Characteristics and Clinical Outcomes in Antiretroviral Treated HIV-HBV Co-infection

Urvi Rana

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School of Epidemiology and Public Health
Faculty of Medicine
University of Ottawa

Ottawa, Ontario, Canada

ABSTRACT

Objective: The objective of this thesis was to compare demographic and clinical characteristics and factors associated with advanced hepatic fibrosis between HIV and HIV-hepatitis B (HBV) co-infected patients.

Methods: Proportional odds models were developed to investigate socio-demographic and clinical variables' association on liver fibrosis determined by AST-to-Platelet-Ratio-Index (APRI).

Results: HBV status and APRI values were available for 2,419 of 9,289 (26%) participants. 199 (9%) were HBV co-infected. Compared to HIV infected, HIV-HBV co-infected individuals were 2.19 (95% CI: 1.63, 2.90) and 1.68 (95% CI: 1.10, 2.53) times more likely to belong in a higher level of APRI category. Compared to HIV mono-infection, HIV-HBV co-infected participants on ARV therapy were less likely to have clinically significant or advanced fibrosis compared to mild or moderate fibrosis.

Conclusion: We provide evidence in favour of an association between ARV therapy and reduced fibrosis in HIV-HBV co-infected population.

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CHAPTER 1: HEPATITIS B INTRODUCTION

1.1 Epidemiology and Natural Course of HBV Infection

Hepatitis B (HBV) belongs to the hepatotropic DNA virus family and primarily infects liver cells (hepatocytes). HBV causes acute and chronic necroinflammatory liver disease which can range from mild disease to severe or fulminant hepatitis[1-2]. Acute HBV is self-limiting in most adults and resolution of acute infection confers lifelong immunity[2]. In Canada, incidences of acute HBV infection have decreased from 0.97 to 0.49 per 100,000 individuals during 2005 – 2010[3]. This reduction can be attributed to the universal HBV vaccination program implemented by the World Health Organization (WHO) in 1992 [2,4].

Approximately 5% of acutely infected adults do not resolve primary infection and viral replication continues in the liver. These individuals develop chronic hepatitis B (CHB) which can lead to liver damage culminating in cirrhosis, liver failure, and/or hepatocellular carcinoma (HCC)[5,6]. The risk of developing CHB varies with the age at which infection is acquired. The risk is lowest in adults (~5%) and highest (90%) in infants born to HBV infected mothers. Children who acquire HBV between 1 – 5 years of age have a 30 – 50% risk of developing CHB[2,6]. Worldwide, there are 240 million chronically infected persons and an estimated 650,000 people die annually due to CHB. The prevalence of CHB is highest in sub-Saharan Africa and East Asia (~10%) and lowest in Western Europe and North America (~<1%) [2].

1.2 Transmission

Worldwide, the primary mode of HBV transmission is through exposure to infectious blood and bodily fluids via mucous membranes[2,7]. The mode of transmission varies in different parts of the world. In areas with high endemicity (Asia and Africa), perinatal or childhood infections are the

most common modes of transmission. In countries with low endemicity including Canada, HBV infection is mainly present in adults and is transmitted percutaneously or sexually[3,8,9]. High risk groups include those who are HIV-infected, people who inject drugs (PWID), men who have sex with men (MSM), sex workers, transgender individuals, and indigenous populations[3,9].

1.3 Virology

The HBV viral genome is structured as a circular double stranded DNA enveloped in a 42 nm thick layer of lipoproteins. Once the virus enters the hepatocyte nucleus, the genomic DNA is converted to covalently closed circular DNA (cccDNA). Viral RNAs are transcribed from this cccDNA and exported to the cytoplasm for translation of HBV proteins. The HBV genome encodes hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg), and hepatitis B e antigen (HBeAg)[10]. HBcAg, the nucleocapsid protein core of HBV, circulates in serum as a 20 nm spheres coated with the HBsAg envelop protein[1,5]. HBeAg is also present in the core of HBV[6]. The first evidence of liver injury results from the host immune response to the viral antigens[8].

Viral replication itself is not harmful to the host and carriers of the virus usually present with no symptoms of the disease. HBV replication occurs via reverse transcriptase that lacks a proofreading function leading to genetic heterogeneity. At least ten genotypes of HBV (A to J) have been identified; each with ≥8% differences in their genomic sequences. There is evidence that the different genotypes may influence the optimal mode of transmission and disease progression. Genotype B and C are related to perinatal exposure and are primarily observed in high-endemic regions[2,6,11]. Other genotypes are observed with horizontal transmission. A meta-analysis conducted by Wong et al. (2013) demonstrated that the risk of developing HCC is increased with genotype C infection compared to other HBV genotypes[12]. In Canada, genotype

A and D are predominantly found among acute HBV cases and genotype C is mainly associated with chronic infections[4].

1.4 Diagnosis

HBV is diagnosed via highly specific tests that detect HBV antigens and antibodies. The serological markers of HBV differ depending on acute or chronic infection. HBcAg is not easily detected in serum but can be identified in liver tissue of acutely and chronically infected individuals. Antibody to HBcAg (anti-HBc) is produced following HBV infection and its presence denotes past exposure. IgM class antibody to HBcAg (anti-HBc IgM) indicates recent infection with HBV and as such is a marker for acute HBV infection.

HBsAg can be identified in serum 30 – 60 days after exposure to HBV and is used to diagnose acute HBV infection in combination with anti-HBc IgM. Antibodies to hepatitis B surface antigen (anti-HBs) develop in response to acute HBV infection or hepatitis B vaccination and is a serological marker for HBV immunity. Persistence of HBsAg for more than 6 months after detection is indicative of chronic HBV infection. Absence of IgM anti-HBc combined with the presence of anti-HBs also indicates chronic HBV infection.

HBeAg is a soluble viral protein that indicates high levels of HBV replication. HBeAg can be present in both acute and chronic infections and usually indicates that the person is more contagious. Seroconversion of HBeAg to anti-HBe antibodies is associated with lower HBV infectivity. HBV infection may be present with or without HBeAg and its status can be used to differentiate between various stages of chronic HBV infection – immune tolerant, immune active, inactive CHB, immune escape (HBeAg negative CHB), and reactivation. See Appendix A for a detailed table on stages of chronic HBV[2,13].

1.5 Treatment

Treatment for acute HBV mainly focuses on managing symptoms such as nausea, vomiting, fatigue, and jaundice. However, 50% of HBV infections are asymptomatic and most acutely infected adults recover completely by eliminating virus from the blood and liver. Chronic HBV treatment consists of nucleotide/nucleoside antiviral agents including tenofovir, entecavir, lamivudine, adefovir, telbivudine, and emtricitabine as well as interferon therapy. The decision to initiate HBV antiviral treatment is based on laboratory findings (liver enzymes, serology, and HBV DNA levels) and severity of liver fibrosis[13-14].

WHO management guidelines for HIV-HBV co-infection recommend that patients known to be infected with HBV should be tested for HIV[14]. People with HIV-HBV co-infection should be effectively treated for both HIV and HBV infections. HIV is generally treated with highly active antiretroviral therapy. In the context of HIV-HBV co-infection, HAART therapy is tailored to consist of drugs active against HBV as well. In Canada, patients with HIV-HBV co-infection are generally treated using HAART in combination with tenofovir and nucleoside analogues (lamivudine or emtricitabine)[2,9,14,15]. HBV vaccination is recommended for people with HIV infection who are negative for HBsAg and anti-HBs. The HBV vaccine is of reduced immunogenicity for patients with HIV who have low CD4+ counts (<500 cells/ml)[8].

1.6 Assessment of Liver Status/Severity

The METAVIR scoring system can be used to assess stages of liver fibrosis and inflammatory activity. Hepatic fibrosis is divided into five stages (F0 to F4) according to METAVIR scores where F0 = no fibrosis, F1 = mild fibrosis, F2 = moderate fibrosis, F3 = severe fibrosis, and F4 = cirrhosis. Inflammatory activity ranges from A0 to A3 where A0 = no histologic

necroinflammatory activity, A1 = minimal activity, A2 = moderate activity, and A3 = severe activity[16-17].

Transient elastography (FibroScan) is one non-invasive marker used to predict hepatic fibrosis in patients with chronic liver disease. FibroScan is an ultrasound-based technique which measures the elasticity of the liver[18]. FibroScan results range from 2 to 75 kiloPascal's (kPa) and results less than 6.0 kPa are indicative of minimal to no liver disease. Concordance between FibroScan results and fibrosis stage according to METAVIR score varies depending on the type of liver disease present. Liver fibrosis for HBV is classified according to these cut-off values[19-21]:

Fibrosis Stage	Transient elastography results (kPa)
F0 to F1	0 to 6
F2	6-7
F3	7-8/8-11
F4	8-11/11 or higher

Figure 1. Comparing classification of liver fibrosis stage according to METAVIR[16-17] scoring system with Transient elastography results.

The serum alanine aminotransferase (ALT) levels and aspartate aminotransferase (AST) to platelet ratio index (APRI) score is another non-invasive marker used to assess liver fibrosis (Wai, 2003). Higher APRI scores are indicative of more advanced fibrosis/cirrhosis. APRI scores ≤0.5 indicate the absence of clinically significant fibrosis, APRI score 0.51 – 1.49 = presence of intermediate fibrosis, APRI score ≥ 1.50 = presence of clinically significant fibrosis, and APRI score ≥ 2.0 = presence of cirrhosis[16].

CHAPTER 2: INTRODUCTION AND LITERATURE REVIEW

2.1 Introduction

Human Immunodeficiency Virus (HIV) and HBV are among the leading causes of infectious disease deaths worldwide. Approximately 40 million individuals are infected with HIV and an estimated 350 million people are infected with HBV (WHO, 2014). Due to shared routes of transmission including sexual contact, body fluid exposure, needle sharing, and vertical transmission, co-infection of HIV and HBV is common[22-23]. Over 75,000 Canadians live with HIV/AIDS and 6 – 8% are HBV co-infected[3]. In Canada, both infections are primarily observed among high risk adult populations that include MSM and PWID[9]. Both infections are notifiable in Canada through the Canadian Notifiable Disease Surveillance System[3]. The prevalence of HBV is low in Canada due to routine HBV vaccination programs implemented in the mid-90s. HBV remains an important public health issue due to high prevalence rate in Canadian immigrants from HBV-endemic areas[9].

The effect of HIV on HBV infection and disease progression is well described. Liver disease caused by HBV infection is a leading cause of non-AIDS related morbidity and mortality among HIV-infected individuals on antiretroviral therapy (ARV). Approximately 30% of CHB individuals develop cirrhosis and/or liver cancer, resulting in 887,000 deaths from liver related complications [2,21]. Co-infection with HIV is associated with an increased risk of hepatitis B chronicity, cirrhosis, and liver-related mortality among HBV infected individuals[9,24-25].

The effect of HBV on HIV disease has been described previously, albeit with conflicting results. Some studies suggest that HBV infection increases the rate of HIV progression, while others conclude that HBV does not play a role in progression to AIDS[26-27]. Few studies have explored the interactions between HIV and HBV infection in Canada. The epidemiology of HIV and HBV

co-infection has been described in Northern Alberta[9]. However, these local results cannot be generalized to all of Canada. Additionally, this study lacked data regarding liver related mortality, HBV antiviral and HIV antiretroviral virologic efficacy, and socioeconomic status.

2.2 Literature Review

This section critically appraises existing literature on HIV-HBV co-infection. The objective of this literature review was to utilize cohort studies to assess the demographic and clinical characteristics of HIV-HBV co-infection. This first section reviews the impact of HIV on HBV natural history and vice versa, including the influence on hepatic fibrosis and ARV virologic efficacy. The second section focuses on the epidemiology of HIV-HBV co-infection worldwide and in Canada. The final section provides variable justification from previous cohort studies. The review was conducted prior to the initiation of this present thesis analysis (August 2016) and updated upon completion of the analysis.

2.2.1 Methodology

Observational study designs (i.e. mainly cohort studies) were included in this review. Only studies published in English were included. Selected full-text journal articles, relevant reviews, and internet articles were critically reviewed using electronic databases including MEDLINE, Google scholar, PUBMED, and EMBASE. The references of selected articles were hand-searched for relevant material on HIV, HBV, and HIV-HBV co-infection.

2.2.2 Impact of HIV on HBV

Several studies have examined the impact of HIV co-infection on the natural history of HBV infection in European, Asian, African and American countries[27-32].

Studies exploring HIV-HBV co-infection in men who have sex with men have demonstrated that lower CD4 T cell count is associated with higher risk of HBV chronicity[24]. A retrospective analysis conducted by Bodsworth et al. (1991) demonstrated that CHB is more commonly observed in HIV infected men compared to HIV uninfected men (23% vs. 4%, p=0.03)[32]. Thio et al (2013) demonstrated that HIV-HBV co-infected individuals with low CD4 T cell counts (<50 cell/mm3) had high levels of HBV replication (HBV DNA > 200,000 IU/mL) compared to those with higher CD4 T cell counts[33]. There is some evidence that lower CD4 T cell counts may be associated with increased risk of developing HCC in HIV-HBV co-infected individuals[34].

