infection due to occupation or health condition or having a documented history of COVID-19 natural infection confirmed by PCR and/or serology testing were eligible.<sup>8</sup> Recruitment efforts prioritised inclusion of individuals at-risk of SARS-CoV-2 infection based on occupation (healthcare, long-term care facilities, air travel cabin crew, dental care, teachers, daycare) or a pre-existing health condition. Participants were classified as having a

compromised immune system if they had a primary or secondary immunodeficiency, rheumatological condition, autoimmune disease, inflammatory condition, cancer, diabetes, asthma or chronic obstructive pulmonary disease requiring medication, organ/bone marrow transplant and/or received immunosuppressive treatment. Those reporting excessive alcohol consumption (men: 15 or more standard drinks a week; women: 8 or more standard drinks) were also considered immune compromised.<sup>9</sup>

Participants completed electronic questionnaires at baseline; at months 3, 10, 16, 22, 28 and 34; and following documented SARS-CoV-2 infection(s).<sup>8</sup> Information on demographics, medical history, socioeconomic and psychosocial status, exposure risk factors, acute and persistent COVID-19 symptoms and vaccine reactogenicity was collected. Self-reported COVID-19 vaccinations and positive SARS-CoV-2 diagnostic results were tracked by the study coordinator and SARS-CoV-2 infection and vaccination histories were confirmed through medical records, where available. At baseline, participants provided blood samples for serum and peripheral blood mononuclear cell (PBMC) isolation.<sup>8</sup> Convalescent participants completed monthly serum and bimonthly PBMC draws for 10 months. During months 11–34, extension participants provided blood specimens every other month. All biological specimens are stored in the Coronavirus Variants Rapid Response Network biobank, following the highest standards of biobanking practices in sample collection, processing, storage, access and distribution (on application).

IgG titers (BAU/mL) against SARS-CoV-2 spike (S), receptor-binding domain (RBD) and nucleocapsid (N) proteins in participant serum samples were quantified using a high-throughput chemiluminescent direct ELISA as previously described.<sup>8,10,11</sup> A surrogate neutralisation ELISA assay using the full-trimeric S protein from the Wuhan Hu-1 strain was performed to determine neutralisation efficiency in sera. This surrogate protein-based assay enables the quantification of spike-ACE2 interaction in a high-throughput fashion. This

approach has been validated with WHO reference serum and internal control.<sup>10</sup> Incident SARS-CoV-2 infections were identified through self-report of positive PCR and RAT results to the study coordinator and through serology testing. Anti-N IgG titers were used to distinguish between vaccine-acquired immunity and infection-acquired immunity from a prior SARS-CoV-2 infection. Participants were classified as having vaccine-based immunity if they were positive (signal-to-cut-off (SCO)>1) for anti-S and anti-RBD IgG and infection-acquired immunity if they were positive for anti-N IgG and either anti-S or anti-RBD IgG. In the case of one-off or intermittent positive serum sample results for infection-acquired immunity for individuals with no self-reported positive test results, natural immunity was determined by examining the signal strength (the magnitude of SCO) and the preceding and subsequent serum results. The date of the first positive serum sample was taken as the best available estimate of date of infection for participants with SARS-CoV-2 infections identified through serum testing.

Participant characteristics and titers were compared according to study cohort at baseline, vaccine type and immune status. Student's t-test and  $\chi^2$  statistical tests were employed for continuous and categorical variables when assumptions were met. Fisher's exact test as well as non-parametric Mann-Whitney U and Kruskal-Wallis tests were used in the case of low cell values or non-normal distribution. Monte Carlo simulation (n=1 000 000 samples) was employed to estimate p values for large contingency tables with high computational loads. Univariate and multivariable quantile regression analyses were conducted to compare log-adjusted anti-S and anti-RBD levels due to non-normal distribution of the log transformed data. Time points included week 6 post first vaccine dose (data from specimens collected from 4 to 8 weeks post first dose included in analysis), 3 months post second dose (2–4 months data included) and 6 months post second dose (4–6 months data included). Statistical tests and regression analyses were conducted using Statistical Analysis System (SAS) V.9.4 (copyright 2016 by SAS Institute, Cary, North Carolina, USA). Nested models which included or excluded the main term and interaction term were compared through a joint Wald test of the overall

models using the R package ‘quantreg’ to obtain joint p values. Plots were constructed using RStudio (ggplot2 package).

### *Patient and public involvement*

Consultation with members of the public at risk for exposure to SARS-CoV-2 was conducted during the design and implementation of this cohort study. This resulted in the creation of several ‘participant friendly’ pathways for providing serial blood specimens. A web-based, user-friendly study portal was designed which enabled participation and direct access to personal study data was created based on patient and public input. Feedback was sought and implemented throughout the conduct of this study to facilitate patient participation and retention. Consented participants assisted in recruitment as they were provided the opportunity to direct interested individuals to our study team and/or study website to explore enrolling in the SSO cohort study. Patient-focused webinars led by Dr Cooper and his study team were held during the course of the study conduct to provide SSO updates and the opportunity to ask questions related to the study and COVID-19 in general. Study updates were also provided on the SSO website. Our study coordinators and Dr Cooper also provided individualise SSO updates to participants on request. We remain deeply grateful for the considerable time, commitment, data and biological specimens provided by our SSO participants.

## **Results**

### *Participant characteristics*

A total of 1,112 participants were consented and enrolled in SSO (online Supplemental Figure 1). Participant retention was high with 67% of participants completing the initial 10 months of follow-up despite the challenges of public health measures and an arduous study protocol. Participant protocol compliance was high and the number of missing samples was low. Baseline questionnaire and serum draws were completed by 730 surveillance and 257

convalescent participants. Of participants in the surveillance cohort, 20.9% had at least one incident SARS-CoV-2 infection after enrolment, identified through self-report of a positive test result and/or serum IgG antibodies. At least one SARS-CoV-2 infection was identified in 10% of all convalescent SSO participants during the study period. Of note, assay failure rates were low (0.6%, 0.6% and 0.1% for anti-N, anti-RBD and anti-S IgG).

During the initial 10-month follow-up period, 195 individuals transitioned from the surveillance cohort into the convalescent cohort following SARS-CoV-2 infection (n=22) or vaccination (n=173). Thirty-three per cent of participants were lost to follow-up (13%) or withdrew (20%).

A high proportion of participants were women (67.1%), White (89.0%) and Canada-born (87.1%) (Table 1). A high proportion of participants (63.0%) were engaged in public facing occupations. Nearly two-thirds (64.2%) had obtained an undergraduate or advanced degree, 83.3% were employed at baseline and 65% earned at least \$C90 000 annually. Those with a history of a prior SARS-CoV-2 infection at baseline were older (mean age 46.8 vs 44.4 years) and more likely to be obese (19.1% obese vs 12.6%) than those without prior infection. The most prevalent comorbidities at baseline included allergy (42.7%), obesity (14.1%), hypertension (10.4%), dyslipidemia (9.0%), endocrine disorder (10.1%), heart disease (4.0%) and chronic neurological condition (3.0%). Current or past smoking was reported in 27.3% of participants. Just over 30% of participants were classified as immune compromised.

### *Vaccination and infection summary*

At the time of data analysis, 98.5% and 97.7% of participants had received at least one or two vaccine doses, respectively, and 70.9% had received one or more booster shot(s) (Table 2). The majority of participants (87.4%) were vaccinated with mRNA vaccines (Pfizer-BioNTech (Pfizer) (77.6% first dose and 73.7% second dose); Moderna (10.1% for dose 1 and 25.0% dose 2). Adenovirus-based ChAdOx1 vaccine (AstraZeneca) was administered to 12.3% of dose 1 and

1.2% of dose 2 recipients. The median days between the first and second COVID-19 vaccine doses was 58 (IQR: 35–76) and ranged from 11 to 344 days. The length of time in days between second and third vaccine doses was 185 IQR (IQR: 174–212). Post COVID-19 condition symptoms were reported in 224 (21.7%) participants.

#### *Humoral immune response following first and second vaccine doses*

After excluding those with history of COVID-19, 95.9% tested positive for humoral antibody evidence of vaccine-based immunity 6 weeks after dose 1. This proportion increased after full vaccination (97.7% 3 months after dose 2 and 100% 6 months after dose 2). These proportions were similar by vaccine type. Participants who tested negative for vaccine-based immunity 6 weeks after dose 1 were older (median age 59.0 (IQR: 44.0–72.5) vs 54.0 (41.0–60.0),  $p=0.03$ ) and were more likely to be immune compromised (87.5% vs 29.1%,  $p<0.01$ ) to a severe degree (85.7% vs 38.2%,  $p=0.04$ ) than those who tested positive.

An increase in log-adjusted anti-S and anti-RBD IgG titers were observed following vaccination across the cohort (Table 3, online Supplemental Figure 2). Median anti-S and anti-RBD IgG titers ( $\log_{10}$  BAU/mL) 6 weeks post dose 1 were higher with mRNA vaccination (Moderna (S: 3.348 (IQR: 3.189–3.776), RBD: 2.950 (2.645–3.461))) and Pfizer doses (S: 3.101 (IQR: 2.564–3.634), RBD: 2.643 (2.252–3.767)) compared with adenovirus vector vaccine (AstraZeneca (S: 1.893 (1.737–2.582), RBD: 1.939 (1.751–2.550))) (S:  $p<0.0001$ , RBD:  $p<0.001$ ). Median anti-S and anti-RBD IgG titers were comparable regardless of vaccine type 3 months following dose 1.

Maximum median log-adjusted anti-S and anti-RBD titers were observed following completion of the second dose schedule after which median titers declined over time until the next vaccination or natural infection (Table 3, online Supplemental Figure 2). Median anti-S and anti-RBD IgG titers were comparable regardless of vaccine type 6 months following dose 2. Receiving two mRNA vaccines resulted in a higher humoral response compared with receiving



first adenovirus vector vaccine and then an mRNA vaccine 6 weeks post dose 1 (anti-S: 3.118 (IQR: 2.573–3.637) vs 1.893 (IQR: 1.737–2.582);  $p < 0.0001$ ) but not 6 weeks post dose 2 (anti-S: 3.539 (IQR: 3.286–3.706) vs 3.680 (IQR: 3.512–4.053);  $p < 0.01$ ). The humoral response was comparable by vaccine type 3 months following dose 2 as well.

At all time points, those with a history of COVID-19 prior to first vaccination had higher median log-adjusted anti-S and anti-RBD IgG titers (Table 3).

#### *Predictors of humoral immune responses by regression analysis*

Univariate analysis was used to identify variables potentially influencing vaccine immunogenicity following the initial two doses in those without a history of SARS-CoV2 infection (data not shown). After adjusting for immune compromised status, sex, obesity, vaccine type(s) administered for dose 1 and 2, and days between first and second doses by multivariable quantile regression, older age was identified as a predictor of lower median anti-S and anti-RBD IgG titers 3 months after dose 2 for participants with no history of infection (Table 4). By 6 months following vaccine dose 2, age no longer was associated with anti-S and anti-RBD IgG titers. Immune compromised status also predicted lower median log-adjusted anti-S IgG and anti-RBD titers 3 months post dose 2. Increased time between first and second doses predicted higher anti-RBD IgG titers at month 3 and anti-S IgG titers at 6 months after dose 2.

Shorter duration of time from SARS-CoV-2 infection to dose 1 of vaccine predicted higher IgG S and RBD titer at month 6 post second dose of vaccine. No other covariates consistently predicted higher or lower anti-S or anti-RBD IgG titers for participants with SARS-CoV-2 infection history prior to vaccination (Table 4).

Consistent with IgG antibody titer findings, older age and longer time between first and second vaccine dose predicted higher neutralisation at 3 months after vaccine dose 2 in those

without SARS-CoV-2 infection prior to vaccination (Table 5, online Supplemental Figure 3). Immune compromised state also predicted diminished vaccine neutralisation at months 3 and 6 post second vaccine dose. Similar to IgG antibody titer findings, duration of time from SARS-CoV-2 infection to dose 1 of vaccine predicted higher IgG neutralisation at month 6 post second dose of vaccine.

#### *Vaccine tolerability and reactogenicity*

Results are reported for the initial two doses plus the first booster dose. No serious adverse events (SAEs) related to vaccination were reported by SSO participants. One participant died due to malignancy deemed unrelated to vaccination. Commonly reported vaccination symptoms following dose 1 included fatigue (64.7%), pain at the injection site or arm soreness (47.5%), headache (27.2%), fever or chills (26.2%) and body aches (25.3%) (online Supplemental Table 1). The type and proportion of symptoms was similar with subsequent vaccinations. Symptoms lasted the longest after dose 1 (median of 2.0 days (IQR: 1.0–3.0)) compared with subsequent vaccinations.

## **Discussion**

Large scale administration of novel COVID-19 vaccines was deployed to combat the SARS-CoV-2 pandemic. In Canada, supply constraints during initial roll-out lead to staged administration in which high-risk groups were prioritised (e.g., healthcare workers, older adults, adults in Indigenous communities), an increase in the length of time between priming and boosting doses beyond the recommended 21 days (for Pfizer) or 28 days (for Moderna) up to 4 months, and the mixing of different vaccine types.<sup>3,4,12</sup> Our analysis suggest that these strategies all ultimately achieved a potent induction of humoral immunity. In fact, a longer interval than recommended by the manufacturer between dose 1 and 2 increased durability of humoral responses.

Most SSO participants had received two mRNA vaccines at the time of analysis. mRNA and adenovirus vector COVID-19 vaccines elicited robust humoral responses that were sustained 3 months after dose 2. Crude median anti-S and anti-RBD titers ( $\log_{10}$  BAU/mL) peaked 6 weeks after the second dose and then generally decreased over time. All vaccine types demonstrated acceptable safety and tolerability profiles with no reported vaccine-specific SAEs. Mild reactogenicity symptoms were commonly reported but generally lasted a few days. Our results are consistent with prior studies which have found the mRNA COVID-19 vaccine to be overall safe and effective.<sup>13</sup>

In our analysis, vaccine-elicited antibody responses persisted 6 months post vaccination. Of note, all participants without a history of infection at baseline were found to possess evidence of humoral immunity at 6 months following the second vaccine dose. However, to date the correlates of protection for COVID-19 remains poorly defined and it is unclear if the titers reported in this study 6 months post vaccination are sufficient to protect individuals from infection, especially variants of concern, or how long this protection will last beyond 6 months.<sup>14,15</sup> Other studies have observed a gradual decline in titers following initial two dose vaccination which is consistent with our findings.<sup>6,14</sup> There is evidence that humoral responses decline at the 4–6 months point after vaccination, especially in immune compromised individuals.<sup>16,17</sup> Podrazil et al observed a sharp decline in humoral and cellular immune responses 6 months after vaccination with the mRNA Pfizer vaccine in asthma patients receiving biological therapy.<sup>16</sup> Breakthrough SARS-CoV-2 infections further raise concerns regarding the durability of protection following two doses.<sup>18</sup>

Vaccine immunogenicity was robust in young and middle age participants; many of which worked in public-facing occupations including as healthcare providers, teachers, daycare and in the transport sector. Achieving protective immunity with these occupation groups is a critical public health piece in minimising community transmission of SARS-CoV-2. Older age

predicted lower anti-S and anti-RBD IgG titers and neutralisation at 3 months following vaccination in those without prior SARS-CoV-2 infection. Immune senescence is a phenomenon that leads to chronic inflammation as well as suboptimal T cell, B cell, neutrophil and dendritic cell activity and counts.<sup>19</sup> These changes increase the risk of acquiring infections and reduce vaccine immunogenicity (e.g., as shown for influenza and herpes zoster vaccines) in the elderly.<sup>19,20</sup> Immune senescence is likely a contributing factor to the decrease in SARS-CoV-2 immunogenicity with older age, although the specific mechanism as it relates to COVID-19 remain to be characterised. The elderly are at higher risk of severe outcomes from COVID-19.<sup>21</sup> These findings, in combination with the observed accelerated decline in anti-S and anti-RBD IgG titers over time, support current recommendations to receive a booster dose 4–6 months after full vaccination.<sup>15</sup>

High level immune compromised status was associated with diminished COVID-19 vaccine humoral responses in those without prior SARS-CoV-2 infection. Adjusted median anti-S and anti-RBD titers 3 months after the second vaccine dose as well as anti-RBD and neutralisation at month 6 were lower for immune compromised participants. Our cohort includes a heterogeneous collection of immune compromising conditions including cancer, chronic inflammatory, autoimmune or rheumatological disease, HIV and those receiving immune suppressants medications (e.g., transplant recipients). Our results are consistent with other COVID-19 vaccination studies focused on immune compromised populations.<sup>6,16,22,23</sup>

There is emerging evidence that extending the time interval between the first and second COVID-19 vaccine doses improves immune responses.<sup>15,24</sup> Due to the variation in the number of days between vaccine doses received by SSO participants, we were able to prospectively investigate the impact of the time interval on vaccine immunogenicity. In those without pre-vaccine SARS-CoV-2 infection, increasing the number of days between vaccine doses was associated with higher anti-S and RBD IgG titers in the 6-month period vaccination and higher

anti-S IgG neutralisation at month 3 following vaccine dosing. These results show that concerns regarding decreased efficacy from extending the interval between doses are unwarranted and suggest a potential benefit.<sup>25</sup> Extending the vaccine schedule may improve long-term durability of COVID-19 vaccine immunogenicity and prolong protection from SARS-CoV-2 infection and severe symptoms.

SARS-CoV-2 infection prior to initial vaccination was a consistent predictor of anti-S and anti-RBD IgG antibody titers and neutralisation identified in this study. At all time points, having a history of infection increased humoral response to the vaccine. The time interval between SARS-CoV-2 infection and first vaccine predicted anti-S and anti-RBD titers as well as neutralisation in the 6-month period following the second vaccine dose. The crude difference in IgG titers compared with those with no prior infection gradually increased over time following the initial two doses of vaccine suggesting that the slope of antibody decay may be less marked in those with hybrid immunity. Recent research has found that breakthrough infections increase humoral response and neutralisation of SARS-CoV-2 variants.<sup>26,27</sup> Our results warrant further investigation into the joint effect of vaccine-mediated and infection-acquired immunity compared with either independently.

The findings in this study are subject to several strengths and limitations. The SSO cohort has a large sample size of over 1000 participants at baseline with high retention. Prospective, longitudinal data collection commenced prior to widespread vaccine roll-out enabling the collection of pre-vaccination baseline data.<sup>8</sup> Information on many different variables was collected allowing for many key covariates to be included in multivariable analyses. Self-reported infection and vaccination histories were validated using medical records. Multiple testing methods (rapid antigen tests, PCR and serology testing) were used to confirm present and past SARS-CoV-2 infections.

SSO participants are primarily White, characterised by a high level of employment and many reported high-income status. The study was conducted in a developed nation with a robust public health infrastructure. With this in mind, our results may not be fully generalisable to all settings. Adults over 80 years of age are not well represented.<sup>8</sup> Because non-White individuals comprised only 11% of our cohort, we could not explore this important variable in our evaluation of vaccine safety and immunogenicity. Blood draws occurred based on time of enrolment and were not fixed to vaccination dates. Therefore, blood samples for humoral immune assessment were not always available for each post-vaccination time point evaluated. The high-throughput chemiluminescent direct ELISA antibody assay used is subject to a range of uncertainty in the quantification method (3% false discovery rate). Natural infection histories of participants with intermittent or one-off positive results for infection-acquired immunity were manually adjusted but misclassification is possible. There is also a risk of misclassification when relying on self-reported data. Selection bias may have been introduced by the 33% of participants who were lost to follow-up or withdrew. However, we believe that the potential for selection bias is minimal as those who provided serum data and those who were lost to follow-up were similar in terms of baseline data. Our sample size was not of sufficient size to identify rare side effects of vaccination.

Despite these limitations, our analysis of a large, heterogeneous cohort followed for a prolonged duration during the SARS-CoV-2 pandemic demonstrates that the initially administered COVID-19 vaccinations were safe, well-tolerated and highly immunogenic across a broad spectrum of recipients including those employed in public-facing occupations. Our analysis continues to assess durability of vaccine immunogenicity as well as the influence of additional vaccine boosters and natural infection.

## Tables

Table 1: Characteristics of study participants (n=1,034) at baseline in the surveillance cohort (no history of COVID-19 at baseline) and convalescent cohort (history of a COVID-19 at baseline).

| Variable                                     | All participants<br>(n=1,034) | Surveillance<br>Cohort (n=803) | Convalescent<br>Cohort (n=231) | P value |
|----------------------------------------------|-------------------------------|--------------------------------|--------------------------------|---------|
| Age                                          |                               |                                |                                |         |
| Mean (SD)                                    | 45.0 (13.9)                   | 44.4 (13.7)                    | 46.8 (14.6)                    | 0.02    |
| Range (min, max)                             | 61 (18, 79)                   | 61 (18, 79)                    | 54 (22, 76)                    |         |
| Sex, n (%)                                   |                               |                                |                                |         |
| Male                                         | 340 (32.9)                    | 254 (31.6)                     | 86 (37.2)                      | 0.11    |
| Female                                       | 694 (67.1)                    | 549 (68.4)                     | 145 (62.8)                     |         |
| Missing                                      | 7                             | 7                              | 0                              |         |
| Race, n (%)                                  |                               |                                |                                |         |
| Arab/West Asian                              | 20 (1.9)                      | 15 (1.9)                       | 5 (2.2)                        | 0.49    |
| Black                                        | 9 (0.9)                       | 5 (0.6)                        | 4 (1.7)                        |         |
| Indigenous                                   | 19 (1.9)                      | 15 (1.9)                       | 4 (1.7)                        |         |
| Latin American                               | 9 (0.9)                       | 5 (0.6)                        | 4 (1.7)                        |         |
| South Asian                                  | 15 (1.5)                      | 12 (1.5)                       | 3 (1.3)                        |         |
| Southeast Asian                              | 17 (1.7)                      | 14 (1.8)                       | 3 (1.3)                        |         |
| White                                        | 916 (89.0)                    | 715 (89.6)                     | 201 (87.0)                     |         |
| Other                                        | 24 (2.33)                     | 17 (2.1)                       | 7 (3.0)                        |         |
| Missing                                      | 5                             | 5                              | 0                              |         |
| Immigration status, n (%)                    |                               |                                |                                |         |
| Born in Canada                               | 879 (87.1)                    | 682 (87.2)                     | 197 (86.8)                     | 0.87    |
| Immigrated to Canada                         | 130 (12.9)                    | 100 (12.8)                     | 30 (13.2)                      |         |
| Missing                                      | 25                            | 21                             | 4                              |         |
| Education level, n (%)                       |                               |                                |                                |         |
| Elementary school                            | 1 (0.1)                       | 0 (0)                          | 1 (0.5)                        | 0.13    |
| High school                                  | 70 (7.1)                      | 56 (7.3)                       | 14 (6.5)                       |         |
| Trade / Technical Degree /<br>Apprenticeship | 35 (3.6)                      | 25 (3.3)                       | 10 (4.6)                       |         |
| Community College                            | 219 (22.2)                    | 160 (20.8)                     | 59 (27.2)                      |         |
| University Certificate                       | 28 (2.8)                      | 23 (3.0)                       | 5 (2.3)                        |         |
| Bachelor's Degree                            | 407 (41.2)                    | 330 (42.9)                     | 77 (34.5)                      |         |
| Graduate Degree (MSc,<br>MBA, MD, PhD)       | 227 (23.0)                    | 176 (22.9)                     | 51 (23.5)                      |         |
| Prefer not to answer                         | 18                            | 11                             | 7                              |         |
| Missing                                      | 29                            | 22                             | 7                              |         |

|                                                                 |                   |                   |                   |         |
|-----------------------------------------------------------------|-------------------|-------------------|-------------------|---------|
| Annual income before taxes in the past 12 months, n (%)         |                   |                   |                   |         |
| \$Co-\$C29,999                                                  | 42 (4.6)          | 30 (4.2)          | 12 (6.1)          | 0.07    |
| \$C30,000-\$C59,999                                             | 98 (10.8)         | 68 (9.5)          | 30 (15.3)         |         |
| \$C60,000-\$C89,999                                             | 179 (19.7)        | 147 (20.6)        | 32 (16.3)         |         |
| \$C90,000-\$C119,999                                            | 197 (21.7)        | 163 (22.8)        | 34 (17.4)         |         |
| \$C120,000-\$C149,999                                           | 111 (12.2)        | 89 (12.5)         | 22 (11.2)         |         |
| \$C150,000+                                                     | 283 (31.1)        | 217 (30.4)        | 66 (33.7)         |         |
| Prefer not to answer/<br>Unknown                                | 92                | 65                | 27                |         |
| Missing                                                         | 32                | 24                | 8                 |         |
| Employed, n (%)                                                 |                   |                   |                   |         |
| Yes                                                             | 837 (83.3)        | 657 (83.8)        | 180 (81.5)        | 0.41    |
| No                                                              | 168 (16.7)        | 127 (16.2)        | 41 (18.6)         |         |
| Missing                                                         | 29                | 19                | 10                |         |
| Smoking history*, n (%)                                         |                   |                   |                   |         |
| Current Smoker                                                  | 45 (4.4)          | 37 (4.7)          | 8 (3.5)           | 0.70    |
| Former Smoker                                                   | 234 (22.9)        | 179 (22.6)        | 55 (23.8)         |         |
| Never Smoker                                                    | 745 (72.8)        | 577 (72.8)        | 168 (72.7)        |         |
| Missing                                                         | 10                | 10                | 0                 |         |
| Average number of standard drinks of alcohol consumed per week† |                   |                   |                   |         |
| Median (IQR)                                                    | 2.0 (0, 4)        | 2.0 (0, 4)        | 2.0 (0.5, 5.0)    | 0.08    |
| Range (min, max)                                                | 30 (0, 30)        | 25 (0, 25)        | 30 (0, 30)        |         |
| Missing                                                         | 11                | 8                 | 3                 |         |
| Allergies, n (%)                                                |                   |                   |                   |         |
| Yes                                                             | 437 (42.7)        | 333 (42.0)        | 104 (45.2)        | 0.38    |
| No                                                              | 586 (57.3)        | 460 (58.0)        | 126 (54.8)        |         |
| Missing                                                         | 11                | 10                | 1                 |         |
| Obesity (self-reported), n (%)                                  |                   |                   |                   |         |
| Yes                                                             | 143 (14.1)        | 99 (12.6)         | 44 (19.1)         | 0.01    |
| No                                                              | 874 (85.9)        | 688 (87.4)        | 186 (80.9)        |         |
| Missing                                                         | 17                | 16                | 1                 |         |
| Body Mass Index (BMI)‡                                          |                   |                   |                   |         |
| Median (IQR)                                                    | 26.6 (23.4, 30.4) | 26.1 (23.0, 30.1) | 28.2 (25.1, 32.8) | <0.0001 |
| Range (min, max)                                                | 38.7 (16.3, 55.1) | 38.7 (16.4, 55.1) | 33.6 (19.4, 53.0) |         |
| Missing                                                         | 528               | 404               | 124               |         |
| Immune Compromised§, n (%)                                      |                   |                   |                   |         |
| Yes                                                             | 316 (30.6)        | 247 (30.8)        | 69 (29.9)         | 0.80    |



|                                   |            |            |            |      |
|-----------------------------------|------------|------------|------------|------|
| No                                | 718 (69.4) | 556 (69.2) | 162 (70.1) |      |
| Number of Co-Morbidities          |            |            |            |      |
| Median (IQR)                      | 1 (0, 2)   | 1 (0, 2)   | 1 (0, 2)   | 0.35 |
| Range (min, max)                  | 8 (0, 8)   | 7 (0, 8)   | 8 (0, 9)   |      |
| Two or more Co-Morbidities, n (%) |            |            |            |      |
| Yes                               | 388 (37.5) | 292 (36.4) | 96 (41.6)  | 0.15 |
| No                                | 646 (62.5) | 511 (63.6) | 135 (58.4) |      |

\* Number of packs are reported for current and former smokers. Six smokers reported zero packs due to smoking a product other than cigarettes (e.g., cigars).

† One standard drink equals: 341 mL (12 oz.) bottle of 5% beer, cider, or cooler; 142 mL (5 oz.) glass of 12% wine; 43 mL (1.5 oz.) serving of 40% distilled alcohol; (e.g., rye, gin, rum, vodka).

‡ BMI is categorized as per Centers for Disease Control and Prevention: BMI<18.5, underweight; 18.5≤BMI<25 healthy weight; 25≤BMI<30, overweight; BMI≥30.0 obese.

§ Participants were classified as immune compromised if they had a primary immunodeficiency, rheumatological condition, organ-specific autoimmune disease or immune dysfunction, neuromuscular autoimmune disease, inflammatory condition, cancer, HIV, diabetes, asthma requiring medication, or were receiving immunosuppressive treatment, were an organ/bone marrow recipient, or reported excessive alcohol consumption (15+ standard drinks a week for males, 8+ standard drinks a week for females).

Table 2: Participant COVID-19 vaccination information as of analysis cut-off data (September 2022) according to cohort allocation at baseline.

| Variable                                                     | All participants<br>(n=1,034) | Surveillance<br>Cohort (n=803) | Convalescent<br>Cohort* (n=231) | P value |
|--------------------------------------------------------------|-------------------------------|--------------------------------|---------------------------------|---------|
| Vaccinated for COVID-19, n (%)                               |                               |                                |                                 |         |
| Yes                                                          | 952 (98.7)                    | 739 (99.2)                     | 213 (96.8)                      | 0.01    |
| No                                                           | 13 (1.4)                      | 6 (0.8)                        | 7 (3.2)                         |         |
| Missing                                                      | 69                            | 58                             | 11                              |         |
| Number of COVID-19 vaccine dose received, n (%)              |                               |                                |                                 |         |
| None                                                         | 13 (1.4)                      | 6 (0.8)                        | 7 (3.2)                         | <0.01   |
| 1                                                            | 8 (0.8)                       | 6 (0.8)                        | 2 (0.9)                         |         |
| 2                                                            | 259 (26.8)                    | 215 (28.9)                     | 44 (20.0)                       |         |
| 3                                                            | 562 (58.2)                    | 416 (55.8)                     | 146 (66.4)                      |         |
| 4                                                            | 119 (12.3)                    | 98 (13.2)                      | 21 (9.6)                        |         |
| 5                                                            | 4 (0.4)                       | 4 (0.5)                        | 0 (0)                           |         |
| Missing                                                      | 69                            | 58                             | 11                              |         |
| Types of COVID-19 vaccines received, dose 1, n (%)           |                               |                                |                                 |         |
| ChAdOx1                                                      | 117 (12.3)                    | 93 (12.6)                      | 24 (11.3)                       | <0.01   |
| Moderna                                                      | 96 (10.1)                     | 62 (8.4)                       | 34 (16.0)                       |         |
| Pfizer                                                       | 737 (77.6)                    | 582 (79.0)                     | 155 (72.8)                      |         |
| Missing                                                      | 84                            | 66                             | 18                              |         |
| Types of COVID-19 vaccines received, dose 2, n (%)           |                               |                                |                                 |         |
| ChAdOx1                                                      | 11 (1.2)                      | 8 (1.1)                        | 3 (1.4)                         | 0.15    |
| Pfizer                                                       | 696 (73.7)                    | 552 (75.3)                     | 144 (68.3)                      |         |
| Moderna                                                      | 236 (25.0)                    | 172 (23.5)                     | 64 (30.3)                       |         |
| Sinovac                                                      | 1 (0.1)                       | 1 (0.1)                        | 0 (0)                           |         |
| Missing                                                      | 90                            | 70                             | 20                              |         |
| Types of COVID-19 vaccine received, dose 1 and dose 2, n (%) |                               |                                |                                 |         |
| ChAdOx1-ChAdOx1                                              | 10 (1.1)                      | 7 (1.0)                        | 3 (1.4)                         | 0.051   |
| Pfizer-Pfizer                                                | 629 (67.8)                    | 501 (69.6)                     | 128 (61.5)                      |         |
| Moderna-Moderna                                              | 90 (9.7)                      | 59 (8.2)                       | 31 (14.9)                       |         |
| ChAdOx1-Pfizer                                               | 52 (5.6)                      | 42 (5.8)                       | 10 (4.8)                        |         |
| ChAdOx1-Moderna                                              | 54 (5.8)                      | 44 (6.1)                       | 10 (4.8)                        |         |
| Pfizer-Moderna                                               | 92 (9.9)                      | 66 (9.2)                       | 26 (12.5)                       |         |
| Moderna-Sinovac                                              | 1 (0.1)                       | 1 (0.1)                        | 0 (0)                           |         |

|                                                                  |               |               |               |      |
|------------------------------------------------------------------|---------------|---------------|---------------|------|
| Missing                                                          | 106           | 83            | 23            |      |
| Time between first and second<br>COVID-19 vaccine doses, in days |               |               |               |      |
| Median (IQR)                                                     | 58 (35, 76)   | 58 (35, 75)   | 60 (37, 77)   | 0.10 |
| Range (min, max)                                                 | 333 (11, 344) | 114 (11, 276) | 323 (21, 344) |      |
| Missing                                                          | 106           | 86            | 20            |      |

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\* Infection history was determined by self-reported positive COVID-19 PCR or RAT test results and serology testing. Participants tested positive for prior natural SARS-CoV-2 infection by serology if they were positive (SCO>1) for anti-N IgG antibodies and anti-S or anti-RBD IgG antibodies.

Table 3: Median log-adjusted IgG titers ( $\log_{10}$  BAU/mL) against the SARS-CoV-2 spike (S) and receptor-binding domain (RBD) proteins at time points prior to and following COVID-19 vaccination, according to COVID-19 history.<sup>1</sup>

| IgG titer<br>( $\log_{10}$ BAU/mL) | All participants<br>(n=981) | Prior Infection<br>(n=399) | No Prior Infection<br>(n=582) | P value |
|------------------------------------|-----------------------------|----------------------------|-------------------------------|---------|
| Anti-S, 6 weeks pre-dose 1         | n=224                       | n=116                      | n=108                         |         |
| Median (IQR)                       | 1.1994 (0.4433, 2.0795)     | 2.0210 (1.6920, 2.7933)    | 0.4503 (-3.0000, 0.7838)      | <0.0001 |
| Range (min, max)                   | 6.5204 (-3.0000, 3.5204)    | 6.5204 (-3.0000, 3.5204)   | 6.4761 (-3.0000, 3.4761)      |         |
| Anti-RBD, 6 weeks pre-dose 1       | n=216                       | n=116                      | n=100                         |         |
| Median (IQR)                       | 1.4395 (0.8062, 2.0255)     | 1.8986 (1.5322, 2.2269)    | 0.8318 (0.6523, 1.1498)       | <0.0001 |
| Range (min, max)                   | 3.5378 (-0.0862, 3.4517)    | 3.4261 (-0.0186, 3.4074)   | 3.5378 (-0.0862, 3.4517)      |         |
| Anti-S, 6 weeks post dose 1        | n=353                       | n=148                      | n=205                         |         |
| Median (IQR)                       | 3.4180 (2.6530, 3.9675)     | 3.9060 (3.2394, 4.2761)    | 3.0783 (2.1175, 3.6114)       | <0.0001 |
| Range (min, max)                   | 7.4508 (-3.0000, 4.4508)    | 3.4068 (1.0440, 4.4508)    | 7.4508 (-3.0000, 4.4508)      |         |
| Anti-RBD, 6 weeks post dose 1      | n=353                       | n=148                      | n=205                         |         |
| Median (IQR)                       | 3.3422 (2.4154, 3.9307)     | 3.8701 (3.3684, 4.1049)    | 2.5966 (2.2046, 3.6282)       | <0.0001 |
| Range (min, max)                   | 4.8330 (0.2533, 5.0863)     | 3.9276 (1.1587, 5.0863)    | 4.1702 (0.2533, 4.4235)       |         |
| Anti-S, 6 weeks post dose 2        | n=325                       | n=129                      | n=196                         |         |
| Median (IQR)                       | 3.5929 (3.2829, 3.8164)     | 3.7251 (2.1201, 4.0435)    | 3.5500 (3.3009, 3.7308)       | <0.01   |
| Range (min, max)                   | 7.4508 (-3.0000, 4.4508)    | 2.7421 (1.7087, 4.4508)    | 7.4508 (-3.0000, 4.4508)      |         |
| Anti-RBD, 6 weeks post dose 2      | n=326                       | n=129                      | n=197                         |         |
| Median (IQR)                       | 3.6301 (3.1780, 3.8589)     | 3.7788 (3.4144, 4.0226)    | 3.5115 (3.0347, 3.7573)       | <0.0001 |
| Range (min, max)                   | 4.6903 (0.3960, 5.0863)     | 2.8444 (2.2419, 5.0863)    | 4.0184 (0.3960, 4.4144)       |         |
| Anti-S, 3 months post dose 2       | n=576                       | n=209                      | n=367                         |         |
| Median (IQR)                       | 3.4289 (3.1848, 3.6445)     | 3.6476 (3.4323, 3.9300)    | 3.3489 (3.1217, 3.5024)       | <0.0001 |
| Range (min, max)                   | 7.4508 (-3.0000, 4.4508)    | 2.6478 (1.8030, 4.4508)    | 7.4508 (-3.0000, 4.4508)      |         |
| Anti-RBD, 3 months post dose 2     | n=571                       | n=209                      | n=362                         |         |
| Median (IQR)                       | 3.4356 (2.9762, 3.6750)     | 3.6541 (3.4112, 3.9773)    | 3.3167 (2.6394, 3.5268)       | <0.0001 |
| Range (min, max)                   | 4.4834 (0.6029, 5.0863)     | 2.7512 (2.3351, 5.0863)    | 3.8854 (0.6029, 4.4883)       |         |
| Anti-S, 6 months post dose 2       | n=214                       | n=67                       | n=147                         |         |
| Median (IQR)                       | 3.1559 (2.9350, 3.3431)     | 3.4017 (3.2046, 3.6560)    | 3.0556 (2.7678, 3.2170)       | <0.0001 |
| Range (min, max)                   | 3.1706 (1.2802, 4.4508)     | 2.4141 (2.0368, 4.4508)    | 2.4594 (1.2802, 3.7396)       |         |
| Anti-RBD, 6 months post dose 2     | n=212                       | n=65                       | n=147                         |         |
| Median (IQR)                       | 3.0262 (2.4833, 3.2788)     | 3.3432 (3.1691, 3.5881)    | 2.6349 (2.4059, 3.1500)       | <0.0001 |
| Range (min, max)                   | 3.0380 (1.1729, 4.2109)     | 2.0123 (2.1987, 4.2109)    | 2.5881 (1.1729, 3.6929)       |         |

<sup>1</sup> 399 participants had a positive COVID-19 RAT or PCR test prior to baseline or during follow-up or were positive for natural immunity by serum testing during follow-up (SCO>1 for anti-N IgG titers and either anti-S or anti-RBD titers). Samples obtained after the administration of a third vaccine dose were excluded.

Table 4: Difference in median log-adjusted IgG titers (log10 BAU/mL) against the SARS-CoV-2 spike and receptor-binding domain proteins following the initial two COVID-19 vaccine doses by multivariable quantile regression, stratified by SARS-CoV-2 infection history: A) Participants without history of SARS-CoV-2 infection<sup>1</sup>; B) Participants with a history of one SARS-CoV-2 infection prior to vaccination.<sup>2,3</sup>

| <b>A) Participants without a history of SARS-CoV-2 infection</b> |                                 |         |                                  |         |
|------------------------------------------------------------------|---------------------------------|---------|----------------------------------|---------|
| IgG Spike (log10 BAU/mL)                                         |                                 |         |                                  |         |
| Variable                                                         | 3 months post dose 2 (±1 month) |         | 6 months post dose 2 (±2 months) |         |
|                                                                  | Difference (95% CI)             | P value | Difference (95% CI)              | P value |
| Age (per 1 year increase)                                        | -0.0055 (-0.0105, -0.0006)      | 0.03    | -0.0006 (-0.0055, 0.0044)        | 0.82    |
| Sex                                                              |                                 |         |                                  |         |
| Male                                                             | 0.0567 (-0.0494, 0.1627)        | 0.29    | 0.0257 (-0.1630, 0.2143)         | 0.79    |
| Female                                                           | Referent                        |         | Referent                         |         |
| Obese                                                            |                                 |         |                                  |         |
| Yes                                                              | 0.0180 (-0.1372, 0.1732)        | 0.82    | -0.0200 (-0.2291, 0.1891)        | 0.85    |
| No                                                               | Referent                        |         | Referent                         |         |
| Immune Compromised                                               |                                 |         |                                  |         |
| Yes - High                                                       | -0.1852 (-0.3472, -0.0232)      | 0.03    | -0.1027 (-0.5690, 0.3636)        | 0.66    |
| Yes - Low                                                        | -0.1030 (-0.2205, 0.0145)       | 0.09    | -0.0706 (-0.1979, 0.0567)        | 0.28    |
| No                                                               | Referent                        |         | Referent                         |         |
| Types of COVID-19 vaccines received, dose 1 & 2 <sup>4</sup>     |                                 |         |                                  |         |
| ChAdOx1-ChAdOx1                                                  | -                               |         | -                                |         |
| mRNA-ChAdOx1                                                     | -                               |         | -                                |         |
| ChAdOx1-mRNA                                                     | 0.1114 (-0.0447, 0.2676)        | 0.16    | -                                |         |
| mRNA-mRNA                                                        | Referent                        |         | -                                |         |
| Time between first and second doses, in days                     | -0.0002 (-0.0024, 0.0020)       | 0.85    | 0.00239 (0.0007, 0.0071)         | 0.02    |
| IgG RBD (log10 BAU/mL)                                           |                                 |         |                                  |         |
| Variable                                                         | 3 months post dose 2 (±1 month) |         | 6 months post dose 2 (±2 months) |         |
|                                                                  | Difference (95% CI)             | P value | Difference (95% CI)              | P value |
| Age (per 1-year increase)                                        | -0.0145 (-0.0214, -0.0077)      | <0.0001 | -0.0003 (-0.0062, 0.0056)        | 0.91    |
| Sex                                                              |                                 |         |                                  |         |
| Male                                                             | 0.1460 (-0.0092, 0.3011)        | 0.07    | 0.5675 (0.3992, 0.7358)          | <0.0001 |
| Female                                                           | Referent                        |         | Referent                         |         |
| Obesity                                                          |                                 |         |                                  |         |

|                                                             |                            |      |                           |      |
|-------------------------------------------------------------|----------------------------|------|---------------------------|------|
| Yes                                                         | -0.0088 (-0.2615, 0.2439)  | 0.95 | -0.0697 (-0.4325, 0.2932) | 0.71 |
| No                                                          | Referent                   |      | Referent                  |      |
| Immune compromised                                          |                            |      |                           |      |
| Yes - High                                                  | -0.3890 (-0.7290, -0.0491) | 0.03 | -0.1485 (-0.5020, 0.2049) | 0.41 |
| Yes - Low                                                   | 0.0125 (-0.1849, 0.2098)   | 0.90 | -0.0880 (-0.2516, 0.0756) | 0.29 |
| No                                                          | Referent                   |      | Referent                  |      |
| Type of COVID-19 vaccines received, dose 1 & 2 <sup>4</sup> |                            |      |                           |      |
| ChAdOx1-ChAdOx1                                             | -                          |      | -                         |      |
| mRNA-ChAdOx1                                                | -                          |      | -                         |      |
| ChAdOx1-mRNA                                                | 0.2340 (-0.0526, 0.5206)   | 0.11 | -                         |      |
| mRNA-mRNA                                                   | Referent                   |      | -                         |      |
| Time between first and second doses, in days                | 0.0033 (0.0008, 0.0058)    | 0.01 | 0.0046 (-0.0047, 0.0139)  | 0.33 |

### **B) Participants with a history of SARS-CoV-2 infection**

| IgG Spike (log10 BAU/mL)                                     |                                 |         |                                  |         |
|--------------------------------------------------------------|---------------------------------|---------|----------------------------------|---------|
| Variable                                                     | 3 months post dose 2 (±1 month) |         | 6 months post dose 2 (±2 months) |         |
|                                                              | Difference (95% CI)             | P value | Difference (95% CI)              | P value |
| Age (per 1 year increase)                                    | 0.0058 (-0.0004, 0.0120)        | 0.07    | 0.0042 (-0.0028, 0.0111)         | 0.23    |
| Sex                                                          |                                 |         |                                  |         |
| Male                                                         | 0.0162 (-0.1426, 0.1751)        | 0.84    | 0.0874 (-0.0523, 0.2271)         | 0.22    |
| Female                                                       | Referent                        |         | Referent                         |         |
| Obesity                                                      |                                 |         |                                  |         |
| Yes                                                          | 0.1646 (-0.1166, 0.4458)        | 0.25    | -                                |         |
| No                                                           | Referent                        |         | -                                |         |
| Immune Compromised                                           |                                 |         |                                  |         |
| Yes - High                                                   | 0.0636 (-0.3079, 0.4351)        | 0.74    | -0.1055 (-0.4373, 0.2262)        | 0.53    |
| Yes - Low                                                    | 0.0838 (-0.0963, 0.2639)        | 0.36    | 0.2391 (-0.0414, 0.5195)         | 0.09    |
| No                                                           | Referent                        |         | Referent                         |         |
| Types of COVID-19 vaccines received, dose 1 & 2 <sup>4</sup> |                                 |         |                                  |         |
| ChAdOx1-mRNA                                                 | -0.1497 (-0.5913, 0.2918)       | 0.50    | -                                |         |
| mRNA-mRNA                                                    | Referent                        |         | -                                |         |
| Time between first and second doses, in days                 | -0.0012 (-0.0050, 0.0025)       | 0.52    | 0.0008 (-0.0027, 0.0044)         | 0.64    |
| Time from SARS-CoV-2 infection to dose 1                     | -0.0025 (-0.0055, 0.0005)       | 0.11    | -0.0021 (-0.0047, 0.0006)        | 0.12    |

| Quadratic term                                              | 8.0E-6 (6.0E-7, 2.0E-5)<br>0.18 (joint) <sup>5</sup> | 0.07    | 7.1E-6 (-3.5E-7, 1.5E5)<br>0.03 (joint) <sup>5</sup> | 0.06    |
|-------------------------------------------------------------|------------------------------------------------------|---------|------------------------------------------------------|---------|
| IgG RBD (log10 BAU/mL)                                      |                                                      |         |                                                      |         |
| Variable                                                    | 3 months post dose 2 (±1 month)                      |         | 6 months post dose 2 (±2 months)                     |         |
|                                                             | Difference (95% CI)                                  | P value | Difference (95% CI)                                  | P value |
| Age (per 1-year increase)                                   | 0.0072 (-0.0003, 0.0147)                             | 0.06    | 0.0037 (-0.0024, 0.0098)                             | 0.23    |
| Sex                                                         |                                                      |         |                                                      |         |
| Male                                                        | 0.1040 (-0.0735, 0.2814)                             | 0.25    | -0.0057 (-0.1852, 0.1739)                            | 0.95    |
| Female                                                      | Referent                                             |         | Referent                                             |         |
| Obesity                                                     |                                                      |         |                                                      |         |
| Yes                                                         | 0.1213 (-0.2529, 0.4954)                             | 0.52    | -                                                    |         |
| No                                                          | Referent                                             |         | -                                                    |         |
| Immune compromised                                          |                                                      |         |                                                      |         |
| Yes - High                                                  | 0.1029 (-0.1950, 0.4008)                             | 0.50    | -0.0216 (-0.3941, 0.3510)                            | 0.91    |
| Yes - Low                                                   | 0.0474 (-0.2144, 0.3093)                             | 0.72    | 0.2766 (0.0791, 0.4741)                              | <0.01   |
| No                                                          | Referent                                             |         | Referent                                             |         |
| Type of COVID-19 vaccines received, dose 1 & 2 <sup>4</sup> |                                                      |         |                                                      |         |
| ChAdOx1-mRNA                                                | -0.1625 (-0.5915, 0.2665)                            | 0.45    | -                                                    |         |
| mRNA-mRNA                                                   | Referent                                             |         | -                                                    |         |
| Time between first and second doses, in days                | -0.0013 (-0.0055, 0.0028)                            | 0.53    | -0.0008 (-0.0044, 0.0029)                            | 0.68    |
| Time from SARS-CoV-2 infection to dose 1                    | -0.0038 (-0.0075, -0.0001)                           | 0.04    | -0.0016 (-0.0043, 0.0010)                            | 0.22    |
| Quadratic term                                              | 4.6E-6 (1.0E-6, 1.9E-5)<br>0.11 (joint) <sup>5</sup> | 0.03    | 7.0E-6 (2.5E-8, 1.4E5)<br>0.02 (joint) <sup>5</sup>  | 0.05    |

<sup>1</sup> Samples obtained after the administration of a third vaccine dose, a self-reported positive COVID-19 RAT or PCR test, or natural immunity identified by serum testing (SCO>1 for anti-N IgG titers and either anti-S or anti-RBD titers) were excluded.

<sup>2</sup> Samples obtained after the administration of a third vaccine dose, prior to a self-reported positive COVID-19 RAT or PCR test, natural immunity identified by serum testing (SCO>1 for anti-N IgG titers and either anti-S or anti-RBD titers) or following a second positive COVID-19 test were excluded.

<sup>3</sup> Participants were classified as obese if they had a BMI of 35 or greater. IC severity was classified high if participants were receiving immunosuppressive treatment, had active HIV, cancer, had received an organ/bone marrow transplant, had a primary immunodeficiency, a neuromuscular autoimmune diseases. IC was low if the participant had localized autoimmune disease, excessive alcohol consumption, fully suppressed HIV, or diabetes.

<sup>4</sup> Participant who received AstraZeneca vaccines for dose 1 and 2 was excluded due to small sample size.

<sup>5</sup> Nested models which included or excluded the main term and interaction term were compared through a joint Wald test of the overall models using the R package 'quantreg' to obtain joint p values.

Table 5: Difference in median IgG neutralization percentage (%) against the Wuhan-Hu-1 SARS-CoV-2 spike protein following two COVID-19 vaccine doses in those without a history of SARS-CoV-2 infection<sup>1</sup> (A) and those with a history of one SARS-CoV-2 infection prior to vaccination<sup>2</sup> (B) by multivariable quantile regression.<sup>3</sup>

| <b>A) Participants without a history of SARS-CoV-2 infection</b> |                                 |         |                                  |         |
|------------------------------------------------------------------|---------------------------------|---------|----------------------------------|---------|
| Neutralization percentage against Spike protein (%)              |                                 |         |                                  |         |
| Variable                                                         | 3 months post dose 2 (±1 month) |         | 6 months post dose 2 (±2 months) |         |
|                                                                  | Difference (95% CI)             | P value | Difference (95% CI)              | P value |
| Age (per 1 year increase)                                        | -1.1677 (-1.7221, -0.6133)      | <0.01   | -0.0689 (-0.5069, 0.3690)        | 0.77    |
| Sex                                                              |                                 |         |                                  |         |
| Male                                                             | 18.2007 (5.0952, 31.3061)       | <0.01   | 9.5861 (-10.2553, 29.4276)       | 0.34    |
| Female                                                           | Referent                        |         | Referent                         |         |
| Obese                                                            |                                 |         |                                  |         |
| Yes                                                              | -18.9974 (-45.9127, 7.9179)     | 0.17    | -4.0507 (-25.0579, 16.9564)      | 0.70    |
| No                                                               | Referent                        |         | Referent                         |         |
| Immune Compromised                                               |                                 |         |                                  |         |
| Yes – High                                                       | 0.7463 (-22.7513, 24.2439)      | 0.95    | -7.8661 (-32.8323, 17.1000)      | 0.54    |
| Yes – Low                                                        | -25.3594 (-42.7558, -7.9631)    | <0.01   | -20.8856 (-34.2326, -7.5386)     | <0.01   |
| No                                                               | Referent                        |         | Referent                         |         |
| Types of COVID-19 vaccines received, dose 1 & 2 <sup>4</sup>     |                                 |         |                                  |         |
| ChAdOx1-mRNA                                                     | 19.9578 (-1.0877, 41.0033)      | 0.06    | -                                |         |
| mRNA-mRNA                                                        | Referent                        |         | -                                |         |
| Time between first and second doses, in days                     | 0.4735 (0.2418, 0.7052)         | <0.01   | 0.0591 (-0.4208, 0.5390)         | 0.81    |
| <b>B) Participants with a history of SARS-CoV-2 infection</b>    |                                 |         |                                  |         |
| Neutralization percentage against Spike protein (%)              |                                 |         |                                  |         |
| Variable                                                         | 3 months post dose 2 (±1 month) |         | 6 months post dose 2 (±2 months) |         |
|                                                                  | Difference (95% CI)             | P value | Difference (95% CI)              | P value |
| Age (per 1 year increase)                                        | 0.1001 (-0.1575, 0.3577)        | 0.44    | 0.0948 (-0.3506, 0.5403)         | 0.67    |
| Sex                                                              |                                 |         |                                  |         |
| Male                                                             | 1.0307 (-4.8217, 6.8832)        | 0.73    | -0.9827 (-11.6283, 9.6629)       | 0.86    |
| Female                                                           | Referent                        |         | Referent                         |         |
| Obese                                                            |                                 |         |                                  |         |
| Yes                                                              | -2.7891 (-13.2144, 7.6362)      | 0.60    | -                                |         |
| No                                                               | Referent                        |         | -                                |         |
| Immune Compromised                                               |                                 |         |                                  |         |



|                                                              |                             |      |                             |      |
|--------------------------------------------------------------|-----------------------------|------|-----------------------------|------|
| Yes – High                                                   | -2.7280 (-11.5589, 6.1029)  | 0.54 | -33.4104 (-67.5840, 0.7633) | 0.06 |
| Yes – Low                                                    | 3.4292 (-3.5250, 10.3835)   | 0.33 | 7.3644 (-6.1602, 20.8891)   | 0.28 |
| No                                                           | Referent                    |      | Referent                    |      |
| Types of COVID-19 vaccines received, dose 1 & 2 <sup>4</sup> |                             |      |                             |      |
| ChAdOx1-mRNA                                                 | -7.6800 (-31.4146, 16.0546) | 0.52 | -                           |      |
| mRNA-mRNA                                                    | Referent                    |      | -                           |      |
| Time between first and second doses, in days                 | -0.0158 (-0.1803, 0.1487)   | 0.85 | -0.1695 (-0.5095, 0.1704)   | 0.33 |
| Time from prior SARS-CoV-2 infection to dose 1               | -0.0411 (-0.1532, 0.0710)   | 0.47 | 0.0839 (-0.1171, 0.2848)    | 0.41 |
|                                                              | 0.0001 (-0.0001, 0.0004)    | 0.28 | 0.0001 (-0.0004, 0.0006)    | 0.83 |
| Quadratic term                                               | 0.231 (joint) <sup>5</sup>  |      | <0.01 (joint) <sup>5</sup>  |      |

<sup>1</sup> Samples obtained after the administration of a third vaccine dose, a self-reported positive COVID-19 RAT or PCR test, or natural immunity identified by serum testing (SCO>1 for anti-N IgG titers and either anti-S or anti-RBD titers) were excluded.

<sup>2</sup> Samples obtained after the administration of a third vaccine dose, prior to a self-reported positive COVID-19 RAT or PCR test, or natural immunity identified by serum testing (SCO>1 for anti-N IgG titers and either anti-S or anti-RBD titers) or following a second positive COVID-19 test were excluded.

<sup>3</sup> Participants were classified as obese if they had a BMI of 35 or greater. IC severity was classified high if participants were receiving immunosuppressive treatment, had active HIV, cancer, had received an organ/bone marrow transplant, had a primary immunodeficiency, or a neuromuscular autoimmune diseases. IC was low if the participant had localized disease, excessive alcohol consumption, fully suppressed HIV, or diabetes.

<sup>4</sup> Participants who received Sinovac for dose 2 and 3 and participants who received AstraZeneca for dose 1 and dose 2 were excluded due to small sample size.

<sup>5</sup> Nested models which included or excluded the main term and interaction term were compared through a joint Wald test of the overall models using the R package ‘quantreg’ to obtain joint p values.

Supplemental Table 1: IgG titers against the SARS-CoV-2 spike (S) and receptor-binding domain (RBD) proteins at time points prior to and following COVID-19 vaccination according to COVID-19 history and data transformation and treatment (means of raw [A; BAU/mL] and log-adjusted titers [B; log<sub>10</sub> BAU/mL] and geometric mean titers [C; BAU/mL] are presented).<sup>†</sup>

| A. Mean IgG Titer (BAU/mL)     |                               |                                 |                             |         |
|--------------------------------|-------------------------------|---------------------------------|-----------------------------|---------|
| IgG Titer (BAU/mL)             | All participants (n=981)      | Prior Infection (n=399)         | No Prior Infection (n=582)  | P value |
| Anti-S, 6 weeks pre-dose 1     | n=224                         | n=116                           | n=108                       |         |
| Mean (SD)                      | 226.42 (501.27)               | 395.68 (584.99)                 | 44.62 (302.41)              | <0.0001 |
| Range (min, max)               | 3,314.63 (0.00, 3,314.63)     | 3,314.63 (0.00, 3,314.63)       | 2,993.07 (0.00, 2,993.07)   |         |
| Anti-RBD, 6 weeks pre-dose 1   | n=216                         | n=116                           | n=100                       |         |
| Mean (SD)                      | 139.59 (363.11)               | 209.74 (397.55)                 | 58.21 (300.48)              | <0.0001 |
| Range (min, max)               | 2,828.28 (0.82, 2,829.10)     | 2,554.22 (0.96, 2,555.18)       | 2,828.28 (0.82, 2,829.10)   |         |
| Anti-S, 6 weeks post dose 1    | n=353                         | n=148                           | n=205                       |         |
| Mean (SD)                      | 6,838.11 (9,040.06)           | 11,457.11 (10,504.54)           | 3,503.42 (5,902.95)         | <0.0001 |
| Range (min, max)               | 28,237.10 (0.00, 28,237.10)   | 28,226.03 (11.07, 28,237.10)    | 28,237.10 (0.00, 28,237.10) |         |
| Anti-RBD, 6 weeks post dose 1  | n=353                         | n=148                           | n=205                       |         |
| Mean (SD)                      | 6,474.00 (12,984.89)          | 11,259.06 (18,245.70)           | 3,019.42 (4,736.92)         | <0.0001 |
| Range (min, max)               | 121,985.61 (1.79, 121,987.40) | 121,972.99 (14.41, 121,987.40)  | 26,514.61 (1.79, 26,516.40) |         |
| Anti-S, 6 weeks post dose 2    | n=325                         | n=129                           | n=196                       |         |
| Mean (SD)                      | 6,127.24 (7,284.35)           | 8,745.08 (9,633.77)             | 4,404.27 (4,441.35)         | <0.01   |
| Range (min, max)               | 28,237.10 (0.00, 28,237.10)   | 28,185.97 (51.13, 28,237.10)    | 28,237.10 (0.00, 28,237.10) |         |
| Anti-RBD, 6 weeks post dose 2  | n=326                         | n=129                           | n=197                       |         |
| Mean (SD)                      | 6,518.78 (11,522.30)          | 10,323.45 (17,038.59)           | 4,027.40 (3,845.10)         | <0.0001 |
| Range (min, max)               | 121,984.91 (2.49, 121,987.40) | 121,812.84 (174.56, 121,987.40) | 25,962.91 (2.49, 25,965.40) |         |
| Anti-S, 3 months post dose 2   | n=576                         | n=209                           | n=367                       |         |
| Mean (SD)                      | 4,530.05 (6,260.28)           | 8,011.67 (8,818.77)             | 2,547.33 (2,551.34)         | <0.0001 |
| Range (min, max)               | 28,237.10 (0.00, 28,237.10)   | 28,173.57 (63.53, 28,237.10)    | 28,237.07 (0.00, 28,237.07) |         |
| Anti-RBD, 3 months post dose 2 | n=571                         | n=209                           | n=362                       |         |
| Mean (SD)                      | 5,793.95 (14,999.06)          | 11,605.19 (23,467.67)           | 2,438.85 (2,657.20)         | <0.0001 |
| Range (min, max)               | 121,982.99 (4.01, 121,987.00) | 121,770.68 (216.32, 121,987.00) | 30,780.79 (4.01, 30,784.8)  |         |

|                                               |                              |                               |                            |         |
|-----------------------------------------------|------------------------------|-------------------------------|----------------------------|---------|
| Anti-S, 6 months post dose 2                  | n=214                        | n=67                          | n=147                      |         |
| Mean (SD)                                     | 1,933.77 (2,616.42)          | 3,542.01 (4,062.97)           | 1,200.77 (892.67)          | <0.0001 |
| Range (min, max)                              | 28,218.01 (19.06, 28,237.07) | 28,128.23 (108.84, 28,237.07) | 5,471.36 (19.06, 5,490.42) |         |
| Anti-RBD, 6 months post dose 2                | n=212                        | n=65                          | n=147                      |         |
| Mean (SD)                                     | 1,571.27 (2,003.64)          | 3,071.05 (2,822.86)           | 908.10 (929.86)            | <0.0001 |
| Range (min, max)                              | 16,238.11 (14.89, 16,253.00) | 16,094.99 (158.01, 16,253.00) | 4,915.31 (14.89, 4,930.2)  |         |
| B. Mean Log-adjusted IgG Titer (log10 BAU/mL) |                              |                               |                            |         |
| IgG Titer (log10 BAU/mL)                      | All participants (n=981)     | Prior Infection (n=399)       | No Prior Infection (n=582) | P value |
| Anti-S, 6 weeks pre-dose 1                    | n=224                        | n=116                         | n=108                      |         |
| Mean (SD)                                     | 0.88 (1.88)                  | 2.02 (1.07)                   | -0.36 (1.78)               | <0.0001 |
| Range (min, max)                              | 6.52 (-3.00, 3.52)           | 6.52 (-3.00, 3.52)            | 6.48 (-3.00, 3.48)         |         |
| Anti-RBD, 6 weeks pre-dose 1                  | n=216                        | n=116                         | n=100                      |         |
| Mean (SD)                                     | 1.46 (0.77)                  | 1.88 (0.64)                   | 0.97 (0.59)                | <0.0001 |
| Range (min, max)                              | 3.54 (-0.09, 3.45)           | 3.43 (-0.02, 3.41)            | 3.54 (-0.09, 3.45)         |         |
| Anti-S, 6 weeks post dose 1                   | n=353                        | n=148                         | n=205                      |         |
| Mean (SD)                                     | 3.22 (0.98)                  | 3.65 (0.84)                   | 2.92 (0.96)                | <0.0001 |
| Range (min, max)                              | 7.45 (-3.00, 4.45)           | 3.41 (1.04, 4.45)             | 7.45 (-3.00, 4.45)         |         |
| Anti-RBD, 6 weeks post dose 1                 | n=353                        | n=148                         | n=205                      |         |
| Mean (SD)                                     | 3.15 (0.92)                  | 3.63 (0.74)                   | 2.80 (0.88)                | <0.0001 |
| Range (min, max)                              | 4.83 (0.25, 5.09)            | 3.93 (1.16, 5.09)             | 4.17 (0.25, 4.42)          |         |
| Anti-S, 6 weeks post dose 2                   | n=325                        | n=129                         | n=196                      |         |
| Mean (SD)                                     | 3.38 (0.85)                  | 3.41 (0.94)                   | 3.36 (0.80)                | <0.01   |
| Range (min, max)                              | 7.45 (-3.00, 4.45)           | 2.74 (1.71, 4.45)             | 7.45 (-3.00, 4.45)         |         |
| Anti-RBD, 6 weeks post dose 2                 | n=326                        | n=129                         | n=197                      |         |
| Mean (SD)                                     | 3.44 (0.67)                  | 3.63 (0.66)                   | 3.32 (0.66)                | <0.0001 |
| Range (min, max)                              | 4.69 (0.40, 5.09)            | 2.84 (2.24, 5.09)             | 4.02 (0.40, 4.41)          |         |
| Anti-S, 3 months post dose 2                  | n=576                        | n=209                         | n=367                      |         |
| Mean (SD)                                     | 3.34 (0.65)                  | 3.59 (0.65)                   | 3.20 (0.61)                | <0.0001 |
| Range (min, max)                              | 7.45 (-3.00, 4.45)           | 2.65 (1.80, 4.45)             | 7.45 (-3.00, 4.45)         |         |
| Anti-RBD, 3 months post dose 2                | n=571                        | n=209                         | n=362                      |         |
| Mean (SD)                                     | 3.32 (0.64)                  | 3.65 (0.60)                   | 3.13 (0.59)                | <0.0001 |
| Range (min, max)                              | 4.48 (0.60, 5.09)            | 2.75 (2.34, 5.09)             | 3.89 (0.60, 4.49)          |         |
| Anti-S, 6 months post dose 2                  | n=214                        | n=67                          | n=147                      |         |

| Mean (SD)                            | 3.02 (0.56)                   | 3.34 (0.50)                     | 2.88 (0.52)                 | <0.0001              |
|--------------------------------------|-------------------------------|---------------------------------|-----------------------------|----------------------|
| Range (min, max)                     | 3.17 (1.28, 4.45)             | 2.41 (2.04, 4.45)               | 2.46 (1.28, 3.74)           |                      |
| Anti-RBD, 6 months post dose 2       | n=212                         | n=65                            | n=147                       |                      |
| Mean (SD)                            | 2.91 (0.54)                   | 3.31 (0.43)                     | 2.73 (0.48)                 | <0.0001              |
| Range (min, max)                     | 3.04 (1.17, 4.21)             | 2.01 (2.20, 4.21)               | 2.59 (1.17, 3.69)           |                      |
| C. Geometric Mean IgG Titer (BAU/mL) |                               |                                 |                             |                      |
| IgG Titer (BAU/mL)                   | All participants (n=981)      | Prior Infection (n=399)         | No Prior Infection (n=582)  | P value <sup>‡</sup> |
| Anti-S, 6 weeks pre-dose 1           | n=224                         | n=116                           | n=108                       |                      |
| Geometric Mean (Geometric SD)        | 7.50 (75.46)                  | 104.67 (11.81)                  | 0.44 (60.16)                | <0.0001              |
| Range (min, max)                     | 3,314.63 (0.00, 3,314.63)     | 3,314.63 (0.00, 3,314.63)       | 2,993.07 (0.00, 2,993.07)   |                      |
| Anti-RBD, 6 weeks pre-dose 1         | n=216                         | n=116                           | n=100                       |                      |
| Geometric Mean (Geometric SD)        | 28.83 (5.84)                  | 76.46 (4.34)                    | 9.30 (3.90)                 | <0.0001              |
| Range (min, max)                     | 2,828.28 (0.82, 2,829.10)     | 2,554.22 (0.96, 2,555.18)       | 2,828.28 (0.82, 2,829.10)   |                      |
| Anti-S, 6 weeks post dose 1          | n=353                         | n=148                           | n=205                       |                      |
| Geometric Mean (Geometric SD)        | 1,670.21 (9.49)               | 4,420.39 (6.90)                 | 827.20 (9.08)               | <0.0001              |
| Range (min, max)                     | 28,237.10 (0.00, 28,237.10)   | 28,226.03 (11.07, 28,237.10)    | 28,237.10 (0.00, 28,237.10) |                      |
| Anti-RBD, 6 weeks post dose 1        | n=353                         | n=148                           | n=205                       |                      |
| Geometric Mean (Geometric SD)        | 1,403.47 (8.34)               | 4,253.35 (5.48)                 | 630.33 (7.65)               | <0.0001              |
| Range (min, max)                     | 121,985.61 (1.79, 121,987.40) | 121,972.99 (14.41, 121,987.40)  | 26,514.61 (1.79, 26,516.40) |                      |
| Anti-S, 6 weeks post dose 2          | n=325                         | n=129                           | n=196                       |                      |
| Geometric Mean (Geometric SD)        | 2,381.13 (7.11)               | 2,549.49 (8.61)                 | 2,276.43 (6.23)             | <0.01                |
| Range (min, max)                     | 28,237.10 (0.00, 28,237.10)   | 28,185.97 (51.13, 28,237.10)    | 28,237.10 (0.00, 28,237.10) |                      |
| Anti-RBD, 6 weeks post dose 2        | n=326                         | n=129                           | n=197                       |                      |
| Geometric Mean (Geometric SD)        | 2,774.82 (4.71)               | 4,309.36 (4.51)                 | 2,079.92 (4.54)             | <0.0001              |
| Range (min, max)                     | 121,984.91 (2.49, 121,987.40) | 121,812.84 (174.56, 121,987.40) | 25,962.91 (2.49, 25,965.40) |                      |
| Anti-S, 3 months post dose 2         | n=576                         | n=209                           | n=367                       |                      |

|                                   |                                  |                                    |                                |         |
|-----------------------------------|----------------------------------|------------------------------------|--------------------------------|---------|
| Geometric Mean<br>(Geometric SD)  | 2,205.53 (4.50)                  | 3,912.03 (4.43)                    | 1,591.38 (4.11)                | <0.0001 |
| Range (min, max)                  | 28,237.10 (0.00,<br>28,237.10)   | 28,173.57 (63.53,<br>28,237.10)    | 28,237.07 (0.00,<br>28,237.07) |         |
| Anti-RBD, 3 months post<br>dose 2 | n=571                            | n=209                              | n=362                          |         |
| Geometric Mean<br>(Geometric SD)  | 2,077.90 (4.40)                  | 4,459.06 (3.95)                    | 1,337.10 (3.88)                | <0.0001 |
| Range (min, max)                  | 121,982.99 (4.01,<br>121,987.00) | 121,770.68 (216.32,<br>121,987.00) | 30,780.79 (4.01,<br>30,784.80) |         |
| Anti-S, 6 months post dose 2      | n=214                            | n=67                               | n=147                          |         |
| Geometric Mean<br>(Geometric SD)  | 1,053.39 (3.60)                  | 2,178.80 (3.16)                    | 756.37 (3.32)                  | <0.0001 |
| Range (min, max)                  | 28,218.01 (19.06,<br>28,237.07)  | 28,128.23 (108.84,<br>28,237.07)   | 5,471.36 (19.06,<br>5,490.42)  |         |
| Anti-RBD, 6 months post<br>dose 2 | n=212                            | n=65                               | n=147                          |         |
| Geometric Mean<br>(Geometric SD)  | 807.73 (3.44)                    | 2,057.91 (2.69)                    | 534.16 (3.02)                  | <0.0001 |
| Range (min, max)                  | 16,238.11 (14.89,<br>16,253.00)  | 16,094.99 (158.01,<br>16,253.00)   | 4,915.31 (14.89,<br>4,930.20)  |         |

† 399 participants had a positive COVID-19 RAT or PCR test prior to baseline or during follow-up or were positive for natural immunity by serum testing during follow-up (SCO>1 for anti-N IgG titers and either anti-S or anti-RBD titers). Samples obtained after the administration of a third vaccine dose were excluded.

