Inflammation in atherosclerotic cardiovascular disease:

A Heart on Fire

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DEDICATION

For Kathryn—thank you for your love, patience and unwavering belief in me. Through sleep deprivation, border crossings, diaper changes, and fellowship(s), your love, patience, and superhuman multitasking made it all possible. You have been my rock during this long journey. While this thesis may have my name on it, it has your fingerprints all over it (and probably some crayon marks from the kids too). This work is as much yours as it is mine.

ABSTRACT

Interest in the role of inflammation in the pathogenesis of atherosclerotic cardiovascular disease (ASCVD) has continued to grow over the past several decades. As the population continues to age, and the burden of ASCVD continues to climb, researchers and clinicians continue to look for novel treatments and targets to reduces the burden and mitigate the effects of ASCVD. In this thesis, the role of inflammation in the pathogenesis of ASCVD was further assessed along with an assessment of potential therapies that could be used to mitigate the effects of inflammation on the cardiovascular system. This thesis comprises six main sections: (1) a systematic review and network meta-analysis looking at the relative efficacy of anti-inflammatory therapies for the treatment of cardiovascular disease; (2) an observational cross-sectional study to evaluate inflammatory biomarkers in patients with acute cardiovascular events and remote cardiovascular events; (3) a protocol for a double-blind randomized controlled trial to evaluate the efficacy of the anti-inflammatory agent, colchicine, in reducing vascular inflammation; (4) a cost-effectiveness analysis of the use of colchicine for ASCVD; (5) a cohort study evaluating the effect of biologic therapy on vascular inflammation in patients with psoriatic-arthritis/dermatologic; and (6) a cohort study looking at the effect of low-dose statin therapy on vascular inflammation in patients with human-immunodeficiency virus (HIV). While inflammation has long been recognized to be an etiological player in the pathogenesis of ASCVD, until recently, studies exploring inflammation as a potential treatment target have been lacking. This has changed in recent years, with an explosion of studies evaluating anti-inflammatory therapies for the treatment of CV disease. The relative efficacy of these therapies remains unknown. Additionally, it remains unknown whether therapies targeting the inflammatory pathway should be used in all patients with ASCVD (or at high-risk of ASCVD) or if we should specifically target high-risk subgroups. Lastly, information with respect to the cost-efficacy of these therapies from a Canadian health-care system perspective remain needed.

EXECUTIVE SUMMARY

Atherosclerotic cardiovascular disease and its associated complications including stroke, myocardial infarction, and coronary artery disease are a leading cause of morbidity and mortality around the world. With an aging population, the burden of ASVD continues to grow. Thus, a major push for a better understanding of the disease process and push for novel therapeutic agents is underway. Inflammation is an increasingly recognized etiological factor in the pathogenesis of atherosclerotic plaque. Until recently, however, its role as a potential therapeutic target has been underrecognized. In recent years, this has dramatically changed, and now there is a flurry of activity to discover novel agents to harness the potential benefits of reducing vascular inflammation to mitigate the effects of ASCVD.

This thesis explores novel therapeutics for ASCVD with an overall aim of providing usable evidence to health care providers and policy makers to inform their decisions about the use of anti-inflammatory treatments to reduce CV risk. To meet this aim, six research questions were addressed:

> ***Question 1***: What are the available anti-inflammatory therapies for ASCVD risk reduction and what is the relative efficacy of these therapies?
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> ***Question 2:*** Do patients with diabetes have elevated systemic inflammatory levels?
>
> ***Question 3:*** What is the mechanism of action of colchicine in the reduction of ASCVD?
>
> ***Question 4:*** Is colchicine a cost-effective treatment for reduction of ASCVD compared to current therapeutic options?
>
> ***Question 5:*** Could biologic therapy be an effective option to reduce vascular inflammation in patients with psoriasis or psoriatic arthritis?
>
> ***Question 6:*** Could low-dose statin therapy be an effective option to reduce vascular inflammation in patients with human immunodeficiency virus (HIV)?

This thesis contains six main sections, each with a study to address these research questions: (1) a systematic review and network meta-analysis to summarize the evidence available on anti-inflammatory

therapies available for the treatment of ASCVD and to compare their relative efficacy; (2) a cross-sectional study to evaluate inflammatory levels in patients with diabetes after a CV-event; (3) a protocol for a double-blinded randomized controlled trial to evaluate the efficacy of colchicine to reduce vascular inflammation in patients with (pre-)diabetes; (4) an economic evaluation of the cost-effectiveness of colchicine compared to standard of care for ASCVD; (5) a prospective cohort study evaluating the effect of biologic therapy on the reduction of vascular inflammation in patients with psoriasis or psoriatic arthritis; and (6) a prospective randomized controlled study evaluating the effect of low-dose statin therapy on the reduction of vascular inflammation in patients with HIV.

Overview of methods and findings

1. What are the available anti-inflammatory therapies available for the prevention of CV disease and what are the relative risks and benefits? A systematic review and network meta-analysis methodology was used to identify, appraise, and synthesize the available studies evaluating anti-inflammatory therapies for the reduction of CV disease. Unlike traditional systematic reviews, which can only evaluate the effectiveness of one therapy against another, a network meta-analysis can compare the relative efficacy of a multitude of different therapies. In the review, 15,627 studies were screened; 61 randomized controlled trials (RCTs) met inclusion criteria. Bayesian network meta-analysis was performed to calculate risk estimates using random effects analyses in two separate analyses: (1) in patients with ACS and (2) in patients with stable CAD. 41,647 patients were included in the stable CAD network analysis and 29,388 patients were included in the ACS network analysis. In the acute CAD analysis, both non-steroidal anti-inflammatory (NSAID) use and colchicine use were associated with a significant reduction in major adverse cardiac events (MACE) compared to control. In the chronic CAD analysis, both corticosteroids and colchicine were associated with a significant reduction in MACE compared to control.

2. Do patients with diabetes have elevated systemic inflammatory levels? While one approach of CV risk reduction may be to treat everyone with CAD with anti-inflammatory therapies, another possibly more effective strategy may be to target high-risk populations. The purpose of this study was to try to determine

if patients with diabetes have a higher inflammatory burden following CV events. If so, they may be a population of interest to target with effective anti-inflammatory therapies to reduce the burden of CV disease. We conducted an observational cross-sectional study in 2 cohorts of patients: i) those with a recent CV event (ACS, TIA or stroke) within 30-60 days and ii) those with remote prior CV events (>1 year). We then divided these patients into 3 groups: Group 1: recent CV event in patients with prediabetes/diabetes (as defined by the Canadian Diabetes Association Guidelines); group 2: recent CV event in patients without prediabetes/diabetes; and group 3: patients who suffered remote CV event (>1 year). In this observational cohort study, we aimed to compare serum inflammatory biomarker levels between groups to determine if patients with diabetes had higher levels of inflammation following a CV event. We found that IL-6 values were higher in patients with prediabetes/diabetes who had experienced recent CV events, compared to patients with remote CV events, and there was a numerical but non-statistically significant trend of higher median values of hsCRP in patients with prediabetes/diabetes with recent CV vents compared to patients with remote CV events. There was a trend for higher IL-6 and hsCRP levels in patients with prediabetes/diabetes and recent CV events compared to those without dysglycemia who also experienced recent CV events. There was also a trend for higher IL-6 and hsCRP levels in patients with prediabetes/diabetes and a recent CV event compared to the subgroup of patients with prediabetes/diabetes and a remote CV, although this did not reach statistical significance. Finally, there was also a numerical, but non-significant trend for higher IL-6 and hsCRP levels in patients without prediabetes/diabetes and a recent CV event compared to the subgroup of patients with no prediabetes/diabetes and a remote CV. This study suggested that patients with diabetes who suffer a CV event may in fact have a higher burden of inflammation following their event, and may be a potential target for novel anti-inflammatory therapies aimed at curbing future CV risk.

3. What is the mechanism of action of colchicine in the reduction of ASCVD? Inflammation is a key mediator in the development and progression of the atherosclerotic disease process as well as its resultant complications, like myocardial infarction (MI), stroke, and cardiovascular (CV) death, and is emerging as a novel treatment target. Trials involving anti-inflammatory medications have demonstrated outcome

benefit in patients with known CV disease. In this regard, colchicine appears to hold great promise. However, there are potential drawbacks to colchicine use, as some studies have identified an increased risk of infection, and a non-significant trend for increased all-cause mortality. Thus, a more thorough understanding of the underlying mechanism of action of colchicine is needed to enable a better patient selection for this novel CV therapy. A randomized controlled trial, the Canadian Study of Arterial Inflammation in Patients with Diabetes and Recent Vascular Events, Evaluation of Colchicine Effectiveness (CADENCE) will assess the effect of colchicine on vascular inflammation in the carotid arteries and ascending aorta measured with ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET)/CT in patients with type 2 diabetes mellitus (T2DM) or pre-diabetes who have experienced a recent vascular event (acute coronary syndrome (ACS)/MI, transient ischaemic attack (TIA) or stroke). CADENCE is a multicentre, prospective, randomised, double-blinded, placebo-controlled study to determine the effect of colchicine on arterial inflammation as assessed with imaging and circulatory biomarkers, specifically carotid arteries and aortic FDG uptake as well as hs-CRP and IL-6 among others. Patients with T2DM or pre-diabetes who have recently experienced a CV event (within 30-120 days after an ACS (ie, ST-elevation MI (STEMI) or non-STEMI)) or TIA/stroke with documented large vessel atherosclerotic disease will be randomised to treatment with either colchicine 0.6 mg oral daily or placebo. Participants will undergo baseline clinical evaluation including EQ5D assessment, blood work for inflammatory markers and FDG PET/CT scan of the ascending aorta and left and right carotid arteries. Patients will undergo treatment for 6 months and have repeat clinical evaluation including EQ5D assessment, blood work for inflammatory markers and FDG PET/CT scan at the conclusion of the study. The primary outcome will be the change in the maximum target to background ratio (TBR_{max}) in the ascending aorta (or carotid arteries) from baseline to follow-up on FDG PET/CT imaging. Importantly, the CADENCE study will determine whether its anti-inflammatory action is an indirect systemic effect, or a more local plaque action that decreases inflammation. The results will also help identify patients who will benefit most from such therapy.

4. Is colchicine a cost-effective treatment for reduction of ASCVD compared to current therapeutic options? Although some studies have suggested potential efficacy in reducing cardiovascular events, the

cost-effectiveness of colchicine in this context is not clear. A decision model was developed to estimate the healthcare costs in Canadian dollars and the clinical outcomes among patients who have suffered an MI and are treated with colchicine. Probabilistic Markov modelling was used in combination with Monte Carlo simulation to derive expected lifetime costs and quality-adjusted life-years, which permitted the calculation of incremental cost-effectiveness ratios. Models were derived for both short-term (20 months) and long-term (lifelong) colchicine use in this population. In the analyses, long-term colchicine use was dominant over standard of care, with lower average lifetime costs per patient (CAD$91,552.80 vs $97,085.84) and a higher average number of quality-adjusted life-years per patient (19.92 vs 19.80). Short-term colchicine use also dominated over standard of care.

5. Could biologic therapy be an effective option to reduce vascular inflammation in patients with psoriasis or psoriatic arthritis? The aim of the study was to evaluate the changes in central vascular inflammation measured by FDG PET and myocardial blood flow reserve (MFR) determined by ^{82}Rb PET following therapy with biologic agents for 6 months in patients with psoriatic arthritis (PsA) and/or cutaneous psoriasis (PsO) (group 1) and compare with PsO subjects receiving non-biologic therapy (group 2) and controls (group 3). n a cohort of psoriasis patients treated with biologics, FDG uptake in the thoracic aorta decreased over the study period. Patients who demonstrated a significant anti-inflammatory response on FDG PET imaging maintained their MFR compared to non-responders.

6. Could low-dose statin therapy be an effective option to reduce vascular inflammation in patients with HIV? This study aimed to evaluate markers of systemic as well as imaging markers of inflammation in the ascending aorta, bone marrow, and spleen measured by 18F-FDG PET/CT, in persons living with HIV (HIV+) patients at baseline and following therapy with rosuvastatin. HIV+ patients had significantly markers of systemic inflammation including monocyte activation. Treatment with low-dose rosuvastatin in the HIV+ cohort significantly reduced bone marrow, spleen and thoracic aortic FDG uptake.

Conclusion

Inflammation is an increasingly important risk factor for cardiovascular disease. In recent years there has been growing attention on trying to identify novel treatments to mitigate CV risk by curbing vascular

inflammation. While several potential anti-inflammatory agents have emerged as effective for CV risk reduction, careful work is needed to fully evaluate mechanism of action, potential negative effects of these therapies, and cost-efficacy of these therapies. Furthermore, the relative cost-efficacy of novel anti-inflammatory therapeutics may be higher if we can identify specific inflammatory subgroups that would benefit most from anti-inflammatory medications.

CONTRIBUTION OF THE AUTHORS

Six manuscripts were prepared as part of this thesis (presented in Chapters 3-8), all of which have been published or submitted for publication.

All manuscripts were led by Kevin Boczar and were co-authored by at least one supervisor (George A Wells; co-supervisor Rob Beanlands) and members of the thesis advisory committee (Thais Coutinho, Rob deKemp, Doug Coyle). Kevin is first author on all publications, having been responsible for study design, data collection, analysis, and writing of the manuscripts. All studies were designed in consultation and discussion with members of the thesis advisory committee.

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Chapter 1: Introduction

Section 1.1 Statement of the problem

Cardiovascular (CV) diseases are a leading cause of death worldwide and pose a huge burden to healthcare costs around the world.[1] [2] Atherosclerotic cardiovascular disease (ASCVD) refers to an array of cerebral, cardiac, and peripheral vascular conditions involving the build-up and sequelae of atherosclerotic plaque in the blood vessels of the body.[3] Downstream consequences of ASCVD include dementia, chronic kidney disease, peripheral arterial disease, and chief amongst these sequelae, myocardial infarction (MI), MI a leading cause of hospitalization and health care utilization across Canada.[3] Furthermore, studies have shown that the prevalence rates for all ASCVD in Canada have increased over the past 10-year period.[3]

Given the increasing burden of ASCVD on patients and on our healthcare system, much focus has shifted towards mitigating the detrimental disease effects. Notably, while incident ASCVD and specifically MI are high, recurrent MI events are extremely high, with one Canadian study showing up to 50% of patients with incident CV events will potentially experience multiple recurrent ASCVD events in the future.[3] Left untreated, ASCVD can be a progressive condition leading to greater risk of CV events and eventually death.[4] With such dire consequences, and such a high burden of disease, much attention has shifted towards targeting both primary and secondary prevention strategies and treatments for ASCVD. Traditional risk factors such as dyslipidemia, hypertension, diabetes, and smoking have been well-understood for decades, and there is a plethora of pharmacologic and non-pharmacologic measures available for treatment of these well-known ASCVD risk factors. In recent years, the pathological role of inflammation in the development of ASCVD has garnered more attention. Scientists and clinicians alike have realized that the inflammatory cascade is the great instigator in the atherogenic process that takes place within the arterial walls. Inflammation begets plaque, and plaque begets inflammation – a vicious cycle of atherogenesis that left unchecked, can wreak havoc on the arterial health of the body. Thus, the medical community has spent much effort in recent years trying to identify therapies that can break this

cycle. Therapies that can get the proverbial "runaway train" of inflammation back to the station. This has certainly not been an easy task. Firstly, any theoretical benefit in ASCVD risk reduction of an anti-inflammatory therapy must be weighed against the potential negative consequences. While inflammation is often painted as the definitive "villain" in the latest superhero story, the truth is far less clear cut and much more nuanced. The inflammatory process is actually the immune system's helpful response to harmful stimuli, such as external pathogens or damaged cells.[5] It acts by signalling to our body to remove these deleterious stimuli and triggers the initiation of the healing process.[6] Thus, anti-inflammatory therapies to reduce CV risk must also be acceptable from a side-effect perspective. With respect to maximizing the ratio of benefit to risk, identifying high-risk "CV inflammatory" phenotypes may be an avenue of exploration that is needed. Finally, the cost of these medications for our health system must also be weighed to determine if they are beneficial from a societal perspective.

Section 1.2 Objective

The overall objective of this thesis is to provide an overview of the evaluation of anti-inflammatory therapies for the treatment of ASCVD. The aim is two-fold: 1) to provide information to healthcare professionals that could aid in medical-decision making regarding the use of anti-inflammatory therapies in this population as well as, 2) to provide an overall framework on the evaluation of anti-inflammatory treatments for ASCVD.

Section 1.3 Research questions

In order to meet these objectives, this thesis addresses six research questions:

> ***Question 1***: What are the available anti-inflammatory therapies for ASCVD risk reduction and what is the relative efficacy of these therapies?
>
> ***Question 2:*** Do patients with diabetes have elevated systemic inflammatory levels?
>
> ***Question 3:*** What is the mechanism of action of colchicine in the reduction of ASCVD?

Question 4: Is colchicine a cost-effective treatment for reduction of ASCVD compared to current therapeutic options?

Question 5: Could biologic therapy be an effective option to reduce vascular inflammation in patients with psoriasis or psoriatic arthritis?

Question 6: Could low-dose statin therapy be an effective option to reduce vascular inflammation in patients with HIV?

Section 1.4 Chapter outlines

This thesis comprises 8 chapters. Chapter 1 provides an overall introduction and rationale for the thesis, while Chapter 2 provides an overview of ASCVD, the etiological role of inflammation in the pathogenesis of atherosclerosis, and the research methodology. Chapters 3, 4, 5, 6, 7, and 8 present manuscripts that address the research questions. The final chapter of the thesis, Chapter 9, integrates the major findings from the thesis and considers the implications for clinical practice and identifies existing knowledge gaps for future research.

The following section presents a brief overview of each chapter:

Chapter 1. Introduction: This introductory chapter provides a summary of the rationale for the thesis, its overall objectives, and a brief description of each chapter.

Chapter 2. Background: This chapter provides background regarding ASCVD and the role that inflammation plays in its pathogenesis. It also provides an overview of the methodological framework used to guide the thesis.

Chapter 3. Existing therapies for ASCVD treatment: This chapter pertains to a systematic review and network meta-analysis intended to summarize and compare the effectiveness of the available anti-inflammatory therapies that have been evaluated for the treatment of ASCVD. The title of these manuscripts is below:

Section 3.1 Examining anti-inflammatory therapies in the prevention of cardiovascular events: protocol for a systematic review and network meta-analysis of randomised controlled trials

Section 3.2 Anti-inflammatory therapies to prevent cardiovascular events: systematic review and network meta-analysis of randomised controlled trials

Chapter 4. Evaluating if diabetes represents a high-risk inflammatory population for targeted anti-inflammatory therapy: This chapter presents the findings of an observational study that examines the burden of inflammatory biomarkers in patients with diabetes or prediabetes to determine if they are potentially at risk for future CV events. Title of the study is below:

Section 4.1 Observational Cross-Sectional Study of Inflammatory Markers After Transient Ischemic Attacks, Acute Coronary Syndromes, and Vascular Stroke Events.

Chapter 5. Mechanism and efficacy of colchicine for ASCVD: This chapter presents the protocol for a randomized placebo-controlled trial evaluating the mechanism and efficacy of colchicine in the reduction of ASCVD events in patients with prediabetes or diabetes. The title of the study is below:

Section 5.1 The Canadian Study of Arterial Inflammation in Patients with Diabetes and Recent Vascular Events, Evaluation of Colchicine Effectiveness (CADENCE): protocol for a randomised, double-blind, placebo-controlled trial

Chapter 6. Cost-efficacy of colchicine for ASCVD: This chapter presents the results of a manuscript that explores the cost-effectiveness of colchicine in a cost-utility analysis presented in Section 6.1. The title of the manuscript is listed below:

Section 6.1 Cost-Effectiveness of Colchicine for Recurrent Cardiovascular Events.

Chapter 7Anti-inflammatory effect of biologic therapy in patients with psoriatic disease: A prospective cohort FDG PET study.

Chapter 8. Anti-inflammatory effect of rosuvastatin in patients with HIV infection: An FDG-PET pilot study.

Chapter 9. Discussion and Conclusion: This summary chapter will integrate the findings of the individual manuscripts and will place them in the context of current medical practice and policy. Limitations of the thesis are discussion and recommendations for future directions of research is proposed.

The overarching goal of the work provided herein, is to provide a detailed assessment of inflammation as a therapeutic target in ASCVD. Throughout the eight chapters presented, this work seeks to identify high-risk inflammatory patient phenotypes as well as evaluate treatment effectiveness with the use of both clinical as well as imaging-based biomarkers. By covering a wide breadth of research questions—ranging from evaluating the comparative efficacy of available anti-inflammatory agents, to examining their cost-effectiveness and efficacy in specific high-risk populations—this thesis aims to generate practical, evidence-based insights for both knowledge-users as well as policymakers on this timely topic. In addition, it provides an effective framework for testing anti-inflammatory cardiovascular drug efficacy with the use of imaging surrogate markers in a resource-sparing manner. By beginning with a broad review of current anti-inflammatory therapies and concluding in imaging studies of targeted interventions in high-risk subgroup populations, this thesis ultimately seeks to contribute a conceptual and methodological framework for the evaluation and potential implementation of anti-inflammatory treatments in cardiovascular medicine.

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Chapter 2: Background

Overview of Chapter 2.

As introduced in Chapter 1, this thesis focuses on the use of anti-inflammatory therapies for the treatment of atherosclerotic cardiovascular disease (ASCVD) in adults. Chapter 2 provides background information about ASCVD and the etiological role of inflammation in the development and progression of atherosclerotic plaque within the body. Chapter 2 also describes the overall framework of the thesis and the methodologies employed to address each of the research questions outlined in Chapter 1.

Section 2.1.1. Atherosclerosis

The term atherosclerosis is of Greek origin and means thickening of the intimal layer of the arteries and accumulation of fat. It is an apt name, as atherosclerosis itself is the resultant consequence of hyperlipidemia and lipid oxidative processes.[1] The term, atherosclerosis, is composed of two separate parts; atherosis (meaning accumulation of fat) and sclerosis (meaning fibrosis).[2] Atherosclerosis is a common disease in which fatty deposits arise within the innermost layer of the arterial wall. These deposits, termed atheromatous plaques, start with the deposition of cholesterol within the intima and its surrounding smooth muscle tissue.[1] Lipid rich plaques then begin to develop and grow with confluent proliferation of the surrounding fibrous tissues.[1] Fatty material is located at the core of the plaque, which is covered by a thin fibrous cap.

Most life-threatening sequelae from the atherosclerotic disease process results from acute thrombus formation on the surface of a plaque within the lumen of an artery. This can occur when the plaque acutely ruptures, with rupture of the fragile thin fibrous cap, exposure of the underlying lipid-rich core to the arterial blood, and subsequent thrombus formation.[3] The thin-cap fibroatheroma is characterized by a large necrotic core underneath a thin, fibrous cap of tissue that is less than 65 micrometers thick. The thin-cap atheroma contains a multitude of macrophages with evidence of neovascularization and intraplaque hemorrhage.[4] These features all predispose this thin-cap atheroma to rupture. Following rupture, when thrombus forms within the lumen of the vessel, it may completely occlude the lumen, or even embolize

distally to occlude further down in the vascular bed. In doing so, it can cause infarction to the tissues that are distally fed by the culprit blood vessel; leading to myocardial infarction if this occlusion is within a coronary artery, or stroke if the occlusion is within a cerebral artery.[4]

Inflammation is now well-recognized to play a crucial role in the pathogenesis of CAD. However, inflammation itself is integral to the functioning of our body's immune system. The inflammatory process is a critical component of the immune system's response to potentially noxious stimuli such as external pathogens or damaged cells.[5] During an acute inflammatory reaction, cellular and molecular events occur that contribute to minimizing impending injury or injection.[6] However, the concept "too much of a good thing" certainly applies to the immune system's inflammatory response. As described earlier, chronic inflammation can have extremely deleterious effects on the cardiovascular system and is a well-established risk factor for ASCVD.

Inflammation causes altered vessel biology, accelerated atherogenesis, plaque formation, and increases plaque instability. Thus, atherosclerosis itself is now recognized as a chronic inflammatory disorder. The atherosclerotic process is initiated by immune-mediated endothelial disfunction and structural alterations of the arterial wall.[7] These alterations include the absence of a confluent luminal elastin layer as well as the exposure of proteoglycans which causes the recruitment of T cells and monocytes after the expression of proinflammatory and prothrombotic cytokines and chemokines.[8 9] This endothelial dysfunction then permits the subendothelial accumulation of lipoproteins.[10] Lipoprotein particles are made of proteins, phospholipids, and lipids like cholesterol and triglycerides.[1] One of the key atherogenic lipoprotein mediators is the cholesterol-rich low-density lipoprotein (LDL).[1 11] Lipoprotein can accumulate within the vascular intima in several manners, as it can directly infiltrate into the endothelium or can also adhere to extracellular matrix components like proteoglycan.[1] Elevated circulating levels of cholesterol transported by apolipoprotein B100 (ApoB100)-containing LDL binds to negative charged extracellular matrix proteoglycans thereby leading to the retention of LDL particle in the intima. Once within the intima, these LDL particles are susceptible to oxidative modification by reactive oxygen species or enzymes like

myeloperoxidase or lipoxygenase that are released from inflammatory cells.[7] Oxidized LDL (oxLDL) triggers the secretion of chemokines by endothelial cells and also cause increase expression of adhesion molecules – these combine to drive intimal immune cell infiltration.[7] With this comes the early formation of a "fatty-streak" lesion, consisting of T cells and monocyte-derived lipid-laden foam cells. As more apoptotic cells, debris, and cholesterol accumulate within the lesion, a necrotic core is then formed. These plaques are covered by a fibrous cap which is composed of collagen and smooth muscle cells (SMCs). Over time, the collagen and SMCs can become replaced by macrophages resulting in a thinning of the cap and predisposing the lesion to subsequent rupture. In particular, the "shoulder" regions of the lesions are most heavily infiltrated by T cells and mast cells, which results in the production of proinflammatory mediators and contributes to adventitial inflammation of the plaque.[12] Neutrophils play an important role in the pathogenesis of the atherosclerotic disease process. Early neutrophil inflammatory signals trigger the intimal recruitment of monocytes, which go on to differentiate into macrophages. These macrophages then ingest native and modified LDL, leading to the formation of lipid-laden foam cells. Macrophages have several deleterious effects within the arterial wall, including the amplification of LDL modification, the secretion of cytokynes, proteases or procoagulant factors (which all promote the vulnerability of atherosclerotic plaques), and when not effectively cleared, contribute to necrotic core formation within the nucleus of the plaque.[7] Furthermore, oxLDL may actually induce neutrophil transmigration and degranulation to bring more classical monocytes into the plaque. Neutrophils are short lived cells and very quickly become apoptotic.[13] Their death triggers the release of an inflammatory milieu of signalling molecules that further attracts monocytes/macrophages for scavenging.[14] Thus, the inflammatory cascade of atherosclerosis continues if left unchecked.