HIV infection also decreases the rate of HBeAg clearance. Vento S et al. (1989) demonstrated that HIV infected persons with protective antibodies to HBV (anti-HBs) are at higher risk of reactivation of HBV infection compared to HIV uninfected individuals[35]. Cirrhosis tends to be more common in HIV-HBV co-infected individuals despite lower ALT levels than observed in HBV mono-infection[36-37].

Matthews et al (2009) reported that initiating combination ARV (tenofovir and lamivudine) among HIV-HBV co-infected individuals reduces the risk of liver-related disease and mortality compared to HBV monotherapy[38]. Dore et al (2004) also reported greater HBV DNA suppression in HIV-HBV co-infected individuals initiating ARV therapy compared to HBV monotherapy[39]. These studies demonstrate that HIV adversely impacts HBV infection with rapid progression to liver fibrosis[40-41]. Specifically, fibrosis progression is dependent on age, HBV DNA level, CD4 count and use of antiretrovirals.

2.2.3 Impact of HBV on HIV

There is evidence that HBV co-infection hastens the progression of HIV to AIDS. The multinational AIDS Cohort study (MACs) conducted in USA suggested that prior to HAART initiation, HIV-HBV co-infected patients had lower CD4 T cell counts compared to HIV mono-infected patients[33,42]. Other studies analysing HIV-HBV co-infection among patients receiving ARV have also reported lower CD4 cell counts among co-infected individuals compared to HIV mono-infected patients [43]. However, this has been challenged in recent years. A retrospective cohort study conducted by Coffin et al. (2013) analysing the characteristics of HIV-HBV co-infected individuals on long-term ARV did not find clinically significant differences between the median CD4 T cell counts[44].

Several studies have analysed the impact of HBV co-infection on HIV virologic response to ARV. These studies have reported that the time to HIV virologic suppression is independent of HBV status[27,45-46]. However, studies examining the association between HBV co-infection on HIV immunological recovery during ARV have presented conflicting results. A cohort study conducted by the EuroSIDA group did not find any association between HBV co-infection and immunological response to ARV[25]. On the contrary, the Swiss HIV Cohort Study reported that the presence of HBV infection (past or present) adversely impacts CD4 cell recovery during the first three years of ARV[46-47]. These studies have several key limitations including large populations of unknown HBV status and lack of liver disease cofactors such as alcohol use

2.2.4 Epidemiology of HIV-HBV Co-infection

HIV-HBV co-infection epidemiology varies depending on the different geographical regions due to varying dominant modes of transmission. In Canada, HIV-HBV co-infection is primarily

observed among MSM and PWID[8-9,48-49]. A retrospective analysis conducted by Pittman et al. (2014) focuses on describing the HBV natural history among HIV positive patients in Northern Alberta[9]. This study population had a 5.5% prevalence of HIV-HBV co-infection which is lower compared to other low endemic countries. More males were co-infected than females and tended to be White whereas females with HIV-HBV co-infection were mainly Black ($p<0.0001$) followed by Indigenous women. Using the Public Health Agency of Canada surveillance system, Pittman et al. (2014) identified that the HIV-HBV co-infected females were primarily from HIV-endemic countries[3]. HBV infection was considered endemic among the Canadian Indigenous Peoples prior to the wide availability of the HBV vaccines. According to PHAC, the rate of acute hepatitis B infection is approximately three times higher among Canadian Indigenous persons compared to non-indigenous persons[3]. Socioeconomic factors within this population including high unemployment rates and limited housing may contribute to the higher prevalence of HBV. While vaccine implementation programs have been successful, living in remote communities with poor access to medical care can impede this success. Viral hepatitis remains an important concern within Canadian Indigenous populations[3-4].

An interesting finding from this study was that the prevalence of HBV co-infection has decreased in more recent years. Participants that were HIV positive in the 1980s had a higher prevalence of HBsAg compared to those infected in the 1990s. This can be attributed to the introduction of HBV vaccinations in the early 90s. Pittman et al. (2014) also concluded that there was a higher mortality rate in HIV-HBV co-infected individuals diagnosed with HIV before 1997[9]. They also demonstrated that mortality rates were twice as high among HIV-HBV co-infected individuals compared to HIV mono-infected patients following the introduction of HAART (23.5% versus 9.5%; $P<0.001$). As informative as this study was in describing the HIV-

HBV population in North Alberta, there are key limitations. Approximately 10% of participants had unknown HBsAg status. This could skew the prevalence of co-infected individuals in the cohort and lead to distorted results.

Gillis et al. (2013) conducted a cohort study utilizing the Ontario HIV Treatment Network Cohort (OCS) to evaluate the health-related quality of life in HIV positive individuals[48]. This study revealed similar results as the one conducted by Pittman et al (2014)[9]. Co-infected patients were mostly MSM. Participants with HIV-HBV co-infection had been infected with HIV longer than patients with HIV mono-infection HIV patients. The analysis revealed no relationship with diminished mental health related quality of life scores. These results were adjusted for potential confounders including PWID, recent drug, and alcohol use. To date, this is the only study that has examined health related quality of life in a Canadian HIV-HBV co-infected population. Similar to the previous study, this analysis is based on co-infection status abstracted from medical charts and thus, may have underestimated or misclassified HBV participants. The timing of hepatitis infection is also uncertain in this study and was assumed to have occurred before or concurrent with the HIV infection. These studies provide limited information regarding the severity of liver disease in HIV-HBV co-infected individuals.

CHAPTER 3: RATIONALE & OBJECTIVES

This thesis aims to investigate the characteristics and clinical outcomes of HIV-HBV co-infection in a Canadian cohort of antiretroviral treated HIV individuals.

3.1 Rationale

Despite advances in HIV and HBV mono-infection prevention and treatment, research on HIV-HBV co-infection remains sparse. Studies exploring the impact of HIV-HBV co-infection on individuals receiving ARV are limited and inconclusive. This can be attributed to small sample size, misclassification of HBV status, and large heterogeneity in study populations.

There are currently two cohort studies that have described the HIV-HBV co-infection population in Canada. However, there are no studies examining the relationship between HBV status and hepatic fibrosis or the impact of HBV infection on HIV virologic suppression and rebound.

This proposed analysis will use a large national cohort (CANOC)[49] with documented HIV status, known dates of HIV infection, detailed ARV therapy regimen, and longer follow-up duration to explore the clinical characteristics of HIV and HBV co-infection in Canada. Liver disease is one of the leading causes of non-AIDS related mortality among HIV patients, and by evaluating the impact of HIV-HBV co-infection on liver fibrosis, the findings of this study may have relevant clinical implications. By examining the relationship between HIV, HBV and antiretroviral therapy, HIV and HBV care may be improved. This analysis will contribute new knowledge to the demographic and epidemiology of HIV-HBV co-infection in Canada.

3.2 Objectives and Hypothesis

The objectives and hypothesis of the thesis are:

1) To describe the demographic characteristics of HIV infected and HIV-HBV co-infected individuals in the CANOC cohort.

Hypothesis: HIV-HBV co-infected participants will more likely be male, PWID, MSM, and have higher APRI scores at baseline prior to ARV initiation compared to people living with HIV.

2) To evaluate the association of HBV co-infection on hepatic fibrosis among a cohort of people living with HIV.

Hypothesis: Participants with known HBV status will be independently associated with having hepatic fibrosis and cirrhosis. HIV-HBV co-infected subjects will more likely have significant fibrosis/cirrhosis compared to moderate/no fibrosis prior to initiating ARV therapy and at end of follow-up period.

3) To assess the impact of HIV-HBV co-infection on HIV virologic efficacy (virologic suppression and rebound) among ARV treated HIV patients.

Hypothesis: HBV status will be independently associated with virologic suppression (less likely to achieve suppression) and more likely to rebound compared to HIV mono-infection.

CHAPTER 4: METHODS

4.1 Study Design

A longitudinal retrospective cohort analysis was conducted using data from the Canadian Observation Cohort (CANOC) Collaboration. CANOC is an interprovincial collaboration of eight cohorts from British Columbia (BC), Quebec, and Ontario. A detailed description of the CANOC profile has been previously published[49].

4.2 Study Population

The eligibility criteria for CANOC includes documented HIV infection, resident of Canada, aged 18 years and over, ARV therapy naïve (should have initiated first ARV regiment on or after January 1, 2000, and participants are required to have at least 1 measurement of HIV RNA viral load and CD4 cell count within 6 months of initiating combination ARV (use of three of more antiretroviral drugs) between January 1, 2000 and December 14, 2014. As of December 31st, 2014, the CANOC cohort comprised of 10,477 ARV initiators.

For this analysis, starting with the CANOC cohort, we included only those participants with complete HBV infection status and complete liver enzyme data (APRI scores). We defined baseline as any time within 1 year prior to initiating first ARV therapy. Baseline values for CD4 cell count and HIV viral load was obtained within 6 months prior to first ARV date.

End of follow-up period was defined as any time within 1 year before the patient's last follow date or December 31, 2014 (for example, if study end date for participant is Jan 1, 2010 then the recent APRI score is calculated using AST/platelet values between Jan 1, 2009 and Jan 1, 2010). This analysis includes data collected up to December 31, 2014.

4.3 Data Collection

Data collection and extraction of clinical and demographic data of eligible participants is conducted bi-annually at the participating cohort data centres. Personally identifiable information is removed before submitting demographic and clinical data to the CANOC data-coordinating site – British Columbia Centre for Excellence in HIV/AIDS in Vancouver, BC.

4.4 Outcomes

1. Liver fibrosis was defined using APRI scores, a non-invasive marker. APRI was calculated using ([AST/ (upper limit of normal AST)/platelets] x 100)[16]. The upper limit of normal AST was set to 40 IU/L. Values above this threshold were considered 'elevated levels' of AST. The APRI score was categorized as:

APRI score	**Category**
<0.50	Mild/no fibrosis
0.51 – 1.49	Moderate/intermediate fibrosis
1.50 – 1.99	Clinically significant fibrosis
≥2.0	Advanced fibrosis/cirrhosis

APRI ratios ≥ 1.50 were used to denote clinically significant fibrosis. APRI scores have been validated as a non-invasive marker for liver fibrosis. It has been previously used to identify advanced liver fibrosis and cirrhosis in patients infected with HCV and HIV[16,50-52].

2. The primary outcome measurements for assessing response to HIV antiretroviral therapy in HBV-HIV co-infected individuals were time to HIV RNA suppression from date of first ARV initiation and time to virologic rebound (VR).

a. Virologic suppression (VS) was defined as first of two consecutive HIV RNA measurements <50 copies/ml at least three months apart.

b. Virologic rebound was defined as first of two consecutive HIV-RNA viral loads >200 copies/mL at least three months apart after VS.

Participants who did not achieve either of the outcome end-points were censored at their last available viral load collection date or December 31, 2014 (whichever came first).

4.5 Variables

Our primary variable of interest was HBV infection status. Participants were classified as 'ever HBV co-infected' if identified as HBV-positive. HBV infection was defined as surface antigen positive, HBV DNA positive, and/or by physician report. Since all HIV positive patients are required to undergo HBV testing, participants were tested for HBV infection at cohort entry. If participants were HBV negative, they were offered hepatitis B vaccination and categorized as HBV negative.

Demographic variables of interest included: age, sex, race, province of residence, known HIV transmission risk factors including MSM, PWID, and deceased. Hepatitis C infection status was ascertained through antibody test, PCR, or physician report. If a participant was HCV RNA positive, they were classified as being 'ever hepatitis C co-infected'. AIDS-defining illnesses were based on the classification reported by 1993 Centers for Disease Control and Prevention[53](Appendix B). Baseline AIDS-defining illness (ADI) was recorded prior to, or on first ARV date. Only confirmed ADI cases were included. 'No ADI ever' refers to no recorded ADI's during study period. 'No ADI before/or first ARV date' refers to no recoded ADI prior to study enrollment.

These are standard variables evaluated in studies describing HIV-HBV or HIV-HCV co-infected individuals. Additionally, they have also been analyzed by previous cohort studies conducted by Pittman et al., the EuroSIDA, Swiss HIV Cohort Study, and the MAC cohort studies [9,25-28,47].

4.6 Missing data

A complete case analysis strategy was employed to handle missing data for all variables of interest except for race (due to large proportion of missing data). Missing race data was categorized as 'unknown' category within the variable. Those with missing or unknown data regarding viral hepatitis infection status and other important covariates (HIV risk factors, liver enzyme levels, baseline CD4+ cell counts, and HIV viral load measurements) were excluded from all analysis including descriptive study.