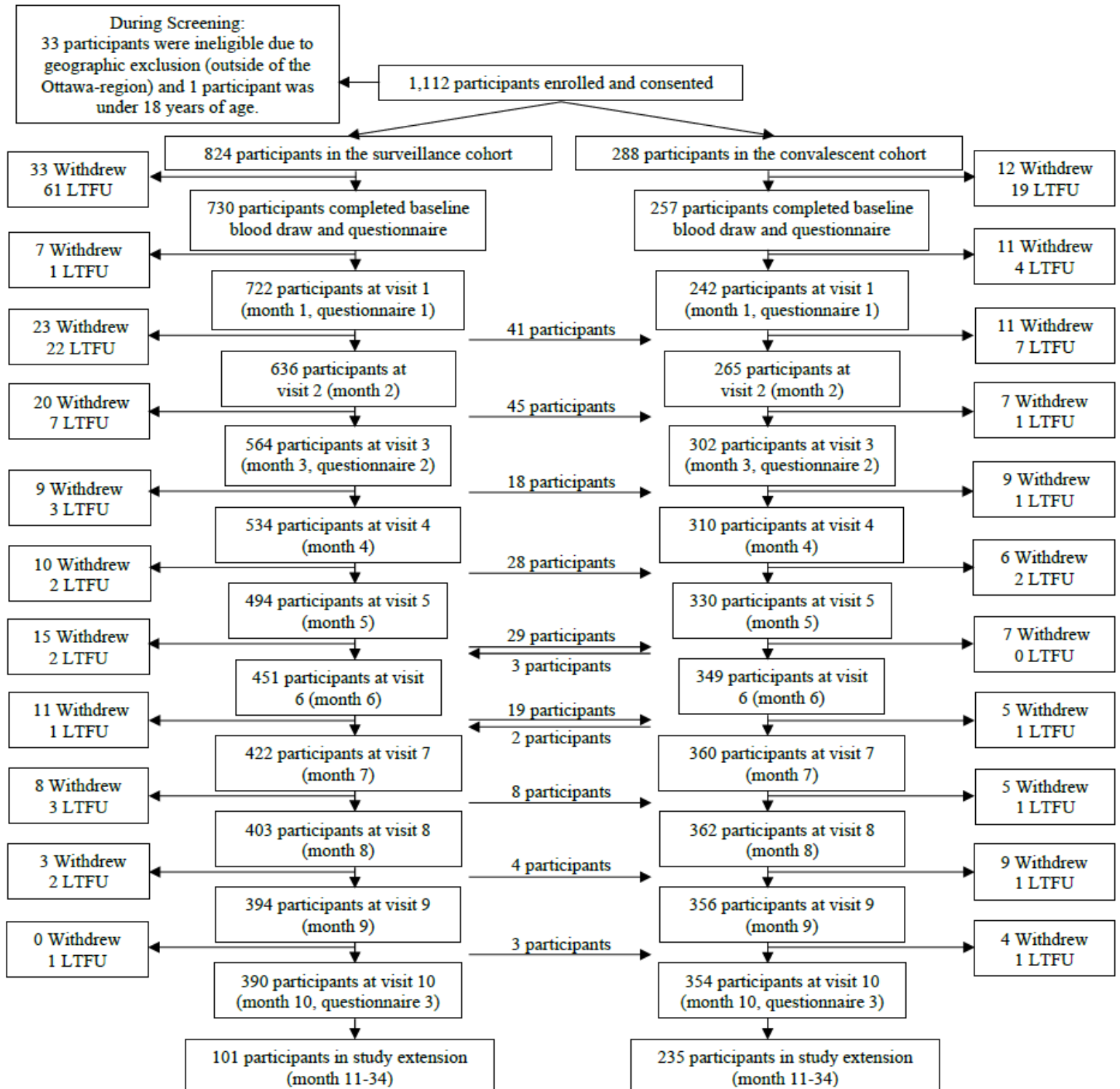
‡ For comparing geometric mean titers, the Mann-Whitney U test was conducted using natural log-adjusted titers.

Supplemental Table 2: Reported signs and symptoms following COVID-19 vaccination.

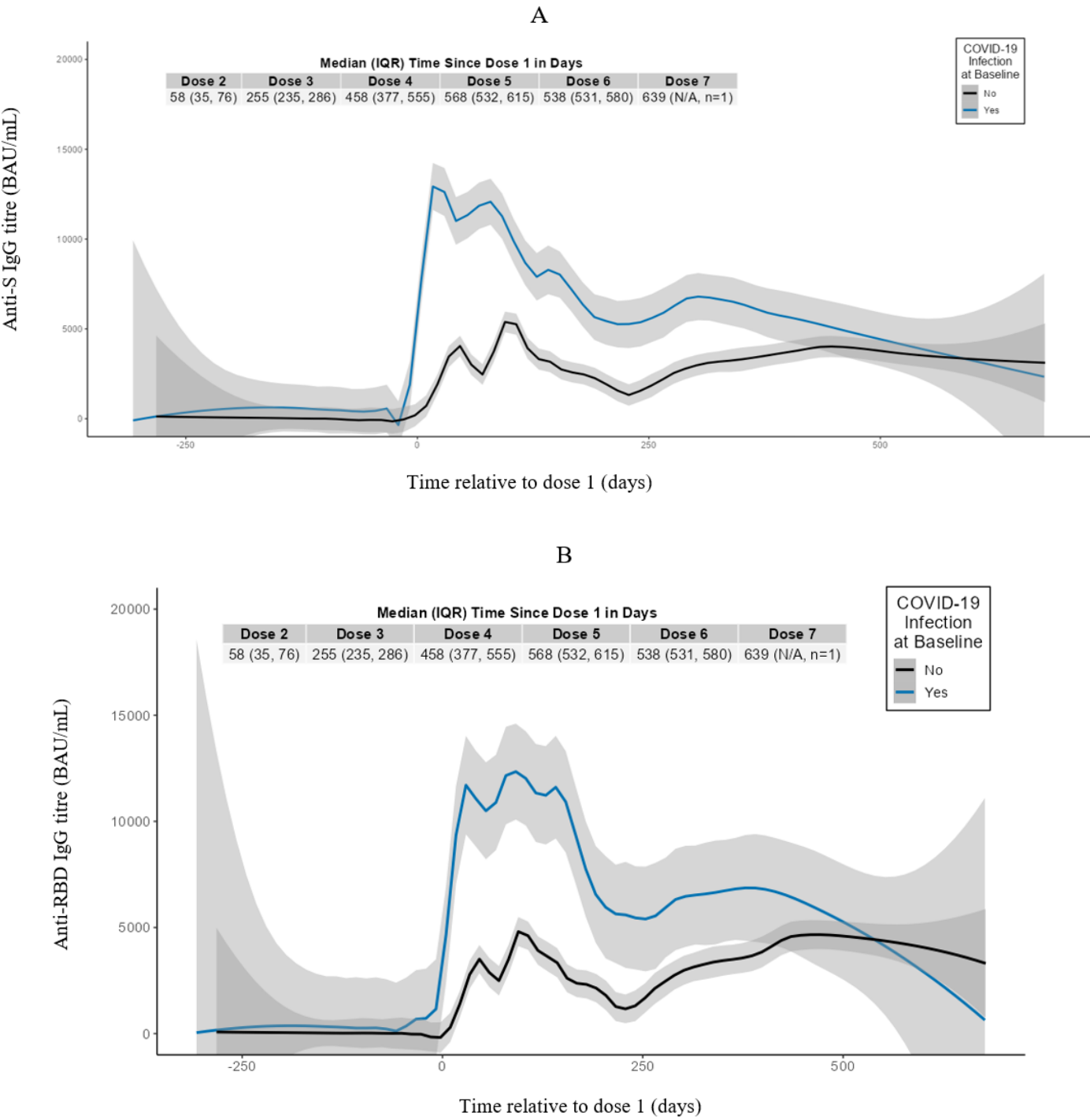
| a) Signs and Symptoms                                               |                  |                |                |
|---------------------------------------------------------------------|------------------|----------------|----------------|
|                                                                     | Frequency, n (%) |                |                |
| Sign or Symptom                                                     | Dose 1 (n=221)   | Dose 2 (n=292) | Dose 3 (n=187) |
| Fatigue                                                             | 143 (64.7)       | 216 (74.0)     | 130 (69.5)     |
| Pain at injection site/sore arm                                     | 105 (47.5)       | 111 (38.0)     | 80 (42.8)      |
| Headache                                                            | 60 (27.2)        | 87 (29.8)      | 52 (27.8)      |
| Fever/Chills                                                        | 58 (26.2)        | 90 (30.8)      | 47 (25.1)      |
| Body aches/muscle soreness/Joint Pain                               | 56 (25.3)        | 93 (31.9)      | 55 (29.4)      |
| Swelling/Redness at injection site                                  | 17 (7.7)         | 15 (5.1)       | 17 (9.1)       |
| Nausea and/or Vomiting                                              | 17 (7.7)         | 29 (9.9)       | 7 (3.7)        |
| Lymphadenopathy/Axillary swelling and tenderness                    | 7 (3.2)          | 11 (3.8)       | 7 (3.7)        |
| Itchiness                                                           | 6 (2.7)          | 6 (2.1)        | 3 (1.6)        |
| Sore throat or runny/stuffy nose                                    | 6 (2.7)          | 2 (0.7)        | 5 (2.7)        |
| Brain fog                                                           | 5 (2.3)          | 10 (3.4)       | 3 (1.6)        |
| Diarrhea/Intestinal discomfort                                      | 5 (2.3)          | 3 (1.0)        | 2 (1.1)        |
| b) Length of symptoms (days)                                        |                  |                |                |
| Length of time of symptoms (days)                                   | Dose 1 (n=200)   | Dose 2 (n=265) | Dose 3 (n=176) |
| Median (IQR)                                                        | 2.0 (1.0, 3.0)   | 1.0 (1.0, 2.0) | 1.0 (1.0, 2.0) |
| Range (min, max)                                                    | 91.5 (0.5, 92)   | 61.5 (0.5, 62) | 31.5 (0.5, 32) |
| c) Self-reported severity of symptoms compared to expected severity |                  |                |                |
|                                                                     | Dose 1 (n=219)   | Dose 2 (n=297) | Dose 3 (n=187) |
| Worse                                                               | 66 (30.1)        | 90 (30.3)      | 38 (20.3)      |
| Better                                                              | 43 (19.6)        | 84 (28.3)      | 46 (24.6)      |
| Same                                                                | 103 (47.0)       | 115 (38.7)     | 101 (54.0)     |
| Other                                                               | 7 (3.2)          | 8 (2.7)        | 2 (1.1)        |

## Figures

Supplemental Figure 1: Flow chart of study participation according to visit date, data collection schedule, and study cohort.



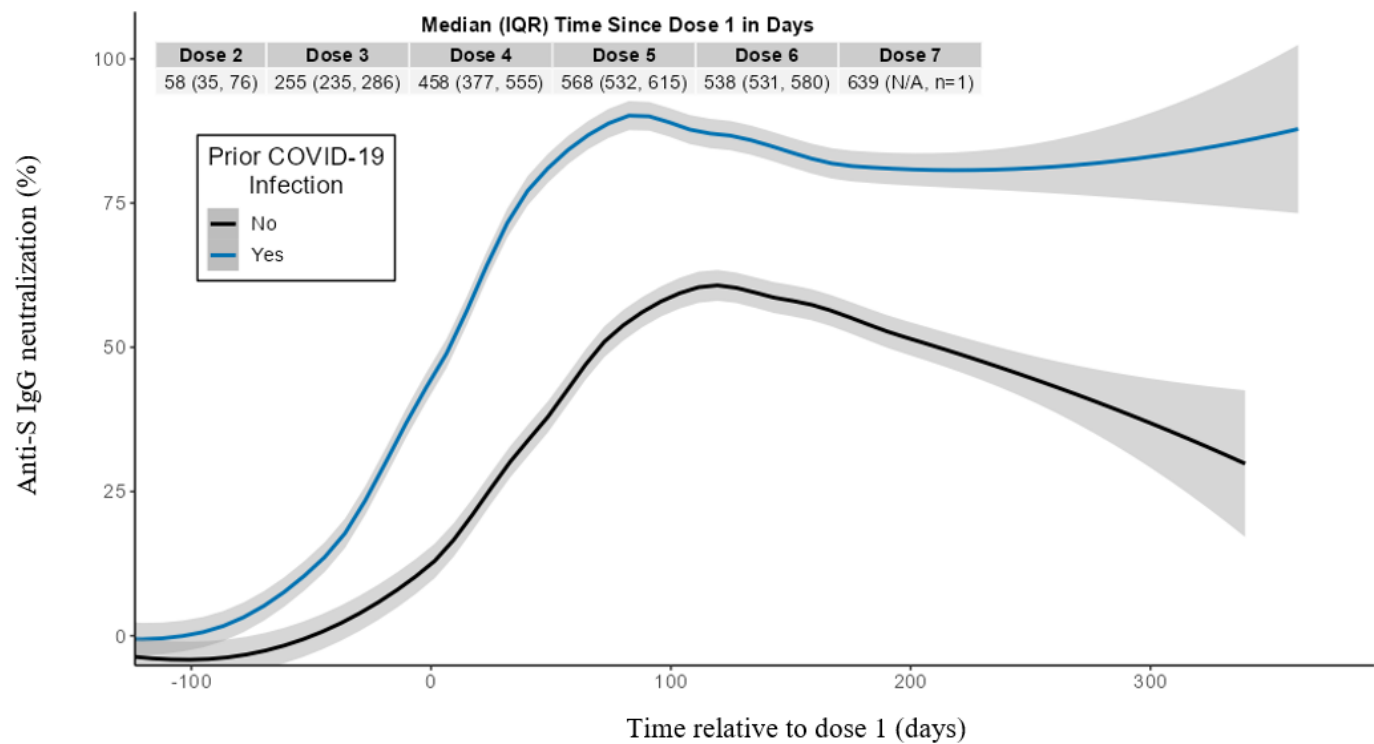
Supplemental Figure 2: Loess smooth plots of IgG titers (BAU/mL) against (A) the SARS-CoV-2 spike (S) and (B) receptor-binding domain (RBD) proteins over time according to COVID-19 history at baseline.<sup>1</sup>



<sup>1</sup> Data following reinfections (in the convalescent cohort) were excluded. Bands indicate 95% confidence intervals for the smoothed line.



Supplemental Figure 3: Loess smooth plot of IgG neutralization (snELISA) percentage (%) against the Wuhan-Hu-1 SARS-CoV-2 spike protein over time according to COVID-19 history at time of sample collection by PCR, rapid antigen test, or serology testing.<sup>1</sup>



<sup>1</sup>Data following reinfections (in the convalescent cohort) were excluded. Bands indicate 95% confidence intervals for the smoothed line.

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# Chapter 3 – Immune Compromising States Impair SARS-CoV-2 Vaccine Humoral Immunogenicity Despite Multiple Booster Doses

## Preface

The study presented in this chapter focuses on the humoral immune response to COVID-19 vaccination in the first clinical population listed in objective III of this thesis: the immune compromised (IC). We categorized the IC conditions and medications in the SSO cohort as either high-level or low-level (based on prior evidence in the literature regarding immunosuppressive effects and documented associations with increased risk of respiratory virus infection) and assessed the influence of high-level or low-level IC on antibody production after up to five doses of the COVID-19 vaccine through multilevel robust linear regression with restricted cubic splines. We completed objective IIIa (to longitudinally analyze antibodies produced by COVID-19 vaccination according to IC condition or medication) by conducting subgroup analyses according to IC condition or medication.

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## Abstract

The healthcare burden of SARS-CoV-2 infection among immune compromised (IC) individuals remains high as variants continue to emerge and circulate. High-quality observational research in this vulnerable and heterogeneous population is needed to characterize the long-term durability of humoral immunity elicited by initial COVID-19 vaccination and by additional boosting with original vaccines and/or updated vaccines targeting newer variants. The “Stop the Spread Ottawa” cohort was prospectively followed up to 46 months starting in September 2020. Data from 932 participants was utilized to elucidate the influence of various IC states on COVID-19 vaccine humoral immunogenicity. IC conditions and medications were categorized a priori as high-level (n=104) or low-level (n=158). We conducted multilevel linear regression to model  $\log_{10}$ -transformed anti-spike and anti-receptor-binding domain IgG, IgA and IgM antibodies after four (vaccine only immunity) or five (hybrid immunity) doses. Cubic regression splines and robust linear regression accounted for non-linear trends and outliers. IC status was associated with older age, obesity, comorbidities, greater healthcare burden, higher booster uptake and shorter time intervals between vaccine doses. Prior to vaccination, non-IC participants experienced a higher incidence of SARS-CoV-2 infections, whereas the incidence of breakthrough infections was greater for IC participants following doses 4 to 7. Antibody production was similar for low-level and non-IC participants. High-level IC participants exhibited diminished antibody response overall. Effect modification by prior infection history and time was present. Antibody waning was faster for high-level IC participants compared to low-level IC and non-IC participants for anti-S after dose 1, 3 (IgG), 4 (IgA) for those with no prior infection and anti-S IgA after dose 5 for those with hybrid immunity. High-level IC conditions (cancer, organ/bone marrow transplant, use of a systemic immunosuppressant, rheumatological conditions) and suppressed HIV predicted attenuated antibody production compared to NIC participants. Low-level IC (asthma/COPD requiring medication, Celiac disease, diabetes) did not. These results suggest that individuals with high-level IC conditions



should be prioritized by strategies to bolster and maintain humoral immunity against SARS-CoV-2 (e.g., boosting with updated vaccines).

## **Introduction**

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in December 2019. The subsequent COVID-19 pandemic resulted in millions of deaths worldwide.<sup>1</sup> Immune compromised (IC) individuals have an increased risk of severe illness and poor infection outcomes, such as post-acute sequelae or death, and are disproportionately impacted by COVID-19.<sup>2,3</sup> Newly developed COVID-19 vaccines reduce SARS-CoV-2 virus infection severity in the general population but a blunted humoral immune response was observed after initial vaccination among individuals with pre-existing health conditions that impair immune function.<sup>1</sup> IC individuals may also experience faster waning of vaccine-elicited immunity, raising concerns regarding the degree of protection provided by vaccination over months and years.<sup>1,4,5</sup> Although COVID-19 is now considered an endemic pathogen, IC populations remain at a heightened risk. In Ontario, Canada the healthcare burden of SARS-CoV-2 infection for immune compromised individuals remains high despite vaccine rollout and the emergence of less virulent variants.<sup>6</sup>

Clinical trials of SARS-CoV-2 vaccines employed strict eligibility criteria that often excluded immune compromising conditions.<sup>7</sup> Given this void of clinical trial data, high-quality prospective longitudinal observational research may be valuable in characterizing the long-term durability of the immune response to SARS-CoV-2 vaccination in immune compromised populations. However, methodological limitations in observational research, such as residual confounding, small sample sizes, limited follow-up over a small number of doses and cross-sectional comparisons, introduce systematic error which obfuscates our understanding of the intricate immune response to SARS-CoV-2.<sup>1,2,8-13</sup> Furthermore, analyses aimed at specific immune compromising conditions may have limited generalizability to a highly heterogeneous

overall population of immune compromised individuals. Furthermore, a high prevalence of various comorbidities interact to influence immune profiles and degree of immune impairment.<sup>14–17</sup> There is considerable variability in the observed humoral immune response to COVID-19 vaccination, both when comparing different immune compromising conditions or when investigating the response within individual conditions.<sup>2,17</sup> For example, differential responses were observed among individuals with hematological malignancies after the second vaccine dose and among kidney transplant recipients after the third vaccine dose. Together, these factors lead to inconsistencies in the literature regarding the impact of different immune compromising conditions and medications on COVID-19 vaccine humoral immunogenicity in the long-term.

Opposing evidence has been generated by observational research conducted to date. There are numerous epidemiological studies reporting an association between immune compromising states (e.g., Celiac disease, kidney disease and/or transplant, HIV infection, systemic lupus erythematosus, rheumatic disease, malignancy) and reduced antibody titers and/or neutralizing capacity following vaccination against COVID-19.<sup>2,4,8,15,18–20</sup> However, the magnitude—and in some cases the direction or the presence—of the association varies depends on the specific disease or immunosuppressant medication and the number and type of vaccine doses. Indeed, conflicting observational studies indicate no impact by some immune compromising conditions on SARS-CoV-2 vaccine humoral immunogenicity (e.g., Celiac disease, HIV infection, type 1 and type 2 diabetes).<sup>9–12,21,22</sup> As an example, an observational study in British Columbia, Canada reported that neutralization activity and the rate of antibody decay up to 6 months following dose two were similar for 99 people living with HIV (PLWH) and 152 healthy controls, regardless of CD4+ T-cell count.<sup>22</sup>

As highlighted by Höft et al. and Razonable et al., the long-term dynamics of humoral immunity elicited by SARS-CoV-2 vaccination in immune compromised individuals requires

further characterization.<sup>3,23</sup> Longitudinal analyses with extended follow-up of large cohorts which are representative of real-world IC populations are needed.<sup>5,23</sup> Stop the Spread Ottawa (SSO) is a prospective longitudinal study of a heterogeneous cohort of adults living in an urban-setting followed throughout the COVID-19 pandemic. We utilized this cohort to shed light on the dynamics of humoral immunity after multiple boosting doses according to different IC states. Many different immune compromising states are represented in this cohort, including primary immunodeficiencies, rheumatological and inflammatory conditions, autoimmune diseases, chronic kidney or liver disease, malignancies, HIV, organ/bone marrow transplant, and use of various immunosuppressant medications. Our work provides insights regarding the humoral immune response in this vulnerable population over a prolonged period of observation. We identified specific immune compromised subgroups which experience diminished production and retention of humoral immunity against SARS-CoV-2. Our findings may be valuable in informing vaccination strategies aimed at maximizing protection in light of seasonal SARS-CoV-2 circulation and the emergence of newer variants. We hypothesize that immune compromising conditions and medications blunt the humoral immune response to SARS-CoV-2 vaccination across vaccine doses compared to non-immune compromised and that antibody production and decay are negatively impacted in immune compromised populations compared to non-immune compromised participants.

## **Methods**

### *Study Design*

Stop the Spread Ottawa (SSO) commenced in the fall of 2020 to prospectively and longitudinally characterize the humoral and cellular immune response to SARS-CoV-2 infection and vaccination in a cohort of adults in the region of Ottawa, Canada.<sup>24</sup> Recruitment efforts targeted individuals at a heightened risk of SARS-CoV-2 infection due to pre-existing health conditions or occupational exposure, and/or individuals with documented seropositivity against

SARS-CoV-2 or a positive SARS-CoV-2 quantitative reverse transcription polymerases chain reaction test (PCR) result. The data collection protocol has been reported previously.<sup>24,25</sup> Briefly, participants were recruited from September 2020 to February 2022 and followed for a minimum of 10 months and optionally up to 34 (extension phase one) or 46 months (extension phase two). Serial blood draws and serial questionnaires were completed.

Participants with complete data regarding baseline immune status, antibody results, and vaccination dates were included in this analysis. Pregnant participants were excluded. Samples provided by participants prior to vaccine rollout in the region with no reported vaccinations were utilized as pre-vaccination baseline data. Immune compromising conditions and medications with prior evidence of immunosuppressive effects which may influence the humoral immune response to SARS-CoV-2 vaccination were identified in baseline questionnaires based on clinical expertise and review of the scientific literature (Supplemental Table 1). These states were categorized as high-level or low-level depending on the strength of previously reported immunosuppressive effects. Many participants reported multiple IC conditions, in which case their classification was determined by their highest-level condition. Systemic immunosuppressant medication use was also identified and compared to NIC participants in a separate subgroup analysis irrespective of what IC conditions were reported.

#### *SARS-CoV-2 Vaccination and Infection Data*

IgG, IgA and IgM antibodies against the Wuhan SARS-CoV-2 spike (S), receptor-binding domain (RBD) and nucleocapsid (N) antigens were quantified by a high-throughput direct chemiluminescent ELISA assay as previously reported.<sup>26</sup> IgG titer are reported in international binding antibody units (BAU) per mL according to the World Health Organization (WHO) international reference standard. IgA and IgM titers are reported in micrograms (µg) per mL.

Self-reported COVID-19 vaccination dates were confirmed by medical record review when available. Incident SARS-CoV-2 infections were identified in the absence of a positive PCR

or rapid antigen test (RAT) result (e.g., asymptomatic infections) by IgG seropositivity against the N antigen, which is not included in SAR-CoV-2 vaccines and therefore indicates natural immunity. Participants were classified as having hybrid immunity if they had received at least one SARS-CoV-2 vaccine dose and had a recorded positive PCR/RAT test result or were seropositive for natural immunity by serology at the date of sample collection. Infections occurring up to 14 days after vaccination were classified as prior to vaccination due to the time required to generate vaccine-elicited immunity.<sup>27</sup>

### *Statistical Analysis*

Demographics, vaccination and infection characteristics, and crude antibody titers were compared between immune compromised (IC) and non-immune compromised (NIC) participants using two-sided statistical tests. Statistical significance was determined by an alpha level of 0.05. Parametric or non-parametric tests were utilized according to data distribution (assessed by histograms and Q-Q plots) and test assumptions. Crude antibody data from low-level IC participants were pooled with either high-level IC or NIC participants to determine if the classification of IC states attenuated the impact of IC conditions on the humoral response to vaccination.

Antibody quantification data was non-normal and skewed. Antibody titers were shift up by one unit to prevent the generation of undefined values from zero values and then linearized by log<sub>10</sub>-transformation. The influence of immune compromising conditions on antibody titers up to five doses was assessed by univariable and multivariable linear regression analysis. Data was stratified by vaccine dose and SARS-CoV-2 infection history and models were generated according to antibody isotype (IgG, IgM, IgA) and anti-S/RBD antibody specificity. Regions of sparse data (e.g., ~300 days since dose was received) were not included. Random effects were included for individual intercepts (and slopes subject to sample size constraints) to account for autocorrelation that arises between repeated measures for the same individual. In the case of

outliers, robust linear regression was conducted and the Student's t-distribution linked the predictors and the outcome.

Cubic regression splines were applied to the term for "time since vaccination dose" to model the non-linear antibody titer trend observed after log-transformation. Separate smooths were generated according to IC status to determine if antibody slope varies according to IC status and investigate potential effect modification by IC conditions. If model fit was improved by separate IC smooths, models with separate trends for IC groups were reported. Model building decisions were guided by the Akaike information criterion (AIC). Model comparisons for fixed effects and random effects were generated by maximum likelihood (ML) and restricted maximum likelihood (REML) estimation, respectively. Final models were generated by REML.

Multivariable models adjusted for the following covariates where applicable: age at date of vaccine dose administration, sex, ethnicity, vaccine type, time between doses, time since last SARS-CoV-2 infection. Contrasts were calculated at different time points to test the difference in predicted values between IC and NIC participants. A large number of models were produced. Therefore, conclusions regarding the overall influence of IC conditions on SARS-CoV-2 were made cautiously to reduce the risk of type I error arising from multiplicity.<sup>28</sup> A balanced approach was employed to adjust for multiple comparisons in which the trade off between type I and type II error and type I error and power were considered.<sup>29</sup> The ramifications of identifying spurious associations between vaccine immunogenicity and immune compromising conditions were deemed less harmful than failing to identify reduced immunogenicity in this vulnerable population. Hypotheses were specified a priori and were specifically aimed at testing the time-varying rate of antibody production and decay, thereby justifying multiple contrasts between IC groups over time. Weighing these factors together, the highly conservative Bonferroni correction for multiplicity was not utilized and an alpha level of 0.025 was used to determine statistical significance.<sup>29</sup>

Statistical tests were conducted using Statistical Analysis System (SAS) version 9.4. Regression analyses were completed (package mgcv) and figures were generated (package ggplot2) using RStudio version 2023.06.1.

## **Results**

1,033 participants were recruited and 932 eligible participants were included in this study. Reasons for exclusion included: no serology sample collected (n=46); no completed baseline questionnaire (n=8); no reported vaccine doses and no blood samples provided prior to vaccine rollout (n=35); and pregnancy (n=12).

Of 262 participants identified as IC, 104 were categorized as high-level and 158 as low-level (Table 1). The mean age was highest in the high-level IC (HIC) group (54.2 years, SD: 13.0), followed by low-level IC (LIC; 49.5 years, SD: 13.8) and lowest in the non-immune compromised (NIC) group (43.3 years, SD: 13.4;  $P < 0.0001$ ). The proportion of females was highest in the NIC group, followed by the high-level IC group (68.2% [n=670], 62.5% [n=104];  $P = 0.04$ ). IC participants were more likely to be obese (31.4% high-level, 29.3% low-level, 20.5% NIC;  $P = 0.008$ ) and have comorbidities (e.g., heart disease, dyslipidemia, hypertension, chronic hematological disorder). They were less likely to be employed (57.4% high-level IC, 74.8% low-level IC, 88.7% NIC;  $P < 0.0001$ ). The overall health burden was greater for IC participants: 28.4% of high-level and 5.1% of low-level IC participants were receiving regular medical treatment at baseline compared to 0.2% of NIC participants (n=670;  $P < 0.0001$ ). Asthma requiring medication (43.9%), high-level immunosuppressant medication/treatment (24.1%), diabetes (15.6%), rheumatological condition (15.6%), HIV (11.1%), cancer (9.9%), and organ/bone marrow transplant (9.2%) were the largest subgroups represented in the IC cohort (n=262).

### *SARS-CoV-2 Vaccinations and Infections*

Overall uptake of SARS-CoV-2 vaccines was high (98.0%), with only five of 158 low-level IC and 13 of 670 NIC participants unvaccinated at the time of analysis (Table 2). Of the 18 unvaccinated participants, three low-level IC and nine NIC participants were lost to follow-up before COVID-19 vaccines became available. The remaining low-level IC (n=2) and NIC (n=4) participants completed follow-up questionnaires and reported no vaccinations. IC participants were more likely to have received boosting doses compared to NIC (e.g., 18.3% vs 9.4% for four doses;  $P<0.0001$ ). Vaccine types were similar for IC and NIC participants for the majority of doses. Consistent with vaccine rollout in Ontario, Canada, the proportion of AstraZeneca (AZ) doses was greatest for dose one and AZ was not administered for doses 3 to 9. mRNA vaccines were the most common type and the majority of fifth doses were bivalent vaccines (85.7%). The number of days between dose two and three & three and four differed between IC groups participants ( $P<0.0001$ ,  $P<0.0001$ ; Table 2). The time intervals between doses were shorter for high-level IC participants than low-level and NIC participants.

Although the total number of SARS-CoV-2 infections was similar for high- and low-level IC and NIC participants, the crude incidence of infections relative to dose number varied according to IC status ( $P<0.0001$ ; Table 2). The incidence of SARS-CoV-2 infection occurring prior to vaccination was highest among NIC participants (41.4%). However, after doses 4 to 7 the incidence of breakthrough infections was greater for IC participants (e.g., 6.0% for high-level IC, 7.1% for low-level IC and 1.3% NIC after dose 6,  $P=<0.0001$ ). All later doses (4+) in the cohort were administered after the Omicron variant had emerged and began circulating in Ontario, Canada. To adjust for differences in transmissibility between Omicron and earlier variants, the infection data was stratified in a post hoc analysis pre- and post-November 28th, 2021 (the start of the Omicron wave in Ontario). Prior to the spread of the Omicron variant, the risk of breakthrough infections according to dose number was comparable between analysis groups (Table 3). However, after Omicron began circulating in Ontario high-level IC



participants experienced a greater incidence of breakthrough infection after dose five compared to low-level IC or NIC participants.

### *Crude Humoral Immune Response to SARS-CoV-2 Vaccination*

IgG, IgA, and IgM titers against the SARS-CoV-2 spike (S) and receptor binding domain (RBD) antigens relative to the date of first SARS-CoV-2 vaccine dose are shown in Figure 1. Vaccination generated a strong humoral response and titers rose across all analysis groups after the initial dose. Participants with a prior history of SARS-CoV-2 infection at the time of vaccination produced higher titers than those with vaccine-derived immunity only. Crude antibody titers were compared between various IC groupings at multiple time points following five doses. Antibody production in low-level IC participants was more similar to NIC participants than high-level IC conditions and the influence of IC status on anti-S and anti-RBD IgG titers was strongest when low-level IC participants were pooled with NIC participants. Therefore, this grouping was used for the remainder of analyses.

High-level IC status was associated with impaired unadjusted antibody response among participants with no prior infection. Median anti-S and anti-RBD IgG titers were lower for high-level IC participants than low-level IC and NIC participants 14 and 45 days after dose one and 14, 45, and 90 days after dose two and 90 days after dose three. Median anti-S IgA, anti-RBD IgA, and anti-RBD IgM were lower for high-level IC participants 14 days after dose one and 90 days after dose two. For participants with hybrid immunity, no consistent influence of high-level IC status was observed. For both infection groups, small sample sizes hindered cross-sectional statistical analyses concerning later doses four and five.

The demographic and clinical characteristics of individuals who produced low titers (defined as  $\log_{10}$  anti-S IgG titers  $< 3.13$  for all samples) following the second vaccine dose were analyzed (n=94; data not shown). Low titer response was associated with older age, male sex, AstraZeneca vaccine type, as well as organ/bone marrow transplant, HIV infection (suppressed),

rheumatological condition, cancer, and high-level immunosuppressant medication. Low titers were not predicted by ethnicity or time interval between dose one and two.

### *Multivariable Regression Analysis of the Humoral Immune Response to SARS-CoV-2 Vaccination*

High-level IC (HIC) status was associated with impaired SARS-CoV-2 vaccine humoral response for up to five doses (Table 4A and 4B). The negative impact of HIC on humoral immunogenicity was greatest for IgG antibodies (compared to IgA or IgM) and for antibodies targeting the S antigen (compared to RBD antigen).

Some heterogeneity was observed depending on the specific vaccine dose and SARS-CoV-2 infection history suggesting that prior immunity acquired through SARS-CoV-2 infection modifies the relationship between immune compromising states and the humoral response to vaccination (Table 4A and 4B). For the first three SARS-CoV-2 vaccine doses, the detrimental impact of HIC on IgG, IgA, and IgM antibody titer production and durability was less consistently observed among participants with a prior infection compared to those without a prior infection. For example, no statistically significant association between HIC status and antibody titers was detected after dose 2 across all three isotypes for participants with a history of SARS-CoV-2 infection. In contrast, in participants with no prior infection HIC predicted lower anti-S and anti-RBD IgG, IgA and IgM titer peaks (and in some cases faster antibody decay) after dose two compared to LIC and NIC participants. Over time participants acquired SARS-CoV-2 infections and crossed-over from the vaccine-only group to the convalescent cohort. As a result, there was insufficient power to assess humoral COVID-19 vaccine immunogenicity after dose five among those with vaccine-elicited immunity only. Lower anti-S IgG, IgA, IgM and lower anti-RBD IgG was observed after dose 5 for HIC participants compared to LIC and NIC participants among those with hybrid immunity.

The slope of antibody production and decay differed between HIC and LIC/NIC participants and modelling different smooths for according to IC status improved regression fit for multiple doses, antibody isotypes, and target SARS-CoV-2 antigens. Faster antibody waning was observed for anti-S IgG (after dose 1, 3) and IgA (after dose 4) for those with no prior infection as well as anti-S IgA (after dose 5) for those with hybrid immunity (Table 4A and 4B).

To evaluate the vaccine humoral immune response associated with specific IC states, additional anti-S IgG multivariable models were generated for participants with no prior SARS-CoV-2 infection using data corresponding to the second COVID-19 vaccine dose (Table 6). Cancer, organ/bone marrow transplant, suppressed HIV, use of a systemic immunosuppressant, and rheumatological conditions predicted attenuated production of anti-S IgG titers after the second vaccine dose compared to NIC participants. In addition, there was effect modification by time on the relationship between the humoral immune response and immunosuppressant medication use, rheumatological condition, suppressed HIV, or organ/bone marrow transplant. Organ/bone marrow transplant recipients exhibited the largest magnitude reduction in peak antibodies relative to NIC participants after receipt of the second vaccine dose ( $-1.992 \log_{10}$  BAU/mL,  $P < 0.001$  at day 15 after dose 2), followed by participants on immunosuppressant medications ( $-1.602 \log_{10}$  BAU/mL,  $P = 0.007$  at day 15 after dose 2). With the exception of suppressed HIV infection, the low-level IC conditions analyzed were not observed to impair humoral COVID-19 vaccine immunogenicity. These findings support the classification of these conditions (asthma/COPD requiring medication, Celiac disease, diabetes) as low-level IC and provide further evidence indicating that these participants are comparable to NIC participants. Sample size limitations prevented analyses aimed at other high- and low-level IC conditions.

Overall, IgG, IgA, and IgM titer production and retention was impaired in participants with high-level immune compromising conditions compared to LIC or NIC participants despite multiple boosting doses.

## Discussion

### *Classification of Immune Compromising States*

#### High-Level IC

We developed a system of classification for immune compromising states and determined the impact of various states on the humoral immune response following COVID-19 vaccination. Immune compromising states with strong evidence of immune function impairment and increased risk of respiratory infections in the literature were categorized a priori as high-level immune impairment (Supplemental Table 1). We observed that the use of systemic immunosuppressant medication (e.g., corticosteroids, biologics, disease-modifying anti-rheumatic drugs, calcineurin inhibitors, or purine nucleoside analogs) was associated with impaired humoral immunogenicity upon receipt of the second COVID-19 vaccine dose, as well as malignancy (current or past), rheumatological diseases (e.g., rheumatoid arthritis, active systemic lupus erythematosus, polymyalgia rheumatica, polymyositis, ankylosing spondylitis, scleroderma/CREST syndrome) and organ/bone marrow transplant.

These results are consistent with previous studies reporting a negative impact of immunosuppressant medication, organ/bone marrow transplant, rheumatological disease, and cancer on vaccine-elicited antibodies. Immunosuppressant medication use has been demonstrated to impair B cell-mediated vaccine immunogenicity after the first three COVID-19 vaccine doses in association with a spectrum of conditions (e.g., kidney, liver, and other solid organ transplantation, hematopoietic stem cell transplantation, autoimmune diseases, rheumatological diseases, hemodialysis, cancer).<sup>1,2,19,30-32</sup> For example, seropositivity after two mRNA COVID-19 vaccine doses was negatively associated with various immunosuppressant medications (e.g., rituximab, mycophenolate mofetil, and prednisone) for patients with systemic autoimmune rheumatic diseases (defined as anti-S IgG > 150 AU/mL) and myasthenia gravis (defined as > anti-RBD IgG 225 AU/mL).<sup>33,34</sup> It has been postulated that the

immunosuppressant therapies such as rituximab, mycophenolate, mycophenolate mofetil, and glucocorticoids curb antibody production in patients with rheumatological diseases in comparison to healthy controls.<sup>7,18,19</sup> Similarly, there is evidence linking immunosuppressant therapies with diminished antibody production following COVID-19 vaccination in transplant recipients.<sup>15</sup> All of these medications are represented in the SSO cohort (Supplemental Table 1). The prevalence of systemic immunosuppressant medication use in the SSO cohort was higher in transplant recipients (67%) and participants with rheumatological disease (58%) than cancer patients (15%). Reduced humoral immunogenicity observed for participants in the cohort with rheumatological diseases or an organ/bone marrow transplant may be at least partially explained by medication use, in addition to disease-mediated drivers of immunosuppression.<sup>15,35</sup>

A large cohort study in England of 108,215 cancer patients in remission and 523,541 controls matched on age and sex found that the risk of hospitalization or death from influenza infection was higher in cancer survivors than those without history of cancer.<sup>36</sup> Accordingly, we pooled five participants in cancer remission at baseline with active cancer patients in the high-level IC group rather than allocating these participants to the low-level IC group. This decision may have slightly attenuated the observed influence of cancer on humoral COVID-19 vaccine response in our study. In an overview of systematic reviews Sun et al. indicated that seropositivity following SARS-CoV-2 vaccination was lower in cancer patients actively undergoing treatment and that among the cancer patients on treatment, the seropositivity proportion differed between immunosuppressant cancer therapies (e.g., anti-CD20 and CAR-T therapy were associated with lower seropositivity rates than chemotherapy or hormonal therapy).<sup>20</sup> Interestingly, despite a low proportion of systemic immunosuppressant treatment reported among cancer patients in the SSO cohort, these participants still demonstrated lower vaccine-elicited antibody levels compared to NIC participants. Given the small number of cancer participants in remission and the overall poor vaccine response observed for participants with current or past cancer, we presume that the risk of misclassification is low and that the

immunosuppressive effects of cancer persists regardless of treatment status. Cancer type was also identified in multiple studies by Sun et al. as a factor by which the seroconversion proportion varied.<sup>20</sup> For example, patients with hematologic malignancies responded more poorly to a second COVID-19 vaccine than those with solid cancers. We did not consider cancer type in this work. Although the magnitude of cancer seropositivity reported by Sun et al. varied according to cancer type, the overall response was worse in cancer patients than non-cancer controls, which is consistent with our findings. Along with the growing body of evidence in the literature, our results suggest that cancer patients remain a vulnerable patient population that mounts an inferior immune response to vaccination, regardless of immunosuppressive treatment or cancer type.

#### Low-Level IC

Conditions with weak or mixed evidence of immunosuppressant effects reported in the literature were classified as low-level IC (Supplemental Table 1). Of these, Celiac disease, diabetes and use of inhaled corticosteroids/intranasal corticosteroid sprays were not associated with lower antibody titers compared to NIC participants after the second COVID-19 vaccine, but the humoral vaccine response was impaired for participants with a suppressed HIV infection.

Inhaled corticosteroids exert local anti-inflammatory effects in the respiratory tract and prolonged use is associated with an increased risk of pneumonia.<sup>37-39</sup> It is unclear if the effects of inhaled corticosteroids extend beyond the respiratory tract and weaken the adaptive immune response following intramuscular COVID-19 vaccination; therefore, non-systemic corticosteroid medications for the treatment of chronic obstructive pulmonary disease (COPD) and asthma were classified as low-level IC and use of inhaled medications (e.g., Advair, Alvesco, Breo Ellipta, Flovent, Symbicort, Zenhale) and nasal sprays (e.g., apo-mometasone, azelastine and fluticasone, ciclesonide, budesonide, fluticasone furoate) were grouped together (Supplemental Table 1). We found by multivariable regression that inhaled corticosteroids and nasal

corticosteroid sprays did not significantly impair anti-S IgG production after COVID-19 vaccination, which suggests that vaccine-elicited immunogenicity in patients receiving local corticosteroid treatment is equivalent to NIC participants. These findings are consistent with work published by Nakajima et al.<sup>40</sup> We were unable to assess the impact of the route of administration for corticosteroids (inhalation vs. intranasal spray) due to the small size of participants taking intranasal steroids. There is a lack of research regarding the influence of both inhaled and intranasal corticosteroids on the humoral immune response to vaccination and these findings provide much needed information regarding this topic.

There is limited information in the literature regarding the humoral response to SARS-CoV-2 vaccination among individuals with Celiac disease. Of three studies found, one cross-sectional analysis reported lower IgG titers after vaccination for those with Celiac disease compared to those without.<sup>8</sup> Two other studies were in agreement with our findings and detected no difference in anti-S IgG titers after vaccination according to Celiac disease.<sup>9,41</sup> Our study provides much needed information in this under-researched area.

We found no difference in anti-S IgG antibody levels between diabetics and NIC participants after the second vaccine dose. This observation may be explained by the high proportion of mRNA vaccines administered in our cohort as the second dose: other studies demonstrated a stronger humoral immune response for mRNA vaccines compared to other types (e.g., inactivated or vector-based vaccines) among type 1 or type 2 diabetics.<sup>21,42</sup> Diminished anti-RBD IgG production and neutralization activity was observed for type 2 diabetics 3 to 6 months after vaccination with CoronaVac but not the BNT162b2 vaccine.<sup>21</sup> No difference in B cell activity following mRNA vaccination was detected for type 1 diabetics and for a pooled cohort of both type 1 and type 2 diabetics compared to non-diabetics.<sup>10,43</sup> Whereas a cohort of type 2 diabetics exhibited impaired anti-RBD IgG titer production and neutralization activity against the wild type SARS-CoV-2 virus compared to healthy controls after receipt of an

inactivated vaccine for their third dose.<sup>44</sup> Our analyses did not account for glycemic control, which may be an important factor that mediates or modifies the relationship between diabetes and B cell activity following SARS-CoV-2 vaccination. A study completed in 2022 by Cheng et al. observed diminished anti-RBD IgG production in type 2 diabetic patients with poor glycemia control after two doses of an inactivated vaccine compared to non-type 2 individuals but not for type 2 diabetic patients with well-controlled glycemia.<sup>45</sup> The unadjusted effects of glycemia control may hinder interpretation of our results and we note this as a study limitation. Overall, our results, along with previous findings, suggest mRNA vaccines generate strong humoral immunity in diabetic patients.

In contrast to other LIC conditions, we observed that suppressed HIV infection was associated with lower anti-S IgG titers after dose two compared to NIC participants. There are conflicting results in the literature regarding the strength of COVID-19 vaccine immunogenicity in people living with HIV (PLWH), as highlighted by review papers published in 2022 and 2024.<sup>5,23</sup> Observational research has found both comparable vaccine immunogenicity between HIV-positive individuals and HIV-negative controls<sup>11,12,16,17,22</sup> and impaired titer production for HIV-positive individuals.<sup>46-48</sup> These contrasting results may be due to unadjusted confounding factors or effect modification in PLWH. Multiple studies suggest that nadir or current CD4 T cell count modifies the humoral response in PLWH following two or three vaccine doses,<sup>49,50</sup> as illustrated by Antinori et al., who demonstrated impaired humoral immune response to two COVID-19 vaccine doses compared to controls for PLWH with a current CD4 T cell count  $< 500/\text{mm}^3$  but not for PLWH with a current CD4 T cell count  $> 500/\text{mm}^3$ .<sup>51</sup> We adjusted for confounding by many key factors in our multivariable regression analyses but did not account for nadir or current CD4 count. Our results indicate that the HIV patients with suppressed viral load exhibit impaired humoral immunity following vaccination and may experience reduced protection. However, nadir and current CD4 count may require consideration when



administering tailored vaccine strategies for PLWH given indications of heterogeneity in vaccine response in PLWH according to this factor.

In summary, these results support the classification of cancer, use of systemic immunosuppressant medication, history of organ/bone marrow transplant, and rheumatological disease as high-level immune compromising and indicate that low-level IC conditions, with the exception of suppressed HIV infection, demonstrate comparable humoral immune vaccine response to NIC participants.

#### Overall IC Response

Subsample sizes were too small to support additional subgroup analyses related to other high- or low-level IC conditions in the cohort, such as chronic kidney disease or primary immunodeficiencies. However, inferences regarding the combined influence of high or low-level conditions can be made. Crude titer comparisons indicated that vaccine-elicited immunogenicity was similar between low-level IC participants and NIC participants. Multivariable regression analysis revealed inferior IgG, IgA, and IgM antibody production and durability for HIC participants relative to low-level IC or NIC participants after five COVID-19 vaccine doses. These results affirm the decision to group LIC and NIC participants together and suggest that other LIC conditions (chronic kidney disease, autoimmune hepatitis, Graves' disease, mild liver fibrosis, SLE in remission, and inflammatory bowel disease not requiring medication) do not impair the humoral immune response to SARS-CoV-2 vaccination but other HIC conditions (neuromuscular autoimmune diseases and severe liver cirrhosis) do.

#### *Hybrid Immunity vs. Vaccine-Derived Immunity*

Participants with a prior SARS-CoV-2 infection at the time of vaccination achieved higher titers than those with vaccine-derived immunity only (Figure 1). This is consistent with a collective body of evidence describing heightened antibody production and enhanced clinical

protection from hybrid immunity than either vaccination or natural infection alone.<sup>52-54</sup> The observed negative influence of HIC on anti-S and anti-RBD IgG, IgA and IgM after three vaccine doses was weaker for participants with hybrid immunity than participants with a prior infection at time of vaccination. This suggests that vaccination boosted antibody titers substantially in both HIC and LIC/NIC participants and provides further evidence regarding the strong immune response elicited by hybrid immunity. We did not adjust for disease severity of SARS-CoV-2 infection in our analysis. More severe illness associated with older age has been linked with higher circulating antibodies, greater memory B cell activation and therefore heightened humoral immune activity upon subsequent immunization.<sup>54</sup> Consequently, a more severe clinical presentation during prior SARS-CoV-2 infection may contribute to a more robust recall of humoral immunity among HIC participants within the hybrid immunity group relative to those with no history of SARS-CoV-2 infection.

We found that the relationship between HIC status and the humoral vaccine response varied across time and according to infection history but effect modification by other variables, such as age, was not assessed. Feuchtenberger et al. observed that methotrexate use predicted impaired SARS-CoV-2 vaccination humoral response among older individuals with rheumatoid arthritis but not younger individuals with the same condition, suggesting that age may be a effect modifier for the suppressive effects of IC medications or conditions on the humoral immune response to SARS-CoV-2 vaccines.<sup>56</sup> Effect modification by age should be considered in future research regarding the trajectory of antibody titers in immune compromised individuals.

#### *Clinical Protection from SARS-CoV-2 Vaccination*

A higher incidence of SARS-CoV-2 infection occurred among unvaccinated NIC participants than unvaccinated high-level or low-level IC participants. This observation contravenes expectations, as IC individuals are at higher risk of contracting COVID-19 than NIC individuals.<sup>3</sup> Differences in health behaviours likely explain this finding.<sup>32,57</sup> Immune

compromised individuals tend to be more health-conscious and have higher adherence to masking and social distancing recommendations and travel restrictions, especially prior to vaccine rollout when no other protective measures were available. Disparate levels of exposure to community spread between analysis groups may also have contributed to lower rates of SARS-CoV-2 infection among IC participants prior to vaccine development. Fewer immune compromised individuals in the SSO cohort were employed at baseline, thereby limiting close contact with others in the workplace or during transit to and from the workplace.

The risk of SARS-CoV-2 infection is not static over time but rather changes as level of community spread fluctuates and lockdowns are put in place or lifted. Additionally, clinical protection provided by vaccination differs between SARS-CoV-2 variants. The Omicron variant possesses immune escape mutations that reduce the effectiveness of vaccination in preventing COVID-19.<sup>58</sup> Accordingly, we stratified infection data according to the start of the Omicron wave in Ontario to adjust for the effect of variant on SARS-CoV-2 communicability. After the spread of Omicron, high-level IC individuals experienced a higher incidence of breakthrough infections after dose 5, 6, and 7, whereas the proportion of breakthrough infections occurring after dose two or three was higher for NIC participants. The explanation for these observations is multifaceted and complex. Alignment between calendar date and dose number, dose type, variant wave, and public health restrictions prevents us from disentangling these factors when characterizing the clinical protection provided by later doses in the post-Omicron period. All first, second, and third doses were administered prior to the emergence and spread of the Omicron variant. Additional booster doses (which included bivalent and monovalent vaccines containing mRNA encoding antigens from the Omicron variant) were received when Omicron predominated. However, the calendar dates of dose four and five are not aligned for HIC and LIC or NIC participants. Uptake of additional boosters was higher among IC participants and prioritization of individuals with impaired immune activity led to earlier administration of fourth and fifth doses. As demonstrated by shorter time intervals between doses for HIC

participants compared to NIC participants, IC individuals obtained their fourth and fifth doses at an earlier calendar date. Analysis groups were therefore exposed to differing levels of community transmission following these doses, which systemically altered the apparent impact of HIC status on the risk of acquiring a SARS-CoV-2 infection relative to dose number. Longer intervals between dose two and three and dose three and four for NIC participants meant that they experienced greater waning of humoral immunity following their second and third doses than IC individuals, who were more likely to obtain additional boosters at an earlier date. Moreover, reduced booster uptake among NIC participants and longer intervals between doses increased cumulative exposure time after dose two and three during which a SARS-CoV-2 infection may be acquired. All these factors likely account for the higher observed incidence of breakthrough infections for NIC participants than IC participants after dose two and three (Table 3).

In summary, during and after the Omicron wave in Ontario IC participants experienced a greater incidence of SARS-CoV-2 infection despite multiple boosting doses. This finding is consistent with multiple published studies, such as the INFORM study in England which reported higher rates of hospitalisation and mortality for immune compromised individuals compared to non-immune compromised after four or more COVID-19 vaccine doses.<sup>3,59,60</sup> This finding supports the classification of HIC states as high-level in this study, but are limited by concurrent secular trends and the resulting systematic differences between high-level IC and LIC or NIC participants, which obfuscate the interpretation of breakthrough infection risk.<sup>32</sup>

### *Challenges in IC Status Classification*

The classification of IC status in this real-world cohort of adults was complicated by the high degree of heterogeneity in IC conditions, medications, comorbidities represented and the large number of participants who reported multiple immune compromising conditions and medications. For example, all participants in our cohort with chronic kidney disease also

reported another qualifying IC condition, making it impossible to assess the isolated impact of chronic kidney disease on SARS-CoV-2 vaccine immunogenicity. Heterogeneity and high prevalence of comorbidities are consistent challenges for research regarding IC populations.<sup>14,15</sup>

To address this issue, we classified participants according to the highest-level IC condition and interactions between conditions were not assessed. It is possible that underlying combinations of low-level IC conditions and high-level IC conditions may exacerbate immunosuppression and diminished humoral immune response to SARS-CoV-2 vaccination to a greater magnitude than would be otherwise observed. Principal component analysis previously conducted by Kreutmair et al. has revealed clustering between comorbidities resulting in distinct immune signatures profiles in the context of severe SARS-CoV-2 infection.<sup>61</sup> Future research should be conducted to add to the work done by Kreutmair et al. and investigate the influence of overlapping IC states on SARS-CoV-2 vaccine immunity and describe the profiles of IC subpopulations demonstrating the weakest immune response to vaccination.

The classification of IC status was further complicated by the time-varying nature of immune function. IC status was ascertained at baseline and not assessed during follow-up. Study follow-up was long, increasing the risk that participants may have developed a new immune compromising disease or initiated immunosuppressant medication over time. Many high-level IC conditions identified in the cohort require lifelong immunosuppressant use (e.g., organ transplantation) and/or are associated long term disease-mediated immunosuppression (e.g., cancer).<sup>13,15,35,36</sup> Thus, high-level IC participants are unlikely to change in classification over time. Transition from low-level IC status to high-level IC, or from NIC to low- or high-level IC is a more consequential source of misclassification. Changes in immunosuppressant medication use were captured in follow-up questionnaires. However, the overall the quality of follow-up questionnaire data regarding immune compromising conditions was low. We identified eight instances in which participants classified as NIC at baseline became high-level IC later in the

study and two cases where low-level IC participants became HIC. Two of these participants provided no blood draws after the date that their status changed. Given this fact and the low number of participants who became HIC after baseline, we assume that the risk of misclassification due to changes in IC status is low.

### *Study Strengths and Limitations*

SSO is a large, heterogeneous cohort of adults living in an urban setting. The representation of many different immune compromising conditions and medications in the cohort is a major strength of this study and improves generalizability to diverse, real-world immune compromised populations. By prioritizing individuals with immune compromising conditions during recruitment, we improved our overall power and obtained sufficiently large subsample sizes to facilitate granular analyzes of specific conditions or states within both high-level and low-level categories. We collected extensive data on demographic and health-related variables, facilitating thorough adjustment for confounding in multivariable modelling. Comprehensive clinical data collection also allowed for the identification of immune compromising medications and conditions that were not known to be immune compromising by participants, thereby reducing the risk of misclassification of IC status in self-reported data. We are confident in the high quality of vaccination and infection data in this study. COVID-19 vaccination dates and types were verified through direct communication with participants and by medical record review where available and anti-N IgG seropositivity was used to capture incident SARS-CoV-2 infections for which a positive test result was unavailable (e.g., asymptomatic infections, periods of limited testing availability). Prospective data collection using a standardized protocol reduced the risk of recall bias and observer bias. However, it is important to note that at times months passed between study questionnaires and recall bias was not entirely absent. Follow-up was long, spanning four years of the COVID-19 pandemic, and included a pre-vaccination baseline period and a high number of COVID-19 vaccine doses. Our

statistical approach adjusted for many sources of bias. The longitudinal multilevel models we produced accounted for autocorrelation between serial observations, non-linear trends in antibody production and decay, outliers, and effect modification by time. We employed flexible modelling with restricted cubic splines, which performs well in the context of non-linear data and is preferred over older approaches (such as quadratic regression) when characterizing antibody titers over time; however, splines are currently underutilized in epidemiological research, including SARS-CoV-2 vaccine research.<sup>55,62</sup>

We acknowledge the following limitations. Determination of time since SARS-CoV-2 infection was not exact. The date of first positive RAT/PCR test results and/or anti-N seropositivity was used as the date of SARS-CoV-2 infection start; however, this approach does not address false negative test results that may have occurred in early stages of infection or the time necessary to mount a humoral immune response against a pathogen. Prior to symptom onset, the probability of a false negative PCR test result was previously estimated to range from 100% on day one after infection to 67% on day four.<sup>63</sup> As a result, the first reported positive PCR/RAT test result may occur days after the true date of infection. Similarly, it takes an average of 12 days for B cells to undergo clonal expansion and plasma cells to secrete antibodies against SARS-CoV-2 antigens.<sup>64</sup> This delay introduces a gap between the true date of infection and the first date of anti-N seropositivity. These factors prevent precise determination of time since infection and may result in incomplete adjustment for confounding by this variable.

We conducted subgroup analyses for five high- and three low-level IC categories (Table 6). Small subgroup sample sizes prevented analyses concerning other IC conditions, such as chronic kidney disease, primary immunodeficiency and neuromuscular autoimmune disease. We investigated the influence of IC conditions and medications on IgG, IgA and IgM antibody titers but did not examine the impact on neutralization activity. Antibody quantity and quality are reportedly correlated in the general population and both measures have been used to gauge

vaccine effectiveness.<sup>58,65,66</sup> However, there is some conflicting evidence regarding the association between antibody titers and neutralization capacity among immune compromised individuals.<sup>46</sup> Heftdal et al. observed that after two vaccine doses antibody titers were lower in PLWH compared to healthy controls, but there was no difference in neutralization activity.<sup>46</sup> Additional regression analyses are planned in which we will model neutralization activity (ID<sub>50</sub>) against the Wuhan and Omicron XBB.1.5 spike antigens over time in order to ascertain if the findings in this study regarding the quantity of antibodies produced after COVID-19 vaccination also hold true for antibody quality.

Humoral immunity encompasses one branch of adaptive immunity, the other being cell-mediated immunity. The two branches are intertwined and function together to target and eliminate foreign antigens. As a consequence, IgG antibody titers and T cell activity have been observed to be correlated.<sup>54</sup> However, some discrepancy between T cell and B cell activity may exist in the case of primary immunodeficiencies or specific immunosuppressant medications that target either humoral or cell-mediated activity. For example, rituximab selectively inhibited B cell response following SARS-CoV-2 vaccination but not T cell activity.<sup>4,67,68</sup> Conversely, T cell function may alleviate deficits in the humoral immune response among immune compromised patients. Our findings may not apply to cellular immunity and T cell activity elicited by SARS-CoV-2 vaccination may not be impaired in immune compromised individuals. To obtain a complete characterization of vaccine-elicited immunity and protection, T cell activity should also be investigated in future research.

A large proportion of the SSO cohort is White and most vaccine doses administered were mRNA vaccines. This limits generalizability and our findings may not hold true for non-White ethnicities or vaccination by non-mRNA types. No children were included in the cohort and we are unable to make any conclusions regarding the impact of immune compromising conditions or medications on COVID-19 vaccine immunogenicity humoral for individuals under the age of



18. Volunteer bias precluded the inclusion of severely ill IC individuals who were unable to complete samples collection or study questionnaires. Loss to follow-up (LTFU) is another source of selection bias in prospective longitudinal cohort studies which may distort our results. For example, if the burden of completing study visits was higher for IC participants than NIC participants or if socioeconomic barriers disproportionately affected IC participants, LTFU would introduce systemic bias over time. However, IC individuals in the SSO cohort were engaged and dedicated and we observed a high rate of retention of IC participants in the extension cohorts (~40%).

### *Conclusion*

In conclusion, in this prospective longitudinal observational cohort study we observed an association between high-level IC conditions and impaired anti-S and anti-R antibody production and durability following SARS-CoV-2 vaccination despite multiple boosting doses. Cancer, systemic immunosuppressant medication use, organ/bone marrow transplant, rheumatological conditions, and suppressed HIV infection were associated with blunted vaccine-elicited humoral immunity. Individuals with these conditions should be prioritized for additional boosting to bolster and maintain humoral immunity against SARS-CoV-2 and reduce the risk of breakthrough infections as new variants continue to emerge and circulate. Tailored vaccination strategies in which vaccine type (mRNA vs. inactivated, monovalent vs. bivalent), infection history, and co-occurring IC conditions and medications are considered should be utilized to improve protection in this vulnerable group.<sup>2</sup> Other low-level IC states investigated in this study, such as asthma/COPD requiring inhaled or intranasal corticosteroids, Celiac disease, and diabetes, did not impair vaccine immunogenicity, suggesting that these conditions may not require additional methods for improving vaccine effectiveness compared to the general population.

## Tables

Table 1: Participant demographics and clinical characteristics at baseline, according to immune compromised status (n=932).

| Variable                                            | All Participants<br>(n=932) | Immune<br>compromised –<br>High level<br>(n=104) | Immune<br>compromised –<br>Low level<br>(n=158) | Non-immune<br>compromised<br>(n=670) | P       |
|-----------------------------------------------------|-----------------------------|--------------------------------------------------|-------------------------------------------------|--------------------------------------|---------|
| Age                                                 |                             |                                                  |                                                 |                                      |         |
| Mean (SD)                                           | 45.6 (13.9)                 | 54.2 (13.0)                                      | 49.5 (13.8)                                     | 43.3 (13.4)                          | <0.0001 |
| Range (min, max)                                    | 61.0 (18, 79)               | 55.0 (24, 79)                                    | 58.0 (18, 76)                                   | 58.0 (18, 76)                        |         |
| Gender, n (%)                                       |                             |                                                  |                                                 |                                      |         |
| Male                                                | 314 (34.0)                  | 39 (37.9)                                        | 63 (40.4)                                       | 212 (31.8)                           | 0.049   |
| Female                                              | 606 (65.5)                  | 63 (61.2)                                        | 91 (58.3)                                       | 452 (67.9)                           |         |
| Gender diverse                                      | 5 (0.5)                     | 1 (1.0)                                          | 2 (1.3)                                         | 2 (0.3)                              |         |
| Missing                                             | 7                           | 1                                                | 2                                               | 4                                    |         |
| Race, n (%)                                         |                             |                                                  |                                                 |                                      |         |
| Arab/West Asian                                     | 20 (2.2)                    | 0 (0)                                            | 4 (2.6)                                         | 16 (2.4)                             | 0.46    |
| Black                                               | 5 (0.5)                     | 0 (0)                                            | 1 (0.6)                                         | 4 (0.6)                              |         |
| East or Southeast Asian                             | 16 (1.7)                    | 2 (1.9)                                          | 6 (3.8)                                         | 8 (1.2)                              |         |
| Indigenous                                          | 14 (1.5)                    | 0 (0)                                            | 2 (1.3)                                         | 12 (1.8)                             |         |
| Latin American                                      | 9 (1.0)                     | 1 (1.0)                                          | 0 (0)                                           | 8 (1.2)                              |         |
| South Asian                                         | 13 (1.4)                    | 2 (1.9)                                          | 2 (1.3)                                         | 9 (1.4)                              |         |
| White                                               | 830 (89.2)                  | 97 (93.3)                                        | 141 (89.2)                                      | 592 (88.5)                           |         |
| Other                                               | 24 (2.6)                    | 2 (1.9)                                          | 2 (1.3)                                         | 20 (3.0)                             |         |
| Missing                                             | 1                           | 0                                                | 0                                               | 1                                    |         |
| Income before taxes in the past<br>12 months, n (%) |                             |                                                  |                                                 |                                      |         |
| 0-\$29,999                                          | 37 (4.5)                    | 5 (5.4)                                          | 11 (7.9)                                        | 21 (3.5)                             | 0.001   |
| \$30,000-\$59,999                                   | 88 (10.7)                   | 20 (21.5)                                        | 12 (8.6)                                        | 56 (9.4)                             |         |
| \$60,000-\$89,999                                   | 162 (19.7)                  | 19 (20.4)                                        | 31 (22.1)                                       | 112 (18.9)                           |         |
| \$90,000-\$119,999                                  | 180 (21.8)                  | 16 (17.2)                                        | 37 (26.4)                                       | 128 (21.6)                           |         |
| \$120,000-\$149,999                                 | 95 (11.5)                   | 7 (7.5)                                          | 21 (15.0)                                       | 67 (11.3)                            |         |
| \$150,000+                                          | 263 (31.9)                  | 26 (28.0)                                        | 28 (20.0)                                       | 209 (35.2)                           |         |
| Missing                                             | 106                         | 11                                               | 18                                              | 77                                   |         |
| Employed, n (%)                                     |                             |                                                  |                                                 |                                      |         |
| Yes                                                 | 755 (82.9)                  | 58 (57.4)                                        | 116 (74.8)                                      | 581 (88.7)                           | <0.0001 |
| No                                                  | 156 (17.1)                  | 43 (42.6)                                        | 39 (25.2)                                       | 74 (11.3)                            |         |
| Missing                                             | 21                          | 3                                                | 3                                               | 15                                   |         |
| Smoking history, n (%)                              |                             |                                                  |                                                 |                                      |         |

|                                                                             |            |           |              |              |         |
|-----------------------------------------------------------------------------|------------|-----------|--------------|--------------|---------|
| Current Smoker                                                              | 36 (3.9)   | 8 (7.7)   | 4 (2.6)      | 24 (3.6)     | 0.06    |
| Former Smoker                                                               | 218 (23.6) | 31 (29.8) | 41 (26.3)    | 146 (22.0)   |         |
| Never Smoker                                                                | 671 (72.5) | 65 (62.5) | 111 (71.2)   | 495 (74.4)   |         |
| Missing                                                                     | 7          | 0         | 2            | 5            |         |
| Average number of standard drinks of alcohol consumed per week <sup>1</sup> |            |           |              |              |         |
| Median (IQR)                                                                | 2 (0, 4)   | 1 (0, 3)  | 1.5 (0, 4.0) | 2 (0.5, 4.5) | 0.001   |
| Range                                                                       | 0-30       | 0-15      | 0-25         | 0-30         |         |
| Missing                                                                     | 4          | 0         | 1            | 3            |         |
| Allergies, n (%)                                                            |            |           |              |              |         |
| Yes                                                                         | 400 (43.3) | 56 (54.4) | 98 (62.8)    | 246 (37.0)   | <0.0001 |
| No                                                                          | 524 (56.7) | 47 (45.6) | 58 (37.2)    | 419 (63.0)   |         |
| Missing                                                                     | 8          | 1         | 2            | 5            |         |
| Obesity (self-reported or BMI≥30), n (%) <sup>2</sup>                       |            |           |              |              |         |
| Yes                                                                         | 215 (23.2) | 32 (31.4) | 46 (29.3)    | 137 (20.5)   | 0.008   |
| No                                                                          | 710 (76.8) | 70 (68.6) | 111 (70.7)   | 530 (79.5)   |         |
| Missing                                                                     | 6          | 2         | 1            | 3            |         |
| Heart disease, n (%)                                                        |            |           |              |              |         |
| Yes                                                                         | 39 (4.2)   | 10 (9.8)  | 12 (7.7)     | 17 (2.6)     | 0.0003  |
| No                                                                          | 881 (95.8) | 92 (90.2) | 144 (92.3)   | 645 (97.4)   |         |
| Missing                                                                     | 12         | 2         | 2            | 8            |         |
| Receiving regular medical treatment, n (%)                                  |            |           |              |              |         |
| Yes                                                                         | 38 (4.1)   | 29 (28.4) | 8 (5.1)      | 1 (0.2)      | <0.0001 |
| No                                                                          | 883 (95.9) | 73 (71.6) | 148 (94.9)   | 662 (99.9)   |         |
| Missing                                                                     | 11         | 2         | 2            | 7            |         |
| Immune compromising conditions, n (%) <sup>3</sup>                          |            |           |              |              |         |
| Asthma or COPD requiring medication                                         | 115 (43.9) | 16 (15.4) | 99 (62.7)    |              |         |
| Cancer                                                                      | 26 (9.9)   | 26 (25.0) | 0 (0.0)      |              |         |
| Celiac                                                                      | 7 (2.7)    | 0 (0.0)   | 7 (4.4)      |              |         |
| Chronic kidney disease                                                      | 10 (3.8)   | 7 (6.7)   | 3 (1.9)      |              |         |
| Diabetes                                                                    | 41 (15.6)  | 12 (11.5) | 29 (18.4)    |              |         |
| HIV, active and inactive                                                    | 29 (11.1)  | 5 (4.8)   | 24 (15.2)    |              |         |
| High-Level IC medication/treatment                                          | 63 (24.1)  | 63 (60.6) | 0 (0.0)      |              |         |
| Inflammatory bowel disease                                                  | 9 (3.4)    | 4 (3.8)   | 5 (3.2)      |              |         |

|                                   |           |           |         |
|-----------------------------------|-----------|-----------|---------|
| Neuromuscular autoimmune disorder | 4 (1.5)   | 4 (3.8)   | 0 (0.0) |
| Organ/tissue transplant recipient | 24 (9.2)  | 24 (23.1) | 0 (0.0) |
| Primary immunodeficiency          | 7 (2.7)   | 7 (6.7)   | 0 (0.0) |
| Rheumatological condition         | 41 (15.6) | 36 (34.6) | 5 (3.2) |

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Two-sided parametric (Student's t-test) or non-parametric (Mann-Whitney U test, chi-square test) statistical tests were used. Fisher tests were used when assumptions for the chi-square test were not met. an alpha level of 0.05 determined statistical significance. SD: standard deviation; IQR: interquartile range.