Section 2.1.2. Epidemiology of atherosclerotic cardiovascular disease

Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of death worldwide.[15] In 2019, heart disease was the number one cause of death in the United States.[16] Despite a concerted effort to treat and control traditional risk factors such as hypertension, dyslipidemia, smoking, and diabetes, as our

population in Canada continues to age, the burden of CV disease continues to climb. In recent years, more attention has also been garnered by a risk factor that has historically been not in the limelight, inflammation.

The World Health Organization considers multimorbidity as a condition in which a person suffers from two or more chronic conditions simultaneously.[17] With the aging population, multimorbidity is becoming increasingly common, and many of the chronic diseases that afflict individuals share common underlying biological pathways. One of these common underpinnings is inflammation. CVD is a leading cause of morbidity and mortality for many of the patients that suffer from various chronic inflammatory conditions. Thus, it may be worthwhile to target these high-risk patients with co-morbid inflammatory conditions to reduce the burden of ASCVD.

While it has been well established that inflammation plays an important etiologic role in the pathogenesis of ASCVD, until recently, establishing treatments to target inflammation to reduce CV events had been elusive. In recent years, there has been a plethora of recent studies that have sought to find novel treatments that target various aspects of the inflammatory cascade to improve the CV health of patients. In order to inform clinicians regarding the use of these potential therapies, an up-to-date assessment of the evidence is needed. In this thesis, various aspects of the inflammatory hypothesis of ASCVD are evaluated:

Question 1: What are the benefits and harms of anti-inflammatory therapies for ASCVD?

Question 2: Do patients with diabetes have elevated systemic inflammatory levels?

Question 3: What is the mechanism of action of colchicine in the reduction of ASCVD?

Question 4: Is colchicine a cost-effective treatment for reduction of ASCVD compared to current therapeutic options?

Question 5: Could biologic therapy be an effective option to reduce vascular inflammation in patients with psoriasis or psoriatic arthritis?

Question 6: Could low-dose statin therapy be an effective option to reduce vascular inflammation in patients with HIV?

The following section provides a brief overview of the methodologies used to address these research questions.

Section 2.1.3. Treatment of inflammation to reduce cardiovascular disease

There are thousands of articles which have described experimental studies of inflammation in ASCVD. Furthermore, thousands more preclinical research studies have explored the relationship between inflammation and CV events.[18] However, up until recently we lacked solid clinical evidence that an anti-inflammatory therapy could reduce CV events. In recent years, this has dramatically shifted. Clinical evidence has now closed the gap between experiment and clinical practice. There are a multitude of recent clinical trials which have evaluated the efficacy of various anti-inflammatory agents for the treatment of ASCVD.

The first trial to validate the concept that targeting inflammation could theoretically lower CV events was the CANTOS trial. CANTOS was a large clinical trial of over 10,000 patients that evaluated the efficacy of Canakinumab, an IL-1B antibody, at reducing CV events in well-treated patients with stable CAD and a persistently elevated hsCRP (>2 mg/L). Notably, CANTOS preselected a patient population that was somewhat "high-risk" – that is, patients with known CAD and with elevated residual inflammation. Indeed, Canakinumab, a targeted therapy for inflammation, was found to reduce major adverse cardiovascular events by 15%. Furthermore, a sub analysis of the CANTOS data showed that participants that responded to the anti-inflammatory therapy by a greater than median reduction in hsCRP levels had a 26% reduction in the endpoint of myocardial infarction, stroke, or CV death – as well as a reduction in total mortality. However it is also important to note that it was not all good news. Importantly, there was a small but statistically significant increase in infections, including fatal infections, in those taking canakinumab. These infections were from common viral and bacterial agents – not opportunistic organisms.[19] The other detractive aspect of many of the anti-inflammatory agents is the cost. In the

CANTOS trial, Canakinumab was given 4 times per year. At a cost of $16,000 per dose, this works out to a cost of $64,000 per patient per year of treatment. Given the burden of atherosclerotic disease in Canada, one can see how the societal cost of this medication would become astronomically high. Rather than simply searching for medications that are efficacious from a CV risk reduction perspective, we need to think broader from a health-systems perspective and look for medications that are cost-effective for the treatment of CV disease.

While the inflammatory hypothesis of ASCVD is well established, the clinical use and effectiveness of anti-inflammatory agents is much less so. While there have been a plethora of anti-inflammatory medications that have been tested for efficacy, their relative effectiveness is much less well known. Most systematic reviews present comparisons between pairs of interventions (i.e. an experimental intervention and a comparator) for a specific condition and setting, such as ASCVD. However, in the case of establishing relative efficacy of anti-inflammatory agents for ASCVD, the problem lies in the fact that it is not a single comparison that is required. Owing to the fact that there are so many different anti-inflammatory agents that have been tested, establishing their relative efficacy is much more difficult. Thus, for decision-makers who need to decide between alternative interventions, there is great appeal to a single review that includes all relevant interventions, and presents their comparative effectiveness and potential for harm. Network meta-analysis provides an analysis that yields this information.[20 21]

Any set of studies that links at lest three interventions via direct comparisons forms a network of interventions. A potential powerful advantage of creating an established network of interventions is the ability to make indirect comparisons between the interventions. These are comparisons between interventions that have not been made directly within the original studies. These indirect comparisons can be estimated by utilizing mathematical combinations of the available direct intervention effect estimates that are available from the clinical trials of effectiveness. Network meta-analysis then combines direct and indirect estimates across a network of interventions in a single analysis. In a field where multiple treatment options exist for a single condition, this study design is quite attractive. In addition to the

obvious advantage of being able to compare multiple treatments, empiric evidence has suggested that it actually gives more precise estimates of the intervention effects when compared with a single direct or indirect estimate.[22 23] In addition, network meta-analysis can actually provide comparative information regarding pairs of interventions that have never been directly evaluated against one another within an individual randomized controlled trial.[21]

While leveraging the advantages that network analyses afford can be extremely beneficial, indirect comparisons made within these networks are ultimately observational evidence and therefore are subject to the risk of biases that all observational studies can suffer from including confounding. Additionally, the validity of an indirect comparison made between two interventions is reliant on the fact that the various sets of randomized trials that are being used to make the indirect comparisons are in fact similar in all important factors aside from the intervention comparison being made.[24-27] This is in essence, the concept of transitivity. In other words, studies can differ in a wide range of characteristics. If these characteristics are associated with the effect of an intervention, then they are considered effect modifiers. Thus, if the effect modifiers differ between studies, it is not appropriate to use those studies to make an indirect comparison.

Section 2.2.1. Cardiovascular disease in Diabetes

A major drawback to many of the anti-inflammatory therapies that are being considered for the treatment of ASCVD are the potential side-effects. Inflammation is the immune system's natural response to noxious stimuli like pathogens or damaged cells.[5] It acts to trigger the removal of injurious stimuli and also initiates the body's healing response.[28] Thus, inflammation is a helpful defense mechanism that helps us maintain health.[29] One can see how using medications to potentially dampen this vital immune response could potentially carry dangerous consequences. Sure enough, studies have shown potential side effects (depending on the anti-inflammatory medication) of increased infections, gastrointestinal side effects, and even potentially increased mortality. Therefore, careful consideration of the right populations to use these medications will be very important. Identification of high-risk "inflammatory" population

that would derive the most benefits from these potential therapies may be the best way to proceed. A major focus of this thesis will be trying to identify potential inflammatory populations that may benefit from anti-inflammatory treatments to reduce ASCVD risk.

A cross-sectional study design will be used to assist in answering the second question of the thesis. The purpose of this chapter is to identify the prevalence of elevated inflammatory blood biomarkers in patients with diabetes who have suffered a recent myocardial infarction (MI). This will allow us to determine if patients with diabetes who have established CAD (i.e. they have suffered an MI) have an increased inflammatory burden, and therefore if they could potentially be a vulnerable high-inflammatory, high-risk population for further anti-inflammatory therapies. A cross-sectional study design is a simple, elegant, and efficient means to answer this question. In a cross-sectional study, participants are selected based solely on the inclusion and exclusion criteria set by the investigator.[30] Once the participants have been identified, the outcome of interest can be identified for each participant. Because it is a one-time measurement of simultaneous exposure and outcome, causality cannot be ascertained from a cross-sectional study design, however as mentioned above, it is a useful tool to determine prevalence of a condition or disease (or prevalence of elevated inflammatory biomarkers, in this case) in a specific population of interest. Thus, it can be informative for future cohort or randomized controlled trials.

Similar to other diseases that have been highlighted, CV disease is the leading cause of morbidity and mortality for patients with type 2 diabetes.[31] Compared with people without type 2 diabetes, those with the disease are at increased risk of both cardiovascular morbidity and mortality.[32-34] Evidence exists that up to one third of persons living with diabetes have established CV disease.[35] CV events typically occur earlier in life in patients with type 2 diabetes and also are typically more severe.[36 37] While traditional risk factors such as hypertension, dyslipidemia and hyperglycemia are thought to play the majority of the etiologic role.[38] However, even when these risk factors are treated according to guidelines, a substantial cardiovascular risk remains.[39] Thus it is hypothesized that low grade systemic inflammation may play an important role in mediating this risk.[39] The underlying pathologic process of type 2 diabetes is insulin

resistance; this leads not only to hyperglycemia, but also potentiates low-grade inflammation.[38] It has been well established that increased adiposity leads to hyperglycemia. However, when excess adipose tissue becomes dysfunctional, it also produces pro-inflammatory cytokines like TNF-alpha and interleukin-6.[40] Furthermore, as this excess adipose tissue becomes dysfunctional, anti-inflammatory adipokine production such as adiponectin is reduced.[40]

Despite improvements in CV risk factor management and concomitant improvements in diabetes-related mortality, excess all-cause mortality as well as CV-related mortality continue to occur at unacceptable rates.[41] Given the prevalence of the disease in the population, the clinical impact of CV disease in patients with type 2 diabetes is high and is associated with increased healthcare costs.[42] This again highlights type 2 diabetes as an chronic inflammatory condition with high CV morbidity and mortality. Similar to patients with psoriasis and HIV, this population deserves special attention to try to determine therapies that can potentially target inflammation to reduce CV disease burden.

Section 2.2.2. Mechanism of Action of Colchicine in Cardiovascular Disease

A randomized controlled trial (RCT) will be used to determine the mechanism of action of colchicine in potentially reducing ASCVD events in patients with (pre)diabetes. RCTs have oft been considered the gold standard for medical evidence because of their ability to eliminate bias due to confounding and therefore their ability to ensure internal validity.[43] By prospectively assigning participants to the study arms (in this case, placebo or colchicine), selection bias can be minimized.[44] When performed properly, the randomization process involved in an RCT helps to ensure that study groups are well balanced on baseline factors that are known and even unknown prognostic factors (i.e. confounders).[45] Requirements for the pre-registration of RCT protocols also minimizes the risk of bias arising from deviations from intended interventions and selective outcome reporting.[46] Furthermore the requirement of pre-registration of RCT protocols promotes consistency of treatments and measurements that may be challenging in observational trials.[47]

Randomized imaging trials offer a whole set of additional advantages over traditional clinical randomized controlled trials. We are in a golden era of cardiac imaging. Cardiac imaging has revolutionized the diagnosis, prognostication, and treatment of all forms of heart disease. We have a plethora of imaging options at our fingertips.[48] Imaging technology is advancing at a breakneck pace. This is certainly true in the field of nuclear cardiology. Nuclear imaging is being used in more ways than ever before, with applications ranging from diagnosis of CAD, prognostication of ischemic heart disease, detection of hibernation, detection of infection, diagnosing sarcoidosis, to amyloid imaging. Artificial intelligence is pushing the field to new frontiers. Similarly, there has been an equally momentous push for more high-quality cardiovascular clinical trials to answer important questions in our field. However, with this push for more trials, comes a responsibility to utilize our trial resources in an effective manner. Clinical trials are held to high-standards, and with this, there are high expectations to balance the exponentially increasing costs of performing high-quality research, maximize the efficiency of our research, increase the representation of typically under-represented populations within CV research, all while producing premium quality studies. With the broad range of applications of nuclear cardiology, its ability to image molecular targets, and its global availability, it is uniquely poised to be able to answer clinically relevant questions in meaningful, potentially novel, and efficient clinical designs. With this comes an ever-increasing number of trials utilizing nuclear cardiology as a tool to answer clinically relevant questions.

We will utilize a cardiac imaging outcome as a surrogate outcome endpoint in several studies within this thesis. The first study is to determine the mechanism of action of colchicine in reducing CV events. This study will leverage the strength of an RCT and will also leverage the strength of using an imaging surrogate endpoint. The largest strength of this type of endpoint is that it can test clinical effectiveness in a smaller population, acting as an efficient means to test hypotheses.

Section 2.2.3. Cost-effectiveness of colchicine for cardiovascular disease

Cost-effectiveness analyses allow for the exploration of both costs as well as health outcomes of one or more interventions. They compare treatments to either other therapies or the status quo, by determining

how much it costs to gain a unit of a health outcome, such as a life year gained. Ultimately, health economic analyses are designed to inform decisions. They involve the assessment of the cost and effect trade-offs of potential interventions that impact health outcomes. The evaluations can incorporate and synthesize evidence from multiple sources to inform the analysis. The analysis undertaken for this study in particular took a social decision-making viewpoint; this is based on the premise that the health care decision-maker is seeking to maximize the degree to which a policy objective is achieved subject to the available resources. In this case, the explicit objective is improving the overall health of the Canadian population. There are two key central concepts that are considered are central tenets to the economic evaluation of novel therapies. First is the concept of decision-making under scarcity. In other words, this assumes that healthcare resources are not limitless, and that trade-offs and decisions are required to determine which technologies are worthy of funding in a healthcare system. Often in healthcare there is a mismatch between what is theoretically feasible and what is economically possible.[49] Therefore, the goal of the economic analysis is to inform this decision. The second concept relates to the need for the efficient allocation of resources within the healthcare system. Efficiency refers to obtaining the maximal possible improvement in an outcome from a given set of resources available.[50]

A cost-utility analysis is one of four different types of "full" economic evaluations. These different types of economic evaluations share a common underlying theme, in that they all involve a comparative analysis of alternative courses of action based on both costs as well as consequences.[51] However compared with the other types of evaluations, a cost-utility analysis allows for the most broad consideration of health benefits. This is because it uses an overall summary measure, the quality-adjusted life year (QALY), that can be compared across different outcomes or disease states.[51] Additionally, cost-utility analyses allow for preferences for health states to be incorporated into the analysis by use of utility weights in the calculation of the QALY.[51]

There are two main forms of cost-utility analyses: trial-based or model based. A trial-based model utilizes a single RCT to provide the necessary information to guide the evaluation, including the costs and

outcomes associated with a particular treatment strategy.[52] There are several limitations to this approach. Firstly, this approach can be limited by the possibility of that not all relevant treatment options are considered within the model. Given the model is based on a single clinical trial, only treatment options considered within the trial are included within the model. Secondly, data available for treatment efficacy and patient outcomes are based on the length of follow-up of the trial being used. Therefore, there is a possibility that there is a short duration of treatment and observation available for modeling if the trial utilized did not have a long period of follow-up. Thirdly, there is a risk that the results of a randomized-controlled trial are not directly applicable to the population being considered for funding or the decision context. Lastly, there may be a failure to consider to measure important costs or outcomes within the utilized RCT.[52] In contrast to this, model-based evaluations consider all available relevant evidence, thereby mitigating the concerns associated with trial-based evaluations.[52] Thus, a model-based approach will be used as the form of economic evaluation undertaken for this thesis.

Section 2.2.4. Cardiovascular disease in HIV

With over 45 million people living with human immunodeficiency virus-1 infection (PLWH) around the world, HIV continues to pose a huge public health hurdle.[53] To further highlight this, over 37 million lives have been lost over the past 4 decades associated with HIV – and to this day, there remains no reliable cure.[54] Fortunately, increasing access to effective prevention measures, disease screening, and treatment with antiretroviral therapy (ART) has transformed HIV from a death sentence into a chronic illness. PLWH are now living to older ages, and with that, are experiencing the disease states commonly associated with older ages. Notably rates of myocardial infarction (MI), heart failure (HF), and sudden cardiac death (SCD) are all higher than that observed in the general population.[55-58] As a result, ASCVD remains a leading cause of morbidity and mortality among PLWH in the Americas and Europe.[59-61] A meta-analysis and systematic review demonstrated that PLWH have a relative risk for MI that is 1.4 to 2.1 compared to controls.[59]

As previously mentioned, the central role of inflammation in the pathogenesis of ASCVD is well established. Within atheromatous plaque, CD4+ and CD8+ T cells are key drivers of intimal tissue inflammation.[62] Epidemiological data supports the notion that chronic HIV disease may promote ASCVD through abnormalities in both adaptive and innate immune mechanisms. This is true even in PLWH that are well-treated with ART. Evidence over the past decade has demonstrated that even despite adequate viral suppression with ART, inflammation persists at supranormal levels.[63-65] Although ART treatment improves the HIV-induced deficiency of adaptative immunity, the degree of restoration is often incomplete.[63] Levels of CD4+ T cells may remain abnormally low, and abnormalities of CD8+ T cell activation can also persist.[63 66 67] In one cohort study of PLWH, abnormal CD4+ T cell recovery was present even after 6 years of ART treatment.[66] The relevance of persistent T-cell abnormalities to the pathogenesis of ASCVD and its subsequent complications was highlighted in a recent study that demonstrated that low CD4+ T cells (<200 cells/mm^3), high CD8+ T cells (reflecting ongoing abnormal activation), and a low CD4:CD8 ratio were all associated with ischemic heart disease events.[68] Persistent immune activation despite adequate viral suppression with ART may be driven in party by the persistence of HIV in lymphatic tissues, microbial translocation, and coinfection with organisms (e.g. cytomegalovirus and tuberculosis).[69] Interestingly, epidemiological studies have demonstrated that PLWH[70] that have elevated markers of inflammation (C-reactive protein or interleukin-6), monocyte activation (soluble CD14 and CD163), and increased coagulation activity (D-dimers) have an increased risk of ASCVD events.[59 71-74] In an analysis of 755 PLWH who were at low-to-moderate risk of CAD, elevated levels of IL-6 and lipoprotein-associated phospholipase A2 (reflecting systemic inflammation and atherosclerotic plaque inflammation, respectively) were associated with subclinical atherosclerotic coronary artery plaque, independently of other traditional risk factors.[75] There is also evidence to support the pathogenic role of monocytes in HIV-associated atherogenesis.[74 76 77] Data has demonstrated higher levels of circulating sCD163 in PLWH and both arterial wall inflammation as well as noncalcified plaque. Furthermore, there has also been associations described between higher frequences of intermediate (CD14+CD16+) or nonclassical (CD14dimCD16+) monocyte phenotypes which traffic to sites of injury

like the vascular intimal layer.[74 76 77] Much of the work done to establish the mechanisms underlying the role of monocyte/macrophage-mediated atherogenesis often utilize cross-sectional study designs, which unfortunately limits causal inference drawn from the study.[53] This highlights the need for randomized interventional studies to validate the immune mediators of HIV-associated ASCVD risk as well as identify potential therapies for this high-risk subgroup.

Section 2.2.5. Cardiovascular disease in Psoriasis

Psoriasis is a chronic inflammatory condition that can affect both the skin and joints. It is one of the most common skin diseases that presents in dermatology practice and is characterized by a complex underlying inflammatory pathogenic process.[78] The prevalence of psoriasis has been described as being approximately 2% worldwide.[79] It is a disease of young people, with 50% of the cases of psoriasis presenting within the first three decades of life.[79] An overly robust inflammatory response in various body systems provoked by a dysregulated immune system is thought to underpin a pivotal role in the pathogenesis of this disease.[70 80 81] Notably, the life expectancy of people with psoriasis has been found to be close to 5 years lower compared to the general population, and cardiovascular disease is thought to be the main cause of this with the majority of deaths in people with psoriasis attributable to CVD.[82] Importantly, this dysregulated immune response is thought to play an important role in the link between psoriasis and its association with ASCVD.[83] Interestingly, the risk of major cardiovascular events (MACE) in patients with psoriasis has a direct and linear relationship with the underlying psoriasis disease severity.[84] Evidence has demonstrated that the risk of cardiovascular-related death is higher amongst persons with severe psoriasis (RR 1.39; 95% CI [1.11-1.74]) compared to those with a mild form of psoriasis (RR 1.03; 95% CI [0.86-1.25]).[85]

Psoriasis is a chronic Th1/Th17 inflammatory skin disease. In psoriatic dermatologic plaques, Th1 cytokines, interferon-y (INF-y), IL-2, and TNF-α all stimulate the production of inflammatory cytokines (TNF-α, IL-1B, and IL-6) and chemokines ligands 8, 9, 10, 11, and 20.[86] This results in migration of T-cells to the dermis and upregulation of adhesion molecules. Binding of the T-cells to the adhesion

molecules in the dermis results in the migration of more T-cells to the dermis and thereby provokes more inflammation. Additionally, the elevated levels of circulating Th1 cytokines like TNF-α also lead to endothelial dysfunction and extravasation of T-cells into the site of atherosclerotic plaques.[85] Activation of Th1 enhanced by IL-12 is a well-recognized pathogenic step in both psoriasis and atherosclerosis – thus it is likely that the pathogenic downstream release of cytokines is shared between these two disease entities.[87]

Recent work has demonstrated that persons with psoriasis have increased non-calcified lipid-rich atherosclerotic plaque burden. This is thought to be mediated by immunologic abnormalities and impaired HDL function.[88] Patients with psoriasis have been found to have significantly elevated levels of oxidation-modified lipids, including oxidised LDL, HDL, and lipoprotein (a) which all contribute to promote a proatherogenic lipoprotein profile and an impaired HDL-cholesterol efflux capacity.[88] Additionally, oxidized LDL starts a cascade of biochemical reactions that results in endothelial cell dysfunction and atherosclerotic plaque formation.[89] In vitro studies have shown that even low levels of oxidized LDL can activate macrophages and mast cells to increase monocyte-endothelium adhesion, thereby contributing to endothelial dysfunction and early atherogenesis.[90]

Platelets are also immune cells that trigger and regulate immune and inflammatory processes.[91] It has been shown that platelet dysfunction plays an important pathogenic role in psoriasis, with patients with psoriasis demonstrating an increased percentage of platelet aggregation (which is also correlated with psoriatic disease severity).[91] In psoriasis, activated platelets can release biologically active materials to create an inflammatory environment.[92]

Inflammatory blood biomarkers such as C-reactive protein (CRP), tumor necrosis factor (TNF) alpha, interleukin (IL)-6, and glycoprotein acetylation (GlycA) are increased in psoriasis, are associated with psoriasis disease severity, and are also associated with future cardiovascular risk in healthy individuals without psoriasis.[93-95] Consistent with the epidemiological as well as translational studies that have emphasized the link between psoriasis and CV disease, studies have also shown that patients with

psoriasis have increased vascular inflammation as measured by 18F-fluorodeoxyglucose positron emission tomography (18F-FDG PET/CT).[96 97] Thus, psoriasis is a high-yield disease to evaluate anti-inflammatory therapies to reduce vascular inflammation, and ultimately potentially reduce CV burden in this population.

Section 2.3.1. Summary

In this chapter, background information about the role of inflammation in atherosclerotic cardiovascular disease has been described. The following chapters (Chapters 3-8) present a series of six manuscripts that each address of the six research questions by utilizing the methodologies introduced within this chapter.

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Chapter 3 Existing therapies for ASCVD treatment

CHAPTER OVERVIEW

This chapter addresses the first research question: *"What are the benefits and harms of anti-inflammatory therapies for ASCVD?"*

Before this thesis, there had been no comprehensive study looking at the relative efficacy of the available anti-inflammatory therapies for coroanry artery disease. Thus, the objective of this chapter was to identify and assess all randomized controlled trials, involving anti-inflammatory therapies to treat coronary artery disease in adults, in order to gain an understanding of the relative efficacy of these therapies.

Chapter 3 contains two manuscripts (Sections 3.1-3.2). Each manuscript is briefly described below, with a more thorough description on the first page of each section.

CHAPTER CONTENTS

Section 3.1. presents the protocol for a systematic review and network meta-analysis that evaluates the relative efficacy of the available anti-inflammatory therapies for coronary artery disease. This manuscript was published in the *British Medical Journal Open*.

Section 3.2. presents the results of the systematic review and network meta-analysis that evaluates the relative efficacy of the available anti-inflammatory therapies for coronary artery disease. This manuscript was submitted to *Canadian Journal of Cardiology Open*.

Section 3.1 Protocol for a systematic review and network meta-analysis of randomised controlled trials examining anti-inflammatory therapies in the prevention of cardiovascular events

SECTION OVERVIEW

This section presents a protocol for a systematic review and network meta-analysis that evaluates the relative efficacy of the available anti-inflammatory therapies for coronary artery disease. The findings of this review are described in **Section 3.2**.