4.7 Statistical Analysis

For all analysis, we used backward selection to build adjusted models and the final model selection was based on the overall goodness of fit as assessed by the Akaike information criterion (AIC). Data were analyzed using SAS software (version 9.4, SAS Institute)[54].

4.7.1 Objective I

Demographic and clinical characteristics were compared between HIV-HBV co-infected and HIV infected groups using chi-square or Fisher exact tests for categorical variables, and Wilcoxon's Rank Sum test for continuous variables. Differences in characteristics across different categories of liver fibrosis determined by APRI scores were compared using Kruskal-Wallis rank test or Chi-square/Fisher exact tests. Characteristics were presented using frequencies and proportions for categorical variables and median and interquartile ranges for

continuous variables. Cochran-Armitage trend test was performed for ordinal group comparisons (APRI scores by HBV status).

4.7.2 Objective II

Logistic regression models were developed to assess the independent association of HBV infection status on severe fibrosis stage. Chi-square tests and correlation coefficients were used to assess collinearity among categorical and continuous variables respectively. Additionally, variable inclusion/exclusion for further analysis was determined based on significance ($p<0.05$). The variance inflation factor (VIF) was used to assess for multicollinearity among the predictor variables in the model. VIF quantifies how much the variance is inflated due to correlation between the covariates. A VIF value of more than 10 (consequently, a tolerance value of less than 0.1) was considered as predicting multicollinearity. None of the predictor variables exceeded a tolerance value ≤ 0.1 indicating lack of multicollinearity among the variables of interest.

Primary analysis compared clinically significant fibrosis/cirrhosis (APRI$\geq$1.5) to mild/moderate fibrosis (APRI $\leq$1.5) using HBV status as the primary predictor at both baseline and end of study follow-up. Unadjusted associations between covariates of interest and APRI>1.5 were first assessed in simple logistic regression models. Variables that were statistically significant ($p<0.05$) were then adjusted for in multivariable models.

Secondary analyses were conducted to assess the relationship between HBV infection status across all categories of APRI values. Since this is an ordinal outcome, a proportional odds regression model was developed. This model is based on the proportional odds assumption that states that the relationship between different categories of the outcome is the same. In other

words, the outputted odds ratio accurately describes the relationship between the lowest category versus all the next higher categories. The proportional odds assumption was checked using the score test for the proportional odds assumption. A significant test statistic ($p<0.05$) indicates that the proportional odds assumption has been violated. For both baseline and end of study follow-up period, univariate analyses were conducted between APRI value and covariates of interest. HBV status did not violate the proportional odds assumption and was included in multivariate analyses. All other covariates were included in multivariable analyses based on statistical significance ($p<0.05$).

We explored the interaction between HIV-HBV co-infection and years of ARV therapy on APRI scores. We therefore built logistic regression models stratified by HBV infection status, using APRI>1.5 as the outcome and years of ARV therapy as the primary predictor. Unadjusted odds ratios (OR) and adjusted odds ratios (aOR) are presented along with 95% CI.

4.7.3 Objective III

Cox proportional hazards models were developed to describe the factors associated with 1) time to HIV RNA suppression after ARV initiation and 2) time to virologic rebound following VS. Rebound analysis were conducted only among those that achieved suppression. The proportional hazards assumption was checked both graphically and statistically. Variables that violated this assumption were excluded from multivariable analyses to avoid introducing any biases. Variables that met the assumption were included in multivariable analysis if they were significant ($p<0.05$) by univariate analyses. Unadjusted and adjusted hazard ratios (aHR) are presented along with 95% CI.

4.8 Ethics

Simon Fraser University Research Ethics Board and the University of British Columbia Research Ethics Board have approved the activities of CANOC. All of the participating cohorts have received ethics approval from their local institutional review boards: Providence Health Care Research Institute Office of Research Services, The Ottawa Hospital Research Ethics Board, University Health Network (UHN) Research Ethics Board, Véritas Institutional Review Board (IRB), Biomedical C (BMC) Research Ethics Board of the McGill University Heath Centre (MUHC), University of Toronto HIV Research Ethics Board (HIV REB), and Women's College Hospital Research Ethics Board.

An amendment to the existing CANOC ethics application at the Ottawa Hospital Research Institute was submitted on August 23rd, 2016. This application was approved by the Ottawa Health Science Network Research Ethics Board (OHSN-REB) to conduct secondary data analysis at the Ottawa Hospital Research Institute (Appendix C).

CHAPTER 5: RESULTS

5.1 Study Population

The CANOC cohort consists of a total of 10,477 HIV positive participants. However, a single site – Maple Leaf Medical Clinic (MLMC) - misclassified HBV vaccine antibody response as evidence of HBV infection. Following the exclusion of participants from the MLMC site, a total of 9,289 HIV-positive participants were eligible for this analysis, 2,476 (27%) of whom were excluded due to missing/unknown HBV status. A comparison of the baseline characteristics between those included for consideration in our study cohort (N=6,813) and the excluded participants [MLMC) (N=1,158)] did not reveal any significant differences to impact our objectives (Appendix D).

Of the 6,813 remaining participants, 4,394 were excluded based on missing liver enzyme data including baseline and end of study AST levels. Our final analytical population was 2,419 HIV positive participants (Figure 2).

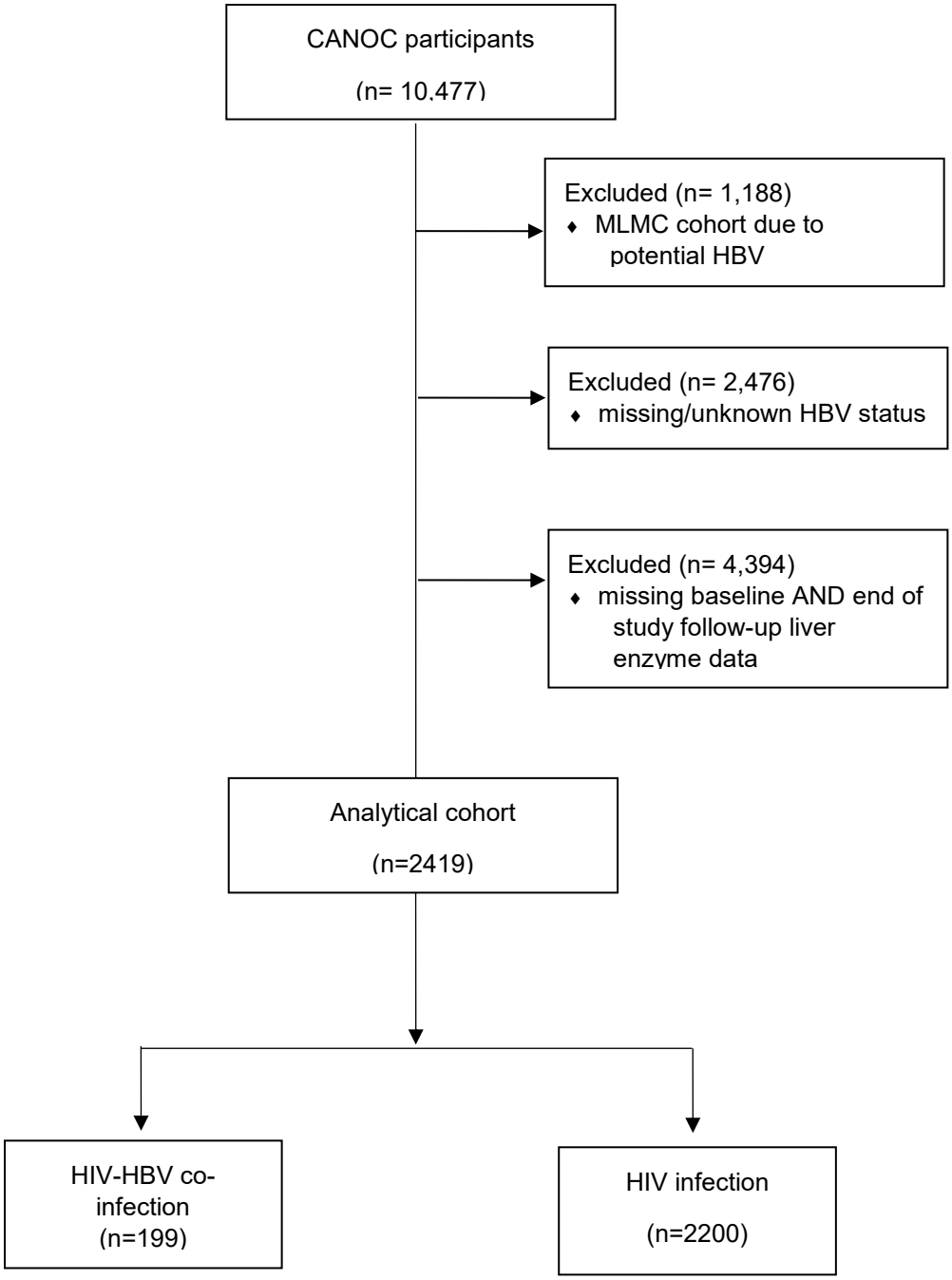

Figure 2. STROBE diagram for the progress of analytical cohort selection.

5.2 Descriptive Analysis

Of the 2,419 HIV positive individuals with known HBV test results, 199 (8%) were HIV-HBV co-infected. Demographic characteristics including age, sex, race, MSM, and province of

residence did not differ between HIV infected and HIV-HBV co-infected groups **(Table 1)**. The prevalence of HIV-HCV co-infection was significantly higher among those who were also hepatitis B co-infected (31%, vs. 23%, p=0.02). Compared to HIV infected participants, HIV-HBV co-infected participants were more likely to acquire HIV infection through injection drug use (28% vs. 21%, p=0.03). The proportions of individuals having experienced a baseline AIDS-defining illness (ADI) on or before their first ARV treatment date were significantly different between HIV-HBV co-infected and HIV infected individuals (p=0.01), with co-infected participants less likely to have a baseline ADI (62% vs. 72%). Mortality was higher among patients with HIV-HBV co-infection: 11% compared with 7% in patients without HBV infection (p=0.02) for participants included in our study.

The estimated median duration of ARV therapy exposure was longer among HIV-HBV co-infected group (5.97 years; IQR: 3.11 – 9.94 years) compared to HIV infected individuals (5.01 years; IQR: 2.50 – 8.75 years) (p=0.003). The median CD4 cell count at baseline was 188 cells/mm^3 in HIV-HBV co-infected participants (IQR: 120 – 360) versus 235 cells/mm^3 in HBV-negative participants (IQR: 85 – 294) (p=0.0002) **(Table 2)**. HIV-HBV co-infected participants also had higher median APRI scores at baseline (0.50 vs. 0.37, p<0.0001) and at end of study follow-up (0.32 vs. 0.30, p=0.03) compared to those without HBV infection. There were no statistically significant differences between the baseline and end-of-follow up HIV viral load measurements between the two sub-populations.

Table 1. Demographic characteristics of the HIV infected study participants by HBV infection status (N=2,419).

Demographic Characteristics	HBV negative N=2,220	HBV positive N=199	p value[a]
Age	40 (32.0-46.0)	39 (33.0-45.0)	0.69
Male sex	1,806 (81)	164 (82)	0.71
Deceased	150 (7)	22 (11)	0.02
Race			0.11
White	967 (44)	78 (39)	
Black	408 (18)	50 (25)	
Indigenous	138 (6)	10 (5)	
Asian	147 (7)	20 (10)	
Hispanic	116 (5)	9 (5)	
Other	82 (4)	5 (3)	
Unknown	362 (16)	27 (14)	
Province			0.49
BC	946 (43)	88 (44)	
ON	870 (39)	70 (35)	
QC	404 (18)	41 (21)	
Hepatitis C co-infection	520 (23)	61 (31)	0.02
Risk Factors			
MSM	998 (45)	93 (47)	0.63
PWID	467 (21)	55 (28)	0.03
Baseline ADI[b]			0.01
≥1 before/at FARVDT	1,596 (72)	123 (62)	
None before/at FARVDT	446 (20)	55 (28)	
No ADI ever	178 (8)	21 (11)	
Years on ARV therapy	5.01 (2.50 – 8.75)	5.97 (3.11 – 9.94)	0.003

BC: British Colombia, ON: Ontario, QC: Quebec; MSM: Men who have sex with men; PWID: people who inject drugs; ADI: AIDS defining illness; ARV: anti-retroviral therapy; FARVDT: first naïve ARV date.

[a] Data shown are frequencies and proportions for categorical variables and median and interquartile ranges for continuous variables. p values for continuous variables were calculated using chi-square or Fisher exact tests and for continuous variables were calculated using Wilcoxon's Rank Sum tests.

[b]'No ADI ever' refers to no recorded ADI's during study period. 'None before/or first ARV date' refers to no recoded ADI prior to study enrollment.

Table 2. Clinical characteristics of the HIV infected study participants by HBV infection status (N=2,419).