<sup>1</sup> A standard drink of alcohol is defined a 341 mL bottle of 5% beer, cider, or cooler; a 142 mL glass of 12% wine; or a 43 mL serving of 40% distilled alcohol (e.g., rye, gin, rum, vodka).

<sup>2</sup> Participants with self-reported obesity or BMI  $\geq 30.0$ .

<sup>3</sup> Immune compromising conditions included cancer, organ/tissue transplant, primary immunodeficiencies, rheumatological conditions, neuromuscular autoimmune diseases, active HIV, serious liver disorder (e.g., severe cirrhosis) (high-level immune compromised), respiratory conditions requiring medication (e.g. asthma), local autoimmune diseases, fully suppressed HIV, mild liver disorder (e.g., mild cirrhosis) and kidney disorders (i.e. chronic kidney disease), diabetes, and inactive SLE (low-level immune compromised). Use of immunosuppressants were included but the level was dependent on the strength of immunosuppression caused by the specific medications.

Table 2: COVID-19 vaccination and infection data according to immune compromised status (n=932).

| Variable                                                             | All Participants<br>(n=932) | Immune<br>compromised –<br>High level (n=104) | Immune<br>compromised –<br>Low level<br>(n=158) | Non-immune<br>compromised<br>(n=670) | P       |
|----------------------------------------------------------------------|-----------------------------|-----------------------------------------------|-------------------------------------------------|--------------------------------------|---------|
| Vaccinated at time of analysis, n (%)                                |                             |                                               |                                                 |                                      |         |
| Yes                                                                  | 913 (98.0)                  | 104 (100)                                     | 153 (96.8)                                      | 656 (97.9)                           | 0.18    |
| No                                                                   | 19 (2.0)                    | 0 (0)                                         | 5 (3.2)                                         | 14 (2.1)                             |         |
| Missing                                                              | 0                           | 0                                             | 0                                               | 0                                    |         |
| Number of COVID-19 vaccine doses received at time of analysis, n (%) |                             |                                               |                                                 |                                      |         |
| 0                                                                    | 18 (1.9)                    | 0 (0)                                         | 5 (3.2)                                         | 13 (1.9)                             | <0.0001 |
| 1                                                                    | 4 (0.4)                     | 1 (1.0)                                       | 0 (0)                                           | 3 (0.5)                              |         |
| 2                                                                    | 237 (25.4)                  | 15 (14.4)                                     | 30 (19.0)                                       | 192 (28.7)                           |         |
| 3                                                                    | 460 (49.4)                  | 44 (42.3)                                     | 80 (50.6)                                       | 336 (50.2)                           |         |
| 4                                                                    | 111 (11.9)                  | 24 (23.1)                                     | 24 (15.2)                                       | 63 (9.4)                             |         |
| 5                                                                    | 50 (5.4)                    | 9 (8.7)                                       | 9 (5.7)                                         | 32 (4.8)                             |         |
| 6                                                                    | 36 (3.9)                    | 4 (3.9)                                       | 8 (5.1)                                         | 24 (3.6)                             |         |
| 7                                                                    | 10 (1.1)                    | 2 (1.9)                                       | 2 (1.3)                                         | 6 (0.9)                              |         |
| 8                                                                    | 4 (0.4)                     | 3 (2.9)                                       | 0 (0)                                           | 1 (0.2)                              |         |
| 9                                                                    | 1 (0.1)                     | 1 (1.0)                                       | 0 (0)                                           | 0 (0)                                |         |
| 10                                                                   | 1 (0.1)                     | 1 (1.0)                                       | 0 (0)                                           | 0 (0)                                |         |
| COVID-19 vaccine type, dose 1, n (%)                                 |                             |                                               |                                                 |                                      |         |
| AstraZeneca                                                          | 115 (12.6)                  | 8 (7.7)                                       | 23 (15.0)                                       | 84 (12.8)                            | 0.33    |
| Moderna                                                              | 92 (10.1)                   | 13 (12.5)                                     | 18 (11.8)                                       | 61 (9.3)                             |         |
| Pfizer                                                               | 706 (77.3)                  | 83 (79.8)                                     | 112 (73.2)                                      | 511 (77.9)                           |         |
| Missing                                                              | 0                           | 0                                             | 0                                               | 0                                    |         |
| COVID-19 vaccine type, dose 2, n (%)                                 |                             |                                               |                                                 |                                      |         |
| AstraZeneca                                                          | 11 (1.2)                    | 2 (1.9)                                       | 1 (0.7)                                         | 8 (1.2)                              | 0.46    |
| Moderna                                                              | 229 (25.2)                  | 23 (22.3)                                     | 48 (31.4)                                       | 158 (24.2)                           |         |
| Pfizer                                                               | 668 (73.5)                  | 78 (75.7)                                     | 104 (68.0)                                      | 486 (74.4)                           |         |
| Sinovac                                                              | 1 (0.1)                     | 0 (0)                                         | 0 (0)                                           | 1 (0.2)                              |         |
| Missing                                                              | 0                           | 0                                             | 0                                               | 0                                    |         |
| COVID-19 vaccine type, dose 3, n (%)                                 |                             |                                               |                                                 |                                      |         |
| Moderna                                                              | 186 (27.8)                  | 27 (31.0)                                     | 40 (32.8)                                       | 119 (25.9)                           | 0.64    |
| Pfizer                                                               | 480 (71.9)                  | 60 (69.0)                                     | 82 (67.2)                                       | 338 (73.6)                           |         |
| Pfizer (bivalent)                                                    | 1 (0.2)                     | 0 (0)                                         | 0 (0)                                           | 1 (0.2)                              |         |
| Sinovac                                                              | 1 (0.2)                     | 0 (0)                                         | 0 (0)                                           | 1 (0.2)                              |         |

| Missing                                     | 5                             | 1                            | 0                             | 4                       |         |
|---------------------------------------------|-------------------------------|------------------------------|-------------------------------|-------------------------|---------|
| COVID-19 vaccine type, dose 4, n (%)        |                               |                              |                               |                         |         |
| Moderna (half dose)                         | 3 (1.4)                       | 0 (0)                        | 1 (2.3)                       | 2 (1.6)                 | 0.0003  |
| Moderna                                     | 77 (36.3)                     | 27 (61.4)                    | 12 (27.9)                     | 33 (30.4)               |         |
| Moderna (bivalent)                          | 32 (15.1)                     | 1 (2.3)                      | 5 (11.6)                      | 26 (20.8)               |         |
| Moderna (monovalent, Omicron XBB)           | 1 (0.5)                       | 1 (2.3)                      | 0 (0)                         | 0 (0)                   |         |
| Novavax                                     | 1 (0.5)                       | 0 (0)                        | 1 (2.3)                       | 0 (0)                   |         |
| Pfizer                                      | 69 (32.6)                     | 14 (31.8)                    | 14 (32.6)                     | 41 (32.8)               |         |
| Pfizer (monovalent, Omicron XBB)            | 1 (0.5)                       | 0 (0)                        | 0 (0)                         | 1 (0.8)                 |         |
| Pfizer (bivalent)                           | 28 (13.2)                     | 1 (2.3)                      | 10 (23.3)                     | 17 (13.6)               |         |
| Missing                                     | 1                             | 0                            | 0                             | 1                       |         |
| COVID-19 vaccine type, dose 5, n (%)        |                               |                              |                               |                         |         |
| Moderna                                     | 8 (8.2)                       | 6 (30.0)                     | 0 (0)                         | 2 (3.3)                 | 0.01    |
| Moderna (monovalent, Omicron XBB)           | 2 (2.0)                       | 0 (0)                        | 0 (0)                         | 2 (3.3)                 |         |
| Moderna (bivalent)                          | 30 (30.6)                     | 6 (30.0)                     | 8 (44.4)                      | 16 (26.7)               |         |
| Novavax                                     | 1 (1.0)                       | 0 (0)                        | 1 (5.6)                       | 0 (0)                   |         |
| Pfizer                                      | 1 (1.0)                       | 0 (0)                        | 0 (0)                         | 1 (1.7)                 |         |
| Pfizer (monovalent, Omicron XBB)            | 1 (1.0)                       | 0 (0)                        | 1 (5.6)                       | 0 (0)                   |         |
| Pfizer (bivalent)                           | 54 (55.1)                     | 8 (40.0)                     | 8 (44.4)                      | 38 (63.3)               |         |
| VBI-2901a (trivalent)                       | 1 (1.0)                       | 0 (0)                        | 0 (0)                         | 1 (1.7)                 |         |
| Missing                                     | 4                             | 0                            | 1                             | 3                       |         |
| Time between first and second doses in days |                               |                              |                               |                         |         |
| Median (IQR)                                | n=903<br>58.0 (35, 76)        | n=103<br>58 (28, 69)         | n=153<br>59.0 (37.0, 72.0)    | n=647<br>58 (35, 77)    | 0.24    |
| Range                                       | 19, 344                       | 21, 125                      | 21, 147                       | 19, 344                 |         |
| Missing                                     | 11                            | 1                            | 0                             | 10                      |         |
| Time between second and third doses in days |                               |                              |                               |                         |         |
| Median (IQR)                                | n=669<br>185.0 (174.0, 211.5) | n=88<br>158 (115, 185)       | n=122<br>185.5 (175.0, 206.0) | n=459<br>187 (176, 268) | <0.0001 |
| Range                                       | 22, 712                       | 56, 288                      | 68, 342                       | 22, 712                 |         |
| Missing                                     | 4                             | 0                            | 1                             | 3                       |         |
| Time between third and fourth doses in days |                               |                              |                               |                         |         |
| Median (IQR)                                | n=212<br>220.5 (142.0, 294.0) | n=44<br>133.5 (116.0, 158.0) | n=42<br>238 (156, 309)        | n=126<br>249 (155, 297) | <0.0001 |
| Range                                       | 28, 891                       | 28, 891                      | 89, 393                       | 90, 722                 |         |
| Missing                                     | 1                             | 0                            | 1                             | 0                       |         |

|                                                                                                        |                      |                      |                |                |         |
|--------------------------------------------------------------------------------------------------------|----------------------|----------------------|----------------|----------------|---------|
| Time between fourth and fifth doses in days                                                            | n=101                | n=20                 | n=18           | n=63           |         |
| Median (IQR)                                                                                           | 175.0 (143.0, 240.0) | 199.5 (160.5, 263.0) | 157 (143, 187) | 175 (126, 243) | 0.24    |
| Range                                                                                                  | 10, 517              | 140, 389             | 89, 406        | 10, 517        |         |
| Missing                                                                                                | 1                    | 0                    | 1              | 0              |         |
| SARS-CoV-2 Infection Y/N, n (%)                                                                        |                      |                      |                |                |         |
| Yes                                                                                                    | 454 (48.7)           | 53 (51.0)            | 89 (56.3)      | 312 (46.6)     | 0.08    |
| No                                                                                                     | 478 (51.3)           | 51 (49.0)            | 69 (43.7)      | 358 (53.4)     |         |
| Number of SARS-CoV-2 Infection, n (%)                                                                  |                      |                      |                |                |         |
| 0                                                                                                      | 478 (51.3)           | 51 (49.0)            | 69 (43.7)      | 358 (53.4)     | 0.36    |
| 1                                                                                                      | 351 (37.7)           | 42 (40.4)            | 69 (43.7)      | 240 (35.8)     |         |
| 2                                                                                                      | 85 (9.1)             | 8 (7.7)              | 17 (10.8)      | 60 (9.0)       |         |
| 3                                                                                                      | 18 (1.9)             | 3 (2.9)              | 3 (1.9)        | 12 (1.8)       |         |
| Self-reported and serology-based SARS-CoV-2 infection in relation to vaccine doses, n (%) <sup>§</sup> |                      |                      |                |                |         |
| COVID-19 prior to dose 1                                                                               | 227 (39.5)           | 20 (29.9)            | 43 (38.4)      | 164 (41.4)     | <0.0001 |
| COVID-19 after dose 1                                                                                  | 5 (0.9)              | 0 (0)                | 0 (0)          | 5 (1.3)        |         |
| COVID-19 after dose 2                                                                                  | 61 (10.6)            | 4 (6.0)              | 7 (6.3)        | 50 (12.6)      |         |
| COVID-19 after dose 3                                                                                  | 165 (28.7)           | 18 (26.9)            | 37 (33.0)      | 110 (27.8)     |         |
| COVID-19 after dose 4                                                                                  | 72 (12.5)            | 9 (13.4)             | 14 (12.5)      | 49 (12.4)      |         |
| COVID-19 after dose 5                                                                                  | 23 (4.0)             | 9 (13.4)             | 2 (1.8)        | 12 (3.0)       |         |
| COVID-19 after dose 6                                                                                  | 17 (3.0)             | 4 (6.0)              | 8 (7.1)        | 5 (1.3)        |         |
| COVID-19 after dose 7                                                                                  | 5 (0.9)              | 3 (4.5)              | 1 (0.9)        | 1 (0.9)        |         |
| Total                                                                                                  | 575                  | 67                   | 112            | 396            |         |

Two-sided parametric (Student's t-test) or non-parametric (Mann-Whitney U test, chi-square test) statistical tests were used. Fisher tests were used when assumptions for the chi-square test were not met. an alpha level of 0.05 determined statistical significance. SD: standard deviation; IQR: interquartile range.

<sup>§</sup> SARS-CoV-2 infections that occurred within 14 days of a vaccine dose were classified as breakthrough infections corresponding to the preceding dose and infections that occurred within 14 days of the initial dose were considered an infection prior to vaccination.

Table 3: Incidence of COVID-19 before and after the start of the Omicron wave in Ontario, Canada, according to immune compromised status (n=932). SARS-CoV-2 infections that occurred within 14 days of a vaccine dose were classified as breakthrough infections corresponding to the preceding dose and infections that occurred within 14 days of the initial dose were considered an infection prior to vaccination.

| Variable                                                                                      | All<br>Participants<br>(n=932) | Immune<br>compromised –<br>High level (n=104) | Low level immune<br>compromised & non-<br>immune compromised<br>(n=828) | P       |
|-----------------------------------------------------------------------------------------------|--------------------------------|-----------------------------------------------|-------------------------------------------------------------------------|---------|
| Self-reported and serology-based COVID-19 in relation to vaccine doses, n (%)                 |                                |                                               |                                                                         |         |
| Prior to November 28 <sup>th</sup> , 2021 (the start of the Omicron wave in Ontario, Canada): |                                |                                               |                                                                         |         |
| COVID-19 prior to dose 1                                                                      | 224 (91.1)                     | 20 (90.9)                                     | 204 (91.1)                                                              | 0.17    |
| COVID-19 after dose 1                                                                         | 5 (2.0)                        | 0 (0)                                         | 5 (2.2)                                                                 |         |
| COVID-19 after dose 2                                                                         | 16 (6.5)                       | 1 (4.6)                                       | 15 (6.7)                                                                |         |
| COVID-19 after dose 3                                                                         | 1 (0.4)                        | 1 (4.6)                                       | 0 (0)                                                                   |         |
| COVID-19 after dose 4                                                                         | 0 (0)                          | 0 (0)                                         | 0 (0)                                                                   |         |
| COVID-19 after dose 5                                                                         | 0 (0)                          | 0 (0)                                         | 0 (0)                                                                   |         |
| COVID-19 after dose 6                                                                         | 0 (0)                          | 0 (0)                                         | 0 (0)                                                                   |         |
| COVID-19 after dose 7                                                                         | 0 (0)                          | 0 (0)                                         | 0 (0)                                                                   |         |
| Total                                                                                         | 246                            | 22                                            | 224                                                                     |         |
| After November 28 <sup>th</sup> , 2021 (the start of the Omicron wave in Ontario, Canada):    |                                |                                               |                                                                         |         |
| COVID-19 prior to dose 1                                                                      | 3 (0.9)                        | 0 (0)                                         | 3 (1.1)                                                                 | 0.0008* |
| COVID-19 after dose 1                                                                         | 0 (0)                          | 0 (0)                                         | 0 (0)                                                                   |         |
| COVID-19 after dose 2                                                                         | 45 (13.7)                      | 3 (6.7)                                       | 42 (14.8)                                                               |         |
| COVID-19 after dose 3                                                                         | 164 (49.9)                     | 17 (37.8)                                     | 147 (51.8)                                                              |         |
| COVID-19 after dose 4                                                                         | 72 (21.9)                      | 9 (20.0)                                      | 63 (22.2)                                                               |         |
| COVID-19 after dose 5                                                                         | 23 (7.0)                       | 9 (20.0)                                      | 14 (4.9)                                                                |         |
| COVID-19 after dose 6                                                                         | 17 (5.2)                       | 4 (8.9)                                       | 13 (4.6)                                                                |         |
| COVID-19 after dose 7                                                                         | 5 (1.5)                        | 3 (6.7)                                       | 2 (0.7)                                                                 |         |
| Total                                                                                         | 329                            | 45                                            | 284                                                                     |         |



Table 4A: Multivariable linear regression of anti-spike (S) and anti-receptor-binding domain (RBD) IgG, IgA, and IgM antibodies up to five SARS-CoV-2 vaccine doses among participants with no prior SARS-CoV-2 infection. For models that include separate smooths for IC groups, the difference of predicted titers between high-severity immune compromised participants (IC) and low-level IC & non-immune compromised (NIC) participants are shown below model estimates (see column “IC – NIC”). High-level IC referent: low-level & NIC. Male sex referent: female sex. Non-White race referent: White race. Time=Days since dose date. Dose 1-4 vaccine type referent: Pfizer.

| Anti-S IgG (log10 BAU/mL) |          |         | Anti-R IgG (log10 BAU/mL) |          |         | Anti-S IgA (log10 µg/mL) |          |         | Anti-R IgA (log10 µg/mL) |          |         | Anti-S IgM (log10 µg/mL) |          |         | Anti-R IgM (log10 µg/mL) |          |         |
|---------------------------|----------|---------|---------------------------|----------|---------|--------------------------|----------|---------|--------------------------|----------|---------|--------------------------|----------|---------|--------------------------|----------|---------|
| Dose 1                    |          |         |                           |          |         |                          |          |         |                          |          |         |                          |          |         |                          |          |         |
|                           | Estimate | P       |                           | Estimate | P       |                          | Estimate | P       |                          | Estimate | P       |                          | Estimate | P       |                          | Estimate | P       |
| Intercept                 | 3.051    | <0.0001 |                           | 2.661    | <0.0001 |                          | 0.370    | <0.0001 |                          | 0.127    | <0.0001 |                          | 0.387    | <0.0001 |                          | 0.252    | <0.0001 |
| High-level IC             | -0.515   | <0.0001 |                           | -0.313   | 0.0017  |                          | -0.119   | 0.002   |                          | -0.050   | 0.05    |                          | -0.024   | 0.59    |                          | -0.044   | 0.15    |
| Age at dose 1             | -0.016   | <0.0001 |                           | -0.013   | <0.0001 |                          | -0.003   | 0.005   |                          | -0.001   | 0.04    |                          | -0.003   | 0.004   |                          | -0.002   | 0.01    |
| Male sex                  | -0.035   | 0.66    |                           | 0.021    | 0.75    |                          | 0.044    | 0.10    |                          | 0.030    | 0.09    |                          | 0.018    | 0.56    |                          | 0.042    | 0.04    |
| Non-White Ethnicity       | -0.071   | 0.59    |                           | -0.176   | 0.12    |                          | -0.011   | 0.79    |                          | -0.011   | 0.72    |                          | -0.054   | 0.32    |                          | -0.055   | 0.12    |
| Dose Type Moderna         | 0.309    | 0.07    |                           | 0.551    | 0.0001  |                          | 0.117    | 0.04    |                          | 0.114    | 0.003   |                          | 0.071    | 0.29    |                          | 0.039    | 0.38    |
| Dose Type AZ              | -0.324   | 0.006   |                           | -0.133   | 0.17    |                          | -0.054   | 0.16    |                          | -0.008   | 0.74    |                          | 0.032    | 0.46    |                          | -0.008   | 0.78    |
| Time                      | IC - NIC | P       | Time                      | IC - NIC | P       | Time                     | IC - NIC | P       | Time                     | IC - NIC | P       | Time                     | IC - NIC | P       | Time                     | IC - NIC | P       |
| 0                         | -0.159   | 0.69    | 5                         | 0.434    | 0.04    | 0                        | 0.107    | 0.15    | 0                        | 0.051    | 0.42    | Not applicable           |          |         | 0                        | 0.013    | 0.86    |
| 15                        | -1.064   | <0.001  | 15                        | -0.707   | <0.001  | 15                       | -0.356   | <0.001  | 15                       | -0.167   | <0.001  |                          |          |         | 15                       | -0.052   | 0.24    |
| 25                        | -0.515   | 0.04    | 25                        | -0.536   | 0.006   | 25                       | -0.117   | 0.09    | 25                       | -0.074   | 0.10    |                          |          |         | 30                       | -0.031   | 0.66    |
| 35                        | -0.396   | 0.09    | 50                        | -0.218   | 0.24    | 50                       | -0.005   | 0.94    | 40                       | -0.017   | 0.75    |                          |          |         | 60                       | -0.114   | 0.05    |
| 45                        | -0.492   | 0.02    | 70                        | -0.594   | 0.24    | 70                       | -0.241   | 0.06    | 70                       | -0.037   | 0.65    |                          |          |         |                          |          |         |
| 65                        | -0.807   | 0.01    |                           |          |         |                          |          |         |                          |          |         |                          |          |         |                          |          |         |
| Dose 2                    |          |         |                           |          |         |                          |          |         |                          |          |         |                          |          |         |                          |          |         |
|                           | Estimate | P       |                           | Estimate | P       |                          | Estimate | P       |                          | Estimate | P       |                          | Estimate | P       |                          | Estimate | P       |
| Intercept                 | 3.499    | <0.0001 |                           | 3.335    | <0.0001 |                          | 0.446    | <0.0001 |                          | 0.235    | <0.0001 |                          | 0.275    | <0.0001 |                          | 0.192    | <0.0001 |
| High-level IC             | -0.479   | <0.0001 |                           | -0.463   | <0.0001 |                          | -0.111   | 0.0001  |                          | -0.050   | 0.01    |                          | -0.082   | 0.004   |                          | -0.026   | 0.09    |
| Age at dose 2             | -0.008   | <0.0001 |                           | -0.011   | <0.0001 |                          | -0.001   | 0.09    |                          | -0.002   | 0.003   |                          | -0.001   | 0.20    |                          | -0.001   | 0.005   |
| Male sex                  | 0.003    | 0.94    |                           | 0.040    | 0.35    |                          | 0.049    | 0.02    |                          | 0.047    | 0.0007  |                          | 0.033    | 0.10    |                          | -0.0003  | 0.97    |
| Non-White Ethnicity       | 0.020    | 0.69    |                           | -0.033   | 0.62    |                          | 0.055    | 0.09    |                          | 0.040    | 0.07    |                          | -0.035   | 0.26    |                          | -0.004   | 0.80    |
| Dose Type Moderna         | 0.164    | <0.0001 |                           | 0.221    | <0.0001 |                          | 0.053    | 0.03    |                          | 0.062    | 0.0001  |                          | 0.057    | 0.02    |                          | 0.023    | 0.057   |
| Days between dose 1 & 2   | 0.001    | 0.02    |                           | 0.004    | <0.0001 |                          | -0.0001  | 0.74    |                          | -0.001   | <0.0001 |                          | -0.001   | 0.057   |                          | -0.001   | 0.001   |
| Time                      | IC - NIC | P       | Time                      | IC - NIC | P       | Time                     | IC - NIC | P       | Time                     | IC - NIC | P       | Time                     | IC - NIC | P       | Time                     | IC - NIC | P       |
| -30                       | -1.172   | < 0.001 | Not applicable            |          |         | Not applicable           |          |         | -20                      | -0.015   | 0.55    | Not applicable           |          |         | -25                      | -0.096   | <0.001  |
| 0                         | -1.221   | < 0.001 |                           |          |         |                          |          |         | 0                        | -0.039   | 0.13    |                          |          |         | 0                        | -0.072   | <0.001  |
| 15                        | -1.488   | < 0.001 |                           |          |         |                          |          |         | 15                       | -0.327   | <0.001  |                          |          |         | 15                       | -0.061   | <0.001  |
| 50                        | -0.080   | 0.40    |                           |          |         |                          |          |         | 50                       | -0.061   | 0.007   |                          |          |         | 50                       | -0.038   | 0.02    |

|                              |          |         |      |                                                                                                                                                                                      |         |      |                |         |      |          |        |          |          |        |          |          |      |
|------------------------------|----------|---------|------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|------|----------------|---------|------|----------|--------|----------|----------|--------|----------|----------|------|
| 100                          | -0.082   | 0.45    |      |                                                                                                                                                                                      |         | 75   | -0.050         | 0.03    |      | 75       | -0.046 | 0.004    |          |        |          |          |      |
| 150                          | -0.095   | 0.52    |      |                                                                                                                                                                                      |         | 100  | -0.019         | 0.43    |      | 100      | -0.034 | 0.05     |          |        |          |          |      |
|                              |          |         |      |                                                                                                                                                                                      |         | 150  | 0.006          | 0.84    |      | 150      | 0.042  | 0.06     |          |        |          |          |      |
|                              |          |         |      |                                                                                                                                                                                      |         | 250  | 0.045          | 0.27    |      | 225      | 0.033  | 0.32     |          |        |          |          |      |
| Dose 3                       |          |         |      |                                                                                                                                                                                      |         |      |                |         |      |          |        |          |          |        |          |          |      |
|                              | Estimate | P       |      | Estimate                                                                                                                                                                             | P       |      | Estimate       | P       |      | Estimate | P      |          |          |        |          |          |      |
| Intercept                    | 3.504    | <0.0001 |      | 3.838                                                                                                                                                                                | <0.0001 |      | 0.367          | <0.0001 |      | 0.211    | 0.04   | 0.103    | 0.20     |        |          |          |      |
| High-level IC                | -0.264   | <0.01   |      | -0.202                                                                                                                                                                               | 0.07    |      | 0.014          | 0.75    |      | -0.028   | 0.58   | 0.007    | 0.87     |        |          |          |      |
| Age at dose 3                | -0.006   | 0.04    |      | -0.010                                                                                                                                                                               | 0.001   |      | 0.001          | 0.54    |      | -0.0004  | 0.76   | 0.0004   | 0.67     |        |          |          |      |
| Male sex                     | 0.073    | 0.31    |      | 0.047                                                                                                                                                                                | 0.55    |      | 0.029          | 0.37    |      | -0.003   | 0.92   | -0.022   | 0.42     |        |          |          |      |
| Non-White Ethnicity          | -0.045   | 0.73    |      | -0.137                                                                                                                                                                               | 0.31    |      | 0.059          | 0.30    |      | -0.047   | 0.45   | 0.001    | 0.98     |        |          |          |      |
| Dose Type Moderna            | 0.262    | 0.001   |      | 0.294                                                                                                                                                                                | 0.001   |      | 0.061          | 0.09    |      | 0.083    | 0.04   | 0.068    | 0.03     |        |          |          |      |
| Days between dose 2 & 3      | -0.0002  | 0.75    |      | -0.001                                                                                                                                                                               | 0.07    |      | -0.0001        | 0.76    |      | 0.00002  | 0.95   | -0.00001 | 0.95     |        |          |          |      |
| Time                         | IC - NIC | P       | Time | IC - NIC                                                                                                                                                                             | P       | Time | IC - NIC       | P       | Time | IC - NIC | P      | Time     | IC - NIC | P      |          |          |      |
| -30                          | -0.098   | 0.46    |      | Not applicable                                                                                                                                                                       |         |      | Not applicable |         |      | -25      | 0.044  | 0.51     | -25      | 0.037  | 0.59     |          |      |
| 0                            | 0.135    | 0.35    |      |                                                                                                                                                                                      |         |      |                |         |      | 0        | 0.063  | 0.30     | 0        | 0.034  | 0.49     |          |      |
| 15                           | -0.225   | 0.14    |      | Note: when separate smooths were generated for IC groups, high-level IC predicted titers were lower than low-level IC & NIC predicted titers at 25, 50, 150 days after vaccine dose. |         |      |                |         |      | 15       | 0.076  | 0.24     | 15       | 0.031  | 0.50     |          |      |
| 35                           | -0.510   | 0.002   |      |                                                                                                                                                                                      |         |      |                |         |      | 75       | -0.040 | 0.56     | 75       | 0.015  | 0.80     |          |      |
| 50                           | -0.402   | 0.005   |      |                                                                                                                                                                                      |         |      |                |         |      | 125      | -0.198 | 0.04     | 150      | -0.021 | 0.78     |          |      |
| 75                           | -0.212   | 0.11    |      |                                                                                                                                                                                      |         |      |                |         |      | 150      | -0.269 | 0.11     | 200      | -0.049 | 0.70     |          |      |
| 100                          | -0.396   | 0.006   |      |                                                                                                                                                                                      |         |      |                |         |      |          |        |          | 240      | -0.072 | 0.70     |          |      |
| 125                          | -0.454   | 0.003   |      |                                                                                                                                                                                      |         |      |                |         |      |          |        |          |          |        |          |          |      |
| 150                          | -0.502   | 0.002   |      |                                                                                                                                                                                      |         |      |                |         |      |          |        |          |          |        |          |          |      |
| 175                          | -0.642   | 0.002   |      |                                                                                                                                                                                      |         |      |                |         |      |          |        |          |          |        |          |          |      |
| Dose 4                       |          |         |      |                                                                                                                                                                                      |         |      |                |         |      |          |        |          |          |        |          |          |      |
|                              | Estimate | P       |      | Estimate                                                                                                                                                                             | P       |      | Estimate       | P       |      | Estimate | P      |          | Estimate | P      |          |          |      |
| Intercept                    | 3.240    | <0.0001 |      | 3.540                                                                                                                                                                                | <0.0001 |      | 0.002          | 0.99    |      | -0.122   | 0.56   |          | 0.095    | 0.77   | 0.165    | 0.0497   |      |
| High-level IC                | -0.106   | 0.24    |      | -0.218                                                                                                                                                                               | 0.11    |      | -0.113         | 0.09    |      | -0.061   | 0.36   |          | 0.019    | 0.85   | 0.012    | 0.66     |      |
| Age at dose 4                | 0.002    | 0.61    |      | -0.001                                                                                                                                                                               | 0.78    |      | 0.004          | 0.07    |      | 0.002    | 0.42   |          | 0.0003   | 0.93   | -0.001   | 0.30     |      |
| Male sex                     | -0.024   | 0.73    |      | -0.025                                                                                                                                                                               | 0.82    |      | 0.008          | 0.87    |      | 0.004    | 0.94   |          | 0.020    | 0.81   | -0.034   | 0.08     |      |
| Dose Type Moderna            | 0.089    | 0.21    |      | 0.023                                                                                                                                                                                | 0.83    |      | 0.112          | 0.03    |      | 0.054    | 0.29   |          | 0.161    | 0.051  | -0.005   | 0.82     |      |
| Dose Type Moderna (Bivalent) | 0.177    | 0.16    |      | 0.210                                                                                                                                                                                | 0.28    |      | -0.093         | 0.30    |      | -0.062   | 0.50   |          | 0.034    | 0.82   | 0.065    | 0.07     |      |
| Dose Type Pfizer (Bivalent)  | -0.247   | 0.23    |      | -0.327                                                                                                                                                                               | 0.30    |      | -0.327         | 0.02    |      | -0.296   | 0.048  |          | -0.069   | 0.78   | -0.035   | 0.52     |      |
| Days between dose 3 & 4      | 0.0002   | 0.78    |      | -0.0001                                                                                                                                                                              | 0.91    |      | 0.001          | 0.19    |      | 0.001    | 0.06   |          | 0.0003   | 0.78   | -0.00004 | 0.86     |      |
| Time                         | IC - NIC | P       | Time | IC - NIC                                                                                                                                                                             | P       | Time | IC - NIC       | P       | Time | IC - NIC | P      | Time     | IC - NIC | P      | Time     | IC - NIC | P    |
| -25                          | -0.306   | 0.01    |      | Not applicable                                                                                                                                                                       |         | -30  | -0.289         | 0.002   | -35  | -0.149   | 0.11   | -15      | -0.368   | 0.006  | -25      | -0.035   | 0.28 |
| 0                            | -0.137   | 0.18    |      |                                                                                                                                                                                      |         | 0    | -0.188         | 0.027   | 0    | -0.084   | 0.28   | 0        | -0.180   | 0.23   | 0        | -0.025   | 0.38 |

|     |        |      |                                                                                                                                                                                                                      |     |        |      |     |        |      |     |        |      |     |        |      |
|-----|--------|------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|--------|------|-----|--------|------|-----|--------|------|-----|--------|------|
| 25  | -0.009 | 0.94 | Note: when separate smooths were generated for IC groups, high-level IC predicted titers were lower than low-level IC & NIC predicted titers at 20 days prior to vaccine dose and 0, 25, 50 days after vaccine dose. | 25  | -0.109 | 0.23 | 25  | -0.047 | 0.54 | 25  | 0.184  | 0.14 | 25  | -0.015 | 0.56 |
| 50  | 0.054  | 0.62 |                                                                                                                                                                                                                      | 50  | -0.038 | 0.69 | 50  | -0.015 | 0.86 | 50  | 0.163  | 0.24 | 50  | -0.005 | 0.84 |
| 100 | 0.021  | 0.84 |                                                                                                                                                                                                                      | 100 | 0.003  | 0.98 | 100 | 0.018  | 0.84 | 100 | -0.067 | 0.71 | 100 | 0.015  | 0.62 |
| 150 | -0.086 | 0.44 |                                                                                                                                                                                                                      | 150 | -0.020 | 0.84 | 150 | -0.019 | 0.84 | 200 | 0.094  | 0.50 | 200 | 0.054  | 0.26 |
| 200 | -0.208 | 0.06 |                                                                                                                                                                                                                      | 250 | -0.028 | 0.83 | 250 | -0.146 | 0.28 | 300 | 0.060  | 0.72 | 250 | 0.074  | 0.22 |
| 225 | -0.272 | 0.03 |                                                                                                                                                                                                                      | 325 | -0.400 | 0.02 | 300 | -0.097 | 0.53 | 340 | -0.051 | 0.77 | 300 | 0.094  | 0.20 |
| 250 | -0.338 | 0.03 |                                                                                                                                                                                                                      | 340 | -0.518 | 0.01 |     |        |      |     |        |      |     |        |      |

An alpha level of 0.025 was used to determine statistical significance to account for bias from multiplicity.

Table 4B: Multivariable linear regression of anti-spike (S) and anti-receptor-binding domain (RBD) IgG, IgA, and IgM antibodies up to five SARS-CoV-2 vaccine doses among participants with at least one prior SARS-CoV-2 infection. For models that include separate smooths for IC groups, the difference of predicted titers between high-severity immune compromised participants (IC) and low-level IC & non-immune compromised (NIC) participants are shown below model estimates (see column “IC – NIC”). High-level IC referent: Low-level & NIC. Male sex referent: female sex. Non-White race referent: White race. Time=Days since dose date. Dose 1-4 vaccine type referent: Pfizer. Note, for dose 5 the vaccine type referent is Pfizer (bivalent) for anti-S and anti-RBD IgG (log10 BAU/mL) and Moderna (bivalent) for anti-S and anti-RBD IgA (log10 µg/mL) and IgM (log10 µg/mL).

| Anti-S IgG (log10 BAU/mL)            |          |         | Anti-R IgG (log10 BAU/mL) |          |         | Anti-S IgA (log10 µg/mL) |                                                                                                                                                                                   |         | Anti-R IgA (log10 µg/mL) |                                                                                                                                                                             |         | Anti-S IgM (log10 µg/mL) |          |         | Anti-R IgM (log10 µg/mL) |          |         |
|--------------------------------------|----------|---------|---------------------------|----------|---------|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|--------------------------|----------|---------|--------------------------|----------|---------|
| Dose 1                               |          |         |                           |          |         |                          |                                                                                                                                                                                   |         |                          |                                                                                                                                                                             |         |                          |          |         |                          |          |         |
|                                      | Estimate | P       |                           | Estimate | P       |                          | Estimate                                                                                                                                                                          | P       |                          | Estimate                                                                                                                                                                    | P       |                          | Estimate | P       |                          | Estimate | P       |
| Intercept                            | 2.639    | <0.0001 |                           | 2.369    | <0.0001 |                          | 0.338                                                                                                                                                                             | <0.0001 |                          | 0.234                                                                                                                                                                       | 0.0004  |                          | 0.370    | <0.0001 |                          | 0.269    | <0.0001 |
| High-level IC                        | -0.163   | 0.26    |                           | 0.006    | 0.97    |                          | -0.112                                                                                                                                                                            | 0.09    |                          | -0.028                                                                                                                                                                      | 0.66    |                          | -0.047   | 0.53    |                          | -0.056   | 0.36    |
| Age at dose 1                        | 0.011    | <0.0001 |                           | 0.013    | <0.0001 |                          | 0.003                                                                                                                                                                             | 0.03    |                          | 0.002                                                                                                                                                                       | 0.08    |                          | 0.001    | 0.37    |                          | 0.001    | 0.19    |
| Male sex                             | -0.099   | 0.23    |                           | -0.100   | 0.32    |                          | -0.023                                                                                                                                                                            | 0.53    |                          | 0.019                                                                                                                                                                       | 0.57    |                          | -0.059   | 0.15    |                          | -0.026   | 0.43    |
| Non-White Ethnicity                  | 0.222    | 0.08    |                           | 0.292    | 0.06    |                          | 0.092                                                                                                                                                                             | 0.11    |                          | -0.010                                                                                                                                                                      | 0.85    |                          | 0.052    | 0.41    |                          | 0.079    | 0.12    |
| Dose Type Moderna                    | 0.019    | 0.85    |                           | 0.021    | 0.87    |                          | -0.051                                                                                                                                                                            | 0.27    |                          | -0.045                                                                                                                                                                      | 0.32    |                          | 0.023    | 0.66    |                          | 0.037    | 0.38    |
| Dose Type AZ                         | -0.582   | <0.0001 |                           | -0.589   | 0.0001  |                          | -0.198                                                                                                                                                                            | 0.0003  |                          | -0.192                                                                                                                                                                      | 0.0005  |                          | -0.066   | 0.28    |                          | 0.009    | 0.86    |
| Days since last SARS-CoV-2 infection | -0.001   | 0.0007  |                           | -0.001   | 0.17    |                          | -0.0005                                                                                                                                                                           | 0.001   |                          | -0.0002                                                                                                                                                                     | 0.08    |                          | 0.0002   | 0.17    |                          | 0.0003   | 0.008   |
| Time                                 | IC - NIC | P       | Time                      | IC - NIC | P       | Time                     | IC - NIC                                                                                                                                                                          | P       | Time                     | IC - NIC                                                                                                                                                                    | P       | Time                     | IC - NIC | P       | Time                     | IC - NIC | P       |
| -15                                  | -0.570   | 0.04    | -10                       | -0.280   | 0.34    |                          | Not applicable                                                                                                                                                                    |         |                          | Not applicable                                                                                                                                                              |         | -10                      | -0.081   | 0.40    | -15                      | -0.135   | 0.10    |
| 0                                    | 0.381    | 0.13    | 0                         | 0.416    | 0.14    |                          | Note: when separate smooths were generated for IC groups, high-level IC predicted titers were lower than low-level IC & NIC predicted titers at 10 to 20 days after vaccine dose. |         |                          | Note: when separate smooths were generated for IC groups, high-level IC predicted titers were lower than low-level IC & NIC predicted titers at 10 days after vaccine dose. |         | 0                        | 0.030    | 0.77    | 0                        | -0.022   | 0.77    |
| 20                                   | -0.043   | 0.86    | 15                        | -0.182   | 0.54    |                          |                                                                                                                                                                                   |         |                          |                                                                                                                                                                             |         | 15                       | -0.001   | 0.99    | 15                       | -0.035   | 0.64    |
| 50                                   | -0.622   | 0.02    | 40                        | -0.046   | 0.89    |                          |                                                                                                                                                                                   |         |                          |                                                                                                                                                                             |         | 40                       | -0.073   | 0.48    | 40                       | -0.078   | 0.33    |
| 60                                   | -0.154   | 0.64    | 60                        | 0.544    | 0.13    |                          |                                                                                                                                                                                   |         |                          |                                                                                                                                                                             |         | 60                       | -0.048   | 0.68    | 60                       | -0.022   | 0.83    |
| Dose 2                               |          |         |                           |          |         |                          |                                                                                                                                                                                   |         |                          |                                                                                                                                                                             |         |                          |          |         |                          |          |         |
|                                      | Estimate | P       |                           | Estimate | P       |                          | Estimate                                                                                                                                                                          | P       |                          | Estimate                                                                                                                                                                    | P       |                          | Estimate | P       |                          | Estimate | P       |
| Intercept                            | 3.518    | <0.0001 |                           | 3.327    | <0.0001 |                          | 0.560                                                                                                                                                                             | <0.0001 |                          | 0.331                                                                                                                                                                       | <0.0001 |                          | 0.308    | <0.0001 |                          | 0.286    | <0.0001 |
| High-level IC                        | 0.001    | 0.99    |                           | 0.051    | 0.61    |                          | -0.050                                                                                                                                                                            | 0.44    |                          | -0.020                                                                                                                                                                      | 0.74    |                          | 0.069    | 0.23    |                          | -0.003   | 0.95    |
| Age at dose 2                        | 0.005    | 0.002   |                           | 0.007    | 0.0004  |                          | -0.001                                                                                                                                                                            | 0.62    |                          | -0.001                                                                                                                                                                      | 0.69    |                          | 0.001    | 0.37    |                          | -0.0001  | 0.96    |
| Male sex                             | 0.011    | 0.83    |                           | 0.070    | 0.24    |                          | 0.020                                                                                                                                                                             | 0.61    |                          | 0.053                                                                                                                                                                       | 0.15    |                          | -0.014   | 0.69    |                          | -0.022   | 0.50    |
| Non-White Ethnicity                  | 0.086    | 0.24    |                           | 0.062    | 0.49    |                          | 0.096                                                                                                                                                                             | 0.09    |                          | 0.032                                                                                                                                                                       | 0.55    |                          | 0.074    | 0.16    |                          | 0.024    | 0.61    |
| Dose Type Moderna                    | 0.030    | 0.55    |                           | 0.059    | 0.35    |                          | 0.030                                                                                                                                                                             | 0.46    |                          | 0.015                                                                                                                                                                       | 0.69    |                          | 0.042    | 0.26    |                          | -0.020   | 0.54    |
| Dose Type AZ                         | -        | -       |                           | -        | -       |                          | -0.024                                                                                                                                                                            | 0.87    |                          | 0.019                                                                                                                                                                       | 0.90    |                          | -        | -       |                          | -        | -       |
| Days between dose 1 & 2              | -0.003   | 0.003   |                           | -0.003   | 0.005   |                          | 0.00003                                                                                                                                                                           | 0.97    |                          | -0.0003                                                                                                                                                                     | 0.63    |                          | -0.001   | 0.05    |                          | -0.001   | 0.27    |

|                                                                                                                                                                                  |          |         |                                 |         |                                 |         |                                 |       |                                 |       |                                 |         |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|---------|---------------------------------|---------|---------------------------------|---------|---------------------------------|-------|---------------------------------|-------|---------------------------------|---------|
| Days since last SARS-CoV-2 infection                                                                                                                                             | -0.001   | <0.0001 | -0.001                          | 0.003   | -0.0004                         | 0.002   | -0.0003                         | 0.004 | -0.00002                        | 0.87  | 0.0001                          | 0.31    |
| Time IC - NIC<br>Not applicable                                                                                                                                                  | P        |         | Time IC - NIC<br>Not applicable | P       | Time IC - NIC<br>Not applicable | P       | Time IC - NIC<br>Not applicable | P     | Time IC - NIC<br>Not applicable | P     | Time IC - NIC<br>Not applicable | P       |
|                                                                                                                                                                                  |          |         |                                 |         |                                 |         |                                 |       |                                 |       | -15                             | 0.37    |
|                                                                                                                                                                                  |          |         |                                 |         |                                 |         |                                 |       |                                 |       | 0                               | 0.86    |
|                                                                                                                                                                                  |          |         |                                 |         |                                 |         |                                 |       |                                 |       | 15                              | 0.59    |
|                                                                                                                                                                                  |          |         |                                 |         |                                 |         |                                 |       |                                 |       | 40                              | 0.24    |
|                                                                                                                                                                                  |          |         |                                 |         |                                 |         |                                 |       |                                 |       | 75                              | 0.60    |
|                                                                                                                                                                                  |          |         |                                 |         |                                 |         |                                 |       |                                 |       | 100                             | 0.58    |
|                                                                                                                                                                                  |          |         |                                 |         |                                 |         |                                 |       |                                 |       | 150                             | 0.47    |
|                                                                                                                                                                                  |          |         |                                 |         |                                 |         |                                 |       |                                 |       | 175                             | 0.97    |
| Dose 3                                                                                                                                                                           |          |         |                                 |         |                                 |         |                                 |       |                                 |       |                                 |         |
| Intercept                                                                                                                                                                        | Estimate | P       | Estimate                        | P       | Estimate                        | P       | Estimate                        | P     | Estimate                        | P     | Estimate                        | P       |
| High-level IC                                                                                                                                                                    | 3.239    | <0.0001 | 3.276                           | <0.0001 | 0.349                           | 0.003   | 0.260                           | 0.03  | 0.022                           | 0.86  | 0.247                           | <0.0001 |
| Age at dose 3                                                                                                                                                                    | -0.014   | 0.85    | -0.053                          | 0.56    | -0.058                          | 0.32    | -0.030                          | 0.62  | -0.023                          | 0.73  | -0.002                          | 0.93    |
| Male sex                                                                                                                                                                         | 0.005    | 0.003   | 0.006                           | 0.006   | 0.002                           | 0.14    | 0.0002                          | 0.89  | 0.002                           | 0.22  | -0.002                          | 0.009   |
| Non-White                                                                                                                                                                        | 0.030    | 0.52    | 0.037                           | 0.51    | 0.072                           | 0.051   | 0.053                           | 0.17  | -0.040                          | 0.33  | -0.012                          | 0.5     |
| Ethnicity                                                                                                                                                                        | -0.022   | 0.78    | -0.095                          | 0.32    | 0.127                           | 0.04    | 0.072                           | 0.26  | 0.004                           | 0.96  | 0.00003                         | 0.999   |
| Dose Type                                                                                                                                                                        |          |         |                                 |         |                                 |         |                                 |       |                                 |       |                                 |         |
| Moderna                                                                                                                                                                          | 0.105    | 0.02    | 0.164                           | 0.004   | 0.042                           | 0.26    | 0.053                           | 0.17  | 0.081                           | 0.04  | 0.023                           | 0.20    |
| Days between dose 2 & 3                                                                                                                                                          | 0.001    | 0.01    | 0.001                           | 0.03    | 0.001                           | 0.07    | 0.0005                          | 0.23  | 0.001                           | 0.02  | -0.0002                         | 0.29    |
| Days since last SARS-CoV-2 infection                                                                                                                                             | 0.0005   | <0.0001 | 0.001                           | <0.0001 | 0.0002                          | 0.01    | 0.0001                          | 0.07  | 0.0001                          | 0.38  | -                               | 0.32    |
|                                                                                                                                                                                  |          |         |                                 |         |                                 |         |                                 |       |                                 |       | 0.00003                         |         |
| Time IC - NIC<br>Not applicable                                                                                                                                                  | P        |         | Time IC - NIC<br>Not applicable | P       | Time IC - NIC<br>0              | 0.08    | Time IC - NIC<br>Not applicable | P     | Time IC - NIC<br>Not applicable | P     | Time IC - NIC<br>-15            | 0.59    |
| Note: when separate smooths were generated for IC groups, high-level IC predicted titers were lower than low-level IC & NIC predicted titers at 15 & 35 days after vaccine dose. |          |         |                                 |         | 15                              | 0.02    |                                 |       |                                 |       | 0                               | 0.68    |
|                                                                                                                                                                                  |          |         |                                 |         | 35                              | 0.02    |                                 |       |                                 |       | 15                              | 0.78    |
|                                                                                                                                                                                  |          |         |                                 |         | 50                              | 0.07    |                                 |       |                                 |       | 50                              | 0.95    |
|                                                                                                                                                                                  |          |         |                                 |         | 100                             | 0.19    |                                 |       |                                 |       | 100                             | 0.97    |
|                                                                                                                                                                                  |          |         |                                 |         | 150                             | 0.31    |                                 |       |                                 |       | 200                             | 0.80    |
|                                                                                                                                                                                  |          |         |                                 |         | 200                             | 0.92    |                                 |       |                                 |       | 300                             | 1.00    |
|                                                                                                                                                                                  |          |         |                                 |         | 300                             | 0.68    |                                 |       |                                 |       | 400                             | 0.27    |
|                                                                                                                                                                                  |          |         |                                 |         | 400                             | 0.87    |                                 |       |                                 |       | 485                             | 0.13    |
| Dose 4                                                                                                                                                                           |          |         |                                 |         |                                 |         |                                 |       |                                 |       |                                 |         |
| Intercept                                                                                                                                                                        | Estimate | P       | Estimate                        | P       | Estimate                        | P       | Estimate                        | P     | Estimate                        | P     | Estimate                        | P       |
| High-level IC                                                                                                                                                                    | 3.694    | <0.0001 | 3.697                           | <0.0001 | 0.792                           | <0.0001 | 0.480                           | 0.01  | 0.291                           | 0.02  | 0.172                           | 0.08    |
| Age at dose 4                                                                                                                                                                    | -0.134   | 0.05    | -0.191                          | 0.03    | -0.131                          | 0.11    | -0.111                          | 0.02  | -0.152                          | 0.005 | -0.068                          | 0.11    |
| Non-White                                                                                                                                                                        | 0.001    | 0.47    | 0.002                           | 0.51    | -0.001                          | 0.67    | -0.001                          | 0.46  | 0.001                           | 0.56  | 0.0003                          | 0.80    |
| Ethnicity                                                                                                                                                                        | 0.013    | 0.75    | 0.006                           | 0.91    | 0.040                           | 0.43    | 0.032                           | 0.50  | -0.014                          | 0.67  | -0.013                          | 0.60    |
| Male sex                                                                                                                                                                         | -0.127   | 0.17    | -0.126                          | 0.31    | -0.054                          | 0.64    | -0.090                          | 0.41  | 0.095                           | 0.20  | 0.105                           | 0.06    |
| Dose Type                                                                                                                                                                        |          |         |                                 |         |                                 |         |                                 |       |                                 |       |                                 |         |
| Moderna                                                                                                                                                                          | 0.101    | 0.05    | 0.111                           | 0.11    | -0.077                          | 0.23    | -0.021                          | 0.74  | -0.002                          | 0.97  | 0.053                           | 0.11    |

|                                      |          |         |                |          |         |      |              |       |                |          |       |      |          |        |                |          |      |
|--------------------------------------|----------|---------|----------------|----------|---------|------|--------------|-------|----------------|----------|-------|------|----------|--------|----------------|----------|------|
| Dose Type Moderna (Half dose)        | 0.040    | 0.76    |                | 0.070    | 0.68    |      | -0.272       | 0.09  |                | -0.337   | 0.03  |      | 0.223    | 0.03   |                | -0.028   | 0.72 |
| Dose Type Moderna (Bivalent)         | 0.065    | 0.37    |                | 0.089    | 0.37    |      | -0.038       | 0.68  |                | 0.024    | 0.79  |      | 0.032    | 0.59   |                | 0.086    | 0.06 |
| Dose Type Pfizer (Bivalent)          | 0.043    | 0.60    |                | 0.052    | 0.64    |      | -0.084       | 0.42  |                | -0.016   | 0.87  |      | 0.037    | 0.57   |                | 0.125    | 0.02 |
| Days between dose 3 & 4              | -0.0003  | 0.50    |                | 0.0001   | 0.83    |      | -0.001       | 0.34  |                | 0.0004   | 0.50  |      | -0.0004  | 0.23   |                | -0.0003  | 0.37 |
| Days since last SARS-CoV-2 infection | 0.0003   | <0.0001 |                | 0.0005   | <0.0001 |      | -<br>0.00002 | 0.81  |                | 0.00002  | 0.84  |      | 0.0001   | 0.16   |                | 0.0001   | 0.17 |
| Time                                 | IC - NIC | P       | Time           | IC - NIC | P       | Time | IC - NIC     | P     | Time           | IC - NIC | P     | Time | IC - NIC | P      | Time           | IC - NIC | P    |
| -25                                  | -0.113   | 0.25    | -30            | -0.276   | 0.01    | -25  | -0.245       | 0.004 | -30            | -0.118   | 0.14  | -25  | -0.204   | <0.001 | Not applicable |          |      |
| 0                                    | -0.135   | 0.15    | 0              | -0.205   | 0.03    | 0    | -0.224       | 0.007 | 0              | -0.095   | 0.23  | 0    | -0.209   | <0.001 |                |          |      |
| 25                                   | -0.205   | 0.02    | 25             | -0.156   | 0.11    | 25   | -0.202       | 0.01  | 25             | -0.083   | 0.29  | 15   | -0.210   | <0.001 |                |          |      |
| 45                                   | -0.175   | 0.04    | 50             | -0.120   | 0.22    | 50   | -0.181       | 0.03  | 50             | -0.077   | 0.33  | 50   | -0.205   | <0.001 |                |          |      |
| 100                                  | -0.024   | 0.81    | 150            | -0.139   | 0.21    | 75   | -0.159       | 0.049 | 100            | -0.085   | 0.28  | 75   | -0.195   | <0.001 |                |          |      |
| 200                                  | -0.171   | 0.07    | 200            | -0.202   | 0.06    | 150  | -0.095       | 0.26  | 150            | -0.111   | 0.16  | 100  | -0.181   | 0.001  |                |          |      |
| 300                                  | -0.187   | 0.12    | 250            | -0.254   | 0.03    | 250  | -0.009       | 0.93  | 200            | -0.128   | 0.12  | 150  | -0.142   | 0.01   |                |          |      |
| 400                                  | -0.221   | 0.10    | 300            | -0.275   | 0.04    |      |              |       | 250            | -0.134   | 0.12  | 200  | -0.101   | 0.09   |                |          |      |
| 500                                  | -0.209   | 0.18    | 350            | -0.269   | 0.05    |      |              |       |                |          |       | 250  | -0.062   | 0.34   |                |          |      |
|                                      |          |         | 400            | -0.244   | 0.11    |      |              |       |                |          |       |      |          |        |                |          |      |
|                                      |          |         | 450            | -0.205   | 0.27    |      |              |       |                |          |       |      |          |        |                |          |      |
| Dose 5                               |          |         |                |          |         |      |              |       |                |          |       |      |          |        |                |          |      |
|                                      | Estimate | P       |                | Estimate | P       |      | Estimate     | P     |                | Estimate | P     |      | Estimate | P      |                | Estimate | P    |
| Intercept                            | 3.773    | <0.0001 |                | 3.770    | <0.0001 |      | 0.650        | 0.003 |                | 0.533    | 0.003 |      | 0.108    | 0.58   |                | 0.013    | 0.93 |
| High-level IC                        | -0.299   | 0.006   |                | -0.435   | 0.0007  |      | -0.223       | 0.06  |                | -0.156   | 0.09  |      | -0.119   | 0.23   |                | -0.099   | 0.21 |
| Age at dose 5                        | -0.001   | 0.66    |                | 0.000    | 0.97    |      | 0.001        | 0.86  |                | 0.00004  | 0.99  |      | 0.002    | 0.47   |                | 0.003    | 0.21 |
| Male sex                             | 0.010    | 0.90    |                | 0.017    | 0.86    |      | -0.017       | 0.84  |                | -0.018   | 0.79  |      | -0.027   | 0.72   |                | -0.099   | 0.1  |
| Dose Type Moderna (Bivalent)         | 0.133    | 0.10    |                | 0.141    | 0.15    |      | Referent     | -     |                | Referent | -     |      | Referent | -      |                | Referent | -    |
| Dose Type Pfizer (Bivalent)          | Referent | -       |                | Referent | -       |      | -0.118       | 0.16  |                | 0.037    | 0.58  |      | 0.049    | 0.5    |                | 0.103    | 0.08 |
| Days between dose 4 & 5              | 0.00003  | 0.95    |                | 0.0004   | 0.54    |      | -0.0001      | 0.90  |                | 0.0003   | 0.54  |      | 0.00001  | 0.98   |                | 0.0002   | 0.7  |
| Days since last SARS-CoV-2 infection | 0.0001   | 0.32    |                | 0.0002   | 0.20    |      | 0.0002       | 0.10  |                | 0.0001   | 0.35  |      | 0.0001   | 0.22   |                | 0.0002   | 0.09 |
| Time                                 | IC - NIC | P       | Time           | IC - NIC | P       | Time | IC - NIC     | P     | Time           | IC - NIC | P     | Time | IC - NIC | P      | Time           | IC - NIC | P    |
| Not applicable                       |          |         | Not applicable |          |         | -15  | -0.168       | 0.19  | Not applicable |          |       | -15  | -0.072   | 0.51   | Not applicable |          |      |
|                                      |          |         |                |          |         | 0    | -0.171       | 0.17  |                |          |       | 0    | -0.077   | 0.48   |                |          |      |
|                                      |          |         |                |          |         | 25   | -0.171       | 0.16  |                |          |       | 25   | -0.085   | 0.42   |                |          |      |
|                                      |          |         |                |          |         | 50   | -0.165       | 0.16  |                |          |       | 50   | -0.092   | 0.37   |                |          |      |
|                                      |          |         |                |          |         | 75   | -0.159       | 0.17  |                |          |       | 100  | -0.104   | 0.30   |                |          |      |
|                                      |          |         |                |          |         | 100  | -0.160       | 0.18  |                |          |       | 200  | -0.146   | 0.15   |                |          |      |
|                                      |          |         |                |          |         | 150  | -0.186       | 0.13  |                |          |       | 275  | -0.210   | 0.06   |                |          |      |
|                                      |          |         |                |          |         | 200  | -0.260       | 0.06  |                |          |       |      |          |        |                |          |      |

|     |        |       |
|-----|--------|-------|
| 250 | -0.395 | 0.01  |
| 275 | -0.467 | 0.005 |

An alpha level of 0.025 was used to determine statistical significance to account for bias from multiplicity .

Table 5: Multivariable linear regression of anti-spike (S) IgG antibodies after the second SARS-CoV-2 vaccine dose among participants with no prior SARS-CoV-2 infection, according to IC subgroup. For models that include separate smooths for IC groups, the difference of predicted titers between immune compromised participants (IC) and non-immune compromised (NIC) participants are shown below model estimates (see column “IC – NIC”). High-level IC referent: low-level & NIC. Male sex referent: female sex. Non-White race referent: White race. Time=Days since dose date. Dose 2 vaccine type referent: Pfizer.

| Anti-S IgG (log <sub>10</sub> BAU/mL) Following Dose 2 |          |          |         |                                |          |          |                              |      |          |                           |         |  |
|--------------------------------------------------------|----------|----------|---------|--------------------------------|----------|----------|------------------------------|------|----------|---------------------------|---------|--|
| Asthma/COPD<br>(requiring medication)                  |          |          |         | Cancer                         |          |          | Celiac's Disease             |      |          | Diabetes                  |         |  |
|                                                        |          | Estimate | P       |                                |          | Estimate | P                            |      |          | Estimate                  | P       |  |
| Intercept                                              |          | 3.499    | <0.0001 |                                |          | 3.529    | <0.0001                      |      |          | 3.509                     | <0.0001 |  |
| IC                                                     |          | -0.008   | 0.84    |                                |          | -0.243   | 0.007                        |      |          | -0.024                    | 0.83    |  |
| Age at dose 2                                          |          | -0.007   | <0.0001 |                                |          | -0.008   | <0.0001                      |      |          | -0.008                    | <0.0001 |  |
| Male sex                                               |          | 0.018    | 0.56    |                                |          | 0.009    | 0.79                         |      |          | 0.007                     | 0.84    |  |
| Non-White Ethnicity                                    |          | 0.0001   | 0.99    |                                |          | 0.034    | 0.51                         |      |          | 0.023                     | 0.65    |  |
| Dose Type Moderna                                      |          | 0.178    | <0.0001 |                                |          | 0.176    | <0.0001                      |      |          | 0.168                     | <0.0001 |  |
| Days between dose 1 & 2                                |          | 0.0008   | 0.19    |                                |          | 0.0009   | 0.15                         |      |          | 0.001                     | 0.18    |  |
| HIV (suppressed)                                       |          |          |         | IC Medications (high severity) |          |          | Organ/Bone Marrow Transplant |      |          | Rheumatological Condition |         |  |
|                                                        |          | Estimate | P       |                                |          | Estimate | P                            |      |          | Estimate                  | P       |  |
| Intercept                                              |          | 3.535    | <0.0001 |                                |          | 3.518    | <0.0001                      |      |          | 3.572                     | <0.0001 |  |
| IC                                                     |          | -0.641   | <0.0001 |                                |          | -0.433   | <0.0001                      |      |          | -1.146                    | <0.0001 |  |
| Age at dose 2                                          |          | -0.008   | <0.0001 |                                |          | -0.009   | <0.0001                      |      |          | -0.008                    | <0.0001 |  |
| Male sex                                               |          | -0.002   | 0.94    |                                |          | -0.0003  | 0.99                         |      |          | 0.025                     | 0.48    |  |
| Non-White Ethnicity                                    |          | 0.023    | 0.64    |                                |          | 0.036    | 0.51                         |      |          | 0.001                     | 0.99    |  |
| Dose Type Moderna                                      |          | 0.172    | <0.0001 |                                |          | 0.174    | <0.0001                      |      |          | 0.188                     | <0.0001 |  |
| Days between dose 1 & 2                                |          | 0.001    | 0.18    |                                |          | 0.002    | 0.01                         |      |          | 0.001                     | 0.08    |  |
| Time                                                   | IC - NIC |          | P       | Time                           | IC - NIC |          | P                            | Time | IC - NIC |                           | P       |  |
| -25                                                    | -1.813   |          | <0.001  | -25                            | -0.610   |          | <0.001                       | -25  | -2.911   |                           | <0.001  |  |
| 0                                                      | -1.339   |          | <0.001  | 0                              | -1.420   |          | <0.001                       | 0    | -1.909   |                           | <0.001  |  |
| 14                                                     | -1.312   |          | <0.001  | 15                             | -1.602   |          | <0.001                       | 15   | -1.992   |                           | <0.001  |  |
| 25                                                     | -0.451   |          | 0.003   | 25                             | -1.010   |          | <0.001                       | 25   | -1.464   |                           | <0.001  |  |
| 50                                                     | 0.091    |          | 0.61    | 50                             | -0.175   |          | 0.12                         | 50   | -0.787   |                           | <0.001  |  |
| 75                                                     | -0.833   |          | 0.001   | 100                            | -0.096   |          | 0.41                         | 100  | -0.583   |                           | <0.001  |  |
| 100                                                    | 0.144    |          | 0.33    |                                |          |          |                              |      |          |                           |         |  |



|     |        |      |     |        |      |     |        |      |     |       |      |
|-----|--------|------|-----|--------|------|-----|--------|------|-----|-------|------|
| 100 | -0.022 | 0.93 | 150 | -0.096 | 0.62 | 125 | -0.566 | 0.03 | 150 | 0.281 | 0.12 |
| 125 | -0.222 | 0.30 |     |        |      |     |        |      |     |       |      |
| 150 | -0.177 | 0.55 |     |        |      |     |        |      |     |       |      |

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An alpha level of 0.025 was used to determine statistical significance to account for bias from multiplicity .

Supplemental Table 1: Categorization of immune compromising conditions and medications in the Stop the Spread Ottawa (SSO) cohort, in order of the strength of previously reported immunosuppressive effects. In the case of a participant reporting multiple IC states, the classification was determined by the highest-level condition.

| Classification of IC Conditions and Medications                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Justification                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|--------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| High-Level Immune Compromising States                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Systemic immunosuppressant medication (e.g., corticosteroids, biologics) | <p>Abemaciclib<sup>69</sup></p> <p>Adalimumab<sup>70</sup></p> <p>Azathioprine<sup>71</sup></p> <p>Budesonide<sup>72</sup></p> <p>Cladribine<sup>73</sup></p> <p>Cyclosporine<sup>74</sup></p> <p>Dupilumab<sup>75</sup></p> <p>Etanercept<sup>76</sup></p> <p>Glatiramer acetate<sup>77</sup></p> <p>Guselkumab<sup>78</sup></p> <p>Infliximab<sup>79</sup></p> <p>Leflunomide<sup>81</sup></p> <p>Methotrexate<sup>82</sup></p> <p>Mycophenolate mofetil<sup>83</sup></p> <p>Ocrelizumab<sup>84</sup></p> <p>Prednisone<sup>85</sup></p> <p>Risankizumab<sup>86</sup></p> <p>Rituximab<sup>87</sup></p> <p>Secukinumab<sup>88</sup></p> <p>Sirolimus<sup>89</sup></p> <p>Sulfasalazine<sup>90</sup></p> <p>Tacrolimus<sup>91</sup></p> <p>Teriflunomide<sup>92</sup></p> <p>Tocilizumab<sup>93</sup></p> <p>Tofacitinib<sup>94</sup></p> <p>Upadacitinib<sup>95</sup></p> <p>Ustekinumab<sup>96</sup></p> | Product monographs report immunosuppression as an established side effect. Use of these medications is associated with a heightened risk of respiratory tract infections, and other infections.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Conditions                                                               | <p>Malignancy (current or past)<sup>20,36</sup></p> <p>Primary immunodeficiency (antibody deficiency, hypogammaglobulinemia)<sup>97</sup></p> <p>Organ/bone marrow transplant<sup>30</sup></p> <p>HIV (not suppressed)<sup>98</sup></p> <p>Rheumatological condition (rheumatoid arthritis, active systemic lupus erythematosus, polymyalgia rheumatica, polymyositis, ankylosing spondylitis, scleroderma/CREST syndrome)<sup>33</sup></p> <p>Neuromuscular autoimmune diseases (multiple sclerosis, Myasthenia gravis)<sup>99</sup></p> <p>Severe liver cirrhosis<sup>100</sup></p>                                                                                                                                                                                                                                                                                                                       | <p>Sun et al. and Carreira et al. report impaired COVID-19 vaccine response due to malignancy and an increased risk of serious COVID-19 among individuals with a history of cancer.<sup>20,36</sup></p> <p>Primary immunodeficiency is associated with repeated respiratory tract infections.<sup>97</sup></p> <p>Early studies indicated impaired COVID-19 vaccine humoral immune response among organ/bone marrow transplant recipients and individuals with rheumatological disease.<sup>30,33</sup></p> <p>Sheth et al. report that HIV infection is associated with an increased risk of repeated respiratory tract infections, and other infections.<sup>98</sup></p> <p>Iaffaldano et al. report a heightened risk of COVID-19 among individuals with multiple sclerosis (MS).<sup>99</sup> Severe</p> |

|                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | <p>MS was also associated with more serious COVID-19.</p> <p>Albillos et al. describe the systemic immunodeficiency and immune dysfunction that occurs with liver cirrhosis.<sup>100</sup></p>                                                                                                                                                                                                                                                                                                    |
| Low-Level Immune Compromising States               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Asthma/COPD treated with (non-systemic) medication | <p>Inhaled medications:<sup>39</sup></p> <p>Albuterol<br/>Budesonide<br/>Budesonide and formoterol<br/>Ciclesonide<br/>Fluticasone<br/>Fluticasone and salmeterol<br/>Fluticasone and vilanterol<br/>Mometasone furoate and formoterol fumarate dihydrate<br/>Omalizumab</p> <p>Nasal sprays containing corticosteroids:<sup>39</sup></p> <p>Apo-mometasone<br/>Azelastine and fluticasone<br/>Budesonide<br/>Ciclesonide<br/>Fluticasone furoate<br/>Fluticasone propionate<br/>Mometasone</p> | <p>There is a substantial body evidence drawing a link between the chronic use of oral corticosteroids and systemic immunosuppression (e.g., impaired cell-mediated immunity).<sup>39</sup></p> <p>Pandya et al. report possible systemic immunosuppression following the use of inhaled corticosteroids as well. For example, there are case reports and other studies indicating that inhaled corticosteroids may be associated with repeated viral infections, tuberculosis, or pneumonia.</p> |
| Autoimmune conditions                              | <p>Celiac disease<sup>8</sup></p> <p>Autoimmune hepatitis<sup>101</sup></p> <p>Graves' disease<sup>102</sup></p>                                                                                                                                                                                                                                                                                                                                                                                | <p>Early studies indicated impaired COVID-19 vaccine humoral immune response among individuals with Celiac disease and autoimmune hepatitis.<sup>8,101</sup></p> <p>Struja et al. summarize evidence suggesting a possible link between immunodeficiency and Graves' disease.<sup>102</sup></p>                                                                                                                                                                                                   |
| Other conditions                                   | <p>Diabetes mellitus<sup>42</sup></p> <p>Chronic kidney disease<sup>103</sup></p> <p>Systemic Lupus Erythematosus (SLE) in remission<sup>18</sup></p>                                                                                                                                                                                                                                                                                                                                           | <p>Early studies indicated impaired COVID-19 vaccine humoral immune response among individuals with diabetes or SLE.<sup>42,18</sup></p> <p>Syed-Ahmed et al. reported increased risk of infection and impaired</p>                                                                                                                                                                                                                                                                               |

|  |                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                        |
|--|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | Mild liver cirrhosis (fibrosis) <sup>100</sup><br><br>HIV (fully suppressed) <sup>98</sup><br><br>Inflammatory bowel disease (not treated with medication) <sup>104</sup> | immune function among individuals with chronic kidney disease. <sup>103</sup><br><br>Liver fibrosis and suppressed HIV infection were classified as low-level IC relative to cirrhosis or unsuppressed HIV infection.<br><br>Inflammatory bowel disease was investigated as an immune compromised population in an early scoping review of the literature on COVID-19 vaccine response. <sup>104</sup> |
|--|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

The following were categorized as high-level: malignancies (active or in the past); organ/bone marrow transplant; primary immunodeficiencies (e.g., specific antibody deficiencies, hypogammaglobulinemia); rheumatological conditions (e.g., rheumatoid arthritis, active systemic lupus erythematosus [SLE], ankylosing spondylitis); neuromuscular autoimmune diseases (e.g., multiple sclerosis, myasthenia gravis); active HIV (detectable viral load >20 IU/mL); serious liver disease (e.g., severe cirrhosis); use of systemic immunosuppressant medication (e.g., azathioprine, mycophenolate mofetil [CellCept], infliximab, prednisone [by injection]). Low-level IC states included: respiratory conditions requiring inhaled medication or nasal sprays such as corticosteroids (e.g., asthma); local/organ-specific autoimmune diseases (e.g., celiac disease, autoimmune hepatitis, inflammatory bowel disease, Graves' disease, Hashimoto's disease) not treated with a systemic immunosuppressant; mild or resolved liver cirrhosis; inactive SLE; fully suppressed HIV (viral load <20 IU/mL); chronic kidney disease; diabetes.