MANUSCRIPT STATUS

The protocol for this manuscript has been published:

Boczar KE, Shin S, Bezzina KA, Geejo A, Pearson AL, Shahab S, Fehlmann CA, Visintini S, Beanlands R, Wells GA. Examining anti-inflammatory therapies in the prevention of cardiovascular events: protocol for a systematic review and network meta-analysis of randomised controlled trials. BMJ Open. 2022 Jun 27;12(6):e062702. doi: 10.1136/bmjopen-2022-062702. PMID: 35760536; PMCID: PMC9237867.

Supporting material

Supplementary file 1: PRISMA-P checklist

Supplementary file 2: Search strategy

Author roles and contributions:

KEB, RB, and GW conceived the study. SV designed the search strategy. KEB drafted the protocol, which was reviewed and revised by all authors.

RELATED THESIS APPENDICES

Appendix G: Published manuscripts. The PDF of the published manuscript is presented in Appendix G.

Protocol for a systematic review and network meta-analysis of randomised controlled trials examining anti-inflammatory therapies in the prevention of cardiovascular events

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Keywords: Inflammation, Anti-Inflammatory, Myocardial Infarction, Cardiovascular disease

Abstract

Introduction

Inflammation is emerging as an important risk factor for atherosclerotic cardiovascular disease (ASCVD) and has been a recent target for many novel therapeutic agents. However, comparative evidence regarding efficacy of these anti-inflammatory treatment options is currently lacking.

Methods and Analysis

This systematic review will include randomized controlled trials evaluating the effect of anti-inflammatory agents on cardiovascular outcomes in patients with known cardiovascular disease. Studies will be retrieved from Medline, Embase, the Cochrane Central Register of Controlled Trials, as well as clinical trial registry websites, Europe PMC and conference abstract handsearching. Eligible interventions must have some component of anti-inflammatory agent. These include (but are not limited to): NSAIDs, colchicine, prednisone, methotrexate, canakinumab, pexelizumab, anakinra, succinobucol, losmapimod, inclacumab, atreleuton, LP-PLA_2 (Darepladib), and $sPLA_2$ (Varespladib). The primary outcomes will include major adverse cardiac events (MACE), and each individual component of MACE (myocardial infarction, stroke, and cardiovascular death). Key secondary outcomes will include unstable angina, heart failure, all-cause mortality, cardiac arrest, and revascularization. Screening, inclusion, data extraction and quality assessment will be performed independently by two reviewers. Network meta-analysis based on the random-effects model will be conducted to compare treatment effects both directly and indirectly. The evidential quality of the findings will be assessed with appropriate tools including the Grades of Recommendations, Assessment, Development, and Evaluation (GRADE) profiler, Confidence in Network Meta-Analysis (CINeMA) tool, or threshold analysis.

Ethics and Dissemination

The findings of this systematic review will be important to clinicians, patients and health policymakers regarding the use of anti-inflammatory agents for the treatment of ASCVD.

Study Registration

Prospero registration number: CRD42022303289

Keywords

Systematic review, inflammation, myocardial infarction, cardiovascular events, network meta-analysis

Strengths and limitations of this study

- Analysis of data within the structure of an NMA allows pairwise effects to be estimated directly and indirectly, even in the absence of direct evidence
- Rigorous search of published and unpublished data will be conducted
- Article selection process, data extraction, and risk of bias will all be performed by two reviewers in parallel
- We will use an appropriate tool to summarise and assess the quality of evidence across the included studies
- Potential limitations include the possible role of confounding factors in biasing finding estimates

Introduction

Description of Condition

Atherosclerotic cardiovascular disease (ASCVD) is a leading cause of mortality and morbidity around the world.[1, 2] The incidence of myocardial infarctions (MI) has been dramatically lowered in populations that have pursued a strategy of aggressive detection and control of traditional cardiovascular risk factors for coronary artery disease (CAD), like hypertension, diabetes, cigarette smoking, and elevated low-density lipoprotein cholesterol (LDL-C).[2]

Despite adopting a strategy of aggressively controlling traditional risk factors for cardiovascular disease (CVD), major adverse cardiovascular events (MACE) unfortunately continue to occur at high rates.[3] Thus, much attention is now focused on other potentially modifiable risk factors that can be targeted to further reduce the burden of ASCVD.

The pathogenic basis of atherosclerosis is a complex process; we now know that its biological basis is more intricate than simply attributing it to intimal infiltration of LDL-C. Thus, we are beginning to acknowledge that our therapies will need to extend beyond treatment for the traditional risk factors. In particular, recent clinical and experimental evidence has supported inflammation as playing a key role in the initiation, progression, and eventual overt clinical manifestations of ASCVD.[4]

Contribution of Inflammation in the Pathophysiology of Atherosclerosis

ASCVD is now thought of as a chronic inflammatory disease of blood vessels which is initially triggered by intimal LDL-C infiltration. An early mechanism in atherogenesis is the exposure of the intimal endothelium to noxious stimuli, such as hypercholesterolemia, hypertension and

importantly, inflammation. This impairs its ability to act as a functional barrier thereby causing it to become 'activated'. After their activation, intimal endothelial cells increase their expression of leukocyte adhesion molecules.[5, 6] This change in expression allows the migration of leukocytes, particularly neutrophils and monocytes, into the subendothelial space from the circulating blood. Once inside the arterial wall, monocytes then differentiate into macrophages and begin to ingest modified LDL-C particles, eventually becoming lipid-laden foam cells. The aggregation of foam cells results in a yellow coloured 'fatty streak' in the vessel wall and thereby the first overt sign of atherosclerotic disease.[2]

From a clinical perspective, inflammatory mediators have shown pivotal roles in mediating thrombotic complications of atherosclerosis, namely myocardial infarction and ischaemic stroke.[7, 8] This fact has spurred the clinical evaluation of inflammation as a therapeutic target in an attempt to further reduce the burden of CVD.[9-12]

Description of the Intervention

The encouraging results obtained from basic CV research endorsed their early translation into the clinical setting which unfortunately failed on several early investigations.[13, 14] However, since these early clinical trials, there have also been a multitude of successes, and there is a plethora of new research looking at various anti-inflammatory therapies for the mitigation of cardiovascular events.

While these randomized controlled trials (RCTs) have primarily compared these novel agents to placebo (and background of statin therapy), few, if any, have been compared to other inflammatory therapies. Thus, there is currently a lack of understanding of the relative effectiveness of these therapies.

The current review aims to provide an up-to-date analysis of the effectiveness of anti-inflammatory interventions for preventing MACE. We will improve upon previous reviews by comparing a comprehensive list of anti-inflammatory therapies not just with control groups but with other anti-inflammatory interventions, even when they have not been directly compared in a trial, using network meta-analysis (NMA). Additionally, our review will be conducted in accordance with PRISMA recommendations.[15]

Objectives

The primary objective of this review is to assess the effectiveness of anti-inflammatory therapies in cardiac disease, examined in randomized controlled trials (RCTs). The results will provide a clearer understanding of the benefit of each individual anti-inflammatory therapy, and will also allow the comparison of the relative effects of each intervention.

Methods and analysis

This protocol was developed according to the (Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols) PRISMA-P 2015 checklist[15], as shown in the PRISMA-P checklist (see supplementary file 1) and was registered with PROSPERO (number CRD42022303289). Important amendments made to the protocol will be documented and published alongside the results of the systematic review.

Types of studies

Randomized controlled trials will be included in this study.

Population

All patients, either male or female, of any age, with known coronary artery disease will be included in this review. We will include participants with both acute coronary syndromes (ACS), as well as stable coronary artery disease. However, if we find significant differences between

interventions used to treat those with ACS versus those with stable CAD, we will conduct separate/subgroup analyses according to the type of CAD present.

Intervention

Eligible interventions must have some component of anti-inflammatory agent. We will include any medication, the primary effect of which is anti-inflammatory, including (but are not limited to): NSAIDs, colchicine, prednisone, methotrexate, canakinumab, pexelizumab, anakinra, succinobucol, losmapimod, inclacumab, atreleuton, LP-PLA_2 (Darepladib), and $sPLA_2$ (Varespladib). We will not consider any medication which does not have a primary mechanism of action that acts through the inhibition of inflammation (e.g. statins or allopurinol).

Comparisons

All control group types will be included in this systematic review regardless of whether they received or were exposed to any other type of control intervention, or lack thereof.

Primary outcome

The following primary outcome will be extracted:

- MACE and each individual component of MACE:
    - Myocardial infarction
    - Stroke
    - Cardiovascular death

Secondary outcomes and adverse outcomes will be extracted from studies identified as meeting the above-mentioned inclusion criteria.

Key secondary outcomes

- Unstable angina
- Heart failure

- All-cause mortality
- Cardiac arrest
- Revascularization

Key adverse outcomes

These relate to adverse events suggested in previous trials and include but are not limited to:

- Infection/pneumonia
- Diarrhea/GI upset
- Malignancy

Years of publication considered

No limitation on the year of publication will be imposed.

Language

No language restriction will be imposed. In the event that an eligible study has been written in a language other than English, where possible, professional translators will be hired.

Publication status

Published and unpublished trials will be included in this review. Ongoing studies will be searched in the Clinicaltrials.gov and World Health Organization's International Clinical Trials Registry Platform (ICTRP) and considered where relevant.

Search Strategy

The search strategy will be developed using a combination of subject headings and keywords by a medical librarian (SV) in Medline in collaboration with team members, then peer-reviewed by a second librarian as per PRESS guidelines[16]. It will then be run in the various databases listed below from inception. Search results will be exported to Covidence and duplicates will be eliminated using the platform's duplication identification feature. The search will be re-run prior

to the final analysis. A draft of the Medline search strategy is included in the supplementary files (see supplementary file 2).

Filters

Cochrane randomized controlled trial search filters were employed for both Medline and Embase.[17]

Information sources

The following electronic databases will be searched:

- Cochrane Central Register of Controlled Trials (CENTRAL)
- Ovid Medical Literature Analysis and Retrieval System Online (MEDLINE)
- Ovid Excerpta Medica Database (EMBASE)

Where critical information is not reported in published research, study authors will be contacted by email for more information. If no replies are received, two subsequent emails will be sent at 2 and 4 weeks.

The following additional resources will also be searched:

- ***Reference lists*** of relevant systematic reviews and individual studies will be hand-searched.
- ***Grey literature*** will be searched to explore the possibility of any unpublished research. This includes:
    - Clinical trial registries:
        - ClinicalTrials.gov
        - World Health Organization's ICTRP
    - Preprints from Europe PMC

    - Conference abstracts will be included as part of the Embase database search. Gaps in Embase indexation will be addressed with hand searching of select relevant conferences:
        - American Cardiology Conference
        - American Heart Association Conference
        - European Society of Cardiology Conference
        - Canadian Cardiovascular Congress
- RCTs registered but not yet published will be screened and included when eligible.

Selection process

COVIDENCE software will be used for study screening. Deduplication of study records will be carried out using COVIDENCE.

Following the deduplication process in COVIDENCE, the selection process will be conducted independently by two review authors in parallel (KEB, KAB, ShS, ALP, AG, and SS), who will screen and identify studies based on inclusion and exclusion criteria.

The first selection of potentially eligible studies will be conducted by screening titles and abstracts in COVIDENCE. Disagreements between reviewers will be resolved through discussion and consensus. Where consensus is not possible, a third external reviewer (KAB) will resolve any disagreements. After the first round of screening is completed, the full texts of the resulting studies will be screened for eligibility independently by the two reviewers (KEB, KAB, ShS, ALP, AG, and SS) according to the process outlined above.

Detailed reasons for exclusion will be tracked and reported in the PRISMA flow diagram. Multiple reports of the same studies will be considered together. Included articles will receive an identification number before data extraction.

Data extraction and management

For data collection, a pre-designed, standardized data extraction sheet will be used. The reviewers will first test the extraction sheet on two studies. They will then discuss and make amendments as necessary. Finally, data from included studies will be extracted and risk of bias assessments will be performed independently by two reviewers in parallel (KEB, KAB, ShS, ALP, AG, and SS). Data regarding study design, intervention characteristics (type of anti-inflammatory, length of therapy, intensity, outcomes), participant characteristics, comparators, setting, loss to follow-up rates, outcome measures, and effect sizes will be extracted. Unadjusted results will be preferred over adjusted results to improve consistency across studies and reduce the potential for selective reporting bias.

Data Items

Participants Participant characteristics that may modify treatment effects will be extracted and reported. These will include baseline sex, age, comorbidity status, presence of other inflammatory conditions, and concomitant alternative anti-inflammatory medication use. The number of participants at baseline and those lost to follow-up will also be extracted.

Intervention Data on other potential intervention modifiers such as treatment length, length of follow-up, and length of time from acute coronary syndrome (if relevant) will be included.

Comparator Data on the type of comparator used as well as data regarding control group participants will be extracted.

Risk of bias in individual studies

The risk of bias assessment will be conducted independently by two reviewers (KEB, ShS, ALP, AG, and SS) using the Cochrane Collaboration's Risk of Bias Assessment Tool. In cases of

disagreement, consensus will be reached through discussion and where not possible to obtain consensus, a third reviewer (KAB) will be included in the process.

The Cochrane Risk of Bias Assessment Tool assesses the following potential sources of bias: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, other sources of bias. Each category will be scored as at low, high or unclear risk of bias.

Sequence generation We will assess the allocation methods to determine the potential of bias that can be introduced due to the creation of incomparable groups.

Allocation concealment Adequacy of concealment in the participant allocation process will be assessed. If randomized methods of concealment are used, this will be considered adequate. Potential for bias due to inadequate concealment of the allocation process will also be assessed.

Blinding We will evaluate whether participants and personnel were blinded when allocating participants to treatment groups and whether outcome assessors were blinded as to which intervention group participants were in.

Selective outcome reporting Any evidence of attempts by the authors to omit the reporting of relevant outcomes will also be assessed.

Measures of treatment effect

- **Dichotomous outcome data** - For dichotomous outcomes, intervention effectiveness will be summarised as odds ratios (OR) or risk ratios (RR) and presented with 95% confidence intervals (CIs).
- **Continuous outcome data** - For continuous outcomes, data from treatment completers will be pooled by calculating the mean differences (MDs) between groups.

Data Synthesis

Clinical and methodological heterogeneity will be assessed by examining the variation in characteristics across studies in terms of baseline participant characteristics, the use and composition of "optimal medical therapy", outcomes, and other relevant study characteristics.

Network meta-analysis

If at least two studies are judged to be clinically homogeneous, a meta-analysis will be conducted. Our results will be analysed using a network meta-analysis (NMA).[18] An NMA utilises an interconnected network of interventions, which thereby allows the assessment of the comparative effectiveness of several competing interventions for a condition.[19] This can be performed as long as all the trials included in the analysis form a connected network.[20, 21] Analysis of data within the structure of an NMA allows pairwise effects to be estimated directly and indirectly, even in the absence of direct evidence.[22] For our NMA we will fit a model which compares anti-inflammatory interventions.

Data analysis

All statistical analyses will be conducted in a Bayesian framework using OpenBUGS software (www.openbugs.net). OpenBUGS is a commonly used program for conducting NMA in a Bayesian framework.[23] Random effects models, assuming a common between-study variability, will be used to address statistical heterogeneity. The goodness of fit of each model to the data will be assessed using the posterior mean residual deviance. Models will be compared using the Deviance Information Criterion (DIC), calculated by summing the posterior mean of the residual deviance and the effective number of parameters. Satisfying the consistency assumption is critical to ensuring the validity of an NMA model. This implies that there is no intervention effect modification by treatment comparison or that the frequency of effect modifiers is similar across the included studies.[24] This assumption can be examined by assessing the

inclusion/exclusion criteria of every intervention in the network to ensure characteristics are similar in ways that could potentially modify treatment effects.

If quantitative data analysis is not appropriate, a qualitative description and table of the included studies and data will be performed and displayed.

Subgroup analysis

Several pre-specified subgroup analyses will be conducted if data permits. These include:

- Sex
- Setting of ASCVD (acute coronary syndrome (ACS) versus non-ACS setting)
- Time after index event for initiation of anti-inflammatory agent
- Published versus unpublished literature

Assessment of reporting biases

Potential small-study effects will be examined by including study size as a covariate in a meta-regression analysis. Funnel plot asymmetry will be tested[25] to explore potential reporting bias (i.e., publication bias, selective outcomes reporting and selective analysis reporting).

Sensitivity analyses

Sensitivity analyses will be conducted by excluding studies at high or unclear risk of bias on allocation concealment and blinding domains per the Cochrane Risk of Bias Assessment Tool.[26] Sensitivity analyses will include fixed-effect analyses for the pairwise and network meta-analyses

Confidence in cumulative evidence

We will use an appropriate tool to summarise and assess the quality of the evidence across included studies.[27] This might include Grades of Recommendations, Assessment, Development,

and Evaluation (GRADE)[28], Confidence in Network Meta-Analysis (CINeMA)[29], or threshold analysis[30].

Patient and public involvement statement

Due to the nature of this study, patients are not involved in this project.

Ethics and Dissemination

Ethics approval is not required for this systematic review. This meta-analysis will be disseminated through a peer-reviewed journal.

Discussion

The proposed systematic review and network meta-analysis aims to address questions regarding the comparative effectiveness of anti-inflammatory therapies for the treatment of ASCVD. This question is important for several reasons. As mentioned, ASCVD is an extremely common problem, and is a leading cause of morbidity and mortality.[1, 2] With a growing number of therapeutic agents being developed to target the inflammation pathway, it is extremely useful for practitioners to have knowledge regarding the relative comparison of these novel drugs. Thus, this study could be important for clinical practice, providing information regarding relative benefits and harms from these anti-inflammatory agents. Furthermore, with the burden of the potential financial cost of these drugs for healthcare payers, this review will be potentially useful regarding funding decisions.

The major strength of this systematic review and network meta-analysis is that it will comprehensively summarise the evidence regarding anti-inflammatory benefit and harm for the treatment of ASCVD. Moreover, this network meta-analysis will allow both direct and indirect

comparison between these novel anti-inflammatory medications. Additionally, our comprehensive search strategy will attempt to discover both published and unpublished (grey) literature in the field. Limitations, on the other hand, also exist in our analysis. First, the strength of our review will be dependent on the existing evidence base for this topic area. If only one or two RCTs exist for a given comparison in the network, then the strength of the analysis will reflect that. Second, the sample size and the number of included studies may be small due to the novelty and resource-intensive nature of conducting an RCT of this nature on this topic.

Acknowledgements

We thank Becky Skidmore, MLS, Ottawa, ON, for peer review of the MEDLINE search strategy.

Data Availability Statement

Data generated by our research will be made available as possible upon reasonable request.

Contributors

Conceptualization: KE Boczar, GA Wells; Methodology: KE Boczar, SM Visintini, RS Beanlands, GA Wells; Project administration : KE Boczar; Supervision: GA Wells, RS Beanlands; Writing – original draft : KE Boczar; Writing – review & editing : KE Boczar, S Shahab, AL Pearson, A Geejo, S Shin, K Bezzina, CA Fehlmann, SM Visintini, RS Beanlands, GA Wells. KE Boczar is the guarantor of the review.

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Conflicts of Interest

No conflicts of interest to disclose.

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Additional Files

Supplementary file 1: PRISMA-P checklist

Supplementary file 2: Search strategy

Supplementary file 1: PRISMA-P Checklist

Protocol for a systematic review and network meta-analysis of randomised controlled trials examining anti-inflammatory therapies in the prevention of cardiovascular events

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Online Supplemental: PRISMA-P 2015 Checklist

This checklist has been adapted from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

Section/topic	#	Checklist item	Information reported		Section
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	X		Title
Update	1b	If the protocol is for an update of a previous systematic review, identify as such		X	
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	X		Abstract
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	X		Title
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	X		Contributors
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments		X	
Support					
Sources	5a	Indicate sources of financial or other support for the review	X		Funding
Sponsor	5b	Provide name for the review funder and/or sponsor	X		Funding
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	X		Contributors, Funding
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	X		Introduction

Section/topic	#	Checklist item	Information reported		Section
			Yes	No	
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	X		Methods
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	X		Methods
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	X		Methods
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	X		Appendix
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	X		Methods
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	X		Methods
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	X		Methods
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	X		Methods
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	X		Methods
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	X		Methods
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	X		Methods
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	X		Methods
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	X		Methods

Section/topic	#	Checklist item	Information reported		Section
			Yes	No	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	X		Methods
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	X		Methods
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	X		Methods

Supplementary file 2: Search strategy

Table 1. Medline Search

#	Searches
1	exp Arteriosclerosis/ or Plaque, Atherosclerotic/
2	(atherosclero* or athero-sclero* or arteriosclero* or arterial-sclero* or arteriolosclero* or arteriolo-sclero* or vascular sclero* or ASCVD or ASCAD or intima plaque).ti,ab,kf,kw.
3	Acute Coronary Syndrome/ or exp Coronary Disease/
4	((coronary adj3 (arter* or stenos* or disease? or disorder? or syndrom?)) or CAD or SCAD).ti,ab,kf,kw.
5	exp Percutaneous Coronary Intervention/ or exp Myocardial Revascularization/
6	(((aortocoronary or aorto-coronary or coronary) adj3 bypass*) or CABG).ti,ab,kf,kw.
7	exp endarterectomy/ or exp thrombectomy/ or exp Atherectomy/ or exp Embolectomy/
8	(angioplast* or atherectom* or endarterectom* or thrombectom* or thromboendarterectom* or thrombo-endarterectom* or PCI or PTCA or (Percutaneous adj3 (intervent* or revascular*))).ti,ab,kf,kw.
9	or/1-8
10	exp Anti-Inflammatory Agents, Non-Steroidal/
11	(((non-steroidal or nonsteroidal) adj2 (anti-inflammatory or antiinflammatory or analg?esic*)) or NSAID*).ti,ab,kf,kw.
12	(acalabrutinib* or aceclofenac* or acemetacin* or acetaminosalol* or acetylsalicylate* or acetylsalicylic* or aclantate* or actarit* or adalimumab* or afasevikumab* or afimetoran* or alclofenac* or aldafermin* or alminoprofen* or aloxiprin* or amfenac* or aminophenazone* or aminosalicylic* or amlitelimab* or amlodipine* or ampiroxicam* or amtolmetin guacil* or anirolac* or antiflammin* or apadenoson* or apremilast* or araprofen* or ascription* or asivatrep* or astegolimab* or atibuclimab* or atliprofen* or aviptadil* or azathioprine* or azelaic acid* or bakeprofen* or balsalazide* or bardoxolone* or bardoxolone methyl* or belumosudil* or bendazac* or benorilate* or benoxaprofen* or bermoprofen* or bimosiamose* or brazikumab* or brensocatib* or brimonidine* or bromfenac* or broperamole* or bucloxic acid* or bucolome* or bufexamac* or butibufen* or camobucol* or carbasalate* or carotegrast* or carprofen* or cedirogant* or celecoxib* or cibinetide* or cicloprofen* or cimicoxib* or cinmetacin* or cinnoxicam* or clidanac* or clofezone* or clonixin* or clonixin lysine* or cloximate* or crisaborole*).ti,ab,kf,kw.
13	(dagrocorat* or danicopan* or dapansutrile* or dapatifagene navolactibac* or darbufelone* or daxdilimab* or dazodalibep* or dehydrozingerone* or demethoxycurcumin* or deracoxib* or deucravacitinib* or dexibuprofen* or dexketoprofen* or dexpemedolac* or diclofenac* or didemethoxycurcumin* or diflunisal* or diftalone* or dimethyl fumarate* or dimethyl sulfoxide* or diphenpyramide* or ditazole* or droxicam* or duometacin* or ebdarokimab* or ebselen* or edasalonexent* or efruxifermin* or elsibucol* or emavusertib* or emorfazone* or emvododstat* or endolac* or enfenamic acid* or enflicoxib* or enpatoran* or epirizole* or etodolac* or etofenamate* or etoricoxib* or evobrutinib* or felbinac* or fenamic acid* or fenbufen* or fenclofenac* or fenclozic acid* or fendosal* or fenflumizole* or fenoprofen* or fentiazac* or fepradinol* or feprazone* or firategrast* or firocoxib* or flobufen* or flosulide* or flufenamate aluminum* or flufenamic acid* or flunixin* or flunoxaprofen* or fluproquazone* or flurbiprofen*

	or flutiazin* or fosdagrocorat* or fosfosal* or furaprofen* or furcloprofen* or furobufen* or furofenac* or fuzapladib*).ti,ab,kf,kw.
14	(glucametacin* or gluconate zinc* or guacetisal* or guaimesal* or gusacitinib* or hydroxychloroquine* or ibrigampar* or ibufenac* or ibuprofen* or ibuproxam* or icoduline* or icosapentaenoic acid* or iguratimod* or ilonidap* or imidazole salicylate* or imisopasem manganese* or imsidolimab* or incyclinide* or indameth* or indometacin* or indoprofen* or ipsalazide* or iptacopan* or isecarosmab* or isofezolac* or isonixin* or isoxepac* or isoxicam* or itepekimab* or kebuzone* or ketoprofen* or ketoprofen lysine* or ketorolac* or lazertinib* or lazucirnon* or leflunomide* or lenabasum* or licofelone* or lifitegrast* or lirentelimab* or lobuprofen* or lonazolac* or lorecivivint* or lornoxicam* or losmiprofen* or loxoprofen* or lumiracoxib* or lusvertikimab* or lyprinol* or lysine acetylsalicylate*).ti,ab,kf,kw.
15	(mabuprofen* or magnesium salicylate* or manoalide* or mapracorat* or mavacoxib* or meclofenam* or mefenamic acid* or meloxicam* or melrilimab* or mesalazine* or methotrexate* or metiazinic acid* or metoxibutropate* or milategrast* or mipragoside* or mirococept* or miroprofen* or mivavotinib* or mofebutazone* or mofezolac* or mongersen* or morazone* or morniflumate* or mosedipimod* or nabumetone* or nangibotide* or naproxcinod* or naproxen* or navamepent* or nepafenac* or neurofenac* or neurotropin* or nictindole* or niflumic acid* or nimesulide* or ocarocoxib* or odalprofen* or olsalazine* or ordesekimab* or ormeloxifene* or orpanoxin* or otenaproxesul* or oxaceprol* or oxametacin* or oxaprazine* or oxaprozin* or oxicam derivative* or oxindanac* or oxyphenbutazone*).ti,ab,kf,kw.
16	(palifermin* or parcetasal* or parecoxib* or pelubiprofen* or pemedolac* or perisoxal* or phenylbutazone* or phenylbutazone megallate* or picolamine salicylate* or piketoprofen* or pimeprofen* or pipebuzone* or piproxen* or pirazolac* or pirfenidone* or piroxicam* or piroxicam beta cyclodextrin* or pirprofen* or plonmarlimab* or plozalizumab* or polmacoxib* or pralnacasan* or pranoprofen* or prinomide* or prinomide triethanolamine* or proglumetacin* or proquazone* or pyrazinobutazone* or quellor* or rapamycin* or rasagiline* or ravagalimab* or relfovetmab* or reltecimod* or remestemcel L* or resatorvid* or rimacalib* or rimazolium* or risankizumab* or robenacoxib* or rofecoxib* or romazarit* or rosiptor* or rosmarinic acid* or rovazolac* or rozibafusp alfa* or ruxolitinib* or salazosulfapyridine* or salicylic acid* or salnacedin* or salsalate* or satralizumab* or scalaradial* or semapimod* or semorinemab* or sibofimloc* or simufilam* or sudoxicam* or sulfosalicylate samarium* or sulindac* or suprofen* or suxibuzone*).ti,ab,kf,kw.
17	(talniflumate* or tapinarof* or tazofelone* or telazorlimab* or tenidap* or tenosal* or tenosiprol* or tenoxicam* or tepoxalin* or teriflunomide* or tesnatilimab* or tiaprofenic acid* or tiaramide* or tilmacoxib* or tilnoprofen arbamel* or tilomisole* or timegadine* or tioxamast* or tioxaprofen* or tirnovetmab* or tolebrutinib* or tolfenamic acid* or tolmetin* or tomaralimab* or tomicorat* or torudokimab* or tozorakimab* or tralokinumab* or tribuzone* or triethanolamine salicylate* or tropesin* or tryptamide* or ufenamate* or valategrast* or valdecoxib* or valerylsalicylic acid* or vasoactive intestinal polypeptide* or vedaprofen* or velsecorat* or vemircopan* or verramed* or vilobelimab* or vixarelimab* or ximoprofen* or zabedosertib* or zaloglanstat* or zaltoprofen* or zaurategrast* or zidometacin* or zinc salicylate* or zoliprofen* or zomepirac*).ti,ab,kf,kw.
18	exp Colchicine/
19	(Colbenemid* or Colchichin* or colchicum* or colchily* or colchineos* or colchimedio* or colchiquim* or colchisol* or colchysat* or colcin* or colcrys* or colctab* or colgout* or colrefuz* or colsaloid* or Condylon* or gloperba* or goutichine* or goutnil* or kolkicin* or kolkisin* or mitigare* or tolchicine*).ti,ab,kf,kw.