Clinical Characteristics	HBV negative N=2,220	HBV positive N=199	p value[a]
Baseline HIV viral load (Log10 copies/mL)			
<4	332 (15)	30 (15)	0.87
4 – 5	1,011 (46)	94 (47)	
>5	877 (40)	75 (38)	
End of follow-up HIV viral load (Log10 copies/mL)			0.38
<4	2,104 (95)	193 (97)	
4 – 5	69 (3)	4 (2)	
>5	47 (2)	2 (1)	
Baseline CD4 cell count (cells/mm^3)	235 (120-360)	188 (85 – 294)	0.0002
Baseline CD4 count category (cells/mm^3)	897 (40)	105 (53)	
≤199	720 (32)	60 (30)	0.002
200 – 349	367 (17)	24 (12)	
350 – 499	236 (11)	10 (5)	
> 500			
Baseline AST	29 (23-41)	36 (26-62)	<0.0001
Baseline AST category			
Normal (10 - 40 IU/L)	1,649 (74)	120 (60)	<0.0001
Elevated (>40 IU/L)	571 (26)	79 (40)	
End of follow-up AST	25 (20-33)	26 (20-38)	0.14
End of follow-up AST category			
Normal (10 - 40 IU/L)	1,855 (84)	152 (76)	0.01
Elevated (>40 IU/L)	365 (17)	47 (24)	
Baseline APRI	0.37 (0.27-0.59)	0.50 (0.35-1.04)	<0.0001
Baseline APRI category			
≤0.5 (mild)	1,501(68)	99 (50)	<0.0001[b]
0.51-1.49 (moderate)	593 (27)	68 (34)	
1.50 – 1.99 (significant)	34 (2)	8 (4)	
≥2.0 (advanced/cirrhosis)	92 (4)	24 (12)	
End of follow-up APRI	0.30 (0.22-0.43)	0.32 (0.23-0.56)	0.03
End of follow-up APRI category			
≤0.5 (mild)	1,831 (82)	145 (73)	0.005[b]
0.51-1.49 (moderate)	302 (14)	39 (20)	
1.50 – 1.99 (significant)	24 (1)	3 (2)	
≥2.0 (advanced/cirrhosis)	63 (3)	12 (6)	
Suppressed since FARVDT	2,040 (92)	190 (96)	0.07
Rebound (since first VS)	481 (24)	56 (29)	0.07

AST: aspartate aminotransferase; APRI: aspartate aminotransferase to platelet ratio index; VS: virological suppression; FARVDT: first naïve ARV date.

[a] Data shown are frequencies and proportions for categorical variables and median and interquartile ranges for continuous variables. p values for continuous variables were calculated using chi-square or Fisher exact tests and for continuous variables were calculated using Wilcoxon's Rank Sum tests.

[b]p values were calculated using Kruskal-Wallis rank tests.

5.3 Evaluation of Predictors of Liver Fibrosis

5.3.1 Prevalence of Liver Fibrosis

Of the 2,419 study participants, 66% (N=1660) had no or mild fibrosis, 27% (N=661) had moderate fibrosis, 1.7% (N=42) had clinically significant fibrosis, and 4.8% (N=116) had advanced fibrosis/cirrhosis according to the APRI values.

5.3.2 Comparing mild/moderate fibrosis (APRI<1.5) to clinically significant/advanced fibrosis (APRI>1.5)

Among the 2,419 study participants, 159 (6.5%) had an APRI value of >1.5 at baseline, of which 32 (20%) were HIV-HBV co-infected and 90 (57%) were HIV-HCV co-infected. The demographic and clinical characteristics differed among the study participants with varying APRI scores **(Table 3).** Those with higher APRI scores (>1.5) tended to be older at study initiation, were more likely to be males, Caucasian, and more likely to have acquired HIV through injection drug use compared to participants with mild/moderate fibrosis. Participants did not differ with regards to achieving HIV viral load suppression since first ARV treatment date. However, participants with APRI>1.5 were less likely to rebound following VS compared to those with APRI<1.5 (69% vs. 79%, p=0.05). Participants with significant to advanced fibrosis also had higher HIV viral loads and lower median CD4 cell counts at baseline.

Table 3. Characteristics of HIV infected study participants by baseline APRI values (N=2,419)

Variable	Baseline APRI values (N=2419)		p-value[a]
	APRI<1.5 (N=2,261)	APRI>1.5 (N=158)	
Age	39 (32 – 46)	42 (36 – 48)	0.001
Male sex	3,373 (83)	229 (81)	0.46
Deceased	136 (6)	36 (23)	<0.0001
Race			0.006
White	963 (43)	82 (52)	
Black	443 (20)	15 (9)	
Indigenous	135 (6)	13 (8)	
Asian	158 (7)	9 (6)	
Hispanic	122 (5)	3 (2)	
Other	78 (3)	9 (6)	
Unknown	362 (16)	27 (17)	
Province			
BC	938 (41)	96 (61)	
ON	897 (40)	43 (27)	<0.0001
QC	426 (19)	19 (12)	
Hepatitis B coinfection	167 (74)	32 (20)	
Hepatitis C co-infection	491 (22)	90 (57)	<0.0001
Risk Factors			
MSM	1,258 (56)	70 (44)	0.006
PWID	449 (20)	73 (46)	<0.0001
Baseline ADI			
≥1 before/at FARVDT	465 (21)	36 (23)	0.43
None before/at FARVDT	1,606 (71)	113 (72)	
No ADI ever	190 (8)	9 (5)	
Median years on ARV therapy	5.09 (8.81- 14.97)	5.03 (10.15 -14.833)	0.72
Baseline HIV viral load (Log10 copies/mL)	349 (15)	13 (8)	0.003
<4	1,040 (46)	65 (41)	
4 - 5	872 (39)	80 (51)	
>5			
End of follow-up HIV viral load (Log10 copies/mL)			0.61
<4	2,149 (95)	148 (94)	
4 - 5	67 (3)	6 (4)	
>5	45 (2)	4 (3)	
Baseline CD4 count(cells/mm3)			
<199	917 (41)	85 (54)	
200 - 349	735 (33)	45 (28)	0.006
350 -499	371 (16)	20 (13)	
>500	238 (11)	8 (5)	
Suppressed since FARVDT	2,088 (92)	142 (90)	0.26
Rebound (since first VS)	1,595 (76)	98 (69)	0.05

BC: British Colombia, ON: Ontario, QC: Quebec; MSM: Men who have sex with men; PWID: People who inject drugs; ADI: AIDS defining illness; ARV: anti-retroviral therapy; FARVDT: first naïve ARV date.

[a]Data shown are frequencies and proportions for categorical variables and median and interquartile ranges for continuous variables. p values for continuous variables were calculated using chi-square or Fisher exact tests and for continuous variables were calculated using Wilcoxon's Rank Sum tests.

We wanted to further explore whether these factors were associated with having an APRI value >1.5 compared to having lower APRI values (<1.5) at baseline prior to ARV initiation. Compared to HIV infected individuals, HIV-HBV co-infected participants were 3.08 (95% CI: 1.99 – 4.78) times more likely to have an APRI value>1.5 compared to APRI<1.5 at baseline prior to ARV initiation. Similarly, HIV-HCV co-infected participants were more likely (aOR: 4.55, 95% CI: 3.26 – 6.35) to belong in higher APRI categories prior to ARV initiation after adjusting for demographic characteristics (**Table 4)**. Other risk factors such as MSM, IDU, baseline HIV viral load, and CD4 cell counts were also associated with increased odds of having significant/advanced fibrosis. However, in multivariable analyses, only HBV and HCV co-infections, age at first ARV date, and baseline HIV viral load remained significantly associated with having clinically significant/advanced fibrosis prior to ARV therapy.

Table 4. Logistic regression model showing factors associated with having clinically significant or advanced liver fibrosis compared to mild/moderate fibrosis at baseline prior to ARV initiation as determined by APRI values (N=2,419).

Variable	APRI >1.5 (clinically significant/advanced fibrosis) at baseline					
	Univariate Analysis			Multivariable Analysis		
	OR	95% CI	Wald p	OR	95% CI	Wald p
Hepatitis B						
HBV negative		Reference			Reference	
HBV positive	3.18	2.10 - 4.84	<0.0001	3.08	1.99 - 4.78	<0.001
Hepatitis C						
HCV negative		Reference			Reference	
HCV positive	4.77	3.43 - 6.63	<0.0001	4.55	3.26 - 6.35	<0.001
Baseline AIDS					-	
No ADI ever		Reference				
None before/at FARVDT	1.49	0.74 - 2.98	0.44			
≥1 before/at FARVDT	1.63	0.77 - 3.46				
Race					-	
White		Reference				
Black	0.40	0.23 - 0.70				
Indigenous	1.13	0.61 - 2.09				
Asian	0.67	0.33 - 1.36	0.01			
Hispanic	0.29	0.09 - 0.93				
Other	1.35	0.66 - 2.80				
Unknown	0.88	0.56 - 1.38				
Birth sex					-	
Female		Reference				
Male	1.06	0.70 - 1.62	0.78			
Province					-	
BC		Reference				
ON	0.47	0.32 - 0.68	<0.0001			
QC	0.44	0.26 - 0.72				
Years on ARV	1.02	0.98 - 1.06	0.37		-	
Age at first ARV	1.02	1.01 - 1.04	0.003	1.02	1.00 - 1.04	0.01
MSM	0.63	0.46 - 0.88	0.006		-	
PWID	3.47	2.49 - 4.82	<0.0001		-	
Baseline HIV viral load (Log10 copies/mL)						
<4		Reference			Reference	
4 – 5	1.68	0.91 - 3.08	0.004	1.49	0.80 - 2.77	0.21
>5	2.46	1.35 - 4.48		2.15	1.16 - 3.97	0.02
Baseline CD4 count (cells/mm^3)					-	
> 500		Reference				
≤199	2.76	1.32 - 5.77				
200 – 349	1.82	0.85 - 3.92	0.007		-	
350 – 499	1.60	0.70 - 3.70				

BC: British Colombia, ON: Ontario, QC: Quebec; MSM: Men who have sex with men; PWID: People who inject drugs; ADI: AIDS defining illness; ARV: anti-retroviral therapy; FARVDT: first naïve ARV date.

We also examined whether similar factors predicted having APRI values >1.5 after the initiation of ARV therapy. HIV-HBV co-infection was no longer associated with having APRI >1.5 after ARV therapy exposure in adjusted analyses **(Table 5).** HIV-HCV co-infection had increased odds of being associated with having clinically significant /advanced fibrosis after ARV therapy (aOR: 6.35, 95% CI: 4.12 - 9.79) in analyses adjusted for end of study HIV viral load and baseline CD4 cell counts.

Table 5. Logistic regression model showing factors associated with having clinically significant or advanced liver fibrosis compared to mild/moderate fibrosis at end of study follow up after ARV therapy as determined by APRI values (N=2,419).

	APRI >1.5 (clinically significant/advanced fibrosis) at end of study follow up					
	Univariate Analysis			Multivariable Analysis		
Variable	**OR**	**95% CI**	**Wald p**	**OR**	**95% CI**	**Wald p**
Hepatitis B					-	
Never co-infected		Reference				
Ever co-infected	2.00	1.13 -3.53	0.02			
Hepatitis C						
Never co-infected		Reference			Reference	
Ever co-infected	7.03	4.61 - 10.74	<0.0001	6.35	4.12 - 9.79	<0.0001
Baseline AIDS					-	
No ADI ever		Reference				
None before/at FARVDT	1.72	0.69 - 4.31	0.41			
≥1 before/at FARVDT	1.95	0.73 - 5.19				
Race					-	
White		Reference				
Black	0.28	0.12 - 0.65				
Indigenous	1.83	0.95 - 3.54				
Asian	0.77	0.33 - 1.84				
Hispanic	0.51	0.16 - 1.67	0.009			
Other	1.00	0.35 - 2.84				
Unknown	1.31	0.78 - 2.18				
Years on ARV	0.97	0.92 - 1.02	0.26		-	
Birth sex					-	
Female		Reference				
Male	0.69	0.43 - 1.10	0.12			
Province					-	
BC		Reference				
ON	0.44	0.28 - 0.70	0.0001			
QC	0.34	0.17 - 0.67				
Age at first ARV	1.01	0.99 - 1.02	0.21		-	
MSM	0.34	0.26 - 0.60	<0.0001		-	
PWID	5.05	3.37 - 7.56	<0.0001		-	
Baseline viral load					-	
<4		Reference				
4 – 5	1.46	0.72 - 2.93				
>5	1.87	0.94 - 3.74				
End viral load						
<4		Reference			Reference	
4 – 5	3.33	1.54 - 7.16	<0.0001	2.27	1.01 - 5.10	<0.0001
>5	8.80	4.41 - 17.43		7.33	3.46 - 15.51	
Baseline CD4						
> 500		Reference			Reference	
≤199	2.73	1.17 - 6.38		1.82	0.76 - 4.36	
200 – 349	1.49	0.60 - 3.64	0.0001	1.12	0.45 - 2.80	0.004
350 – 499	0.41	0.12 -1.48		0.33	0.09 - 1.22	

BC: British Colombia, ON: Ontario, QC: Quebec; MSM: Men who have sex with men; PWID: People who inject drugs Injection drug users; ADI: AIDS defining illness; ARV: anti-retroviral therapy; FARVDT: first naïve ARV date.