## Figures

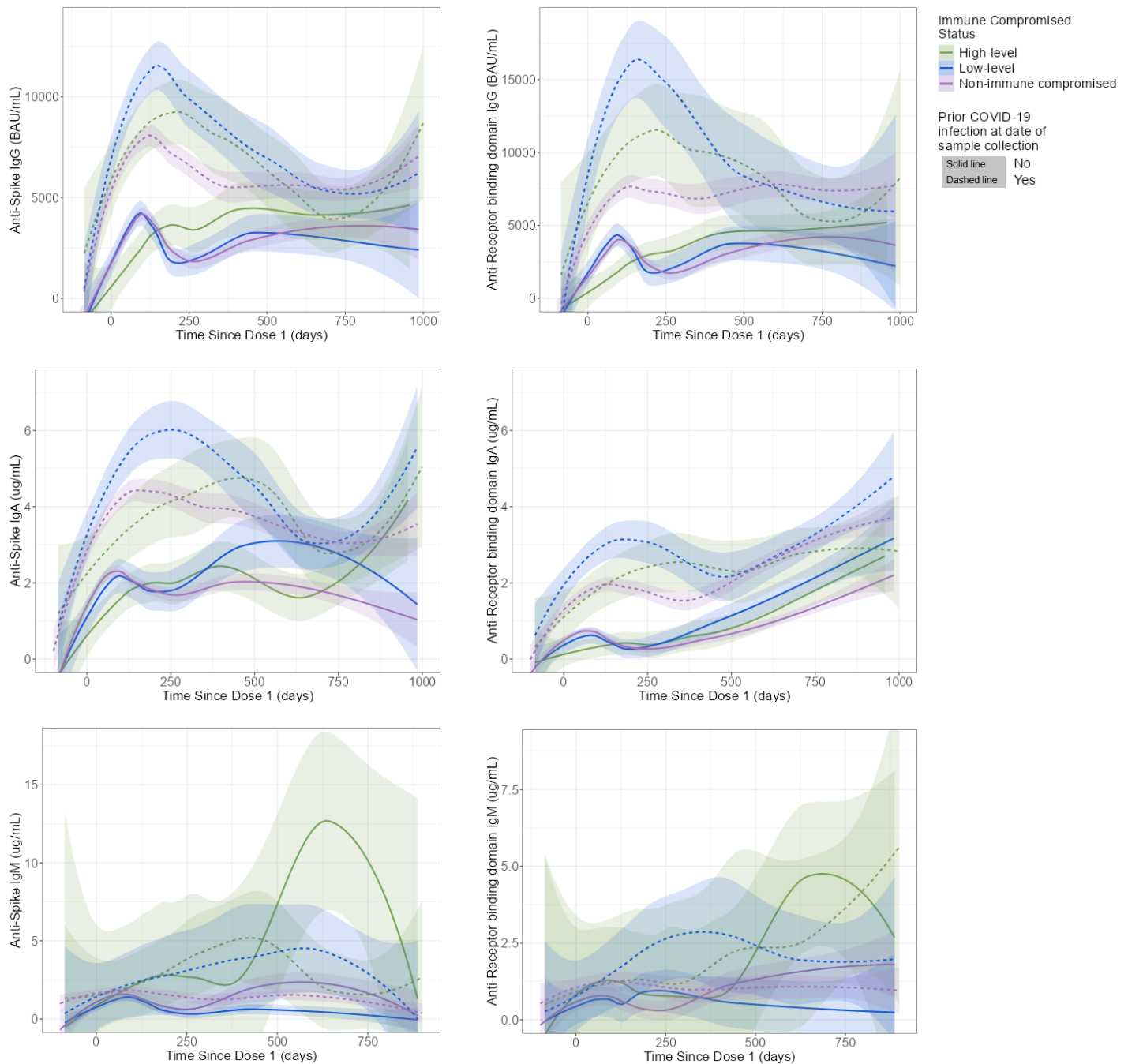


Figure 1: LOESS smoothing curves depicting IgG (BAU/mL), IgM, IgA ( $\mu\text{m}/\text{mL}$ ) antibody titers against the spike and receptor binding domain antigens over time relative to the first dose of COVID-19 vaccination. Separate curves are presented according to immune compromised status and SARS-CoV-2 infection history. Standard error is represented by bands.

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# Chapter 4 – Obesity Does Not Influence SARS-CoV-2 Vaccine Humoral Immunogenicity

## Preface

This article partially accomplishes research objective III: to determine if immune compromised status, obesity, excessive alcohol consumption, or SSRI medication use influence the production and durability and/or neutralization activity of antibodies targeting SARS-CoV-2 following COVID-19 vaccination. In this study, the slope of antibody production and decay following three COVID-19 vaccine doses was compared between obese participants and non-obese participants. Neutralization activity was assessed according to obesity up to 2 doses.

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## **Abstract**

Obesity is a recognized factor influencing immune function and infectious disease outcomes. Characterization of the influence of obesity on SARS-CoV-2 vaccine humoral immunogenicity is required to properly tailor vaccine type (mRNA, viral-vector, protein subunit vaccines) and dosing schedule. Data from a prospective cohort study collected over 34 months was used to evaluate the slope of antibody production and decay and neutralizing capacity following SARS-CoV-2 vaccination in individuals with and without obesity at baseline. Most participants were female (65.4%), white (92.4%), and received mRNA vaccines. 210 were obese and 697 non-obese. Sex and infection-acquired immunity were identified as effect modifiers for the relationship between obesity and COVID-19 vaccine humoral immunogenicity. No consistent influence of obesity on peak titers, titer retention, antibody isotype (IgG, IgM, IgA), or neutralization was identified when controlling for other key variables. It may not be necessary to consider this variable when developing SARS-CoV-2 vaccine dosing strategies.

## **Introduction**

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerging in Wuhan China in December 2019, quickly spread worldwide due to its high level of transmissibility causing millions of deaths and hospitalizations<sup>1</sup>. In response to the novel pandemic, vaccines were developed to reduce dissemination, morbidity and mortality resulting from SARS-CoV-2 virus infection. Vaccine immunogenicity is influenced by host factors including age, gender, gut microbiota, and comorbidities,<sup>2,3</sup> viral variants (i.e., Omicron sublineages), as well as external elements pertaining to vaccine administration, type, number of doses, and dosing interval<sup>3,4</sup>. Across a wide range of other infectious diseases including influenza, hepatitis B, tetanus,<sup>5</sup> and middle eastern respiratory virus (MERS), obesity has been recognized as a variable influencing vaccine immunogenicity<sup>6–8</sup>. The influence of obesity on SARS-COV-2 vaccine immunogenicity has been evaluated but not been fully elucidated<sup>9,10</sup>. Projections from the World Obesity

Federation estimate that by 2035, the international prevalence of obese and overweight individuals will rise from 38% (in 2020) to over 50%<sup>11</sup>. The escalating obesity epidemic,<sup>12</sup> and its multifaceted impacts on public health, emphasizes the importance of gaining a more comprehensive understanding of its influence on both acute and long-term humoral vaccine efficacy.

Individuals living with obesity are at higher risk for severe outcome with COVID-19 infection including increased hospitalization and ICU admission<sup>9,13,14</sup>. Prior studies have suggested lower antibody titers<sup>6–8</sup> accelerated antibody waning, and attrition of neutralizing capacity with obesity<sup>9</sup>. However, the literature is not consistent. In fact, some prior publications report that obese patients exhibited higher peak antibody titers<sup>9</sup>, including neutralizing antibody titres<sup>10</sup>.

The Stop the Spread Ottawa (SSO) cohort study provides insight into the dynamics of the immune response to natural infection and vaccination in a diverse, urban-based population followed over the entirety of the SARS-CoV-2 pandemic. Our robust serial monitoring of participants allows for an in-depth assessment of vaccine immunogenicity in obese and non-obese individuals. To this end, our group assessed quantification of key antibody isotypes against the full trimeric spike protein and receptor binding domain as a function of obesity. We hypothesize that obese individuals will exhibit a reduced rate of antibody production and faster rate of antibody decay following vaccination.

## **Methods**

### *Study Design*

The Stop the Spread Ottawa (SSO) cohort study was implemented to assess SARS-CoV-2 humoral and cellular immune responses following infection and vaccination across multiple at-risk groups. SSO is a prospective study which has collected longitudinal data on individuals

residing in the Ottawa, Canada region, 18 years of age or older, and who are at high risk of exposure to SARS-CoV-2 (due to health conditions or occupation), experiencing severe SARS-CoV-2 infection outcomes, and/or have a previous documented SARS-CoV-2 infection (confirmed by quantitative reverse transcription polymerases chain reaction test (RT-PCR) or serology analyses)<sup>15,16</sup>. Recruitment occurred from September 2020 until February 2022. The initial 1,006 participants were followed for 10 months and underwent serial blood draws and completed serial questionnaires. Three hundred of these participants completed an additional 24-month extension phase.

Individuals meeting SSO eligibility criteria (n=1,006) were recruited and included in a convalescence cohort comprised of individuals with a history of COVID-19 infection (confirmed by RT-PCR or serology analyses) and a surveillance cohort comprised of individuals at high risk of exposure to SARS-CoV-2. Surveillance cohort participants with a confirmed case (by RT-PCR or rapid antigen test) of COVID-19 infection were voluntarily transferred to the convalescent cohort. Specific to our obesity analysis, participants were included with data regarding obesity status, serum antibody results, and vaccination dates. Participants who became pregnant during follow-up were excluded. Participants were classified as obese at baseline if they had a BMI of 30 or greater according to the United States Centers for Disease Control and Prevention (CDC) BMI categories for adults<sup>17</sup> or if they self-identified as being obese in the baseline questionnaire. In cases where self-reported status and BMI classification lead to incongruent obesity classifications, BMI data was used to determine obesity classification. If BMI data was unavailable, study staff contacted the participants directly to obtain the information.

Variables including age, sex, ethnicity, comorbidities, immune compromised status, time between vaccine doses, and time from last COVID-19 infection were adjusted for in the analysis. Immune compromising conditions were identified and classified based on expert opinion and evidence in the literature regarding strength of immunosuppressive effects. Cancer, organ/tissue

transplant, primary immunodeficiencies, rheumatological conditions (e.g., rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis), multiple sclerosis, myasthenia gravis, active HIV (detectable viral load  $\geq 20$  copies/mL), and serious liver disorder (e.g., severe cirrhosis) were considered high-level immune compromising. Respiratory conditions requiring medication (i.e. asthma), autoimmune diseases (e.g., celiac disease, autoimmune hepatitis), fully suppressed HIV (viral load  $< 20$  copies/mL), less severe liver disorder (e.g., mild cirrhosis) and kidney disorders (i.e. chronic kidney disease) as well as excessive alcohol consumption were considered low-level immune compromising. Use of immunosuppressants and inflammatory bowel disease were also included as immune compromising states, but the level was dependent on the strength of immunosuppression caused by the specific medications.

#### *SARS-CoV-2 Vaccination and Infection Data*

SARS-CoV-2 IgG, IgA and IgM antibodies were quantified using a high-throughput direct chemiluminescent ELISA performed on participant serum samples. IgG results were calibrated according to the World Health Organization (WHO) international reference standard and expressed in international binding antibody units (BAU) per mL. IgA and IgM results were expressed in micrograms ( $\mu\text{g}$ ) per mL. ELISA is subject to a limited range of detectability often causing variability among assays. On account of this, serum, and plasma dilutions of 1:1000 of 1:100 were used to ensure that antibody titers fell within a quantifiable range thus providing accurate results<sup>18</sup>. A surrogate neutralization ELISA was performed to ascertain neutralizing capacity (%) for anti-S IgG. Anti-N IgG antibodies were used to identify incident SARS-CoV-2 infections for which a positive test result was not reported. Individuals positive for anti-N IgG in addition to either anti-S or anti-RBD were considered to have acquired immunity through natural infection. Vaccination status at time of serum sample collection was determined using self-reported COVID-19 vaccination dates, which were verified using medical records when available. Participants with hybrid immunity were identified according to date of vaccination

(self-reported and verified) and infection (self-reported or identified by anti-N IgG titers). If an infection occurred within 14 days after vaccination, this infection was considered as prior to vaccination to account the 2-week delay in vaccine-elicited immunity<sup>19</sup>.

### *Statistics and Reproducibility*

The data had a non-normal and skewed distribution and was linearized through the  $\log_{10}$ -transformation. One unit was added to all unadjusted values to eliminate undefined values that would have otherwise been produced by log-transforming 0 values. Outliers and some skewing were still observed for the log-adjusted data. Two-sided non-parametric or parametric statistical tests with an alpha level of 0.05 were used where appropriate (as determined by histograms and Q-Q plots). Given the high number of models generated for different doses and antibody isotypes, statistical significance was interpreted conservatively.

To determine the impact of obesity on humoral immunity following COVID-19 vaccination, the slope of antibody production and decay after each vaccine dose was assessed by univariable and multivariable segmented Bayesian robust linear regression using the Student's t-distribution for the outcome. Both absolute antibody titers and neutralizing capacity for anti-S IgG following SARS-CoV-2 vaccination were assessed. Baseline titers and the slopes of titer production and decay for obese participants were compared to overweight, normal weight, and underweight participants. A linear spline was placed at the titer peak to generate separate slope estimates for the regions of antibody production and decay and the x-axis was restricted to remove data from prior doses and exclude sparse data at extended follow-up. Random effects for intercepts (and slopes where sufficient data was available) were incorporated to address autocorrelation inherent to longitudinal data. The inclusion of an interaction term between obesity and time allowed slopes to vary according to obesity. Data was stratified by antibody isotype (IgG, IgM, IgA) and anti-S/RBD antibody specificity, vaccine dose number, SARS-CoV-2 infection history, and sex due to evidence in the literature indicating that the slope of antibody

decay may vary by sex<sup>20,21</sup>. Sensitivity analyses exploring different BMI groupings were conducted with univariable models.

For some models which failed to converge with the Student's t-distribution, the "skew-normal" family was used<sup>22</sup>. Bayesian models used default non-informative priors, 4 chains and at least 7,000 iterations. Model convergence and chain mixing was verified with Rhat (<1.05) and Bulk-ESS (>400). Statistical tests comparing demographics and vaccination and infection characteristics between obese and non-obese participants were conducted using Statistical Analysis System (SAS) version 9.4. RStudio version 2023.06.1 was used to complete regression analyses (package brms) and generate figures (package ggplot2).

#### *Data Availability*

The datasets used in this study may be acquired from the corresponding author upon reasonable request by qualified researchers. All requests will be subject to limitations implemented to protect participant privacy. Data requests require approval from research ethics committees and data custodians.

## **Results**

#### *Study Participant Characteristics*

Of 907 eligible participants, 210 were classified as obese and 697 as non-obese (Table 1). Of 121 participants with self-reported obesity, 72 had BMI data available. The mean BMI for these participants was 37.1 (standard deviation: 5.8; range: 30.0-55.1). Compared to the non-obese group, those classified as obese were older (mean age; 48.2 vs 44.9 years), had a greater proportion of men (39.1% vs 33.3%) and comorbidities including hypertension (21.0% vs 7.6%) and diabetes (9.0% vs 3.2%). Non-obese individuals consumed more alcohol (mean average number of standard drinks per week; 3.3 vs 2.4). Almost 90% of participants received an mRNA vaccine for their initial dose and 98% plus received a mRNA vaccine for subsequent doses



(Supplemental Table 1). The crude incidence of SARS-CoV-2 infection (46.2% vs 38.2%) and incidence of repeat infection (8.1% vs 4.9%) was higher in obese SSO participants. The incidence of post-vaccination breakthrough SARS-CoV-2 infections were generally similar in obese and non-obese individuals up to 7 doses.

#### *Humoral Response to SARS-CoV-2 Vaccination*

Titers for IgG, IgM, and IgA over up to 24 months post vaccination against the spike (S), receptor binding domain (RBD) proteins are represented in Figure 1. Relative to the initial SARS-CoV-2 vaccine, antibody production and decay trends across doses 1 to 7 is depicted. Overall, individuals with a prior history of SARS-CoV-2 infection achieved higher peak titers and increased rates of antibody production upon vaccination.

Univariable models comparing obese and overweight participants to normal and underweight were generated for anti-S and anti-RBD IgG to explore the impact of different comparison groups on vaccine immunogenicity. This grouping attenuated the effect of obesity and overweight participants were not grouped with obese participants for all study analyses. Sensitivity analyses were conducted with univariable models to investigate BMI cut-offs of 35 and 40 (except a BMI of 40 for males with no prior infection due to limited sample size). The results did not indicate that an effect by obesity on vaccine immunogenicity was present at higher BMI values.

Multivariable models adjusted for confounding by key covariates, such as age, vaccine type, immune compromised status, time between doses and time since last SARS-CoV-2 infection. Overall, our multivariable analysis does not suggest a clear influence of obesity on SARS-CoV-2 vaccine humoral immunogenicity (Figure 2a-c). No consistent pattern regarding obesity emerged across time points, antibody isotype or infection history.

Modelling results for some measures and timepoints differed according to sex (Figure 2a-c). The slope of anti-S IgG, anti-RBD IgG and anti-S IgA antibody production was reduced for obese males with no prior SARS-CoV-2 infection after the first vaccine dose but not with subsequent doses or for IgM antibodies. These observations contrast those of female participants with no prior infection, for which no difference in the slope of titer production was observed following the first vaccine dose. Obese female participants with a history of SARS-CoV-2 infection experienced a less steep increase in neutralizing capacity following dose 1 compared to non-obese females. This was not observed in obese males. Therefore, sex was identified as a potential modifier in obesity's influence over vaccine-elicited titers.

Preexisting infection-acquired immunity against SARS-CoV-2 was also identified as a variable that may modify the relationship between obesity and vaccine humoral response. Obese females who had a prior SARS-CoV-2 infection when the first vaccine dose was administered demonstrated a less steep increase in neutralization activity following vaccination compared to non-obese females, but no difference was seen for obese females without prior SARS-CoV-2 at the time of vaccination.

No reduction in neutralization activity was observed for obese males without history of SARS-CoV-2 infection, suggesting that the observed reduction in the slope of titer production for anti-S IgG, anti-RBD IgG and anti-S IgA may not be clinically meaningful (Supplemental Table 1). Trends in neutralization activity were similar for obese and non-obese females after two vaccination doses, regardless of SARS-CoV-2 history. These observations are supported by comparable incidences of breakthrough infections during follow-up for obese and non-obese participants (Table 2).

Altogether, obesity did not predict the humoral response to SARS-CoV-2 vaccines. We did not observe consistent fluctuations in the slope of anti-S or anti-RBD IgG, IgM, and IgA

titers or anti-S IgG neutralizing capacity increase and decay as a function of obesity following SARS-CoV-2 vaccination after two doses and one booster dose.

## **Discussion**

Overall, our analysis illustrates a lack of consistent influence of obesity on humoral SARS-CoV-2 vaccine immunogenicity assessed by antibody titer production, antibody titer decay rates, and neutralizing capacity. This is in contrast to several other publications which suggest a negative influence of obesity on SARS-CoV-2 immunogenicity<sup>6,7,9,10,23</sup>. However, many of these studies were cross-sectional and only analyzed crude IgG titers against either the S or RBD antigen<sup>24-28</sup>. Small sample sizes (ranging from n=63 to 252), which reduced power and/or precluded subgroup analysis by sex, and short follow-up (e.g., 1 month after the second vaccine dose) are other limiting factors in the literature<sup>24-28</sup>. For example, Phumisantiphong et al. reported lower titers against the RBD after 2 doses of the Sinovac vaccine for obese participants (BMI>30) compared to lower BMI categories in a cohort of 473 healthcare workers<sup>29</sup>. Although the sample size was large, the cross-sectional comparison was limited to 4-5 weeks after dose 2 and no adjustment for any confounders such as sex or age was completed. The vaccine type also differed compared to our study. mRNA based SARS-CoV-2 vaccines tend to be more immunogenic than non-mRNA based vaccines<sup>30-33</sup>. It is plausible that the use of mRNA SARS-CoV-2 vaccines may overwhelm any influence of obesity on vaccine immunogenicity.

Several studies support our findings and did not report an impact by obesity on SARS-CoV-2 vaccine immunogenicity<sup>34-50</sup>. Papaioannidou et al. conducted multivariable analysis of anti-S IgG titers 2-4 weeks after two doses of the Pfizer-BioNTech (BNT162b2) vaccine in a cohort of healthcare workers (n=290)<sup>49</sup>. Before and after adjusting for sex and age, obesity and BMI did not predict titers. Additionally, according to Bayart et al. BMI did not predict anti-S/RBD IgG up to 42 days following complete vaccination with BNT162b2 after adjusting for sex, age and blood type, regardless of SARS-CoV-2 infection history (n=231)<sup>38</sup>. Similar results were

obtained for the AstraZeneca/Oxford vaccine (ChAdOx1) vaccine in multivariable regression analyses of anti-S/RDB and anti-S IgG titers performed by Lee et al. and Singh et al.<sup>44,50</sup>. Some studies have obtained variable results regarding the effect of obesity on SARS-CoV-2 vaccine humoral immunogenicity depending on previous SARS-CoV-2 infection status, the measure of obesity or humoral immunogenicity assessed, sex, age group and time point of assessment<sup>7,9,24,38,51–53</sup>. Many of these reports were also limited by the same biases that impact the studies that suggest that obesity negatively impacts vaccine immunogenicity.

We believe that our analysis is more robust as we considered key confounders, accounted for multiple antibody isotypes, prospectively assessed the durability of immunogenicity longitudinally over an extended follow-up period and evaluated neutralization as well. While evaluation of antibody titer trends provided a quantitative measure of the humoral immune response, additional investigation into neutralizing capacity allowed for a greater representation of functional efficacy leading to a more comprehensive assessment. We adjusted for important factors which reportedly influence antibody titer decline in our analyses, including aging, immune compromised status, vaccine type, and the interval between first and second doses<sup>7,15,54</sup>. Among the research conducted, few have reviewed the long-term effects of obesity spanning up to three doses while also employing a large, heterogeneous population.

Our analysis suggests that sex is a key modifier of the influence of obesity on the slope of IgG titer production following vaccination. This effect was predominantly in male participants leading to a blunted humoral response with the initial doses of vaccine which was not observed with subsequent boosters. Prior COVID-19 studies highlight decreased neutralization capacity and lower antibody titers in male participants<sup>10,54</sup>. Yamamoto et al. also demonstrated effect modification by sex and BMI on anti-S IgG titers after two doses of the BNT162b2 vaccine (n=2,435)<sup>21</sup>. These results are consistent with our findings. The observed difference may be the results of the underlying physiological difference that exist between males and females. Adipose

tissue distribution in men and women differ (e.g., obese males have more visceral fat which leads to higher levels of chronic inflammation) and higher estrogen levels in women are associated with increased T and B cell activity, while testosterone has been linked to diminished antibody production<sup>21,28,55</sup>.

Distinct humoral responses to the initial vaccine dose were observed in this study for obese and non-obese participants according to preexisting infection-acquired immunity against SARS-CoV-2. Differences in the immune response to natural infection and vaccination have been noted for SARS-CoV-2 in previous studies and the characterization of hybrid immunity and its complexities continues to be an important area of research<sup>34,56</sup>. Kara et al. similarly identified infection-acquired immunity as a variable influencing the relationship between obesity and vaccine humoral immunogenicity<sup>7</sup>: obesity was associated with lower anti-S IgG titers after vaccination for those without a prior infection but not those with a history of COVID-19 (n=290). Together with our results, these data suggest that hybrid immunity may also modify the relationship between obesity and vaccine humoral response and consequently should be considered in future work.

Although slower production of some antibody isotypes was observed after dose 1 for obese males without a prior SARS-CoV-2 infection, there was no corresponding reduction in antibody activity in neutralization assays. These results emphasize the importance of investigating antibody activity in addition to antibody concentration to fully characterize the humoral immune response. While often correlated<sup>57,58</sup>, discrepancies may occur between antibody levels and neutralization capacity<sup>59,60</sup>. Neutralization activity for males and females after 2 vaccine doses was similar among obese and non-obese participants up to 3 doses, whether they had vaccine-derived immunity or hybrid immunity. Higher normalized neutralization titers are strongly associated with improved protection against COVID-19<sup>61–63</sup>, and indeed incidences of breakthrough infections during follow-up were similar for obese and

non-obese participants. These findings demonstrate the effectiveness of vaccination against COVID-19 in obese individuals.

Optimizing vaccine type and adjuvants as well as dosing schedule and mode of delivery in vulnerable populations is crucial to reduce COVID-19 morbidity and mortality. Vaccination schedule is a key factor in immunization as antibody waning leaves individuals vulnerable the longer they wait for a booster dose. This phenomenon is exemplified in obese individuals who reportedly possess increased antibody decay rates leaving them more vulnerable to breakthrough infection due to poor long-term immunity/sustained antibody response<sup>53,64</sup>. In our study, we accounted for the time interval between doses and no correlation was found between obesity and poorer antibody waning. However, obese individuals had a higher overall risk of SARS-CoV-2 infection and reinfection. Evidence in the literature indicates that obese individuals are at a higher risk of severe COVID-19<sup>65</sup>. SARS-CoV-2 vaccination is an effective means of improving clinical outcomes<sup>66,67</sup> and we demonstrated that humoral vaccine response is comparable in obese and non-obese individuals. SARS-CoV-2 vaccination and boosting remains a powerful tool for developing and maintaining protection against COVID-19 in obese individuals, especially as immunity wanes and novel variants emerge over time<sup>62</sup>.

Several limitations are acknowledged. Cases of COVID-19 infection and vaccination details were obtained either partly or completely by participant self-report. We believe that our data is of high quality given the standardization of data collection protocols, prospective data collection and verification of vaccination and infection values by use of serology data generated from our participants. We were unable to assess vaccine immunogenicity beyond dose three given sparsity of data. Obesity status was ascertained at baseline by self-report and/or BMI calculation. We did not reassess for weight change over the course of the study which could have influenced our findings in the later months of SSO participation. BMI, a common and easy way of tracking body fat percentage, is not a perfect measure of obesity and can vary due to bone

density, and muscle mass. We did not assess other anthropomorphic measures such as waist circumference or body fat percentage which could have potentially increased the reliability of the results. Diet, exercise, and genetic predispositions could influence the immune system but were not considered in this analysis. A large proportion of the cohort is white and this may limit generalizability. We assessed an adult cohort and cannot comment on the role of obesity on vaccine immunogenicity in childhood.

We did not assess mucosal humoral immunity or measures of cellular immune response. Further research into these domains of immune response in obese individuals following SARS-CoV-2 may provide additional insights as the impact of obesity on vaccine-elicited humoral and cell-mediated immunity may differ. Few studies have been conducted in which both the humoral and cell-mediated immune response to COVID-19 vaccination were analyzed<sup>68</sup>. Animal studies provide some evidence that both classes of adaptive immunity are impaired in obese mice. O'Meara et al. and Chen et al. reported that serum antibody titers and T cell activation following vaccination with the BNT162b2 vaccine were reduced in mice fed a high-fat diet compared to a normal diet<sup>69,70</sup>. Evidence in humans is mixed. In an analysis of a cohort (n=115) in which 67% of participants received two doses of the ChAdOx1 vaccine, BMI did not predict vaccine-elicited anti-S antibody titers but higher BMI was associated with a reduced frequency of TNF<sup>+</sup>CD4<sup>+</sup>, TNF<sup>+</sup>CD8<sup>+</sup>, and IL-2<sup>+</sup>CD4<sup>+</sup> SARS-CoV-2 specific T cells<sup>71</sup>. However, participants with a BMI above 30 were poorly represented (n=13) which may bias these results, and fewer individuals received an mRNA vaccine compared to the SSO cohort, which limits comparability with our study. Another study by Choi et al. reported a negative association between BMI and T cell response but not humoral immunogenicity; however, similarly only four individuals were obese according to their BMI and all 80 received the ChAdOx1 vaccine<sup>72</sup>. Contrasting results were obtained by Herzberg et al. in a longitudinal cohort study of 184 individuals vaccinated with BNT162b2<sup>73</sup>. Obesity was associated with reduced anti-S IgG titers and neutralizing antibodies but not with SARS-CoV-2 specific T cell response. Zhu et al. reported that inactivated vaccines

(BBIBP-CorV or CoronaVac) elicited lower anti-RBD IgG titers, neutralizing antibody titers, and SARS-CoV-2 specific TNF- $\alpha$ <sup>+</sup> T cell activity in those classified as obese or overweight<sup>74</sup>. The literature is limited and conflicted.

Various studies indicate that mRNA vaccines are more immunogenic and effective than alternative types such as ChAdOx1<sup>74,75</sup>. mRNA vaccines may be sufficiently immunogenic as to generate comparable humoral and cell-mediated immune responses in obese and non-obese individuals while other vaccine types may fail to elicit the same response and may impact T cell and B cell functioning differently. In this study, the majority of doses administered were mRNA vaccines. Therefore, a comparison of immunogenicity between mRNA and non-mRNA vaccines was not possible. Humoral and cell-mediated immune responses in obese individuals could not be compared according to vaccine type. Additionally, the findings of this study may not apply to other vaccine types.

### *Conclusion*

In conclusion, our analysis of a large urban-based North American cohort suggests that obesity does not consistently affect SARS-CoV-2 anti-S and anti-R IgG, IgM, and IgA following vaccination. Similarly, obesity does not influence neutralizing antibodies. As such, it may not be necessary to consider this variable when developing SARS-CoV-2 vaccine dosing strategies.



## Tables

Table 1: SSO Study Participant Characteristics.

| Variable                                                | All participants<br>(n=907) | Non-Obese <sup>1</sup><br>(n=697) | Obese <sup>2</sup><br>(n=210) | P     |
|---------------------------------------------------------|-----------------------------|-----------------------------------|-------------------------------|-------|
| Demographics:                                           |                             |                                   |                               |       |
| Age                                                     |                             |                                   |                               |       |
| Mean (SD)                                               | 45.7 (14.0)                 | 44.9 (14.3)                       | 48.2 (12.3)                   | 0.001 |
| Range (min, max)                                        | 61 (18, 79)                 | 61 (18,79)                        | 52 (22,74)                    |       |
| Sex, n (%)                                              |                             |                                   |                               |       |
| Male                                                    | 314 (34.6)                  | 232 (33.3)                        | 82 (39.1)                     | 0.12  |
| Female                                                  | 593 (65.4)                  | 465 (66.7)                        | 128 (61.0)                    |       |
| Immigration status, n (%)                               |                             |                                   |                               |       |
| Born in Canada                                          | 774 (87.0)                  | 594 (86.6)                        | 180 (88.2)                    | 0.54  |
| Immigrated to Canada                                    | 116 (13.0)                  | 92 (13.4)                         | 24 (11.8)                     |       |
| Missing                                                 | 17                          | 11                                | 6                             |       |
| Ethnicity, n (%)                                        |                             |                                   |                               |       |
| White                                                   | 837 (92.4)                  | 639 (91.8)                        | 198 (94.3)                    | 0.004 |
| Indigenous                                              | 14 (1.6)                    | 9 (1.3)                           | 5 (2.4)                       |       |
| Black                                                   | 5 (0.6)                     | 5 (0.7)                           | 0                             |       |
| Asian                                                   | 29 (3.2)                    | 29 (4.2)                          | 0                             |       |
| Latin American                                          | 9 (1.0)                     | 6 (0.9)                           | 3 (1.4)                       |       |
| Mixed                                                   | 12 (1.3)                    | 8 (1.2)                           | 4 (1.9)                       |       |
| Missing                                                 | 1                           | 1                                 | 0                             |       |
| Smoker, n (%)                                           |                             |                                   |                               |       |
| Never Smoker                                            | 653 (72.4)                  | 512 (73.9)                        | 141 (67.5)                    | 0.19  |
| Former Smoker                                           | 213 (23.6)                  | 155 (22.4)                        | 58 (27.8)                     |       |
| Current Smoker                                          | 36 (3.4)                    | 26 (3.8)                          | 10 (4.8)                      |       |
| Missing                                                 | 5                           | 4                                 | 1                             |       |
| Average number of standard drinks per week <sup>3</sup> |                             |                                   |                               |       |
| Median (IQR)                                            | 2.0 (4.0)                   | 2.0 (4.5)                         | 1.0 (4.0)                     | 0.002 |
| Range (min, max)                                        | 30 (0, 30)                  | 30 (0, 30)                        | 20 (0, 20)                    |       |
| Missing                                                 | 1                           | 1                                 | 0                             |       |
| Comorbidities:                                          |                             |                                   |                               |       |
| Dyslipidemia, n (%)                                     |                             |                                   |                               |       |
| No                                                      | 816 (90.0)                  | 634 (91.0)                        | 182 (86.7)                    | 0.07  |

|                                        |            |            |            |         |
|----------------------------------------|------------|------------|------------|---------|
| Yes                                    | 91 (10.0)  | 63 (9.0)   | 28 (13.3)  |         |
| Hypertension, n (%)                    |            |            |            |         |
| No                                     | 810 (89.3) | 644 (92.4) | 166 (79.0) | <0.0001 |
| Yes                                    | 97 (10.7)  | 53 (7.6)   | 44 (21.0)  |         |
| Diabetes, n (%)                        |            |            |            |         |
| No                                     | 856 (95.4) | 666 (96.8) | 190 (90.9) | 0.0004  |
| Yes                                    | 41 (4.6)   | 22 (3.2)   | 19 (9.0)   |         |
| HIV, n (%)                             |            |            |            |         |
| No                                     | 869 (96.9) | 665 (96.5) | 204 (98.1) | 0.26    |
| Yes                                    | 28 (3.1)   | 24 (3.5)   | 4 (2.0)    |         |
| Immune compromised, <sup>4</sup> n (%) |            |            |            |         |
| No                                     | 605 (66.7) | 479 (68.7) | 126 (60.0) | 0.04    |
| Yes – Low Level                        | 200 (22.1) | 148 (21.2) | 52 (24.8)  |         |
| Yes – High Level                       | 102 (11.3) | 70 (10.0)  | 32 (15.2)  |         |

Two-sided parametric (Student's t-test) or non-parametric (Mann-Whitney U test, chi-square test) statistical tests were used in accordance with the data distribution (normality was determined by histograms and Q-Q plots). Fisher tests were used if assumptions for the chi-square test were not met. Statistical significance was determined with an alpha level of 0.05. SD: standard deviation; IQR: interquartile range.

<sup>1</sup> Reported no obesity or had a BMI of  $\leq 29.9$ .

<sup>2</sup> Reported obesity or had a BMI of  $\geq 30.0$ .

<sup>3</sup> A standard drink of alcohol is defined a 341 mL bottle of 5% beer, cider, or cooler; a 142 mL glass of 12% wine; or a 43 mL serving of 40% distilled alcohol (e.g., rye, gin, rum, vodka).

<sup>4</sup> Immune compromising conditions included cancer, organ/tissue transplant, primary immunodeficiencies, rheumatological conditions, multiple sclerosis, myasthenia gravis, active HIV, liver disorder (e.g., severe cirrhosis) (high-level immune compromising), respiratory conditions requiring medication (e.g. asthma), autoimmune diseases, fully suppressed HIV, liver disorder (e.g., mild cirrhosis) and kidney disorders (i.e. chronic kidney disease), excessive alcohol consumption (low level). Use of immunosuppressants and inflammatory bowel disease were also included, but the level was dependent on the strength of immunosuppression caused by the specific medications.

Table 2: SARS-CoV-2 Infection and Vaccination Data.

| Variable                                              | All participants<br>(n=907) | Non-Obese <sup>1</sup><br>(n=697) | Obese <sup>2</sup><br>(n=210) | P     |
|-------------------------------------------------------|-----------------------------|-----------------------------------|-------------------------------|-------|
| Number of doses, n (%)                                |                             |                                   |                               |       |
| Unvaccinated                                          | 17 (1.9)                    | 14 (2.0)                          | 3 (1.4)                       |       |
| Dose 1                                                | 4 (0.4)                     | 4 (0.6)                           | 0 (0.0)                       |       |
| Dose 2                                                | 226 (24.9)                  | 186 (26.7)                        | 40 (19.1)                     |       |
| Dose 3                                                | 452 (49.8)                  | 346 (49.6)                        | 106 (50.5)                    | 0.18  |
| Dose 4                                                | 128 (14.1)                  | 90 (12.9)                         | 38 (18.1)                     |       |
| Dose 5                                                | 65 (7.2)                    | 46 (6.6)                          | 19 (9.1)                      |       |
| Dose 6                                                | 11 (1.2)                    | 8 (1.2)                           | 3 (1.4)                       |       |
| Dose 7                                                | 4 (0.4)                     | 3 (0.4)                           | 1 (0.5)                       |       |
| Average time between doses<br>(in days), median (IQR) |                             |                                   |                               |       |
| Dose 1 – 2                                            | 58.0 (41.0)                 | 58.0 (39.0)                       | 61.0 (40.0)                   | 0.04  |
| Dose 2 – 3                                            | 185.0 (37.0)                | 186.0 (67.0)                      | 182.0 (21.5)                  | 0.008 |
| Dose 3 – 4                                            | 218.0 (152.0)               | 212.0 (153.0)                     | 219.5 (148.5)                 | 0.77  |
| Dose 4 – 5                                            | 161.0 (59.0)                | 164.0 (46.0)                      | 150.0 (57.0)                  | 0.26  |
| Dose 5 – 6                                            | 179.0 (105.0)               | 179.0 (105.0)                     | 141.5 (124.5)                 | 0.95  |
| Dose 6 – 7                                            | 168.5 (79.0)                | 197.0 (69.0)                      | 108 (NA, n=1)                 | -     |
| Breakthrough infection <sup>3</sup> , n (%)           |                             |                                   |                               |       |
| Baseline                                              | 219 (46.5)                  | 155 (45.7)                        | 64 (48.5)                     |       |
| Post Dose 1                                           | 4 (0.9)                     | 3 (0.9)                           | 1 (0.8)                       |       |
| Post Dose 2                                           | 51 (10.8)                   | 43 (12.7)                         | 8 (6.1)                       |       |
| Post Dose 3                                           | 142 (30.2)                  | 98 (28.9)                         | 44 (33.3)                     | 0.34  |
| Post Dose 4                                           | 45 (9.6)                    | 31 (9.1)                          | 14 (10.6)                     |       |
| Post Dose 5                                           | 9 (1.9)                     | 8 (2.4)                           | 1 (0.8)                       |       |
| Post Dose 6                                           | 1 (0.2)                     | 1 (0.3)                           | 0 (0.0)                       |       |
| Post Dose 7                                           | 0 (0.0)                     | 0 (0.0)                           | 0 (0.0)                       |       |
| Total                                                 | 471 (100.0)                 | 339 (100.0)                       | 132 (100.0)                   |       |

Two-sided parametric (Student's t-test) or non-parametric (Mann-Whitney U test, chi-square test) statistical tests were used in accordance with the data distribution (data normality was determined by histograms and Q-Q plots). Fisher tests were used if assumptions for the chi-square test were not met. Statistical significance was determined with an alpha level of 0.05. SD: standard deviation; IQR: interquartile range.

<sup>1</sup> Reported no obesity or had a BMI of  $\leq 29.9$ .

<sup>2</sup> Reported obesity or had a BMI of  $\geq 30.0$ .

<sup>3</sup> SARS-CoV-2 infections that occurred within 14 days of a dosing visit were considered a breakthrough infection corresponding to the prior dose and infections that occurred within 14 days of the initial dose were considered a baseline infection.

Supplemental Table 1: Additional SSO Participant Demographics and SARS-CoV-2 Infection Data.

| Variable                                           | All participants<br>(n=907) | Non-Obese <sup>1</sup><br>(n=697) | Obese <sup>2</sup><br>(n=210) | P    |
|----------------------------------------------------|-----------------------------|-----------------------------------|-------------------------------|------|
| Demographics:                                      |                             |                                   |                               |      |
| Gender, n (%)                                      |                             |                                   |                               |      |
| Male                                               | 311 (34.5)                  | 231 (33.3)                        | 80 (38.7)                     | 0.30 |
| Female                                             | 585 (64.9)                  | 459 (66.1)                        | 126 (60.9)                    |      |
| Gender diverse                                     | 5 (0.6)                     | 4 (0.6)                           | 1 (0.5)                       |      |
| Missing                                            | 6                           | 3                                 | 3                             |      |
| Education, n (%)                                   |                             |                                   |                               |      |
| Elementary School                                  | 1 (0.1)                     | 0                                 | 1 (0.5)                       | 0.03 |
| High School                                        | 62 (7.0)                    | 43 (6.3)                          | 19 (9.4)                      |      |
| Trade / Apprenticeship /<br>Technical CEGEP        | 31 (3.5)                    | 24 (3.5)                          | 7 (3.5)                       |      |
| Community College Diploma                          | 192 (21.6)                  | 137 (19.9)                        | 55 (27.0)                     |      |
| University Certificate                             | 25 (2.8)                    | 17 (2.5)                          | 8 (3.9)                       |      |
| Bachelor's degree                                  | 357 (40.1)                  | 283 (41.2)                        | 74 (36.5)                     |      |
| Graduate Degree                                    | 206 (23.2)                  | 171 (24.9)                        | 35 (17.2)                     |      |
| Prefer not to answer                               | 16 (1.8)                    | 12 (1.8)                          | 4 (2.0)                       |      |
| Missing                                            | 17                          | 10                                | 7                             |      |
| Income (before taxes) in the past 12 months, n (%) |                             |                                   |                               |      |
| \$0 – \$29,999                                     | 37 (4.2)                    | 32 (4.7)                          | 5 (2.5)                       | 0.29 |
| \$30,000-\$59,999                                  | 86 (9.7)                    | 59 (8.6)                          | 27 (13.2)                     |      |
| \$60,000-\$89,999                                  | 155 (17.4)                  | 121 (17.6)                        | 34 (16.7)                     |      |
| \$90,000-\$119,999                                 | 178 (20.0)                  | 133 (19.4)                        | 45 (22.1)                     |      |
| \$120,000-\$149,999                                | 93 (10.5)                   | 70 (10.2)                         | 23 (11.3)                     |      |
| \$150,000 +                                        | 258 (29.0)                  | 204 (29.7)                        | 54 (26.5)                     |      |
| Prefer not to answer / Do not know                 | 83 (9.3)                    | 67 (9.8)                          | 16 (7.8)                      |      |
| Missing                                            | 17                          | 11                                | 6                             |      |
| Employed, n (%)                                    |                             |                                   |                               |      |
| No                                                 | 159 (17.8)                  | 124 (18.1)                        | 35 (16.8)                     | 0.65 |
| Yes                                                | 735 (82.2)                  | 561 (81.9)                        | 174 (83.3)                    |      |
| Missing                                            | 13                          | 12                                | 1                             |      |
| SARS-CoV-2 Vaccination Data:                       |                             |                                   |                               |      |
| Dose 1 type, n (%)                                 |                             |                                   |                               |      |
| AstraZeneca                                        | 112 (12.6)                  | 88 (12.9)                         | 24 (11.7)                     | 0.11 |

|                             |            |            |            |       |
|-----------------------------|------------|------------|------------|-------|
| Moderna                     | 89 (10.0)  | 62 (9.1)   | 27 (13.1)  |       |
| Pfizer                      | 687 (77.3) | 533 (78.0) | 154 (74.8) |       |
| Other                       | 1 (0.1)    | 0 (0)      | 1 (0.5)    |       |
| Dose 2 type, n (%)          |            |            |            |       |
| AstraZeneca                 | 10 (1.1)   | 8 (1.2)    | 2 (1.0)    |       |
| Moderna                     | 225 (25.4) | 166 (24.5) | 59 (28.6)  | 0.60  |
| Pfizer                      | 649 (73.3) | 504 (74.2) | 145 (70.4) |       |
| Other                       | 1 (0.1)    | 1 (0.2)    | 0 (0)      |       |
| Dose 3 type, n (%)          |            |            |            |       |
| Moderna                     | 183 (28.0) | 126 (25.8) | 57 (34.3)  |       |
| Pfizer                      | 470 (71.9) | 361 (74.0) | 109 (65.7) | 0.07  |
| Other                       | 1 (0.2)    | 1 (0.2)    | 0 (0)      |       |
| SARS-CoV-2 Infection Data:  |            |            |            |       |
| Number of infections, n (%) |            |            |            |       |
| 0                           | 493 (54.4) | 397 (57.0) | 96 (45.7)  |       |
| 1                           | 363 (40.0) | 266 (38.2) | 97 (46.2)  | 0.006 |
| 2                           | 46 (5.1)   | 29 (4.2)   | 17 (8.1)   |       |
| 3                           | 5 (0.6)    | 5 (0.7)    | 0          |       |

Two-sided parametric (Student's t-test) or non-parametric (Mann-Whitney U test, chi-square test) statistical tests were used in accordance with the data distribution (data normality was determined by histograms and Q-Q plots). Fisher tests were used if assumptions for the chi-square test were not met. Statistical significance was determined with an alpha level of 0.05.

<sup>1</sup> Reported no obesity or had a BMI of  $\leq 29.9$ .

<sup>2</sup> Reported obesity or had a BMI of  $\geq 30.0$ .

## Figures

Figure 1: LOESS smoothing curves depicting baseline, acute and long-term ( $\geq 24$  month) IgG (BAU/mL), IgM, IgA ( $\mu\text{g/mL}$ ) titers against the spike and receptor binding domain antigens relative to the first dose of COVID-19 vaccination. Participant data is grouped by obesity status and prior history of COVID-19. Confidence interval bands are shown in grey.

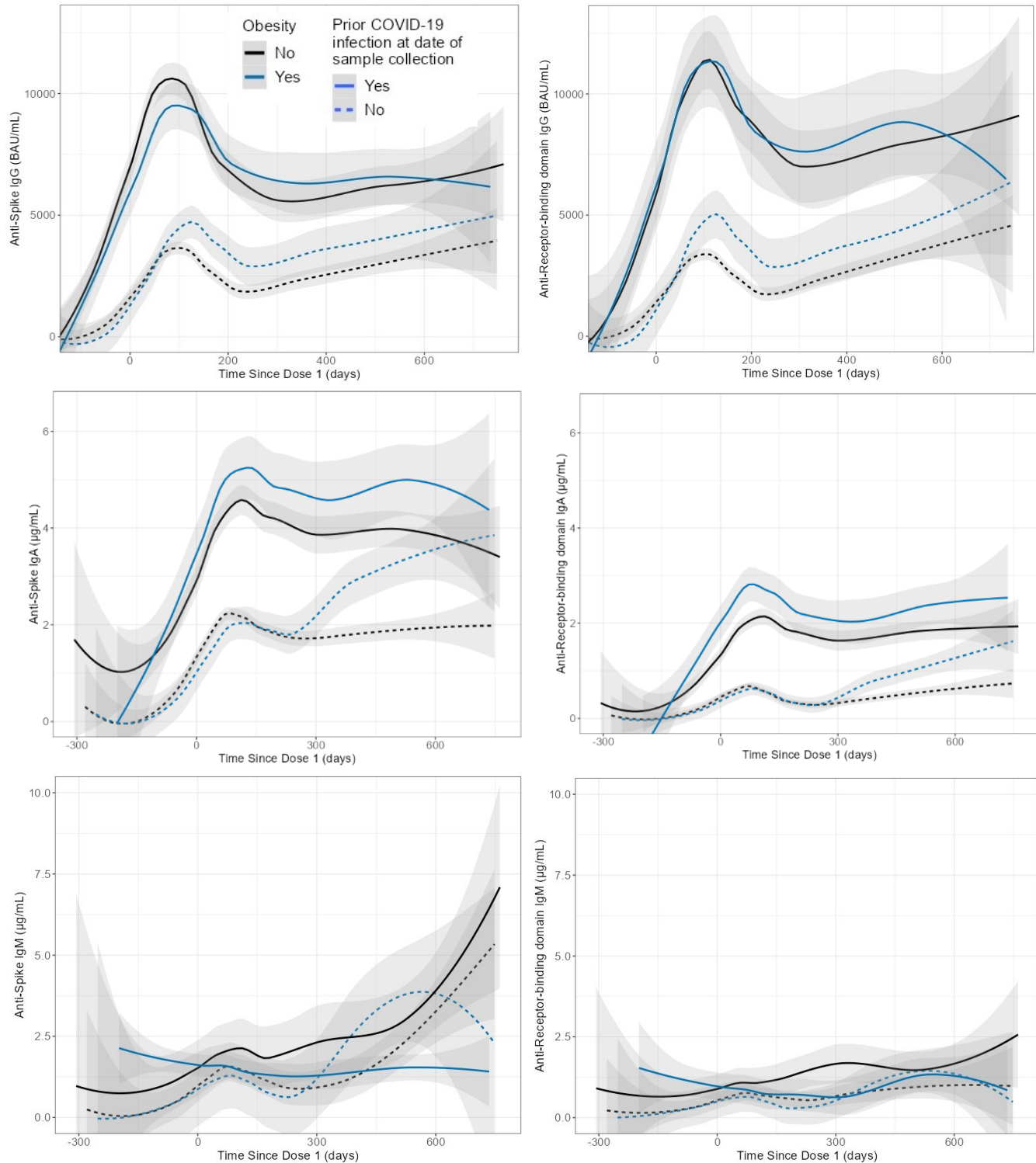
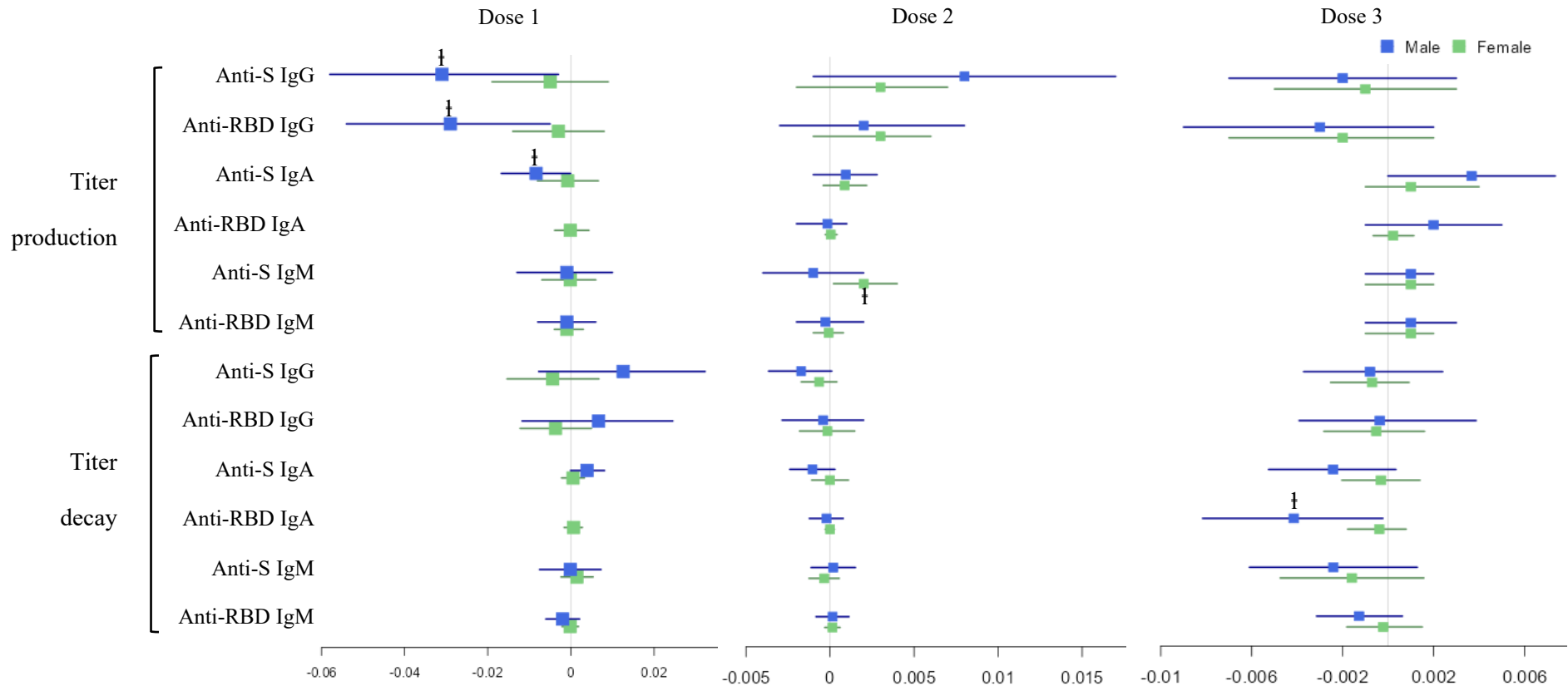


Figure 2a: Forest plots of multivariable modeling estimates for anti-S and anti-RBD IgG, IgM, IgA production and decay following three vaccination doses, comparing obese and non-obese participants with no history of SARS-CoV-2 infection at time of vaccination. Boxes indicate model estimates and bars represent the 95% credible intervals.

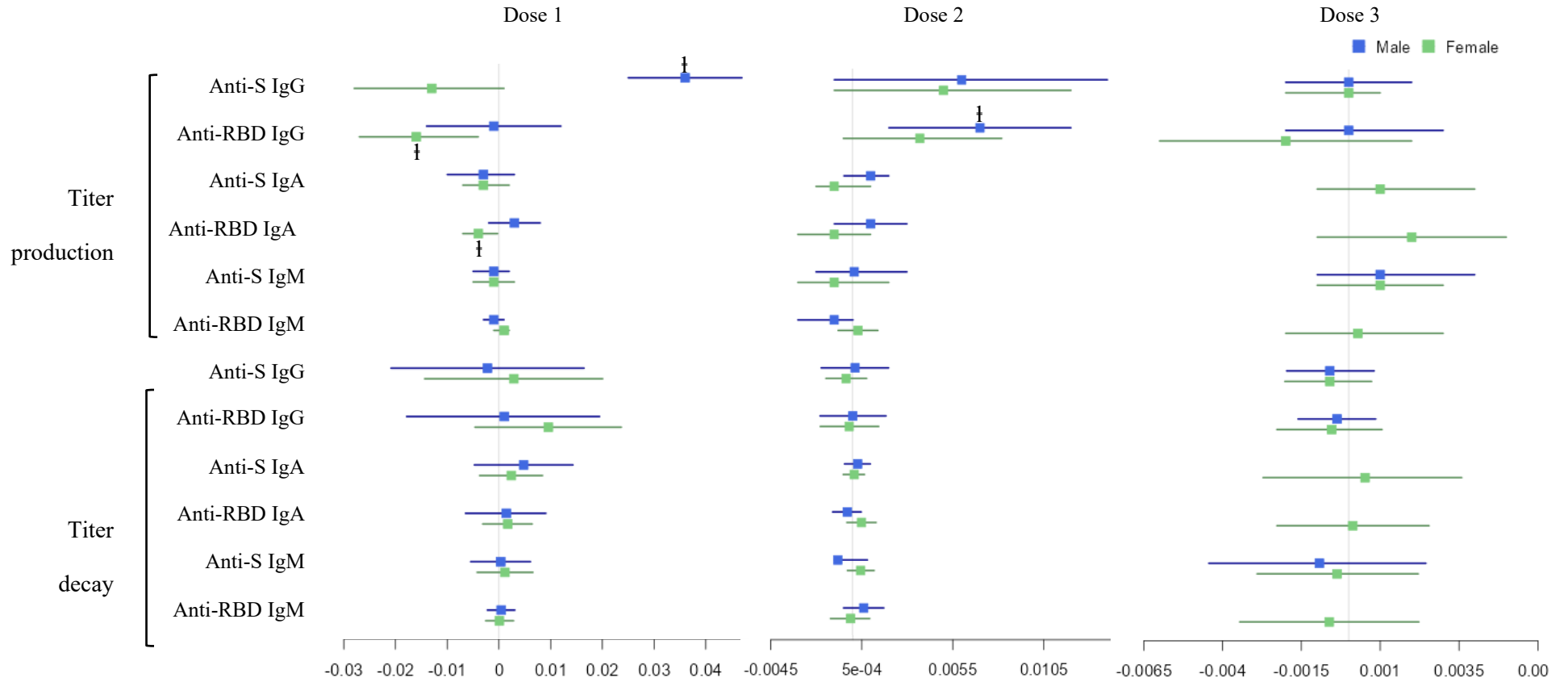


† Indicates differences in estimates for which the 95% credible interval does not include the null value of zero.

Confounders sets for each model were as follows: immune status for anti-S IgA (dose 1), anti-S IgM (dose 2) for male models; age & immune status for anti-S & anti-RBD IgG (dose 1), anti-R IgM (dose 2) anti-S IgA & anti-RBD IgA (dose 3) for male models and anti-R IgM (dose 3) for female models; age, ethnicity, immune status, & vaccine type for anti-S & anti-R IgG, IgA IgM (dose 1), anti-S IgA, IgM (dose 3) for female models; age, immune status, vaccine type, & time between dose and the proceeding dose for anti-S & anti-RBD IgG (dose 3) for male models and anti-R IgA (dose 2, 3) for female models; age, ethnicity, immune status, vaccine type, & time between dose and the proceeding dose for anti-S & anti-RBD IgG, IgA (dose 2) anti-S & anti-RBD IgM (dose 3) for male models and anti-S & anti-RBD IgG (dose 2, 3), anti-S IgA, anti-S & anti-RBD IgM (dose 2) for female models.



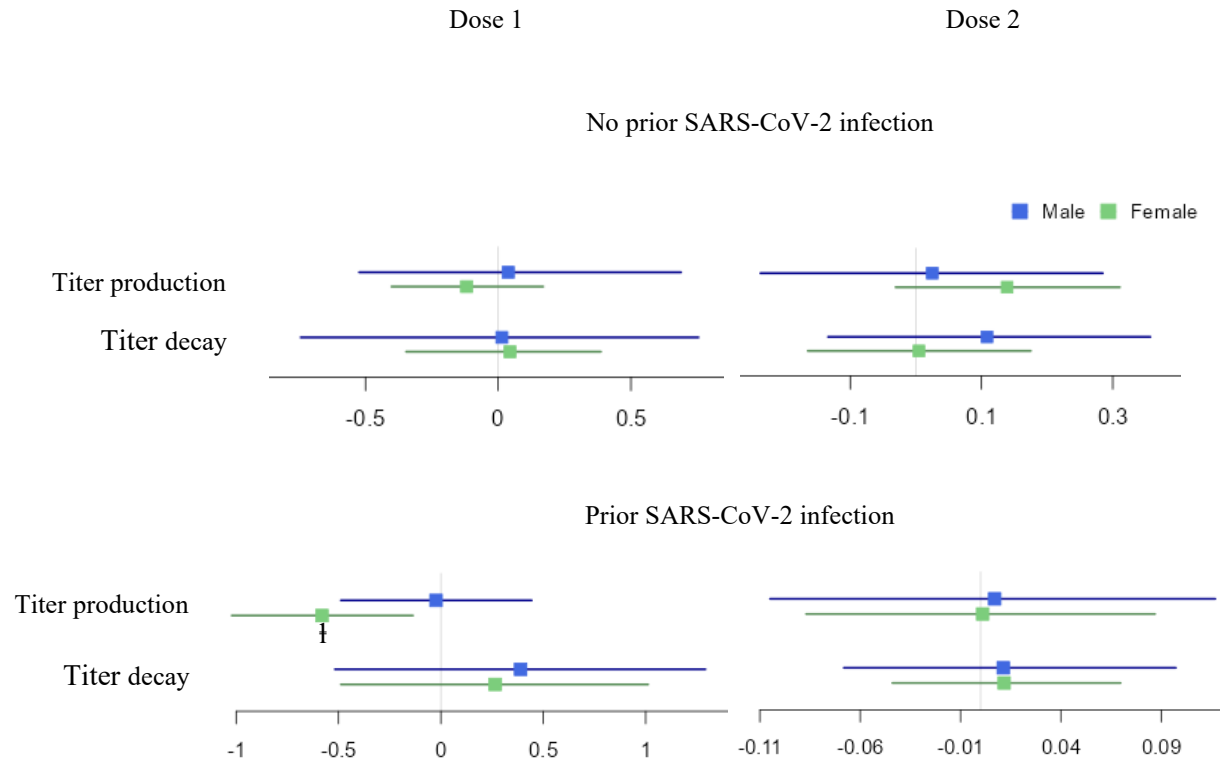
Figure 2b: Forest plots of multivariable modeling estimates for anti-S and anti-RBD IgG, IgM, IgA production and decay following three vaccination doses, comparing obese and non-obese participants with a history of SARS-CoV-2 infection at time of vaccination, resulting in hybrid immunity. Boxes indicate model estimates and bars represent the 95% credible intervals.



† Indicates differences in estimates for which the 95% credible interval does not include the null value of zero.

Confounders sets for each model were as follows: immune status for anti-S IgA, (dose 2) anti-S IgM (dose 3) for male models; age & immune status for anti-RBD IgG (dose 3) for male models and anti-S IgA (dose 3) for female models; age, ethnicity, immune status & vaccine type for anti-S & anti-RBD IgG, IgA (dose 1), anti-S & anti-RBD IgM (dose 1, 2, 3) for male and female models and anti-RBD IgA (dose 2 for males and dose 3 for females); age, ethnicity, immune status, vaccine type, & time between dose and the proceeding dose for anti-S & anti-RBD IgG (dose 2), anti-S & anti-RBD IgA (dose 2), anti-S IgG (dose 3) for male models and anti-S & anti-RBD IgG (dose 2, 3) for female models. All models adjusted for time since last infection at time of vaccination.

Figure 2c: Forest plots of multivariable modeling estimates for anti-S neutralization activity following two vaccination doses, comparing obese and non-obese participants. Boxes indicate model estimates and bars represent the 95% credible intervals.



† Indicates differences in estimates for which the 95% credible interval does not include the null value of zero.

Confounders sets for each model were as follows: immune status for (dose 1, males), age, ethnicity, immune status & vaccine type (dose 1 females), age, ethnicity, immune status, vaccine type & time between dose and the proceeding dose (dose 2 males and females) for models for participants with no prior infection; age, immune status & vaccine type (dose 1 males), age, ethnicity, immune status & vaccine type (dose 1 males), age, ethnicity, immune status, vaccine type, & time between dose and the proceeding dose (dose 2 for males and females). All models for participants with a prior SARS-CoV-2 infection adjusted for time since last infection at time of vaccination.

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# Chapter 5 – Alcohol Consumption Does Not Influence SARS-CoV-2 Vaccine Immunogenicity: A stop the spread Ottawa cohort analysis

## **Preface**

This next study addresses the third exposure listed in research objective III: excessive alcohol consumption. The slope of antibody production and decay following four COVID-19 vaccine doses was compared according to excessive alcohol consumption as defined by the Canadian Centre on Substance Abuse and Addiction. Neutralization activity was assessed up to 3 doses.

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**Ethics Approval:** The study protocol was approved by The Ottawa Health Science Network Research Ethics Board (2020-0481). Informed consent was obtained from all study participants.

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**Relevant Appendices:** N/A.

## **Abstract**

Summary: The effect of alcohol consumption on COVID-19 vaccine immunogenicity was evaluated. Participants from the Stop the Spread Ottawa cohort were categorized by alcohol consumption categorized as excessive or non-excessive based on the Canadian Centre on Substance Abuse and Addiction's (CCSA) 2023 guidelines on alcohol use. Our analyses showed that alcohol consumption did not influence SARS-CoV-2 antibody baseline levels, post vaccine increase or decay over time.

## **Introduction**

COVID-19 vaccination induces spike (S) protein-specific antibody and SARS-CoV-2-specific T cell immune response.<sup>1-3</sup> While absolute antibody titers and neutralizing capacity decline over time, cellular immunity persists following vaccination and infection.<sup>4</sup> Evaluation of modifiable risk factors affecting the extent and durability of vaccine-mediated immunity and risk for breakthrough infections remain necessary to inform vaccine policy.<sup>5</sup>

Chronic alcohol consumption has been shown to negatively affect both humoral and cellular immunity by decreasing lymphocyte and T cell proliferation and function, decreasing dendritic cell and monocyte migration and function, inducing the release of pro-inflammatory cytokines, and altering IgA and IgM production.<sup>6-9</sup> While chronic high level alcohol use dampens immune responses to vaccines and infection in both animal models and human studies<sup>10-13</sup>, the effect, if any, of moderate alcohol use on vaccine immunogenicity remains unresolved.<sup>6,10,14-16</sup>

Factors established to decrease COVID-19 vaccine immunogenicity and increase the risk of severe SARS-CoV-2 infection include older age, obesity, tobacco smoking, and various co-morbidities including diabetes and autoimmune disorders.<sup>16-18</sup> Access alcohol consumption has been identified as a predictor of COVID-19 infection and reinfection, hospitalization, intensive care admission and 30-day mortality.<sup>13,19</sup> Alcohol consumption could also impact COVID-19

vaccine immunogenicity. However, despite several efforts to provide clarity the effect of alcohol on COVID-19 vaccine immunogenicity remains uncertain.<sup>4,12,16,20-22</sup> Although valuable in increasing understanding, many of these studies are limited by small sample size, evaluation of relatively uniform populations, use of a single vaccine type, short duration of follow-up, restricted focus on the initial vaccine dose(s) and limited array of immune measures assessed. We addressed these limitations utilizing a diverse, urban-based population participating in the Stop the Spread Ottawa Cohort. Our evaluation is longitudinal over a multi-year period and evaluates anti-S and anti-R IgG, IgM, and IgA as well as neutralizing antibody responses over four COVID-19 vaccine doses.

## **Methods**

All data used for this study was collected as part of the Stop the Spread Ottawa (SSO) Cohort study.<sup>23</sup> Initiated in September 2020, SSO is a prospective, longitudinal cohort study investigating immune response to SARS-CoV-2 natural infection and vaccination. Conduct of this study was approved by the Ottawa Health Science Network Research Ethics Board (#2020-0481-01H).

Eligible participants were 18 years or older, residing in the Ottawa, Canada-region, and at-risk for SARS-CoV-2 exposure/infection due to occupation or health condition or having a documented history of COVID-19 natural infection confirmed by PCR and/or serology testing. COVID-19 vaccination and positive SARS-CoV-2 diagnostic results were self-reported by participants and/or reported with serology results and confirmed through vaccination histories and medical records where available. Information on demographics and medical history was collected from serial participant surveys. Participants provided serial blood samples for serum and peripheral blood mononuclear cell (PBMC) isolation. Antibody titers from participants lacking recorded vaccination dates from samples obtained prior to vaccine rollout were incorporated in baseline data.