20	(64-86-8* or SML2Y3J35T*).rn.
21	Prednisone/
22	(adasone* or acsis* or ancortone* apo-prednisone* or bicortone* or biocortone* or cartancyl* or colisone* or Cortan* or cortancyl* or cortidelt* or cortiprex* or cotone* or cutason* or dacorten* or dacortin* or decortancyl* or decortin* or de-cortisyl* or decortisyl* or dihydrocortisone* or dihydrocortisone* or dekortin or dellacort* or deltacorten* or delta-cortelan* or delta-cortisone* or deltacortisone* or deltacortone* or delta-dome* or delta-prenovis* or deltasone* or delitisone* or deltison* or deltra* or diadreson* or di-adreson* or drazone* or econosone* or encorton* or encortone* or enkorton* or enkortolon* or fernisone* or fiasone* or hostacortin* or insone* or incocortyl* or juvason* or kortancyl* or liquid pred* or lodotra* or lodtra* or lisacort* or me-korti* or meprison* or metacortandracin* or Meticorten* or meticortine* or nisona* or nizon* or novoprednisone* or nurison* or Orasone* or orisane* or panafcort* or paracort* or panasol* or parmenison* or pehacort* or predeltin* or precort* or precortal* or prednicen* or prednicorm* or prednicort* or prednicot* or predni tablinen* or prednidib* or prednilonga* or predniment* or prednison* or prednitone* or prednizon* or prednovister* or presone* or pronison* or pronizon* or pulmison* or rayos* or rectodelt* or rectrocortine* or servisone* or sone$2 or steerometz* or sterapred* or supercortil* or ultracorten* or urtilone* or winpred* or wojtab* or zenadrid*).ti,ab,kf,kw.
23	(53-03-2 or VB0R961HZT).rn.
24	Methotrexate/
25	(abitextrate* or abitrexate* or amethopterin* or amethopterine* or ametopterine* or antifolan* or biotrexate* or brimexate* or canceren* or emtexate* or emthexat* or emthexate* or emtrexate* or enthexate* or farmitrexat* or farmotrex* or fauldexato* or folex* or ifamet* or imeth$2 or intradose MTX or jylamvo* or lantarel* or ledertrexate* or lumexon* or maxtrex* or medsatrexate* or metatrexan* or metex* or methoblastin* or methohexate* or methotrate* or methotrexat* or methylaminopterin* or metical* or metoject* or metotressato* or metothrexate* or metotrexat* or metotrexin* or metrex* or metrotex* or mexate* or neotrexate* or nordimet* or novatrex* or otrexup* or rasuvo* or reditrex* or reumatrex* or rheumatrex* or texate* or texorate* or tremetex* or trexall* or trexeron* or trixilem* or xaken* or xatmep* or zexate*).ti,ab,kf,kw.
26	(59-05-2 or YL5FZ2Y5U1).rn.
27	Interleukin-1beta/
28	(ACZ-885* or ACZ885* or Canakinumab* or ilaris*).ti,ab,kf,kw.
29	(914613-48-2 or 37CQ2C7X93).rn.
30	Antibodies, Monoclonal, Humanized/
31	(pexelizumab or "h5G1.1-SC*").ti,ab,kf,kw.
32	(219685-93-5 or CHZ6OLQ3UU).rn.
33	Interleukin 1 Receptor Antagonist Protein/
34	(Anakinra* or Antril* or Kineret* or il-1ra* or (interleukin 1 receptor adj1 (antagonist or block* or inhibit*))).ti,ab,kf,kw.
35	(143090-92-0 or 9013DUQ28K).rn.
36	(agi-1067* or agi1067* or agz-1067* or agz1067* or probucol succinate* or Succinobucol*).ti,ab,kf,kw.
37	(216167-82-7 or J1J54V24R4).rn.

38	(ftx-1821* or ftx1821* or gsk-856553* or gsk856553* or gw-856553* or gw856553* or Losmapimod* or sb-856553* or sb856553*).ti,ab,kf,kw.
39	(F2DQF16BXE or 585543-15-3).rn.
40	(lc1004* or lc-1004* or inclacumab* or ro4905417* or ro-4905417*).ti,ab,kf,kw.
41	(1256258-86-2 or A6734I702L).rn.
42	(Atreleuton* or a85761* or a-85761* or abt761* or abt-761* or via-2291* or via2291*).ti,ab,kf,kw.
43	(U301T88E1M or 154355-76-7).rn.
44	(darapladib* or sb-480848* or sb480848*).ti,ab,kf,kw.
45	(356057-34-6 or UI1U1MYH09).rn.
46	(Varespladib* or Varepladib* or ly315920* or ly-315920* or s-5920* or s5920*).ti,ab,kf,kw.
47	(2Q3P98DATH or 172732-68-2).rn.
48	or/10-47
49	randomized controlled trial.pt.
50	controlled clinical trial.pt.
51	randomized.ab.
52	placebo.ab.
53	clinical trials as topic.sh.
54	randomly.ab.
55	trial.ti.
56	49 or 50 or 51 or 52 or 53 or 54 or 55
57	exp animals/ not humans.sh.
58	56 not 57
59	9 and 48 and 58

Section 3.2. Anti-inflammatory therapies to prevent cardiovascular events: systematic review and network meta-analysis of randomised controlled trials

SECTION OVERVIEW

This section presents a manuscript that describes the findings of a systematic review and network meta-analysis of randomised controlled trials to evaluate the comparative efficacy of anti-inflammatory therapies for the prevention of adverse outcomes in patients with coronary artery disease. The protocol listed in **Section 3.1** was followed in order to identify and appraise studies, extract and summarize the data, and assess the certainty of the evidence as well as the confidence in the network analysis results. Literature searching was performed on January 15, 2025.

MANUSCRIPT STATUS

This manuscript has been submitted for publication.

Supporting Material

Appendix 1: Medline search

Appendix 2: Embase search

Appendix 3: Cochrane Central Register of Controlled Trials Search

Appendix 4: ClinicalTrials.Gov Search

Appendix 5: World Health Organization International Clinical Trials Registry Platform (ICTRP) Search

Appendix 6: Europe PMC Search (https://europepmc.org/)

Appendix 7: List of Conferences Hand-searched

Appendix 8: ACS – MACE Evidence Summary Table

Appendix 9. Stable CAD – MACE Evidence Summary Table

Appendix 10. ACS – MACE Evidence Summary Table for Studies with ≥30 Days of Follow Up and ≥30 Days of Treatment

Appendix 11. ACS – MACE Evidence Summary Table for Studies Published 2010 or Later with ≥30 Days of Follow Up and ≥30 Days of Treatment

Appendix 12. Stable CAD – MACE Evidence Summary Table for Studies with ≥30 Days of Follow Up and ≥30 Days of Treatment

Appendix 13. Chronic CAD – MACE Evidence Summary Table for Studies Published 2010 or Later with ≥30 Days of Follow Up and ≥30 Days of Treatment

Appendix 14. Confidence in the results.

Appendix 15. PRISMA Diagram

Appendix 16. Inconsistency plot for stable CAD network. Shows posterior mean deviance of the individual data points from fitted consistency and inconsistency models.

Appendix 17. Inconsistency plot for ACS network. Shows posterior mean deviance of the individual data points from fitted consistency and inconsistency models.

Appendix 18. Secondary outcomes

Author roles and contributions

KEB, RB, and GW designed the stud. SV developed and executed the search strategy. KEB, AP, RH, SS, SS, AG, CF, KAB all assisted with reviewing titles and abstracts, full texts, data extraction, and quality assessment. KEB analyzed the data and wrote the first draft of the manuscript, which was critically reviewed and revised for content by all authors. All authors provided comments and suggestions on the draft and approved the final version of the mauscript for submission.

RELATED THESIS APPENDICES

Appendix A: Abstracts accepted for oral presentation. Findings from Section 3.2 were presented at the American Heart Association Meeting (2023).

Boczar KE, Shin S, Bezzina KA, Geejo A, Pearson AL, Shahab S, Fehlmann CA, Visintini S, Beanlands R, Wells GA. Examining anti-inflammatory therapies in the prevention of cardiovascular events: a systematic review and network meta-analysis of randomised controlled trials. AHA, Philadelphia (October 2023).

Anti-inflammatory therapies to prevent cardiovascular events: systematic review and network meta-analysis of randomised controlled trials

Short title: Anti-inflammatory therapies for cardiovascular protection

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Total Word Count: 5384

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Abstract

Background

Anti-inflammatory therapies have been increasingly investigated for the reduction of cardiovascular (CV) events. The objective of this paper was to summarize and compare the relative effectiveness of anti-inflammatory medications for the reduction of CV events in patients with known coronary artery disease (CAD), either acute coronary syndromes (ACS) or stable CAD.

Methods

Systematic review and network meta-analysis of randomised controlled trials (RCTs) that included at least one anti-inflammatory treatment and involved patients with CAD. Databases searched: Medline, Embase, Cochrane Central Register of Controlled Trials, clinical trial registry websites, Europe PMC, and conference abstracts. Bayesian network meta-analysis was performed to calculate risk estimates using random effects analyses in patients with ACS and stable CAD. Risk of bias assessments were performed using the Cochrane Risk of Bias 2 (RoB2) tool.

Results

15,627 studies were screened; 41 met inclusion criteria. 29,487 patients were included in the ACS network and 41,791 in the stable CAD network. In the ACS network analysis, both non-steroidal anti-inflammatory drugs (OR: 0.30, 95% Credible Limits [CrI]: 0.11-0.74) and colchicine (OR: 0.71, CrI: 0.58-0.88) were associated with a significant reduction in major adverse cardiac events (MACE) compared to control. In the stable CAD analysis, both corticosteroids (OR: 0.44, 95% CrI: 0.26-0.72) and colchicine (OR: 0.65, CrI: 0.54-0.77) were associated with a significant reduction in MACE compared to control.

Conclusions

In patients with ACS, NSAIDs and colchicine were associated with a reduction in MACE. In patients with stable CAD, both colchicine and corticosteroids were associated with a reduction in MACE. Colchicine consistently demonstrated a reduction in MACE regardless of the acuity of coronary disease.

Patient consent statement: The authors confirm that patient consent is not applicable to this article. This is a systematic review and network meta-analysis using de-identified data.

Ethics statement: Our study did not require ethical/IRB approval, as per the standards of our institutions.

Funding sources: none

Disclosures: None of the authors have conflicts of interest to disclose. Dr. Kevin Boczar has received honoraria for advisory board activity for MedTrace, and is PI or site-PI for research grants from MedTrace, Novartis, and Novo Nordisk. He received salary support from the Canadian Institutes of Health Research (FRN: 171284). None of these are considered to pose a conflict of interest for this work.

Main Manuscript

Introduction

CAD is a leading cause of morbidity and mortality worldwide. Great strides have been made in the secondary prevention of CAD, with the goal of mitigating its resultant complications such as myocardial infarction and left ventricular dysfunction. While therapies such as antiplatelets, angiotensin-converting enzyme inhibitors, and medications targeting low-density lipoprotein are well-established parts of routine clinical care, more attention has recently been given to anti-inflammatory therapies.[1,2]

Inflammation has long been recognized to play an integral role in the pathogenesis of atherosclerotic disease. However, it has only been in recent years that inflammation has been regarded as a therapeutic target.[3] Recent guidelines (e.g. Canadian, European, and South American)[4,5,6] now recommend using low-dose colchicine for secondary prevention of cardiovascular events in patients with CAD. Furthermore, numerous trials have shown varying levels of CV benefit using therapies to target several aspects of the inflammatory cascade.

While some anti-inflammatory therapies have demonstrated effectiveness relative to placebo in randomized controlled trials, the relative efficacy of these therapies compared to each other is unclear. Therefore, the objective of this study was to compare anti-inflammatory agents for the secondary prevention of CV disease in patients with known CAD, both in the ACS setting and the stable CAD setting, in terms of their relative effectiveness and side effects.

Methods

This review is reported according to PRISMA-NMA guidelines.[7]

A peer-reviewed search was conducted on January 15, 2025 in MEDLINE, Embase, and the Cochrane Central Register of Controlled Trials via Ovid (Supplementary Tables 1-3).[8] No limits to language or publication date were applied. However, RCT search filters were applied in Medline and Embase.[9] Regular alerts were also set up for Medline. The protocol was published and registered in the PROSPERO International Prospective Register of Systematic Reviews (CRD42022303289).[10]

Grey literature searching included searches of clinical trial registries, a search of Europe PMC for pre-prints, and hand-searching of select conferences for any abstracts not already indexed in Embase (Supplementary Tables 4-7).

Search results were exported to Covidence (Melbourne, Australia) and duplicates were eliminated using the platform's duplicate identification feature.

The population that we studied were individuals with coronary artery disease (both ACS and stable CAD). The following anti-inflammatory treatments were included: corticosteroids, methotrexate, tocilizumab, anakinra, canakinumab, pexelizumab, colchicine, NSAIDs, EA-230, LeukArrest, darapladib, varespladib, losmapimod, succinobucol, and inclacumab.

RCTs with either active- or placebo-control groups were included if they evaluated at least one anti-inflammatory drug under review, reported data for MACE, or any of its subcomponents, and enrolled patients with known coronary artery disease. We included both published and yet-to-be-published research (e.g., posters). Two reviewers independently reviewed the abstracts and full texts of retrieved articles to ensure they met inclusion criteria. For the included articles, data was independently extracted by two reviewers in parallel. Disagreements were resolved by a third investigator. Risk of bias assessment of RCTs was performed independently by two reviewers using the Cochrane Risk of Bias 2 (RoB2) tool.[11]

Bayesian network meta-analysis was performed utilizing WinBUGS software (MRC Biostatistics Unit, Cambridge UK). We used a binomial likelihood model as it accounts for multi-arm trials within the evidence network. A fixed effects network meta-analysis was utilized, as most of the evidence network consisted of single study connections. We modelled OR point estimates as well as 95% credible intervals using Markov Chain Monte Carlo methods. We also calculated the probability that each treatment was the most efficacious agent, the second best, the third best, and so on.[12, 13] Vague priors were used for basic parameters throughout. We assessed model fit based on the deviance information criterion (DIC) and compared the residual deviance to the number of unconstrained data points.[14] We ensured convergence of the models were reached by examining trace plots and the Brooks-Gelman-Rubin statistic.[12]

Three chains were fitted in WinBUGS for each analysis with at least 40 000 iterations and a burn-in of at least 40 000 iterations. A key assumption with network meta-analysis is that there is no conflict between direct and indirect evidence, termed consistency.[15] We assessed consistency

within the model by comparing deviance and DIC statistics in fitted consistency and inconsistency models.[12, 15] We also plotted the posterior mean deviance of the individual study data points in the inconsistency model against the study data points from the consistency model to identify potential loops where inconsistency was present.[12, 15]

To assess whether trials were comparable within a network meta-analysis we examined the baseline patient characteristics to ensure similarity. We found that baseline rates of MACE were higher in trials looking at populations involving ACS relative to stable CAD, so the decision was made to create these networks separately. The WinBUGS code and data needed to replicate our analyses are available online.[14]

We conducted two sensitivity analyses. Network meta-analyses were repeated with 1) only studies that had at least 30 days of treatment and 30 days of follow-up included, and 2) only studies that had at least 30 days of treatment, 30 days of follow-up, and that were published 2010 or later. Both sensitivity analyses were performed for the ACS and stable CAD networks separately.

We used the CINeMA approach to evaluate the credibility of our results.[16]

Results

Our trial selection process is illustrated in a flow chart based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[7] statement (Supplementary Figure 1).

A total of 17,021 studies were identified, and 41 RCTs met the eligibility criteria for inclusion. Within the ACS network for MACE, there were 21 studies included; trials evaluated the efficacy for the reduction of MACE in NSAIDs (1 study[17]), tocilizumab (2 studies[18,19]), colchicine (10 studies[20-29]), anakinra (2 studies[30,31]), darapladib (1 study[32]), LeukArrest (high and low dose; 1 study[33]), losmapimod (high and low dose; 2 studies[34,35]), and varespladib (2 studies[36,37]). Most of the trials in the ACS network for MACE were published (20 studies) with 1 in poster format[26]. Within the stable CAD network for MACE, there were 20 studies included; trials evaluated the efficacy for the reduction of MACE in corticosteroids (3 trials[38-40]), varespladib (2 studies[41,42]), NSAIDs (3 studies[43-45]), inclacumab (1 study[46]), canakinumab (2 studies[47,48]), colchicine (3 studies[22, 49, 50]), EA-230 (1 study[51]), darapladib (2 studies[52,53]), methotrexate (1 study[54]),

pexelizumab (1 study[55]), and succinobucol (1 study[56]). All trials in the stable CAD network for MACE were in published format.

The NSAIDs and doses tested were: meloxicam 15 mg daily (for admission and 30 days following discharge[17]), celecoxib 400 mg before percutaneous angioplasty followed by 200 mg twice daily (for 3-6 months[43,44]), or parecoxib/valdecoxib 40 mg every 12 hours (for 14 days[41]). The corticosteroids and doses used were: prednisone 1 mg/kg for 10-15 days followed by taper (0.5 mg/kg for 15-20 more days, then 0.25 mg/kg for 10-15 days[38,40]), methylprednisolone 125 mg intramuscularly night before and morning of percutaneous angioplasty followed by prednisone 60 mg oral daily for one week[39]. Colchicine was dosed at 0.5 mg daily (with a 2 mg loading dose[23] or without a loading dose[21, 25, 49, 50]), 1 mg daily[20, 24], or 1.8 mg once pre-procedure[22].

Bayesian network meta-analysis was performed to calculate risk estimates using random effects analyses. There were 71,278 patients included in our analyses, with 41,791 patients included in the stable CAD network analysis and 29,487 patients included in the ACS network analysis. In the ACS analysis, colchicine (OR: 0.77, 95% Credible Limits [CrI]: 0.62-0.95) and NSAID use (OR: 0.30, 95% CrI: 0.11-0.74) were associated with a significant reduction in MACE. In the stable CAD analysis, colchicine (OR: 0.65, CrI: 0.54-0.77) and corticosteroid use (OR: 0.44, 95% CrI): 0.26-0.72) were associated with a significant reduction in MACE. Most trials reported MACE as a primary outcome. Follow-up of trials reporting the primary outcome of MACE varied from 30 days to 4 years. Follow-up of trials reporting any outcome varied from 48 hours to 4 years. Trials were published between the years of 1989 to 2021.

Mean age across the included trials varied from 57 to 73 years. All trials included patients of both sexes and most had a higher proportion of male participants. The studies were individually critically appraised. Overall, there was significant variation in study quality. The larger trials comprising the bulk of patients included in the systematic review were judged to be methodologically sound.

Primary Outcome: Major Adverse Cardiac Events (MACE)

Acute Coronary Syndromes (ACS) Network

The evidence network (Figure 1) for the primary analysis of the ACS group was comprised of 18 RCTs representing 10 anti-inflammatory interventions in addition to placebo/control (n=29,487). The posterior mean residual deviance (67.68) was somewhat close to the number of unconstrained data points (55), which is an indication of moderate model fit.

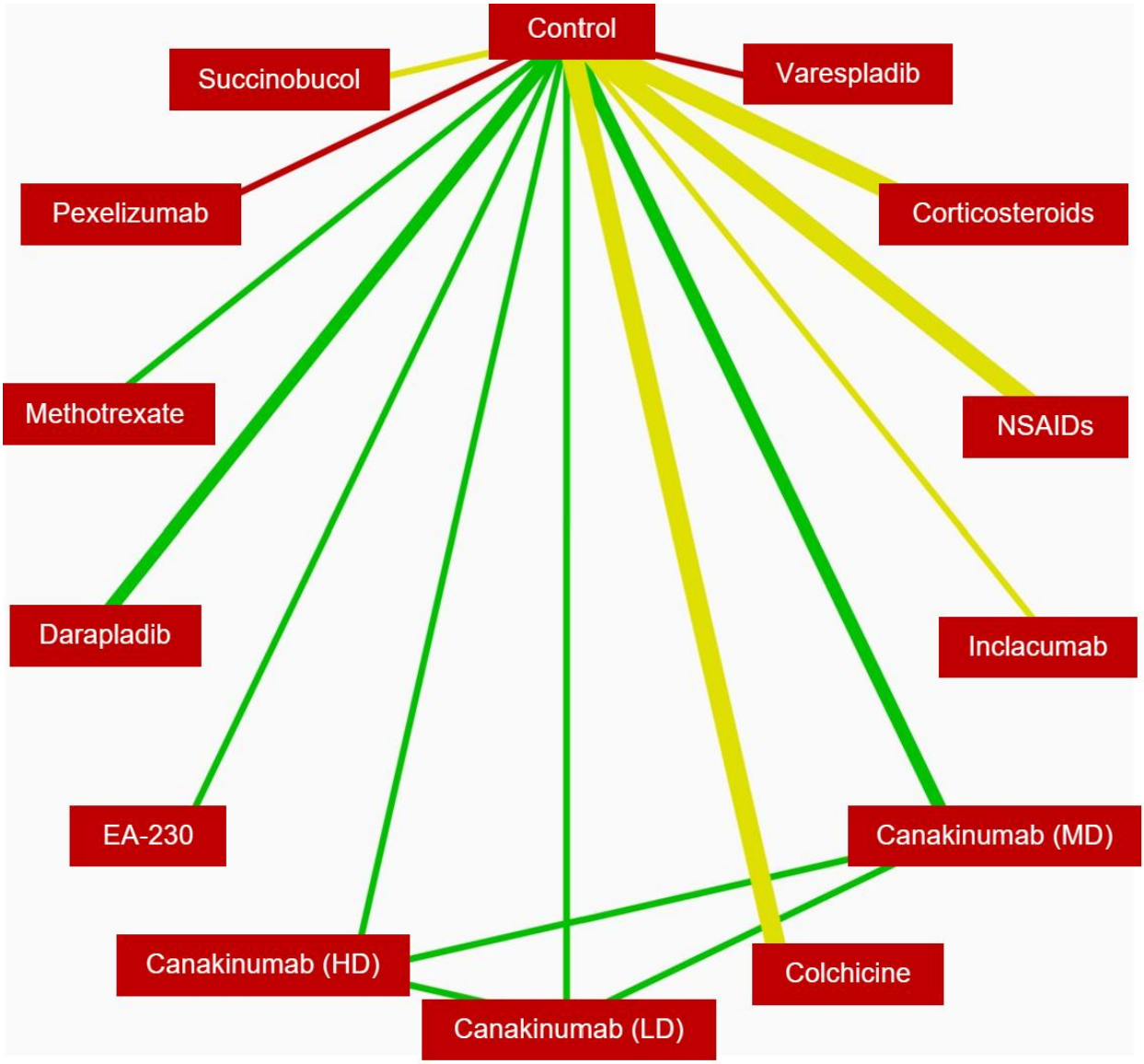

Figure 1. Evidence network for reduction of MACE in patients with ACS. The width of the lines is proportional to the number of randomised controlled trials comparing each pair of treatments, and the color of each line represents the average risk of bias (RoB) of the trials comparing each pair of treatments (red is high RoB, yellow is moderate RoB, green is low RoB). MACE = major adverse cardiac events, ACS = acute coronary syndrome, RoB = risk of bias

NSAIDs and colchicine were associated with a reduction in MACE relative to placebo/control (Figure 2). No differences in MACE were detected between standard therapy and each of the other anti-inflammatory therapies.