HIV-HBV co-infection was associated with APRI values >1.5 both at baseline and end of study follow up. **(Table 4)**. This analysis only compared two subgroups using an APRI cut-off value of 1.5 for advanced fibrosis/cirrhosis. We wanted to compare factors associated with hepatic fibrosis across all categories of APRI classification **(Table 6).** Thus, we performed multivariable proportional odds logistic regression to investigate whether being HIV-HBV co-infected is associated with a greater chance of having more severe hepatic fibrosis. Prior to ARV initiation, HIV-HBV (OR: 2.30, 95% CI: 1.74 - 3.04) and HIV-HCV co-infection (OR: 2.95, 95% CI: 2.45 - 3.55) were independently associated with higher APRI score category compared to HIV-infected participants **(Table 6)**. After adjusting for HIV-HCV co-infection, province of residence, baseline HIV viral load and CD4 cell counts, HIV-HBV co-infection remained significantly associated with increased odds of a higher fibrosis category (aOR: 2.19, 95% CI: 1.64 - 2.93).

Participants with baseline CD4 cell counts <200 cells/mm^3 were more likely to have higher APRI score indicating severe liver fibrosis than those with CD4+ cell counts >499 cells/mm^3 in unadjusted (OR: 2.63, 95% CI: 1.91 – 3.63) and adjusted analyses (aOR: 1.76, 95% CI: 1.27 – 2.50).

Table 6. Multivariable proportional odds logistic regression models showing the odds of more severe hepatic fibrosis compared to milder fibrosis as determined by APRI values in HIV-infected patients prior to ARV initiation (at baseline) (N=2,419).

Variable	Severity of Liver Fibrosis (APRI values) at baseline					
	Univariate			Multivariable		
	POR	95% CI	Wald p	POR	95% CI	Wald p
Hepatitis B						
HBV negative		Reference			Reference	
HBV positive	2.30	1.74 - 3.04	<0.0001	2.19	1.64 - 2.93	<0.0001
Hepatitis C						
HCV negative		Reference			Reference	
HCV positive	2.95	2.45 - 3.55	<0.0001	2.52	2.07 - 3.08	<0.0001
Race					-	
White		Reference				
Black	0.46	0.36 - 0.60				
Indigenous	1.28	0.92 - 1.80	<0.0001			
Other	0.78	0.62 - 1.00				
Unknown	0.67	0.52 - 0.85				
Baseline AIDS					-	
Never		Reference				
None	1.48	1.06 - 2.07	<0.001			
At least one	2.15	1.49 -3.09				
Years on ARV	1.04	1.02 - 1.06	0.0009		-	
Birth sex					-	
Female		Reference				
Male	1.39	1.12 - 1.74	0.004			
Province					-	
BC		Reference			Reference	
ON	0.56	0.47 - 0.67	<0.0001	0.74	0.60 - 0.90	0.001
QC	0.47	0.37 - 0.60		0.68	0.52 - 0.88	
Age at first ARV	1.01	1.01 - 1.02	0.0006		-	
MSM	0.77	0.65 - 0.91	0.002		-	
PWID	2.70	2.23 - 3.27	<0.0001		-	
Baseline HIV viral load (log10 copies/ml)						
<4		Reference			Reference	
4 – 5	1.74	1.30 - 2.33	<0.0001	1.51	1.12 - 2.04	<0.0001
>5	3.38	2.53 - 4.52		2.58	1.90 - 3.52	
Baseline CD4 count (cells/mm^3)						
>499		Reference			Reference	
≤199	2.63	1.91 - 3.63		1.76	1.27 - 2.50	
199 – 349	1.45	1.04 - 2.02	<0.0001	1.26	0.89 - 1.77	<0.0001
350 – 499	1.07	0.73 - 1.55		1.02	0.70 - 1.51	

POR: Proportional odds ratio, BC: British Colombia, ON: Ontario, QC: Quebec; MSM: Men who have sex with men; PWID: People who inject drugs; ADI: AIDS defining illness; ARV: anti-retroviral therapy; FARVDT: first naïve ARV date.

While analyses presented in **Table 5** demonstrate the factors associated with liver fibrosis by comparing APRI values using a cut-off of 1.5 post-ARV, we wanted to examine related factors by comparing across all four categories of APRI values of hepatic fibrosis. Here, we showed that post ARV, HIV-HBV co-infection was also associated with a greater chance of having higher APRI values after ARV therapy in both unadjusted (OR:1.78, 95% CI: 1.28 – 2.46) and in analyses adjusted for age, men who have sex with men, and HIV-HCV co-infection (aOR: 1.68, 95% CI: 1.19 – 2.37) (**Table 7)** compared to HIV infected participants.

Table 7. Multivariable proportional odds logistic regression models showing the odds of having more severe hepatic fibrosis compared to milder fibrosis as determined by APRI values in HIV-infected patients after ARV therapy exposure (at end of study follow-up) (N=2,419).

Variable	Severity of Liver Fibrosis (APRI values) at end-of-study follow-up					
	Univariate			Multivariable		
	POR	95% CI	Wald p	POR	95% CI	Wald p
Hepatitis B						
HBV negative		Reference			Reference	
HBV positive	1.78	1.28 - 2.46	0.0006	1.68	1.19 – 2.37	0.003
Hepatitis C						
HCV negative		Reference			Reference	
HCV positive	5.43	4.37 - 6.75	<0.0001	3.76	2.82 – 5.02	<0.0001
Race						
White		Reference			-	
Black	0.49	0.35 - 0.68				
Indigenous	1.98	1.37 - 2.87	<0.0001			
Other	0.77	0.56 - 1.05				
Unknown	1.08	0.81 - 1.44				
Birth sex			0.77		-	
Female		Reference				
Male	1.04	080. - 1.36				
Years on ARV	1.01	0.98 - 1.04	0.53		-	
Province						
BC		Reference			Reference	
ON	0.52	0.41 - 0.65	<0.0001		-	
QC	0.49	0.36 - 0.67				
Age at first ARV	1.02	1.01 - 1.03	0.0003	1.02	1.01 – 1.03	0.002
MSM	0.57	0.46 - 0.70	0.002			
PWID	4.25	3.41 - 5.29	<0.0001	1.73	1.28 – 2.32	0.0003
Baseline HIV viral load (log10 copies/ml)						
<4		Reference			Reference	
4 – 5	1.30	0.93 – 1.79	0.14		-	
>5	1.39	1.00 – 1.94				
End of follow-up viral load (log10 copies/ml)						
<4		Reference			Reference	
4 – 5	2.42	1.48 - 3.96	<0.0001		-	
>5	4.91	2.86 - 8.45				
Baseline CD4 (cells/mm^3)						
>499		Reference			Reference	
≤199	1.71	1.16 - 2.51				
199 – 349	1.13	0.76 - 1.70	0.0002		-	
350 – 499	1.00	0.64 - 1.57				

POR: Proportional odds ratio, BC: British Colombia, ON: Ontario, QC: Quebec; MSM: Men who have sex with men; PWID: People who inject drugs; ADI: AIDS defining illness; ARV: anti-retroviral therapy; FARVDT: first naïve ARV date.

5.3.3 Comparing mild/moderate fibrosis (APRI<1.5) to clinically significant/advanced fibrosis (APRI>1.5) by HBV status

We were also interested in exploring whether the factors predicting APRI scores after ARV therapy exposure differed according to HBV status only (**Table 8)**. We thus tested for effect modification by including interaction terms in logistic regression models but found no statistically significant interactions (p>0.05). Nonetheless, we stratified our previous models and found that of those with APRI>1.5, only 32 participants with HBV positive. This limited our analysis to fewer covariates. HBV was not a significant effect modifier of the relationship of years on ARV exposure and liver fibrosis outcomes. However, we did observe that HIV-HCV co-infection did not alter the odds of having APRI >1.5 between HIV mono-infection (aOR: 7.19, 95% CI: 4.52 - 11.44) and HIV-HBV co-infection (aOR: 7.95, 95% CI: 2.32 - 27.30). Similarly, we did not observe any differences among the two sub-populations regarding baseline CD4 cell counts or HIV viral load measurements when predicting the probability of having clinically significant/advanced fibrosis compared to mild/moderate fibrosis. Among participants with HIV mono-infection, we observed that ARV therapy exposure reduced the odds of having APRI >1.5 (aOR: 0.93, 95% CI: 0.88 - 0.99). Similarly, those who were HIV-HBV co-infected and had ARV therapy were less likely (aOR: 0.73, 95% CI: 0.59 - 0.89) to have clinically significant or advanced fibrosis compared to mild or moderate fibrosis.

Table 8. Logistic regression models comparing risk factors associated with APRI>1.5 (clinically significant/advanced fibrosis) after ARV therapy among HIV infection and HIV-HBV co-infection participants (N=2419).

Variable	APRI >1.5 at end of study follow up					
	HBV negative (n=2220)			HBV positive (n=199)		
	OR	95% CI	Wald p	OR	95% CI	Wald p
Years on ARV	0.93	0.88 - 0.99	0.02	0.73	0.59 - 0.89	0.002
Hepatitis C status						
Never co-infected		Reference	<0.001		Reference	0.001
Ever co-infected	7.19	4.52 - 11.44		7.95	2.32 - 27.30	
Baseline CD4 count (cells/mm^3)	1.00	1.00 - 1.00	0.0005	1.00	0.99 - 1.00	0.31
Baseline HIV viral load (log10 copies/ml)						
<4		Reference	0.66		Reference	0.41
4 - 5	1.20	0.54 - 2.65		0.45	0.07 - 2.97	
>5	1.20	0.53 - 2.73		0.61	0.09 - 4.30	

ARV: antiretroviral therapy. Model adjusted for: hepatitis C status, baseline CD4, baseline HIV.

5.4 Evaluation of Virologic Efficacy

5.4.1 Virologic Suppression

Virologic suppression was achieved by 2,230 (92%) participants of which 537 (22%) rebounded. Among the 199 HIV-HBV co-infected and 581 HIV-HCV co-infected participants, 190 (95%) and 525 (90%) patients achieved VS respectively. The median time to suppression for HIV-HBV co-infected patients was 0.33 years (IQR: 0.22 - 0.51 years) and for HIV infected participants was 0.66 years (IQR: 0.32 – 2.1 years).

Kaplan-Meier (KM) curves were used to compare time to suppression by HBV status **(Figure 3)** and test for Cox proportionality hazards assumption. There was no significant difference in time to VS between the HIV-HBV co-infection and HIV infected sub-populations. For Cox proportional hazards assumption to be met, the ratio of the hazard rates between the HIV infected and HIV-HBV co-infected groups have to remain constant over time. The KM curves however, depict that the survival curves for both group regarding time to suppression overlap and are not parallel. This is an indication that the hazard ratios are not constant over time and the Cox proportionality hazards assumption has been violated. HBV infection status violated this assumption and was thus not included in multivariable analysis to avoid bias **(Figure 3)**. Furthermore, HBV status and time to virologic suppression was not statistically significant in logistic regression models.

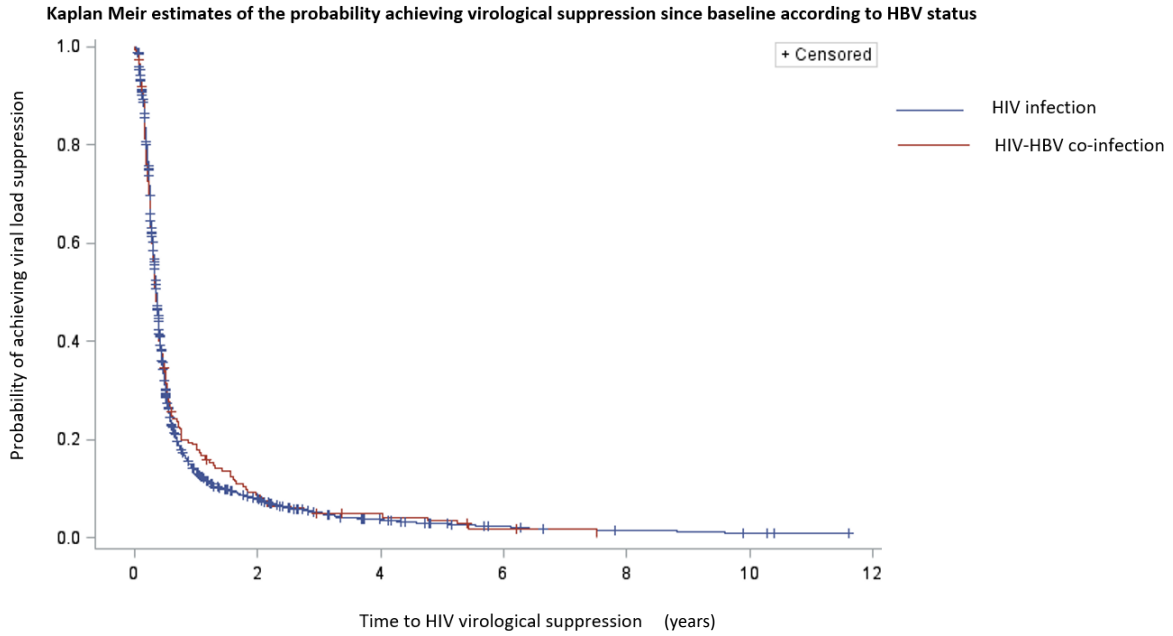

Figure 3. Time to virological suppression or end of follow up period by hepatitis B status (n=2,419).