Alcohol consumption was documented by participants in baseline and follow-up surveys as standard drinks (SD), defined as 13.5 g of alcohol<sup>24</sup>, or more precisely as 341 mL (12 oz.) bottle of 5% beer, cider, or cooler; 142 mL (5 oz.) glass of 12% wine; 43 mL (1.5 oz.) serving of 40% distilled alcohol; (e.g., rye, gin, rum, vodka). Reported alcohol consumption was stratified based on the Canadian Centre on Substance Abuse and Addiction's (CCSA) 2023 guidelines on alcohol use.<sup>25</sup> High level alcohol use was defined as 3 or more SD/week and non-high level alcohol use was defined as 0-2.9 SD/week. Binary stratification was also used in which high level alcohol use was defined as 7 or more SD/week and non-high level alcohol use was defined as 0-6.9 SD/week to analyse the impact of higher alcohol consumption on vaccine immunogenicity.

Humoral immunity to natural SARS-CoV-2 infection and/or COVID-19 vaccination was measured by immunoglobulins G (IgG), M (IgM), A (IgA), and neutralizing antibody activity (%) from serum as previously described.<sup>23,26</sup> Briefly, IgG (BAU/mL and µg/mL), IgM (µg/mL) and IgA (µg/mL) titers against SARS-CoV-2 spike (S) and receptor-binding domain (R) proteins in participant serum samples were quantified using a high-throughput chemiluminescent direct ELISA. Neutralizing percentage of serum anti-S IgG was determined using a surrogate neutralization ELISA assay utilizing the full-trimeric S protein from the Wuhan Hu-1 strain. The presence of anti-N IgG titers was utilized to distinguish natural immunity resulting from SARS-CoV-2 infection from vaccine-acquired immunity. Vaccine-elicited immune responses take approximately 14 days to manifest.<sup>27</sup> To account for this delay, participants with a COVID-19 infection up to 14 days after their vaccination date, were considered as having an infection prior to vaccination.

Participants lacking recorded alcohol consumption and individuals who were pregnant during the study period were excluded from this specific analysis. Participant characteristics and antibody titers and neutralization activity were compared according to reported alcohol



(SD/week) consumption at baseline. The Shapiro-Wilk test, comparison of means and medians and examination of histograms was employed to determine normality of data. The chi-square statistical test was employed for categorical variables when assumptions were met; otherwise, the Fisher's exact test was employed. The Student's t-test or the non-parametric Mann-Whitney U test was used depending on normality. Monte Carlo simulation (n=1,000,000 samples) was employed to estimate p-values for large contingency tables with high computational loads.

Univariable and multivariable multilevel linear regression analyses were conducted to model log-adjusted anti-S and anti-R levels and neutralization activity over time to assess, where sufficient data was available, whether alcohol use (high level alcohol defined as 3 or more standard drinks/week and non-high level alcohol defined as 0 to 2.9 standard drinks/week) was a predictor of peak antibody titer and whether the slopes of antibody titer increase and decay varied according to alcohol consumption with time as an interaction term. Other higher cut-points of alcohol use were assessed for exploratory purposes as well. Antibody titers were shifted up by 1 unit to account for zero values that would have resulted in undefined values following log-transformation. Models were generated at each vaccination time point separately for surveillance and convalescent participants. Convalescent models included samples from participants who had a prior infection at the time of vaccination. Any samples collected following an additional infection during follow-up relative to the dose were excluded. Time points were restricted to isolate the region of linear increase at time of vaccination and to exclude regions of sparse data at longer follow-up times or samples after subsequent vaccine doses. Random effects were included to allow the slopes and intercepts to vary for individuals to account for autocorrelation between serial observations. Linear splines were incorporated with knots located at the antibody peak to separate regions of antibody increase and antibody decay. Interaction terms between time and alcohol consumption were included to assess if the rate of antibody increase and decay varied according to alcohol consumption.

Statistical analyses were conducted using Statistical Analysis System (SAS) version 9.4. Plots were constructed using RStudio version 2023.06.1 (ggplot2 package).

## **Results**

### *Participant Characteristics*

Baseline demographics are described in Table 1. The majority of cohort participants were female (65.3%), white (89.2%) and Canadian-born (87.0%). The median (IQR) age was 45.0 (34, 56) years. The most prevalent baseline co-morbidities in the SSO Cohort included allergies (43.1%), chronic lung conditions (13.4%), hypertension (10.8%), and dyslipidemia (10.1%). Twenty-eight percent of the cohort were classified as immune compromised due to immunocompromising medication use and/or condition(s). Most of the cohort never smoked (72.4%) and 4.0% were current smokers.

Of 909 participants included in this analysis, 387 participants were identified as high level alcohol consumers at baseline (i.e. 3 or more standard drinks per week). Median alcohol consumption was 2.0 (IQR 0.0, 4.0) SD/week. The median (IQR) alcohol consumption was 5.0 (4.0, 7.0) SD/week and 1.0 (0.0, 1.0) SD/week in the group with excessive alcohol use and the group with no excessive alcohol use. Those consuming high level alcohol were more likely to be male, white and Canadian-born. They were less likely to be immune compromised.

There were several imbalances in the baseline characteristic of high level alcohol consumers compared to those without excessive alcohol. We attempted to account for this in our regression analyses (see below).

### *COVID Infection and Vaccination Summary*

Among all participants, 22.4% had at least one incident COVID-19 infection before enrolment, identified through self-report of a positive test result (Table 1). This was higher in those consuming high level alcohol. Forty-five percent of participants had at least one infection

prior to and during the study period identified through self-report and serology. Rates of baseline infection and infection confirmed during the course of the study were similar between alcohol use groups (Table 1).

Among all participants, 34.9% were COVID-19 vaccinated at the time of SSO enrolment (Table 2). At the time of data analysis, 97.8% and 97.2% of the entire cohort received at least one or two COVID-19 vaccine doses, respectively. Additionally, 72.4% of participants received at least one additional vaccine dose. These proportions and for subsequent booster doses did not differ by alcohol consumption level. The median (IQR) days between the first and second COVID-19 vaccine doses was 58 (35, 76) days and ranged from 19 to 344 days. The median (IQR) length of time in days between second and third vaccine doses was 185 (174, 211) days and ranged from 22 and 546 days. There was no clinically relevant difference in time between vaccine doses as a function of alcohol use at these time points.

#### *Humoral Immune Response following Vaccine Doses according to Alcohol Consumption*

Loess smooth plots of log-adjusted anti-N, anti-R, and anti-S IgA, IgG, and IgM levels are represented in Figure 1. IgG titers in BAU/mL are described in Supplemental Figure S1. Higher titers were achieved following vaccination in those with prior SARS-CoV-2 infection. While anti-N antibody levels were relatively stable over time, more distinct peaks were observed in anti-R and anti-S titers, suggesting differences in the immunogenicity imparted by natural infection (anti-N IgG) compared to vaccination. Consistent with prior serum Ig concentrations as a rule, IgG signals were stronger than IgA and IgM signals.

#### *Alcohol as a Predictor of Humoral Immune Responses*

UVA models with interaction terms to account for time were developed (Appendix Tables). These models focused on those with and without a history of SARS-CoV-2 infection prior to vaccination as well as intervals before and after the first, second, third and fourth dose

of vaccination. IgG, IgA and IgM antibodies were considered. Overall, these results did not reveal a consistent difference in anti-S and anti-R antibody titers or neutralization antibody function between alcohol use groups over time. In general, the slopes of antibody decay following vaccination were not influenced by alcohol consumption grouping.

To further explore the influence of alcohol consumption of vaccine immunogenicity, multivariable analyses with time interaction terms and controlling for age, sex, ethnicity, obesity status, immune compromise status, vaccine type, interval between vaccine doses, and time since last COVID-19 infection where applicable, were constructed (Appendix Table 4). No effect of alcohol use on SARS-CoV-2 IgG, IgA and IgM antibody titers or neutralization was identified. The slope of antibody decay following vaccination was not consistently influenced by amount of alcohol use.

Several sensitivity models were developed. Comparable results were obtained when alcohol use of 7 or more SD/week was compared with 0-6.9 SD/week and when alcohol use of 3 or more SD/week and 7 or more SD/week were compared with 0 SD/week (data not shown). A sensitivity analysis was performed to evaluate the potential bias introduced by participants who altered their alcohol consumption during the evaluation period. Participants reporting a change in alcohol consumption that would have resulted in a change in their alcohol consumption classification were removed from analysis of anti-S and anti-R IgG and neutralization models. The results were overall similar (data not shown).

## **Discussion**

Chronic excessive alcohol causes immune impairment and consequently decreases vaccine efficacy.<sup>9-11,17,28</sup> In contrast, low to moderate alcohol use has a neutral effect or may, according to some reports, even potentiate immune function in animal models thereby hypothetically improving vaccine immunogenicity.<sup>10,29</sup> The mechanism behind this is unclear. Existing cohort studies evaluating the effect of alcohol use on COVID-19 vaccine efficacy have

generated conflicting results.<sup>21,22</sup> In our analysis, we studied the effect of high level and non-high level alcohol use on the immunogenicity of COVID-19 vaccines in a North American cohort, specifically focusing on humoral immunity, measured as SARS-CoV-2-targeting antibody titers. We identified no consistent effect of alcohol, using multiple cut-points of use, on COVID-19 vaccine immunogenicity.

It is well understood that multiple factors influence immune response after COVID-19 vaccination including age, immunocompromised status, and tobacco smoking.<sup>16,20</sup> While some studies have found that alcohol consumption is a negative predictor of the titer of certain antibodies<sup>4,18,30</sup> the generalizability of results to the general population and how these results directly translate to immune protection from SARS-CoV-2 remain unclear. Additionally, comparisons made between non-alcohol consumers and active users fail to consider the effect of moderate consumption on vaccine efficacy.

Many studies evaluating the effect of alcohol on COVID-19 vaccine immunogenicity are limited by small sample size, narrow focus of immune measures evaluated, short time periods of evaluation, focus on only the first dose of vaccine, use of a single vaccine types and evaluation of a uniform population types. It is therefore predictable that results from these analyses are discordant. Given the large size and heterogeneous characteristics of our cohort, we were able to address many of these limitations. In our study, we found that alcohol use did not have a consistent effect on humoral immune response over time. This is consistent with at least one other population-based study addressing the influence of alcohol use on COVID-19 vaccine immunogenicity.<sup>21</sup> We assessed multiple antibody subtypes (IgG, IgA, IgM) and neutralizing antibodies; a decidedly robust analytical approach to evaluate potential effects of alcohol consumption on vaccine-induced immunity to SARS-CoV-2.

Our study has many strengths. Notably, alcohol stratification is based on current CCSA guidelines, which highlight the health risks associated with different levels of weekly standard

drink consumption. Some studies only make binary comparisons between non-consumers and participants with alcohol use<sup>4,12</sup>, resulting in a lack of granularity when evaluating the effect of alcohol consumption on the immune system. Our analysis stratified alcohol use in multiple different ways to address this issue. Additional strengths of this analysis include a large sample size enabling evaluation up to the fourth vaccine dose and extensive information on those with immunocompromising conditions in our cohort. We employed multiple methods for capturing and validating COVID-19 infections in the participant population. We utilized robust biostatistics approaches including use of random effects for slopes and intercepts thereby accounting for autocorrelation between repeated measures that occurs with the use of longitudinal data.

We acknowledge several limitations. Firstly, while cellular immunity is considered to be an important part of the immune response to COVID-19 vaccination<sup>31</sup>, it was not evaluated in this analysis. We expect that further study of B-cell and T-cell responses of this cohort will offer knowledge that will compliment our analyses reported here. This study was conducted in a developed nation and consists primarily of white participants. This may impact generalizability. The use of self reported participant questionnaires to quantitate alcohol use could introduce biases which would impact the quality of our analysis. Prospective longitudinal studies have potential selection bias arising from volunteer bias and loss of follow-up. Further evaluation is needed to evaluate alcohol consumption as a risk factor for severe COVID-19<sup>17</sup> and its effect on long-term COVID-19 vaccine effectiveness.<sup>13,32</sup> There were differences in some key baseline characteristics of high level alcohol consumers compared to those without excessive alcohol use (Tables 1 and 2). We attempted to account for this in our regression analyses but acknowledge that we may have fallen short of completely accounting for these imbalances. Assay limitations and sparse data samples at longer follow-up periods (fifth and subsequent vaccine doses) and for some subgroups (non-white individuals, non-mRNA vaccine recipients) also represent study

limitations. The abundance of modeling results in this study for different titers and time points increase the risk of type I error.

### *Conclusion*

In conclusion, our analysis of a large urban-based North American population cohort showed that high level alcohol use (three or more and 7 or more standard drinks/week) did not have a consistent effect on SARS-CoV-2 anti-S and anti-R IgG, IgM, and IgA at baseline or following vaccination. Similarly, alcohol use did not have a consistent effect on increase, decay, or baseline levels of SARS-CoV-2 neutralizing antibodies over time.

## Tables

Table 1: Characteristics of participants at baseline according to alcohol consumption level (n=909).

| Variable                                     | Study cohort<br>(n=909) | High Level<br>Alcohol Use*†<br>(n=387) | Non-High Level<br>Alcohol Use<br>(n=522) | P-value                 |
|----------------------------------------------|-------------------------|----------------------------------------|------------------------------------------|-------------------------|
| Age (years)                                  |                         |                                        |                                          |                         |
| Median (IQR)                                 | 45.0 (34.0, 56.0)       | 46.0 (34.0, 58.0)                      | 45.0 (34.0, 55.0)                        | 0.17                    |
| Range                                        | 18.0, 79.0              | 20.0, 79.9                             | 18.0, 79.0                               | (W=95,632.50)           |
| Sex                                          |                         |                                        |                                          |                         |
| Male, n (%)                                  | 315 (34.7%)             | 154 (39.8%)                            | 161 (30.8%)                              | 0.005**                 |
| Female, n (%)                                | 594 (65.3%)             | 233 (60.2%)                            | 361 (69.2%)                              | (X <sup>2</sup> =7.86)  |
| Ethnicity (self-declared)                    |                         |                                        |                                          |                         |
| Arab/West Asian, n (%)                       | 19 (2.1%)               | 5 (1.3%)                               | 14 (2.7%)                                | 0.02**                  |
| Black, n (%)                                 | 5 (0.6%)                | 0 (0.0%)                               | 5 (1.0%)                                 | (X <sup>2</sup> =19.84) |
| East Asian, n (%)                            | 9 (1.0%)                | 3 (0.8%)                               | 6 (1.1%)                                 |                         |
| Southeast Asian, n (%)                       | 6 (0.7%)                | 0 (0.0%)                               | 6 (1.1%)                                 |                         |
| Indigenous, n (%)                            | 14 (1.5%)               | 3 (0.8%)                               | 11 (2.1%)                                |                         |
| Latin American, n (%)                        | 8 (0.9%)                | 2 (0.5%)                               | 6 (1.1%)                                 |                         |
| South Asian, n (%)                           | 13 (1.4%)               | 3 (0.8%)                               | 10 (1.9%)                                |                         |
| White, n (%)                                 | 810 (89.2%)             | 363 (94.0%)                            | 447 (85.6%)                              |                         |
| Other, n (%)                                 | 25 (2.6%)               | 8 (1.8%)                               | 17 (3.3%)                                |                         |
| Immigration Status                           |                         |                                        |                                          |                         |
| Immigrated to Canada, n (%)                  | 116 (13.0%)             | 33 (8.7%)                              | 83 (16.2%)                               | 0.001**                 |
| Unknown                                      | 16                      | 7                                      | 9                                        | (X <sup>2</sup> =10.85) |
| Smoking history                              |                         |                                        |                                          |                         |
| Current Smoker, n (%)                        | 36 (4.0%)               | 19 (4.9%)                              | 17 (3.3%)                                | 0.14                    |
| Former Smoker, n (%)                         | 214 (23.6%)             | 100 (25.9%)                            | 114 (22.0%)                              | (X <sup>2</sup> =3.92)  |
| Never Smoker, n (%)                          | 655 (72.4%)             | 267 (69.2%)                            | 388 (74.8%)                              |                         |
| Missing                                      | 4                       | 1                                      | 3                                        |                         |
| Alcohol consumption (standard<br>units/week) |                         |                                        |                                          |                         |
| Median (IQR)                                 | 2.0 (0.0, 4.0)          | 5.0 (4.0, 7.0)                         | 1.0 (0.0, 1.0)                           | <0.0001**               |
| Range                                        | 0.0, 30.0               | 2.5, 30.0                              | 0.0, 2.0                                 | (W=0.00)                |
| Body mass index (kg/m <sup>2</sup> )         |                         |                                        |                                          |                         |
| Median (IQR)                                 | 26.5 (23.2, 30.4)       | 26.1 (23.1, 29.5)                      | 26.7 (23.2, 31.1)                        | 0.60                    |
| Range                                        | 15.7, 55.1              | 16.3, 49.1                             | 15.7, 55.1                               | (W=101,440.50)          |
| Missing                                      | 329                     | 136                                    | 193                                      |                         |



|                                                                                       |             |             |             |                                    |
|---------------------------------------------------------------------------------------|-------------|-------------|-------------|------------------------------------|
| Immune deficiency                                                                     |             |             |             |                                    |
| Yes, n (%)                                                                            | 207 (22.8%) | 75 (19.4%)  | 132 (25.3%) | 0.04**<br>(X <sup>2</sup> =4.41)   |
| No, n (%)                                                                             | 702 (77.2%) | 312 (80.6%) | 390 (74.7%) |                                    |
| Degree of immunosuppression †                                                         |             |             |             |                                    |
| High immunosuppression, n (%)                                                         | 104 (11.4%) | 31 (8.0%)   | 73 (14.0%)  | 0.01**<br>(X <sup>2</sup> =9.11)   |
| Low immunosuppression, n (%)                                                          | 152 (16.7%) | 61 (15.8%)  | 91 (17.4%)  |                                    |
| No immunosuppression, n (%)                                                           | 653 (71.8%) | 295 (76.2%) | 358 (68.6%) |                                    |
| Number of total self-reported & serology-based SARS-CoV-2 infections during follow-up |             |             |             |                                    |
| 1 infection, n (%)                                                                    | 228 (25.1%) | 93 (24.0%)  | 135 (25.9%) | 0.07<br>(X <sup>2</sup> =7.11)     |
| 2 infections, n (%)                                                                   | 17 (1.9%)   | 12 (3.1%)   | 5 (1.0%)    |                                    |
| 3 infections, n (%)                                                                   | 1 (0.1%)    | 1 (0.3%)    | 0 (0.0%)    |                                    |
| No infections, n (%)                                                                  | 663 (72.9%) | 281 (72.6%) | 382 (73.2%) |                                    |
| COVID-19 prior to dose 1, n (%)                                                       | 215 (23.7%) | 103 (26.6%) | 112 (21.5%) |                                    |
| COVID-19 after dose 1, n (%)                                                          | 3 (0.3%)    | 1 (0.3%)    | 2 (0.4%)    |                                    |
| COVID-19 after dose 2, n (%)                                                          | 40 (4.4%)   | 24 (6.2%)   | 16 (3.1%)   |                                    |
| COVID-19 after dose 3, n (%)                                                          | 133 (14.6%) | 52 (13.4%)  | 81 (15.5%)  |                                    |
| COVID-19 after dose 4, n (%)                                                          | 48 (5.3%)   | 23 (5.9%)   | 25 (4.8%)   |                                    |
| COVID-19 after dose 5, n (%)                                                          | 7 (0.8%)    | 4 (1.0%)    | 3 (0.6%)    |                                    |
| Baseline COVID-19 (self-reported)                                                     |             |             |             |                                    |
| Yes, n (%)                                                                            | 204 (22.4%) | 101 (26.1%) | 103 (19.7%) | p=0.02**<br>(X <sup>2</sup> =5.18) |
| No, n (%)                                                                             | 705 (77.6%) | 286 (73.9%) | 419 (80.3%) |                                    |
| COVID-19 prior to/during study period (self-reported/serology)                        |             |             |             |                                    |
| Yes, n (%)                                                                            | 413 (45.4%) | 187 (48.3%) | 226 (43.3%) | p=0.13<br>(X <sup>2</sup> =2.26)   |
| No, n (%)                                                                             | 496 (54.6%) | 200 (51.7%) | 296 (56.7%) |                                    |

\* High level alcohol use was characterized as 3 or more standard drinks per week. Missing values excluded from percentage calculation and statistical tests.

† Participants were classified as immune compromised if they had a primary immunodeficiency, rheumatological condition, organ-specific autoimmune disease or immune dysfunction, neuromuscular autoimmune disease, inflammatory condition, cancer, HIV, diabetes, asthma requiring medication, were receiving immunosuppressive treatment, or were an organ/bone marrow recipient. Alcohol consumption was not considered for immune status classification.

\*\* Indicates statistical significance.

Table 2: Participant COVID-19 vaccination information as of analysis cut-off data according to cohort allocation at baseline.

| Variable                                                 | Study cohort<br>(n=909) | High Level Alcohol<br>Use* (n=387) | Non-High Level<br>Alcohol Use<br>(n=522) | P-value                 |
|----------------------------------------------------------|-------------------------|------------------------------------|------------------------------------------|-------------------------|
| Vaccinated at baseline (first blood draw, self-reported) |                         |                                    |                                          |                         |
| Yes, n (%)                                               | 311 (34.9%)             | 123 (32.2%)                        | 188 (37.0%)                              | 0.12                    |
| No, n (%)                                                | 579 (65.1%)             | 259 (67.8%)                        | 320 (63.0%)                              | (X <sup>2</sup> =4.32)  |
| Missing                                                  | 19                      | 5                                  | 14                                       |                         |
| Received dose 1                                          |                         |                                    |                                          |                         |
| Yes, n (%)                                               | 889 (97.8%)             | 380 (98.2%)                        | 509 (97.5%)                              | 0.49                    |
| No, n (%)                                                | 20 (2.2%)               | 7 (1.8%)                           | 13 (2.5%)                                | (X <sup>2</sup> =0.48)  |
| Received dose 2                                          |                         |                                    |                                          |                         |
| Yes, n (%)                                               | 884 (97.2%)             | 377 (97.4%)                        | 507 (97.1%)                              | 0.79                    |
| No, n (%)                                                | 25 (2.8%)               | 10 (2.6%)                          | 15 (2.9%)                                | (X <sup>2</sup> =0.070) |
| Received dose 3                                          |                         |                                    |                                          |                         |
| Yes, n (%)                                               | 658 (72.4%)             | 277 (71.6%)                        | 381 (73.0%)                              | 0.64                    |
| No, n (%)                                                | 251 (27.6%)             | 110 (28.4%)                        | 141 (27.0%)                              | (X <sup>2</sup> =0.22)  |
| Received dose 4                                          |                         |                                    |                                          |                         |
| Yes, n (%)                                               | 207 (22.8%)             | 87 (22.5%)                         | 120 (23.0%)                              | 0.86                    |
| No, n (%)                                                | 702 (77.2%)             | 300 (77.5%)                        | 402 (77.0%)                              | (X <sup>2</sup> =0.033) |
| Received dose 5                                          |                         |                                    |                                          |                         |
| Yes, n (%)                                               | 80 (8.8%)               | 32 (8.3%)                          | 48 (9.2%)                                | 0.63                    |
| No, n (%)                                                | 829 (91.2%)             | 355 (91.7%)                        | 474 (90.8%)                              | (X <sup>2</sup> =0.24)  |
| Time between first and second doses in days              |                         |                                    |                                          |                         |
| Median (IQR)                                             | 58.0 (35.0, 76.0)       | 61 (35.8, 77.0)                    | 57 (25.0, 75.0)                          | 0.046**                 |
| Range                                                    | 19.0, 344.0             | 19.0, 147.0                        | 20.0, 344.0                              | (W=87,668)              |
| Missing data/Missing dose                                | 27                      | 11                                 | 16                                       |                         |
| Time between first and second doses in days, n (%)       |                         |                                    |                                          |                         |
| <35                                                      | 249 (28.2%)             | 94 (25.0%)                         | 155 (30.6%)                              | 0.18                    |
| 35-75                                                    | 410 (46.5%)             | 182 (48.4%)                        | 228 (45.1%)                              | (X <sup>2</sup> =3.39)  |
| >75                                                      | 223 (25.3%)             | 100 (26.6%)                        | 123 (24.3%)                              |                         |
| Missing data/Missing dose                                | 27                      | 11                                 | 16                                       |                         |
| Time between second and third doses in days              |                         |                                    |                                          |                         |
| Median (IQR)                                             | 185.0 (174.0, 211.0)    | 187.0 (176.0, 228.0)               | 184 (172.0, 206.0)                       | 0.03**                  |

|                                                    |             |             |             |                        |
|----------------------------------------------------|-------------|-------------|-------------|------------------------|
| Range                                              | 22.0, 546.0 | 65.0, 541.0 | 22.0, 546.0 | (W=47,194)             |
| Missing data/Missing dose                          | 254         | 112         | 142         |                        |
| Time between second and third doses in days, n (%) |             |             |             |                        |
| <175                                               | 187 (28.5%) | 66 (24.0%)  | 121 (31.8%) | 0.09                   |
| 175-210                                            | 303 (46.3%) | 135 (49.1%) | 168 (44.2%) | (X <sup>2</sup> =4.81) |
| >210                                               | 165 (25.2%) | 74 (26.9%)  | 91 (23.9%)  |                        |
| Missing data/Missing dose                          | 254         | 112         | 142         |                        |

\* High level alcohol use was characterized as 3 or more standard drinks per week. Missing values excluded from percentage calculation and statistical tests.

\*\* Indicates statistical significance.

Supplemental Table 1: Univariable multilevel linear regression models of anti-S and anti-R IgG titers in participants with no previous infection prior to vaccination. Interaction terms between time and excessive alcohol consumption are included. Excessive alcohol use was defined as  $\geq 3$  drinks/week and was compared to non-high level alcohol defined as 0-2.9 drinks/week (the referent category).

| Anti-S IgG (log BAU/mL)                                                                |          |                 |          | Anti-R IgG (log BAU/mL)                                                                |          |                 |          |
|----------------------------------------------------------------------------------------|----------|-----------------|----------|----------------------------------------------------------------------------------------|----------|-----------------|----------|
| Dose 1                                                                                 |          |                 |          |                                                                                        |          |                 |          |
| Effect                                                                                 | Estimate | 95% CI          | P        | Effect                                                                                 | Estimate | 95% CI          | P        |
| Intercept                                                                              | 1.13     | 0.97, 1.29      | <0.0001* | Intercept                                                                              | 1.26     | 1.14, 1.37      | <0.0001* |
| Time since dose 1 (in days)                                                            | 0.064    | 0.058, 0.070    | <0.0001* | Time since dose 1 (in days)                                                            | 0.043    | 0.038, 0.048    | <0.0001* |
| Linear spline (knot at day 30)                                                         | -0.086   | -0.096, -0.076  | <0.0001* | Linear spline (knot at day 30)                                                         | -0.057   | -0.065, -0.048  | <0.0001* |
| Excessive alcohol                                                                      | -0.18    | -0.44, 0.072    | 0.16     | Excessive alcohol                                                                      | -0.023   | -0.21, 0.16     | 0.80     |
| Time since dose 1 interaction                                                          | 0.0059   | -0.0042, 0.016  | 0.25     | Time since dose 1 interaction                                                          | 0.0025   | -0.0058, 0.011  | 0.56     |
| Linear spline interaction                                                              | -0.0028  | -0.018, 0.013   | 0.73     | Linear spline interaction                                                              | -0.0010  | -0.015, 0.013   | 0.89     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                 | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                 | P        |
| Difference in intercepts                                                               | -0.18    |                 | 0.16     | Difference in intercepts                                                               | -0.023   |                 | 0.80     |
| Difference in slope before knot                                                        | 0.0059   |                 | 0.25     | Difference in slope before knot                                                        | 0.0025   |                 | 0.56     |
| Difference in slope after knot                                                         | 0.0031   |                 | 0.48     | Difference in slope after knot                                                         | 0.0015   |                 | 0.70     |
| Dose 2                                                                                 |          |                 |          |                                                                                        |          |                 |          |
| Effect                                                                                 | Estimate | 95% CI          | P        | Effect                                                                                 | Estimate | 95% CI          | P        |
| Intercept                                                                              | 2.72     | 2.61, 2.82      | <0.0001* | Intercept                                                                              | 2.65     | 2.57, 2.73      | <0.0001* |
| Time since dose 2 (in days)                                                            | 0.016    | 0.014, 0.018    | <0.0001* | Time since dose 2 (in days)                                                            | 0.018    | 0.016, 0.019    | <0.0001* |
| Linear spline (knot at day 40)                                                         | -0.019   | -0.022, -0.017  | <0.0001* | Linear spline (knot at day 40)                                                         | -0.022   | -0.024, -0.020  | <0.0001* |
| Excessive alcohol                                                                      | 0.15     | -0.014, 0.31    | 0.07     | Excessive alcohol                                                                      | 0.15     | 0.022, 0.28     | 0.022*   |
| Time since dose 2 interaction                                                          | 0.00051  | -0.0030, 0.0041 | 0.78     | Time since dose 2 interaction                                                          | -0.00024 | -0.0027, 0.0022 | 0.85     |
| Linear spline interaction                                                              | -0.0017  | -0.0059, 0.0026 | 0.44     | Linear spline interaction                                                              | -0.00074 | -0.0039, 0.0024 | 0.64     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                 | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                 | P        |
| Difference in intercepts                                                               | 0.15     |                 | 0.07     | Difference in intercepts                                                               | 0.15     |                 | 0.02*    |
| Difference in slope before knot                                                        | 0.00051  |                 | 0.78     | Difference in slope before knot                                                        | -0.00024 |                 | 0.85     |
| Difference in slope after knot                                                         | -0.0011  |                 | 0.08     | Difference in slope after knot                                                         | -0.0010  |                 | 0.09     |

| Dose 3                                                                                 |          |                  |          |                                                                                        |          |                 |          |
|----------------------------------------------------------------------------------------|----------|------------------|----------|----------------------------------------------------------------------------------------|----------|-----------------|----------|
| Effect                                                                                 | Estimate | 95% CI           | P        | Effect                                                                                 | Estimate | 95% CI          | P        |
| Intercept                                                                              | 3.08     | 2.99, 3.17       | <0.0001* | Intercept                                                                              | 3.02     | 2.92, 3.12      | <0.0001* |
| Time since dose 3 (in days)                                                            | 0.0079   | 0.0065, 0.0093   | <0.0001* | Time since dose 3 (in days)                                                            | 0.0094   | 0.0080, 0.011   | <0.0001* |
| Linear spline (knot at day 60)                                                         | -0.011   | -0.013, -0.0085  | <0.0001* | Linear spline (knot at day 60)                                                         | -0.013   | -0.016, -0.011  | <0.0001* |
| Excessive alcohol                                                                      | 0.11     | -0.047, 0.26     | 0.17     | Excessive alcohol                                                                      | 0.072    | -0.095, 0.24    | 0.40     |
| Time since dose 3 interaction                                                          | -0.0014  | -0.0035, 0.00082 | 0.22     | Time since dose 3 interaction                                                          | -0.0012  | -0.0034, 0.0010 | 0.28     |
| Linear spline interaction                                                              | 0.0020   | -0.0014, 0.0053  | 0.25     | Linear spline interaction                                                              | 0.0023   | -0.0012, 0.0057 | 0.20     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                  | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                 | P        |
| Difference in intercepts                                                               | 0.11     |                  | 0.17     | Difference in intercepts                                                               | 0.072    |                 | 0.40     |
| Difference in slope before knot                                                        | -0.0014  |                  | 0.22     | Difference in slope before knot                                                        | -0.0012  |                 | 0.28     |
| Difference in slope after knot                                                         | 0.00062  |                  | 0.49     | Difference in slope after knot                                                         | 0.0011   |                 | 0.30     |
| Dose 4                                                                                 |          |                  |          |                                                                                        |          |                 |          |
| Effect                                                                                 | Estimate | 95% CI           | P        | Effect                                                                                 | Estimate | 95% CI          | P        |
| Intercept                                                                              | 3.17     | 3.00, 3.34       | <0.0001* | Intercept                                                                              | 3.064    | 2.85, 3.28      | <0.0001* |
| Time since dose 4 (in days)                                                            | 0.0060   | 0.0035, 0.0084   | <0.0001* | Time since dose 4 (in days)                                                            | 0.0071   | 0.0044, 0.0097  | <0.0001* |
| Linear spline (knot at day 75)                                                         | -0.0095  | -0.014, -0.0054  | <0.0001* | Linear spline (knot at day 75)                                                         | -0.010   | -0.15, -0.0060  | <0.0001* |
| Excessive alcohol                                                                      | 0.21     | -0.081, 0.50     | 0.15     | Excessive alcohol                                                                      | 0.29     | -0.084, 0.65    | 0.13     |
| Time since dose 4 interaction                                                          | -0.0022  | -0.0065, 0.0021  | 0.31     | Time since dose 4 interaction                                                          | -0.0015  | -0.0062, 0.0032 | 0.52     |
| Linear spline interaction                                                              | 0.0022   | -0.0060, 0.010   | 0.59     | Linear spline interaction                                                              | -0.0019  | -0.011, 0.0069  | 0.67     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                  | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                 | P        |
| Difference in intercepts                                                               | 0.21     |                  | 0.15     | Difference in intercepts                                                               | 0.29     |                 | 0.13     |
| Difference in slope before knot                                                        | -0.0022  |                  | 0.31     | Difference in slope before knot                                                        | -0.0015  |                 | 0.52     |
| Difference in slope after knot                                                         | 0.000022 |                  | 0.99     | Difference in slope after knot                                                         | -0.0034  |                 | 0.28     |

Intercept estimates were converted back from log<sub>10</sub>(IU/mL) to IU/mL to better demonstrate baseline unadjusted antibody levels.

\* Indicates a statistically significant P value.

Supplemental Table 2: Univariable multilevel linear regression models of anti-S and anti-R IgG titers in participants with previous infection prior to vaccination. Interaction terms between time and excessive alcohol consumption are included. Excessive alcohol use was defined as  $\geq 3$  drinks/week and was compared to non-high level alcohol defined as 0-2.9 drinks/week (the referent category).

| Anti-S IgG (log BAU/mL)                                                                |           |                 |          | Anti-R IgG (log BAU/mL)                                                                |          |                   |          |
|----------------------------------------------------------------------------------------|-----------|-----------------|----------|----------------------------------------------------------------------------------------|----------|-------------------|----------|
| Dose 1                                                                                 |           |                 |          |                                                                                        |          |                   |          |
| Effect                                                                                 | Estimate  | 95% CI          | P        | Effect                                                                                 | Estimate | 95% CI            | P        |
| Intercept                                                                              | 2.82      | 2.67, 2.98      | <0.0001* | Intercept                                                                              | 2.67     | 2.52, 2.81        | <0.0001* |
| Time since dose 1 (in days)                                                            | 0.033     | 0.028, 0.038    | <0.0001* | Time since dose 1 (in days)                                                            | 0.035    | 0.030, 0.039      | <0.0001* |
| Linear spline (knot at day 25)                                                         | -0.032    | -0.044, -0.020  | <0.0001* | Linear spline (knot at day 25)                                                         | -0.034   | -0.044, -0.023    | <0.0001* |
| Excessive alcohol                                                                      | 0.13      | -0.088, 0.35    | 0.24     | Excessive alcohol                                                                      | 0.14     | -0.064, 0.34      | 0.18     |
| Time since dose 1 interaction                                                          | -0.0044   | -0.012, 0.0028  | 0.23     | Time since dose 1 interaction                                                          | -0.0064  | -0.013, -0.000017 | 0.047*   |
| Linear spline interaction                                                              | 0.0010    | -0.016, 0.017   | 0.91     | Linear spline interaction                                                              | 0.0050   | -0.0096, 0.019    | 0.50     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                 | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                   | P        |
| Difference in intercepts                                                               | 0.13      |                 | 0.21     | Difference in intercepts                                                               | 0.138    |                   | 0.18     |
| Difference in slope before knot                                                        | -0.0044   |                 | 0.23     | Difference in slope before knot                                                        | -0.0064  |                   | 0.047*   |
| Difference in slope after knot                                                         | -0.0034   |                 | 0.56     | Difference in slope after knot                                                         | -0.0015  |                   | 0.78     |
| Dose 2                                                                                 |           |                 |          |                                                                                        |          |                   |          |
| Effect                                                                                 | Estimate  | 95% CI          | P        | Effect                                                                                 | Estimate | CI                | P        |
| Intercept                                                                              | 3.58      | 3.48, 3.68      | <0.0001* | Intercept                                                                              | 3.60     | 3.49, 3.71        | <0.0001* |
| Time since dose 2 (in days)                                                            | -0.00070  | -0.0035, 0.0022 | 0.65     | Time since dose 2 (in days)                                                            | 0.0058   | 0.0034, 0.0081    | <0.0001* |
| Linear spline (knot at day 25)                                                         | -0.000031 | -0.0038, 0.0036 | 0.97     | Linear spline (knot at day 25)                                                         | -0.0078  | -0.011, -0.0047   | <0.0001* |
| Excessive alcohol                                                                      | 0.040     | -0.10, 0.18     | 0.58     | Excessive alcohol                                                                      | 0.054    | -0.10, 0.21       | 0.49     |
| Time since dose 2 interaction                                                          | 0.0024    | -0.0016, 0.0064 | 0.24     | Time since dose 2 interaction                                                          | 0.00025  | -0.0031, 0.0036   | 0.89     |
| Linear spline interaction                                                              | -0.0031   | -0.0082, 0.0021 | 0.24     | Linear spline interaction                                                              | -0.0010  | -0.0053, 0.0033   | 0.65     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                 | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                   | P        |
| Difference in intercepts                                                               | 0.040     |                 | 0.58     | Difference in intercepts                                                               | 0.054    |                   | 0.49     |
| Difference in slope before knot                                                        | 0.0024    |                 | 0.24     | Difference in slope before knot                                                        | 0.00025  |                   | 0.89     |

|                                                                                        |          |                  |          |                                                                                        |          |                  |          |
|----------------------------------------------------------------------------------------|----------|------------------|----------|----------------------------------------------------------------------------------------|----------|------------------|----------|
| Difference in slope after knot                                                         | -0.00070 |                  | 0.45     | Difference in slope after knot                                                         | -0.00070 |                  | 0.40     |
| Dose 3                                                                                 |          |                  |          |                                                                                        |          |                  |          |
| Effect                                                                                 | Estimate | 95% CI           | P        | Effect                                                                                 | Estimate | CI               | P        |
| Intercept                                                                              | 3.68     | 3.59, 3.77       | <0.0001* | Intercept                                                                              | 3.71     | 3.60, 3.82       | <0.0001* |
| Time since dose 3 (in days)                                                            | 0.0028   | 0.0013, 0.0042   | 0.00010* | Time since dose 3 (in days)                                                            | 0.0031   | 0.0015, 0.0046   | 0.00010* |
| Linear spline (knot at day 60)                                                         | -0.0039  | -0.0056, -0.0021 | <0.0001* | Linear spline (knot at day 60)                                                         | -0.0041  | -0.0060, -0.0023 | <0.0001* |
| Excessive alcohol                                                                      | -0.087   | -0.21, 0.034     | 0.16     | Excessive alcohol                                                                      | -0.14    | -0.29, 0.011     | 0.07     |
| Time since dose 3 interaction                                                          | 0.00013  | -0.0018, 0.0021  | 0.90     | Time since dose 3 interaction                                                          | 0.00035  | -0.0018, 0.0025  | 0.75     |
| Linear spline interaction                                                              | 0.00022  | 0.0021, 0.0036   | 0.85     | Linear spline interaction                                                              | 0.00018  | -0.0024, 0.0028  | 0.89     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                  | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                  | P        |
| Difference in intercepts                                                               | -0.09    |                  | 0.16     | Difference in intercepts                                                               | -0.14    |                  | 0.07     |
| Difference in slope before knot                                                        | 0.00013  |                  | 0.90     | Difference in slope before knot                                                        | 0.0034   |                  | 0.75     |
| Difference in slope after knot                                                         | 0.00035  |                  | 0.38     | Difference in slope after knot                                                         | 0.00053  |                  | 0.24     |
| Dose 4                                                                                 |          |                  |          |                                                                                        |          |                  |          |
| Effect                                                                                 | Estimate | CI               | P        | Effect                                                                                 | Estimate | CI               | P        |
| Intercept                                                                              | 3.73     | 3.64, 3.82       | <0.0001* | Intercept                                                                              | 3.773    | 3.66, 3.89       | <0.0001* |
| Time since dose 4 (in days)                                                            | 0.0028   | 0.0011, 0.0046   | 0.0019*  | Time since dose 4 (in days)                                                            | 0.0032   | 0.0012, 0.0052   | 0.0020*  |
| Linear spline (knot at day 45)                                                         | -0.0051  | -0.0075, -0.0026 | <0.0001* | Linear spline (knot at day 45)                                                         | -0.006   | -0.0083, -0.0027 | 0.00020* |
| Excessive alcohol                                                                      | -0.019   | -0.14, 0.10      | 0.76     | Excessive alcohol                                                                      | 0.01     | -0.16, 0.17      | 0.94     |
| Time since dose 4 interaction                                                          | 0.00068  | -0.0018, 0.0032  | 0.59     | Time since dose 4 interaction                                                          | 0.0007   | -0.0021, 0.0036  | 0.61     |
| Linear spline interaction                                                              | 0.00053  | -0.0032, 0.0043  | 0.78     | Linear spline interaction                                                              | 0.0004   | -0.0039, 0.0046  | 0.86     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                  | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                  | P        |
| Difference in intercepts                                                               | -0.019   |                  | 0.76     | Difference in intercepts                                                               | 0.0060   |                  | 0.94     |
| Difference in slope before knot                                                        | 0.00068  |                  | 0.59     | Difference in slope before knot                                                        | 0.00073  |                  | 0.61     |
| Difference in slope after knot                                                         | 0.0012   |                  | 0.20     | Difference in slope after knot                                                         | 0.0011   |                  | 0.32     |

Intercept estimates were converted back from log10(IU/mL) to IU/mL to better demonstrate baseline unadjusted antibody levels.

\* Indicates a statistically significant P value.

Supplemental Table 3: Univariable multilevel linear regression models of neutralizing antibodies (%). Interaction terms between time and excessive alcohol consumption are included. Excessive alcohol use was defined as  $\geq 3$  drinks/week and was compared to non-high level alcohol defined as 0-2.9 drinks/week (the referent category).

| Neutralization % - No Prior Infection                                                  |          |                  |          | Neutralization % - Prior Infection                                                     |          |                   |          |
|----------------------------------------------------------------------------------------|----------|------------------|----------|----------------------------------------------------------------------------------------|----------|-------------------|----------|
| Dose 1                                                                                 |          |                  |          |                                                                                        |          |                   |          |
| Effect                                                                                 | Estimate | 95% CI           | P        | Effect                                                                                 | Estimate | 95% CI            | P        |
| Intercept                                                                              | 3.82     | 1.4822, 6.1666   | 0.0014*  | Intercept                                                                              | 37.56    | 31.9655, 43.1595  | <0.0001* |
| Time since dose 1 (in days)                                                            | 0.265    | 0.1496, 0.3795   | <0.0001* | Time since dose 1 (in days)                                                            | 1.189    | 1.0269, 1.3503    | <0.0001* |
| Linear spline (knot at day 30)                                                         | -0.406   | -0.6253, -0.1865 | 0.00030* | Linear spline (knot at day 50)                                                         | -1.549   | -2.114, -0.9846   | <0.0001* |
| Excessive alcohol                                                                      | 2.07     | -1.8272, 5.9691  | 0.30     | Excessive alcohol                                                                      | 1.483    | -6.5844, 9.5502   | 0.72     |
| Time since dose 1 interaction                                                          | -0.0005  | -0.1875, 0.1865  | 1.00     | Time since dose 1 interaction                                                          | 0.0700   | -0.1627, 0.3027   | 0.55     |
| Linear spline interaction                                                              | -0.1251  | -0.4747, 0.2245  | 0.48     | Linear spline interaction                                                              | -0.9635  | -1.9026, -0.02438 | 0.04     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                  | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                   | P        |
| Difference in intercepts                                                               | 2.07     |                  | 0.30     | Difference in intercepts                                                               | 1.483    |                   | 0.72     |
| Difference in slope before knot                                                        | -0.0005  |                  | 1.00     | Difference in slope before knot                                                        | 0.0700   |                   | 0.55     |
| Difference in slope after knot                                                         | -0.1256  |                  | 0.26     | Difference in slope after knot                                                         | -0.8935  |                   | 0.03*    |
| Dose 2                                                                                 |          |                  |          |                                                                                        |          |                   |          |
| Effect                                                                                 | Estimate | 95% CI           | P        | Effect                                                                                 | Estimate | 95% CI            | P        |
| Intercept                                                                              | 36.65    | 33.8018, 39.505  | <0.0001* | Intercept                                                                              | 81.74    | 76.9614, 86.5169  | <0.0001* |
| Time since dose 2 (in days)                                                            | 0.634    | 0.5765, 0.691    | <0.0001* | Time since dose 2 (in days)                                                            | 0.196    | 0.09102, 0.3009   | <0.0001* |
| Linear spline (knot at day 50)                                                         | -0.828   | -0.9123, -0.7434 | <0.0001* | Linear spline (knot at day 35)                                                         | -0.360   | -0.5588, -0.1616  | 0.00040* |
| Excessive alcohol                                                                      | 1.13     | -3.4416, 5.6972  | 0.63     | Excessive alcohol                                                                      | 0.76     | -6.0642, 7.5869   | 0.83     |
| Time since dose 2 interaction                                                          | -0.01785 | -0.1107, 0.07502 | 0.71     | Time since dose 2 interaction                                                          | -0.02586 | -0.1762, 0.1245   | 0.74     |
| Linear spline interaction                                                              | -0.0021  | -0.1389, 0.1348  | 0.98     | Linear spline interaction                                                              | 0.07710  | -0.2055, 0.3597   | 0.59     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                  | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                   | P        |
| Difference in intercepts                                                               | 1.13     |                  | 0.63     | Difference in intercepts                                                               | 0.76     |                   | 0.83     |
| Difference in slope before knot                                                        | -0.01785 |                  | 0.71     | Difference in slope before knot                                                        | -0.02586 |                   | 0.74     |
| Difference in slope after knot                                                         | -0.0199  |                  | 0.55     | Difference in slope after knot                                                         | 0.0512   |                   | 0.59     |



| Dose 3                                                                                 |          |                   |          |                                                                                        |          |                  |          |
|----------------------------------------------------------------------------------------|----------|-------------------|----------|----------------------------------------------------------------------------------------|----------|------------------|----------|
| Effect                                                                                 | Estimate | 95% CI            | P        | Effect                                                                                 | Estimate | 95% CI           | P        |
| Intercept                                                                              | 44.44    | 31.2671, 57.6043  | <0.0001* | Intercept                                                                              | 71.62    | 60.6865, 82.5484 | <0.0001* |
| Time since dose 3 (in days)                                                            | 0.1920   | -0.3385, 0.7226   | 0.46     | Time since dose 3 (in days)                                                            | 0.6357   | 0.2756, 0.9958   | 0.0008*  |
| Linear spline (knot at day 50)                                                         | -0.581   | -1.6482, 0.4863   | 0.26     | Linear spline (knot at day 35)                                                         | -0.791   | -1.4114, -0.1707 | 0.01*    |
| Excessive alcohol                                                                      | 2.95     | -19.3781, 25.2745 | 0.79     | Excessive alcohol                                                                      | 2.095    | -12.2451, 16.435 | 0.77     |
| Time since dose 3 interaction                                                          | 0.7673   | -0.1831, 1.7177   | 0.11     | Time since dose 3 interaction                                                          | 0.3346   | -0.1876, 0.8568  | 0.21     |
| Linear spline interaction                                                              | -0.7522  | -2.7246, 1.2202   | 0.44     | Linear spline interaction                                                              | -0.7865  | -1.7649, 0.1919  | 0.11     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                   | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                  | P        |
| Difference in intercepts                                                               | 2.95     |                   | 0.79     | Difference in intercepts                                                               | 2.095    |                  | 0.77     |
| Difference in slope before knot                                                        | 0.7673   |                   | 0.11     | Difference in slope before knot                                                        | 0.3346   |                  | 0.21     |
| Difference in slope after knot                                                         | 0.01509  |                   | 0.98     | Difference in slope after knot                                                         | -0.4519  |                  | 0.12     |

Intercept estimates were converted back from log<sub>10</sub>(IU/mL) to IU/mL to better demonstrate baseline unadjusted antibody levels.

\* Indicates a statistically significant P value.

Supplemental Table 4: Univariable multilevel linear regression models of anti-S and anti-R IgA titers in participants with no previous infection prior to vaccination. Interaction terms between time and excessive alcohol consumption are included. Excessive alcohol use was defined as  $\geq 3$  drinks/week and was compared to non-high level alcohol defined as 0-2.9 drinks/week (the referent category).

| Anti-S IgA (log µg/mL)                                                                 |           |                  |          | Anti-R IgA (log µg/mL)                                                                 |           |                   |          |
|----------------------------------------------------------------------------------------|-----------|------------------|----------|----------------------------------------------------------------------------------------|-----------|-------------------|----------|
| Dose 1                                                                                 |           |                  |          |                                                                                        |           |                   |          |
| Effect                                                                                 | Estimate  | 95% CI           | P        | Effect                                                                                 | Estimate  | 95% CI            | P        |
| Intercept                                                                              | -0.21     | -0.29, -0.14     | <0.0001* | Intercept                                                                              | -0.79     | -0.92, -0.68      | <0.0001* |
| Time since dose 1 (in days)                                                            | 0.0082    | 0.0070, 0.0094   | <0.0001* | Time since dose 1 (in days)                                                            | 0.0030    | 0.0022, 0.0037    | <0.0001* |
| Linear spline (knot at day 20)                                                         | -0.014    | -0.016, -0.012   | <0.0001* | Linear spline (knot at day 20)                                                         | -0.0053   | -0.0067, -0.0040  | <0.0001* |
| Excessive alcohol                                                                      | 0.0013    | -0.045, 0.048    | 0.96     | Excessive alcohol                                                                      | -0.0047   | -0.032, 0.023     | 0.74     |
| Time since dose 1 interaction                                                          | -0.00071  | -0.0026, 0.0012  | 0.45     | Time since dose 1 interaction                                                          | -0.00029  | -0.0016, 0.0010   | 0.66     |
| Linear spline interaction                                                              | 0.00070   | -0.0026, 0.0040  | 0.68     | Linear spline interaction                                                              | 0.00037   | -0.0018, 0.0026   | 0.74     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                  | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                   | P        |
| Difference in intercepts                                                               | 0.0013    |                  | 0.96     | Difference in intercepts                                                               | -0.0047   |                   | 0.74     |
| Difference in slope before knot                                                        | -0.00071  |                  | 0.45     | Difference in slope before knot                                                        | -0.00029  |                   | 0.66     |
| Difference in slope after knot                                                         | -0.000010 |                  | 0.99     | Difference in slope after knot                                                         | 0.000083  |                   | 0.90     |
| Dose 2                                                                                 |           |                  |          |                                                                                        |           |                   |          |
| Effect                                                                                 | Estimate  | 95% CI           | P        | Effect                                                                                 | Estimate  | 95% CI            | P        |
| Intercept                                                                              | 0.16      | 0.12, 0.21       | <0.0001* | Intercept                                                                              | -0.26     | -0.32, -0.20      | <0.0001* |
| Time since dose 2 (in days)                                                            | 0.0025    | 0.0020, 0.0030   | <0.0001* | Time since dose 2 (in days)                                                            | 0.0018    | 0.0014, 0.0022    | <0.0001* |
| Linear spline (knot at day 33)                                                         | -0.0034   | -0.0041, -0.0028 | <0.0001* | Linear spline (knot at day 25)                                                         | -0.0034   | -0.0041, -0.0028  | <0.0001* |
| Excessive alcohol                                                                      | 0.00044   | -0.042, 0.043    | 0.98     | Excessive alcohol                                                                      | -0.018    | -0.053, 0.017     | 0.32     |
| Time since dose 2 interaction                                                          | 0.00029   | -0.00052, 0.0011 | 0.48     | Time since dose 2 interaction                                                          | -0.000030 | -0.00073, 0.00067 | 0.93     |
| Linear spline interaction                                                              | -0.00036  | -0.0014, 0.00065 | 0.48     | Linear spline interaction                                                              | 0.000051  | -0.00096, 0.0011  | 0.92     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                  | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                   | P        |
| Difference in intercepts                                                               | 0.00044   |                  | 0.98     | Difference in intercepts                                                               | -0.018    |                   | 0.32     |
| Difference in slope before knot                                                        | 0.00029   |                  | 0.48     | Difference in slope before knot                                                        | -0.000030 |                   | 0.93     |
| Difference in slope after knot                                                         | -0.000070 |                  | 0.70     | Difference in slope after knot                                                         | 0.000018  |                   | 0.94     |

| Dose 3                                                                                 |          |                   |          |                                                                                        |          |                   |          |
|----------------------------------------------------------------------------------------|----------|-------------------|----------|----------------------------------------------------------------------------------------|----------|-------------------|----------|
| Effect                                                                                 | Estimate | 95% CI            | P        | Effect                                                                                 | Estimate | 95% CI            | P        |
| Intercept                                                                              | 0.12     | 0.04, 0.20        | <0.0001* | Intercept                                                                              | -0.59    | -0.74, -0.47      | <0.0001* |
| Time since dose 3 (in days)                                                            | 0.0014   | 0.00081, 0.0021   | <0.0001* | Time since dose 3 (in days)                                                            | 0.0016   | 0.00097, 0.0022   | <0.0001* |
| Linear spline (knot at day 80)                                                         | -0.0016  | -0.0026, -0.00060 | 0.0020*  | Linear spline (knot at day 60)                                                         | -0.0019  | -0.0028, -0.00093 | 0.00010* |
| Excessive alcohol                                                                      | -0.022   | -0.099, 0.056     | 0.58     | Excessive alcohol                                                                      | -0.023   | -0.071, 0.025     | 0.34     |
| Time since dose 3 interaction                                                          | 0.00018  | -0.00086, 0.0012  | 0.73     | Time since dose 3 interaction                                                          | -0.00020 | -0.0012, 0.00080  | 0.70     |
| Linear spline interaction                                                              | -0.00013 | -0.0017, 0.0015   | 0.87     | Linear spline interaction                                                              | 0.00070  | -0.00079, 0.0022  | 0.36     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                   | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                   | P        |
| Difference in intercepts                                                               | -0.022   |                   | 0.58     | Difference in intercepts                                                               | -0.023   |                   | 0.34     |
| Difference in slope before knot                                                        | 0.00018  |                   | 0.73     | Difference in slope before knot                                                        | -0.00020 |                   | 0.70     |
| Difference in slope after knot                                                         | 0.000050 |                   | 0.91     | Difference in slope after knot                                                         | 0.00050  |                   | 0.20     |
| Dose 4                                                                                 |          |                   |          |                                                                                        |          |                   |          |
| Effect                                                                                 | Estimate | 95% CI            | P        | Effect                                                                                 | Estimate | 95% CI            | P        |
| Intercept                                                                              | 0.27     | 0.14, 0.38        | <0.0001* | Intercept                                                                              | -0.47    | -0.90, -0.22      | 0.0013   |
| Time since dose 4 (in days)                                                            | 0.0013   | -0.0012, 0.0038   | 0.30     | Time since dose 4 (in days)                                                            | 0.0019   | 0.00018, 0.0035   | 0.030    |
| Linear spline (knot at day 35)                                                         | -0.0021  | -0.0053, 0.0010   | 0.18     | Linear spline (knot at day 55)                                                         | -0.0021  | -0.0046, 0.00035  | 0.091    |
| Excessive alcohol                                                                      | 0.027    | -0.11, 0.16       | 0.70     | Excessive alcohol                                                                      | 0.012    | -0.12, 0.14       | 0.85     |
| Time since dose 4 interaction                                                          | -0.0022  | -0.0070, 0.0027   | 0.37     | Time since dose 4 interaction                                                          | -0.00080 | -0.0036, 0.0020   | 0.57     |
| Linear spline interaction                                                              | 0.0028   | -0.0037, 0.0092   | 0.39     | Linear spline interaction                                                              | -0.00020 | -0.0044, 0.0040   | 0.93     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                   | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                   | P        |
| Difference in intercepts                                                               | 0.027    |                   | 0.70     | Difference in intercepts                                                               | 0.012    |                   | 0.85     |
| Difference in slope before knot                                                        | -0.0022  |                   | 0.37     | Difference in slope before knot                                                        | -0.00080 |                   | 0.57     |
| Difference in slope after knot                                                         | 0.00061  |                   | 0.60     | Difference in slope after knot                                                         | -0.0010  |                   | 0.34     |

Intercept estimates were converted back from log10(IU/mL) to IU/mL to better demonstrate baseline unadjusted antibody levels.

\* Indicates a statistically significant P value.

Supplemental Table 5: Univariable multilevel linear regression models of anti-S and anti-R IgA titers in participants with previous infection prior to vaccination. Interaction terms between time and excessive alcohol consumption are included. Excessive alcohol use was defined as  $\geq 3$  drinks/week and was compared to non-high level alcohol defined as 0-2.9 drinks/week (the referent category).

| Anti-S IgA (log µg/mL)                                                                 |          |                  |          | Anti-R IgA (log µg/mL)                                                                 |          |                   |          |
|----------------------------------------------------------------------------------------|----------|------------------|----------|----------------------------------------------------------------------------------------|----------|-------------------|----------|
| Dose 1                                                                                 |          |                  |          |                                                                                        |          |                   |          |
| Effect                                                                                 | Estimate | 95% CI           | P        | Effect                                                                                 | Estimate | 95% CI            | P        |
| Intercept                                                                              | 0.24     | 0.15, 0.32       | <0.0001* | Intercept                                                                              | -0.056   | -0.16, 0.035      | <0.0001* |
| Time since dose 1 (in days)                                                            | 0.0077   | 0.0060, 0.0094   | <0.0001* | Time since dose 1 (in days)                                                            | 0.0086   | 0.0070, 0.010     | <0.0001* |
| Linear spline (knot at day 40)                                                         | -0.011   | -0.016, -0.0054  | <0.0001* | Linear spline (knot at day 28)                                                         | -0.012   | -0.016, -0.0082   | <0.0001* |
| Excessive alcohol                                                                      | 0.00020  | -0.077, 0.077    | 1.00     | Excessive alcohol                                                                      | -0.013   | -0.077, 0.052     | 0.70     |
| Time since dose 1 interaction                                                          | -0.0017  | -0.0042, 0.00076 | 0.17     | Time since dose 1 interaction                                                          | -0.0012  | -0.0035, 0.0012   | 0.33     |
| Linear spline interaction                                                              | 0.0013   | -0.0062, 0.0089  | 0.73     | Linear spline interaction                                                              | 0.0013   | -0.0040, 0.0065   | 0.64     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                  | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                   | P        |
| Difference in intercepts                                                               | 0.00020  |                  | 1.00     | Difference in intercepts                                                               | -0.013   |                   | 0.70     |
| Difference in slope before knot                                                        | -0.0017  |                  | 0.17     | Difference in slope before knot                                                        | -0.0012  |                   | 0.33     |
| Difference in slope after knot                                                         | -0.00037 |                  | 0.90     | Difference in slope after knot                                                         | 0.000085 |                   | 0.96     |
| Dose 2                                                                                 |          |                  |          |                                                                                        |          |                   |          |
| Effect                                                                                 | Estimate | 95% CI           | P        | Effect                                                                                 | Estimate | 95% CI            | P        |
| Intercept                                                                              | 0.57     | 0.50, 0.64       | <0.0001* | Intercept                                                                              | 0.27     | 0.19, 0.34        | <0.0001* |
| Time since dose 2 (in days)                                                            | 0.00058  | -0.00053, 0.0017 | 0.30     | Time since dose 2 (in days)                                                            | 0.00034  | -0.00036, 0.0010  | 0.34     |
| Linear spline (knot at day 30)                                                         | 0.00058  | -0.0025, 0.00020 | 0.01*    | Linear spline (knot at day 30)                                                         | -0.0018  | -0.0028, -0.00089 | 0.0002*  |
| Excessive alcohol                                                                      | -0.044   | -0.12, 0.030     | 0.24     | Excessive alcohol                                                                      | -0.0094  | -0.07956, 0.06084 | 0.79     |
| Time since dose 2 interaction                                                          | 0.0012   | -0.00039, 0.0028 | 0.14     | Time since dose 2 interaction                                                          | 0.0013   | 0.00033, 0.0023   | 0.001*   |
| Linear spline interaction                                                              | -0.0011  | -0.0029, 0.00077 | 0.25     | Linear spline interaction                                                              | -0.0016  | -0.0029, -0.00025 | 0.02*    |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                  | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                   | P        |
| Difference in intercepts                                                               | -0.044   |                  | 0.24     | Difference in intercepts                                                               | -0.0094  |                   | 0.79     |
| Difference in slope before knot                                                        | 0.0012   |                  | 0.14     | Difference in slope before knot                                                        | 0.0013   |                   | 0.01*    |
| Difference in slope after knot                                                         | 0.00010  |                  | 0.77     | Difference in slope after knot                                                         | -0.00027 |                   | 0.48     |

| Dose 3                                                                                 |           |                  |          |                                                                                        |          |                    |          |
|----------------------------------------------------------------------------------------|-----------|------------------|----------|----------------------------------------------------------------------------------------|----------|--------------------|----------|
| Effect                                                                                 | Estimate  | 95% CI           | P        | Effect                                                                                 | Estimate | 95% CI             | P        |
| Intercept                                                                              | 0.52      | 0.42, 0.62       | <0.0001* | Intercept                                                                              | 0.16     | 0.042, 0.28        | <0.0001* |
| Time since dose 3 (in days)                                                            | 0.00062   | -0.00036, 0.0016 | 0.21     | Time since dose 3 (in days)                                                            | 0.0014   | 0.00053, 0.0022    | 0.0018*  |
| Linear spline (knot at day 65)                                                         | -0.00055  | -0.0039, 0.0028  | 0.74     | Linear spline (knot at day 65)                                                         | -0.0026  | -0.0050, -0.00029  | 0.028*   |
| Excessive alcohol                                                                      | -0.011    | -0.11, 0.091     | 0.83     | Excessive alcohol                                                                      | -0.061   | -0.15, 0.032       | 0.20     |
| Time since dose 3 interaction                                                          | -0.000050 | -0.0014, 0.0013  | 0.94     | Time since dose 3 interaction                                                          | -0.00040 | -0.0016, 0.00076   | 0.50     |
| Linear spline interaction                                                              | -0.0016   | -0.0062, 0.0031  | 0.50     | Linear spline interaction                                                              | 0.00089  | -0.00242, 0.004193 | 0.59     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                  | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                    | P        |
| Difference in intercepts                                                               | -0.011    |                  | 0.83     | Difference in intercepts                                                               | -0.061   |                    | 0.20     |
| Difference in slope before knot                                                        | -0.00005  |                  | 0.94     | Difference in slope before knot                                                        | -0.0004  |                    | 0.50     |
| Difference in slope after knot                                                         | -0.0016   |                  | 0.38     | Difference in slope after knot                                                         | 0.00049  |                    | 0.72     |
| Dose 4                                                                                 |           |                  |          |                                                                                        |          |                    |          |
| Effect                                                                                 | Estimate  | 95% CI           | P        | Effect                                                                                 | Estimate | 95% CI             | P        |
| Intercept                                                                              | 0.53      | 0.41, 0.63       | <0.0001* | Intercept                                                                              | 0.20     | 0.067, 0.32        | <0.0001* |
| Time since dose 4 (in days)                                                            | -0.00024  | -0.0035, 0.0030  | 0.87     | Time since dose 4 (in days)                                                            | 0.00017  | -0.0015, 0.0018    | 0.84     |
| Linear spline (knot at day 15)                                                         | 0.00055   | -0.0034, 0.0045  | 0.76     | Linear spline (knot at day 15)                                                         | 0.000094 | -0.0021, 0.0023    | 0.93     |
| Excessive alcohol                                                                      | -0.016    | -0.14, 0.10      | 0.79     | Excessive alcohol                                                                      | -0.0048  | -0.11, 0.10        | 0.93     |
| Time since dose 4 interaction                                                          | 0.00016   | -0.0043, 0.0046  | 0.94     | Time since dose 4 interaction                                                          | -0.00035 | -0.0029, 0.0021    | 0.78     |
| Linear spline interaction                                                              | -0.00013  | -0.0054, 0.0052  | 0.96     | Linear spline interaction                                                              | 0.00059  | -0.0026, 0.0038    | 0.72     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                  | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                    | P        |
| Difference in intercepts                                                               | -0.016    |                  | 0.79     | Difference in intercepts                                                               | -0.0048  |                    | 0.93     |
| Difference in slope before knot                                                        | 0.00016   |                  | 0.94     | Difference in slope before knot                                                        | -0.00035 |                    | 0.78     |
| Difference in slope after knot                                                         | 0.000032  |                  | 0.96     | Difference in slope after knot                                                         | 0.00023  |                    | 0.73     |

Intercept estimates were converted back from log10(IU/mL) to IU/mL to better demonstrate baseline unadjusted antibody levels.

\* Indicates a statistically significant P value.

Supplemental Table 6: Univariable multilevel linear regression models of anti-S and anti-R IgM titers in participants with no previous infection prior to vaccination. Interaction terms between time and excessive alcohol consumption are included. Excessive alcohol use was defined as  $\geq 3$  drinks/week and was compared to non-high level alcohol defined as 0-2.9 drinks/week (the referent category).

| Anti-S IgM (log µg/mL)                                                                 |           |                   |          | Anti-R IgM (log µg/mL)                                                                 |           |                   |          |
|----------------------------------------------------------------------------------------|-----------|-------------------|----------|----------------------------------------------------------------------------------------|-----------|-------------------|----------|
| Dose 1                                                                                 |           |                   |          |                                                                                        |           |                   |          |
|                                                                                        | Estimate  | 95% CI            | P        | Effect                                                                                 | Estimate  | 95% CI            | P        |
| Dose 1 (in days)                                                                       | -0.36     | -0.47, -0.26      | <0.0001* | Intercept                                                                              | -0.54     | -0.64, -0.46      | <0.0001* |
|                                                                                        | 0.0047    | 0.0034, 0.0060    | <0.0001* | Time since dose 1 (in days)                                                            | 0.0028    | 0.0020, 0.0036    | <0.0001* |
| Time (knot at day 30)                                                                  | -0.0055   | -0.0083, -0.0027  | <0.0001* | Linear spline (knot at day 30)                                                         | -0.0039   | -0.0055, -0.0023  | <0.0001* |
| Excessive alcohol                                                                      | 0.014     | -0.036, 0.065     | 0.57     | Excessive alcohol                                                                      | 0.019     | -0.015, 0.052     | 0.27     |
| Time since dose 1 interaction                                                          | 0.0015    | -0.00064, 0.0036  | 0.17     | Time since dose 1 interaction                                                          | 0.0015    | 0.00018, 0.0029   | 0.03*    |
| Linear spline interaction                                                              | -0.0041   | -0.0086, 0.00031  | 0.07     | Linear spline interaction                                                              | -0.0027   | -0.0053, -0.00019 | 0.04*    |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                   | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                   | P        |
| Difference in intercepts                                                               | 0.014     |                   | 0.57     | Difference in intercepts                                                               | 0.019     |                   | 0.27     |
| Difference in slope before knot                                                        | 0.0015    |                   | 0.17     | Difference in slope before knot                                                        | 0.0015    |                   | 0.03*    |
| Difference in slope after knot                                                         | -0.0027   |                   | 0.09     | Difference in slope after knot                                                         | -0.0012   |                   | 0.15     |
| Dose 2                                                                                 |           |                   |          |                                                                                        |           |                   |          |
|                                                                                        | Estimate  | 95% CI            | P        | Effect                                                                                 | Estimate  | 95% CI            | P        |
| Dose 2 (in days)                                                                       | -0.042    | -0.10, 0.014      | <0.0001* | Intercept                                                                              | -0.33     | -0.40, -0.26      | <0.0001* |
|                                                                                        | 0.00015   | -0.00053, 0.00083 | 0.67     | Time since dose 2 (in days)                                                            | 0.000061  | -0.00058, 0.00071 | 0.85     |
| Time (knot at day 17)                                                                  | -0.0010   | -0.0018, -0.00024 | 0.01*    | Linear spline (knot at day 17)                                                         | -0.00046  | -0.0012, 0.00030  | 0.23     |
| Excessive alcohol                                                                      | -0.0067   | -0.051, 0.038     | 0.77     | Excessive alcohol                                                                      | 0.0066    | -0.027, 0.040     | 0.70     |
| Time since dose 2 interaction                                                          | 0.000017  | -0.0011, 0.0011   | 0.98     | Time since dose 2 interaction                                                          | -0.00042  | -0.0015, 0.00061  | 0.42     |
| Linear spline interaction                                                              | -0.00001  | -0.0013, 0.0013   | 0.98     | Linear spline interaction                                                              | 0.00035   | -0.00088, 0.0016  | 0.58     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                   | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                   | P        |
| Difference in intercepts                                                               | -0.0067   |                   | 0.77     | Difference in intercepts                                                               | 0.0066    |                   | 0.70     |
| Difference in slope before knot                                                        | 0.000017  |                   | 0.98     | Difference in slope before knot                                                        | -0.00042  |                   | 0.42     |
| Difference in slope after knot                                                         | 0.0000029 |                   | 0.99     | Difference in slope after knot                                                         | -0.000080 |                   | 0.61     |

| Dose 3                                                                                 |          |                   |          |                                                                                        |            |                  |          |
|----------------------------------------------------------------------------------------|----------|-------------------|----------|----------------------------------------------------------------------------------------|------------|------------------|----------|
| Effect                                                                                 | Estimate | 95% CI            | P        | Effect                                                                                 | Estimate   | 95% CI           | P        |
| Intercept                                                                              | -0.31    | -0.45, -0.20      | <0.0001* | Intercept                                                                              | -0.42      | -0.54, -0.32     | <0.0001* |
| Time since dose 3 (in days)                                                            | 0.0012   | 0.00076, 0.0016   | <0.0001* | Time since dose 3 (in days)                                                            | 0.00039    | -0.00025, 0.0010 | 0.23     |
| Linear spline (knot at day 115)                                                        | -0.0016  | -0.0025, -0.00060 | 0.002    | Linear spline (knot at day 60)                                                         | -0.00087   | -0.0019, 0.00018 | 0.10     |
| Excessive alcohol                                                                      | -0.020   | -0.086, 0.047     | 0.56     | Excessive alcohol                                                                      | -0.046     | -0.096, 0.0041   | 0.07     |
| Time since dose 3 interaction                                                          | -0.00026 | -0.00096, 0.00045 | 0.47     | Time since dose 3 interaction                                                          | -0.0000028 | -0.0010, 0.0010  | 1.00     |
| Linear spline interaction                                                              | 0.00036  | -0.0012, 0.0019   | 0.64     | Linear spline interaction                                                              | 0.00083    | -0.00075, 0.0024 | 0.30     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                   | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate   |                  | P        |
| Difference in intercepts                                                               | -0.020   |                   | 0.56     | Difference in intercepts                                                               | -0.046     |                  | 0.072    |
| Difference in slope before knot                                                        | -0.00026 |                   | 0.47     | Difference in slope before knot                                                        | -0.0000028 |                  | 1.00     |
| Difference in slope after knot                                                         | 0.00010  |                   | 0.86     | Difference in slope after knot                                                         | 0.00083    |                  | 0.04*    |
| Dose 4                                                                                 |          |                   |          |                                                                                        |            |                  |          |
| Effect                                                                                 | Estimate | 95% CI            | P        | Insufficient sample size                                                               |            |                  |          |
| Intercept                                                                              | -0.15    | -0.49, 0.075      | <0.0001* |                                                                                        |            |                  |          |
| Time since dose 4 (in days)                                                            | 0.0016   | 0.00028, 0.0029   | 0.019*   |                                                                                        |            |                  |          |
| Linear spline (knot at day 75)                                                         | -0.0025  | -0.0046, -0.00051 | 0.015*   |                                                                                        |            |                  |          |
| Excessive alcohol                                                                      | 0.0043   | -0.18, 0.19       | 0.96     |                                                                                        |            |                  |          |
| Time since dose 4 interaction                                                          | -0.0013  | -0.0036, 0.0011   | 0.29     |                                                                                        |            |                  |          |
| Linear spline interaction                                                              | 0.0021   | -0.0020, 0.0063   | 0.31     |                                                                                        |            |                  |          |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                   | P        |                                                                                        |            |                  |          |
| Difference in intercepts                                                               | 0.0043   |                   | 0.96     |                                                                                        |            |                  |          |
| Difference in slope before knot                                                        | -0.0013  |                   | 0.29     |                                                                                        |            |                  |          |
| Difference in slope after knot                                                         | 0.00089  |                   | 0.48     |                                                                                        |            |                  |          |

Intercept estimates were converted back from log10(IU/mL) to IU/mL to better demonstrate baseline unadjusted antibody levels.