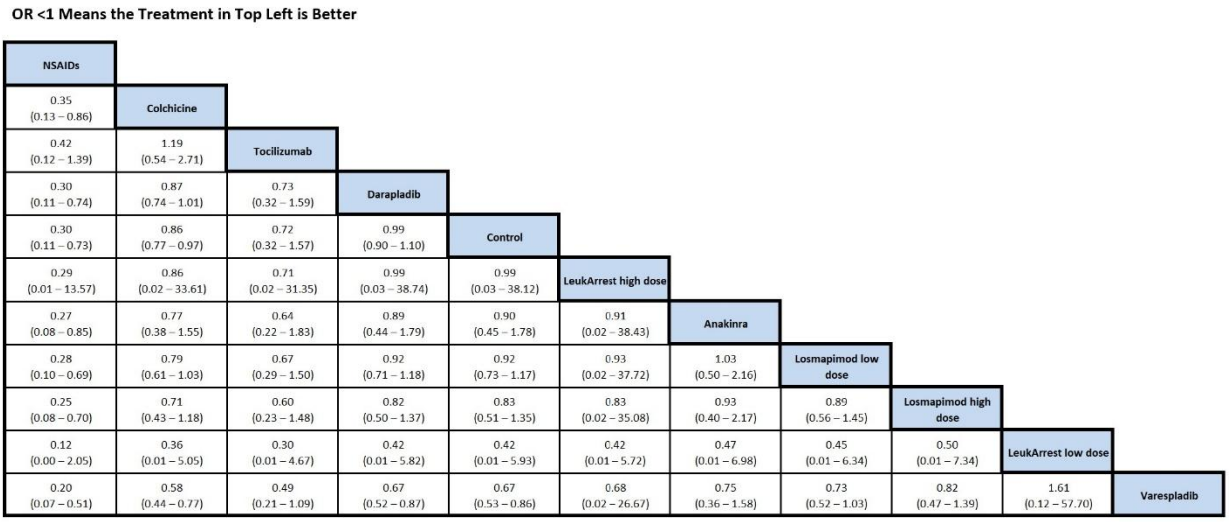

Figure 2. OR from network meta-analysis for MACE comparison in ACS network. Results between individual treatments should be interpreted with caution given the limitations associated with using a fixed-effects model. See Supplementary Table 8 for additional details. MACE = major adverse cardiac events, ACS = acute coronary syndrome, OR = odds ratio

Stable CAD Network

The evidence network (Figure 3) for the primary analysis for the stable coronary artery disease analysis was comprised of 19 RCTs representing 14 anti-inflammatory interventions in addition to placebo/control (n=41,791). The posterior mean residual deviance (61.8) is less than the number of unconstrained data points (105), which is an indication of good model fit.

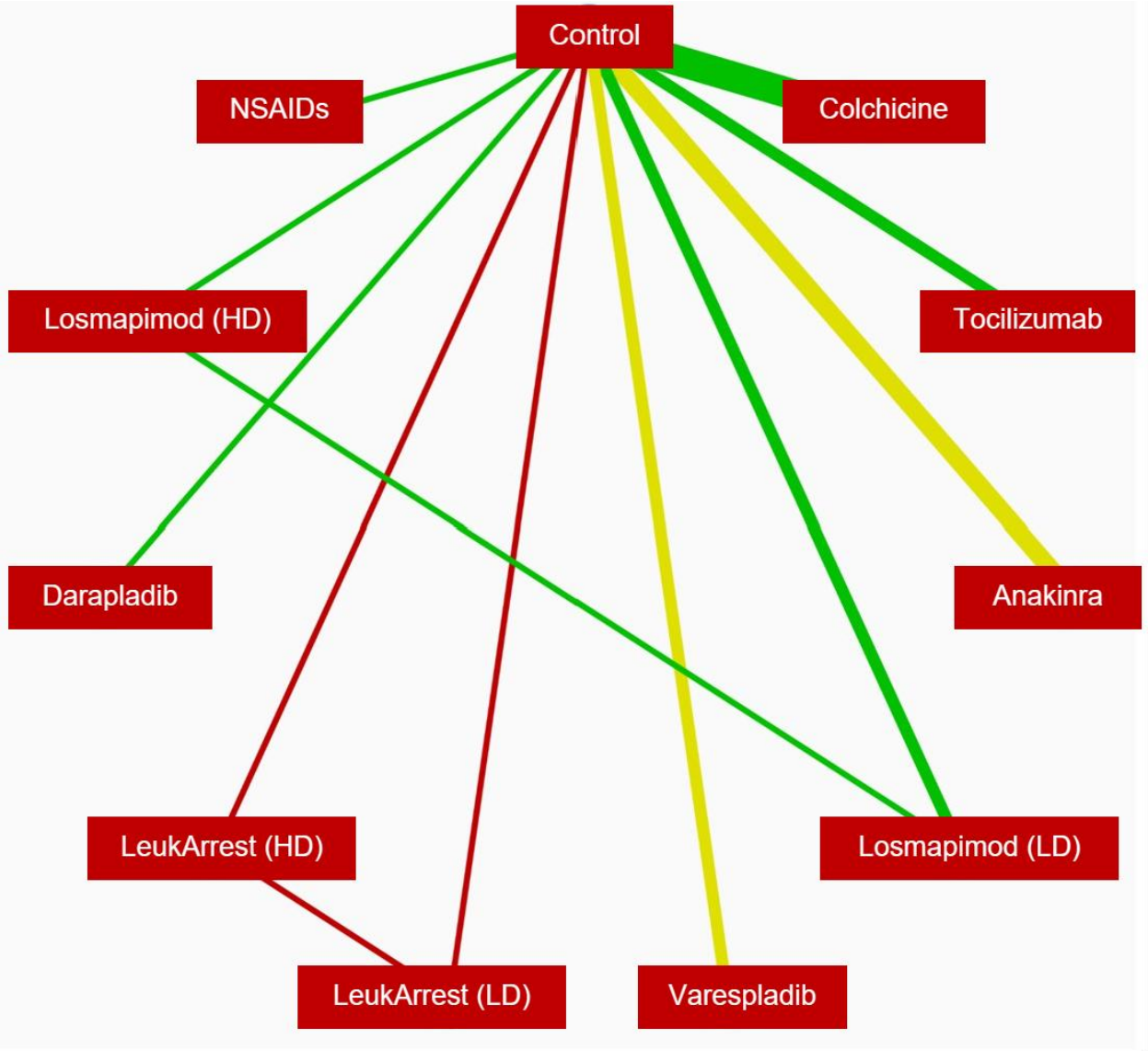

Figure 3. Evidence network for reduction of MACE in patients with stable CAD. The width of the lines is proportional to the number of randomised controlled trials comparing each pair of treatments, and the color of each line represents the average risk of bias (RoB) of the trials comparing each pair of treatments (red is high RoB, yellow is moderate RoB, green is low RoB). MACE = major adverse cardiac events, CAD = coronary artery disease, RoB = risk of bias

Both colchicine and corticosteroids were associated with a reduction in MACE relative to placebo/control (Figure 4). No differences in MACE were detected between standard therapy and each of the other anti-inflammatory therapies.

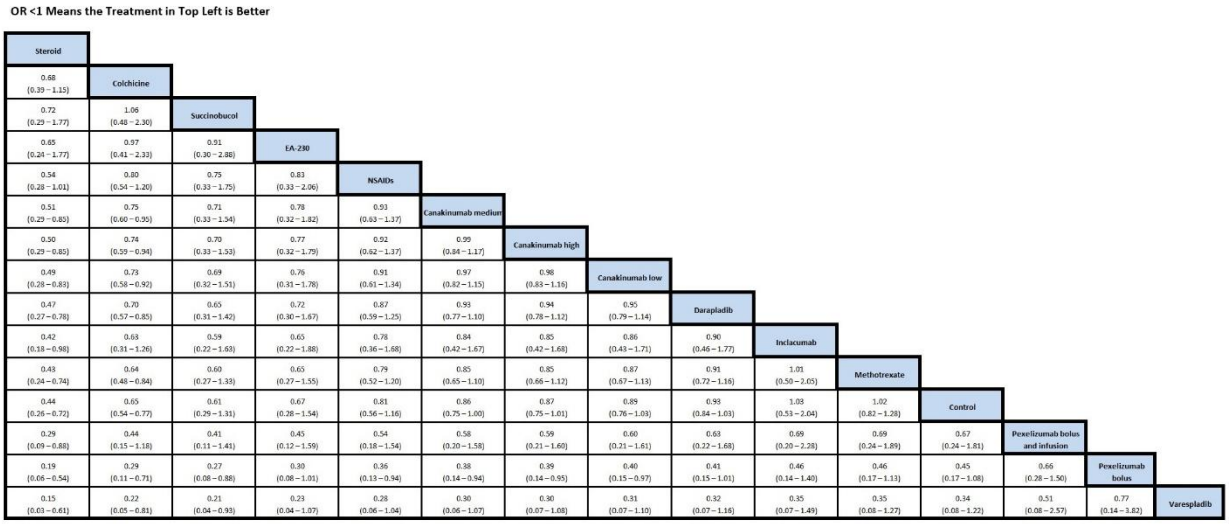

Figure 4. OR from network meta-analysis for MACE comparison in stable CAD network. Results between individual treatments should be interpreted with caution given the limitations associated with using a fixed-effects model. See Supplementary Table 9 for additional details. MACE = major adverse cardiac events, CAD = coronary artery disease, OR = odds ratio

For both the ACS and stable CAD networks, estimates that were derived from the direct pairwise comparison of treatments were similar to those obtained in indirect comparisons. Additionally, comparison of the posterior mean deviance of the individual data points from fitted consistency and inconsistency models revealed no evidence of loops within the treatment network where inconsistency could be present in both the stable CAD and ACS networks, respectively.

Confidence in the Results

The CINeMA approach was used to assess the confidence in the results of this network meta-analysis.[16] The full results are included in Supplementary Table 14. In both the ACS network and stable CAD network, the overall confidence in the results for each comparison made within the network was generally rated as either low or very low. This was largely secondary to the presence of significant imprecision and significant heterogeneity between included studies.

Sensitivity analyses

ACS Network

In the sensitivity analysis for the ACS network considering studies with ≥30 days of follow up and ≥30 days of treatment (12 studies, 35,286 patients; see Supplementary Table 10), only NSAID use was associated with a significant reduction in MACE (OR: 0.28, 95% CrI: 0.10-0.70). When further restricting to studies published 2010 or later (11 studies, 35,166 patients; see Supplementary Table 11), the one study evaluating NSAID use was excluded (Atman 2002), and only colchicine was associated with a significant reduction in MACE.

Stable CAD Network

In the sensitivity analysis for the stable CAD network, there were 14 studies with ≥30 days of follow up and ≥30 days of treatment including 38,959 patients (Supplementary Table 12). Corticosteroids (OR: 0.37, 95% CrI: 0.21-0.65) and colchicine (OR: 0.63, 95% CrI: 0.52-0.76), were associated with a significant reduction in MACE. When restricting to studies published 2010 or later (11 studies with 38,088 patients; see Supplementary Table 13), corticosteroids (OR: 0.37, 95% CrI: 0.21-0.65) and colchicine (OR: 0.63, 95% CrI: 0.52-0.76), remained significantly associated with reduction in MACE.

Discussion

We identified 41 RCTs comparing anti-inflammatory agents for the prevention of MACE in patients with CAD. Of these, 18 RCTs were included in the ACS network analysis and 19 RCTs were included in the stable CAD network. To the best of our knowledge, this is the most up-to-date and complete systematic review and network meta-analysis to evaluate the currently available evidence and summarize the benefits and harms of anti-inflammatory therapies in the prevention of MACE in patients with known CAD. Our findings align with prior reports on the efficacy of colchicine for this indication, but this analysis expands upon that knowledge and allows for the comparison of colchicine to other anti-inflammatory therapies. We have also provided detailed evidence networks[80] which allow for the visualization of available evidence on this topic. We have provided a summary of the evidence for anti-inflammatory therapies for patients in both the ACS setting and the stable CAD setting.

In the ACS setting, NSAIDs and colchicine were associated with reductions of MACE relative to standard of care (no anti-inflammatory therapies). Conversely, Varespladib was associated with an increased risk of MACE compared to standard of care. In the stable CAD setting, both corticosteroids and colchicine were associated with a reduction in MACE compared to standard

of care. None of the investigated anti-inflammatory therapies were associated with an increased risk of MACE relative to the standard of care in the stable CAD setting. Of note, in the sensitivity analyses for the ACS network, only colchicine use remained significantly associated with a reduction in MACE when limiting to studies with a minimum of 30 days of treatment and 30 days of follow-up. For stable CAD, in the sensitivity analysis, colchicine and corticosteroid use remained associated with a significant reduction in MACE.

Adverse therapy effects are discussed in further detail in the secondary outcomes section, in the supplementary material. To further elaborate, prednisone was not observed to result in increased rates of gastrointestinal bleeding, clinically significant glucose intolerance, or increased risk of infection. Meloxicam use was not associated with an increased risk of bleeding or impairment of the cardiovascular, pulmonary, renal, or autonomic nervous systems. Celecoxib was not associated with an increased risk of bleeding, but some patients discontinued it due to gastrointestinal discomfort, skin rashes, pruritis, or headache. Regarding colchicine, a variable proportion of patients complained of gastrointestinal intolerance due to diarrhea, nausea, or vomiting. As with any treatment, physicians should counsel their patients about the expected benefits and harms of potential therapies and weigh them when making decisions.

Guidelines have increasingly recommended colchicine for the secondary prevention of atherosclerotic cardiovascular disease. In both the ACS and stable CAD networks, colchicine demonstrated significant reductions in MACE compared to control (ACS: OR 0.71, 95% CrI 0.58-0.88; Stable CAD: OR 0.65, 95% CrI 0.54-0.77). Interestingly the European and South American CV prevention guidelines have endorsed colchicine as an anti-inflammatory therapy for the prevention of MACE in adult patients with CAD.[5,6] Likewise, both the FDA and Health Canada have followed suit, and both have endorsed colchicine for use in adult patients with known CAD for the prevention of MACE.[4] No other anti-inflammatory therapies have been recommended for the prevention of cardiovascular disease yet. However, the clinical use of anti-inflammatory therapies has lagged behind guideline recommendations. While our analysis identified potential benefits from other agents like NSAIDs in ACS (OR 0.30, 95% CrI 0.11-0.74) and corticosteroids in stable CAD (OR 0.44, 95% CrI 0.26-0.70), these results should be interpreted with caution. It is our hope that this study will inform further research and into the use of these, and other anti-inflammatory agents, for the secondary prevention of cardiovascular disease in patients with known CAD.

The present analysis must be interpreted in the context of historical data on anti-inflammatory agents. While our findings suggest potential benefits with certain agents like colchicine, it's crucial to acknowledge the uncertainty and even documented harm associated with other anti-inflammatory strategies in cardiovascular disease. Early enthusiasm for selective COX-2 inhibitors was tempered by evidence of increased MACE. More broadly, non-aspirin NSAIDs as a class have been linked to increased cardiovascular risk, with strong recommendations against their use in patients with established or high risk for CVD.[81] This context highlights the importance of cautious interpretation and the need for rigorous safety assessments of any anti-inflammatory agent considered for long-term cardiovascular protection. Our findings suggest colchicine as a promising agent with sufficient evidence to support its use both in acute and stable CAD settings. Future research should prioritize identifying patient subgroups who are most likely to benefit from anti-inflammatory therapies while minimizing potential harms.

Limitations

There are several limitations to our analysis. Firstly, we used a fixed-effects model for analysis. This was done as the evidence networks were primarily connected by single studies and random-effects models yielded inappropriately high credible limits. However, using fixed-effects models will likely bias the results in favour of the anti-inflammatory therapies. This is due to the fact that anti-inflammatory therapies that demonstrated statistically significant findings in the original trial will retain statistical significance when incorporated in a fixed-effects model which is not necessarily true with a random-effects model.[80] Secondly, there was considerable variability in the length of follow-up of the studies. Studies with a longer length of follow-up would be more likely to detect differences in rates of events between treatment groups, if a difference did exist. Thirdly, many of the studies included in the study had a small sample size (less than 200 patients). Overestimation of treatment effect because of a small sample size should not be overlooked. These limitations should caution the use of the present study’s findings for informing the role of anti-inflammatory agents for the secondary prevention of cardiovascular disease in patients with known CAD.

A majority of the data for corticosteroids was in patients who received bare-metal stents (BMS). A benefit may have been observed in this population because the corticosteroids could have affected the rates of in-stent restenosis. However, drug-eluting stents (DES) are predominantly

being used at the time of this study. Future studies could consider re-examining the utility of corticosteroids in patients with DES.

Furthermore, another concern is publication bias, which occurs when studies with significant or positive findings are more likely to be published than those with non-significant or negative results. As a result, the evidence base available for analysis may overrepresent favorable outcomes, skewing the results and leading to erroneous conclusions. Furthermore, excluding unpublished or less accessible studies might make the findings less representative of real-world outcomes. Consequently, the review could favor interventions that seem effective only in select populations or under specific conditions. Moreover, underreporting of adverse events in unpublished studies can underestimate risks associated with certain interventions. Particular to network meta-analyses, publication bias can result in fewer or no connections for interventions with less favorable results. This distorts the relative ranking of treatments, as comparisons may favor interventions with disproportionately positive evidence. In addition, network meta-analyses combine direct and indirect evidence. If publication bias affects the direct evidence, it propagates through the network and compromises indirect comparisons, leading to biased treatment rankings. Publication bias was combatted in our study by searching for unpublished studies, conference abstracts, trial registries, and grey literature.

Finally, network meta-analyses are most valuable when comparing multiple active treatments across different studies. In the present study, most comparisons are between a single anti-inflammatory agent and placebo/control, and only two anti-inflammatory drugs of limited clinical relevance in cardiology have dosing studies that create actual network connections (Anakinra (high and low dose) and Losmapimod (high and low dose)). However, this study provides a comprehensive overview of anti-inflammatory agent treatments in CAD patients and provides hypothesis generating findings to inform future research in the field.

Conclusions

After sensitivity analyses, compared with standard of care, only colchicine was associated with reductions in MACE in patients with ACS, whereas varespladib increased the risk relative to standard of care. In patients with stable CAD, corticosteroids and colchicine were associated with a reduction in MACE compared to standard of care. The results of the current review suggest that anti-inflammatory therapies have relative benefit in patients with CAD. Comparative

studies or network meta-regression analyses of patient-level data are now needed to further evaluate the comparative risks and harms of these anti-inflammatory agents in these populations.

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Appendix 1. Medline Search

Database: Medline Search (Ovid MEDLINE® ALL 1946 to February 17, 2022) Platform: Ovid Date Searched: February 18, 2022		
#	**Searches**	**Results**
1	exp Arteriosclerosis/ or Plaque, Atherosclerotic/	197274
2	(atherosclero* or athero-sclero* or arteriosclero* or arterial-sclero* or arteriolosclero* or arteriolo-sclero* or vascular sclero* or ASCVD or ASCAD or intima plaque).ti,ab,kf,kw.	184426
3	Acute Coronary Syndrome/ or exp Coronary Disease/	243706
4	((coronary adj3 (arter* or stenos* or disease? or disorder? or syndrom?)) or CAD or SCAD).ti,ab,kf,kw.	328530
5	exp Percutaneous Coronary Intervention/ or exp Myocardial Revascularization/	116164
6	(((aortocoronary or aorto-coronary or coronary) adj3 bypass*) or CABG).ti,ab,kf,kw.	57086
7	exp endarterectomy/ or exp thrombectomy/ or exp Atherectomy/ or exp Embolectomy/	30081
8	(angioplast* or atherectom* or endarterectom* or thrombectom* or thromboendarterectom* or thrombo-endarterectom* or PCI or PTCA or (Percutaneous adj3 (intervent* or revascular*))).ti,ab,kf,kw.	127048
9	or/1-8	704058
10	exp Anti-Inflammatory Agents, Non-Steroidal/	208246
11	(((non-steroidal or nonsteroidal) adj2 (anti-inflammatory or antiinflammatory or analg?esic*)) or NSAID*).ti,ab,kf,kw.	50432
12	(acalabrutinib* or aceclofenac* or acemetacin* or acetaminosalol* or acetylsalicylate* or acetylsalicylic* or aclantate* or actarit* or adalimumab* or afasevikumab* or afimetoran* or alclofenac* or aldafermin* or alminoprofen* or aloxiprin* or amfenac* or aminophenazone* or aminosalicylic* or amlitelimab* or amlodipine* or ampiroxicam* or amtolmetin guacil* or anirolac* or antiflammin* or apadenoson* or apremilast* or araprofen* or ascription* or asivatrep* or astegolimab* or atibuclimab* or atliprofen* or aviptadil* or azathioprine* or azelaic acid* or bakeprofen* or balsalazide* or bardoxolone* or bardoxolone methyl* or belumosudil* or bendazac* or benorilate* or benoxaprofen* or bermoprofen* or bimosiamose* or brazikumab* or brensocatib* or brimonidine* or bromfenac* or broperamole* or bucloxic acid* or bucolome* or bufexamac* or butibufen* or camobucol* or carbasalate* or carotegrast* or carprofen* or cedirogant* or celecoxib* or cibinetide* or ciclopROfen* or cimicoxib* or cinmetacin* or cinnoxicam* or clidanac* or clofezone* or clonixin* or clonixin lysine* or cloximate* or crisaborole*).ti,ab,kf,kw.	59208
13	(dagrocorat* or danicopan* or dapansutrile* or dapatifagene navolactibac* or darbufelone* or daxdilimab* or dazodalibep* or dehydrozingerone* or demethoxycurcumin* or deracoxib* or deucravacitinib* or dexibuprofen* or dexketoprofen* or dexpemedolac* or diclofenac* or didemethoxycurcumin* or diflunisal* or diftalone* or dimethyl fumarate* or dimethyl sulfoxide* or	36777

	diphenpyramide* or ditazole* or droxicam* or duometacin* or ebdarokimab* or ebselen* or edasalonexent* or efruxifermin* or elsibucol* or emavusertib* or emorfazone* or emvododstat* or endolac* or enfenamic acid* or enflicoxib* or enpatoran* or epirizole* or etodolac* or etofenamate* or etoricoxib* or evobrutinib* or felbinac* or fenamic acid* or fenbufen* or fenclofenac* or fenclozic acid* or fendosal* or fenflumizole* or fenoprofen* or fentiazac* or fepradinol* or feprazone* or firategrast* or firocoxib* or flobufen* or flosulide* or flufenamate aluminum* or flufenamic acid* or flunixin* or flunoxaprofen* or fluproquazone* or flurbiprofen* or flutiazin* or fosdagrocorat* or fosfosal* or furaprofen* or furcloprofen* or furobufen* or furofenac* or fuzapladib*).ti,ab,kf,kw.	
14	(glucametacin* or gluconate zinc* or guacetisal* or guaimesal* or gusacitinib* or hydroxychloroquine* or ibrigampar* or ibufenac* or ibuprofen* or ibuproxam* or icoduline* or icosapentaenoic acid* or iguratimod* or ilonidap* or imidazole salicylate* or imisopasem manganese* or imsidolimab* or incyclinide* or indameth* or indometacin* or indoprofen* or ipsalazide* or iptacopan* or isecarosmab* or isofezolac* or isonixin* or isoxepac* or isoxicam* or itepekimab* or kebuzone* or ketoprofen* or ketoprofen lysine* or ketorolac* or lazertinib* or lazucirnon* or leflunomide* or lenabasum* or licofelone* or lifitegrast* or lirentelimab* or lobuprofen* or lonazolac* or lorecivivint* or lornoxicam* or losmiprofen* or loxoprofen* or lumiracoxib* or lusvertikimab* or lyprinol* or lysine acetylsalicylate*).ti,ab,kf,kw.	33747
15	(mabuprofen* or magnesium salicylate* or manoalide* or mapracorat* or mavacoxib* or meclofenam* or mefenamic acid* or meloxicam* or melrilimab* or mesalazine* or methotrexate* or metiazinic acid* or metoxibutropate* or milategrast* or mipragoside* or mirococept* or miroprofen* or mivavotinib* or mofebutazone* or mofezolac* or mongersen* or morazone* or morniflumate* or mosedipimod* or nabumetone* or nangibotide* or naproxcinod* or naproxen* or navamepent* or nepafenac* or neurofenac* or neurotropin* or nictindole* or niflumic acid* or nimesulide* or ocarocoxib* or odalprofen* or olsalazine* or ordesekimab* or ormeloxifene* or orpanoxin* or otenaproxesul* or oxaceprol* or oxametacin* or oxaprazine* or oxaprozin* or oxicam derivative* or oxindanac* or oxyphenbutazone*).ti,ab,kf,kw.	62386
16	(palifermin* or parcetasal* or parecoxib* or pelubiprofen* or pemedolac* or perisoxal* or phenylbutazone* or phenylbutazone megallate* or picolamine salicylate* or piketoprofen* or pimeprofen* or pipebuzone* or piproxen* or pirazolac* or pirfenidone* or piroxicam* or piroxicam beta cyclodextrin* or pirprofen* or plonmarlimab* or plozalizumab* or polmacoxib* or pralnacasan* or pranoprofen* or prinomide* or prinomide triethanolamine* or proglumetacin* or proquazone* or pyrazinobutazone* or quellor* or rapamycin* or rasagiline* or ravagalimab* or relfovetmab* or reltecimod* or remestemcel L* or resatorvid* or rimacalib* or rimazolium* or risankizumab* or robenacoxib* or rofecoxib* or romazarit* or rosiptor* or rosmarinic acid* or rovazolac* or rozibafusp alfa* or ruxolitinib* or salazosulfapyridine* or salicylic acid* or salnacedin* or salsalate* or satralizumab* or scalaradial* or semapimod* or semorinemab* or sibofimloc*	67671