Table 9 presents the unadjusted and adjusted HR for achieving HIV VS. The adjusted HR for achieving VS was not significantly different by race or province of residence, whereas, MSM, PWID, baseline and end of follow-up viral load, and baseline CD4 cell count were significantly associated with achieving HIV VS. In adjusted multivariable analyses, injection drug users (aHR: 0.77, 95% CI: 0.68 - 0.88) and participants with low CD4+ cell counts (<199 cells/mm^3) at baseline (aHR: 0.76, 95% CI: 0.65 - 0.88, p=0.0004) compared to those with >499 cells/mm^3 were less likely to achieve VS.

Table 9. Unadjusted and adjusted Cox regression proportional hazards ratio (HR) examining factors associated with virologic suppression since first ARV initiation (N=2,419).

	Time to Suppression since first ARV initiation (years)					
	Univariate Analyses			Multivariate Analyses		
Variable	**HR**	**95% CI**	**Wald p**	**HR**	**95% CI**	**Wald p**
Hepatitis B						
HBV negative		Reference				
HBV positive	0.96	0.83 - 1.12	0.62		-	
Hepatitis C						
HCV negative		Reference				
HCV positive	0.69	0.63 - 0.77	<0.0001		-	
Race			<0.0001			<0.0001
White		Reference			Reference	
Black	1.17	1.04 - 1.31	0.007	1.12	0.98 - 1.29	0.11
Indigenous	0.65	0.54 - 0.79	<0.0001	0.74	0.61 - 0.90	0.003
Asian	1.13	0.95 - 1.34	0.16	1.07	0.91 - 1.27	0.41
Hispanic	1.13	0.93 - 1.37	0.23	1.04	0.85 - 1.26	0.74
Other	1.04	0.98 - 1.25	0.72	1.14	0.91 - 1.43	0.25
Unknown	1.00	0.89 - 1.13	0.99	0.93	0.81 - 1.06	0.25
Birth sex						
Female		Reference	0.0007			
Male	1.21	1.08 - 1.35			-	
Province						
BC		Reference			Reference	
ON	1.11	1.01 - 1.22	0.03	0.88	0.79 - 0.98	0.01
QC	1.27	1.13 - 1.42	<0.0001	0.95	0.83 - 1.07	0.38
Age at FARVDT	1.00	0.99 - 1.00	0.19		-	
MSM	1.29	1.19 - 1.41	<0.0001	1.24	1.12 - 1.37	<0.0001
PWID	0.65	0.59 - 0.73	<0.0001	0.77	0.68 - 0.88	<0.0001
Baseline HIV viral load (log10 copies/ml)						
<4		Reference			Reference	
4 – 5	0.64	0.57 - 0.72	<0.0001	0.69	0.61 - 0.79	<0.0001
>5	0.46	0.40 - 0.52	<0.0001	0.48	0.42 - 0.55	<0.0001
End of follow-up HIV viral load (log10 copies/ml)						
<4		Reference			Reference	
4 – 5	0.38	0.28 - 0.52	<0.0001	0.39	0.29 - 0.55	<0.0001
>5	0.19	0.12 - 0.30	<0.0001	0.22	0.14 - 0.34	<0.0001
Baseline CD4 (cells/mm^3)						
>499		Reference			Reference	
≤199	0.60	0.52 - 0.70	<0.0001	0.76	0.65 - 0.88	0.0004
199 – 349	0.71	0.61 - 0.83	<0.0001	0.74	0.64 - 0.86	0.0001
350 – 499	0.81	0.68 - 0.95	0.01	0.79	0.67 - 0.94	0.006

HR: Hazards ratio; BC: British Colombia, ON: Ontario, QC: Quebec; MSM: Men who have sex with men; PWID: People who inject drugs; ARV: anti-retroviral therapy; FARVDT: first naïve ARV date.

5.4.2 Virologic Rebound

Following VS, 56 (30%) of the 190 HIV-HBV co-infected and 224 (42%) of the 525 HIV-HCV co-infected participants experienced virological rebound. The median time to rebound among HIV infected patients was 4.5 years (IQR: 2.31-7.79) and 1.93 years (IQR: 0.99 - 3.76) among HIV - HBV co-infected participants.

Kaplan-Meier curves depicting time to virologic rebound stratified by HBV status demonstrate that there are no significant differences between the HIV-HBV co-infected and HIV infected sub-population **(Figure 4)**. **Table 10** shows the factors that were independently associated with time to virologic rebound. Race, province of residence, and baseline CD4 cell counts were not predictive of HIV VR, whereas, age at first ARV, sex, and HCV infection as significantly associated with experiencing HIV VR following VS in adjusted analyses.

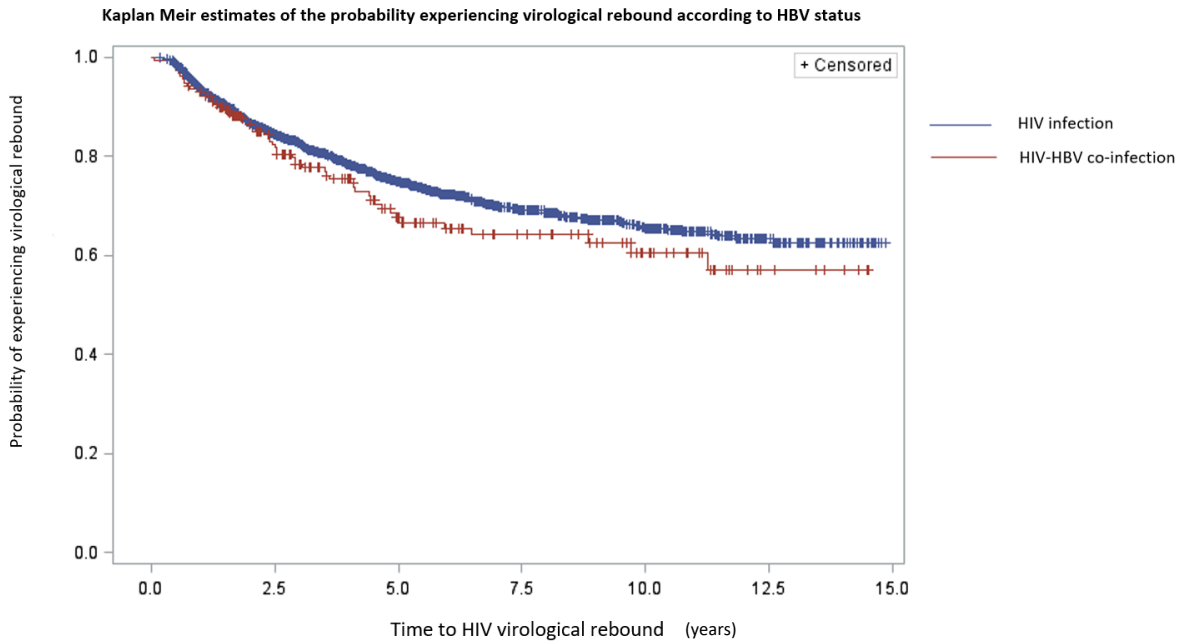

Figure 4. Time to virological rebound (after virological suppression) or end of follow up period by hepatitis B status (n=2,230).

Table 10. Unadjusted and adjusted Cox regression proportional hazards ratio (HR) examining factors associated with virologic rebound since virologic suppression (N=2,230).

	Time to rebound since VS					
	Univariate Analyses			Multivariate Analyses		
Variable	**HR**	**95% CI**	**Wald p**	**HR**	**95% CI**	**Wald p**
Hepatitis B						
HBV negative		Reference				
HBV positive	1.21	0.92 - 1.60	0.14		-	
Hepatitis C						
HCV negative		Reference			Reference	
HCV positive	2.45	2.06 - 2.90	<0.0001	2.12	1.73 - 2.61	<0.0001
Race						
White		Reference			Reference	
Black	1.26	1.00 - 1.57	0.05	1.28	0.98 - 1.68	0.007
Indigenous	2.95	2.26 - 3.56	<.0001	1.49	1.11 - 1.99	0.08
Asian	0.64	0.41 - 0.98	0.04	0.68	0.43 - 1.05	0.83
Hispanic	0.91	0.60 - 1.39	0.66	0.95	0.62 - 1.47	0.009
Other	1.57	1.05 - 2.22	0.03	1.71	1.14 - 2.56	0.12
Unknown	0.86	0.63 - 1.15	0.30	0.78	0.57 - 1.07	
Birth sex						
Female		Reference			Reference	
Male	0.55	0.46 - 0.67	<0.0001	0.66	0.53 - 0.82	0.0001
Province						
BC		Reference			Reference	
ON	0.68	0.56 - 0.82	<0.0001	0.71	0.57 - 0.88	0.002
QC	0.74	0.58 - 0.95	0.006	0.89	0.68 - 1.16	0.39
Age at first ARV	0.98	0.97 - 0.99	<0.0001	0.98	0.97 - 0.99	<0.0001
MSM	0.59	0.50 - 0.70	<0.0001			
PWID	2.40	2.01 - 2.85	<0.0001			
Baseline HIV viral load (log10 copies/ml)						
<4		Reference			-	
4 – 5	1.08	0.83 - 1.39	0.60			
>5	1.01	0.83 - 1.31	0.97			
End of follow-up HIV viral load (log10 copies/ml)						
<4		Reference			Reference	
4 – 5	6.86	4.92 - 9.56	<0.0001	4.91	3.49 - 6.91	
>5	4.54	2.76 - 7.50	<0.0001	4.83	2.91 - 8.02	
Baseline CD4 (cells/mm^3)						
>499		Reference			Reference	
≤199	1.31	0.91 - 1.87	0.14	0.98	0.68 - 1.42	0.93
199 – 349	0.99	0.68 - 1.43	0.95	0.81	0.55 - 1.18	0.27
350 – 499	0.6	0.39 - 0.96	0.03	0.56	0.36 - 0.88	0.01

HR: Hazards ratio; BC: British Colombia, ON: Ontario, QC: Quebec; MSM: Men who have sex with men; PWID: People who inject drugs; ARV: anti-retroviral therapy; FARVDT: first naïve ARV date.

CHAPTER 6: DISCUSSION

HIV and hepatitis B virus share transmission routes making co-infection quite common. Our study is the first to describe the epidemiology of HIV-HBV co-infection in Canada at the time of ARV initiation.

6.1 HIV-HBV Co-infection

The Public Health Agency of Canada reports that of the 75,000 Canadians living with HIV, approximately 6 - 8 % are HIV-HBV co-infected[3]. The prevalence of HIV-HBV co-infection in our analysis was 8%, which is consistent with co-infection rates among the general Canadian population and other North American HIV cohorts[9,28,44-47,]. The median duration of follow-up period in our cohort was 5.01 (IQR: 2.50 - 8.75) and 5.97 (IQR: 3.11 - 9.94) years among HIV infected and HIV-HBV co-infected participants respectively**.**

The prevalence of HBV infection has diminished substantially in Canada in the last two decades due to the universal HBV vaccination program. However, the number of reported cases of HBV infection continues to increase, mainly in non-Canadian born and older population[3,9]. In our study population, the majority of HIV-HBV co-infected participants were White followed by individuals of Black ethnicity. A large proportion of our study population were missing data on race due to incomplete recording on enrolment forms within the BC cohort. This may mask any differences that exist between the HIV-HBV co-infected and HIV infected participants with regards to race.

In countries with low HIV and HBV endemicity like Canada, sexual and percutaneous transmission is the most common route of HBV infection[28,55]. We observed similar patterns in our analysis with 55% of the cohort reporting ‘men who have sex with men’ as an HIV

transmission risk factor. Among MSM with HIV, 8% were HIV-HBV co-infected. The prevalence of HCV infection and injection drug use was significantly higher among participants with HIV-HBV co-infection compared to HIV patients without HBV co-infection. These findings are consistent with other viral hepatitis-HIV co-infection studies[28,56-58].