\* Indicates a statistically significant P value.

Supplemental Table 7: Univariable multilevel linear regression models of anti-S and anti-R IgM titers in participants with previous infection prior to vaccination. Interaction terms between time and excessive alcohol consumption are included. Excessive alcohol use was defined as  $\geq 3$  drinks/week and was compared to non-high level alcohol defined as 0-2.9 drinks/week (the referent category).

| Anti-S IgM (log µg/mL)                                                                 |           |                   |          | Anti-R IgM (log µg/mL)                                                                 |           |                   |          |
|----------------------------------------------------------------------------------------|-----------|-------------------|----------|----------------------------------------------------------------------------------------|-----------|-------------------|----------|
| Dose 1                                                                                 |           |                   |          |                                                                                        |           |                   |          |
| Effect                                                                                 | Estimate  | 95% CI            | P        | Effect                                                                                 | Estimate  | 95% CI            | P        |
| Intercept                                                                              | 0.0057    | -0.094, 0.095     | <0.0001* | Intercept                                                                              | -0.11     | -0.20, -0.024     | <0.0001* |
| Time since dose 1 (in days)                                                            | 0.0036    | 0.0020, 0.0051    | <0.0001* | Time since dose 1 (in days)                                                            | 0.0031    | 0.0020, 0.0041    | <0.0001* |
| Linear spline (knot at day 28)                                                         | -0.0057   | -0.0092, -0.0022  | 0.002*   | Linear spline (knot at day 17)                                                         | -0.0047   | -0.0066, -0.0028  | <0.0001* |
| Excessive alcohol                                                                      | 0.015     | -0.054, 0.083     | 0.67     | Excessive alcohol                                                                      | 0.0092    | -0.046, 0.064     | 0.74     |
| Time since dose 1 interaction                                                          | -0.00027  | -0.0025, 0.0020   | 0.81     | Time since dose 1 interaction                                                          | -0.00069  | -0.0021, 0.00070  | 0.33     |
| Linear spline interaction                                                              | -0.00010  | -0.0051, 0.0049   | 0.97     | Linear spline interaction                                                              | 0.00039   | -0.0023, 0.0030   | 0.77     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                   | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                   | P        |
| Difference in intercepts                                                               | 0.015     |                   | 0.67     | Difference in intercepts                                                               | 0.0092    |                   | 0.74     |
| Difference in slope before knot                                                        | -0.00027  |                   | 0.81     | Difference in slope before knot                                                        | -0.00069  |                   | 0.33     |
| Difference in slope after knot                                                         | -0.00037  |                   | 0.83     | Difference in slope after knot                                                         | -0.00031  |                   | 0.73     |
| Dose 2                                                                                 |           |                   |          |                                                                                        |           |                   |          |
| Effect                                                                                 | Estimate  | 95% CI            | P        | Effect                                                                                 | Estimate  | 95% CI            | P        |
| Intercept                                                                              | 0.12      | 0.025, 0.20       | <0.0001* | Intercept                                                                              | -0.082    | -0.17, 0.0011     | <0.0001* |
| Time since dose 2 (in days)                                                            | -0.00040  | -0.0010, 0.00022  | 0.20     | Time since dose 2 (in days)                                                            | -0.00080  | -0.0014, -0.00023 | 0.006*   |
| Linear spline (knot at day 40)                                                         | -0.000050 | -0.00094, 0.00085 | 0.92     | Linear spline (knot at day 45)                                                         | 0.00078   | -0.00010, 0.0017  | 0.08     |
| Excessive alcohol                                                                      | -0.022    | -0.091, 0.047     | 0.54     | Excessive alcohol                                                                      | -0.038    | -0.094, 0.019     | 0.19     |
| Time since dose 2 interaction                                                          | -0.00032  | -0.0012, 0.00056  | 0.47     | Time since dose 2 interaction                                                          | -0.000090 | -0.00089, 0.00071 | 0.82     |
| Linear spline interaction                                                              | 0.00034   | -0.00090, 0.0016  | 0.59     | Linear spline interaction                                                              | -0.00036  | -0.0016, 0.00088  | 0.57     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                   | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate  |                   | P        |
| Difference in intercepts                                                               | -0.022    |                   | 0.54     | Difference in intercepts                                                               | -0.038    |                   | 0.19     |
| Difference in slope before knot                                                        | -0.00032  |                   | 0.47     | Difference in slope before knot                                                        | -0.00009  |                   | 0.82     |
| Difference in slope after knot                                                         | 0.000018  |                   | 0.95     | Difference in slope after knot                                                         | -0.00045  |                   | 0.21     |



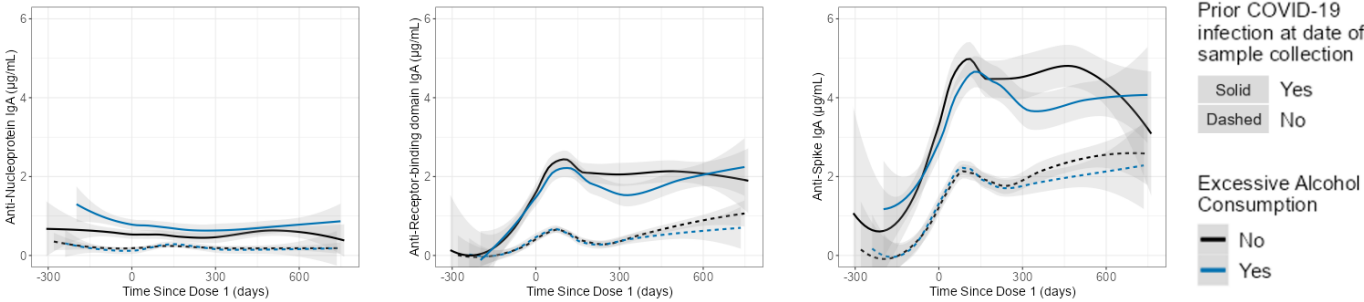
| Dose 3                                                                                 |          |                    |          |                                                                                        |          |                   |          |
|----------------------------------------------------------------------------------------|----------|--------------------|----------|----------------------------------------------------------------------------------------|----------|-------------------|----------|
| Effect                                                                                 | Estimate | 95% CI             | P        | Effect                                                                                 | Estimate | 95% CI            | P        |
| Intercept                                                                              | 0.075    | -0.029, 0.17       | <0.0001* | Intercept                                                                              | -0.17    | -0.29, -0.058     | <0.0001* |
| Time since dose 3 (in days)                                                            | 0.0012   | 0.00062, 0.0018    | 0.0001*  | Time since dose 3 (in days)                                                            | -0.00014 | -0.00088, 0.00060 | 0.71     |
| Linear spline (knot at day 65)                                                         | -0.0016  | -0.0023, -0.00083  | <0.0001* | Linear spline (knot at day 65)                                                         | -0.0001  | -0.0010, 0.00081  | 0.83     |
| Excessive alcohol                                                                      | -0.076   | -0.15, -0.0018     | 0.045*   | Excessive alcohol                                                                      | -0.11    | -0.17, -0.043     | 0.001*   |
| Time since dose 3 interaction                                                          | -0.00085 | -0.0017, -0.000030 | 0.04*    | Time since dose 3 interaction                                                          | 0.0001   | -0.00090, 0.0011  | 0.84     |
| Linear spline interaction                                                              | 0.0012   | 0.00016, 0.0022    | 0.03*    | Linear spline interaction                                                              | 0.00041  | -0.00084, 0.0017  | 0.52     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                    | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                   | P        |
| Difference in intercepts                                                               | -0.076   |                    | 0.045*   | Difference in intercepts                                                               | -0.11    |                   | 0.001*   |
| Difference in slope before knot                                                        | -0.00085 |                    | 0.04*    | Difference in slope before knot                                                        | 0.00010  |                   | 0.84*    |
| Difference in slope after knot                                                         | 0.00034  |                    | 0.045*   | Difference in slope after knot                                                         | 0.00051  |                   | 0.02*    |
| Dose 4                                                                                 |          |                    |          |                                                                                        |          |                   |          |
| Effect                                                                                 | Estimate | 95% CI             | P        | Effect                                                                                 | Estimate | 95% CI            | P        |
| Intercept                                                                              | 0.21     | 0.022, 0.37        | <0.0001* | Intercept                                                                              | -0.21    | -0.54, 0.015      | <0.0001* |
| Time since dose 4 (in days)                                                            | 0.00019  | -0.00061, 0.00098  | 0.64     | Time since dose 4 (in days)                                                            | 0.0023   | -0.00070, 0.0052  | 0.13     |
| Linear spline (knot at day 75)                                                         | -0.00032 | -0.0017, 0.0011    | 0.65     | Linear spline (knot at day 75)                                                         | -0.0034  | -0.0075, 0.00068  | 0.10     |
| Excessive alcohol                                                                      | -0.15    | -0.30, 0.0037      | 0.06     | Excessive alcohol                                                                      | -0.042   | -0.19, 0.10       | 0.57     |
| Time since dose 4 interaction                                                          | 0.00023  | -0.00088, 0.0013   | 0.68     | Time since dose 4 interaction                                                          | -0.0021  | -0.0064, 0.0022   | 0.34     |
| Linear spline interaction                                                              | -0.00085 | -0.0028, 0.0011    | 0.39     | Linear spline interaction                                                              | 0.0031   | -0.0029, 0.0090   | 0.31     |
| Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                    | P        | Comparisons for excessive alcohol before (titer increase) and after (titer decay) knot | Estimate |                   | P        |
| Difference in intercepts                                                               | -0.15    |                    | 0.06     | Difference in intercepts                                                               | -0.042   |                   | 0.57     |
| Difference in slope before knot                                                        | 0.00023  |                    | 0.68     | Difference in slope before knot                                                        | -0.0021  |                   | 0.34     |
| Difference in slope after knot                                                         | -0.00061 |                    | 0.31     | Difference in slope after knot                                                         | 0.0010   |                   | 0.42     |

Intercept estimates were converted back from log<sub>10</sub>(IU/mL) to IU/mL to better demonstrate baseline unadjusted antibody levels.

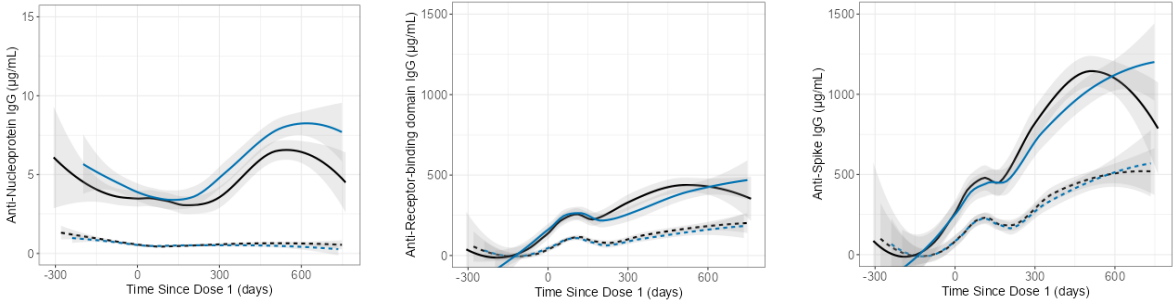
\* Indicates a statistically significant P value.

Figures

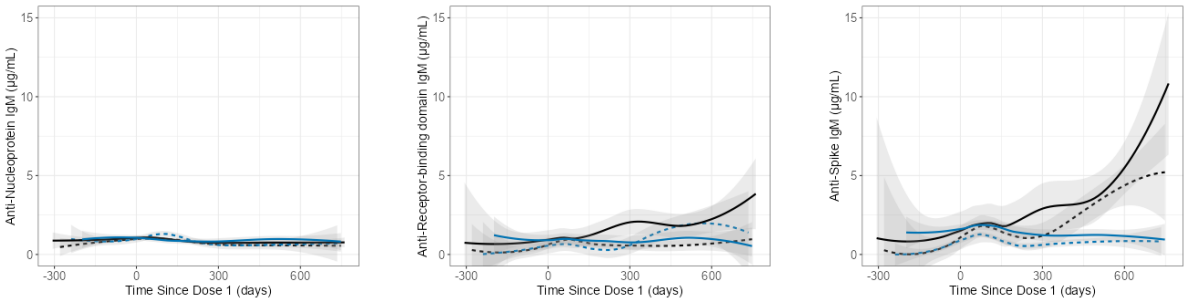
(A)



(B)



(C)

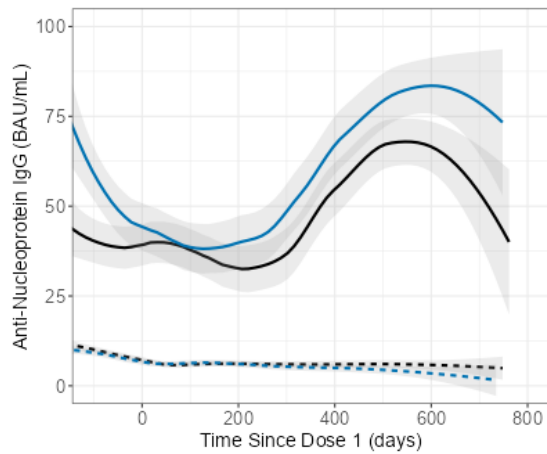


(D)

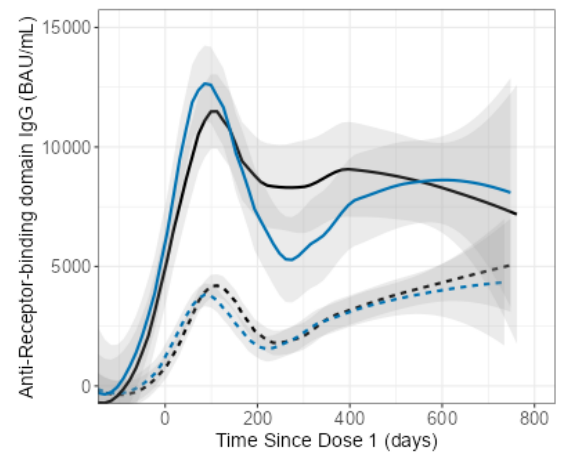
| Median (IQR) Time Since Dose 1 in Days |                      |                      |                      |                      |                      |
|----------------------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Dose 2 (n=882)                         | Dose 3 (n=658)       | Dose 4 (n=207)       | Dose 5 (n=80)        | Dose 6 (n=15)        | Dose 7 (n=4)         |
| 58.0 (35.0, 76.0)                      | 255.0 (235.0, 286.0) | 463.0 (380.0, 560.0) | 582.5 (536.5, 644.0) | 713.0 (580.0, 734.0) | 727.5 (679.5, 737.5) |

Figure 1: Loess smooth plots of (A) IgA titers ( $\mu\text{g/mL}$ ), (B) IgG titers ( $\mu\text{g/mL}$ ), and (C) IgM titers ( $\mu\text{g/mL}$ ) against the SARS-CoV-2 nucleocapsid (N), receptor-binding domain (R), and spike (S) proteins over time according to COVID-19 history at the date of sample collection and baseline alcohol consumption. Separate lines are presented according to COVID-19 history and excessive alcohol consumption groupings. Solid lines are used for those with a prior infection at sample collection and dashed lines indicate no prior infection. Blue lines represent data for participants with excessive alcohol consumption at baseline and black lines represent no excessive alcohol consumption. Excessive alcohol consumption was defined as 3 or more standard drinks/week. Panel (D) shows median time between dose 1 and subsequent doses. These results are descriptive in nature due to variability in the number and timing of vaccination doses and SARS-CoV-2 infections during follow-up and show the crude antibody titers over time in the cohort.

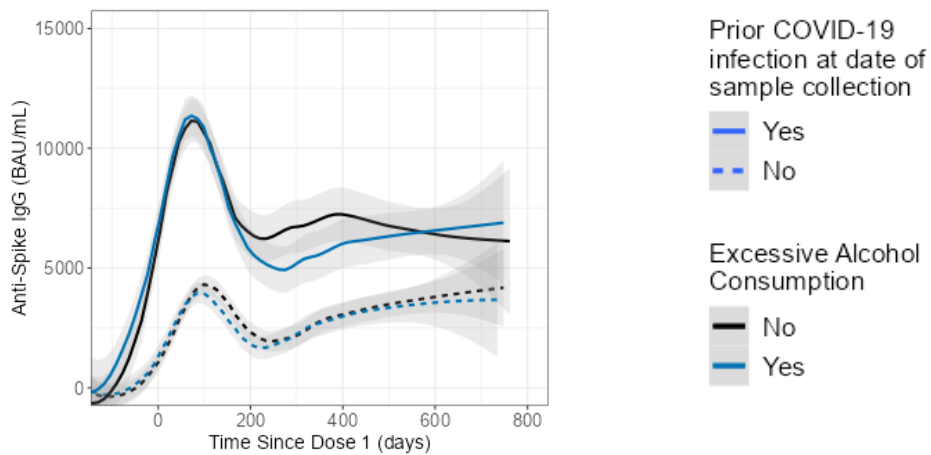
(A)



(B)



(C)



Supplemental Figure S1: Loess smooth plots of (A) anti-SARS-CoV-2 nucleocapsid (N) IgG titers (BAU/mL), (B) anti-SARS-CoV-2 receptor-binding domain (RBD) IgG titers (BAU/mL), and (C) anti-SARS-CoV-2 spike (S) IgG titers (BAU/mL) over time according to COVID-19 history at the date of sample collection and baseline alcohol consumption. Excessive alcohol consumption was defined as 3 or more standard drinks/week.

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# Chapter 6 – Selective Serotonin Reuptake Inhibitors Do Not Impact SARS-CoV-2 Vaccine Humoral Immunogenicity nor the Odds of Post-COVID-19 Condition

## **Preface**

The completion of the following study fulfills the final component of research objective III. The final exposure of interest specified in objective III—SSRI medication use—was assessed. The impact of SSRI use on the slope of antibody production and decay following three COVID-19 vaccine doses was analyzed by flexible, multilevel modeling. Objective IV was also completed and the odds of developing PCC (as defined by the WHO clinical case definition ICD-11 code RAO2) following SARS-CoV-2 infection according to SSRI use was investigated by multivariable regression analysis.

**Citation:** Keeshan A, Saeed S, Langlois M-A, Cooper C.L. Selective Serotonin Reuptake Inhibitors Do Not Impact SARS-CoV-2 Vaccine Humoral Immunogenicity nor the Odds of Post-COVID-19 Condition.

**Status:** Not yet submitted.

**Author Contributions:** A.K., S.S., M-A.L., C.L.C. conceptualized and designed the study and reviewed the manuscript. A.K., C.L.C. jointly prepared the manuscript. A.K. conducted the data analysis. M-A.L. completed the laboratory testing and related data analysis. C.L.C. completed the study recruitment.

**Ethics Approval:** Informed consent was obtained from all study participants and the study was reviewed and approved by The Ottawa Health Science Network Research Ethics Board (2020-0481).

**Declaration of Competing Interest:** The authors declare no competing interests.

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**Relevant Appendices:** N/A.

## Abstract

Immunomodulatory effects of selective serotonin reuptake inhibitors (SSRIs), including anti-inflammatory and lymphocyte suppression, have been reported by *in vitro* and animal model studies. Preliminary observational research indicates that SSRI use is associated with less severe COVID-19, a lower risk of developing post-COVID-19 condition (PCC) after SARS-CoV-2 infection, and improvements in PCC symptoms, but there is a paucity of information. We utilized the prospective longitudinal Stop the Spread Ottawa cohort to address these gaps. The influence of SSRI medication use on IgG, IgA and IgM anti-S and anti-RBD log<sub>10</sub>-adjusted antibody titers after three COVID-19 vaccine doses was analyzed through multilevel multivariable linear regression. Models incorporated penalized cubic smoothing splines and random effects. Multivariable logistic regression was conducted to assess the risk of PCC according to SSRI use. PCC cases were identified in accordance with the WHO clinical case definition (ICD-11 code RAO2). 929 participants were included. SSRI use was associated with female sex and obesity. SSRI use did not predict antibody titers and the rate of titer production and decay was similar for SSRI users and non-SSRI users. The odds of PCC was similar for SSRI users, adults taking non-SSRI antidepressants or anxiolytics, and those not on medication to treat anxiety or depression. In conclusion, SSRI use does not influence the humoral immune response to COVID-19 vaccination, nor does SSRI use impact the risk of PCC following SARS-CoV-2 infection. To our knowledge, this is the first analysis investigating the impact on SSRI medication use on the humoral immune response to COVID-19 vaccination.

## Introduction

The COVID-19 pandemic has caused the deaths of millions of people around the world to date.<sup>1</sup> Newly developed COVID-19 vaccines helped to curb the spread of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and reduced the severity of SARS-CoV-2 infection.<sup>2</sup> However, COVID-19 remains a serious threat to public health as newer variants emerge and breakthrough infections and/or repeated infections continue to add to the high burden of morbidity and post-acute sequelae resulting from the COVID-19 pandemic.<sup>3</sup> Post-COVID-19 condition (PCC) is a complex chronic disease that develops in some individuals following acute SARS-CoV-2 infection.<sup>4</sup> There are many symptoms of PCC involving multiple organ systems and together leading to poor health and poor quality of life, disability, economic hardship and social isolation.<sup>4,5</sup> Examples include fatigue, post-exertional malaise, coughing, shortness of breath, joint pain, poor sleep, brain fog and cognitive dysfunction.<sup>4,5</sup> It is estimated that over 100 million individuals globally suffer from PCC and yet the current understanding regarding the etiology of PCC is very limited and there is a lack of effective prevention and treatment approaches.<sup>5</sup>

Preliminary published evidence indicates that selective serotonin reuptake inhibitors (SSRIs) may lower the severity of COVID-19 illness and reduce the risk of developing PCC. Early in the COVID-19 pandemic, SSRIs were put forward as a potential treatment for SARS-CoV-2 infection based on previously documented antiviral effects against Ebola, HIV and Coxsackie B4 viruses.<sup>6</sup> SSRIs have demonstrated immunosuppressive effects in murine and rats models of autoimmune diseases such as rheumatoid arthritis and inflammatory bowel disease.<sup>7,8</sup> Multiple mechanisms of anti-inflammatory effects have been proposed, including decreased lymphocyte proliferation, lowered proinflammatory cytokines production (e.g., interleukin [IL]-1 $\beta$ , IL-6, IL-2, interferon [IFN]- $\gamma$ , tumor necrosis factor alpha [TNF- $\alpha$ ]), and activation of the sigma-1 receptor (S1R) pathway thereby downregulating the inflammatory response mediated by the

endoplasmic reticulum.<sup>7–11</sup> It was hypothesized that through these immunomodulatory effects SSRIs may reduce the risk of life-threatening hyperinflammation (e.g., cytokine storm or sepsis) during SARS-CoV-2 infection.<sup>7,8</sup> A scoping review published in 2022 identified 14 clinical trials investigating SSRIs, selective norepinephrine reuptake inhibitors (SNRIs) or trazodone for the treatment of SARS-CoV-2 infection, and 10 reported a beneficial impact on severity of COVID-19 illness.<sup>12</sup> For example, two randomized placebo-controlled trials found that SSRI use among COVID-19 patients lowered morbidity and mortality. Observational research involving large cohorts (n=83,584 and n=40,459) and propensity score matching also reported an association between SSRI use and reduced severity of COVID-19.<sup>13,14</sup> Of note, an opposing retrospective nationwide study utilizing Danish registries reported a higher risk of mortality associated with SSRI use.<sup>15</sup> Despite evidence of immunomodulatory effects and studies demonstrating both positive and detrimental impacts on clinical outcomes of SARS-CoV-2 infection, no work has been done to determine the impact on SSRI medication use on the humoral immune response to COVID-19 vaccination. This represents a significant gap in the scientific literature.

Similarly, there are reports indicating that SSRI use may be associated with lower incidence of PCC following SARS-CoV-2 infection. It has been postulated that SSRIs reduce the risk of PCC through immunomodulatory and neuropsychiatric effects, for example by balancing neurotransmitter systems and the kynurenine pathway which are dysregulated in PCC patients.<sup>5,16</sup> However, limited work has been done to investigate SSRI medication use and its impact on PCC incidence. Two retrospective studies reported a lower risk of presumed or diagnosed PCC (defined by ICD-10 code U09.9) associated with SSRI use.<sup>16–18</sup> Improvements in PCC symptoms (such as brain fog and chronic fatigues and dysautonomia) were observed following treatment with SSRIs in two other studies; however, one of these analyses lacked a control group comparison.<sup>5,19</sup> In contrast, other analyses identified no influence of SSRIs on the cumulative incidence of PCC.<sup>20,21</sup> For example, fluvoxamine demonstrated no beneficial impact

on PCC prevention in a post hoc modified intention-to-treat placebo clinical trial conducted by Bramante et al.<sup>20</sup>

To our knowledge no study has been conducted to date concerning the influence of SSRIs on COVID-19 vaccine humoral immunogenicity. Many researchers have called attention to the dearth of knowledge regarding SSRIs and the risk of PCC and stressed the need for high-quality randomized and observational studies.<sup>16,22</sup> The Stop the Spread Ottawa (SSO) cohort was utilized to address these knowledge gaps. SSO is a heterogeneous cohort of adults in an urban-setting who were followed prospectively throughout and after the SARS-CoV-2 pandemic. Extensive collection of demographic, clinical, and serological data facilitated a thorough assessment of SSRI medication use in relation to vaccine humoral immunogenicity and the risk of PCC. The following research objectives were met: i) assess the influence of SSRI medication use on the humoral immune response to vaccination against COVID-19; ii) determine if SSRI medication use is associated with the development of post-COVID conditions (PCC). We hypothesized that SSRI medication use predicts higher vaccine-elicited antibody titers and improved antibody durability compared to no SSRI use and that SSRI use is associated with a lower risk of PCC.

## **Methods**

### *Study Design*

The Stop the Spread Ottawa (SSO) cohort was created in the fall of 2020 and followed participants for a minimum of 10 months and up to a maximum of 46 months. Adults at an increased risk of SARS-CoV-2 infection due to occupation or pre-existing health conditions were targeted during recruitment, as well as adults with documented history of COVID-19 by quantitative reverse transcription polymerases chain reaction test (PCR) or by serology testing.<sup>23</sup> Serial blood draws and detailed questionnaires were collected. The data collection protocol was published previously.<sup>23,24</sup>

Eligible participants with available questionnaire data and vaccination information who provided at least one blood draw were included. Participants who reported being pregnant at baseline were excluded and data collected after imputed pregnancy dates occurring during follow-up was not utilized. Blood samples provided prior to vaccine rollout in the National Capital Region by participants with no reported vaccinations were included as pre-vaccination baseline data. SSRI medication use was determined at baseline and during follow-up. Participants who were taking an SSRI at baseline or initiated SSRI use during follow-up prior to their first COVID-19 vaccine dose were allocated to the SSRI group; otherwise they were allocated to the non-SSRI group. If a participant began taking an SSRI after initial COVID-19 vaccination, they were allocated to the non-SSRI group and blood samples collected following SSRI initiation were not utilised.

#### *SARS-CoV-2 Vaccinations and Infections*

IgG, IgA and IgM antibodies targeting the Wuhan SARS-CoV-2 spike (S), receptor-binding domain (RBD) and nucleocapsid (N) antigens were measured using a high-throughput direct chemiluminescent ELISA assay, the details of which were described previously.<sup>25</sup> IgG titer units (international binding antibody units [BAU] per mL) were standardized to the World Health Organization (WHO) international reference. IgA and IgM titers were expressed in micrograms (µg) per mL. Medical records were used to verify self-reported COVID-19 vaccination dates when available. Incident SARS-CoV-2 infections that occurred outside of positive PCR or rapid antigen test (RAT) (e.g., asymptomatic infections) were captured by anti-nucleocapsid (anti-N) IgG seropositivity. To account for the time required to generate vaccine-elicited immunity, participants were classified as having natural immunity prior to vaccination if an infection occurred up to 14 days after the date of vaccination.<sup>26</sup>

#### *Statistical Analysis*



Antibody titers were linearized through log<sub>10</sub>-transformation after shifting each value up by one unit to eliminate the production of undefined values from zeros. Multivariable linear regression analysis was conducted to assess the influence of SSRI medication use on IgG, IgA and IgM anti-S and anti-RBD antibody production and durability after three COVID-19 vaccine doses. Data was stratified according to dose number and immunity type (vaccine-only or hybrid immunity). Non-linear trends in log<sub>10</sub>-transformed antibody titers over time were addressed with flexible modeling and penalized cubic smoothing splines were applied to the variable for time since vaccination. Models with separate smooths for each SSRI group were generated to establish if effect modification by time on the relationship between SSRI use and humoral vaccine immune response was present and if the slope varied according to SSRI use. Random effects were included for individual intercepts to adjust for autocorrelation between repeated measures. Random effects for individual slopes were also included depending on the sample size and goodness of fit statistics. Robust linear regression utilizing the Student's t-distribution was employed in the case of outliers. Model building was steered by the Akaike information criterion (AIC) statistic and final models were estimated by restricted maximum likelihood (REML). Due to the large number of models generated for different strata, antibody isotypes and SARS-CoV-2 antigens, we applied a cautious approach when interpreting study findings to mitigate the increased risk of type I error from multiplicity.<sup>27</sup> Statistical significance of linear regression model estimates was determined using an alpha level of 0.025.

Odds ratios were calculated to measure the association of i. SSRIs, ii. non-SSRI medications used for the treatment of depression or anxiety, iii. both SSRIs and non-SSRI antidepressants or anxiolytics, with the development of PCC following SARS-CoV-2 infection. PCC cases were identified according to the WHO clinical case definition and the corresponding ICD-11 code RA02. Diagnosis criteria include: a) confirmed or presumptive SARS CoV-2 infection; b) symptoms lasting a minimum of 2 months and affecting daily functioning; c) there is no alternative diagnosis.<sup>28,29</sup> Adjusted odds ratios were obtained through multivariable logistic

regression modelling. Goodness of fit diagnostics (plots of standardized residuals against fitted values), goodness of fit statistics (AIC) and collinearity diagnostics (Variance Inflation Factor, tolerance, condition index) were examined during model building. We selected covariates based on a priori evidence in the literature of confounding on the relationship between PCC and SSRI use and ensured that there were at least 10 outcome events per parameter as per standard practice.<sup>30</sup> Penalized logistic regression was implemented using Firth's correction to adjust for quasi-complete separation resulting from the near perfect determination of PCC development according to vaccination status. Confidence intervals were generated using profile likelihood rather than the Wald's test, which is biased in the case of Frith's correction.<sup>31</sup>

Statistical tests and logistic regression analyses were completed with Statistical Analysis System (SAS) version 9.4. RStudio version 2023.06.1 was used to generate linear regression models (package mgcv & ggplot2).

## **Results**

### *Participant Characteristics*

The eligibility criteria were met by 929 participants. Individuals were excluded if they provided no blood samples (n=46), did not completed a baseline questionnaire (n=8), had no reported vaccine doses and provided no blood samples prior to vaccine rollout (n=35) or were pregnant at baseline or at time of vaccination (n=14). The following SSRI medications were identified in the cohort: citalopram; escitalopram; fluoxetine; fluvoxamine; paroxetine; sertraline; unspecified SSRI. Other antidepressants and anxiolytics included serotonin-norepinephrine reuptake inhibitors (SNRIs; desvenlafaxine, duloxetine, levomilnacipran, venlafaxine), norepinephrine-dopamine reuptake inhibitors (NDRIs; bupropion), serotonin antagonist and reuptake inhibitors (SARIs; trazodone), tricyclic antidepressants (amitriptyline, nortriptyline), serotonin modulators and stimulators (SMSs; vortioxetine), and an oxidase

inhibitor (tranylcypromine). One participant in the SSRI group did not provide any blood samples following SSRI initiation and was excluded.

We stratified participants according to their reported SSRI medication use (Table 1). Demographic and clinical characteristics of study groups were similar for nearly all factors assessed, except for sex and obesity. The proportion of males was higher in the non-SSRI group (35.2%) than in the SSRI group (22.0%) and females made up almost three quarters of SSRI users compared to less than two thirds of non-SSRI users ( $P=0.005$ ; Table 1). Participants taking an SSRI were more likely to be obese (35.4% vs 22.0%,  $P=0.009$ ) than those in the non-SSRI group. Other variables (age, ethnicity, immigration status, income, employment status, smoking history, alcohol consumption) and the prevalence of various comorbidities (allergy, heart disease, hypertension, diabetes, chronic lung disease, liver disease, hematological disorder and neurological impairment, dyslipidemia, rheumatological disorder, organ/bone marrow transplant, cancer) were comparable between SSRI and non-SSRI groups.

The number, timing, and types of COVID-19 vaccinations and the incidence of SARS-CoV-2 infections were similar for SSRI users and non-SSRI users (Table 2). The most common vaccine type across all doses was mRNA-based vaccine. The AstraZeneca vaccine was only received for dose one (12.6%) or dose two (1.2%). In a post-hoc analysis, we separated SARS-CoV-2 infection data before and after November 28th 2021, the start of the Omicron wave in Ontario, Canada, to adjust for differences in transmissibility between Omicron and previous SARS-CoV-2 variants. We found no difference in the proportion of breakthrough infections relative to vaccine dose number between SSRI users and non-SSRI participants during both periods.

#### *Humoral Response to SARS-CoV-2 Vaccination According to SSRI Use*

We did not detect a consistent influence by SSRI medication use on SARS-CoV-2 vaccine humoral immunogenicity up to three doses through multivariable multilevel linear regression

analysis (Supplemental Table 1 & 2). Among participants with no prior SARS-CoV-2 infection and those with hybrid immunity, there was no persistent pattern regarding the impact of SSRI use on the production and retention of all three antibody isotypes targeting the S and RBD SARS-CoV-2 antigens after adjusting for key confounding variables. In addition, there was no clear evidence of effect modification by time on the relationship between the SARS-CoV-2 antibody titers and SSRI medication. Generally, the rate of titer production and decay was similar for both SSRI groups and the slope did not vary according to SSRI use.

We investigated the impact of using different groupings concerning the classification of non-SSRI antidepressants and anxiolytics on the regression analysis results and generated additional models for anti-S IgG levels after dose two. In the first scenario, we combined participants taking medications that target serotonin levels in addition to other neurotransmitters with SSRI-users (SNRIs, SARIs, SMSs, tricyclic antidepressants, and oxidase inhibitors) and combined those taking NDRIs with the non-SSRIs group. In the second scenario, participants taking SNRIs, SARIs, SMSs, tricyclic antidepressants, and oxidase inhibitors were pooled in a third separate group and participants taking NDRIs were included in the non-SSRIs group. In both cases, neither SSRIs nor other medications that also reduce serotonin uptake predicted anti-S titers after dose two.

#### *Risk of Post-COVID-19 Condition Among SSRI Users and Non-SSRI Users*

One hundred and forty-seven participants with a history of COVID-19 at baseline or during follow-up developed PCC (32.9% of 447 participants; Table 2). We were unable to confirm PCC status in 22 participants who were missing dates needed to calculate the duration of PCC symptoms. The crude odds of PCC among SSRI users was comparable to the crude odds of PCC among participants not on antidepressants or anxiolytics (OR: 1.81; 95% CI: 0.91, 3.64). Similarly, the odds of PCC for participants taking a non-SSRI anxiolytic or antidepressant was

similar to the odds of PCC among participants not taking an antidepressant or anti-anxiety medication (OR: 2.00; 95% CI: 0.86, 4.66).

The same findings were obtained after adjusting for confounding for age, sex, obesity, smoking status, and vaccination status through multivariable logistic regression. No association between SSRI use (adjusted OR: 0.65; 95% CI: 0.07, 4.63) or non-SSRI medication use for depression or anxiety (adjusted OR: 2.53; 95% CI: 0.24, 14.37) and the development of PCC was observed (Table 3). Comparable odds of PCC was still observed whether SSRI and non-SSRI medications were combined (adjusted OR: 1.66; 95% CI: 0.47, 5.96) or SSRI use was compared to participants on a non-SSRI medication or not taking any medication to treat anxiety or depression (adjusted OR: 1.71; 95% CI: 0.34, 8.63; data not shown). These findings suggest that individuals on treatment for anxiety or depression are not at a higher or lower risk of PCC following SARS-CoV-2 infection compared to those not taking an SSRI or non-SSRI medication.

## **Discussion**

We observed no consistent influence by SSRI use on the humoral immune response to COVID-19 vaccination up to three doses by multivariable multilevel linear regression analysis. IgG, IgA, and IgM titers against the spike and receptor-binding domain antigens were not determined by SSRI use, indicating that COVID-19 vaccines stimulate a robust B cell response regardless of SSRI use. Despite evidence in the literature of immunosuppressive effects of SSRIs—such as the inhibition of lymphocyte proliferation and cytokine production in vitro and in animal models and reports of lower IL-2, IL-4, IL-6, IL-10, IL-17a and IFN- $\gamma$  plasma levels in patients with depression on sertraline treatment—our findings indicate that these effects do not extend to B cell activity following COVID-19 vaccination.<sup>7,32</sup> To our knowledge, this is the first analysis on the influence of SSRI medication use on COVID-19 vaccine humoral immunogenicity.

SSRI use was not associated with the risk of breakthrough SARS-CoV-2 infections or the odds of developing PCC after SARS-CoV-2 infection in both crude and multivariable analyses. This finding is in contrast to observational studies suggesting that SSRI medication use may protect against the development of PCC and improve prognosis when utilized as a treatment. A positive association between a depression or anxiety diagnosis and chronic fatigue following SARS-CoV-2 infection was reported for a cohort of patients receiving care at the post-COVID-19 outpatient clinic at St James's Hospital in Dublin, Ireland.<sup>33,34</sup> However, this analysis was cross-sectional and temporality between poor mental health and PCC could not be established. A survey study utilizing electronic health records corresponding to over one million individuals in the United Kingdom identified a higher risk of PCC among those with anxiety or depression prior to the COVID-19 pandemic.<sup>35</sup> Conversely, Sidky et al. analyzed data from the retrospective National COVID Cohort Collaborative study and reported lower prevalence of PCC (as defined by the code ICD-10 U09.9) among those taking an SSRI at baseline.<sup>17</sup> In a study of 95 PCC patients, cognitive symptoms (e.g., brain fog, sensory processing issues), fatigue and dysautonomia improved relative to baseline following treatment with an SSRI.<sup>5</sup> However, no comparison to a control group was conducted and there was no blinding to treatment status, which increases the risk of bias and diminishes the reliability of these findings.

Our findings are consistent with one of two published randomized controlled trials (RCTs). A multicenter placebo-controlled clinical trial of adults with a high BMI in which PCC was investigated as a secondary outcome and fluvoxamine was included in a subgroup analysis failed to detect an association between fluvoxamine and the incidence of PCC.<sup>20</sup> However, the authors acknowledge low power as a constraint and recommended careful interpretation of the results of subgroup analyses. Additionally, these findings may not apply to non-obese or non-overweight individuals. Conversely, a second randomized placebo-controlled study of adult PCC patients found that cognitive function improved at a faster rate among those receiving vortioxetine after adjusting for baseline c-reactive protein.<sup>36</sup>

Our findings do not support the hypothesis that SSRI use protects against PCC development. Although compelling preliminary evidence has been generated by in vitro and animal model studies, the literature regarding the impact of SSRI medication use on the risk of PCC or the use of SSRIs as a potential treatment for PCC is sparse and restricted by limitations in study design.<sup>5,21</sup> The discrepancy between our findings and those of other observational studies may be due to bias resulting from unadjusted confounding (e.g., systemic differences in the characteristics of patients prescribed SSRIs and individuals without anxiety or depression), a lack of blinding, and small sample sizes. Our results are in agreement with one of two randomized controlled trials conducted previously and we did not discern a clinical impact of SSRI use on the risk of PCC in humans.<sup>20</sup> However, one RCT demonstrated that SSRIs improved cognitive symptoms of PCC.<sup>36</sup> SSRIs may yet prove to be an effective treatment for PCC patients through multiple proposed mechanism, such as balancing neurotransmitter systems, decreasing chronic inflammation, and regulating the hypothalamic-pituitary-adrenal axis.<sup>5,37</sup>

### *Strengths and Limitations*

Our approach has numerous strengths which bolster the validity of our findings. Multiple methods were utilized to capture incident SARS-CoV-2 infections, including self-reported positive PCR/RAT test results and anti-N IgG seropositivity. This enabled the identification of asymptomatic infections and limited the impact of false-negative test results. Vaccine dates and types were verified through direct outreach to participants or medical record review, reducing the risk of misclassification regarding these variables. Extensive data collection facilitated the adjustment of many key factors related to COVID-19 vaccine immunogenicity and PCC. (e.g., hybrid immunity vs. vaccine-only immunity, immune compromising conditions and medications, dose type, obesity, smoking status). We adjusted for confounding by multiple key predictors of SARS-CoV-2 vaccine humoral immunogenicity and PCC, including older age, female sex, ethnicity, obesity and a history of smoking.<sup>33,38,39</sup> Our approach corrected for sources

of statistical bias, such as autocorrelation, non-linearity, and outliers. In contrast to prior cross-sectional studies, our study is longitudinal; therefore, temporality between SSRI medication use and outcomes can be established. Recall bias is a major limitation of earlier retrospective studies. Our data was collected prospectively and the risk of recall bias was lower compared to these studies. Our large sample size and the inclusion of a non-SSRI comparison group further bolster the validity of our findings.

The clinical manifestation of PCC is heterogeneous and involves multiple organ systems.<sup>40</sup> In the absence of biomarkers or diagnostic tests, case identification relies on symptomology. The wide range of PCC phenotypes further complicates the accurate diagnosis of PCC.<sup>20,41,42</sup> We included presumptive PCC cases for which SARS-CoV-2 infections were not confirmed but were inferred based on contact-tracing or respiratory symptoms. This decision increased power and lowered the risk of misclassification of PCC arising from infections that occurred during a period of time when access to testing was limited, such as early in the pandemic. Furthermore, the use of an objective immunological measure of COVID-19 vaccine humoral immunogenicity mitigated sources of systematic error impacting self-reported data and ensured accurate outcome ascertainment.

We acknowledge the following limitations which should be considered when interpreting our findings. Demographic and clinical data was self-reported and is subject to various sources of information bias. Recall bias may be present if PCC patients were more likely to accurately report all their medications compared to non-PCC patients who may have been less engaged and failed to report use of an SSRI or non-SSRI anti-anxiety medication or antidepressant. Personal hypotheses regarding the etiology of PCC among participants with PCC may have led to reporting bias in certain areas, such as in listing specific medications and supplements.<sup>43</sup> Standardized data collection protocols and outcome status ascertainment helped to alleviate these sources of bias.



The time-varying nature of SSRI use is an important aspect of this study. Antidepressant and anxiolytic use was primarily determined at baseline and follow-up data regarding medication use was limited. Exposure misclassification may have occurred if a participant initiated a medication for depression or anxiety during follow-up and failed to report it. Secular trends were present during the course of data collection and the risk of PCC development was not consistent over follow-up. Previous research comparing the risk of PCC following infection by different variants indicates that the probability of developing PCC is higher following infection by older variants (e.g., Wuhan wildtype variant, Alpha or Delta variants) relative to Omicron.<sup>44,45</sup> Vaccination status is another factor that alters the risk of PCC development and vaccination against COVID-19 has been demonstrated to protect against PCC.<sup>38</sup> Different SARS-CoV-2 variants predominated over time and the risk of PCC was greater early in the pandemic when vaccination was unavailable and Wuhan variant was circulating. We did not account for these time-varying factors in our logistic regression modelling and assumed that the SSRI and non-SSRI groups did not differ according to variant exposure or vaccination status during follow-up. Nearly all demographic and clinical variables were similar for SSRI users and non-SSRIs users, including socioeconomic status, employment status, immune compromised status, and timing and type of vaccine doses, suggesting that SSRI and non-SSRI groups were exposed to comparable levels of variant circulation pre- and post-vaccination.

Sample size constraints restricted our analysis on vaccine immunogenicity to three doses. The influence of SSRI medication use on the cell-mediated immune response to COVID-19 vaccination was not measured. Research on the T cell response to COVID-19 vaccination among SSRI users and non-SSRI users is needed to obtain a complete picture of the immune response to COVID-19 vaccination.

Our prospective longitudinal cohort study is limited by volunteer bias and loss to follow-up (LTFU). Study participation required travel to the hospital in order to provide blood samples.

As a direct consequence, enrollment was restricted to individuals who were healthy enough to travel and the inclusion severely ill or disabled PCC patients or individuals with debilitating anxiety or depression was precluded. Similarly, the duration of follow-up was long (up to 46 months) which increased the risk of selection bias from LTFU, potentially impacting power, generalizability and internal validity.<sup>41</sup> A higher burden of study participation on individuals with anxiety or depression or PCC may introduce systemic bias if differential LTFU with respect to SSRI use and PCC status occurred as a result. However, enrolled participants with PCC and/or anxiety were likely more motivated to continue their participation in SSO due to heightened concerns for their health and we indeed observed high retention of immune compromised individuals in later phases of the study. This fact, along with engaged study staff, help to minimize LTFU. White females are disproportionately represented in our cohort and the majority of vaccines administered were mRNA vaccines. This limits generalizability to non-white, non-female populations and to other vaccine types. All participants were 18 years or older and we can make no inferences for youth or children regarding the impact of SSRI medications on vaccine humoral immunogenicity or the risk of developing PCC.

Residual confounding by unadjusted factors (e.g., ethnicity, low socioeconomic status, comorbidities such as asthma or type 2 diabetes) may influence our findings.<sup>39,46</sup> The incorporation of additional factors in future research such as specific SSRI type and dosage would provide additional clarification in this research topic.<sup>7,16,21</sup> As highlighted by Gobin et al. and Prasanth et al., the therapeutic dose required to produce a clinical impact in PCC patients may be higher than that prescribed for the treatment of depression.<sup>7,21</sup>

### *Conclusion*

In this prospective longitudinal observational study, SSRI medication use did not predict antibody titers following three COVID-19 vaccine doses. COVID-19 vaccination elicited comparable humoral immunity in adults treated with SSRIs and those not taking an SSRI. The

risk of PCC following SARS-CoV-2 infection was not associated with SSRI use nor the use of non-SSRI treatments for anxiety or depression. Our study indicates that humoral immunity elicited by vaccination is robust among SSRI users. Without any proven or effective treatments for PCC, vaccination against COVID-19 is currently the best strategy to prevent long-term morbidity and sequelae following SARS-CoV-2 infection, regardless of SSRI medication use.<sup>5</sup> High-quality randomized controlled clinical trials are needed to determine the utility of SSRI medication as a treatment of PCC.

## Tables

Table 1: Participant demographics and clinical characteristics at baseline, according to SSRI medication use classification (n=929).

| Variable                                         | All Participants<br>(n=929) | SSRI Use<br>(n=83) | No SSRI Use<br>(n=846) | P     |
|--------------------------------------------------|-----------------------------|--------------------|------------------------|-------|
| Age                                              |                             |                    |                        |       |
| Mean (SD)                                        | 45.6 (13.9)                 | 43.2 (13.2)        | 45.8 (14.0)            | 0.10  |
| Range (min, max)                                 | 61.0 (18.0, 79.0)           | 51.0 (22.0, 73.0)  | 61.0 (18.0, 79.0)      |       |
| Gender, n (%)                                    |                             |                    |                        |       |
| Male                                             | 314 (34.1)                  | 18 (22.0)          | 296 (35.2)             | 0.005 |
| Female                                           | 603 (65.4)                  | 62 (75.6)          | 541 (64.4)             |       |
| Gender diverse                                   | 5 (0.5)                     | 2 (2.4)            | 3 (0.4)                |       |
| Missing                                          | 7                           | 1                  | 6                      |       |
| Race, n (%)                                      |                             |                    |                        |       |
| Arab/West Asian                                  | 20 (2.2)                    | 0 (0)              | 20 (2.4)               | 0.77  |
| Black                                            | 5 (0.5)                     | 0 (0)              | 5 (0.6)                |       |
| East or Southeast Asian                          | 16 (1.7)                    | 1 (1.2)            | 15 (1.8)               |       |
| Indigenous                                       | 14 (1.5)                    | 2 (2.4)            | 12 (1.4)               |       |
| Latin American                                   | 9 (1.0)                     | 1 (1.2)            | 8 (1.0)                |       |
| South Asian                                      | 13 (1.4)                    | 0 (0)              | 13 (0)                 |       |
| White                                            | 827 (89.1)                  | 77 (92.8)          | 750 (88.8)             |       |
| Other                                            | 24 (2.6)                    | 2 (2.4)            | 22 (2.6)               |       |
| Missing                                          | 1                           | 0                  | 1                      |       |
| Income before taxes in the past 12 months, n (%) |                             |                    |                        |       |
| 0-\$29,999                                       | 37 (4.5)                    | 5 (6.9)            | 32 (4.3)               | 0.15  |
| \$30,000-\$59,999                                | 88 (10.7)                   | 9 (12.3)           | 79 (10.5)              |       |
| \$60,000-\$89,999                                | 161 (19.5)                  | 19 (26.0)          | 142 (18.9)             |       |
| \$90,000-\$119,999                               | 181 (21.9)                  | 14 (19.2)          | 167 (22.2)             |       |
| \$120,000-\$149,999                              | 95 (11.5)                   | 11 (15.1)          | 84 (11.2)              |       |
| \$150,000+                                       | 263 (31.9)                  | 15 (20.6)          | 248 (33.0)             |       |
| Missing                                          | 104                         | 10                 | 94                     |       |
| Employed, n (%)                                  |                             |                    |                        |       |
| Yes                                              | 752 (82.8)                  | 70 (87.5)          | 682 (82.4)             | 0.28  |
| No                                               | 146 (17.6)                  | 10 (12.5)          | 146 (17.6)             |       |
| Missing                                          | 21                          | 3                  | 18                     |       |
| Obesity (self-reported or BMI $\geq$ 30), n (%)  |                             |                    |                        |       |

|                                            |            |           |            |       |
|--------------------------------------------|------------|-----------|------------|-------|
| Yes                                        | 214 (23.2) | 29 (35.4) | 185 (22.0) | 0.009 |
| No                                         | 709 (76.8) | 53 (64.6) | 656 (78.0) |       |
| Missing                                    | 6          | 1         | 5          |       |
| <hr/>                                      |            |           |            |       |
| Receiving regular medical treatment, n (%) |            |           |            |       |
| Yes                                        | 38 (4.1)   | 5 (6.1)   | 33 (4.0)   | 0.38  |
| No                                         | 880 (95.9) | 77 (93.9) | 803 (96.1) |       |
| Missing                                    | 11         | 1         | 10         |       |
| <hr/>                                      |            |           |            |       |
| Immune compromised, n (%) <sup>§</sup>     |            |           |            |       |
| Yes - High level                           | 104 (11.2) | 7 (8.4)   | 97 (11.5)  | 0.45  |
| Yes - Low level                            | 157 (16.9) | 17 (20.5) | 140 (16.6) |       |
| No                                         | 668 (71.9) | 59 (71.1) | 609 (72.0) |       |
| Missing                                    | 0          | 0         | 0          |       |

Two-sided parametric (Student's t-test) or non-parametric (Mann-Whitney U test, chi-square test) statistical tests were used. Normality was determined by histogram and Q-Q plot inspection. The Fisher's exact test was used when chi-square test assumptions were not met. Statistical significance was determined using an alpha level of 0.05. SD: standard deviation; IQR: interquartile range.

<sup>§</sup> High-level immune compromising conditions included malignancies, organ/tissue transplant, primary immunodeficiencies, rheumatological conditions, multiple sclerosis, myasthenia gravis, active HIV, serious liver disease (e.g., severe cirrhosis) and systemic immunosuppressant medication use. Low-level conditions include respiratory conditions treatment with corticosteroids and other immune compromising medications (e.g. asthma), topical corticosteroid use, organ-specific autoimmune disease (e.g., celiac disease, autoimmune hepatitis), diabetes, fully suppressed HIV, mild liver disorder (e.g., mild cirrhosis), inactive SLE and kidney disorders (i.e. chronic kidney disease).

Table 2: COVID-19 vaccination and infection data according to SSRI medication use (n=929).

| Variable                                                                | All Participants<br>(n=929) | SSRI Use<br>(n=83) | No SSRI Use<br>(n=846) | P    |
|-------------------------------------------------------------------------|-----------------------------|--------------------|------------------------|------|
| Vaccinated at time of analysis, n (%)                                   |                             |                    |                        |      |
| Yes                                                                     | 911 (98.1)                  | 82 (98.8)          | 829 (98.0)             | 1.00 |
| No                                                                      | 18 (1.9)                    | 1 (1.2)            | 17 (2.0)               |      |
| Missing                                                                 | 0                           | 0                  | 0                      |      |
| Number of COVID-19 vaccine doses<br>received at time of analysis, n (%) |                             |                    |                        |      |
| 0                                                                       | 18 (1.9)                    | 1 (1.2)            | 17 (2.0)               | 0.09 |
| 1                                                                       | 4 (0.4)                     | 1 (1.2)            | 3 (0.4)                |      |
| 2                                                                       | 235 (25.3)                  | 25 (30.1)          | 210 (24.8)             |      |
| 3                                                                       | 459 (49.4)                  | 49 (59.0)          | 410 (48.5)             |      |
| 4                                                                       | 111 (12.0)                  | 4 (4.8)            | 107 (12.7)             |      |
| 5                                                                       | 50 (5.4)                    | 2 (2.4)            | 48 (5.7)               |      |
| 6                                                                       | 36 (3.9)                    | 0 (0)              | 36 (4.3)               |      |
| 7                                                                       | 10 (1.1)                    | 1 (1.2)            | 9 (1.1)                |      |
| 8                                                                       | 4 (0.4)                     | 0 (0)              | 4 (0.5)                |      |
| 9                                                                       | 1 (0.1)                     | 0 (0)              | 1 (0.1)                |      |
| 10                                                                      | 1 (0.1)                     | 0 (0)              | 1 (0.1)                |      |
| COVID-19 vaccine type, dose 1, n (%)                                    |                             |                    |                        |      |
| AstraZeneca                                                             | 115 (12.6)                  | 8 (9.8)            | 107 (12.9)             | 0.60 |
| Moderna                                                                 | 91 (10.0)                   | 10 (12.2)          | 81 (9.8)               |      |
| Pfizer                                                                  | 704 (77.3)                  | 64 (78.1)          | 640 (77.3)             |      |
| Missing                                                                 | 1                           | 0                  | 1                      |      |
| COVID-19 vaccine type, dose 2,<br>n (%)                                 |                             |                    |                        |      |
| AstraZeneca                                                             | 11 (1.2)                    | 2 (2.5)            | 9 (1.1)                | 0.33 |
| Moderna                                                                 | 228 (25.2)                  | 23 (28.4)          | 205 (24.9)             |      |
| Pfizer                                                                  | 666 (73.5)                  | 56 (69.1)          | 610 (74.9)             |      |
| Sinovac                                                                 | 1 (0.1)                     | 0 (0)              | 1 (0.1)                |      |
| Missing                                                                 | 5                           | 1                  | 4                      |      |
| COVID-19 vaccine type, dose 3,<br>n (%)                                 |                             |                    |                        |      |
| Moderna                                                                 | 186 (27.9)                  | 20 (35.7)          | 166 (27.2)             | 0.34 |
| Pfizer                                                                  | 479 (71.8)                  | 36 (64.3)          | 443 (72.5)             |      |
| Pfizer (bivalent)                                                       | 1 (0.2)                     | 0 (0)              | 1 (0.2)                |      |
| Sinovac                                                                 | 1 (0.2)                     | 0 (0)              | 1 (0.2)                |      |

|                                                                                                           |                      |                      |                      |      |
|-----------------------------------------------------------------------------------------------------------|----------------------|----------------------|----------------------|------|
| Missing                                                                                                   | 6                    | 0                    | 6                    |      |
| COVID-19 vaccine type, dose 4,<br>n (%)                                                                   |                      |                      |                      |      |
| Moderna (half dose)                                                                                       | 3 (1.4)              | 0 (0)                | 3 (1.5)              | 0.61 |
| Moderna                                                                                                   | 77 (36.3)            | 3 (42.9)             | 74 (36.1)            |      |
| Moderna (bivalent)                                                                                        | 32 (15.1)            | 2 (28.6)             | 30 (14.6)            |      |
| Moderna (monovalent, Omicron XBB)                                                                         | 1 (0.5)              | 0 (0)                | 1 (0.5)              |      |
| Novavax                                                                                                   | 1 (0.5)              | 0 (0)                | 1 (0.5)              |      |
| Pfizer                                                                                                    | 69 (32.6)            | 1 (14.3)             | 68 (33.2)            |      |
| Pfizer (monovalent, Omicron XBB)                                                                          | 1 (0.5)              | 0 (0)                | 1 (0.5)              |      |
| Pfizer (bivalent)                                                                                         | 28 (13.2)            | 1 (14.3)             | 27 (13.2)            |      |
| Missing                                                                                                   | 1                    | 0                    | 1                    |      |
| Time between first and second doses<br>in days                                                            |                      |                      |                      |      |
|                                                                                                           | n=900                | n=81                 | n=819                |      |
| Median (IQR)                                                                                              | 58 (35, 76)          | 57.0 (35, 77)        | 59.0 (35.0, 76.0)    | 0.95 |
| Range                                                                                                     | 19, 344              | 21, 122              | 19.0, 344.0          |      |
| Missing                                                                                                   | 4                    | 0                    | 4                    |      |
| Time between second and third<br>doses in days                                                            |                      |                      |                      |      |
|                                                                                                           | n=668                | n=56                 | n=612                |      |
| Median (IQR)                                                                                              | 185 (174, 211)       | 182.5 (173.5, 192.0) | 185.0 (174.0, 216.5) | 0.22 |
| Range                                                                                                     | 22, 712              | 88.0, 320.0          | 22.0, 712.0          |      |
| Missing                                                                                                   | 3                    | 0                    | 3                    |      |
| Time between third and fourth doses<br>in days                                                            |                      |                      |                      |      |
|                                                                                                           | n=212                | n=7                  | n=205                |      |
| Median (IQR)                                                                                              | 220.5 (142.0, 294.0) | 231.0 (146.0, 302.0) | 220.0 (142.0, 294.0) | 0.84 |
| Range                                                                                                     | 28.0, 891.0          | 131.0, 306.0         | 28.0, 891.0          |      |
| Missing                                                                                                   | 2                    | 0                    | 2                    |      |
| Number of SARS-CoV-2 Infection,<br>n (%)                                                                  |                      |                      |                      |      |
| 0                                                                                                         | 475 (51.1)           | 46 (55.4)            | 429 (50.7)           | 0.62 |
| 1                                                                                                         | 351 (37.8)           | 31 (37.4)            | 320 (37.8)           |      |
| 2                                                                                                         | 85 (9.2)             | 6 (7.2)              | 79 (9.3)             |      |
| 3                                                                                                         | 18 (1.9)             | 0 (0)                | 18 (2.1)             |      |
| Time of self-reported and serology-<br>based COVID-19 in relation to<br>vaccine doses, n (%) <sup>§</sup> |                      |                      |                      |      |
| COVID-19 prior to dose 1                                                                                  | 227 (39.5)           | 23 (53.5)            | 204 (38.4)           | 0.17 |
| COVID-19 after dose 1                                                                                     | 5 (0.9)              | 1 (2.3)              | 4 (0.8)              |      |
| COVID-19 after dose 2                                                                                     | 61 (10.6)            | 5 (11.6)             | 56 (10.5)            |      |

|                                                                                                                                             |             |           |            |      |
|---------------------------------------------------------------------------------------------------------------------------------------------|-------------|-----------|------------|------|
| COVID-19 after dose 3                                                                                                                       | 165 (28.7)  | 9 (20.9)  | 156 (29.3) |      |
| COVID-19 after dose 4                                                                                                                       | 72 (12.5)   | 2 (4.7)   | 70 (13.2)  |      |
| COVID-19 after dose 5                                                                                                                       | 23 (4.0)    | 3 (7.0)   | 20 (3.8)   |      |
| COVID-19 after dose 6                                                                                                                       | 17 (3.0)    | 0 (0)     | 17 (13.2)  |      |
| COVID-19 after dose 7                                                                                                                       | 5 (0.9)     | 0 (0)     | 5 (0.9)    |      |
| Total                                                                                                                                       | 575 (100.0) | 43 (7.5)  | 532 (92.5) |      |
| Post-COVID-19 Condition meeting WHO clinical case definition and ICD-11 code RA02 among those with a history of SARS-CoV-2 infection, n (%) |             |           |            |      |
| Yes                                                                                                                                         | 147 (32.9)  | 14 (38.9) | 133 (32.4) | 0.42 |
| No                                                                                                                                          | 300 (67.1)  | 22 (61.1) | 278 (67.6) |      |
| Missing                                                                                                                                     | 22          | 0         | 22         |      |

Two-sided parametric (Student's t-test) or non-parametric (Mann-Whitney U test, chi-square test) statistical tests were used. Normality was determined by histogram and Q-Q plot inspection. The Fisher's exact test was used when chi-square test assumptions were not met. Statistical significance was determined using an alpha level of 0.05. SD: standard deviation; IQR: interquartile range.

§ SARS-CoV-2 infections occurring within 14 days of a COVID-19 vaccine dose were classified as a breakthrough infection corresponding to the previous dose and infections occurring within 14 days of the first COVID-19 vaccine dose were classified as a baseline infection.



Table 3: Penalised logistic regression model of post-COVID-19 condition development (PCC) among participants with a SARS-CoV-2 infection at baseline or during follow-up according to the use of an antidepressant or anxiolytic medication that targets serotonin uptake (n=415). The Firth's correction was implemented and confidence intervals were generated using profile likelihood.

| Variable                                                             | Estimate | 95% CI           | P             |
|----------------------------------------------------------------------|----------|------------------|---------------|
| Intercept                                                            | 1.541    | (-0.542, 3.808)  | 0.14          |
| Antidepressant medications influencing serotonin levels <sup>1</sup> |          |                  | Overall: 0.56 |
| SSRIs                                                                | -0.430   | (-2.605, 1.533)  | 0.66          |
| Others                                                               | 0.929    | (-1.411, 2.665)  | 0.34          |
| No depression/anxiety medication use                                 | Referent | -                | -             |
| Age at baseline                                                      | 0.023    | (-0.016, 0.064)  | 0.22          |
| Sex                                                                  |          |                  |               |
| Female                                                               | 0.388    | (-0.672, 1.502)  | 0.46          |
| Male                                                                 | Referent | -                | -             |
| Obesity <sup>2</sup>                                                 |          |                  |               |
| Yes                                                                  | 1.793    | (0.666, 3.066)   | 0.002         |
| No                                                                   | Referent | -                | -             |
| Smoker, ever                                                         |          |                  |               |
| Yes                                                                  | 0.412    | (-0.805, 1.583)  | 0.47          |
| No                                                                   | Referent | -                | -             |
| Vaccinated prior to PCC or study during follow-up                    | -6.902   | (-8.368, -5.746) | <0.0001       |
| Yes                                                                  | Referent | -                | -             |
| No                                                                   |          |                  |               |
| Odds Ratio (OR) of PCC Development                                   |          | 95% CI           |               |
| OR for SSRIs                                                         | 0.65     | (0.07, 4.63)     | 0.66          |
| OR for other antidepressant medications influencing serotonin levels | 2.53     | (0.24, 14.37)    | 0.34          |

<sup>1</sup> Other antidepressant medications included serotonin-norepinephrine reuptake inhibitors (SNRIs), serotonin antagonist and reuptake inhibitors (SARIs), serotonin modulators and stimulators (SMSs), tricyclic antidepressants and oxidase inhibitors. Four participants taking a norepinephrine-dopamine reuptake inhibitor (NDRI) were not included in this analysis.

<sup>2</sup> Obese participants self-reported obesity or had a reported BMI equal to or greater than 30 kg/m<sup>2</sup>.