	or simufilam* or sudoxicam* or sulfosalicylate samarium* or sulindac* or suprofen* or suxibuzone*).ti,ab,kf,kw.	
17	(talniflumate* or tapinarof* or tazofelone* or telazorlimab* or tenidap* or tenosal* or tenosiprol* or tenoxicam* or tepoxalin* or teriflunomide* or tesnatilimab* or tiaprofenic acid* or tiaramide* or tilmacoxib* or tilnoprofen arbamel* or tilomisole* or timegadine* or tioxamast* or tioxaprofen* or tirnovetmab* or tolebrutinib* or tolfenamic acid* or tolmetin* or tomaralimab* or tomicorat* or torudokimab* or tozorakimab* or tralokinumab* or tribuzone* or triethanolamine salicylate* or tropesin* or tryptamide* or ufenamate* or valategrast* or valdecoxib* or valerylsalicylic acid* or vasoactive intestinal polypeptide* or vedaprofen* or velsecorat* or vemircopan* or verramed* or vilobelimab* or vixarelimab* or ximoprofen* or zabedosertib* or zaloglanstat* or zaltoprofen* or zaurategrast* or zidometacin* or zinc salicylate* or zoliprofen* or zomepirac*).ti,ab,kf,kw.	8947
18	exp Colchicine/	15610
19	(Colbenemid* or Colchichin* or colchicum* or colchily* or colchineos* or colchimedio* or colchiquim* or colchisol* or colchysat* or colcin* or colcrys* or colctab* or colgout* or colrefuz* or colsaloid* or Condylon* or gloperba* or goutichine* or goutnil* or kolkicin* or kolkisin* or mitigare* or tolchicine*).ti,ab,kf,kw.	340
20	(64-86-8* or SML2Y3J35T*).rn.	14615
21	Prednisone/	40606
22	(adasone* or acsis* or ancortone* apo-prednisone* or bicortone* or biocortone* or cartancyl* or colisone* or Cortan* or cortancyl* or cortidelt* or cortiprex* or cotone* or cutason* or dacorten* or dacortin* or decortancyl* or decortin* or de-cortisyl* or decortisyl* or dihydrocortisone* or dihydrocortisone* or dekortin or dellacort* or deltacorten* or delta-cortelan* or delta-cortisone* or deltacortisone* or deltacortone* or delta-dome* or delta-prenovis* or deltasone* or delitisone* or deltison* or deltra* or diadreson* or di-adreson* or drazone* or econosone* or encorton* or encortone* or enkorton* or enkortolon* or fernisone* or fiasone* or hostacortin* or insone* or incocortyl* or juvason* or kortancyl* or liquid pred* or lodotra* or lodtra* or lisacort* or me-korti* or meprison* or metacortandracin* or Meticorten* or meticortine* or nisona* or nizon* or novoprednisone* or nurison* or Orasone* or orisane* or panafcort* or paracort* or panasol* or parmenison* or pehacort* or predeltin* or precort* or precortal* or prednicen* or prednicorm* or prednicort* or prednicot* or predni tablinen* or prednidib* or prednilonga* or predniment* or prednison* or prednitone* or prednizon* or prednovister* or presone* or pronison* or pronizon* or pulmison* or rayos* or rectodelt* or rectrocortine* or servisone* or sone$2 or steerometz* or sterapred* or supercortil* or ultracorten* or urtilone* or winpred* or wojtab* or zenadrid*).ti,ab,kf,kw.	32967
23	(53-03-2 or VB0R961HZT).rn.	40606
24	Methotrexate/	39859
25	(abitextrate* or abitrexate* or amethopterin* or amethopterine* or ametopterine* or antifolan* or biotrexate* or brimexate* or canceren* or emtexate* or emthexat*	45598

	or emthexate* or emtrexate* or enthexate* or farmitrexat* or farmotrex* or fauldexato* or folex* or ifamet* or imeth$2 or intradose MTX or jylamvo* or lantarel* or ledertrexate* or lumexon* or maxtrex* or medsatrexate* or metatrexan* or metex* or methoblastin* or methohexate* or methotrate* or methotrexat* or methylaminopterin* or metical* or metoject* or metotressato* or metothrexate* or metotrexat* or metotrexin* or metrex* or metrotex* or mexate* or neotrexate* or nordimet* or novatrex* or otrexup* or rasuvo* or reditrex* or reumatrex* or rheumatrex* or texate* or texorate* or tremetex* or trexall* or trexeron* or trixilem* or xaken* or xatmep* or zexate*).ti,ab,kf,kw.	
26	(59-05-2 or YL5FZ2Y5U1).rn.	39859
27	Interleukin-1beta/	26436
28	(ACZ-885* or ACZ885* or Canakinumab* or ilaris*).ti,ab,kf,kw.	814
29	(914613-48-2 or 37CQ2C7X93).rn.	498
30	Antibodies, Monoclonal, Humanized/	48944
31	(pexelizumab or "h5G1.1-SC*").ti,ab,kf,kw.	95
32	(219685-93-5 or CHZ6OLQ3UU).rn.	70
33	Interleukin 1 Receptor Antagonist Protein/	5581
34	(Anakinra* or Antril* or Kineret* or il-1ra* or (interleukin 1 receptor adj1 (antagonist or block* or inhibit*))).ti,ab,kf,kw.	9416
35	(143090-92-0 or 9013DUQ28K).rn.	0
36	(agi-1067* or agi1067* or agz-1067* or agz1067* or probucol succinate* or Succinobucol*).ti,ab,kf,kw.	58
37	(216167-82-7 or J1J54V24R4).rn.	52
38	(ftx-1821* or ftx1821* or gsk-856553* or gsk856553* or gw-856553* or gw856553* or Losmapimod* or sb-856553* or sb856553*).ti,ab,kf,kw.	52
39	(F2DQF16BXE or 585543-15-3).rn.	0
40	(lc1004* or lc-1004* or inclacumab* or ro4905417* or ro-4905417*).ti,ab,kf,kw.	10
41	(1256258-86-2 or A6734I702L).rn.	6
42	(Atreleuton* or a85761* or a-85761* or abt761* or abt-761* or via-2291* or via2291*).ti,ab,kf,kw.	25
43	(U301T88E1M or 154355-76-7).rn.	20
44	(darapladib* or sb-480848* or sb480848*).ti,ab,kf,kw.	143
45	(356057-34-6 or UI1U1MYH09).rn.	91
46	(Varespladib* or Varepladib* or ly315920* or ly-315920* or s-5920* or s5920*).ti,ab,kf,kw.	101
47	(2Q3P98DATH or 172732-68-2).rn.	52
48	or/10-47	554747
49	randomized controlled trial.pt.	558953
50	controlled clinical trial.pt.	94700
51	randomized.ab.	551166
52	placebo.ab.	225750
53	clinical trials as topic.sh.	199220
54	randomly.ab.	376371

55	trial.ti.	256986
56	49 or 50 or 51 or 52 or 53 or 54 or 55	1427763
57	exp animals/ not humans.sh.	4960445
58	56 not 57	1313386
59	9 and 48 and 58	3919

Appendix 2: Embase Search

Database: Embase Classic+Embase 1947 to 2022 February 17 Platform: Ovid Date Searched: February 18, 2022		
#	**Searches**	**Results**
1	exp arteriosclerosis/	293364
2	(atherosclero* or athero-sclero* or arteriosclero* or arterial-sclero* or arteriolosclero* or arteriolo-sclero* or vascular sclero* or ASCVD or ASCAD or intima plaque).ti,ab,kf,kw.	276214
3	exp coronary artery disease/	375951
4	((coronary adj3 (arter* or stenos* or disease? or disorder? or syndrom?)) or CAD or SCAD).ti,ab,kf,kw.	494904
5	exp interventional cardiovascular procedure/ or coronary artery bypass graft/ or exp coronary artery surgery/	268451
6	(((aortocoronary or aorto-coronary or coronary) adj3 bypass*) or CABG).ti,ab,kf,kw.	84156
7	exp atherectomy/ or exp endarterectomy/ or exp embolectomy/ or exp thrombectomy/	73525
8	(angioplast* or atherectom* or endarterectom* or thrombectom* or thromboendarterectom* or thrombo-endarterectom* or PCI or PTCA or (Percutaneous adj3 (intervent* or revascular*))).ti,ab,kf,kw.	210906
9	or/1-8	1092932
10	exp *nonsteroid antiinflammatory agent/	335168
11	(((non-steroidal or nonsteroidal) adj2 (anti-inflammatory or antiinflammatory or analg?esic*)) or NSAID*).ti,ab,kf,kw.	80348
12	(acalabrutinib* or aceclofenac* or acemetacin* or acetaminosalol* or acetylsalicylate* or acetylsalicylic* or aclantate* or actarit* or adalimumab* or afasevikumab* or afimetoran* or alclofenac* or aldafermin* or alminoprofen* or aloxiprin* or amfenac* or aminophenazone* or aminosalicylic* or amlitelimab* or amlodipine* or ampiroxicam* or amtolmetin guacil* or anirolac* or antiflammin* or apadenoson* or apremilast* or araprofen* or ascription* or asivatrep* or astegolimab* or atibuclimab* or atliprofen* or aviptadil* or azathioprine* or azelaic acid* or bakeprofen* or balsalazide* or bardoxolone* or bardoxolone methyl* or belumosudil* or bendazac* or benorilate* or benoxaprofen* or bermoprofen* or bimosiamose* or brazikumab* or brensocatib* or brimonidine* or bromfenac* or broperamole* or bucloxic acid* or bucolome* or bufexamac* or butibufen* or camobucol* or carbasalate* or carotegrast* or carprofen* or cedirogant* or celecoxib* or cibinetide* or cicloprofen* or cimicoxib* or cinmetacin* or cinnoxicam* or clidanac* or clofezone* or clonixin* or clonixin lysine* or cloximate* or crisaborole*).ti,ab,kf,kw.	100591
13	(dagrocorat* or danicopan* or dapansutrile* or dapatifagene navolactibac* or darbufelone* or daxdilimab* or dazodalibep* or dehydrozingerone* or demethoxycurcumin* or deracoxib* or deucravacitinib* or dexibuprofen* or	49581

	dexketoprofen* or dexpemedolac* or diclofenac* or didemethoxycurcumin* or diflunisal* or diftalone* or dimethyl fumarate* or dimethyl sulfoxide* or diphenpyramide* or ditazole* or droxicam* or duometacin* or ebdarokimab* or ebselen* or edasalonexent* or efruxifermin* or elsibucol* or emavusertib* or emorfazone* or emvododstat* or endolac* or enfenamic acid* or enflicoxib* or enpatoran* or epirizole* or etodolac* or etofenamate* or etoricoxib* or evobrutinib* or felbinac* or fenamic acid* or fenbufen* or fenclofenac* or fenclozic acid* or fendosal* or fenflumizole* or fenoprofen* or fentiazac* or fepradinol* or feprazone* or firategrast* or firocoxib* or flobufen* or flosulide* or flufenamate aluminum* or flufenamic acid* or flunixin* or flunoxaprofen* or fluproquazone* or flurbiprofen* or flutiazin* or fosdagrocorat* or fosfosal* or furaprofen* or furcloprofen* or furobufen* or furofenac* or fuzapladib*).ti,ab,kf,kw.	
14	(glucametacin* or gluconate zinc* or guacetisal* or guaimesal* or gusacitinib* or hydroxychloroquine* or ibrigampar* or ibufenac* or ibuprofen* or ibuproxam* or icoduline* or icosapentaenoic acid* or iguratimod* or ilonidap* or imidazole salicylate* or imisopasem manganese* or imsidolimab* or incyclinide* or indameth* or indometacin* or indoprofen* or ipsalazide* or iptacopan* or isecarosmab* or isofezolac* or isonixin* or isoxepac* or isoxicam* or itepekimab* or kebuzone* or ketoprofen* or ketoprofen lysine* or ketorolac* or lazertinib* or lazucirnon* or leflunomide* or lenabasum* or licofelone* or lifitegrast* or lirentelimab* or lobuprofen* or lonazolac* or lorecivivint* or lornoxicam* or losmiprofen* or loxoprofen* or lumiracoxib* or lusvertikimab* or lyprinol* or lysine acetylsalicylate*).ti,ab,kf,kw.	52974
15	(mabuprofen* or magnesium salicylate* or manoalide* or mapracorat* or mavacoxib* or meclofenam* or mefenamic acid* or meloxicam* or melrilimab* or mesalazine* or methotrexate* or metiazinic acid* or metoxibutropate* or milategrast* or mipragoside* or mirococept* or miroprofen* or mivavotinib* or mofebutazone* or mofezolac* or mongersen* or morazone* or morniflumate* or mosedipimod* or nabumetone* or nangibotide* or naproxcinod* or naproxen* or navamepent* or nepafenac* or neurofenac* or neurotropin* or nictindole* or niflumic acid* or nimesulide* or ocarocoxib* or odalprofen* or olsalazine* or ordesekimab* or ormeloxifene* or orpanoxin* or otenaproxesul* or oxaceprol* or oxametacin* or oxaprazine* or oxaprozin* or oxicam derivative* or oxindanac* or oxyphenbutazone*).ti,ab,kf,kw.	102431
16	(palifermin* or parcetasal* or parecoxib* or pelubiprofen* or pemedolac* or perisoxal* or phenylbutazone* or phenylbutazone megallate* or picolamine salicylate* or piketoprofen* or pimeprofen* or pipebuzone* or piproxen* or pirazolac* or pirfenidone* or piroxicam* or piroxicam beta cyclodextrin* or pirprofen* or plonmarlimab* or plozalizumab* or polmacoxib* or pralnacasan* or pranoprofen* or prinomide* or prinomide triethanolamine* or proglumetacin* or proquazone* or pyrazinobutazone* or quellor* or rapamycin* or rasagiline* or ravagalimab* or relfovetmab* or reltecimod* or remestemcel L* or resatorvid* or rimacalib* or rimazolium* or risankizumab* or robenacoxib* or rofecoxib* or romazarit* or rosiptor* or rosmarinic acid* or rovazolac* or rozibafusp alfa* or ruxolitinib* or salazosulfapyridine* or salicylic acid* or	89655

	salnacedin* or salsalate* or satralizumab* or scalaradial* or semapimod* or semorinemab* or sibofimloc* or simufilam* or sudoxicam* or sulfosalicylate samarium* or sulindac* or suprofen* or suxibuzone*).ti,ab,kf,kw.	
17	(talniflumate* or tapinarof* or tazofelone* or telazorlimab* or tenidap* or tenosal* or tenosiprol* or tenoxicam* or tepoxalin* or teriflunomide* or tesnatilimab* or tiaprofenic acid* or tiaramide* or tilmacoxib* or tilnoprofen arbamel* or tilomisole* or timegadine* or tioxamast* or tioxaprofen* or tirnovetmab* or tolebrutinib* or tolfenamic acid* or tolmetin* or tomaralimab* or tomicorat* or torudokimab* or tozorakimab* or tralokinumab* or tribuzone* or triethanolamine salicylate* or tropesin* or tryptamide* or ufenamate* or valategrast* or valdecoxib* or valerylsalicylic acid* or vasoactive intestinal polypeptide* or vedaprofen* or velsecorat* or vemircopan* or verramed* or vilobelimab* or vixarelimab* or ximoprofen* or zabedosertib* or zaloglanstat* or zaltoprofen* or zaurategrast* or zidometacin* or zinc salicylate* or zoliprofen* or zomepirac*).ti,ab,kf,kw.	11952
18	*colchicine/	12844
19	(Colbenemid* or Colchichin* or colchicum* or colchily* or colchineos* or colchimedio* or colchiquim* or colchisol* or colchysat* or colcin* or colcrys* or colctab* or colgout* or colrefuz* or colsaloid* or Condylon* or gloperba* or goutichine* or goutnil* or kolkicin* or kolkisin* or mitigare* or tolchicine*).ti,ab,kf,kw.	473
20	(64-86-8* or SML2Y3J35T*).rn.	34251
21	*prednisone/	43283
22	(adasone* or acsis* or ancortone* apo-prednisone* or bicortone* or biocortone* or cartancyl* or colisone* or Cortan* or cortancyl* or cortidelt* or cortiprex* or cotone* or cutason* or dacorten* or dacortin* or decortancyl* or decortin* or de-cortisyl* or decortisyl* or dihydrocortisone* or dihydrocortisone* or dekortin or dellacort* or deltacorten* or delta-cortelan* or delta-cortisone* or deltacortisone* or deltacortone* or delta-dome* or delta-prenovis* or deltasone* or delitisone* or deltison* or deltra* or diadreson* or di-adreson* or drazone* or econosone* or encorton* or encortone* or enkorton* or enkortolon* or fernisone* or fiasone* or hostacortin* or insone* or incocortyl* or juvason* or kortancyl* or liquid pred* or lodotra* or lodtra* or lisacort* or me-korti* or meprison* or metacortandracin* or Meticorten* or meticortine* or nisona* or nizon* or novoprednisone* or nurison* or Orasone* or orisane* or panafcort* or paracort* or panasol* or parmenison* or pehacort* or predeltin* or precort* or precortal* or prednicen* or prednicorm* or prednicort* or prednicot* or predni tablinen* or prednidib* or prednilonga* or predniment* or prednison* or prednitone* or prednizon* or prednovister* or presone* or pronison* or pronizon* or pulmison* or rayos* or rectodelt* or rectrocortine* or servisone* or sone$2 or steerometz* or sterapred* or supercortil* or ultracorten* or urtilone* or winpred* or wojtab* or zenadrid*).ti,ab,kf,kw.	61627
23	(53-03-2 or VB0R961HZT).rn.	178929
24	*methotrexate/	53338

25	(abitextrate* or abitrexate* or amethopterin* or amethopterine* or ametopterine* or antifolan* or biotrexate* or brimexate* or canceren* or emtexate* or emthexat* or emthexate* or emtrexate* or enthexate* or farmitrexat* or farmotrex* or fauldexato* or folex* or ifamet* or imeth$2 or intradose MTX or jylamvo* or lantarel* or ledertrexate* or lumexon* or maxtrex* or medsatrexate* or metatrexan* or metex* or methoblastin* or methohexate* or methotrate* or methotrexat* or methylaminopterin* or metical* or metoject* or metotressato* or metothrexate* or metotrexat* or metotrexin* or metrex* or metrotex* or mexate* or neotrexate* or nordimet* or novatrex* or otrexup* or rasuvo* or reditrex* or reumatrex* or rheumatrex* or texate* or texorate* or tremetex* or trexall* or trexeron* or trixilem* or xaken* or xatmep* or zexate*).ti,ab,kf,kw.	78910
26	(59-05-2 or YL5FZ2Y5U1).rn.	180823
27	canakinumab/	3930
28	(ACZ-885* or ACZ885* or Canakinumab* or ilaris*).ti,ab,kf,kw.	1881
29	(914613-48-2 or 37CQ2C7X93).rn.	2417
30	pexelizumab/	360
31	(pexelizumab or "h5G1.1-SC*").ti,ab,kf,kw.	112
32	(219685-93-5 or CHZ6OLQ3UU).rn.	355
33	anakinra/	4590
34	(Anakinra* or Antril* or Kineret* or il-1ra* or (interleukin 1 receptor adj1 (antagonist or block* or inhibit*))).ti,ab,kf,kw.	14433
35	(143090-92-0 or 9013DUQ28K).rn.	7368
36	succinobucol/	75
37	(agi-1067* or agi1067* or agz-1067* or agz1067* or probucol succinate* or Succinobucol*).ti,ab,kf,kw.	76
38	(216167-82-7 or J1J54V24R4).rn.	152
39	losmapimod/	207
40	(ftx-1821* or ftx1821* or gsk-856553* or gsk856553* or gw-856553* or gw856553* or Losmapimod* or sb-856553* or sb856553*).ti,ab,kf,kw.	83
41	(F2DQF16BXE or 585543-15-3).rn.	201
42	inclacumab/	50
43	(lc1004* or lc-1004* or inclacumab* or ro4905417* or ro-4905417*).ti,ab,kf,kw.	20
44	(1256258-86-2 or A6734I702L).rn.	46
45	atreleuton/	161
46	(Atreleuton* or a85761* or a-85761* or abt761* or abt-761* or via-2291* or via2291*).ti,ab,kf,kw.	37
47	(U301T88E1M or 154355-76-7).rn.	156
48	darapladib/	411
49	(darapladib* or sb-480848* or sb480848*).ti,ab,kf,kw.	207
50	(356057-34-6 or UI1U1MYH09).rn.	376
51	varespladib/	233

52	(Varespladib* or Varepladib* or ly315920* or ly-315920* or s-5920* or s5920*).ti,ab,kf,kw.	125
53	(2Q3P98DATH or 172732-68-2).rn.	218
54	or/10-53	870554
55	crossover procedure/	69817
56	double blind procedure/	194935
57	exp randomized controlled trial/	700059
58	single blind procedure/	45231
59	(random$ or factorial$ or crossover$ or cross over$ or cross-over$ or placebo$ or (doubl$ adj blind$) or (singl$ adj blind$) or assign$ or allocat$ or volunteer$).mp.	2947243
60	55 or 56 or 57 or 58 or 59	2947906
61	exp animal/	30333319
62	exp human/	24637922
63	61 not 62	5695397
64	60 not 63	2682255
65	9 and 54 and 64	7316

Appendix 3. Cochrane Central Register of Controlled Trials Search

Database: EBM Reviews - Cochrane Central Register of Controlled Trials January 2022 **Platform: Ovid** **Date Searched: February 18, 2022**		
#	**Searches**	**Results**
1	exp Arteriosclerosis/ or Plaque, Atherosclerotic/	11471
2	(atherosclero* or athero-sclero* or arteriosclero* or arterial-sclero* or arteriolosclero* or arteriolo-sclero* or vascular sclero* or ASCVD or ASCAD or intima plaque).ti,ab.	12951
3	Acute Coronary Syndrome/ or exp Coronary Disease/	16154
4	((coronary adj3 (arter* or stenos* or disease? or disorder? or syndrom?)) or CAD or SCAD).ti,ab.	42037
5	exp Percutaneous Coronary Intervention/ or exp Myocardial Revascularization/	11573
6	(((aortocoronary or aorto-coronary or coronary) adj3 bypass*) or CABG).ti,ab.	12770
7	exp endarterectomy/ or exp thrombectomy/ or exp Atherectomy/ or exp Embolectomy/	1084
8	(angioplast* or atherectom* or endarterectom* or thrombectom* or thromboendarterectom* or thrombo-endarterectom* or PCI or PTCA or (Percutaneous adj3 (intervent* or revascular*))).ti,ab.	22547
9	or/1-8	73860
10	exp Anti-Inflammatory Agents, Non-Steroidal/	20078
11	(((non-steroidal or nonsteroidal) adj2 (anti-inflammatory or antiinflammatory or analg?esic*)) or NSAID*).ti,ab.	11105
12	(acalabrutinib* or aceclofenac* or acemetacin* or acetaminosalol* or acetylsalicylate* or acetylsalicylic* or aclantate* or actarit* or adalimumab* or afasevikumab* or afimetoran* or alclofenac* or aldafermin* or alminoprofen* or aloxiprin* or amfenac* or aminophenazone* or aminosalicylic* or amlitelimab* or amlodipine* or ampiroxicam* or amtolmetin guacil* or anirolac* or antiflammin* or apadenoson* or apremilast* or araprofen* or ascription* or asivatrep* or astegolimab* or atibuclimab* or atliprofen* or aviptadil* or azathioprine* or azelaic acid* or bakeprofen* or balsalazide* or bardoxolone* or bardoxolone methyl* or belumosudil* or bendazac* or benorilate* or benoxaprofen* or bermoprofen* or bimosiamose* or brazikumab* or brensocatib* or brimonidine* or bromfenac* or broperamole* or bucloxic acid* or bucolome* or bufexamac* or butibufen* or camobucol* or carbasalate* or carotegrast* or carprofen* or cedirogant* or celecoxib* or cibinetide* or cicloprofen* or cimicoxib* or cinmetacin* or cinnoxicam* or clidanac* or clofezone* or clonixin* or clonixin lysine* or cloximate* or crisaborole*).ti,ab.	17081
13	(dagrocorat* or danicopan* or dapansutrile* or dapatifagene navolactibac* or darbufelone* or daxdilimab* or dazodalibep* or dehydrozingerone* or demethoxycurcumin* or deracoxib* or deucravacitinib* or dexibuprofen* or dexketoprofen* or dexpemedolac* or diclofenac* or didemethoxycurcumin* or diflunisal* or diftalone* or dimethyl fumarate* or dimethyl sulfoxide* or diphenpyramide* or ditazole* or droxicam* or duometacin* or ebdarokimab* or	8122

	ebselen* or edasalonexent* or efruxifermin* or elsibucol* or emavusertib* or emorfazone* or emvododstat* or endolac* or enfenamic acid* or enflicoxib* or enpatoran* or epirizole* or etodolac* or etofenamate* or etoricoxib* or evobrutinib* or felbinac* or fenamic acid* or fenbufen* or fenclofenac* or fenclozic acid* or fendosal* or fenflumizole* or fenoprofen* or fentiazac* or fepradinol* or feprazone* or firategrast* or firocoxib* or flobufen* or flosulide* or flufenamate aluminum* or flufenamic acid* or flunixin* or flunoxaprofen* or fluproquazone* or flurbiprofen* or flutiazin* or fosdagrocorat* or fosfosal* or furaprofen* or furcloprofen* or furobufen* or furofenac* or fuzapladib*).ti,ab.	
14	(glucametacin* or gluconate zinc* or guacetisal* or guaimesal* or gusacitinib* or hydroxychloroquine* or ibrigampar* or ibufenac* or ibuprofen* or ibuproxam* or icoduline* or icosapentaenoic acid* or iguratimod* or ilonidap* or imidazole salicylate* or imisopasem manganese* or imsidolimab* or incyclinide* or indameth* or indometacin* or indoprofen* or ipsalazide* or iptacopan* or isecarosmab* or isofezolac* or isonixin* or isoxepac* or isoxicam* or itepekimab* or kebuzone* or ketoprofen* or ketoprofen lysine* or ketorolac* or lazertinib* or lazucirnon* or leflunomide* or lenabasum* or licofelone* or lifitegrast* or lirentelimab* or lobuprofen* or lonazolac* or lorecivivint* or lornoxicam* or losmiprofen* or loxoprofen* or lumiracoxib* or lusvertikimab* or lyprinol* or lysine acetylsalicylate*).ti,ab.	11607
15	(mabuprofen* or magnesium salicylate* or manoalide* or mapracorat* or mavacoxib* or meclofenam* or mefenamic acid* or meloxicam* or melrilimab* or mesalazine* or methotrexate* or metiazinic acid* or metoxibutropate* or milategrast* or mipragoside* or mirococept* or miroprofen* or mivavotinib* or mofebutazone* or mofezolac* or mongersen* or morazone* or morniflumate* or mosedipimod* or nabumetone* or nangibotide* or naproxcinod* or naproxen* or navamepent* or nepafenac* or neurofenac* or neurotropin* or nictindole* or niflumic acid* or nimesulide* or ocarocoxib* or odalprofen* or olsalazine* or ordesekimab* or ormeloxifene* or orpanoxin* or otenaproxesul* or oxaceprol* or oxametacin* or oxaprazine* or oxaprozin* or oxicam derivative* or oxindanac* or oxyphenbutazone*).ti,ab.	16052
16	(palifermin* or parcetasal* or parecoxib* or pelubiprofen* or pemedolac* or perisoxal* or phenylbutazone* or phenylbutazone megallate* or picolamine salicylate* or piketoprofen* or pimeprofen* or pipebuzone* or piproxen* or pirazolac* or pirfenidone* or piroxicam* or piroxicam beta cyclodextrin* or pirprofen* or plonmarlimab* or plozalizumab* or polmacoxib* or pralnacasan* or pranoprofen* or prinomide* or prinomide triethanolamine* or proglumetacin* or proquazone* or pyrazinobutazone* or quellor* or rapamycin* or rasagiline* or ravagalimab* or relfovetmab* or reltecimod* or remestemcel L* or resatorvid* or rimacalib* or rimazolium* or risankizumab* or robenacoxib* or rofecoxib* or romazarit* or rosiptor* or rosmarinic acid* or rovazolac* or rozibafusp alfa* or ruxolitinib* or salazosulfapyridine* or salicylic acid* or salnacedin* or salsalate* or satralizumab* or scalaradial* or semapimod* or semorinemab* or sibofimloc* or simufilam* or sudoxicam* or sulfosalicylate samarium* or sulindac* or suprofen* or suxibuzone*).ti,ab.	6447