APRI, a non-invasive marker of hepatic fibrosis was significantly associated with HIV-HBV co-infection. As expected, individuals with HIV-HBV co-infection had higher median APRI values and elevated AST levels both at baseline and end of follow-up compared to HIV-infected participants. Our study is unique in that it compared fibrosis both prior to, and after ARV therapy among HIV-HBV co-infected participants accounting for potential confounding effects of ARV toxicity on the relationship between HIV-HBV co-infection and liver disease.

This analysis was the first to describe the epidemiology of anti-retroviral naïve HIV-HBV co-infected participants in Canada. We describe the overall demographic and clinical characteristics of HIV-HBV co-infection and compare it to HIV infection. Our findings are similar to nationally reported incidence of HBV infection and also corroborate with other North American and European cohorts with similar HIV and HBV endemicity.

In Canada, there has been a declining trend of HBV cases due to universal vaccination programs implemented for preadolescent children (aged 9 – 13). While vaccination programs have been responsible for the declining rate of HBV infection among preadolescents, the rate of infection has remained consistent among people at high-risk for HBV and among foreign-born Canadian residents.

Studies have reported that 70% of chronically infected Canadians are born in HBV endemic regions. The increasing burden of chronic HBV infection among adults reflects the need to

improve screening programs, especially among those newly immigrated to Canada. Currently, serological screening for chronic HBV is not routinely conducted among individuals prior to entering Canada. Only those with risk factors including HIV infection and past HBV infection are screened prior to entry.

HBV infection is primarily asymptomatic and approximately 30% of infections have no prior identifiable risk factors[59-61]. It is easier to identify individuals by pre-entry screening rather than through hospital visits or community-based programmes where compliance is poor, especially among PWID, persons with high-risk sexual behavior, and people infected with HIV. PWID and persons engaging in high-risk sexual behaviour are at the highest risk for acquiring HBV infection. As immigration policies tend to be complex, increasing pre-entry screening requirements can intimidate potential newcomers due to fear of inadmissibility. However, with the increased incidence of HBV infection among newcomers and its impact on the healthcare system, it is crucial that individuals are identified sooner[62]. By increasing pre-entry screening for HBV, especially from HBV endemic regions, we can better identify infected individuals who require monitoring and medical management. This can also lead to increased vaccination among susceptible contacts, including young children entering from endemic countries where HBV routine vaccination is not yet fully implemented. By doing so, we can aim to reduced HBV associated mortality and morbidity and prevent further transmission.

6.2 Evaluation of Hepatic Fibrosis Pre- and Post-ARV Therapy

With the advent of antiretroviral therapies, HIV infected individuals are living longer allowing more time for chronic liver disease-related outcomes to emerge. Liver disease, especially progression to cirrhosis and HCC remains one of the leading causes of mortality among HIV positive individuals[41,63]. Hepatic fibrosis tends to rapidly progress in individuals with viral

hepatitis and HIV co-infections. We used ARPI to assess the impact of HIV-HBV co-infection on liver fibrosis progression both before and after ARV therapy. We found that when comparing across all stages of hepatic fibrosis predicted by APRI (proportional odds model), HIV-HBV co-infection was significantly associated with higher APRI values both before and after ARV therapy.

Several studies have analyzed the impact of HIV-HBV co-infection on fibrosis progression using non-invasive methods. A study conducted by Price et al., utilizing the MAC cohort found a strong association between HIV-HBV co-infection and clinically significant liver fibrosis using APRI, but the number of study participants examined was small[64]. Two other studies have provided evidence of HIV-HBV co-infection on fibrosis progression using non-invasive markers of liver disease [44,65]. Our study is the first to describe the impact of HIV-HBV co-infection on liver fibrosis using APRI values after ARV therapy initiation in a Canadian population. In our analysis, we report that clinically significant/advanced hepatic fibrosis was prevalent in 20% of HIV-HBV co-infected participants prior to or at ARV initiation. We also found that those with HIV-HBV co-infection were three times more likely to have clinically significant/advanced fibrosis (APRI>1.5) compared to HIV infected participants at baseline. When comparing across all stages of liver fibrosis, HIV-HBV co-infected participants had increased odds of higher APRI values at the end of follow-up period.

Histological evaluation remains the gold standard for assessing hepatic fibrosis and necroinflammation. Other non-invasive measures including transient elastography (FibroScan) and serum fibrosis markers have been validated with a high degree of accuracy to detect varying levels of fibrosis[66-68]. Alternatively, APRI, a surrogate marker for liver fibrosis can also be used and has been validated in previous HIV-viral hepatitis co-infected studies in accurately detecting

clinically significant fibrosis and cirrhosis[16, 68-70]. It has been shown to be a predictor of mortality among HIV-hepatitis co-infected patients[71]. Our study showed that co-infected patients tended to have higher APRI values both before and after ARV therapy compared to HIV infected patients. Serial APRI testing can be beneficial in the surveillance of liver disease among HIV-HBV co-infected patients in combination with imaging tests. It can provide excellent diagnostic accuracy and prognosis of liver-related mortality reducing the number of liver biopsies performed. However, liver biopsy is still irreplaceable as treatment for chronic HBV is dependent on the degree of necroinflammation and not fibrosis[72-73].

Studies have reported a strong association between low baseline CD4 cell counts and liver fibrosis among HIV infected patients. Results from the MAC cohort showed that HIV induced immunosuppression may be an important predictor of liver fibrosis[33]. Similarly, findings from the EUROSIDA cohort reported that HIV-viral hepatitis co-infected participants with baseline CD4 cell counts <300 cells/mm^3 were more likely to progress to clinically significant hepatic fibrosis[25]. Our study reports the association between liver fibrosis and low baseline CD4 cell counts both prior to and after ARV initiation. We found that individuals with baseline CD4 cell counts <199 cells/mm^3 are twice as likely to have higher APRI values at the end of follow-up period compared to those with baseline CD4 cell counts >499 cells/mm^3.

HBV disease tends to be more aggressive in HIV-HBV co-infection with co-infected individuals exhibiting higher levels of HBV DNA replication, elevations in liver enzyme levels, and faster progression to cirrhosis. Studies have demonstrated that HIV-HBV co-infected individuals on antiretroviral therapy including active agents against HBV have greater HBV DNA suppression. This is especially true in individuals with baseline CD4 counts >200 cells/ul[74]. It would be

interesting to report similar findings in a Canadian population. However, the CANOC dataset does not contain HBV DNA data.

While our findings regarding the association between hepatic fibrosis and HIV-viral hepatitis co-infection are consistent with majority of the studies, there are conflicting reports on whether years of ARV therapy is associated with a reduced fibrosis progression rate[75-76]. Combination ARV therapy, particularly those with protease inhibitors (PIs) have been associated with liver-related complications[77-79]. In our cohort, the majority of the HIV-HBV co-infected participants were on nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) with boosted PIs. We aimed to stratify our population by HIV-HBV co-infection vs. HIV mono-infection and found that ARV therapy exposure was associated with decreased odds of having advanced fibrosis at the end of follow-up period after adjusting for HCV coinfection, baseline CD4 and HIV viral load. HIV-HBV co-infected participants received ARV therapy for a median duration of 5.97 years. There are several possible mechanisms by which ARV therapy decreases risk of fibrosis progression. It is possible that the inclusion of HBV-active antiretrovirals may be responsible for diminished liver scarring by suppressing HBV-induced fibrosis production. Puoti et al. (2006) reported that exposure to antiretrovirals with HBV active ARV including lamivudine was associated with suppression of HBV DNA replication and decreased liver related mortality compared to ARV therapy lacking anti-HBV activity[59].

Even though HIV-HBV co-infection is associated with more severe fibrosis during follow-up, we provide evidence that ARV therapy is associated with a decreased risk of fibrosis among HIV-HBV co-infected participants compared to HIV infected participants at the end of follow-up. Survival bias may cofound the association between ARV therapy and its impact on fibrosis

progression as HIV-infected patients without any liver-related morbidities are likely to live longer and initiate ARV therapy[80].

6.3 HIV Virological Outcomes in HIV-HBV Co-infected patients

Assessing the impact of HBV infection on HIV infected patients is critical in determining therapeutic measures and for the prognosis of both infections. In this retrospective analysis of 2230 HIV infected participants with a median of ~6 years of follow-up on ARV, we evaluated the predictors of HIV virological suppression and rebound. Our findings suggest that co-infection with HBV did not significantly affect response to ARV as measured by time to HIV virological suppression and subsequent rebound.

These findings are consistent with other studies conducted in North America reporting that HBV status does not influence rate of HIV virologic suppression or rebound[47,81-84]. Additionally, studies conducted in Europe and China have demonstrated that the rate of virological failure was similar in participants affected with both HIV and HBV compared to those infected with HIV only[25,27,85]. The risk of viral rebound among CANOC participants also did not differ according to HBV status.

6.3.1 CD4+ cell count

While there is consensus surrounding the lack of an association between HBV infection status and virologic response to ARV in HIV infected patients, there is considerable debate regarding HBV infection and immunological recovery during ART. A study conducted by Wandeler et al. (2013) reported that HBV infection can adversely impact CD4 cell count recovery within the first three years of ARV therapy[46]. A possible mechanism for the association of lower CD4 T cell counts and hepatic fibrosis could be immune activation. Participants with HBV co-infection

may have increased T-cell apoptosis which can impair immunological recovery among HIV infected patients[46,86-87]. Early initiation of ARV has been found to improve CD4 cell recovery and prevent HIV disease progression[25,57]. In our analysis, low CD4 cell count was associated with a reduced likelihood of achieving virologic suppression. However, we did not find a significant association between CD4 cell count and virologic rebound. For this analysis, we only considered baseline CD4 cell count measurements and thus, were unable to explore the impact of HIV-HBV co-infection on immunological recovery following ARV therapy. Future CANOC research using time-updated CD4 cell counts may be useful to assess whether ARV therapy can improve immunological recovery in HIV-HBV co-infected participants.

6.4 Strengths

The CANOC cohort is largely representative of the HIV population who have initiated ART in Canada since 2000[49]. Approximately 25% of all HIV Canadians are included in the CANOC cohort. Our study includes data from three provinces - British Columbia, Ontario, and Quebec. There is considerable heterogeneity between these cohorts as data from ON and QC are based on participating clinics whereas data from BC represent the entire sample of HIV positive patients on ARV in the province. The criteria for antiretroviral medication reimbursement differs in each of the three provinces which could be a potential limitation in our study as it can reflect varying levels of access to healthcare among participants. Efforts to include more provinces have been made and the next CANOC dataset will include data from Saskatchewan and Newfoundland as well to allow for more robust future assessments of HIV-HBV co-infection in Canada. Despite these limitations, we are confident of our findings.

Another strength of our cohort is the long 14-year follow-up period and consistency among our study participants with regards to initiation of naïve ARV regimen. Our analyses comprised of

2419 participants with varying levels of liver disease, including 66% with mild fibrosis, 27% with moderate fibrosis, 1.7% with clinically significant fibrosis, and 4.8% with advanced fibrosis/cirrhosis according to APRI values. Currently, in Canada, liver health is monitored via many different techniques: ultrasound (TE), blood tests (Fibrosis 4, Fibrotest, liver enzyme tests etc), and liver biopsy. Our study is the first to provide national evidence on HIV-HBV co-infection using non-invasive markers to analyze fibrosis progression. We show that APRI may be a useful marker to study liver disease progression in large populations. This expands the generalizability of our study results to HIV-HBV co-infected participants in Canada that are currently exposed to ARV. Another strength of our study is that it allows us to analyze the influence of HIV-HBV co-infection on fibrosis progression pre-and post ARV therapy initiation. This is a strength of our study as it allows us to exclude the potentially cofounding effects of ARV therapy on liver disease.

6.5 Limitations

Our study has several limitations that the reader should consider. First, there was a large proportion of missing data due to incomplete or unknown HBV infection testing and lack of baseline APRI measurements for all participants. As APRI scores were our primary study outcome, we opted for complete case analysis to handle missing data. This resulted in a small sample size for our proposed analysis. The data from Maple Leaf Medical Clinic also had to be excluded due to possibility of misclassification of HBV infection status. The excluded participants from MLMC did not differ for the variables of interest from those included for consideration in our analysis (n=6,813) (Appendix D). There was a higher proportion of Black and Indigenous population, and HCV infected participants in our study compared to MLMC but

in order to avoid incorrect HBV status inclusion in our study, we had to exclude this cohort despite the minor differences.

Second, participants who initiated ARV therapy prior to January 2000 were ineligible to participate in CANOC. This limits the generalizability of our findings to patients who have initiated therapy more recently. The participants included in CANOC are already involved with health care facilities, therefore the results from this study may not be representative of the overall HIV-HBV co-infected population across Canada.