Supplemental Table 1: Multivariable linear regression of anti-spike (S) and anti-receptor-binding domain (RBD) IgG, IgA, and IgM antibodies up to three SARS-CoV-2 vaccine doses for participants with no prior SARS-CoV-2 infection. For models that include separate smooths for SSRI groups, the difference of predicted titers between SSRI users and non-SSRI users are shown below model estimates (see column “Difference”). SSRI Use referent: no SSRI use. Male sex referent: female sex. Non-white ethnicity referent: non-white ethnicity. IC referent: non-IC. Time=Days since dose date. Vaccine type referent: Pfizer-BioNTech.

| Anti-S IgG (log <sub>10</sub> BAU/mL) |        |         | Anti-R IgG (log <sub>10</sub> BAU/mL) |         | Anti-S IgA (log <sub>10</sub> µg/mL) |         | Anti-R IgA (log <sub>10</sub> µg/mL) |         | Anti-S IgM (log <sub>10</sub> µg/mL) |            | Anti-R IgM (log <sub>10</sub> µg/mL) |         |            |       |
|---------------------------------------|--------|---------|---------------------------------------|---------|--------------------------------------|---------|--------------------------------------|---------|--------------------------------------|------------|--------------------------------------|---------|------------|-------|
| Estimate                              | P      |         | Estimate                              | P       | Estimate                             | P       | Estimate                             | P       | Estimate                             | P          | Estimate                             | P       |            |       |
| Dose 1                                |        |         |                                       |         |                                      |         |                                      |         |                                      |            |                                      |         |            |       |
| Intercept                             | 2.872  | <0.0001 | 2.395                                 | <0.0001 | 0.360                                | <0.0001 | 0.138                                | 0.001   | 0.320                                | <0.0001    | 0.190                                | <0.0001 |            |       |
| SSRI Use                              | 0.021  | 0.89    | 0.088                                 | 0.46    | -0.088                               | 0.07    | -0.033                               | 0.35    | -0.007                               | 0.89       | 0.007                                | 0.84    |            |       |
| Age at dose 1                         | -0.015 | <0.0001 | -0.012                                | <0.0001 | -0.003                               | 0.002   | -0.002                               | 0.02    | -0.003                               | 0.006      | -0.002                               | 0.01    |            |       |
| Male sex                              | -0.019 | 0.82    | 0.026                                 | 0.72    | 0.044                                | 0.10    | 0.035                                | 0.09    | 0.023                                | 0.45       | 0.041                                | 0.045   |            |       |
| White Ethnicity                       | 0.106  | 0.46    | 0.186                                 | 0.13    | 0.029                                | 0.54    | 0.022                                | 0.54    | 0.063                                | 0.23       | 0.061                                | 0.08    |            |       |
| IC                                    | -0.421 | 0.0009  | -0.235                                | 0.03    | -0.115                               | 0.004   | -0.048                               | 0.12    | -0.030                               | 0.52       | -0.044                               | 0.14    |            |       |
| Dose Type AZ                          | -0.278 | 0.02    | -0.112                                | 0.26    | -0.049                               | 0.20    | -0.008                               | 0.80    | 0.014                                | 0.75       | -0.022                               | 0.46    |            |       |
| Dose Type Moderna                     | 0.297  | 0.10    | 0.528                                 | 0.0006  | 0.133                                | 0.03    | 0.127                                | 0.004   | 0.068                                | 0.32       | 0.033                                | 0.46    |            |       |
| Dose 2                                |        |         |                                       |         |                                      |         |                                      |         |                                      |            |                                      |         |            |       |
| Intercept                             | 3.485  | <0.0001 | 3.216                                 | <0.0001 | 0.500                                | <0.0001 | 0.270                                | <0.0001 | 0.250                                | <0.0001    | 0.196                                | <0.0001 |            |       |
| SSRI Use                              | -0.023 | 0.71    | -0.046                                | 0.52    | 0.010                                | 0.79    | 0.018                                | 0.45    | -0.043                               | 0.22       | -0.035                               | 0.045   |            |       |
| Age at dose 2                         | -0.009 | <0.0001 | -0.011                                | <0.0001 | -0.001                               | 0.12    | -0.002                               | 0.002   | -0.001                               | 0.12       | -0.001                               | 0.0005  |            |       |
| Male sex                              | 0.010  | 0.77    | 0.039                                 | 0.34    | 0.048                                | 0.02    | 0.047                                | 0.0007  | 0.029                                | 0.14       | -0.002                               | 0.86    |            |       |
| White Ethnicity                       | -0.042 | 0.44    | 0.043                                 | 0.51    | -0.056                               | 0.08    | -0.039                               | 0.07    | 0.039                                | 0.21       | 0.008                                | 0.60    |            |       |
| IC                                    | -0.300 | <0.0001 | -0.464                                | <0.0001 | -0.112                               | 0.0001  | -0.060                               | 0.002   | -0.078                               | 0.006      | -0.033                               | 0.02    |            |       |
| Dose Type Moderna                     | 0.184  | <0.0001 | 0.224                                 | <0.0001 | 0.052                                | 0.03    | 0.061                                | 0.0002  | 0.057                                | 0.02       | 0.019                                | 0.10    |            |       |
| Days between dose 1 & 2               | 0.002  | 0.003   | 0.004                                 | <0.0001 | -0.0002                              | 0.67    | -0.001                               | <0.0001 | -0.001                               | 0.08       | -0.001                               | 0.009   |            |       |
| N/A                                   |        |         | N/A                                   |         | N/A                                  |         | N/A                                  |         | Time                                 | Difference | P                                    | Time    | Difference | P     |
|                                       |        |         |                                       |         |                                      |         |                                      |         | -25                                  | 0.029      | 0.56                                 | -25     | -0.003     | 0.92  |
|                                       |        |         |                                       |         |                                      |         |                                      |         | 0                                    | -0.020     | 0.65                                 | 0       | -0.021     | 0.36  |
|                                       |        |         |                                       |         |                                      |         |                                      |         | 15                                   | -0.046     | 0.26                                 | 25      | -0.037     | 0.09  |
|                                       |        |         |                                       |         |                                      |         |                                      |         | 50                                   | -0.021     | 0.58                                 | 50      | -0.055     | 0.02  |
|                                       |        |         |                                       |         |                                      |         |                                      |         | 100                                  | -0.088     | 0.03                                 | 100     | -0.076     | 0.002 |
|                                       |        |         |                                       |         |                                      |         |                                      |         | 150                                  | -0.017     | 0.74                                 | 150     | 0.007      | 0.84  |
|                                       |        |         |                                       |         |                                      |         |                                      |         | 200                                  | -0.150     | 0.02                                 | 200     | -0.027     | 0.53  |
|                                       |        |         |                                       |         |                                      |         |                                      |         | 250                                  | -0.153     | 0.05                                 | 250     | -0.014     | 0.84  |
| Dose 3                                |        |         |                                       |         |                                      |         |                                      |         |                                      |            |                                      |         |            |       |
| Intercept                             | 3.421  | <0.0001 | 3.544                                 | <0.0001 | 0.407                                | 0.0001  | 0.119                                | 0.12    | 0.197                                | 0.005      | 0.065                                | 0.10    |            |       |
| SSRI Use                              | -0.024 | 0.86    | -0.150                                | 0.30    | 0.021                                | 0.73    | -0.003                               | 0.94    | -0.034                               | 0.41       | -0.012                               | 0.57    |            |       |

|                         |         |       |        |        |         |       |        |        |                   |       |        |      |
|-------------------------|---------|-------|--------|--------|---------|-------|--------|--------|-------------------|-------|--------|------|
| Age at dose 3           | -0.004  | 0.11  | -0.008 | 0.004  | 0.001   | 0.67  | -0.001 | 0.44   | -0.001            | 0.41  | 0.0002 | 0.67 |
| Male sex                | 0.075   | 0.30  | 0.052  | 0.50   | 0.037   | 0.24  | 0.040  | 0.09   | -0.030            | 0.18  | -0.017 | 0.16 |
| White Ethnicity         | 0.039   | 0.76  | 0.126  | 0.35   | -0.057  | 0.30  | -0.064 | 0.14   | 0.018             | 0.65  | -0.004 | 0.84 |
| IC                      | -0.277  | 0.007 | -0.224 | 0.04   | 0.022   | 0.62  | 0.061  | 0.07   | -0.023            | 0.46  | 0.026  | 0.12 |
| Dose Type Moderna       | 0.261   | 0.001 | 0.316  | 0.0002 | 0.068   | 0.053 | 0.096  | 0.0005 | 0.071             | 0.004 | 0.021  | 0.13 |
| Days between dose 2 & 3 | -0.0003 | 0.62  | -0.001 | 0.29   | 0.00002 | 0.95  | 0.0004 | 0.10   | 4x10 <sup>7</sup> | 0.998 | 0.0001 | 0.61 |

Statistical significance is indicated by a p-value <0.025 to account for bias from multiplicity.

Supplemental Table 2: Multivariable linear regression of anti-spike (S) and anti-receptor-binding domain (RBD) IgG, IgA, and IgM antibodies up to three SARS-CoV-2 vaccine doses for participants with at least one prior SARS-CoV-2 infection. For models that include separate smooths for SSRI groups, the difference of predicted titers between SSRI users and non-SSRI users are shown below model estimates (see column “Difference”). SSRI Use referent: no SSRI use. Male sex referent: female sex. Non-white ethnicity referent: non-white ethnicity. IC referent: non-IC. Time=Days since dose date. Vaccine type referent: Pfizer-BioNTech.

| Anti-S IgG (log <sub>10</sub> BAU/mL) |        |         | Anti-R IgG (log <sub>10</sub> BAU/mL) |            |        | Anti-S IgA (log <sub>10</sub> µg/mL) |         | Anti-R IgA (log <sub>10</sub> µg/mL) |         | Anti-S IgM (log <sub>10</sub> µg/mL) |            | Anti-R IgM (log <sub>10</sub> µg/mL) |            |        |      |
|---------------------------------------|--------|---------|---------------------------------------|------------|--------|--------------------------------------|---------|--------------------------------------|---------|--------------------------------------|------------|--------------------------------------|------------|--------|------|
| Estimate                              | P      |         | Estimate                              | P          |        | Estimate                             | P       | Estimate                             | P       | Estimate                             | P          | Estimate                             | P          |        |      |
| Dose 1                                |        |         |                                       |            |        |                                      |         |                                      |         |                                      |            |                                      |            |        |      |
| Intercept                             | 3.112  | <0.0001 | 2.825                                 | <0.0001    |        | 0.469                                | <0.0001 | 0.234                                | 0.002   | 0.393                                | <0.0001    | 0.333                                | <0.0001    |        |      |
| SSRI Use                              | -0.082 | 0.55    | -0.340                                | 0.07       |        | -0.081                               | 0.25    | -0.077                               | 0.23    | 0.098                                | 0.19       | 0.040                                | 0.51       |        |      |
| Age at dose 1                         | 0.011  | <0.0001 | 0.012                                 | 0.0008     |        | 0.002                                | 0.14    | 0.002                                | 0.09    | 0.0003                               | 0.80       | 0.001                                | 0.64       |        |      |
| Male sex                              | -0.100 | 0.20    | -0.110                                | 0.30       |        | -0.026                               | 0.51    | 0.016                                | 0.66    | -0.026                               | 0.53       | -0.010                               | 0.76       |        |      |
| White Ethnicity                       | -0.239 | 0.048   | -0.262                                | 0.11       |        | -0.073                               | 0.23    | 0.009                                | 0.87    | -0.026                               | 0.69       | -0.064                               | 0.21       |        |      |
| IC                                    | -0.233 | 0.09    | 0.056                                 | 0.77       |        | -0.078                               | 0.27    | -0.036                               | 0.58    | 0.003                                | 0.97       | -0.022                               | 0.72       |        |      |
| Dose Type AZ                          | -0.687 | <0.0001 | -0.575                                | 0.0003     |        | -0.191                               | 0.001   | -0.184                               | 0.0009  | -0.058                               | 0.36       | 0.017                                | 0.73       |        |      |
| Dose Type Moderna                     | 0.115  | 0.23    | 0.072                                 | 0.59       |        | -0.039                               | 0.44    | -0.043                               | 0.35    | 0.034                                | 0.53       | 0.044                                | 0.31       |        |      |
| Days since last SARS-CoV-2 infection  | -0.001 | 0.002   | -0.001                                | 0.11       |        | -0.001                               | 0.0003  | -0.0003                              | 0.08    | 0.0001                               | 0.49       | 0.0002137                            | 0.10       |        |      |
|                                       |        |         | Time                                  | Difference | P      |                                      |         |                                      |         | Time                                 | Difference | P                                    |            |        |      |
|                                       |        |         | 0                                     | 0.274      | 0.34   |                                      |         |                                      |         | 0                                    | -0.073     | 0.47                                 | 0          | -0.030 | 0.68 |
|                                       |        |         | 15                                    | -0.839     | 0.003  |                                      |         |                                      |         | 15                                   | 0.126      | 0.21                                 | 15         | 0.087  | 0.23 |
|                                       |        |         | 50                                    | -0.464     | 0.13   |                                      |         |                                      |         | 30                                   | 0.214      | 0.04                                 | 30         | 0.109  | 0.17 |
|                                       |        |         |                                       |            |        |                                      |         |                                      |         | 60                                   | 0.134      | 0.33                                 | 60         | 0.013  | 0.91 |
| Dose 2                                |        |         |                                       |            |        |                                      |         |                                      |         |                                      |            |                                      |            |        |      |
| Intercept                             | 3.569  | <0.0001 | 3.334                                 | <0.0001    |        | 0.650                                | <0.0001 | 0.360                                | <0.0001 | 0.405                                | <0.0001    | 0.341                                | <0.0001    |        |      |
| SSRI Use                              | 0.101  | 0.22    | -0.059                                | 0.56       |        | -0.097                               | 0.14    | -0.108                               | 0.09    | 0.012                                | 0.82       | -0.021                               | 0.71       |        |      |
| Age at dose 2                         | 0.005  | 0.005   | 0.008                                 | 0.0003     |        | -0.001                               | 0.50    | -0.0003                              | 0.82    | -0.0001                              | 0.96       | 0.0002                               | 0.90       |        |      |
| Male sex                              | 0.007  | 0.89    | 0.055                                 | 0.37       |        | 0.029                                | 0.47    | 0.047                                | 0.21    | 0.010                                | 0.75       | -0.017                               | 0.60       |        |      |
| White Ethnicity                       | -0.090 | 0.22    | -0.051                                | 0.57       |        | -0.065                               | 0.27    | -0.009                               | 0.88    | -0.065                               | 0.17       | -0.045                               | 0.35       |        |      |
| IC                                    | -0.001 | 0.99    | 0.126                                 | 0.21       |        | -0.030                               | 0.65    | -0.006                               | 0.92    | 0.068                                | 0.20       | 0.001                                | 0.99       |        |      |
| Dose Type AZ                          | -      | -       | -                                     | -          |        | -0.034                               | 0.83    | 0.007                                | 0.96    | -0.178                               | 0.16       | -0.146                               | 0.28       |        |      |
| Dose Type Moderna                     | 0.018  | 0.74    | 0.040                                 | 0.54       |        | 0.026                                | 0.54    | 0.026                                | 0.52    | 0.060                                | 0.07       | -0.026                               | 0.44       |        |      |
| Days between dose 1 & 2               | -0.003 | 0.03    | -0.002                                | 0.10       |        | 0.001                                | 0.57    | -0.0001                              | 0.88    | -0.001                               | 0.25       | -0.001                               | 0.31       |        |      |
| Days since last SARS-CoV-2 infection  | -0.001 | <0.0001 | -0.0005                               | 0.02       |        | -0.0002                              | 0.04    | -0.0002                              | 0.13    | 0.0001                               | 0.56       | 0.0001                               | 0.35       |        |      |
|                                       |        |         | Time                                  | Difference | P      |                                      |         |                                      |         |                                      |            | Time                                 | Difference | P      |      |
|                                       |        |         | -15                                   | -0.839     | <0.001 |                                      |         |                                      |         |                                      |            | 0                                    | 0.043      | 0.47   |      |
|                                       |        |         | 15                                    | 0.191      | 0.19   |                                      |         |                                      |         |                                      |            | 15                                   | 0.022      | 0.72   |      |

|                                      |        |         |  |        |         |      |                    |            |      |        |        |     |         |            |
|--------------------------------------|--------|---------|--|--------|---------|------|--------------------|------------|------|--------|--------|-----|---------|------------|
|                                      |        |         |  | 50     | 0.114   | 0.41 |                    |            |      |        |        | 50  | -0.024  | 0.69       |
|                                      |        |         |  | 100    | 0.168   | 0.19 |                    |            |      |        |        | 100 | -0.056  | 0.38       |
|                                      |        |         |  | 150    | 0.156   | 0.26 |                    |            |      |        |        | 150 | -0.047  | 0.53       |
|                                      |        |         |  | 200    | -0.039  | 0.81 |                    |            |      |        |        | 200 | -0.133  | 0.17       |
|                                      |        |         |  | 250    | -0.357  | 0.09 |                    |            |      |        |        | 250 | -0.090  | 0.52       |
| Dose 3                               |        |         |  |        |         |      |                    |            |      |        |        |     |         |            |
| Intercept                            | 3.199  | <0.0001 |  | 3.215  | <0.0001 |      | 0.524              | <0.0001    |      | 0.454  | 0.0004 |     | 0.006   | 0.97       |
| SSRI Use                             | 0.127  | 0.08    |  | 0.098  | 0.31    |      | -0.026             | 0.73       |      | -0.083 | 0.23   |     | 0.033   | 0.65       |
| Age at dose 3                        | 0.006  | 0.0006  |  | 0.005  | 0.03    |      | 0.001              | 0.60       |      | -0.001 | 0.69   |     | 0.002   | 0.17       |
| Male sex                             | 0.040  | 0.34    |  | 0.018  | 0.75    |      | 0.048              | 0.23       |      | 0.053  | 0.17   |     | -0.038  | 0.34       |
| White Ethnicity                      | 0.042  | 0.55    |  | 0.061  | 0.51    |      | -0.118             | 0.08       |      | -0.109 | 0.09   |     | -0.003  | 0.97       |
| IC                                   | -0.041 | 0.53    |  | -0.077 | 0.37    |      | -0.064             | 0.31       |      | 0.026  | 0.67   |     | -0.020  | 0.75       |
| Dose Type Moderna                    | 0.108  | 0.01    |  | 0.146  | 0.009   |      | 0.059              | 0.14       |      | 0.064  | 0.10   |     | 0.069   | 0.09       |
| Days between dose 2 & 3              | 0.001  | 0.004   |  | 0.001  | 0.02    |      | 0.001              | 0.21       |      | 0.0003 | 0.44   |     | 0.001   | 0.03       |
| Days since last SARS-CoV-2 infection | 0.0005 | <0.0001 |  | 0.001  | <0.0001 |      | -4x10 <sup>6</sup> | 0.96       |      | 0.0001 | 0.23   |     | 0.00003 | 0.60       |
|                                      |        |         |  |        |         |      | Time               | Difference | P    |        |        |     | Time    | Difference |
|                                      |        |         |  |        |         |      | -25                | -0.153     | 0.06 |        |        |     | -20     | 0.065      |
|                                      |        |         |  |        |         |      | 25                 | -0.130     | 0.09 |        |        |     | 0       | 0.077      |
|                                      |        |         |  |        |         |      | 50                 | -0.100     | 0.20 |        |        |     | 50      | 0.101      |
|                                      |        |         |  |        |         |      | 100                | -0.039     | 0.64 |        |        |     | 100     | 0.106      |
|                                      |        |         |  |        |         |      | 200                | 0.147      | 0.14 |        |        |     | 200     | 0.073      |
|                                      |        |         |  |        |         |      | 300                | 0.060      | 0.63 |        |        |     | 300     | 0.013      |
|                                      |        |         |  |        |         |      | 400                | 0.024      | 0.87 |        |        |     | 400     | -0.046     |
|                                      |        |         |  |        |         |      | 500                | 0.004      | 0.98 |        |        |     | 500     | -0.087     |
|                                      |        |         |  |        |         |      | 550                | -0.073     | 0.68 |        |        |     | 550     | -0.103     |

Statistical significance is indicated by a p-value <0.025 to account for bias from multiplicity.

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## Chapter 7 – Immune imprinting: The persisting influence of the first antigenic encounter with rapidly evolving viruses

### **Preface**

**Chapters 2 – 6** encompass multiple analyses conducted to evaluate COVID-19 vaccine humoral immunity according to different exposures. In this final component chapter, we present a review article concerning immune imprinting (also referred to as original antigenic sin) and discuss the potential for this phenomenon to impact the humoral immune response to COVID-19 vaccination. Information relevant to epidemiologists and bench scientists alike is included. We highlight the dearth of information regarding this important subject and hope to inform future work in this area. This article completes the last objective of this dissertation (objective V).

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## **Abstract**

Immune imprinting is a phenomenon that stems from the fundamentals of immunological memory. Upon recurrent exposures to an evolving pathogen, the immune system must weigh the benefits of rapidly recalling established antibody repertoires with greater affinity to the initial variant or invest additional time and energy in producing de novo responses specific to the emerging variant. In this review, we delve into the mechanistic complexities of immune imprinting and its role in shaping subsequent immune responses, both de novo and recall, against rapidly evolving respiratory viruses such as influenza and coronaviruses. By exploring the duality of immune imprinting, we examine its potential to both enhance or hinder immune protection against disease, while emphasizing the role of host and viral factors. Finally, we explore how different vaccine platforms may affect immune imprinting and comment on vaccine strategies that can favor de novo variant-specific antibody responses.

## **Immune imprinting**

Constant exposure to evolving microbes represents a lifelong battle between the host and pathogen, beginning in childhood and continuing into old age. The cornerstone of adaptive humoral immunity relies on the induction of progressively compounding and efficient antibody responses following recurrent antigenic exposures, an effective mechanism exploited by seasonal or booster vaccinations.<sup>1</sup> Yet, this prompts the question: To what degree does our first exposure to a virus, through either natural infection or vaccination, shape subsequent immune responses to later encounters with antigenically related variants? This query has perplexed immunologists since a series of pivotal studies in the 1950s examined humoral response dynamics across distinct age ranges before and after infection or vaccination with different influenza strains.<sup>2-4</sup> Key observations were: 1) potent recall antibody responses induced against viral antigens not included in the vaccine formulation, and 2) the dominant antibody responses were against influenza strains encountered earliest in life. Consequently, Thomas Francis

postulated the doctrine of original antigenic sin (OAS), describing an immune “imprint established by the original virus infection [that] governs the antibody response thereafter.”<sup>5</sup>

Although OAS or immune imprinting has primarily been observed with influenza due to its recurrent seasonality and high mutability, it has also been noted in other viral infections, including dengue virus, norovirus, hepatitis C (HCV), respiratory syncytial virus (RSV), and more recently, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).<sup>6–11</sup> Evidence for imprinting in non-respiratory viruses includes the induction of antibody recall responses to the initially encountered antigen and the production of cross-reactive antibodies following reinfection with heterologous noroviruses genotypes or HCV subtypes. Differential infection risks and worsened clinical outcomes have been observed following sequential exposures to heterologous viral strains. For instance, increased hemorrhagic fever risk has been noted in individuals following secondary dengue infections with a heterologous serotype to the primary infecting serotype, suggesting immune imprinting effects.<sup>12</sup> However, antibody-dependent enhancement (ADE) has also been observed where preexisting antibodies form complexes that facilitate viral entry into target immune cells via Fc receptor binding.<sup>6</sup> Enhanced RSV disease severity was observed among children vaccinated against RSV that were subsequently infected.<sup>7</sup> Thus, differential humoral responses following heterologous viral exposures along with specific evidence of immune imprinting in recurrent influenza infections and from recurrent SARS-CoV-2 infections or vaccinations, highlight the critical need for ongoing research on immune imprinting.

### **Mechanistic intricacies of immune imprinting**

Circulating antibodies offer a unique snapshot of an individual’s distinctive history of infection. Following infection, the host’s B cells can directly recognize native (unprocessed) antigens in secondary lymphoid organs via their binding to the B cell receptor (BCR), and processed antigens that are displayed on MHC class II molecules on the surface of antigen-

presenting cells (APC).<sup>13,14</sup> Interactions with the antigen in both of these scenarios initiates B cell activation through receptor-induced signaling, which can be considerably enhanced by cross-linking multiple BCRs with multivalent antigens or repeating epitopes.<sup>13,14</sup> Although most soluble protein antigens do not contain repetitive epitopes, particulate antigens like viruses or viral-like particles (VLP) often have repetitive arrays of antigen on their surface and can promote BCR cross-linking through higher affinity binding that enhances priming of B cells prior to receiving T cell help.<sup>14</sup> Efficient interactions with the T cell receptor (TCR) and CD40 ligand (CD40L) expressed on antigen-specific helper T cells lead to further B cell activation, proliferation, and differentiation. In the early phase of the humoral response, following the first antigenic exposure, a portion of activated B cells differentiate into plasmablasts and provide an immediate but short-lived source of low-affinity antibodies to eliminate acute infections (Figure 1a). As the humoral response develops, more specialized responses are induced. Remaining activated B cells will differentiate into memory B cells (MBC) or migrate to secondary lymphoid tissues to form germinal centers (GC), where they will undergo isotype class switching, somatic hypermutation, and affinity maturation.<sup>15</sup> Selected B cells with high affinity for the antigen can differentiate into plasma cells (LLPC), which are long-lived and continuous secretors of antibodies, or into MBCs, which will rapidly respond upon reencountering the viral antigen. These become the dominant source of antibodies in subsequent exposures to the antigen (Figure 1a). Thus, both LLPCs and MBCs are desirable cell types to elicit by vaccination, as they can rapidly respond following re-exposure. Therefore, with each new exposure to an antigenically distant viral variant, the induced humoral response may comprise both recall and *de novo* antibody responses, depending on the antigenic similarity between prior and subsequent antigens (Figure 1c).

The emergence of SARS-CoV-2 and the characterization of population immunity following repeated infections and vaccine boosters offer a unique opportunity to disentangle immune imprinting. Most infections and vaccines induce antibodies targeting the

immunodominant spike protein, an envelope glycoprotein involved in viral fusion and entry into permissive cells. Influenza's hemagglutinin (HA) protein serves a similar role. Both are trimeric proteins composed of a variable receptor-binding domain (RBD) or receptor-binding site (RBS) and a more conserved stem or stalk, respectively (Figure 1b). Given the roles of the RBD or RBS in receptor binding and cell entry, these regions contain the majority of antigenic sites where antibody binding can directly inhibit infection, leading to virus neutralization. Neutralizing antibodies are considered a strong correlate of protection against SARS-CoV-2 and influenza infection.<sup>16,17</sup> Epitopes within the RBD or RBS that are critical for virus binding are also, generally, highly immunogenic and attract the humoral response toward them. This targeted immunodominance creates a strong selection pressure that favors the emergence of mutations, also called antigenic drift. In new viral variants, immune escape mutations commonly arise at these immunodominant epitopes targeted by neutralizing antibodies (Figure 1c).<sup>18–20</sup> However, some neutralizing antibodies can target epitopes within the stem or stalk regions, which are relatively conserved between viral variants, are less susceptible to immune escape, and can be broadly protective.<sup>19,21,22</sup> Non-neutralizing antibodies that target subdominant epitopes or regions in the stem or stalk play an important role in recruiting innate and cellular antiviral immunity, and for some viral pathogens, this can occur through the effector functions of Fc-receptors.<sup>23,24</sup> The accumulation of mutations, largely detected in the RBD of recent SARS-CoV-2 variants, further demonstrates the immunodominance of this domain and its significant role in humoral responses. This suggests that SARS-CoV -2 antigen evolution is strongly influenced by the selective pressures exerted by neutralizing responses, similar to influenza's antigenic drift.<sup>19,20</sup>

Since the 1950s, the term OAS has been used interchangeably with other terminologies such as antigen imprinting, immunological imprinting, and immune imprinting to describe the lifelong biased reactivity toward the original antigen. Importantly, the intended terminology stemmed from epidemiological observations of differential antibody reactivity shaped by both



exposure history and age, as highlighted in several excellent reviews.<sup>25–29</sup> Several additional concepts are complementary and peripheral to immune imprinting. For instance, antigenic seniority denotes the quantitative hierarchal nature of antibody induction, wherein the highest titers are observed against antigens encountered earlier in life with progressively lower titers against subsequent exposures to antigenically related viruses (Figure 1c). Similarly, the term back-boosting describes an increase or “boosting” of cross-reactive recall responses to conserved epitopes between the ancestral and latest viral exposure, rather than the induction of *de novo* antibody synthesis toward variant-specific epitopes (Figure 1c). Conversely, back-boosting reflects the immune system’s propensity for inducing cross-reactive antibodies with each infection. Indeed, recall responses can be favored over *de novo* antibody production, as MBCs can be activated with significantly lower antigen concentrations compared to naïve B cells, despite the latter having higher affinity for the immunizing antigen, underlying the basis of immune imprinting effects.<sup>30–33</sup> Furthermore, antibody feedback mechanisms can steer the antibody response and restrict the generation of *de novo* responses with similar specificities. This can occur through antigen sequestering (clearance) by established cellular or humoral responses following exposure to closely related strains.<sup>30,32,34–39</sup> One example is epitope masking, where existing antibodies bind to conserved but non-neutralizing epitopes, promoting antigen clearance. This mechanism restricts novel antigen interactions with memory or naïve B cells and hinders the induction of *de novo* antibody synthesis against similar epitopes.<sup>34–36,40–42</sup>

While much attention for deciphering the mechanisms that drive immune imprinting has focused on humoral immunity, T cell-mediated immunity also plays a crucial role. Indeed, recent evidence highlights the role of T cells in shaping antibody responses. Richards et al. show that reduced CD4<sup>+</sup> T cell responses in individuals with recurrent exposures to influenza can lead to diminished protective antibody responses.<sup>37</sup> Regulatory T cells induced by the initial exposure can reduce the amount of available antigen for presentation to APCs, thereby suppressing activation of naïve B cells and the synthesis of variant-specific *de novo* antibodies.<sup>38</sup> Moreover,

Schiepers et al. show that memory T cells assist B cells in targeting subdominant epitopes to access secondary germinal centers (GCs) following boosting with a homologous antigen, even in the presence of preexisting antibodies.<sup>39</sup> Thus, this evidence highlights the interplay between humoral and cellular compartments in favoring imprinting responses, which can be further exacerbated by limiting the amount of antigen available following recurrent exposures to antigenically similar variants.

### **Immune imprinting in recurrent exposures to influenza**

The skewing of memory recall responses to a restricted range of epitopes from the earliest infections has been shown to suppress the induction of variant-specific *de novo* responses, thereby increasing an individual's susceptibility to new infection with antigenically distant viruses.<sup>43,44</sup> This phenomenon of immune imprinting has been described in detail for influenza.<sup>25–29</sup> Multiple studies have shown that the immune response is biased toward influenza strains encountered earlier in life and that during ensuing exposures to antigenically related strains, antibody production toward the ancestral strain predominates. Lessler et al. observed antigenic seniority for influenza in a cross-sectional study where individuals had higher antibody titers against strains encountered earlier in life rather than against strains from recent infections.<sup>44</sup> Back-boosting of neutralizing antibodies against preceding influenza strains following subsequent influenza exposures was observed in a longitudinal analysis of the Framingham Heart Study data.<sup>43</sup> Other studies have also confirmed these findings, reporting an increase in antibodies against previously encountered antigenically related strains following both influenza infection and vaccination.<sup>19,30,43,45–50</sup>

In immune imprinting, back-boosting coincides with a reduction in the *de novo* response to newer strains, with much of the recent evidence arising after the 2009 H1N1 pandemic. Choi et al. determined that vaccination for the 2009 pandemic H1N1 influenza virus induced relatively lower antibody titers among individuals who were vaccinated with the seasonal H1N1

vaccine three months prior.<sup>51</sup> Recalled cross-reactive antibodies against seasonal H1N1 strains were shown by Andrews et al. to block the development of broadly neutralizing antibodies against the HA stalk following vaccination against the 2009 pandemic strain through epitope masking.<sup>19</sup> Imprinting was shown to decrease protection against antigenically related influenza strains and worsen disease outcomes. During the 1918 Spanish flu and 2009 H1N1 pandemics, the mortality rate was higher for those exposed to discordant subtypes in early childhood.<sup>26</sup> Mortality rates varied similarly according to age (and therefore exposure profile) in the 1957 H2N2 pandemic.<sup>52</sup> Similarly, vaccine effectiveness against H1N1 was impaired for middle-aged and older adults during the 2015–2016 flu season when the circulating H1N1 influenza strain acquired a new glycosylation site.<sup>53</sup> Furthermore, reduced protection from vaccination in the 2018/2019 H3N2 epidemic was associated with age and prior exposure to a mutated H3N2 strain during the 1968 pandemic.<sup>54</sup>

Although much attention has been focused on the detrimental impacts of imprinting, it does not solely impair immune function.<sup>25</sup> Indeed, older individuals may benefit from increased protection and lower mortality rates compared to immunologically naive individuals due to immune imprinting.<sup>55</sup> These beneficial impacts of immune imprinting, acquired from infections encountered even several decades earlier, can persist throughout an individual's lifetime. Birth cohort studies reporting increased titers against influenza strains encountered earlier in life suggest that imprinting reinforces immune memory against earlier strains upon exposure to antigenically related strains throughout life.<sup>26</sup> A multi-country study by Gostic et al. found that individuals initially infected with influenza A group 1 (H1, H2, and H5 seasonal subtypes) or group 2 (H3 and H7 seasonal subtypes) had a relatively low risk of mortality and severe illness from subsequent infections with the corresponding HA subtypes.<sup>25,26,46</sup> This protection likely results from antigenic seniority and the back-boosting of cross-reactive antibodies targeting conserved regions between the initial and infecting virus of the same HA subtype. In accordance with this evidence, epidemiological studies have reported lower mortality rates in the 2009

H1N1 pandemic for older generations exposed to the 1918 H1N1 Spanish flu compared to younger generations.<sup>56</sup> For example, in China, 92% of hospitalizations and 77% of deaths resulting from 2009 H1N1 infections occurred in individuals under the age of 50. Similarly, during the 1918 pandemic, older individuals had lower mortality rates relative to younger individuals.<sup>46</sup> These observations indicate that imprinting induces a strong recall response toward the initial viral exposures and the induction of cross-reactive antibodies through back-boosting. This may confer improved protection for older individuals as they are exposed to antigenically similar viruses across their lifetime, thereby strengthening immunity against the initial virus.

### **Effect of previous HCoV infections on the induction of SARS-CoV-2 humoral responses**

Early in the SARS-CoV-2 pandemic, some studies suggested that prior exposure to human seasonal coronaviruses (HCoVs) 229E, HKU-1, NL63, or OC43 could negatively impact the development of humoral responses specific to SARS-CoV-2.<sup>57–60</sup> The shared sequence homology between HCoVs and SARS-CoV-2 hinted at the potential for immune imprinting. Indeed, back-boosting of antibodies cross-reactive to spike proteins of HCoVs has been observed following SARS-CoV-2 infection and vaccination.<sup>57,58,60,61</sup> For example, Yin et al. determined that COVID-19 vaccination was associated with increased titers against OC43 and HKU-1. Other studies report that SARS-CoV-2 infection also stimulated the production of antibodies against OC43 and HKU-1.<sup>60,62,63</sup> However, the literature on the clinical implications of immune imprinting from prior exposure to HCoVs on SARS-CoV-2 immunity is nuanced. Some evidence indicates that such imprinting may worsen clinical outcomes of SARS-CoV-2 infection.<sup>57–60</sup> Indeed, Aguilar-Bretones et al. reported an expansion of MBCs specific for HCoV-OC43 with limited cross-reactivity to SARS-CoV-2 in severely ill SARS-CoV-2 patients, but not in mildly ill patients.<sup>57</sup> Furthermore, they showed relatively low IgG antibody reactivity and neutralization to

SARS-CoV-2 in these patients with severe COVID-19.<sup>57</sup> In contrast, Guo et al. observed that disease severity correlated with a greater induction of back-boosting antibodies against HCoV-OC43, which were also cross-reactive with SARS-CoV-2.<sup>58</sup>

Additionally, some researchers have demonstrated a link between immune imprinting and post-acute sequelae, also called post-COVID-19 condition (PCC).<sup>59</sup> In convalescent SARS-CoV-2 patients with underlying rheumatic disease, those who developed PCC had reduced antibody reactivity to SARS-CoV-2 and increased reactivity to HCoV-OC43 compared to those who did not develop PCC.<sup>59</sup> In turn, Galipeau et al. suggest that the relative ratio of prior antibody responses against all four HCoVs, rather than the absolute titer against a specific HCoV, correlated with increased SARS-CoV-2 neutralization.<sup>64</sup> However, some studies did not observe a correlation between back-boosting of HCoV antibodies upon infection with SARS-CoV-2 and more severe disease.<sup>60,61</sup> This contrasts with some influenza studies that reported the potential detrimental effect of immune imprinting.<sup>25,26,43,44,51</sup> In particular, Anderson et al. and Aydililo et al. report back-boosting of antibodies against HCoVs following SARS-CoV-2 infection in hospitalized adult patients, but this had no bearing on disease severity.<sup>60,61</sup> However, Aydililo et al. noted a delay in *de novo* antibody production against SARS-CoV-2 in severely ill patients.<sup>60</sup> Although these findings reveal conflicting perspectives on the impact of prior seasonal HCoV infections on disease severity following SARS-CoV-2 infection, they suggest the involvement of complex immune imprinting influences through the induction of back-boosting antibodies to these HCoVs.

### **The dynamics of serum antibody responses and the antigenic distance hypothesis**

The extent of immune imprinting and antibody reactivity toward subsequent infections appears to be fundamentally dependent on the antigenic relatedness between the primary and subsequent antigens, as first postulated by Smith et al.<sup>65</sup> Antigenic distance between two antigenically similar viruses can either enhance or hinder immune protection against disease.<sup>65</sup>

When the level of relatedness between the primary and secondary strains is high, significant back-boosting of antibodies can occur (Figure 1b). Although this could still protect from infection when back-boosted antibodies still effectively neutralized the circulating variant strain. Indeed, Hensley et al. eloquently demonstrated that mice heterologously boosted with an antigenically related influenza variant, which differed by 13 amino acid substitutions relative to the priming variant, preferentially induced recall antibody responses.<sup>66</sup> Although approximately 55% of isolated monoclonal antibodies were specific to the priming antigen, adoptive transfer of serum antibodies following boosting still protected mice from infectious challenge with the secondary strain.<sup>66</sup> In contrast, when circulating variants acquire immune escape mutations but are still sufficiently antigenically similar to the primary exposure, recall responses are favored at the expense of *de novo* antibody synthesis and to the detriment of protection as discussed above.<sup>19,30,43,45–50</sup> However, immune imprinting can be overcome by boosting with more distantly related viral antigens, as shown in several mouse studies.<sup>41,67–69</sup>

The proportion of *de novo* serum antibodies synthesized relative to those produced through recall responses, and the compounding effect of subsequent antigen re-exposures have been challenging to study (Figure 1c). Recently, Schiepers and colleagues employed an eloquent molecular fate-mapping technique to decipher antibody kinetics and their cellular origins following repeated antigen exposures.<sup>41</sup> In transgenic mice primed either by infection or vaccination with messenger RNA-Lipid Nanoparticle (mRNA-LNP), they distinguished antibodies generated from initially primed MBCs and those from *de novo* synthesis. In a prime vaccination followed by a two-dose boost regimen with the ancestral SARS-CoV-2 spike vaccine (homologous boosting), Schiepers et al. observed a 55-fold suppression of *de novo* B cell responses as a result of dominant recall responses. However, a heterologous boost with the Omicron BA.1 spike mRNA vaccine, Omicron BA.1 being a variant of SARS-CoV-2 that shares 91% sequence in the RBD with the ancestral strain, resulted in a partial alleviation of immune imprinting. In particular, they observed only a 3.6-fold suppression of *de novo* antibody

responses with the heterologous boost. Following the heterologous boost, Schiepers et al. detected new BA.1-specific B cell clones that were not cross-reactive with the ancestral strain and that showed greater neutralization against the BA.1 variant specifically. Furthermore, by varying the amino acid sequence of influenza boosting antigens, they experimentally demonstrated that immune imprinting is a function of antigenic distance between the primary and subsequently encountered antigens.<sup>41,67,68</sup> Collectively, these findings suggest that immune imprinting can be alleviated when the boosting antigen is sufficiently distinct from the priming antigen.

### **B cell activation outcomes and the dynamics of germinal center reactions**

Given the role of GC reactions in shaping the diversity of the humoral response, understanding B cell clonal selection and expansion following primary or repeated antigen exposures is critical for dissecting the fundamentals of immune imprinting (Figure 2). In GCs, activated B cells will undergo iterative rounds of somatic hypermutation, where only the clones expressing the highest affinity receptors to the antigen are selected for clonal expansion by receiving survival signals from T follicular helper cells.<sup>70</sup> Some of these affinity-matured clones make their way into circulation, serving as a source of antibodies or as MBCs (Figure 2). However, a portion of MBC clones will undergo further affinity maturation, leading to inter-clonal competition and a progressive loss of MBC clonal diversity given that selection favors clones with the highest antigen affinity to immunodominant epitopes (Figure 2).

Immunodominant epitopes for SARS-CoV-2 and influenza viruses are frequently detected in the RBD and RBS, compared to the relatively immuno-subdominant stem or stalk, respectively (Figure 1b). This immunogenicity could result from their increased epitope exposure compared to the more sheltered regions of the stem or stalk, repetitive epitope display, or specific amino acid sequences leading to improved B cell receptor cross-linking.<sup>19,20,71</sup> Nevertheless, GC reactions concurrently lead to the breadth of the humoral response. Through lineage tracing

experiments, Hagglof et al. demonstrated that ongoing GCs continuously recruit activated B cells and up to 30% of late-stage GCs can consist of clones with limited rounds of mutations and low antigen affinity.<sup>72</sup> Their findings suggest that as the GC reactions develop, antibody-mediated feedback can favor the entry and expansion of B cells specific for non-dominant epitopes, thereby contributing to the maintenance of antibody diversity. Indeed, following primary COVID-19 mRNA vaccination in humans, Weber et al. detected early exported MBCs with limited clonal affinity maturation.<sup>73</sup> These MBCs exhibited broad neutralization against currently circulating viral variants, emphasizing the diversity of the early humoral repertoire.

Recent evidence highlights that mRNA-LNP vaccines induce potent GC responses in both mice and humans, which have important implications for vaccine design.<sup>15,74–82</sup> However, the mechanisms by which the distinct mRNA and LNP components of mRNA-LNP vaccines promote robust GC reactions remain largely undefined. Recent findings by Bettini et al. and others suggest that both components promote the differentiation of T follicular helper cells and dendritic cells, thereby enhancing the overall magnitude of GC reactions compared to protein-subunit vaccines adjuvanted with LNP.<sup>74,80,81,83</sup> Moreover, studies from several groups suggest that prolonged antigen expression and availability following mRNA-LNP vaccination may contribute to enhanced and sustained GC reactions.<sup>77,80,82,84–87</sup> Indeed, comparisons between mRNA-LNP vaccines and protein subunit vaccines adjuvanted with LNP or other adjuvants highlight the potent adjuvant properties of LNP, which likely significantly contribute to the impressive immunogenicity of the mRNA-LNP vaccine platform.<sup>74,79–81,83,86,88,89</sup> Overall these findings suggest that the LNP component of vaccines has intrinsic adjuvant activity and is a major driver in promoting durable and protective antibodies through the combined induction of T follicular helper cells, long-lived plasma cells, MBC and prolonged GC reactions.<sup>15,74–82</sup> Although ongoing GCs have been detected over varying periods following SARS-CoV-2 exposure from natural infection or vaccination, these GC reactions are to shown drive clonal



diversification through antibody-mediated feedback to produce antibodies with sufficient breadth to neutralize a range of antigenic variants.<sup>36,77,87,90,91</sup>

Two alternative models exist to explain GC dynamics following re-exposure through subsequent infection or boosting. First, in the conventional model, affinity-matured MBCs can readily enter secondary or recall GCs for further affinity maturation to finetune their affinity for antigenically drifted viruses.<sup>15</sup> However, recent evidence highlights another potential model of GC dynamics and demonstrates a bottleneck in the recruitment of the diverse, affinity-matured MBCs into recall GCs, restricting entry to only a small portion of high-affinity immunodominant clones in mice and humans<sup>34,39,92</sup> (Figure 2). Mesin et al. demonstrate that recall GCs are mostly composed of activated B cells without prior GC experience (GC-naïve B cells) and a limited subset of dominant affinity-matured MBCs regardless of re-infection or boosting in mouse models. Their findings suggest that antibody-mediated feedback suppresses the recruitment of these affinity-matured MBCs, promoting the recruitment and diversification of GC-naïve B cells in recall GCs, thereby circumventing immune imprinting (Figure 2). Similarly, human lymph node analysis by Turner et al. showed substantial recruitment of GC-naïve B cells to recall GCs following vaccination with influenza despite prior exposures.<sup>93</sup> However, their results suggest a higher propensity for humans, relative to mice, for MBC entry into recall GCs, highlighting a greater potential for immune imprinting to occur. Yet, despite the large diversity of MBCs generated by each exposure, recall antibodies seem to consistently derive from a subset of dominant MBCs generated by the original exposure (Figure 2),<sup>8,41,73,94</sup> In a longitudinal analysis of the B cell repertoire in humans following both vaccination and subsequent Omicron infection, Weber et al. found that a substantial proportion of humoral responses stemmed from recall of the original cohort of MBCs produced following the primary vaccination series.<sup>73</sup> Thus, understanding dynamics of germinal center reactions and their role in the humoral response can have important implications for alleviating immune imprinting in the context of recurrent exposures to antigenically distinct viruses.

## **Influence of the vaccination platform in promoting or overriding immune imprinting**

Secondary viral infection and vaccination can result in varying intensities of immune imprinting. The immune system often favors recall responses over *de novo* synthesis given that MBCs can be activated with much lower antigen concentrations than naïve B cells. Indeed, these effects can be further exacerbated when the amount of antigen available during the secondary exposure is limited, favoring recall responses.<sup>30,32,34–39</sup> Several studies demonstrate this phenomenon by reducing the dose of protein subunit vaccines or infectious viruses. In the case of the latter, the humoral and cellular memory responses established during the initial exposure can suppress viral replication, thereby limiting the amount of viral antigens available to stimulate new responses.<sup>30–33,84,85</sup> In the case of protein subunit vaccination, the amount of administered antigen may be more susceptible to antibody feedback mechanisms, potentially exacerbating the detrimental effects of immune imprinting. In contrast, vaccine platforms that are self-replicating, such as some DNA or mRNA vaccines, may bypass preexisting antibodies and effectively overcome the constraints of immune imprinting mechanisms.<sup>27,68</sup>

Indeed, intramuscular administration of mRNA-LNP vaccines leads to uptake and synthesis of encoded antigens primarily by local cells at the injection site and the draining lymph node, with limited spread to other tissues.<sup>82,95,96</sup> APCs, such as monocytes, macrophages, dendritic cells, play a critical role in mRNA-LNP uptake, antigen production, and subsequent antigen presentation for robust induction of humoral and cellular responses.<sup>74,82,86,95,97</sup> Indeed, monocytes, macrophages and dendritic cells can directly uptake and efficiently translate the mRNA of encoded antigens, thereby driving the immune response through presentation of processed antigen on MHC class I and MHC class II molecules to produce both cellular and humoral responses, respectively. Muscle cells can also uptake and produce antigens and depending on the mRNA design, the synthesized antigen will either be membrane-anchored or

secreted.<sup>98,99</sup> Additionally, mRNA-LNP can be passively transported to draining lymph nodes, although this seems to depend on particle size and composition and needs further investigation.<sup>86</sup> While B cells have the potential to directly uptake and synthesize the encoded antigens, this appears to be less common compared to the direct uptake by macrophages, monocytes and dendritic cells.<sup>74,95,97,100</sup> Instead, B cells will likely interact with either secreted soluble antigens or processed antigens presented on the surface of APCs, which may influence the strength of receptor induced signaling, based on the degree of cross-linking.<sup>14,15,101</sup> In fact, membrane-bound antigens have been shown to be more efficiently recognized compared to soluble antigens.<sup>14,102–104</sup>

Anderson et al. demonstrate through absorption assays, that a first SARS-CoV-2 exposure through natural infection induced back-boosted antibodies with high cross-reactivity to the stem region of sCoV spike protein, contrasting with mRNA-LNP vaccination, which predominantly produced antibodies with higher affinity to the RBD of SARS-CoV-2.<sup>62</sup> Furthermore, studies by England et al. and Willis et al. demonstrated that mRNA-LNP vaccines against SARS-CoV-2 or influenza effectively overcame maternal antibody inhibition of *de novo* humoral responses in vaccinated mouse pups.<sup>79,83</sup> Although Willis et al. showed limited *de novo* responses following a live attenuated influenza vaccine (LAIV) or inactivated vaccine immunization in presence of maternal antibodies, England et al. demonstrated that adjuvanted protein subunit vaccines effectively evoked *de novo* responses despite the presence of the maternal antibodies, with mRNA-LNP vaccines inducing the highest neutralizing titers.<sup>79,83</sup> Indeed, the inclusion of adjuvants during initial or secondary vaccination has been shown to alleviate the effects of immune imprinting. Kim et al. showed that adjuvants which preferentially stimulate dendritic cells, such as CpG ODN, Bordetella pertussis toxin, or squalene-based oil-in-water, elicit strain-specific *de novo* total and neutralizing antibody responses.<sup>68</sup>

In contrast, viruses and VLPs can bind to B cells with high affinity and avidity by crosslinking BCRs with repetitive antigens displayed on their surfaces, thereby enhancing B cell responses and retention in the lymph nodes.<sup>100,105</sup> Cohen et al., as well as others groups, have demonstrated that immunization with heterotypic VLPs, which simultaneously display RBDs from eight different SARS-like beta coronaviruses (sarbecovirus), promote crosslinking interactions with high avidity to subdominant, conserved regions of the adjacent RBD antigens.<sup>106–110</sup> This leads to the induction of broadly cross-reactive and neutralizing responses compared to homotypic VLPs that display only one type of RBD antigen. Furthermore, through fate mapping experiments discussed above, Cohen et al. demonstrate that boosting with heterotypic VLPs induces *de novo* antibody synthesis in mice and non-human primates previously primed with two to four doses of DNA, mRNA, or viral vectored SARS-CoV-2 vaccines.<sup>107</sup> Additionally, serum antibody epitope mapping using deep mutational scanning revealed that boosting with heterotypic VLPs, relative to the homotypic VLPs, elicit antibodies with preferential binding to conserved epitopes of the RBD. These epitopes exhibit low variability among sarbecoviruses and SARS-CoV-2 variants. In contrast, antibodies induced by homotypic VLPs vaccination primarily targeted epitopes on the apex of the antigen. Although more accessible, these epitopes of the RBD are more variable likely due to greater selective pressures, consistent with findings by several groups.<sup>107,108,111–114</sup> This highlights the potential of immunization with heterotypic VLPs or “universal vaccines” as a strategy to mitigate the effects of immune imprinting following recurrent infections with antigenically distinct variants.

### **Immune imprinting following recurrent exposures to SARS-CoV-2 antigens through natural infection or vaccination**

The extensive characterization of population immunity following recurrent exposures to SARS-CoV-2 through booster vaccinations or breakthrough infections has provided immunologists with a unique opportunity to dissect the effects of immune imprinting in the

context of SARS-CoV-2. The continuous circulation and transmission of SARS-CoV-2 has led to the emergence of variants of concern (VOC), characterized by substantial antigenic variation, evolved fitness advantages and distinguished from other viral variants by their increased risk of causing severe COVID-19 disease. These changes are primarily driven by selective pressures arising from recurrent vaccinations and infections.<sup>19,20,115,116</sup> Notably, the efficacy of ancestral SARS-CoV-2 mRNA-LNP and viral vector vaccines was reduced against the Beta variant, showing more than a ten-fold reduction in neutralizing titers in sera from vaccinated donors.<sup>117–119</sup> Breakthrough infections with the Alpha or Delta variants resulted in a greater increase in antibody titers against the ancestral strain compared to the VOC strain in individuals vaccinated with three doses of ancestral mRNA-LNP, highlighting the effects of immune imprinting.<sup>87,120</sup> Meanwhile Zar et al. show antibody back-boosting to ancestral, Beta, Delta or Omicron variants without a clear hierarchical order.<sup>121</sup> Efforts to enhance vaccine efficacy by updating vaccines have led to improved VOC neutralization. However, individuals previously vaccinated with the ancestral mRNA vaccines showed dominant recall antibody responses following monovalent Beta or Delta boosters, or bivalent ancestral and Beta/Delta boosters.<sup>8,122,123</sup> In contrast, boosting with a bivalent ancestral and Beta mRNA vaccine, relative to boosting with the ancestral vaccine, led to a reduction in symptomatic infection from 10.7% to 3.4%.<sup>123</sup>

Following the emergence of Omicron and its sub-variants, which possess a greater number of immune escape mutations over earlier variants, varying effects of immune imprinting have been observed.<sup>115</sup> Omicron breakthrough infections predominantly promoted recall responses, leading to reduced neutralization of Omicron variants. Although some *de novo* antibody synthesis has been observed, it remains at a low level.<sup>8,124,125</sup> Vaccination with an Omicron-specific mRNA-LNP booster has been shown to produce *de novo* antibody responses and superior neutralization against Omicron variants compared to individuals boosted with the ancestral vaccine, which demonstrates the benefits of updating the vaccine to match circulating variants.<sup>123,126–129</sup> However, conflicting evidence reported from some studies indicate no

significant differences in neutralizing antibody titers between those boosted with the Omicron bivalent vaccine or the ancestral vaccine, highlighting potential effects of immune imprinting.<sup>130–134</sup> Whereas, Alsoussi et al. demonstrated that in individuals previously vaccinated with the ancestral SARS-CoV-2 mRNA vaccine, variant-specific *de novo* B cell responses were only induced with Omicron-specific boosting, and not with the ancestral or bivalent Beta/Delta variant boosters (which shared 98.7% and 99.1% sequence conservation with the ancestral RBD, respectively).<sup>8</sup> Although being of low frequency, these clones targeting BA.1 variant-specific epitopes were derived from GC-naïve B cells recruited into recall GCs of the draining lymph nodes following heterologous boosting. Yisimayi et al. demonstrate that boosting with two doses of Omicron protein subunit or mRNA vaccines induced robust variant-specific *de novo* humoral responses in both mice and individuals previously immunized with ancestral SARS-CoV-2 viral vector vaccines.<sup>67</sup> These findings suggest that priming with inactivated viral vector vaccines may lead to a reduced degree of immune imprinting compared to mRNA vaccines, potentially due to the stronger immune response elicited by initial mRNA vaccination. Interestingly, breakthrough infections in individuals with diverse exposure histories from vaccination and infection, have been shown to increase the breadth of neutralizing antibody responses against previous and circulating VOCs, as well as elicit *de novo* responses.<sup>120,121,124,125</sup> In fact, Hoffman et al. demonstrated that boosting with ancestral and BA4.5 bivalent vaccines produced enhanced neutralizing responses against circulating Omicron variant in individuals who had previously received two doses of ancestral mRNA-LNP vaccines and subsequently experienced a breakthrough infection. However, the highest neutralizing titers were observed against the ancestral strain, highlighting the effects of immune imprinting.<sup>124</sup> These findings collectively suggest that Omicron-specific booster vaccinations may partially alleviate the effects of immune imprinting caused by repeated exposures to early SARS-CoV-2 variants. However, their findings also highlight that maintaining the ancestral antigen in the bivalent booster vaccine may favor immune imprinting, supporting the collective decision to

proceed with monovalent SARS-CoV-2 variant specific booster roll-out. Indeed, monovalent XBB.1.5 boosters administered to individuals with a prior vaccination history markedly increased serum neutralizing antibody titers against the XBB.1.5 variant, as well as against the more recently emerged EG.5.1 and JN.1 variants.<sup>135,136</sup>

However, these studies emphasize the persistence of immune imprinting, given that the highest neutralizing responses were observed against the ancestral variant. Notably, Tortorici et al. showed that depletion of antibodies that are cross-reactive with the ancestral antigen abrogated the observed neutralization of XBB1.5, suggesting that mRNA vaccination with the XBB1.5 booster primarily induces recall responses.<sup>137</sup> However, Johnston et al. demonstrated the development of variant-specific *de novo* humoral responses, albeit at a low frequency, following boosting with the XBB1.5 monovalent mRNA vaccine in individuals who were previously vaccinated with ancestral mRNA vaccines and experienced breakthrough infections.<sup>135</sup> Indeed, these studies evaluating neutralizing responses following vaccination with updated booster vaccines highlight the persistence of immune imprinting, which can be reduced when the boosting antigen is sufficiently distinct from ancestral SARS-CoV-2. Therefore, comprehensive evaluations of antigenic distance scores between primary and subsequent viral variants, while comparing various vaccine platforms, may help predict their ability to promote recall or *de novo* antibody responses. When interpreting these studies, it is crucial to consider variables such as 1) differences in antigen presentation by different vaccine platforms (and therefore their recognition by the immune system), 2) antigenic distance between primary and subsequent antigens, and 3) the nature of primary exposure and subsequent re-exposures (natural infection or vaccination).

While several comparisons between influenza and SARS-CoV-2 studies regarding immune imprinting have been discussed, it is important to approach these interpretations with caution due to inherent differences in their virology, epidemiology, and vaccination approaches.

Furthermore, this review highlights the importance of investigating whether different vaccine platforms induce varying degrees of immune imprinting following repeated exposures. Collectively, these findings suggest that boosting with a significantly distinct antigen is paramount in steering the humoral immune response away from favoring recall and toward *de novo* responses that target variant-specific epitopes.

## **Conclusion**

Given the role of immune imprinting in shaping humoral responses following recurrent exposures to antigenically distant viruses, its impact should be thoroughly considered when designing vaccination strategies. The evolving landscape of SARS-CoV-2 and the emergence of variants with immune escape mutations have prompted multiple updates to COVID-19 vaccines. These updates aim to improve vaccine efficacy against circulating variants, similar to seasonal updates for influenza vaccines. Recent studies on SARS-CoV-2 and influenza have shown that boosting with sufficiently antigenically distinct vaccine promotes the induction of variant-specific *de novo* responses that are less cross-reactive with previously encountered antigens. Indeed, some of the next-generation vaccine strategies focus on developing universal vaccines, which induce both variant-specific and broadly protective cross-reactive responses by simultaneously expressing multiple surface glycoproteins from distinct influenza subtypes or coronaviruses.<sup>107,138</sup> Ultimately, the effectiveness of these vaccines will depend on their capacity to overcome the constraints of immune imprinting and address age-related differences in disease risk, which are ultimately shaped by individual's distinct infection histories. A comprehensive understanding of immune imprinting is essential for guiding vaccine design and maximizing vaccine effectiveness against both current and future viral infections.



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## Figures

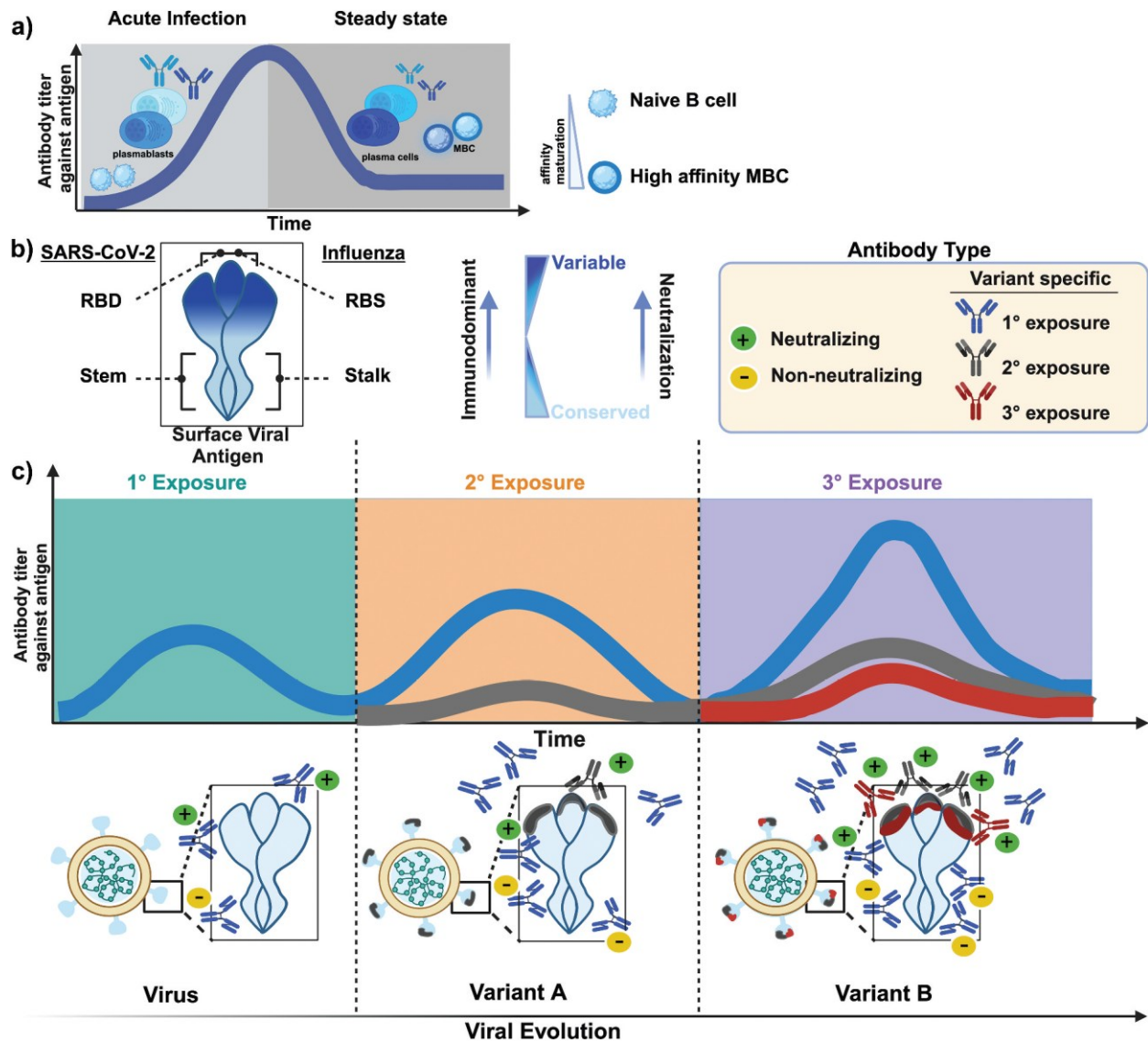


Figure 1. The dynamics of antibody responses following primary and subsequent infections with antigenically distant viruses. a) schematic illustration depicts the dynamics of the humoral response following infection or vaccination. Initially, after the first exposure, a portion of activated B cells differentiate into plasmablasts, which provide an immediate but short-lived source of low-affinity antibodies to limit acute infections. As the humoral response progresses, more specialized responses generate long-lived plasma cells and memory B cells (MBC) which persist at steady state. These cells will rapidly proliferate upon a reencounter with the antigen and become the dominant source of antibodies for subsequent exposures. b) schematic illustration of SARS-CoV-2 and influenza surface viral proteins highlighting the receptor-binding domain (RBD) and/or the receptor-binding site (RBS) as well as the stem and stalk domain, respectively. The gradient represents the more variable, immunodominant domain (dark blue) relative to the more conserved domain (light blue) of the surface viral protein. The

RBD/RBS contains numerous immunodominant epitopes and is an important neutralization target given its role in cell-mediated entry. c) the kinetics of antibody responses following primary and subsequent infections with antigenically distant viruses are depicted. The induced humoral response may compromise both recall and *de novo* responses, depending on the antigenic similarity and cross-reactivity between the infecting variant and previously encountered variants. Primary exposure to an antigen elicits a polyclonal antibody response consisting of both neutralizing and non-neutralizing antibodies. Each subsequent exposure to an antigenically distant viral variant significantly boosts antibodies targeting conserved epitopes while also inducing *de novo* variant-specific neutralizing responses, albeit at much lower magnitudes. Exposure to an antigenically distinct antigen (variant B) alleviates immunological imprinting and promotes *de novo* responses targeted to variant-specific epitopes.

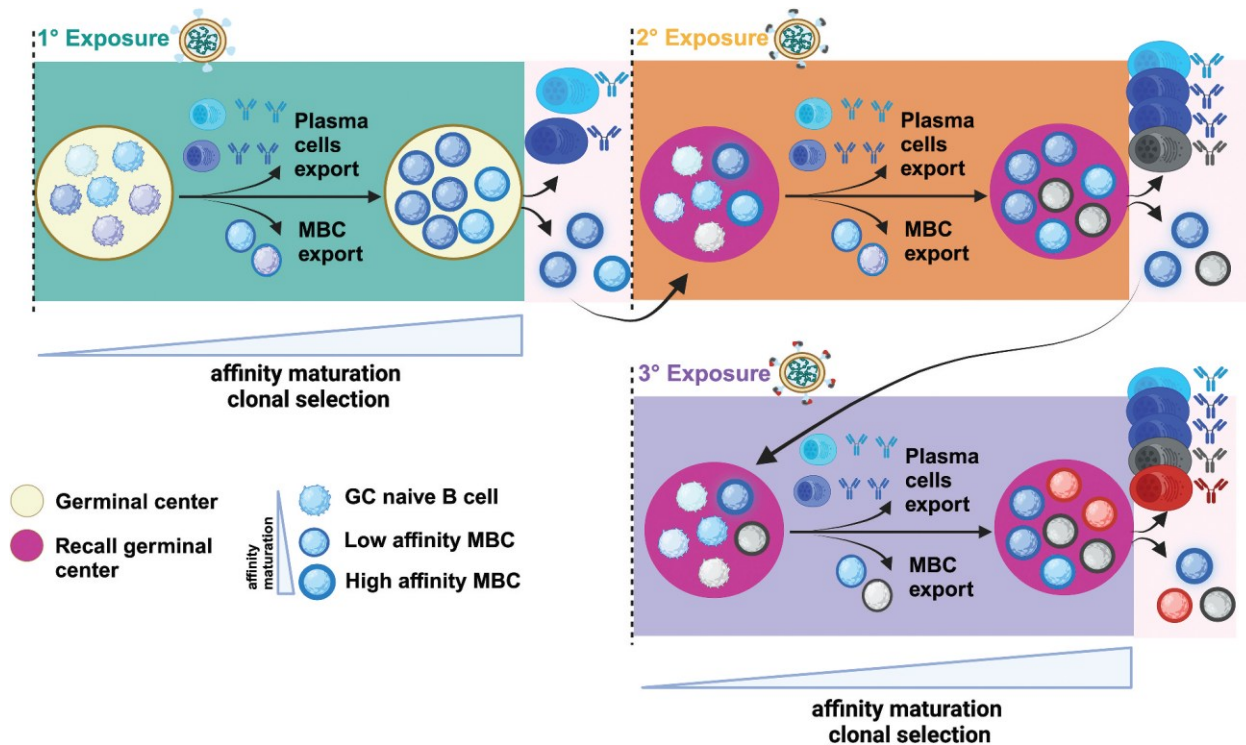


Figure 2. B cell activation outcomes and dynamics of germinal center (GC) reactions following primary and secondary infections with antigenically distant viruses. The schematic illustration depicts the dynamics of GC reactions following primary and secondary infections with antigenically distant viruses. Activated B cells form GCs where they undergo isotype switching, somatic hypermutation, and affinity maturation. Only clones expressing the highest affinity receptors to the antigen are selected for clonal expansion by receiving survival signals from T follicular helper cells. Some of these affinity-matured clones make their way into circulation, serving as a source of antibodies or as low-affinity MBCs. However, a portion of MBC clones will undergo further affinity maturation, leading to a progressive loss of clonal diversity as selection favors clones with the highest antigen affinity to immunodominant epitopes. These high-affinity B cells can differentiate into plasma cells or into MBCs. Following re-exposure through subsequent infection or vaccine boosting, recall GCs are mostly composed of activated B cells without prior GC experience (gc-naïve B cells) and a limited subset of dominant affinity-matured MBCs.

## Chapter 8 – Dissertation Discussion

The COVID-19 pandemic was one of the largest public health crises of the 21<sup>st</sup> century. The emergence of the novel SARS-CoV-2 virus caused extraordinary harm, worsened psychological wellbeing, increased financial difficulties, resulted in substantial disease burden and the deaths of millions of people, and caused the estimated global life expectancy to decrease for the first time since tracking began in 1950.<sup>82–85</sup> The development of COVID-19 vaccines was a vital step in improving population immunity, ending the COVID-19 pandemic and averting millions of deaths.<sup>22,40</sup> The rapid development and rollout of COVID-19 vaccines speak to the unprecedented efforts of medical researchers and healthcare workers around the world and the mobilization of the scientific community to respond to this global threat to public health.

This thesis encompasses six studies completed as a modest part of this global effort to combat the COVID-19 pandemic. Observational research was urgently needed to evaluate the long-term humoral immune response following the administration of novel COVID-19 vaccines in a real-world setting and among vulnerable clinical groups excluded from phase 2/3 RCTs. This goal was met through the completion of the component articles presented in **Chapter 2** to **Chapter 7**. We conducted three studies aimed at characterizing COVID-19 vaccine humoral immunogenicity in vulnerable clinical groups with a heightened risk of SARS-CoV-2 infection and/or severe clinical outcomes of COVID-19, including hospitalization and death: the immune compromised; the obese; and excessive alcohol drinkers. Additionally, we generated knowledge to address a key gap in the scientific literature regarding the impact of SSRI medication use of COVID-19 vaccine humoral immunogenicity and the odds of developing PCC. We also published a review exploring the phenomenon of immune imprinting and its impact on humoral immunity against SARS-CoV-2 and influenza. Our findings inform COVID-19 vaccination



recommendations and will support the public health response to future coronavirus epidemics and pandemics.

## **Summary of Research**

### *Results of the Stop the Spread Ottawa Cohort Study*

The results of the first component article (**Chapter 2**) informed all subsequent studies completed as a part of this thesis. The demographic and clinical profiles of the SSO cohort (n=1,034) as well as the overall humoral immunogenicity of COVID-19 vaccination in this cohort were described. The SSO cohort is primarily white (89.0%), female (66.6%), born in Canada (87.1%), employed (83.2%) and of middle or high SES (65% earned at least \$90,000 CAD per year) at baseline. A high proportion of participants (63.0%) were engaged in public facing occupations. A wide range of ages were represented. Over a third of participants reported two or more comorbidities. Consistent with other studies identifying older age and obesity as risk factors for COVID-19, we observed that SARS-CoV-2 infection at baseline was associated with older age and obesity.<sup>86,87</sup>

The humoral response to vaccination was robust in the majority of participants (~97.7% of 930 participants were seropositive for anti-S and anti-RBD IgG 3 months after dose 2) but older age and immune compromised status were associated with a lower probability of testing seropositive after the first vaccine dose. Previous SARS-CoV-2 infection was associated with higher crude antibody titers across all time points analyzed. Predictors of impaired anti-S and anti-RBD IgG titers and neutralization activity after the second dose were identified by multivariable quantile regression. For participants with no history of SARS-CoV-2 infection, older age and high-level immune compromising conditions were associated with impaired antibody production 3 months after the second dose, whereas a wider time interval between the first and second vaccine doses was associated with higher antibody production 3 and 6 months after dose two. COVID-19 vaccination was safe and well tolerated. Reactogenicity symptoms

were consistent across three doses and included, in descending order of prevalence, fatigue, injection site pain, headache, fever/chills and body aches.