17	(talniflumate* or tapinarof* or tazofelone* or telazorlimab* or tenidap* or tenosal* or tenosiprol* or tenoxicam* or tepoxalin* or teriflunomide* or tesnatilimab* or tiaprofenic acid* or tiaramide* or tilmacoxib* or tilnoprofen arbamel* or tilomisole* or timegadine* or tioxamast* or tioxaprofen* or tirnovetmab* or tolebrutinib* or tolfenamic acid* or tolmetin* or tomaralimab* or tomicorat* or torudokimab* or tozorakimab* or tralokinumab* or tribuzone* or triethanolamine salicylate* or tropesin* or tryptamide* or ufenamate* or valategrast* or valdecoxib* or valerylsalicylic acid* or vasoactive intestinal polypeptide* or vedaprofen* or velsecorat* or vemircopan* or verramed* or vilobelimab* or vixarelimab* or ximoprofen* or zabedosertib* or zaloglanstat* or zaltoprofen* or zaurategrast* or zidometacin* or zinc salicylate* or zoliprofen* or zomepirac*).ti,ab.	1562
18	exp Colchicine/	375
19	(Colbenemid* or Colchichin* or colchicum* or colchily* or colchineos* or colchimedio* or colchiquim* or colchisol* or colchysat* or colcin* or colcrys* or colctab* or colgout* or colrefuz* or colsaloid* or Condylon* or gloperba* or goutichine* or goutnil* or kolkicin* or kolkisin* or mitigare* or tolchicine*).ti,ab.	15
20	Prednisone/	4110
21	(adasone* or acsis* or ancortone* apo-prednisone* or bicortone* or biocortone* or cartancyl* or colisone* or Cortan* or cortancyl* or cortidelt* or cortiprex* or cotone* or cutason* or dacorten* or dacortin* or decortancyl* or decortin* or de-cortisyl* or decortisyl* or dihydrocortisone* or dihydrocortisone* or dekortin or dellacort* or deltacorten* or delta-cortelan* or delta-cortisone* or deltacortisone* or deltacortone* or delta-dome* or delta-prenovis* or deltasone* or delitisone* or deltison* or deltra* or diadreson* or di-adreson* or drazone* or econosone* or encorton* or encortone* or enkorton* or enkortolon* or fernisone* or fiasone* or hostacortin* or insone* or incocortyl* or juvason* or kortancyl* or liquid pred* or lodotra* or lodtra* or lisacort* or me-korti* or meprison* or metacortandracin* or Meticorten* or meticortine* or nisona* or nizon* or novoprednisone* or nurison* or Orasone* or orisane* or panafcort* or paracort* or panasol* or parmenison* or pehacort* or predeltin* or precort* or precortal* or prednicen* or prednicorm* or prednicort* or prednicot* or predni tablinen* or prednidib* or prednilonga* or predniment* or prednison* or prednitone* or prednizon* or prednovister* or presone* or pronison* or pronizon* or pulmison* or rayos* or rectodelt* or rectrocortine* or servisone* or sone$2 or steerometz* or sterapred* or supercortil* or ultracorten* or urtilone* or winpred* or wojtab* or zenadrid*).ti,ab.	8451
22	Methotrexate/	4281
23	(abitextrate* or abitrexate* or amethopterin* or amethopterine* or ametopterine* or antifolan* or biotrexate* or brimexate* or canceren* or emtexate* or emthexat* or emthexate* or emtrexate* or enthexate* or farmitrexat* or farmotrex* or fauldexato* or folex* or ifamet* or imeth$2 or intradose MTX or jylamvo* or lantarel* or ledertrexate* or lumexon* or maxtrex* or medsatrexate* or metatrexan* or metex* or methoblastin* or methohexate* or methotrate* or methotrexat* or methylaminopterin* or metical* or metoject* or metotressato* or metothrexate* or metotrexat* or metotrexin* or metrex* or metrotex* or mexate* or neotrexate* or nordimet* or novatrex* or otrexup* or rasuvo* or reditrex* or	11086

	reumatrex* or rheumatrex* or texate* or texorate* or tremetex* or trexall* or trexeron* or trixilem* or xaken* or xatmep* or zexate*).ti,ab.	
24	Interleukin-1beta/	466
25	(ACZ-885* or ACZ885* or Canakinumab* or ilaris*).ti,ab.	364
26	Antibodies, Monoclonal, Humanized/	4397
27	(pexelizumab or "h5G1.1-SC*").ti,ab.	53
28	Interleukin 1 Receptor Antagonist Protein/	331
29	(Anakinra* or Antril* or Kineret* or il-1ra* or (interleukin 1 receptor adj1 (antagonist or block* or inhibit*))).ti,ab.	1153
30	(agi-1067* or agi1067* or agz-1067* or agz1067* or probucol succinate* or Succinobucol*).ti,ab.	13
31	(ftx-1821* or ftx1821* or gsk-856553* or gsk856553* or gw-856553* or gw856553* or Losmapimod* or sb-856553* or sb856553*).ti,ab.	74
32	(lc1004* or lc-1004* or inclacumab* or ro4905417* or ro-4905417*).ti,ab.	20
33	(Atreleuton* or a85761* or a-85761* or abt761* or abt-761* or via-2291* or via2291*).ti,ab.	18
34	(darapladib* or sb-480848* or sb480848*).ti,ab.	74
35	(Varespladib* or Varepladib* or ly315920* or ly-315920* or s-5920* or s5920*).ti,ab.	22
36	or/10-35	82339
37	9 and 36	3464

Appendix 4. ClinicalTrials.Gov Search

Platform: ClinicalTrials.Gov (https://clinicaltrials.gov/) **Date Searched: March 11, 2022**	
Search	**Results**
(atherosclerosis OR arteriosclerosis OR coronary OR endarterectomy OR thrombectomy OR atherectomy OR embolectomy) | (NSAID OR anti-inflammatory OR colchicine OR prednisone OR methotrexate OR canakinumab OR pexelizumab OR anakinra OR succinobucol OR losmapimod OR inclacumab OR atreleuton OR darapladib OR varespladib)	811

Appendix 5. World Health Organization International Clinical Trials Registry Platform (ICTRP) Search

Platform: World Health Organization International Clinical Trials Registry Platform (ICTRP) (https://www.who.int/clinical-trials-registry-platform) **Date Searched: March 11, 2022**	
Search	**Results**
(atherosclerosis OR arteriosclerosis OR coronary OR endarterectomy OR thrombectomy OR atherectomy OR embolectomy) AND (NSAID OR anti-inflammatory OR colchicine OR prednisone OR methotrexate OR canakinumab OR pexelizumab OR anakinra OR succinobucol OR losmapimod OR inclacumab OR atreleuton OR darapladib OR varespladib)	174 records for 92 trials found!

Appendix 6. Europe PMC Search (https://europepmc.org/)

Platform: Europe PMC (https://europepmc.org/) **Date Searched: March 11, 2022**	
Search	**Results**
TITLE:(athero* OR arteri* OR coronary* OR endarterec* OR thrombect* OR atherect* OR embolect*) OR ABSTRACT:(athero* OR arteri* OR coronary* OR endarterec* OR thrombect* OR atherect* OR embolect*)) AND (TITLE:(NSAID* OR anti-inflamm* OR colchicine* OR prednisone* OR methotrexate* OR canakinumab* OR pexelizumab* OR anakinra* OR succinobucol* OR losmapimod* OR inclacumab* OR atreleuton* OR darapladib* OR varespladib*) OR ABSTRACT:(NSAID* OR anti-inflamm* OR colchicine* OR prednisone* OR methotrexate* OR canakinumab* OR pexelizumab* OR anakinra* OR succinobucol* OR losmapimod* OR inclacumab* OR atreleuton* OR darapladib* OR varespladib*)) AND (PUB_TYPE:"Preprint")	25 results

Appendix 7. List of Conferences Hand-searched

Platforms: Various conference websites **Date searched: March 18, 2022**	
Conferences of Interest	**Indexation Status in Embase**
European Society of Cardiology (ESC) Congress	Indexed up to and including 2021
American College of Cardiology	Indexed up to and including 2021
American Heart Association	Indexed up to and including 2021
Canadian Cardiovascular Congress	Indexed up to and including 2020 2021 conference website searched for the following terms: NSAID (1 result) anti-inflammatory (0 results) colchicine (0 results) prednisone (0 results) methotrexate (0 results) canakinumab (0 results) pexelizumab (0 results) anakinra (0 results) succinobucol (0 results) losmapimod (0 results) inclacumab (0 results) atreleuton (0 results) darapladib (0 results) varespladib (0 results)

Appendix 8. ACS – MACE Evidence Summary Table

Treatment	Pair-wise Meta-Analysis, OR (95% CI) versus placebo	NMA, Odds Ratio (95% CrI) versus placebo	Probability Best (%) for Outcome	Rank for Outcome	SUCRA
Colchicine	0.72 (0.45, 1.15)	0.71 (0.58, 0.88)	0.8%	3.3	0.7850
NSAIDs	0.31 (0.12, 0.77)	0.30 (0.11, 0.73)	66.4%	1.5	0.9546
Anakinra	3.45 (1.08, 11.03)	3.70 (1.26, 13.78)	0%	6.9	0.0718
Tocilizumab	0.72 (0.33, 1.59)	0.72 (0.32, 1.56)	4.5%	4.0	0.7189
Losmapimod low dose	1.01 (0.68, 1.51)	1.09 (0.86, 1.37)	0%	7.0	0.4428
Losmapimod high dose	0.98 (0.56, 1.73)	1.21 (0.75, 1.97)	0%	7.8	0.3748
LeukArrest low dose	1.89 (0.16, 22.75)	2.22 (0.16, 77.17)	4.3%	8.4	0.2916
LeukArrest high dose	0.95 (0.06, 16.31)	0.92 (0.02, 36.30)	23.95%	5.8	0.3748
Varespladib	1.29 (1.00, 1.66)	1.49 (1.16, 1.91)	0%	9.5	0.2261
Darapladib	0.99 (0.90, 1.10)	0.99 (0.90, 1.10)	0%	5.9	0.5501

CI=confidence interval, CrI=credible interval, OR=odds ratio, SUCRA=surface under the cumulative ranking

Appendix 9. Stable CAD – MACE Evidence Summary Table

Treatment	Pair-wise Meta-Analysis, OR (95% CI) versus placebo	NMA, Odds Ratio (95% CrI) versus placebo	Probability Best (%) for Outcome	Rank for Outcome	SUCRA
Colchicine	0.58 (0.35, 0.99)	0.65 (0.54, 0.77)	1.4%	3.5	0.8370
NSAIDs	0.85 (0.31, 2.33)	0.81 (0.56, 1.16)	0.5%	6.4	0.6292
Steroids	0.47 (0.15, 1.50)	0.44 (0.26, 0.72)	51.1%	1.8	0.9577
Succinobucol	0.60 (0.28, 1.28)	0.61 (0.29, 1.31)	15.6%	4.4	0.7843
EA-230	0.68 (0.29, 1.57)	0.67 (0.28, 1.54)	12.7%	5.3	0.7147
Canakinumab medium-dose	0.88 (0.70, 1.10)	0.87 (0.75, 1.00)	0%	7.1	0.5857
Canakinumab high-dose	0.87 (0.75, 1.01)	0.87 (0.75, 1.01)	0%	7.4	0.5656
Canakinumab low-dose	0.88 (0.76, 1.03)	0.89 (0.76, 1.03)	0%	7.6	0.5360
Darapladib	0.93 (0.84, 1.03)	0.93 (0.84, 1.03)	0%	8.9	0.4560
Inclacumab	1.03 (0.53, 1.98)	1.03 (0.53, 2.04)	0.7%	9.6	0.3985
Methotrexate	1.02 (0.82, 1.27)	1.02 (0.82, 1.28)	0%	10.6	0.3306
Pexelizumab bolus and infusion	1.47 (0.55, 3.92)	1.49 (0.55, 4.17)	0.8%	11.8	0.2372
Pexelizumab bolus	2.19 (0.88, 5.44)	2.22 (0.92, 5.88)	0%	14.0	0.0788
Varespladib	2.88 (0.11, 72.41)	2.94 (0.82, 12.5)	17.3%	10.8	0.0723

CI=confidence interval, CrI=credible interval, OR=odds ratio, SUCRA=surface under the cumulative ranking

Appendix 10. ACS – MACE Evidence Summary Table for Studies with ≥30 Days of Follow Up and ≥30 Days of Treatment

Treatment	Pair-wise Meta-Analysis, OR (95% CI) versus placebo	NMA, Odds Ratio (95% CrI) versus placebo	Probability Best (%) for Outcome	Rank for Outcome	SUCRA
NSAIDs	0.31 (0.12, 0.77)	0.28 (0.10 – 0.70)	98.9%	1.0	0.9959
Colchicine	0.74 (0.51, 1.07)	0.80 (0.61 – 1.05)	0.9%	2.2	0.8239
Darapladib	0.99 (0.90, 1.10)	0.91 (0.71 – 1.18)	0%	3.9	0.5920
Losmapimod low dose	1.01 (0.68, 1.51)	1.09 (0.86 – 1.37)	0%	4.8	0.4518
Losmapimod high dose	0.98 (0.56, 1.73)	1.22 (0.74 – 1.96)	0.2%	5.4	0.3676
Varespladib	3.04 (0.47, 19.82)	1.49 (1.16 – 1.89)	0%	6.8	0.1720
Anakinra	3.45 (1.08, 11.03)	3.57 (1.16 – 14.3)	0%	7.8	0.0234

CI=confidence interval, CrI=credible interval, OR=odds ratio, SUCRA=surface under the cumulative ranking

Appendix 11. ACS – MACE Evidence Summary Table for Studies Published 2010 or Later with ≥30 Days of Follow Up and ≥30 Days of Treatment

Treatment	Pair-wise Meta-Analysis, OR (95% CI) versus placebo	NMA, Odds Ratio (95% CrI) versus placebo	Probability Best (%) for Outcome	Rank for Outcome	SUCRA
Colchicine	0.74 (0.51, 1.07)	0.87 (0.77 – 0.99)	80.9%	1.2	0.9594
Darapladib	0.99 (0.90, 1.10)	0.99 (0.90 – 1.10)	4.2%	2.9	0.6911
Losmapimod low dose	1.01 (0.68, 1.51)	1.09 (0.86 – 1.37)	4.0%	3.9	0.5227
Losmapimod high dose	0.98 (0.56, 1.73)	1.20 (0.74 – 1.96)	9.7%	4.4	0.4309
Varespladib	3.04 (0.47, 19.82)	1.47 (1.16 – 1.89)	0%	5.8	0.2009
Anakinra	3.45 (1.08, 11.03)	3.57 (1.18 – 14.3)	0.6%	6.8	0.0267

CI=confidence interval, CrI=credible interval, OR=odds ratio, SUCRA=surface under the cumulative ranking

Appendix 12. Stable CAD – MACE Evidence Summary Table for Studies with ≥30 Days of Follow Up and ≥30 Days of Treatment

Treatment	Pair-wise Meta-Analysis, OR (95% CI) versus placebo	NMA, Odds Ratio (95% CrI) versus placebo	Probability Best (%) for Outcome	Rank for Outcome	SUCRA
Steroid	0.31 (0.09, 1.00)	0.37 (0.21 – 0.65)	68.3%	1.4	0.9616
Colchicine	0.47 (0.21, 1.08)	0.63 (0.52 – 0.76)	1.1%	3.3	0.7922
NSAIDs	1.37 (0.48, 3.97)	0.64 (0.43 – 0.96)	3.0%	3.7	0.7573
Succinobucol	0.60 (0.28, 1.28)	0.61 (0.29 – 1.36)	11.4%	4.1	0.7202
Canakinumab medium	0.88 (0.70, 1.10)	0.87 (0.75 – 1.01)	0%	6.3	0.5152
Canakinumab high	0.87 (0.75, 1.01)	0.88 (0.75 – 1.02)	0%	6.5	0.4962
Canakinumab low	0.88 (0.76, 1.03)	0.89 (0.76 – 1.04)	0%	6.9	0.4662
Inclacumab	1.03 (0.53, 1.98)	1.02 (0.53 – 2.02)	0.4%	8.5	0.3195
Varespladib	3.04 (0.47, 19.82)	2.61 (0.05 – 723.10)	15.8%	9.0	0.2755
Methotrexate	1.02 (0.82, 1.27)	1.02 (0.81 – 1.27)	0%	9.3	0.2418
Darapladib	0.93 (0.84, 1.03)	1.02 (0.92 – 1.14)	0%	9.7	0.2095

CI=confidence interval, CrI=credible interval, OR=odds ratio, SUCRA=surface under the cumulative ranking

Appendix 13. Stable CAD – MACE Evidence Summary Table for Studies Published 2010 or Later with ≥30 Days of Follow Up and ≥30 Days of Treatment

Treatment	Pair-wise Meta-Analysis, OR (95% CI) versus placebo	NMA, Odds Ratio (95% CrI) versus placebo	Probability Best (%) for Outcome	Rank for Outcome	SUCRA
Steroid	0.49 (0.26, 0.91)	0.37 (0.21 – 0.65)	68.3%	1.4	0.9616
Colchicine	0.47 (0.21, 1.08)	0.63 (0.52 – 0.76)	1.1%	3.2	0.7922
NSAIDs	1.37 (0.48, 3.97)	0.64 (0.43 – 0.96)	3.0%	3.7	0.7573
Canakinumab medium	0.88 (0.70, 1.10)	0.87 (0.75 – 1.01)	0%	6.3	0.5152
Canakinumab high	0.87 (0.75, 1.01)	0.88 (0.75 – 1.02)	0%	6.5	0.4962
Canakinumab low	0.88 (0.76, 1.03)	0.89 (0.76 – 1.04)	0%	6.9	0.4662
Inclacumab	1.03 (0.53, 1.98)	1.02 (0.53 – 2.02)	0.4%	8.5	0.3195
Varespladib	2.88 (0.11, 72.41)	2.61 (0.05 – 723.10)	15.8%	9.0	0.2418
Methotrexate	1.02 (0.82, 1.27)	1.02 (0.81 – 1.27)	0%	9.3	0.2095

CI=confidence interval, CrI=credible interval, OR=odds ratio, SUCRA=surface under the cumulative ranking

Appendix 14. Confidence in the results.

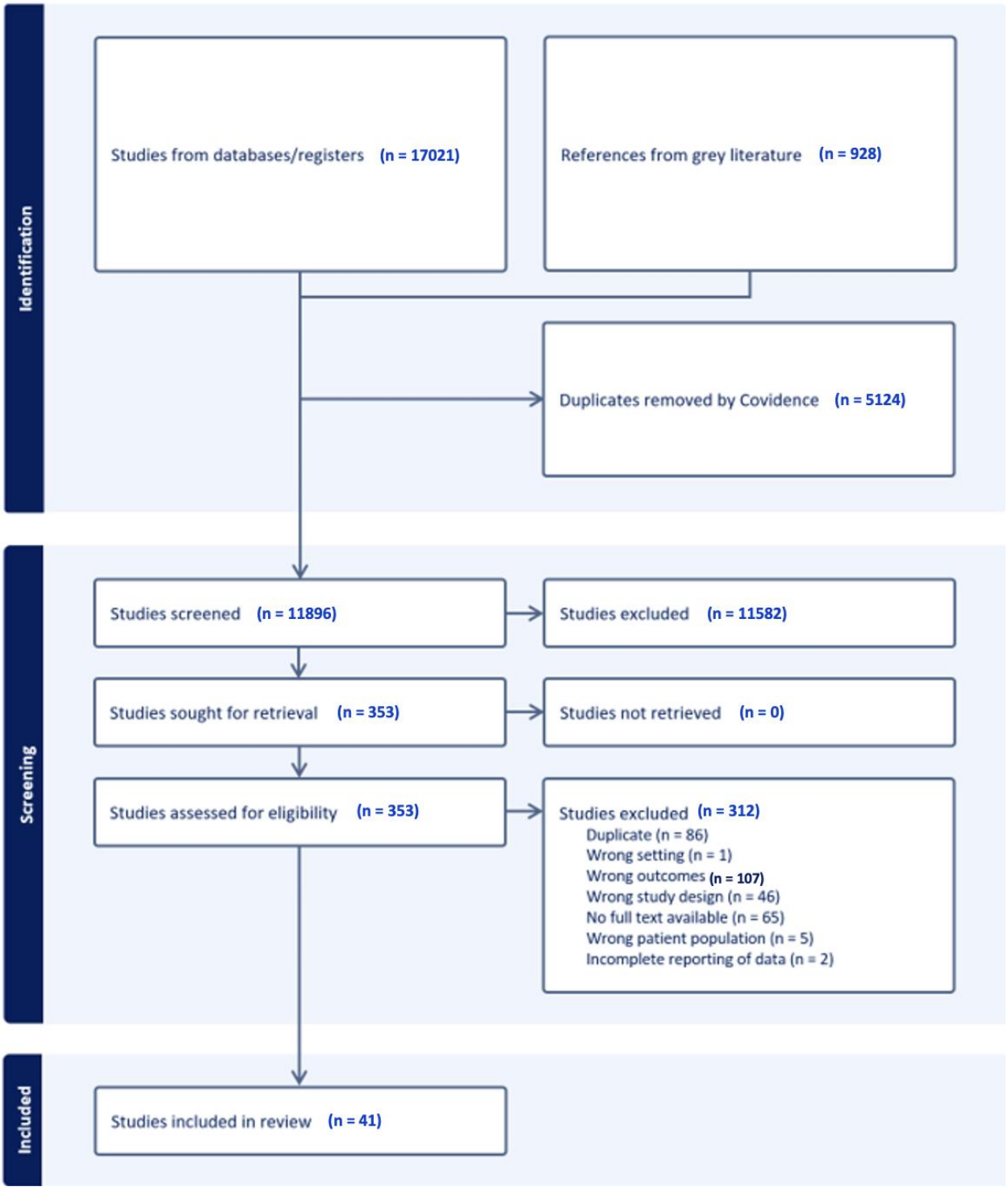
ACS Network

Comparison	Number of studies	Within-study bias	Reporting bias	Indirectness	Imprecision	Heterogeneity	Incoherence	Confidence rating
Canakinumab high dose: Canakinumab low dose	1	No concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Low
Canakinumab high dose: Canakinumab medium dose	1	No concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Low
Canakinumab high dose: Control	1	No concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Low
Canakinumab low dose: Canakinumab medium dose	1	No concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Low
Canakinumab low dose: Control	1	No concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Low
Canakinumab medium dose: Control	2	No concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Low
Colchicine: Control	3	No concerns	Low risk	No concerns	No concerns	Major concerns	No concerns	Low
Control: Darepladib	2	No concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Low
Control: EA-230	1	No concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Low
Control: Inclacumab	1	Some concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Very low
Control: Methotrexate	1	No concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Low
Control: NSAIDs	3	No concerns	Some concerns	No concerns	Major concerns	No concerns	No concerns	Very low
Control: Pexelizumab	1	Major concerns	Some concerns	No concerns	Major concerns	No concerns	No concerns	Very low
Control: Steroids	3	Some concerns	Low risk	No concerns	No concerns	Major concerns	No concerns	Very low
Control: Succinobucol	1	Some concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Very low
Control: Varespladib	1	Major concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Very low