Third, HBV exposure was ascertained by a positive HBV antibody test, HBV PCR analysis, or physician reporting. We did not have repeated HBV infection tests or dates of infection; thus, we were unable to determine if the disease was active or the HBV infection stage (acute vs. chronic). Chronically infected HBV participants can spontaneously clear infection and/or clear with initiation of therapy. Thus, there is a possibility that these participants were misclassified as HBsAg positive. This would bias our results towards the null and underestimate the actual effect HBV co-infection on liver fibrosis among our cohort. However, only about 0.5-2% of chronically infected HBV patients develop antibodies against HBsAg with clearance of HbsAg. If misclassification did occur, this would mean that in our cohort of 199 HIV-HBV co-infected participants, only about 4 patients were incorrectly classified as having HBV infection.

Lastly, since a large proportion of the study participants were also HCV co-infected, we were unable to conduct all our analyses comparing HIV-HBV co-infected to HIV mono-infected patients. This limits our analysis to HBV non-infected vs. HBV infected among HIV positive patients and can lead to residual confounding. We had no information regarding quantitative HBV DNA data to further evaluate the impact of ARV therapy on HBV DNA suppression and liver disease. This has limited our ability to assess the influence of HBV infection on liver-

related mortality among HIV-HBV co-infected patients. Additionally, data was not available for current alcohol use, ARV adherence, and socio-economic determinants of health including access to health care. These have been reported to contribute to the progression of both HIV disease and liver disease [88].

6.6 Conclusion

This work reveals that the prevalence of HBV co-infection was 8% in HIV infected patients in these three provinces, which is similar to other developed countries. HIV-HBV co-infected participants were more likely to present with hepatic fibrosis compared to HIV-infected patients. Our work showed that HBV-coinfection can impact fibrosis progression among HIV infected patients. We also provided evidence of a link between ARV therapy and reduced fibrosis in HIV-HBV co-infected population compared to HIV infected participants. Our study emphasized the importance of ARV therapy among both HIV-infected and HIV-HBV co-infected patients. While our findings suggest that low baseline CD4 cell counts were a strong predictor of severe hepatic fibrosis among both HIV infected and HIV-HBV co-infected participants, it also demonstrated a need for long-term studies with time-updated CD4 counts to evaluate the impact of immunological recovery among HIV-HBV co-infected participants in Canada. Thus, our study highlights the importance of early detection of HBV co-infection so that ARV including HBV-active treatment can initiated. This can impact fibrosis and HIV disease progression by controlling HBV infection and increasing CD4 cell count early during the course of HIV infection.

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APPENDICES

Appendix A: Stages of chronic hepatitis B

Table 11. Stages of chronic hepatitis B (HBsAg positive, Anti-HBs negative, Anti-HBc positive, Anti-HBc IgM negative)

Stage	Characteristics	HBeAg Status
Immune tolerant	- Primarily observed in children born to HBeAg positive mothers. - High levels of HBV DNA > 20,000 IU/mL (>100,000 copies/ml) - Normal liver aminotransferase levels - Minimal liver disease	HBeAg positive
Immune active (HBeAg positive CHB)	- Host immune system elicits a weak cytotoxic response to HBV - HBV DNA levels > 20,000 IU/mL - Elevated or fluctuating levels of liver enzymes (ALT) - Necroinflammatory activity observed	HBeAg positive; may be anti-HBe positive
Inactive CHB	- HBeAg seroconversion - Low HBV DNA (< 2000 IU/mL) - Normal ALT levels - Reduced risk of HCC and cirrhosis	HBeAg negative; anti-HBe positive
Immune escape (HBeAg negative CHB)	- Abnormal or fluctuating ALT levels - HBV DNA level > 20,000 IU/mL (high) - Liver fibrosis may be observed	HBeAg negative; anti-HBe positive or negative
Reactivation	- Reappearance of HBV infection due to immune-suppression (i.e. HIV, chemotherapy, ARV resistance, withdrawal of ARV, etc.) - Abnormal ALT levels - High levels of HBV replication - Higher risk of liver decompensation if cirrhotic.	HBeAg positive or negative

Appendix B: AIDS-defining illness definition

Table 12. AIDS-defining illnesses used for the variable 'Baseline ADI' [51]

AIDS-Defining Illness	Comments
Candidiasis of esophageal, bronchi, trachea, or lungs (one combined definition is used)	
Cervical cancer (understood to be invasive)	
Cytomegalovirus disease (site not specified)	We ask for any CMV, NOT SPECIFIC re Retinitis. So we routinely report only CMV in general, without specifying the type.
Coccidiomycosis - disseminated or extrapulmonary	
Cryptococcosis - extrapulmonary	Not specific to meningitis.
Cryptosporidiosis - chronic intestinal	
HIV encephalopathy (dementia)	
Histoplasmosis - disseminated or extrapulmonary	
Herpes simplex infection - chronic mucocutaneous	Meant to cover chronic ulcer, bronchitis, pneumonitis, or esophagitis as a combined definition
Isosporiasis - chronic intestinal	
Kaposi's sarcoma AIDS-defining unspecified	
Primary lymphoma of brain	
MAC or M kansasii - disseminated or extrapulmonary	
TB AIDS-defining unspecified	Mycobacterium tuberculosis, any site
Mycobacterium other or unspecified species - disseminated or extrapulmonary	Mycobacterium (other than MTb, MAC, or kansasii)
Lymphoma, non-Hodgkins, AIDS defining unspecified	May include Burkitt's, or immunoblastic, or other types of non-Hodgkin's Lymphoma
Pneumocystis carinii pneumonia (PCP)	
Progressive multifocal leukoencephalopathy (PML)	
Pneumonia, recurrent	
Salmonella septicemia	Both current and recurrent included in this definition
Toxoplasmosis of brain	
Unknown as to the specific ADI	Patient is confirmed to have an ADI, just no data as to the name of the specific AIDS defining illness
Wasting syndrome due to HIV	

Appendix C: Ethics

Ottawa Hospital
Research Institute
Institut de recherche
de l'Hôpital d'Ottawa

August 23, 2016

Ottawa Health Science Network Research Ethics Board (OHSN-REB)
Attention: [redacted]
Ottawa Hospital, Civic Campus
[redacted]
Ottawa, Ontario K1Y 4E9

Re: Staff to be added to OHSN-REB #2010-673 01H

To Whom It May Concern:

Please add Urvi Rana to the above mentioned study. Her TCPS-2 is also attached.

[redacted]

Appendix D: Missing data

Maple Leaf Medical clinic reported a much higher percentage of HBV positive participants than any other sites (Table 2). There was a likelihood that MLMC misclassified vaccine response as HBV positive. In order to prevent misclassification bias, we opted to remove MLMC from our study cohort.

Table 13. Classification of HBV status by individual cohort sites

	ACT	*CFE*	*IDTC*	*MLMC*	*MUHC*	*OHTN*	*OTT*	*TGH*	*Total*
Ever HBV +									
No	1177 (80%)	2574 (52%)	142 (87%)	632 (81%)	421 (83%)	1476 (93%)	368 (94%)	258 (75%)	7048 (69%)
Yes	184 (13%)	220 (4%)	7 (4%)	152 (19%)	44 (9%)	109 (7%)	24 (6%)	30 (9%)	770 (8%)
Missing	109 (7%)	2196 (44%)	15 (9%)	0 (0%)	45 (9%)	0 (0%)	1 (0%)	55 (16%)	2421 (24%)
Ever HBV Antigen +									
No	1285 (87%)	2463 (49%)	0 (0%)	0 (0%)	425 (83%)	1399 (88%)	369 (94%)	269 (78%)	6210 (61%)
Yes	66 (4%)	157 (3%)	7 (4%)	31 (4%)	41 (8%)	92 (6%)	23 (6%)	19 (6%)	436 (4%)
Missing	119 (8%)	2370 (47%)	157 (96%)	753 (96%)	44 (9%)	94 (6%)	1 (0%)	55 (16%)	3593 (35%)
Ever HBV PCR +									
No	17 (1%)	534 (11%)	0 (0%)	0 (0%)	440 (86%)	1442 (91%)	3 (1%)	10 (3%)	2446 (24%)
Yes	16 (1%)	156 (3%)	0 (0%)	0 (0%)	12 (2%)	49 (3%)	19 (5%)	10 (3%)	262 (3%)
Missing	1437 (98%)	4300 (86%)	164 (100%)	784 (100%)	58 (11%)	94 (6%)	371 (94%)	323 (94%)	7531 (74%)

ACT: Clinique médicale l'Actuel at Montréal (Quebec)
CFE: Centre for Excellence in HIV/AIDS (British Columbia)
IDTC: Immune Deficiency Treatment Centre (Quebec)
MLMC: Maple Leaf Medical Clinic (Ontario)
OHTN: Ontario HIV Treatment Network (Ontario)
OTT: Ottawa Hospital Immunodeficiency Clinic (Ontario)
TGH: Toronto General Hospital (Ontario)

Table 14. Demographic characteristics of the HIV infected study participants by HBV infection status (N=1,158) for MLMC cohort only.

Demographic Characteristics	HBV negative N = 977	HBV positive N = 181	p value[a]
Age	37.0 (31.0 – 44.0)	41.0 (36.0 – 47.0)	<0.001
Male sex	921 (94)	168 (93)	0.45
Deceased	32 (3)	9 (5)	0.23
Race			0.07
White	627 (64)	102 (56)	
Black	71 (7)	24 (13)	
Indigenous	14 (1)	2 (1)	
Other	201 (21)	42 (23)	
Unknown	64 (7)	11 (6)	
Province			NA*
BC	0 (0)	0 (0)	
ON	977 (100)	181 (100)	
QC	0 (0)	0 (0)	
Hepatitis C co-infection	90 (9)	25 (14)	0.06
Risk Factors			
MSM	711 (73)	129 (71)	0.63
PWID	28 (3)	12 (7)	0.04
Baseline ADI[b]			0.65
≥1 before/at FARVDT	57 (6)	9 (5)	
None before/at FARVDT	920 (94)	172 (95)	
No ADI ever	0 (0)	0 (0)	

BC: British Colombia, ON: Ontario, QC: Quebec; MSM: Men who have sex with men; IDU: Injected drug users; ADI: AIDS defining illness; ARV: anti-retroviral therapy; FARVDT: first naïve ARV date.

[a] Data shown are frequencies and proportions for categorical variables and median and interquartile ranges for continuous variables. p values for continuous variables were calculated using chi-square or Fisher exact tests and for continuous variables were calculated using Wilcoxon's Rank Sum tests.

[b]'No ADI ever' refers to no recorded ADI's during study period. 'None before/or first ARV date' refers to no recoded ADI prior to study enrollment.

* The p-value of chi-square test on province is not available because all the observations come from the same province.

Table 15. Demographic characteristics of the HIV infected study participants by HBV infection status (N=6,813) excluding MLMC cohort.

Demographic Characteristics	HBV negative N=6,211	HBV positive N=702	p value[a]
Age	39 (32.0 – 46.0)	41 (36.0 – 47.0)	<0.001
Male sex	5,042 (81)	522 (87)	0.004
Deceased	467 (8)	77 (13)	<0.001
Race			0.03
White	2,319 (37)	220 (37)	
Black	858 (14)	81 (13)	
Indigenous	388 (6)	22 (4)	
Other	730 (12)	68 (11)	
Unknown	1,905 (31)	211 (35)	
Province			<0.001
BC	2,609 (42)	226 (38)	
ON	1,833 (30)	136 (23)	
QC	1,769 (28)	240 (40)	
Hepatitis C co-infection	1,451 (23)	175 (29)	<0.001
Risk Factors			
MSM	3,097 (50)	331 (55)	0.05
PWID	1,287 (21)	156 (26)	0.006
Baseline ADI[b]			0.18
≥1 before/at FARVDT	1,102 (18)	125 (21)	
None before/at FARVDT	4,770 (77)	445 (74)	
No ADI ever	339 (5)	32 (5)	

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[a] Data shown are frequencies and proportions for categorical variables and median and interquartile ranges for continuous variables. p values for continuous variables were calculated using chi-square or Fisher exact tests and for continuous variables were calculated using Wilcoxon's Rank Sum tests.

[b]'No ADI ever' refers to no recorded ADI's during study period. 'None before/or first ARV date' refers to no recoded ADI prior to study enrollment.

Table 14 compares study participants with known HBV status from one site: MLMC with **Table 15** which presents study participants with known HBV status excluding MLMC. Upon inspection, we find that the mean age is similar between the participants. There is a higher proportion of Black and Indigenous population in our included study participants compared to those excluded. We also observe that there is a larger proportion of PWID in our included cohort compared to MLMC. These changes highlight provincial differences as there is a large proportion of PWID in BC compared to MLMC, which is situated in Ontario. However, in order to prevent misclassification bias with potentially vaccine antibody response as HBV positive, we excluded MLMC from our analysis.