Similar findings are reported by other studies in the literature. There is currently a large body of evidence demonstrating impaired humoral immunogenicity of COVID-19 vaccines among older adults regardless of vaccine type and study design (i.e., observational or experimental).<sup>88–94</sup> Impaired vaccine response among older individuals is likely due to immunosenescence.<sup>88</sup> Multiple review articles and studies also report a more robust humoral response for individuals with hybrid immunity compared to those with immunity derived from vaccination or SARS-CoV-2 infection alone.<sup>95–97</sup> Increased duration between the first and second doses of the COVID-19 vaccine was consistently associated with improved humoral response in multiple studies across a range of extended dosing interval values (e.g., 28 days vs fewer, >10 weeks vs <6 weeks, 89 days vs fewer, 8–16 weeks vs 3–6 weeks, >21 days vs 21 days).<sup>98–104</sup> The time interval between doses was restricted to 6 months or less in most studies; however, two studies observed higher antibody titers after vaccination when the first and second COVID-19 vaccine dose interval was extended beyond 6 months (44–45 weeks or 42–56 months) compared to shorter intervals.<sup>102,103</sup>

Differences in humoral immunogenicity have been noted according to COVID-19 vaccine type. mRNA vaccines are associated with higher antibody titers after dose 2 and dose 3 compared to AstraZeneca vaccines in multiple reports.<sup>105–109</sup> We adjusted for the combination of dose 1 and dose 2 vaccine types in our quantile regression models and but observed no association between the combinations of dose types and antibody titers after vaccination. As demonstrated by Lv et al., multiple studies reported comparable antibodies following homologous mRNA vaccination and heterologous AstraZeneca-mRNA vaccination.<sup>110</sup> This is consistent with our findings and suggests that boosting with mRNA vaccines may overcome deficits associated with homologous AstraZeneca vaccination and boosting.

Sex differences in the COVID-19 vaccine humoral response have been identified by multiple studies. Female sex is generally correlated with a more robust humoral response to COVID-19 vaccination.<sup>88,94,95,111–113</sup> For example, a systematic review of 15 studies found that most reported higher titer production following vaccination among females compared to males.<sup>111</sup> However, sex did not consistently predict antibody titers or neutralization activity following the first two COVID-19 vaccine doses in our study. In fact, compared to female sex, male sex was associated with higher anti-RBD IgG titers 6 months after dose 2 and higher neutralization activity 3 months after dose 2 among participants with no prior SARS-CoV-2 infection. Although not a consistent finding, this result was unexpected. As indicated by Bachmann et al., female sex may be associated with more rapid antibody decay over time than male sex.<sup>111</sup> Inferior antibody durability in females may have resulted in the observed lower absolute antibody titers at extended follow-up after second dose compared to males. Residual confounding due to unadjusted factors such as smoking status, may also be distorting our results.

Other clinical factors have been linked with lower antibody levels after COVID-19 vaccination, such as hypertension, cardiovascular disease, and smoking.<sup>99,114,115</sup> In our study hypertension and cardiovascular disease did not predict antibody titers after vaccination by univariable quantile regression analysis and were not included in multivariable models. A survey of 248 healthcare workers in Italy revealed no association between hypertension and antibody titers 7 days after vaccination; however, antibody titers were measured 7 days after the second dose and insufficient time may have been passed to generate the vaccine-elicited response corresponding to the second dose.<sup>116</sup> A rapid review published in 2022 found 17 of 23 studies which reported impaired antibody production and durability among current smokers<sup>117</sup> and a later systematic review in 2024 confirmed these findings.<sup>118</sup> Interestingly, the population-based longitudinal study COVIDENCE in the United Kingdom did not find an association between smoking and combined anti-S IgG, IgA and IgM antibodies before and after 2 doses of

AstraZeneca or Pfizer-BioNTech vaccines.<sup>99</sup> Our univariable analysis revealed that compared to non-smokers former smoking status predicted lower antibody titers and former/current smoking status predicted lower neutralization capacity among participants with no history of SARS-CoV-2 infection. However, smoking status was not included in our multivariable analyses due to power and overfitting considerations; therefore, unadjusted confounding prevents any inference regarding the impact of smoking on COVID-19 vaccine response.

As stated by Bylica et al., most research conducted to date on SARS-CoV-2 vaccine humoral immune response has been focused on specific clinical populations and there are a limited number of studies on healthy individuals.<sup>106</sup> In addition to informing the other studies completed in this dissertation, the results of this analysis serve an additional function in addressing this knowledge gap.

#### *Immune Compromising States and SARS-CoV-2 Vaccine Humoral Immunogenicity*

Immune compromised individuals remain disproportionately impacted by the COVID-19 pandemic and the continued circulation of variants of concern as the SARS-CoV-2 virus establishes endemicity; however, the long-term humoral immunogenicity of COVID-19 vaccines in the overall immune compromised population after multiple boosting doses remains incompletely understood.<sup>119</sup> Valuable work has been completed to characterize the immunogenicity of two or three COVID-19 vaccine doses among PLWH, those with a primary immune deficiency, malignancy patients, dialysis patients, individuals on immunosuppressants and/or with a history of transplant, or after three doses.<sup>49,51,120</sup> However, data concerning extended follow-up (6 months or longer) or dose four or five is more limited and the extent to which immune compromised individuals require continued boosting to maintain humoral immunity against SARS-CoV-2 is unclear.<sup>48</sup> In addition, there are numerous diseases and medications that may suppress immune function, many of which are represented in the SSO cohort. As a result, the immune compromised population—and the SSO cohort—is highly

heterogeneous in terms of the cause and level of immunosuppression.<sup>121</sup> Many studies conducted on the humoral response to COVID-19 vaccination among the immune compromised are focused on specific immune compromising diseases (e.g., hematological malignancy only) and therefore have limited generalizability to the overall immune compromised population for which vaccine policies and recommendations are created.

Utilizing the SSO cohort, we characterized the overall long-term humoral immune response to five COVID-19 vaccine doses in a diverse sample of immune compromised adults as well as the humoral immune response in specific subgroups defined by IC condition and/or medication (**Chapter 3**) to address these issues. We categorized immune compromising states present in the cohort into two broad categories of high-level or low-level severity based on a prior evidence in the literature of immunosuppressive effects. We observed by multivariable multilevel linear regression analysis with flexible modelling of time since vaccination that antibody response was impaired in high-level IC participants compared to low-level and non-IC participants and that titers decayed more rapidly for high-level IC participants than low-level and non-IC participants. Subgroup analyses revealed that participants with cancer, a history of organ/bone marrow transplant, receiving a systemic immunosuppressant, a rheumatological disease, or suppressed HIV exhibited impaired antibody production compared to NIC participants but participants with asthma/COPD requiring medication, Celiac disease, or diabetes did not. Effect modification by prior infection history was present.

Our findings are consistent with other studies investigating the impact of immune compromising states on the humoral response to COVID-19 vaccination, including: asthma/COPD<sup>122–124</sup>; Celiac disease<sup>125,126</sup>; malignancy<sup>98,127–129</sup>; transplant<sup>88,98</sup>; dialysis<sup>88,98</sup>; rheumatic disease<sup>98,130</sup>; autoimmune disease<sup>131,132</sup>; inborn errors of immunity<sup>98</sup>; inflammatory disease<sup>98,133</sup>; and diabetes<sup>134</sup>.

Our classification of high-level and low-level immune compromising states performed well for all subgroups analyzed, except for patients with suppressed HIV infection which were classified as LIC but exhibited impaired antibody production after the second COVID-19 vaccine dose. There is significant variability in the literature regarding the humoral immunogenicity of COVID-19 vaccines in this population. Low power and/or unadjusted differences in clinical characteristics between samples (e.g., nadir or current CD4 T cell count) may explain these findings obtained for patients with suppressed HIV infections.<sup>135</sup> In addition, impaired humoral response which we observed following dose 2 may be alleviated by additional boosting doses.<sup>51</sup> A 2023 systematic review of 30 studies reported lower seroconversion rates for PLWH compared to health controls after two COVID-19 vaccine doses but not the third dose.<sup>136</sup> Another systematic review of 23 studies published in 2025 reported no association between HIV infection and seroconversion following the third COVID-19 vaccine dose.<sup>135</sup> These observations do not directly contradict our findings and together with our results suggest that the reduced immunogenicity of COVID-19 vaccines may be present after initial doses but may not persist following multiple boosting doses. A more detailed review of the literature can be found in the Discussion section of **Chapter 3**.

Multiple studies demonstrate the benefits of repeated boosting doses for immune compromised individuals.<sup>127,137-140</sup> Although antibody titers are still impaired compared to immunocompetent individuals, additional boosting has been shown to improve antibody titers and seropositivity rates among IC individuals. For example, a third COVID-19 vaccine dose increased seropositivity rates from 39.4% to 66.6% among IC individuals in a sample of 2.7 million Israelis and from 56% to 98% for a sample of liver transplant recipients in Israel (n=61).<sup>127,137</sup> Repeated vaccine boosting is likely an important method for improving protection among IC individuals.

In summary, in this article we classified a diverse range of immune compromising states as high-level or low-level and confirmed that the high-level IC states represented in SSO experience a higher risk of impaired vaccine humoral immunogenicity up to five doses (e.g., cancer, organ/bone marrow transplant history, systemic immunosuppression, rheumatological disease). Patients with suppressed HIV may also experience reduced humoral immunogenicity following vaccination but additional research that incorporates more detailed clinical information (e.g., nadir and current CD4 T cell counts) and utilizes large samples is needed to elucidate trends in antibody titers following multiple boosting doses among PLWH. We generated findings that are applicable to both the general immune compromised population and to specific subgroups and inform COVID-19 vaccination recommendations for these vulnerable clinical groups.

#### *Obesity and SARS-CoV-2 Vaccine Humoral Immunogenicity*

Obese individuals are at a heightened risk of acquiring a SARS-CoV-2 infection and of experiencing poor clinical outcomes of COVID-19, such as hospitalization or death.<sup>59</sup> However, the impact of obesity on vaccine-elicited humoral immunity is unclear due to conflicting reports of both positive and negative associations between obesity and vaccine-elicited antibody titers. We completed a study to improve the understanding of COVID-19 vaccine humoral immunogenicity among obese individuals and to identify if obesity is associated with impaired anti-S and anti-RBD IgG, IgM, and IgA antibody production and long-term durability or anti-S neutralizing capacity. We conducted multivariable segmented Bayesian robust linear regression to compare baseline titers and the slope of titer production and decay between obese and non-obese participants. Random effects were included in regression models to adjust for autocorrelation in longitudinal data. No consistent influence by obesity was observed on the COVID-19 vaccine humoral immune response up to three doses. Hybrid immunity and sex were

identified as possible effect modifiers on the relationship between obesity and vaccine-elicited antibody titers.

A systematic review on obesity and antibody titer production following COVID-19 vaccination was published in 2022.<sup>141</sup> This review synthesized 11 studies and found an overall negative association between obesity and antibody titers after COVID-19 vaccination. A high degree of heterogeneity was noted and subgroup analyses according to vaccine type, days since vaccination, and prior SARS-CoV-2 infection at time of vaccination only partially explained the variation between study findings. This heterogeneity is present across the broader scientific literature on this topic. Our study is one of the few analyses utilizing a large sample published in the years following this review. An analysis of 108 university students in Spain provides further evidence of effect modification by sex on the association between obesity and the humoral response to COVID-19 vaccination.<sup>142</sup> Although limited by the cross-sectional nature of the analysis and small sample size, this study assessed body fat percentage, which may be a more reliable measure of obesity than BMI. A study of 389 adults in Mexico reported higher IgG antibodies among individuals with abdominal obesity after the first COVID-19 vaccine dose but lower IgG titers after the second dose.<sup>143</sup> Consistent with our analysis, no negative association between BMI and vaccine-generated antibodies was identified in a multicentric cohort of 5,470 healthcare workers in Italy.<sup>86</sup>

We longitudinally and prospectively characterized vaccine-elicited antibody titers in a large cohort of adults and produced critical knowledge to support evidence-based strategies to improve the protection of obese individuals from SARS-CoV-2 infection. Together with the results of Spiteri et al., our findings indicate that the administration of three COVID-19 vaccine doses produces comparable humoral response in obese individuals as non-obese individuals.



### *Alcohol Consumption and SARS-CoV-2 Vaccine Humoral Immunogenicity*

Individuals who drink alcohol excessively are at an increased risk of experiencing severe COVID-19.<sup>144</sup> Despite this, the scientific literature on the humoral immune response to COVID-19 vaccination for this vulnerable population is sparse.<sup>145</sup> The aim of this study was to generate knowledge to address this gap and determine the impact of excessive alcohol consumption (as defined by Canadian Centre on Substance Abuse and Addiction's 2023 guidelines) on COVID-19 vaccine humoral immunogenicity after four doses. This was accomplished through segmented multilevel linear regression analysis. Our analyses showed that baseline anti-S and anti-RBD IgG, IgA, and IgM titers and the rate of titer production and decay relative to each dose did not vary according to alcohol consumption. Our findings are in agreement with COVIDENCE, a population-based longitudinal study in the United Kingdom (n=9,101), which did not find an association between anti-S combined IgG, IgA and IgM antibodies before and after 2 doses of the AstraZeneca or Pfizer-BioNTech vaccines and alcohol consumption by multivariable analysis.<sup>99</sup>

Additional research has been conducted in Japan and Spain that is generally inconsistent with our results, although these studies report conflicting results. Alcohol consumption predicted lower anti-S IgG antibody titers and faster antibody waning after a second mRNA COVID-19 vaccine dose in a cohort of healthcare workers in Japan (n=2,964) but not after the third dose.<sup>146</sup> A more in-depth analysis by the same researchers comparing anti-S IgG titers between non-drinkers and regular alcohol drinkers following the administration of a second Pfizer-BioNTech dose (n=5,718) indicated that regular alcohol consumption was associated with lower estimated geometric mean titers (after adjustment for covariates) in a dose dependent manner.<sup>147</sup> Anti-S IgG titers 6 months after a second Pfizer-BioNTech dose were impaired among 94 healthcare workers for those who consumed alcohol compared to those who did not drink alcohol in unadjusted and adjusted analyses.<sup>148</sup> Similarly, univariable and multivariable

analyses identified an association between alcohol consumption and lower anti-S IgG titers 2-5 weeks after a second Pfizer-BioNTech dose among 2,015 healthcare workers in Japan.<sup>149</sup>

Crude peak anti-S IgG titers did not differ between alcohol consumption categories (none,  $\leq 7$  drinks/week,  $> 7$  drinks/week) for 200 healthcare workers in Spain after vaccination with two Pfizer-BioNTech doses.<sup>150</sup> However, excessive alcohol consumption (defined as  $> 7$  drinks/week) predicted lower anti-S IgG titers compared to no alcohol consumption by multivariable regression analyses after the first Pfizer-BioNTech vaccine dose but not after the second. The same findings were obtained when men and women were assessed separately by univariable regression analysis.

In agreement with our findings, daily alcohol consumption did not influence unadjusted anti-S IgG titers 3 months or 6 months after the second Pfizer-BioNTech vaccine dose for a smaller cohort of 337 healthcare workers in Japan.<sup>151</sup> Another study on a different small cohort of healthcare workers in Japan (n=187) showed that daily alcohol consumption was associated with lower crude anti-RBD IgG titers after a third Pfizer-BioNTech dose compared to no alcohol consumption.<sup>145</sup> However, the results from these two studies are limited by a lack of adjustment for confounding.

The results of unadjusted and adjusted analyses in these studies differed. Differences in confounding adjustment approaches and the presence of residual confounding may explain the contrasting results between our study and these analyses. The SSO cohort includes individuals living in the Ottawa region with a diverse range of social and clinical profiles and may not be generalizable to cohorts in Japan and Spain consisting only of healthcare workers.

Unlike our study, none of the other studies in the literature assessed COVID-19 vaccine humoral immunogenicity beyond 2 doses or analyzed the vaccine-elicited response for antibody isotypes other than IgG. To our knowledge, we generated the first analysis in North America focused on the impact of alcohol consumption on COVID-19 vaccine humoral immunogenicity

and did not find that excessive alcohol consumption influenced SARS-CoV-2 antibody baseline levels or anti-S or anti-RBD IgG, IgA, IgM increase or decay over time up to four doses after COVID-19 vaccination.

As stated in the Introduction of this thesis (Chapter 1, page 23), alcohol consumption patterns in Canada differ by age, gender, and urban or rural setting, race, education level, and household income.<sup>62,63</sup> The SSO cohort is not representative of the entire Canadian population and is skewed towards individuals who are female and White with high education or income levels. Our findings therefore may lack applicability to underrepresented groups who self-report excessive alcohol consumption, such as males, Indigenous, Southeast Asian, and Latin American individuals, rural residents, and those without postsecondary education or earning low income. More research is needed to fully characterize the impact of alcohol consumption on COVID-19 vaccine immunogenicity in diverse samples that are generalizable to other populations around the world. However, our results suggest that COVID-19 vaccination generates a robust response among individuals who consume excessive amounts of alcohol.

#### *SSRI Use and SARS-CoV-2 Vaccine Humoral Immunogenicity and the Risk of Post-COVID-19 Condition*

Similar to the topic of alcohol consumption, there is a paucity of information on the influence of SSRI medication use on the humoral immune response to COVID-19 vaccination despite evidence indicating that SSRI use may reduce the severity of COVID-19 and may be a protective factor against the development of PCC.<sup>67,152</sup> The aim of this study was to generate much needed knowledge in this area and determine if SSRI use influenced the production and retention of vaccine-elicited antibodies or the incidence of PCC following SARS-CoV-2 infection. This was achieved through multivariable multilevel linear regression analysis incorporating random effects and penalized cubic smoothing splines for time since vaccination and through multivariable penalized logistic regression with Firth's correction. Our findings indicate that

SSRI use had no impact on these outcomes: SSRI use did not predict antibody titers nor the rate of titer production and decay; the adjusted odds of PCC was similar for users of SSRIs, non-SSRI antidepressants or anxiolytics, and those not on any medication for anxiety or depression.

As the literature on this topic is very limited, there are few studies to which we can compare our results. To our knowledge, this is the first analysis on the influence of SSRI medication use on COVID-19 vaccine humoral immunogenicity. Our findings provide vital evidence regarding the influence of SSRI medication use on COVID-19 vaccination humoral immunogenicity and together suggest that the vaccine response generated among SSRI medication users is comparable to that of non-SSRI users. This novel information is pertinent to the many individuals around the world and in Canada who take an SSRI to manage depression or anxiety.<sup>153</sup>

We observed comparable incidence of breakthrough SARS-CoV-2 infections relative to vaccine dose number for SSRI users and non-SSRI users and comparable odds of PCC. The few observational studies and RCTs available on this topic are conflicted. Our study adds high-quality observation research to the limited literature.

Our approach has multiple methodological strengths compared to some of the current studies which were constrained by cross-sectional comparisons, a lack of a control group, and low power.<sup>68,154–156</sup> In addition, the SSO cohort has strong representation of young, white, females. These individuals are more likely to report mood or anxiety disorders and therefore are an informative population for assessing the impact of SSRI medication use on vaccine humoral immunogenicity and the odds of PCC.<sup>70</sup> However, due to poor representation of other races and individuals with lower socioeconomic status in the SSO cohort, our results may lack generalizability to non-White individuals, individuals earning low income, or those without postsecondary education who report high prevalence of symptoms of anxiety or depression.<sup>71,72</sup> Our analysis did not fully account for time-varying factors such as changes in SSRI use or dosage

or the circulation of different SARS-CoV-2 variants over time. We provide evidence that SSRI medication use does not impact the risk of breakthrough SARS-CoV-2 infections or PCC but further research in which time-varying factors are fully addressed is needed to confirm these findings.

### *Immune Imprinting and SARS-CoV-2 Vaccination*

Immune imprinting is a compelling and meaningful phenomenon which may impact the effectiveness of repeated or seasonal boosting with COVID-19 vaccines.<sup>157</sup> It may also reduce the effectiveness of boosting with updated COVID-19 vaccines that incorporate antigens from newer variants. This topic is vitally important for the clinically vulnerable populations identified in this thesis and elsewhere in the literature (the immune compromised, elderly, and obese) as repeated boosting is a recommended approach to bolster immunity and improve protection in these groups.<sup>158</sup> Should immune imprinting impact the humoral immune response generated by COVID-19 vaccination, it would limit the *de novo* generation of B cells specific to antigenically drifted variants and potentially diminish the humoral response mounted upon exposure to newer variants.<sup>65</sup> However, immune imprinting in the context of COVID-19 is not well understood. There is an urgent need to characterize the potential impacts posed by immune imprinting and develop approaches to overcome any impairments in humoral immunity arising from this phenomenon, if present, as new variants continue to emerge and spread.<sup>159</sup>

In the review article presented in **Chapter 7** we explored the intricate and multifaceted nuances of immune imprinting in the context of influenza virus, human seasonal coronaviruses, and SARS-CoV-2 virus. An interdisciplinary collaboration between bench scientists and epidemiologists, our review article provides researchers with a detailed summary of this important topic. We described the complex dynamics and mechanisms involved in this phenomenon, defined key concepts, outlined some beneficial aspects of imprinting (e.g., sustaining long-term humoral immunity against viruses exposed to many years in the past),

discussed how cell-mediated immune activity contributes to imprinting, and explored strategies that may help to overcome imprinting effects related to COVID-19 vaccination (e.g., selecting vaccine platforms which demonstrate a lower risk of generating imprinting effects and administering SARS-CoV-2 booster vaccines containing distantly related antigens so as to reduce recall of memory B cells produce from prior exposure to the original Wuhan variant and stimulate *de novo* antibody production). This review will support future analyses on immune imprinting and COVID-19.

The body of evidence regarding immune imprinting and COVID-19 in mice models and humans is growing.<sup>160–165</sup> There are multiple recent studies showing that the first SARS-CoV-2 variant to which individuals are exposed, either through vaccination or infection, shapes the immune response to related variants. For example, Abbad et al. reported that boosting with the monovalent KP.2 mRNA vaccine produced cross-reactive antibodies with a strong preference for ancestral variants compared to newer variants, especially among those with a prior SARS-CoV-2 infection.<sup>166</sup>

Furthermore, there are indications that boosting with updated monovalent or bivalent vaccines targeting newer antigens from variants with sufficient antigenic distance from the original variant (e.g., Omicron) at least partially overcomes imprinting and generates antibodies with improved Omicron specificity.<sup>163,167</sup> For example, Yisimayi et al. conducted deep mutational scanning-based epitope characterization of anti-RBD monoclonal antibodies and demonstrated that double Omicron infection stimulated Omicron-specific antibodies which were distinct from antibodies produce following exposure to the Wuhan variant.<sup>163</sup> Studies in the literature are inconsistent and other scientists report that repeated exposure to the Omicron variant through vaccination or infection failed to overcome immune imprinting.<sup>157,162</sup>

These studies underscore the considerable impact that immune imprinting may have on vaccine-elicited immunity and the important implications for vaccine development and public

health recommendations. More work is needed to fully elucidate the complex ways in which imprinting influences the immune response to COVID-19 vaccination and the impact that it may have on clinical protection. In addition, strategies to mitigate the harmful impacts of imprinting need to be developed and assessed in the general population and in vulnerable clinical groups. Our review article will support this future work and calls for more research regarding this important topic.

## **Overarching Strengths and Limitations**

The strengths and limitations of each study are discussed in each component chapter. Below, we briefly emphasize a few strengths and limitations that apply to this thesis as a whole.

Data collection for the SSO cohort occurred prospectively, reducing the risk of recall bias (Appendix Table 1). Detailed demographic and clinical data was obtained through serial questionnaires. The extensive nature of the questionnaires facilitated thorough adjustment of many sources of confounding, such as age, sex, ethnicity, vaccine type, time interval between doses, time since SARS-CoV-2 infection. Anti-N seropositivity facilitated the identification of asymptomatic SARS-CoV-2 infections. Longitudinal data collection allowed for analysis of trends rather than limited comparisons of titers at a few number of time points, which would have limited our power. Study follow-up for the SSO cohort was much longer than that of the COVID-19 vaccine clinical trials, lasting a minimum of 10 months and up to 46 months.<sup>76</sup> This facilitated extended longitudinal assessment of vaccine humoral immunogenicity and the characterization of long-term trends in vaccine-elicited antibody titers. In addition, follow-up encompassed a high number of doses and the administration of various vaccine types and formulations, such as bivalent or monovalent vaccines targeting the Omicron variant. Our modelling approach incorporated biostatistical methods to adjust for autocorrelation between repeated measurements for the same individual over time that is present for longitudinal data.<sup>168</sup> Otherwise the presence of autocorrelation would violate linear regression assumptions

(specifically the independence of errors and homoscedasticity of residuals) and biased the estimation of standard errors of model coefficients. Similarly, outliers and skewed data may bias linear regression results.<sup>169</sup> Our approach incorporated random effects and robust linear regression to prevent these statistical issues.

A high degree of variability in the humoral immune response to COVID-19 vaccination was observed during the completion of this thesis. Multiple systematic reviews of research involving various populations (e.g., adults, the obese, PLWH, healthy young males, older adults) note significant heterogeneity between studies evaluating antibody production and durability following COVID-19 vaccination.<sup>135,141,170–172</sup> We endeavoured through our work to explain this variability and identify what factors contribute to impaired COVID-19 vaccine humoral immunogenicity and may impair the effectiveness of COVID-19 vaccination. Individual variation in humoral responses was addressed by including random intercepts at the level of the participant.

Despite thorough adjustment for confounding, this research is observational and as a consequence any interpretations and conclusions made are restricted to correlation only due to sources of bias inherent to this study design. Our findings presented herein relate to associations only and we make no inferences regarding any causal effects by our exposures on COVID-19 vaccine humoral immunogenicity. Major limitations of observational research include a lack of blinding, residual confounding, exposure status misclassification, and selection bias.<sup>173</sup> For example, adjustment of time-varying factors in our analyses may be incomplete. Changes in body weight, alcohol consumption, SSRI medication use, or immunosuppressant medication use during follow-up may have resulted in misclassification of exposure status over time. We conducted investigations and sensitivity analyses to assess to the degree to which changes in alcohol consumption biased our results and we found that the impact was negligible.



However, the quality of follow-up data regarding these exposures was poor, limiting our sensitivity analyses, and time-varying misclassification may still bias our findings.

Sources of selection bias (e.g., volunteer bias, attrition) and information bias (e.g., misclassification of self-reported data) are explored in detail in the discussion sections of the component articles. Additional information regarding losses to follow-up and withdrawals across the entirety of study follow-up is provided in Appendix Table 3. The most common reasons for study withdrawal were delays in participants accessing their serology results, being too busy (e.g., work and family obligations, the commute to the hospital to provide blood samples took too much time), unspecified reasons, and moving away. IC participants and participants with PCC were more likely to complete all 46 months of follow-up than non-IC participants and non-PCC participants (20.2% of HIC, 10.8% of LIC, and 9.0% of NIC participants completed the entire study; 17.6% of participants with PCC and 8.9% of participant without PCC completed the entire study) and obese participants were more likely to complete the first study extension than non-obese participants (33.3% of obese participants and 19.2% of non-obese participants completed the first study extension).

## **Future Research**

In fulfilling the objectives of this thesis and completing each component article, we contributed novel knowledge to the field of infectious diseases epidemiology and responded to the urgent need to generate information to support the public health response to the COVID-19 pandemic. However, additional research is warranted to continue this work and address remaining knowledge gaps related to COVID-19 vaccination.

The absolute correlates of protection for COVID-19 are unknown.<sup>79</sup> Generally, it is observed that higher antibody titers are associated with a lower risk of SARS-CoV-2 infection, but the exact threshold for preventing infection has not been determined and infection is not always prevented by the presence of high antibodies titers.<sup>106,174,175</sup> A high degree of variability in

immunological measures between individuals following both vaccination or SARS-CoV-2 infection is a significant challenge for defining a specific threshold for protection. Indeed, relative correlates of protection may be more appropriate in light of this heterogeneity.<sup>79,175</sup> In addition, the correlates of protection required to prevent SARS-CoV-2 infection likely differs according to SARS-CoV-2 variant due to differences in infectiousness. Rahmani et al. observed that an anti-RBD antibody concentration of 154-168 BAU/mL was sufficient to prevent infection prior to the spread of Omicron, but the threshold required during Omicron circulation was higher (1,235-3,035 BAU/mL).<sup>176</sup> The SSO cohort was followed during various waves driven by the emergence of new variants and may be utilized to help determine what levels of neutralizing anti-S or anti-RBD antibodies or T or B cells generally confer protection against SARS-CoV-2 infection for different clinical populations while considering the impact of different variants. The correlates of protection for additional outcomes, such as asymptomatic infection or severe COVID-19, may be assessed with the SSO cohort data.<sup>79,175</sup>

Antibody production and durability is a key aspect of the immune response which is used to assess immune protection and the effectiveness of vaccination but it does not fully capture the multifaceted nature of the immune response to COVID-19 vaccination. To obtain a more complete picture of the immune response to COVID-19 vaccination other aspects must also be considered, such as antibody quality and T cell activity.

Antibody neutralization activity is a strong indicator of immune protection against COVID-19.<sup>177</sup> Antibody levels are often strongly correlated with antibody functionality; however, this is not always the case.<sup>112</sup> Neutralization activity against different SARS-CoV-2 variants varies.<sup>178</sup> For example, Favresse et al. noted a lack of neutralization activity against the Omicron variant despite high antibody titers for a subset of individuals in a sample of 155 healthcare workers.<sup>179</sup> Divergence between these two measures is especially important to consider when investigating long-term durability of humoral immunity, as scientists have observed that the

association between antibody quantity and neutralization capacity is not constant over time since vaccination, likely due to differences in kinetics.<sup>112</sup> Neutralization assays are an important method for gauging protection against different SARS-CoV-2 variants.<sup>93</sup> Therefore, in addition to quantifying antibody levels, it is necessary to measure neutralization activity to determine the quality of the antibodies generated by the B cell response upon exposure to COVID-19 vaccines.

Neutralizing activity (%) of anti-S IgG against the S protein from the Wuhan Hu-1 strain was determined using a surrogate neutralization ELISA assay and reported in **Chapter 2, 4 and 5**. This assay utilized a single-point dilution calibration method, which is subject to multiple limitations compared to multi-point dilution assays. A calibration curve cannot be generated for single-point assays and a linear relationship is assumed, which is not always appropriate, especially when the range of possible values is wide, as is the case for antibody data.<sup>180,181</sup> In addition, neutralization percentage is subject to saturation at the upper and lower end of the range of values. Additional neutralization testing was completed in which a 5-point dilution calibration curve was obtained and a more reliable and standardized measure of neutralization capacity, the ID<sub>50</sub> (representing the 50% inhibitory dilution), was obtained for the spike antigen from both the original Wuhan variant and the Omicron XBB.1.5 variant. We will analyze these new results and characterize the ID<sub>50</sub> neutralization capacity over time according to immune compromised status in future work.

The humoral immune response represents only one branch of adaptive immunity; the other is cellular immunity. B cell and T cell activity are interconnected and the humoral and cellular immune response to COVID-19 vaccination is often correlated but is not always congruent.<sup>182</sup> An analysis of the cellular and humoral immune response after a third COVID-19 vaccine dose among healthcare workers in Japan reported an association between B and T cell response at peak periods; however, the distribution of IgG antibody titers shifted from ~54 days after dose 3 to ~145 days after dose 3 but the T cell distribution did not.<sup>182</sup> Additionally, the

change in IgG antibody titers and T cell activity was not similar for older and younger participants. Parry et al. and Nicolas et al. provide additional evidence of heterogeneity in B cell and T cells responses to COVID-19 vaccination.<sup>183,184</sup> Increasing the duration between the first and second COVID-19 vaccine doses improved antibody production but not cellular response for a sample of 175 adults aged 80 or older and two cohorts of healthcare workers. T cell activity is an important component of immune function and should be investigated using SSO cohort data to obtain a complete picture of adaptive response to COVID-19 vaccination.<sup>185</sup>

Future analyses regarding neutralization activity (ID<sub>50</sub>) against both Wuhan and Omicron variants and T cell response are planned. Additional research aimed at identifying predictors of PCC remission and characterizing imprinting effects and the potential impact of imprinting on the clinical protection provided by COVID-19 vaccination would also provide valuable insights.

## **Dissertation Conclusion**

COVID-19 vaccination stimulated robust antibody production among the general population and within groups at high risk of SARS-COV-2 infection and serious COVID-19 clinical outcomes, such as the obese and excessive alcohol consumers, such that comparable antibody titer production and durability was observed compared to non-obese participants and non-excessive drinkers. We identified older age and high-level IC status as predictors of impaired antibody production following COVID-19 vaccination and increased duration between the first and second vaccine doses as a predictor of improved antibody production. Hybrid immunity consistently generated robust humoral immunity. SSRI medication use was not associated with COVID-19 vaccine humoral immunogenicity.

These findings suggest that older adults and high-level immune compromised individuals should be prioritized by public health vaccination guidelines (e.g., the administration of boosting doses or updated vaccines targeting newer variants). Furthermore,

the evidence presented in this dissertation indicates that COVID-19 vaccine humoral immunogenicity is not impaired for obese individuals or individuals reporting excessive alcohol consumption and remains an effective approach for protecting these vulnerable groups.

Early in the COVID-19 pandemic there was a stark lack of information on the novel virus and the newly developed COVID-19 vaccines. We conducted prospective, longitudinal observational research to fill in knowledge gaps arising from the exclusion of vulnerable populations from phase 2/3 RCTs, such as immune compromised individuals. This work has contributed novel knowledge to the rapidly expanding field of infectious disease epidemiology and supported the COVID-19 pandemic response. Our findings inform current SARS-CoV-2 vaccination guidelines and will support the public health response to any future coronavirus pandemics.

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## Appendices

Appendix Table 1: Data collection protocol for the SSO study.

|                   |                              | Surveillance Cohort                                                          | Convalescent Cohort*                                                      |
|-------------------|------------------------------|------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Initial Study     | Month 0 (Baseline visit)     | Baseline questionnaire<br>Serum blood draw (5 mL)<br>PBMC blood draw (20 mL) |                                                                           |
|                   | Month 1, 5, 7, 9             |                                                                              | Serum blood draw (5 mL)<br>PBMC blood draw (20 mL)                        |
|                   | Month 2, 4, 6, 8             |                                                                              | Serum blood draw (5 mL)                                                   |
|                   | Month 3                      | Study questionnaire                                                          | Serum blood draw (5 mL)<br>PBMC blood draw (20 mL)<br>Study questionnaire |
|                   | Month 10                     | Study questionnaire                                                          | Serum blood draw (5 mL)<br>Study questionnaire                            |
| Extension Phase 1 | Month 12, 16, 20, 24, 28, 32 | Serum blood draw (50 mL)                                                     |                                                                           |
|                   | Month 14, 18, 22, 26, 30, 34 | PBMC blood draw (50 mL)<br>Serum blood draw (5 mL)                           |                                                                           |
|                   | Month 16, 22, 28, 34         | Study questionnaire                                                          |                                                                           |
| Extension Phase 2 | Month 38, 46                 | Serum blood draw (50 mL)<br>PBMC blood draw (50 mL)<br>Study questionnaire   |                                                                           |
|                   | Month 42                     | Serum blood draw (50 mL)<br>PBMC blood draw (50 mL)                          |                                                                           |

\* Study questionnaires are also completed upon acquiring a SARS-CoV-2 infection.

Appendix Table 2: Baseline questionnaires for the Stop the Spread Ottawa (SSO) study.

## **DO YOU WANT TO BEGIN QUESTIONNAIRE?**

### **Thank you for participating in the following research study:**

Stop the Spread Ottawa: Analysis of Antibody Neutralization Efficiency and Cellular Immunity to SARS-CoV-2-Positive Individuals Identified in a Surveillance and Convalescence Survey of At-Risk Individuals

Please be advised that the following questionnaire is the longest that you will complete in this study. Most questions are multiple choice/select all that apply. This questionnaire will take approximately 25-30 minutes to complete.

There will be 27 questions on demographics.

There will be 6 questions on your symptoms.

There will be 10 questions on risks of exposure.

Finally, there will be 13 questions on the socioeconomic and psychosocial impacts of COVID19.

To submit a questionnaire for this study, please check the box below and click Submit

☐ I would like to start the questionnaire now

### Category A: Demographics

1. Date of birth: \_\_\_\_\_/\_\_\_\_\_/\_\_\_\_\_ (dd-mm-yyyy)

1a. Age\_\_\_\_\_ (years)

2. Please provide the first 3 characters of your postal code \_\_\_\_\_

3. Biological Sex:

- ☐ Male
- ☐ Female
- ☐ Intersex

4. I identify with the following Gender:

- ☐ Male gender
- ☐ Female gender
- ☐ Gender diverse

5. Country of Birth: Canada

- ☐ Yes *move on to 6*
- ☐ No *will generate 5a, 5b*

5a. Country of origin: \_\_\_\_\_

5b. What year did you become a resident of Canada? \_\_\_\_\_

6. With which ethnic group do you identify?

- ☐ Aboriginal (Inuit, Métis, North American Indian)
- ☐ Arab/West Asian (e.g., Armenian, Egyptian, Iranian, Lebanese, Moroccan)
- ☐ Black (e.g., African, Haitian, Jamaican, Somali)
- ☐ Chinese
- ☐ Filipino
- ☐ Japanese
- ☐ Korean
- ☐ Latin American
- ☐ South Asian
- ☐ South East Asian
- ☐ White (Caucasian)
- ☐ Other, specify: \_\_\_\_\_

7. Did you receive a flu shot during the 2020 / 2021 flu season?

- ☐ Yes *7a will generate*
- ☐ No *move on to 8*  
7a. When did you receive your flu shot? \_\_\_\_\_ (mm/yyyy)
- ☐ Don't know

8. Did you receive a flu shot during the 2019 / 2020 flu season?

- ☐ Yes *8a will generate*
- ☐ No *8b will generate*  
8a. When did you receive your flu shot? \_\_\_\_\_ (mm/yyyy)
- ☐ Don't know
  
- 8b. When did you receive your last flu shot? \_\_\_\_\_ (mm/yyyy)
- ☐ Don't know

9. Are you currently eligible to receive the COVID-19 vaccine?

- ☐ Yes *go to 10, otherwise go to 18*
- ☐ No

☐ Other, *specify* \_\_\_\_\_

10. Have you been vaccinated for COVID-19?

☐ Yes *Go to 11*

☐ No *Go to 18a*

11. How many doses of the COVID-19 vaccine have you received so far?

**Note:** Certain types of vaccines require more than one dose to protect against COVID-19. You would have been informed at the time of vaccination if you needed a second dose.

☐ One dose

☐ Two doses

☐ More than two doses

12. What COVID-19 vaccines have you received so far? Select all that apply:

☐ Pfizer and BioNTech mRNA vaccine

☐ Moderna mRNA vaccine

☐ AstraZeneca Oxford vaccine

☐ Other, *specify the vaccine* \_\_\_\_\_

☐ Don't know

13. When did you receive your **first** dose of the COVID-19 vaccine?

\_\_\_\_\_ (dd-mm-yyyy)

14. Did you have any symptoms (e.g. itching, fatigue, anxiety, swelling at injection site) after receiving your first dose of the COVID-19 vaccine?

☐ Yes *generates 14 a-e*

14a: How soon did you develop symptoms after receiving your first dose of the COVID-19 vaccine?

☐ During or within minutes of injection

☐ Within 15-30 minutes after injection

☐ Within 1-2 hours following injection

☐ Over 2 hours following injection, *specify number of hours* \_\_\_\_

☐ Other, *specify* \_\_\_\_\_

14b: Over what time period did you have these symptoms: when did these symptoms start and when did they stop?

From \_\_\_\_\_ (dd-mm-yyyy) to \_\_\_\_\_ (dd-mm-yyyy)

14c: Please list any symptoms you experienced during this time: \_\_\_\_\_

14d: Were these symptoms better or worse than you expected them to be?

- ☐ My symptoms were worse than I expected
- ☐ My symptoms were better than I expected
- ☐ The symptoms I experienced were what I expected to experience
- ☐ Other, *specify*

14e: Which vaccine did you receive for your first dose?

- ☐ Pfizer and BioNTech mRNA vaccine
- ☐ Moderna mRNA vaccine
- ☐ AstraZeneca Oxford vaccine
- ☐ Other, *specify the vaccine* \_\_\_\_\_
- ☐ Don't know

15. Did you receive a **second** dose of the COVID-19 vaccine?

Note: Certain types of vaccines require more than one dose to protect against COVID-19. You would have been informed at the time of vaccination if you needed a second dose.

- ☐ Yes

When did you receive a second dose of the COVID-19 vaccine? \_\_\_\_\_  
(ddmm-yyyy) *go to 16*

- ☐ No *go to 19*

16. Did you have any symptoms (e.g. itching, fatigue, anxiety, swelling at injection site) after receiving your second dose of the COVID-19 vaccine?

- ☐ Yes *generates 16 a-d*
- ☐ No *go to 17*

16a: How soon did you develop symptoms after receiving your second dose of the COVID-19 vaccine?

- ☐ During or within minutes of injection
- ☐ Within 15-30 minutes after injection
- ☐ Within 1-2 hours following injection
- ☐ Over 2 hours following injection, *specify number of hours* \_\_\_\_
- ☐ Other, *specify* \_\_\_\_\_

16b: Over what time period did you have these symptoms: when did these symptoms start and when did they stop?

From \_\_\_\_\_ (dd-mm-yyyy) to \_\_\_\_\_ (dd-mm-yyyy)

16c: Please list the symptoms you experienced during this time: \_\_\_\_\_

16d: Were these symptoms better or worse than you expected them to be?

- ☐ My symptoms were worse than I expected
- ☐ My symptoms were better than I expected



- ☐ The symptoms I experienced were what I expected to experience ☐ Other, *specify* \_\_\_\_\_

17: Which vaccine did you receive for your **second** dose? *After go to 19*

- ☐ Pfizer and BioNTech mRNA vaccine  
☐ Moderna mRNA vaccine  
☐ AstraZeneca Oxford vaccine  
☐ Other, *specify the vaccine* \_\_\_\_\_  
☐ Don't know

18. Will you be vaccinated for COVID-19 when you are eligible to receive a COVID-19 vaccine?

- ☐ Yes *18a will generate*  
☐ No *18b will generate*

18a. How soon will you go to get vaccinated?

- ☐ As soon as possible after vaccine available *go to 19 otherwise go to 18b*  
☐ 1-2 months after vaccine available ☐ 2-4 months after vaccine available  
☐ More than 4 months after vaccine available

18b. Why would you not go to get vaccinated as soon as possible? \_\_\_\_\_

19. Is there anything you want to share with our research team regarding your thoughts on and/or experiences with the COVID-19 vaccine?  
\_\_\_\_\_

20. Do you have any known allergies?

- ☐ Yes *20a will generate*  
☐ No *move on to 21*  
20 a. What allergies do you have? ☐  
Pollen  
☐ Food, *specify* \_\_\_\_\_  
☐ Medications, *specify* \_\_\_\_\_  
☐ Other, *specify* \_\_\_\_\_

21. Do you live with other people?

- ☐ Yes *will generate 21a, 21b*  
☐ No *move on to 22*  
21a. How many adults? \_\_\_\_\_  
21b. How many children (age<18 years)? \_\_\_\_\_

22. Smoking history:

- ☐ Never Smoker  
☐ Former Smoker  
Age when smoking began (years) \_\_\_\_\_  
Age when smoking discontinued (years) \_\_\_\_\_

Average #packs/day \_\_\_\_\_

☐ Current Smoker

Age when smoking began \_\_\_\_\_

Average # of packs/day \_\_\_\_\_

What do you currently smoke? Select all that apply:

☐ Cigarettes

☐ E-cigarettes/vaping

☐ Marijuana/cannabis

☐ Other, *specify* \_\_\_\_\_

23. How many standard drinks of alcohol do you have in a week, on average? \_\_\_\_\_

One standard drink equals:

341 ml (12 oz.) bottle of 5% beer, cider, or cooler

142 ml (5 oz.) glass of 12% wine

43 ml (1.5 oz.) serving of 40% distilled alcohol

(e.g., rye, gin, rum, vodka)

24. Do you have any of the following conditions? 24a.

Pregnancy

☐ Yes *will generate 24ai*

☐ No *move on to 24b*

☐ Not applicable

☐ Unknown

24 ai. Specify trimester

☐ First

☐ Second

☐ Third

☐ Unknown

24 b. Cancer

☐ Yes

☐ No

24 c. Diabetes

☐ Yes

☐ No

24 d. HIV

☐ Yes *will generate 14di-14diii*

☐ No *move on to 14e*

24 di. Are you being treated with antiretroviral medications?

☐ Yes

☐ No

24 dii. How long have you been living with HIV? \_\_\_\_\_ (years)

24 diii. Is your viral load fully suppressed?

☐ Yes ☐ No

- ☐ Don't know
- 24 e. Other immune deficiency
- ☐ Yes, *specify* \_\_\_\_\_
- ☐ No
- 24 g. Obesity
- ☐ Yes
- ☐ No
- 24 h. Heart disease
- ☐ Yes
- ☐ No
- 24 i. Asthma requiring medication
- ☐ Yes
- ☐ No
- 24 j. Chronic lung disease (non-asthma)
- ☐ Yes
- ☐ No
- 24 k. Chronic liver disease
- ☐ Yes *will generate 24 ki-24 kiii*
- ☐ No *move on to 24 l*
- 24 ki. What type of liver disease do you have \_\_\_\_\_
- 24 kii. How long have you been living with chronic liver disease?  
 \_\_\_\_\_(years)
- 24 kiii. Are you being treated with antiviral medications? \_\_\_\_\_
- ☐ Yes
- ☐ No
- 24 l. Chronic kidney disease
- ☐ Yes
- ☐ No
- 24 m. Chronic hematological disorder
- ☐ Yes
- ☐ No
- 24 n. Chronic neurological impairment/disease
- ☐ Yes
- ☐ No
- 24 o. Organ or bone marrow recipient
- ☐ Yes
- ☐ No
- 24 p. Other health condition(s)
- ☐ Yes, *specify* \_\_\_\_\_
- ☐ No

25. Are you currently receiving treatment that weakens your immune system? (for example chemotherapy, medication for transplants, corticosteroids)

- ☐ Yes, *specify* \_\_\_\_\_  
☐ No

26. Do you regularly go to a hospital or health care setting for a treatment? (for example dialysis or surgery)

- ☐ Yes, *specify* \_\_\_\_\_  
☐ No

27. Are you currently taking any medications or supplements?

- ☐ Yes, *specify* \_\_\_\_\_  
☐ No

#### Category B: Severity of Symptoms

1. Are you having any flu-like symptoms?

- ☐ Yes *will generate 1a-1c*  
☐ No *will generate 1d-1f*  
 1a. When did they start? \_\_\_\_\_ (dd-mm-yyyy)

1b. Did you take medication to manage the above symptoms?

- ☐ Yes, *specify* \_\_\_\_\_  
☐ No

1c. In the last 24 hours what were your symptoms and how would you rate them?

(Note: rate your symptom at its worst for the past 24 hours). *After go to 2*

|                      | None                     | Mild                     | Moderate                 | Severe                   |
|----------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Runny or stuffy nose | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Sinus pain           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Sore/scratchy throat | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Hoarseness           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Cough                | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Shortness of breath  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Wheezing             | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Chest pain           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Ear ache/infection   | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Headache             | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Chills and/or fever  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

|                                      |                          |                          |                          |                          |
|--------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Feeling generally unwell             | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Generalized muscle aches/pains       | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Joint pain (new onset)               | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Abnormally tired                     | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Loss of appetite                     | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Nausea                               | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Vomiting                             | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Diarrhea                             | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Abdominal pain                       | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Abnormal or decreased sense of taste | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Abnormal or decreased sense of smell | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Confusion                            | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Decreased alertness                  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Falling down                         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Nose bleed                           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Rash                                 | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Other, <i>specify</i>                | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Other, <i>specify</i>                | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Other, <i>specify</i>                | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

☐ No

1d. When did you last have any flu-like symptoms? (e.g. runny nose, cough, headache, muscle pains, loss of appetite, decreased alertness, other)

When did they start? \_\_\_\_\_ (dd-mm-yyyy)

When did they stop? \_\_\_\_\_ (dd-mm-yyyy)

☐ Other, *specify* \_\_\_\_\_

1e. Did you take medication to manage these symptoms?

☐ Yes, *specify* \_\_\_\_\_

☐ No

1f. During the time when you last had any flu-like symptoms, what were your symptoms and how would you rate them? (Note: rate your symptom at its worst during the time when you last had symptoms)

|  |      |      |          |        |
|--|------|------|----------|--------|
|  | None | Mild | Moderate | Severe |
|--|------|------|----------|--------|

|                                      |                          |                          |                          |                          |
|--------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Runny or stuffy nose                 | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Sinus pain                           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Sore/scratchy throat                 | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Hoarseness                           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Cough                                | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Shortness of breath                  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Wheezing                             | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Chest pain                           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Ear ache/infection                   | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Headache                             | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Chills and/or fever                  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Feeling generally unwell             | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Generalized muscle aches/pains       | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Joint pain (new onset)               | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Abnormally tired                     | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Loss of appetite                     | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Nausea                               | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Vomiting                             | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Diarrhea                             | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Abdominal pain                       | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Abnormal or decreased sense of taste | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Abnormal or decreased sense of smell | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Confusion                            | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Decreased alertness                  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Falling down                         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Nosebleed                            | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Rash                                 | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Other, <i>specify</i>                | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Other, <i>specify</i>                | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

|                       |                          |                          |                          |                          |
|-----------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Other, <i>specify</i> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|-----------------------|--------------------------|--------------------------|--------------------------|--------------------------|

2. Overall, how were you feeling today?

- ☐ Unable to do regular activities (spent most of the day in bed or on the couch)
- ☐ Able to get by, but not well enough to do regular activities
- ☐ Not feeling well, but able to do most regular activities
- ☐ Annoying symptoms, but able to do all regular activities
- ☐ Feeling well

3. When were you last tested for COVID-19?

Date: \_\_\_\_\_ (dd-mm-yyyy)

- ☐ I have never been tested for COVID-19.

3a. Was this test result:

- ☐ Positive for COVID-19
- ☐ Negative for COVID-19
- ☐ Other, *specify* \_\_\_\_\_

4. Have you had to seek medical attention for symptoms you thought might be caused by COVID-19?

- ☐ Yes *will generate 4a-4d*

- ☐ No *will move on to 5*

4a. Where did you go to seek medical attention? Select all that apply:

- ☐ Family doctor/primary care provider
- ☐ Occupational Health
- ☐ Public Health Testing Centre
- ☐ Walk-in or Urgent Care Clinic
- ☐ Emergency Department
- ☐ Telehealth
- ☐ Other, *specify* \_\_\_\_\_

4b. What were you told by the healthcare team?

- ☐ That you had COVID-19
- ☐ That you had pneumonia
- ☐ That you had the flu
- ☐ Other, *specify* \_\_\_\_\_

\_\_\_\_\_ 4c. Were any medications prescribed to you at this time?

- ☐ Yes, *specify* \_\_\_\_\_
- ☐ No

4d. After seeking medical attention for these symptoms, were you hospitalized?

- ☐ Yes, *if Yes when were you hospitalized?*  
\_\_\_\_\_ (dd-mm-yyyy) to \_\_\_\_\_ (dd/mm/yyyy)
- ☐ No

5. Have you ever tested positive for COVID-19 OR believe you may have had COVID-19?

☐ Yes *questions 5a-5g will generate*

☐ No *move to question 6*

5a. When did you last test positive for COVID-19? \_\_\_\_\_ (dd-mm-yyyy)

☐ I have never tested positive for COVID-19.

5b. At the time when you had/may have had COVID-19, when did your symptoms start and when did they stop?

☐ From \_\_\_\_\_ (dd-mm-yyyy) to \_\_\_\_\_ (dd-mm-yyyy)

☐ I never had symptoms *moves to 5g.*

5c. At this time, what were your symptoms and how would you rate them?

(During the time when you experienced each symptom, how bad was it?)

|                                | None                     | Mild                     | Moderate                 | Severe                   |
|--------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Runny or stuffy nose           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Sinus pain                     | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Sore/scratchy throat           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Hoarseness                     | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Cough                          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Shortness of breath            | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Wheezing                       | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Chest pain                     | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Ear ache/infection             | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Headache                       | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Chills and/or fever            | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Feeling generally unwell       | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Generalized muscle aches/pains | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Joint pain (new onset)         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Abnormally tired               | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Loss of appetite               | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Nausea                         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Vomiting                       | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Diarrhea                       | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Abdominal pain                 | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |



|                                      |                          |                          |                          |                          |
|--------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Abnormal or decreased sense of taste | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Abnormal or decreased sense of smell | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Confusion                            | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Decreased alertness                  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Falling down                         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Nosebleed                            | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Rash                                 | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Other, <i>specify</i>                | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Other, <i>specify</i>                | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Other, <i>specify</i>                | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

5d. After your symptoms began, when was your worst day? (e.g. Day 3 after symptoms started). Day\_\_\_\_\_

5e. On a scale of 1 to 10, 10 being the worst you have ever felt, how would you rate your symptoms overall on this day? \_\_\_\_/10

5f. If these symptoms went away, did you have similar symptoms at a later time?

☐ Yes

When did you have similar symptoms at a later time? From\_\_\_\_\_(dd-mm-yyyy) to \_\_\_\_ (dd-mm-yyyy)

Please list the symptoms you experienced during this time:\_\_\_\_\_

☐ No

☐ All/some symptoms never went away

☐ Other, *specify* \_\_\_\_\_

5g. How many times have you tested positive for COVID-19? \_\_\_\_times If more than once, when was the first time you tested positive for COVID-19? \_\_\_\_\_ (dd/mm/yyyy)

☐ I never tested positive for COVID-19.

6. Have you ever experienced long-term symptoms that were/may have been caused by COVID19?

\*i.e. effects that lasted weeks or months after you first experienced symptoms that may have been caused by COVID-19 (regardless of whether you ever tested positive for COVID-19).

☐ Yes *generates 6a-6e*

6a. What were/are your symptoms?

☐ Fatigue

☐ Shortness of breath

- ☐ Cough
- ☐ Joint pain
- ☐ Muscle pain
- ☐ Chest pain
- ☐ Headache ☐ Dizziness
- ☐ Loss of smell
- ☐ Loss of taste
- ☐ Fast or pounding heartbeat
- ☐ Irritability
- ☐ Difficulties with thinking and concentrating
- ☐ Memory loss
- ☐ Confusion
- ☐ Difficulty sleeping
- ☐ Rash
- ☐ Hair loss
- ☐ Nausea
- ☐ Loss of appetite
- ☐ Diarrhea
- ☐ Other, *please list any other symptoms:*\_\_\_\_\_

6b. When did these symptoms start?  
 \_\_\_\_\_(dd-mm-yyyy)

6c. When did these symptoms stop?  
 \_\_\_\_\_(dd-mm-yyyy)

- ☐ They never stopped, I am currently having symptoms. On a scale of 1 to 10, 10 being the worst you have ever felt, how would you rate your symptoms overall today? \_\_\_\_/10

6d. Why do you believe these symptoms were caused by COVID19?

- ☐ I tested positive for COVID-19
- ☐ Other, *specify*\_\_\_\_\_

6e. Do you believe the quality of life you have now is worse than the quality of life you had before experiencing symptoms that were/may have been caused by COVID-19?

- ☐ Yes
- ☐ No
- ☐ Other, *specify*\_\_\_\_\_

6f. Is there anything you want to share with our research team regarding your thoughts on and/or experiences with long-term symptoms that may have been caused by COVID-19?

---

Category C: Risks of Exposure

1. Have you been in close physical contact with someone who tested positive for COVID19?  
(**Close physical contact** means: less than 2 metres away in the same room/workplace/area OR living in the same house)

☐ Yes *generates 1a-1d*

☐ No *moves on to 2*

1a. When did you last have contact with them? \_\_\_\_\_ (dd-mm-yyyy)

1b. What relationship do you have with this person/these people? Select all that apply:

☐ Parent

☐ Spouse

☐ Sibling

☐ Child

☐ Neighbour

☐ Friend

☐ Sexual partner

☐ Colleague

☐ Healthcare worker

☐ Service provider

☐ Other, please specify \_\_\_\_\_ 1c. Type of contact, select

all that apply:

☐ Nursing home

☐ Retirement home

☐ Dormitory

☐ Shelter

☐ Prison

☐ Emergency Department

☐ Ward

☐ Critical Care Unit

☐ Outpatient Clinic

☐ Household

☐ Friend

☐ Workplace

☐ Non-household sexual contact

☐ Other, please specify \_\_\_\_\_

- 1d. What were the symptoms? \_\_\_\_\_
2. When did you last have close physical contact with a person who returned from outside of Canada?
- ☐ Yes
- ☐ No

3. When did you last travel outside of Canada?

- ☐ Yes *will generate 3a, 3b*  
☐ No *move on to 4*  
 3a. Where did you travel? \_\_\_\_\_  
 3b. Are you an essential worker who must cross the Canada-US border regularly?  
☐ Yes  
☐ No
4. When did you last travel to other Canadian provinces/territories ?  
☐ Yes *if Yes, where?* \_\_\_\_\_  
☐ No
5. Have you used the public transportation system within the last 14 days?  
☐ Yes, *will generate 5a, 5b*  
☐ No, *will just generate 5b*  
 5a. How many times? \_\_\_\_\_  
 5b. On average, how often do you use the public transportation system?  
☐ Never  
☐ Maybe once a week  
☐ 1-3 days a week ☐ 4-6 days a week  
☐ I use it on a daily basis  
☐ I use it multiple times a day
6. Which of the following public spaces have you visited within the last 14 days? Select all that apply:  
*\*checking box will generate "How many visits?" for each entry*  
☐ Pub/bar, *how many visits* \_\_\_\_\_  
☐ Restaurant, *how many visits* \_\_\_\_\_  
☐ Salon, *how many visits* \_\_\_\_\_  
☐ Gym, *how many visits* \_\_\_\_\_  
☐ Public washroom, *how many visits* \_\_\_\_\_  
☐ Mall, *how many visits* \_\_\_\_\_  
☐ Night club, *how many visits* \_\_\_\_\_  
☐ Grocery store, *how many visits* \_\_\_\_\_  
☐ Outdoor events/gatherings with >50 people present, *how many visits* \_\_\_\_\_
7. Please check off all that apply. Are you currently working:  
☐ As a healthcare worker?  
☐ In a long-term care facility?  
☐ On a cabin crew aboard an aircraft?  
☐ As a dentist or allied dental staff member?  
☐ As an elementary/secondary school teacher or staff member?  
☐ In a daycare?  
☐ In the meat packing industry?

- ☐ For a courier?
  - ☐ Other position/workplace at risk of exposure to COVID-19, *specify*
- 

8. Are you currently employed? ☐ Yes *generates 8a-8g*

☐ No *moves on to 9*

8a. Have you ever seen things at work that you thought could increase the risk of spreading COVID-19?

- ☐ All the time
- ☐ Often
- ☐ Sometimes
- ☐ Rarely
- ☐ Never

8b. Would it increase your stress level if you had a duty to report conditions that increased the risk of spreading COVID-19 in your workplace?

- ☐ Yes, a lot
- ☐ Yes, somewhat
- ☐ Not at all

8c. Would you stay home rather than report on risky working conditions?

- ☐ Yes
- ☐ No

8d. If you made a report, what do you think would happen to you?

- ☐ Nothing
- ☐ I would be commended for having done the right thing
- ☐ I would be reassigned/demoted
- ☐ I would be fired

8e. Since the start of the pandemic, is your stress level:

- ☐ Much lower
- ☐ Lower
- ☐ Identical
- ☐ Higher
- ☐ Much higher

8f. Since the start of the pandemic, did you see a doctor because of the stress you were feeling?

- ☐ Yes
- ☐ No

8g. Since the start of the pandemic, has stress affected your work?

- ☐ Yes, I was less productive
- ☐ Yes, I had to be off a few times
- ☐ Yes, I have been on sick leave
- ☐ No

8h. Since the start of the pandemic, did you have to take medication to manage your stress?

- ☐ Yes, I increased a medication I was already taking
- ☐ Yes, I started a new medication
- ☐ No

9. Please check off all that apply. Do you currently live:

- ☐ In a senior's complex? ☐ In assisted living?
- ☐ In a personal care home?
- ☐ In a homeless shelter?

10. The next set of questions is only for participants who have tested positive for COVID-19.

Have you ever tested positive for COVID-19?

- ☐ Yes *will generate questions 10a-10d*
- ☐ No *will go to Category D*

10a. Do you suspect that you were infected by COVID-19 at work?

- ☐ Yes
- ☐ No
- ☐ Unknown

10b. Were you exposed to a confirmed case at work?

- ☐ Yes
- ☐ No
- ☐ Unknown

10c. Did you have direct physical contact with the confirmed case (e.g. hands-on physical contact)?

- ☐ Yes
- ☐ No
- ☐ Unknown

10d. Did you have prolonged face-to-face contact (>15 minutes) with the case?

- ☐ Yes
- ☐ No
- ☐ Unknown

#### Category D: Socioeconomic Impacts of COVID-19

1. What was your employment status before the COVID-19 epidemic?

- ☐ Permanent
- ☐ Contract/temporary
- ☐ Self-employed
- ☐ No paid employment but seeking work

- ☐ No paid employment and not seeking paid work
  - ☐ Informal employment (e.g. working under the table)
2. How many hours of paid work did you perform in an average week before the COVID-19 epidemic? \_\_\_\_\_
3. What was your approximate annual household income before taxes in the past 12 months?
- ☐ \$0 - \$29,999
  - ☐ \$30,000 - \$59,999
  - ☐ \$60,000 to \$89,999
  - ☐ \$90,000 to \$119,999
  - ☐ \$120,000 to \$149,999
  - ☐ \$150,000 or more
  - ☐ Prefer not to answer
  - ☐ Do not know
4. How many people (including yourself) does this income support? \_\_\_\_\_
5. How has your employment status changed in relation to the COVID-19 epidemic?
- ☐ I have lost my job
  - ☐ My income/hours have been reduced
  - ☐ No change
  - ☐ My income/hours have increased
6. Did you work outside the home today?
- ☐ Yes *generates 6a*
- 6a. Please indicate why:
- ☐ My symptoms were mild and I felt well enough to go
  - ☐ I was feeling miserable, but had things I had to do
  - ☐ I was feeling miserable, but felt obligated to work
  - ☐ I could not afford to stay home
  - ☐ I felt well when I left home
  - ☐ Other, *please specify* \_\_\_\_\_
- ☐ No *generates 6b.*
- 6b. Please indicate why:
- ☐ I am self-isolating
  - ☐ I did not feel well enough to work
  - ☐ I was not scheduled to work (e.g. vacation, weekend)
  - ☐ Other, *specify* \_\_\_\_\_

7. Have you lost days of work due to infectious respiratory illness (flu, cold, etc.)?
- ☐ Yes *will generate 7a, 7b*
- ☐ No *move on to 8*
- 7a. How many days of work did you miss due to infectious respiratory illness?
- \_\_\_\_\_
- 7b. Approximately when was the last day you took off due to infectious respiratory illness? \_\_\_\_\_ (dd-mm-yyyy)
8. What is your current level of education?
- ☐ Elementary school
- ☐ High school
- ☐ Trade, technical or vocation school, apprenticeship training or technical CEGEP
- ☐ Diploma from a community college, pre-university CEGEP or non-university certificate
- ☐ University certificate below Bachelor's level
- ☐ Bachelor's degree
- ☐ Graduate degree (MSc, MBA, MD, PhD, etc.) *this answer and above generates 8b.*
- ☐ None *move on to 9*
- ☐ Prefer not to answer *move on to 9*
- 8b. What was your age when you completed this level of education?
- Age when you completed this level of education \_\_\_\_\_.
- ☐ Don't know
- ☐ I prefer not to answer
9. How has the COVID-19 epidemic impacted your ability to meet essential needs and financial commitments e.g. paying rent and buying groceries?
- ☐ Not at all
- ☐ I can still meet most of my essential/financial needs
- ☐ I can meet some of my essential/financial needs
- ☐ I am unable to meet most of my essential/financial needs
10. The next question is only for participants who have tested positive for COVID-19. Have you ever tested positive for COVID-19?
- ☐ Yes *will generate 10 a*
- ☐ No *move on to Category E*

10a. How much has testing positive for COVID-19 impacted your ability to:

|                               | Not Applicable           | No Impact                | Mildly                   | Moderately               | Severely                 |
|-------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Pay for groceries             | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Afford rent/mortgage payments | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Maintain employment           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |



|                                                      |                          |                          |                          |                          |                          |
|------------------------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Obtain new/alternate employment                      | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Afford things you enjoy (but don't necessarily need) | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Advance your career                                  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Pursue a higher level of education/training          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Pay for your family's needs                          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

#### Category E: Psychosocial Impacts of COVID-19

##### 1. K10 Scale

Please tick the answer that is correct for you.

In the past TWO WEEKS:

|                                                                           | All of the time          | Most of the time         | Some of the time         | A little of the time     | None of the time         |
|---------------------------------------------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| About how often did you feel tired out for no good reason?                | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| About how often did you feel nervous?                                     | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| About how often did you feel so nervous that nothing could calm you down? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| About how often did you feel hopeless?                                    | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| About how often did you feel restless or fidgety?                         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| About how often did you feel so restless you could not sit still?         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| About how often did you feel depressed?                                   | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| About how often did you feel that everything was an effort?               | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| About how often did you feel so sad that nothing could cheer you up?      | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| About how often did you feel worthless?                                   | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

Source: Kessler RC, Andrews G, Colpe LJ, Hiripi E, Mroczek DK, Normand SL, Walters EE, Zaslavsky AM. Short screening scales to monitor prevalences and trends in non-specific psychological distress. Psychol Med 2002;32(6):959-76

2. In the past two weeks, have you:

|                                                                                 | Yes                      | No                       |
|---------------------------------------------------------------------------------|--------------------------|--------------------------|
| Decreased face-to-face contact with people?                                     | <input type="checkbox"/> | <input type="checkbox"/> |
| Decreased your work hours because of stress or illness?                         | <input type="checkbox"/> | <input type="checkbox"/> |
| Increased smoking?                                                              | <input type="checkbox"/> | <input type="checkbox"/> |
| Increased how much alcohol you drink?                                           | <input type="checkbox"/> | <input type="checkbox"/> |
| Engaged in any other behaviour that could interfere with work or relationships? | <input type="checkbox"/> | <input type="checkbox"/> |
| Missed >4 work shifts because of stress or illness                              | <input type="checkbox"/> | <input type="checkbox"/> |

3. The next question is only for participants who have tested positive for COVID-19. Have you ever tested positive for COVID-19?

☐ Yes *will generate 3a*

☐ No *move on to 4*

### 3a. Impact of Event Scale-Revised

Testing positive for COVID-19 is a stressful life event.

Below is a list of comments made by people after stressful life events.

Please check each item, indicating how frequently these comments were true for you DURING THE PAST SEVEN DAYS.

|                                                                                                                 | Not at all               | Rarely                   | Sometimes                | Often                    |
|-----------------------------------------------------------------------------------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| I thought about it when I didn't mean to.                                                                       | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| I avoided letting myself get upset when I thought about it or was reminded of it                                | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| I tried to remove it from memory                                                                                | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| I had trouble falling asleep or staying asleep, because of pictures or thoughts about it that came into my mind | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

|                                                                                       |                          |                          |                          |                          |
|---------------------------------------------------------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| I had waves of strong feelings about it                                               | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| I had dreams about it                                                                 | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| I stayed away from reminders about it.                                                | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| I felt as if it hadn't happened or it wasn't real                                     | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| I tried not to talk about it.                                                         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Pictures about it popped into my mind.                                                | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Other things kept making me think about it.                                           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| I was aware that I still had a lot of feelings about it, but I didn't deal with them. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| I tried not to think about it                                                         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Any reminder brought back feelings about it.                                          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| My feelings about it were kind of numb.                                               | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

Source: Weiss D, Marmar C. The Impact of Event Scale -Revised. In J. Wilson & T. Keane (Eds), Assessing psychological trauma and PTSD. New York: Guildford, 1997.

4. Is there any other information you would like to share with us that you think may be relevant to your participation in this study? \_\_\_\_\_

Appendix Table 3: Completion of study phases according to (A) immune compromised (IC) status (n=932), (B) obesity (n=907), and (C) post-COVID-19 condition (PCC) status (n=901). Total study follow-up was 46 months. The initial study lasted 10 months, the first extension phase occurred from month 11 to month 34, and the second extension phase occurred from month 35 to month 46.

(A)

| Study Participation, n (%)           | High-level IC | Low-level IC | Non-IC     | P     |
|--------------------------------------|---------------|--------------|------------|-------|
| Completed all study phases           | 21 (20.2)     | 17 (10.8)    | 60 (9.0)   | 0.002 |
| Completed up to extension 1          | 9 (8.7)       | 23 (14.6)    | 77 (11.5)  |       |
| Completed the first 10 months        | 60 (57.7)     | 79 (50.0)    | 346 (51.6) |       |
| Did not complete the first 10 months | 14 (13.5)     | 39 (24.7)    | 187 (27.9) |       |

(B)

| Study Participation, n (%)           | Obese     | Non-Obese  | P      |
|--------------------------------------|-----------|------------|--------|
| Completed extension 1                | 70 (33.3) | 134 (19.2) | 0.0001 |
| Completed the first 10 months        | 93 (44.2) | 377 (54.1) |        |
| Did not complete the first 10 months | 47 (22.4) | 186 (26.7) |        |

(C)

| Study Participation, n (%)           | PCC        | No PCC    | P       |
|--------------------------------------|------------|-----------|---------|
| Completed all study phases           | 28 (17.6)  | 66 (8.9)  | <0.0001 |
| Completed up to extension 1          | 74 (10.0)  | 31 (19.5) |         |
| Completed the first 10 months        | 391 (52.7) | 78 (49.1) |         |
| Did not complete the first 10 months | 211 (28.4) | 22 (13.8) |         |