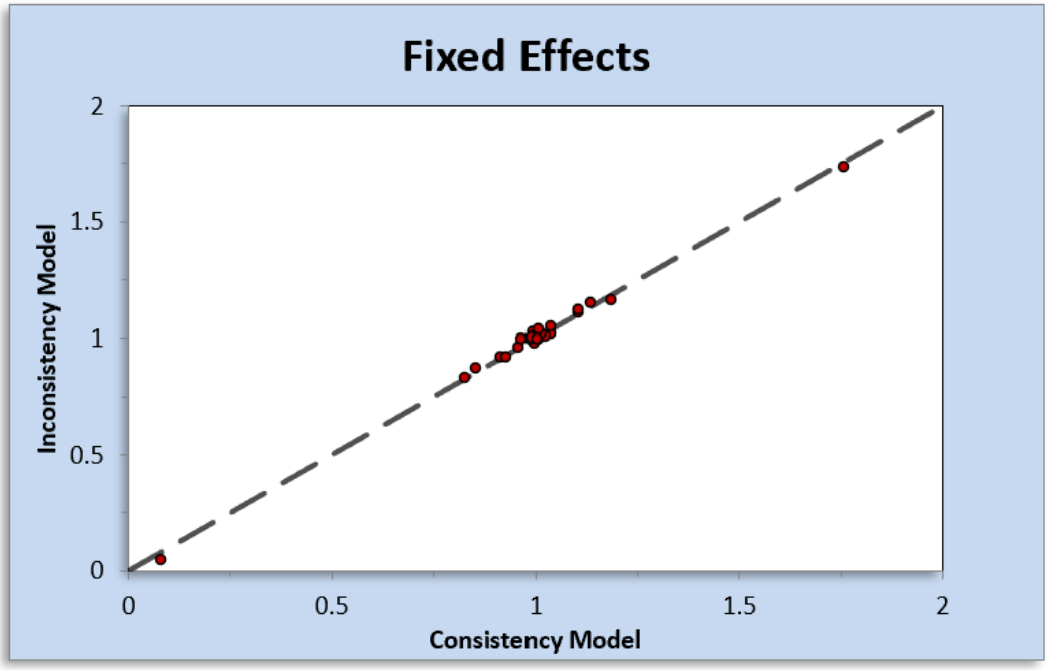
Stable CAD Network

Comparison	Number of studies	Within-study bias	Reporting bias	Indirectness	Imprecision	Heterogeneity	Incoherence	Confidence rating
Anakinra: Control	3	No concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Low
Colchicine:Control	5	No concerns	Low risk	No concerns	No concerns	Major concerns	No concerns	Low
Control:Darapladib	1	No concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Low
Control:LeukArrest high dose	1	Major concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Very low
Control:LeukArrest low dose	1	Major concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Very low
Control:Losmapimod high dose	1	No concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Low
Control:Losmapimod low dose	2	No concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Low
Control:NSAIDs	1	No concerns	Some concerns	No concerns	No concerns	Major concerns	No concerns	Very low
Control:Tocilizumab	2	No concerns	Low risk	No concerns	No concerns	Major concerns	No concerns	Low
Control:Varespladib	2	No concerns	Low risk	No concerns	No concerns	Major concerns	No concerns	Low
LeukArrest high dose:LeukArrest low dose	1	Major concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Very low
Losmapimod high dose:Losmapimod low dose	1	No concerns	Low risk	No concerns	Major concerns	No concerns	No concerns	Low

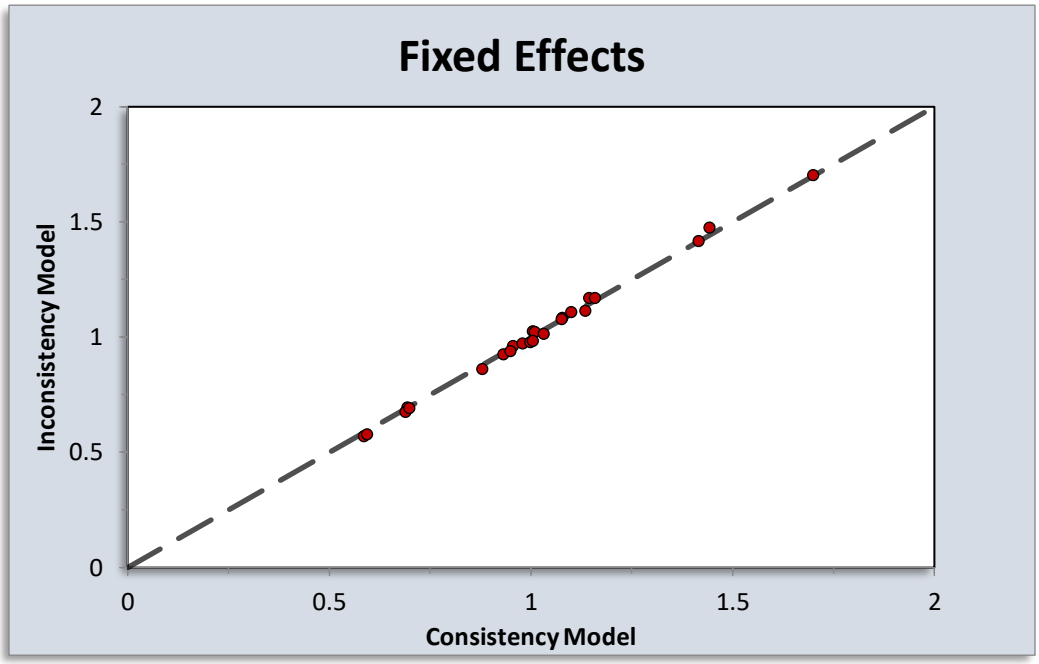
Appendix 15. PRISMA Diagram


Identification
Studies from databases/registers (n = 17021)
References from grey literature (n = 928)
Duplicates removed by Covidence (n = 5124)
Screening
Studies screened (n = 11896)
Studies excluded (n = 11582)
Studies sought for retrieval (n = 353)
Studies not retrieved (n = 0)
Studies assessed for eligibility (n = 353)
Studies excluded (n = 312)
Duplicate (n = 86)
Wrong setting (n = 1)
Wrong outcomes (n = 107)
Wrong study design (n = 46)
No full text available (n = 65)
Wrong patient population (n = 5)
Incomplete reporting of data (n = 2)
Included
Studies included in review (n = 41)

Appendix 16. Inconsistency plot for stable CAD network. Shows posterior mean deviance of the individual data points from fitted consistency and inconsistency models.



Appendix 17. Inconsistency plot for ACS network. Shows posterior mean deviance of the individual data points from fitted consistency and inconsistency models.



Appendix 18. Secondary outcomes

Secondary Outcome: Individual subcomponents of MACE

ACS Network

We evaluated each subcomponent of MACE in a network meta-analysis. The ACS network looking at MI included 23 RCTs[1-23], representing 13 interventions in addition to placebo/control with 41,608 patients in the analysis. The evidence network for MI is similar, albeit larger than for MACE, and included 8 studies (Rosenson 2011[1], Moreira 2017[2], Tardif 2013[4], Brochier 1993[9], Tardif 2008[11], Versaci 2002[13], Armstrong 2007[22], Ulander 2021[20]) that were not in the original network for MACE. Tocilizumab and NSAIDs were associated with a reduced risk of MI relative to placebo/control.

The ACS network looking at non-fatal stroke included 15 RCTs[3,4,6-8,10-12,14,16,21,22,24-26], representing 8 interventions in addition to placebo/control. A total of 41,796 patients were included in the analysis. The evidence network for non-fatal stroke included 6 studies (Tardif 2013[4], Tong 2021[24], Tardif 2008[11], Granger 2003[25], Mahaffey 2003[26], Armstrong 2007[22]) that were not in the original network for MACE. Only colchicine-use was associated with a reduced risk of non-fatal stroke relative to placebo/control.

The acute CAD network looking at cardiovascular death included 12 RCTs[5,6,8,16,18-20,24,27], representing 9 interventions in addition to placebo/control. A total of 34,624 patients were included in the analysis. The evidence network for cardiovascular death was again similar but smaller than for MACE, and included 4 studies (Nakamura 2009[27], Tong 2021[24], Tardif 2008[11], Ulander 2021[20]) that were not in the original network for MACE. No interventions were associated with a reduced risk of cardiac death relative to placebo/control.

Stable CAD Network

The stable CAD network looking at MI included 20 RCTs[1,28-46], representing 12 interventions in addition to placebo/control. The evidence network for MI is similar, albeit larger than for MACE, and included 5 studies (Wong 2006[30], Koo 2007[38], Smith 2011[39], Brown 2004[43], Dzavik 2010[46]) that were not in the original network for MACE. Only colchicine and the 150mg-dose of canakinumab were associated with a reduced risk of MI relative to placebo/control.

The stable CAD network looking at non-fatal stroke included 11 RCTs[33,34,36,37,41,42,44,47-50], representing 8 interventions in addition to placebo/control. The evidence network for non-fatal stroke included 4 studies (Hauser 2016[47], Shah 2020[33], Verrier 2004[41], Cheruku 2004[49]) that were not in the original network for MACE. No interventions were associated with a reduced risk of non-fatal stroke relative to placebo/control.

The stable CAD network looking at cardiovascular death included 10 RCTs[29,34,36-38,40,44,45,48,51], representing 8 interventions in addition to placebo/control. A total of 33,841 patients were included in the analysis. The evidence network for cardiovascular death was again similar but smaller than for MACE and included only one study (Koo 2007[38]) that was not in the original network for MACE. Only colchicine was associated with a reduced risk of cardiac death relative to placebo/control.

Secondary Outcomes: Adverse therapy effects

ACS Network

The ACS network looking at clinically significant infection included 11 RCTs[2,4-8,12,16,52-54] (references), representing 10 interventions in addition to placebo/control. The evidence network for clinically significant infection is similar, albeit smaller than for MACE (with 15,890 patients), and included 4 studies (Moreira 2017[2], Tardif 2013[4], Rusnak 2001[53]) that were not in the original network for MACE. None of the included interventions were associated with an increased risk of infection relative to placebo/control.

The ACS network looking at malignancy included 4 RCTs[6,9,12,14], representing 3 interventions (colchicine, NSAIDs, darapladib) in addition to placebo/control. The evidence network for malignancy is significantly smaller than for MACE and included one study (Brochier 1993[9]) that was not in the original network for MACE. None of the included interventions were associated with an increased risk of malignancy relative to placebo/control.

The ACS network looking at diarrhea and GI upset included 10 RCTs[2,3,6,8,9,12,14,33,37,55], representing 5 interventions (colchicine, NSAIDs, darapladib, methotrexate, losmapimod) in addition to placebo/control. The evidence network for diarrhea/GI upset is significantly smaller than for MACE, and included four studes (Moreira 2017[2], O'Keefe 1992[55], Brochier 1993[9],

Stability 2014[37]) that was not in the original network for MACE. None of the included interventions were associated with an increased risk of diarrhea/GI upset relative to placebo/control.

Stable CAD Network

The Stable CAD network looking at clinically significant infection included 10 RCTs[30,32,34,36,40-42,47,48,56], representing 8 interventions in addition to placebo/control. The evidence network for clinically significant infection is similar, albeit smaller than for MACE, and included 4 studies (Wong 2006[30], Zarpelon 2016[56], Hauser 2016[47], Verrier 2005[41]) that were not in the original network for MACE. None of the included interventions were associated with an increased risk of infection relative to placebo/control.

The stable CAD network looking at malignancy included 3 RCTs[34,36,48], representing 5 interventions (colchicine, canakinumab low dose, canakinumab medium dose, canakinumab high-dose, and methotrexate) in addition to placebo/control. The evidence network for malignancy is significantly smaller than for MACE. Methotrexate use was associated with an increased risk of malignancy relative to placebo/control.

The stable CAD network looking at diarrhea and GI upset included 11 RCTs[1,32-34,36,37,41,42,47,57,58], representing 10 interventions (colchicine, varespladib low dose, varespladib high dose, canakinumab medium-dose, salsalate, darapladib low dose, darapladib medium dose, darapladib high dose, pexelizumab, and methotrexate) in addition to placebo/control. The evidence network for malignancy is similar but smaller than for MACE. Darapladib low and high dose use was associated with a reduced risk of GI upset/diarrhea relative to placebo/control. Methotrexate and colchicine-use were associated with an increased risk of diarrhea/GI upset compared with placebo/control.

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Chapter 4: Systemic inflammation in persons with diabetes

CHAPTER OUTLINE

Chapter 4 addresses the second research question*: Do patients with diabetes have elevated systemic inflammatory levels?*

Patients with diabetes are at increased risk of suffering early atherosclerotic disease related cardiovascular and have an increased risk of recurrent events above that seen in the general population. Systemic inflammation may be one mechanism underlying this association, and if true, this could represent a high-risk group of patients that would potentially benefit from novel anti-inflammatory therapies aimed at curbing cardiovascular risk. In this chapter, the levels of systemic inflammatory markers in patients with diabetes were investigated. The prevalence of elevated high-sensitivity C-reactive protein and interleukin-6 in patients with recent cardiovascular events with or without prediabetes/diabetes, and in a control group of patients with remote cardiovascular events was identified in a cross-sectional study (**Section 4.1**).

Chapter 4 contains one manuscript (**Section 4.1**) which has been published. This manuscript is described below, and a more thorough description is found in the first page of the section.

CHAPTER CONTENTS

Section 4.1 describes the results of a cross-sectional study to evaluate the systemic levels of inflammatory markers in patients with recent cardiovascular events and diabetes compared to patients without diabetes as well as a control group with no recent cardiovascular events. This manuscript was published in the *Canadian Journal of Cardiology Open*.

Section 4.1 Observational Cross-Sectional Study of Inflammatory Markers Following Transient Ischemic Attacks, Acute Coronary Syndromes, and Vascular Stroke Events: a cross-sectional study

SECTION OVERVIEW

This section presents a manuscript that investigates whether systemic inflammatory markers are elevated in patients with diabetes or prediabetes who have suffered a recent cardiovascular event (compared to patients without diabetes as well as patients with no recent cardiovascular events).

MANUSCRIPT STATUS

The manuscript has been published.

Boczar KE, Liu P, Chong AY, So D, Dowlatshahi D, Rayner K, Beanlands R. Observational Cross-Sectional Study of Inflammatory Markers After Transient Ischemic Attacks, Acute Coronary Syndromes, and Vascular Stroke Events. CJC Open. 2020 Dec 31;3(5):675-679. doi: 10.1016/j.cjco.2020.12.020. PMID: 34027372; PMCID: PMC8134934.

Author roles and contributions

KEB and RB designed the study. KEB drafted the manuscript, which was revised by all authors. All authors approved the version of the manuscript submitted for publication.

RELATED THESIS APPENDICES

Appendix G: Published manuscripts. The PDF of published manuscript is presented in Appendix G.

Observational Cross-Sectional Study of Inflammatory Markers Following Transient Ischemic Attacks, Acute Coronary Syndromes, and Vascular Stroke Events

Short title: Inflammatory markers after vascular events

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Brief Summary:

We sought to identify levels of hsCRP and IL-6 in patients with recent cardiovascular events with or without prediabetes/diabetes, and in a control group of patients with remote cardiovascular events. We found hsCRP and IL-6 were elevated in patients with prediabetes/diabetes and a recent cardiovascular event compared to patients with remote events suggesting these patients have a higher inflammatory burden.

Abstract:

We identified the prevalence of elevated high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6) in patients with recent cardiovascular (CV) events with or without prediabetes/diabetes, and in a control group of patients with remote CV events. IL-6 was elevated in patients with prediabetes/diabetes and recent CV events (median 4.84pg/ml (IQR: 3.27, 7.45)) compared to patients with remote events (2.36pg/ml (IQR: 1.09, 4.00)). There was a trend for elevated hsCRP in patients with acute events and prediabetes/diabetes (p=0.147). This supports the notion that patients with prediabetes/diabetes and recent CV events have higher inflammatory burdens than patients without recent CV events or dysglycemia.

Background:

Patients with diabetes are at increased risk of suffering premature atherosclerotic disease related cardiovascular (CV) events [e.g. acute coronary syndromes (ACS), stroke, transient ischemic attacks (TIAs)], and have a higher recurrent event rate than the general population.[1] Systemic and vascular inflammation has been extensively investigated as a promoter of atherosclerosis development and destabilization.[2] Furthermore, several recent trials have demonstrated that therapies focused at blocking the inflammatory cascade directly reduced vascular events.[3-6] The results from these trials raise an intriguing question of how to identify the patients who may benefit the most from these innovative therapies. By examining biomarkers of inflammation, we may be able to identify patients who are at particularly high-risk of vascular events, and may benefit the most from inflammation-targeted therapies to reduce subsequent CV events.

Currently the prevalence of elevated serum high-sensitivity C-reactive protein (hs-CRP) levels post-vascular event has not been widely studied. Identification of the prevalence of patients with elevated inflammatory markers post-event may enable the prospective evaluation of inflammation-targeted therapies and the identification of patients at high-risk for CV events.

We hypothesized that given the link between inflammation and CV events, as well as the higher prevalence of CV events in patients with diabetes, these diabetic or prediabetic patients may have a higher burden of inflammation than a general population of patients with CV disease. We posited that the prevalence of increased hs-CRP and interleukin-6 (IL-6) as markers of vascular inflammation in patients with diabetes or pre-diabetes following recent CV events (ACS, TIA, stroke), would be greater than in a control group of patients without recent acute CV events.

Methods:

We conducted an observational cross-sectional study in 2 cohorts of patients: i) those with a recent CV event (ACS, TIA or stroke) within 30-60 days and ii) those with remote prior CV events (>1 year). Patients were divided into 3 groups: Group 1: recent CV event in patients with prediabetes/diabetes (as defined by the Canadian Diabetes Association Guidelines)[7]; group 2: recent CV event in patients without prediabetes/diabetes; and group 3: patients who suffered remote CV event (>1 year).

Participants were enrolled from either the University of Ottawa Heart Institute or the Stroke Prevention Clinic at The Ottawa Hospital. Enrolled participants for Group 1 and 2 had to: have suffered a recent CV event (ACS, TIA or stroke) within 30-60 days; be 18 years of age or older; and be symptomatically and hemodynamically stable. Group 1 patients had prediabetes/diabetes; Group 3 patients had to have a remote CV event (>1 year prior) and who met the other inclusion criteria were enrolled as a control group. All patients provided written informed consent. Ethics approval was obtained from the Ottawa Health Science Network Research Ethics Board and the study was conducted in accordance with the Helsinki Declaration.

In group 1 and 2, IL-6 and hsCRP were measured within 30-60 days following their CV event. For Group 3, IL-6 and hsCRP were measured >1 year after their CV event. High sensitivity C-reactive protein (hsCRP) was measured with commercially available kit available on a high throughput diagnostic platform (Roche Diagnostics, CZ). IL-6 was assayed using commercially available ELISA kit (R&D Systems).

Demographic data including age, sex, ethnicity, height, weight, waist circumference and blood pressure were recorded as well as current medications, medical history, smoking status, and family history of CV disease. IL-6 and hsCRP were measured within 30-60 days following their vascular event in those in groups 1 and 2 and at time of enrollment for those in group 3.

Demographic characteristics are presented as median (interquartile range (IQR)). Differences between patient groups were evaluated by the Fisher exact test for discrete clinical variables and by the Mann-Whitney U test for continuous variables. Follow-up data were collected as scheduled. All tests were two-sided, and a P value of <0.05 was considered statistically significant. For comparisons of hsCRP and IL-6 values between groups, Kruskal-Wallis tests were conducted to compare the medians between groups. Missing data were considered missing completely at random and pairwise deletion was used to handle the missingness. Statistical analyses were done using MedCalc® Statistical Software version 19.5.3 (MedCalc Software Ltd, Ostend, Belgium).

Results:

Participant characteristics are described in Table 1. In summary, 24 percent of participants were women, with a median age of 64.0 (IQR: 57.0, 71.0) years – these were similar between the three groups. Median BMI was 28.6 (IQR: 25.0, 33.0) and participants in the three groups had a similar proportion of cardiac risk factors except for diabetes (as expected).

Table 1. Baseline Characteristics of the Participants

Variable	Acute Events with (pre-)DM (n=18)	Acute Events without (pre-)DM (n=17)	Remote Events (n=24)
Age, years (median (IQR))	60.5 (57.0, 71.0)	64.0 (55.5, 70.25)	67.0 (60.5, 71.0)
Male sex, n (%)	13 (72%)	13 (76%)	19 (79%)
BMI, kg/m^2 (median (IQR))	28.8 (25.0, 33.3)	26.6 (24.6, 28.8)	29.1 (25.8, 34.2)
Hypertension, n (%)	13 (72%)	8 (47%)	15 (62%)
Dyslipidemia, n (%)	13 (72%)	8 (47%)	23 (96%)
Smoking, n (%)	10 (56%)	6 (32%)	14 (58%)
Diabetes/Pre-Diabetes, n (%)	18 (100%)*	0 (0%)*	12 (50%)*
Baseline medical therapy			
ECASA, n (%)	18 (95%)	16 (94%)	21 (88%)
P2Y12 Inhibitors, n (%)	18 (95%)*	15 (88%)*	12 (50%)*
Anticoagulant, n (%)	1 (5%)	1 (6%)	4 (17%)
Beta-blocker, n (%)	15 (79%)	14 (82%)	18 (75%)
ACEi, n (%)	16 (84%)	15 (88%)	15 (63%)
CCB, n (%)	2 (11%)	2 (12%)	0 (0%)
Statin, n (%)	19 (100%)	15 (88%)	23 (96%)
Metformin, n (%)	6 (32%)	0 (0%)	3 (13%)
Insulin, n (%)	3 (16%)	0 (0%)	2 (8%)
Steroids, n (%)	0 (0%)	0 (0%)	2 (8%)
Any other anti-inflammatory medication, n (%)	0 (0%)	0 (0%)	1 (4%)
Cell count and differential			

Leukocyte, $x10^9$/L (median, (IQR))	8.3 (6.9, 9.6)	6.9 (5.2, 8.1)	8.0 (7.0, 9.3)
Platelet, $x10^9$/L (median, (IQR))	234.5 (177.0, 271.0)	220.2 (170.3, 271.3)	220.0 (181.5, 254.0)
Neutrophils, $x10^9$/L (median, (IQR))	5.2 (4.5, 6.0)	3.9 (2.9, 5.3)	4.8 (4.1, 5.8)
Lymphocytes, $x10^9$/L (median, (IQR))	2.0 (1.7, 2.3)	1.7 (1.2, 2.1)	2.1 (1.6, 2.4)
Monocytes, $x10^9$/L (median, (IQR))	0.6 (0.6, 0.7)	0.6 (0.4, 0.7)	0.6 (0.5, 0.7)
Eosinophils, $x10^9$/L (median, (IQR))	0.3 (0.2, 0.4)	0.2 (0.1, 0.3)	0.2 (0.1, 0.3)
Biomarkers/Biochemistry			
IL-6, pg/mL (median, (IQR)))	4.83 (3.27, 7.45)[#]	2.47 (1.65, 5.61)[#]	2.36 (1.09, 4.00)[#]
hsCRP, mg/mL (median, (IQR))	2.20 (1.20, 7.98) (n=17)	1.00 (0.48, 2.50) (n=17)	1.20 (0.78, 2.10) (n=13)
Random glucose level, mmol/L (median (IQR))	6.10 (5.38, 7.53)	5.30 (5.03, 5.53)	5.50 (4.88, 7.18)
Creatinine, µmol/L (median (IQR))	82.0 (73.0, 97.0)	79.0 (73.0, 92.5)	81.50 (73.0, 93.5)
HbA1c, % (median (IQR))	6.25 (5.90, 7.20)	5.50 (5.40, 5.70)	5.95 (5.50, 6.65)

*Distribution of variables differed significantly across groups on Fisher's exact testing ($p<.05$)

[#]Median values differed across groups during Kruskal-Wallis testing.

Abbreviations: ACEi: angiotensin converting enzyme inhibitor, ACS: acute coronary syndrome. BMI: body mass index. CCB: Calcium channel blocker, DM: diabetes mellitus. HbA1c: hemoglobin A1c. hsCRP: high-sensitivity C-reactive protein. IL-6: interleukin-6, P2Y12: purinergic signaling receptor Y_{12}.

IL-6 median values for groups 1, 2 and 3 were 4.84pg/ml (IQR: 3.27, 7.45), 2.47pg/ml (IQR: 1.65, 5.61), and 2.36pg/ml (IQR: 1.09, 4.00), respectively with a significant difference between IL-6 levels across the three groups (p= 0.034). A post-hoc Mann-Whitney test revealed the median between group 1 and 3 was significantly different (p=0.009). hsCRP median values were 2.20mg/ml (IQR: 1.20, 7.98), 1.00mg/ml (IQR: 0.48, 2.50), and 1.20mg/ml (0.78, 2.10) respectively, and demonstrated a trend towards significant differences across the three groups (p=0.147). Results shown in Figure 1.

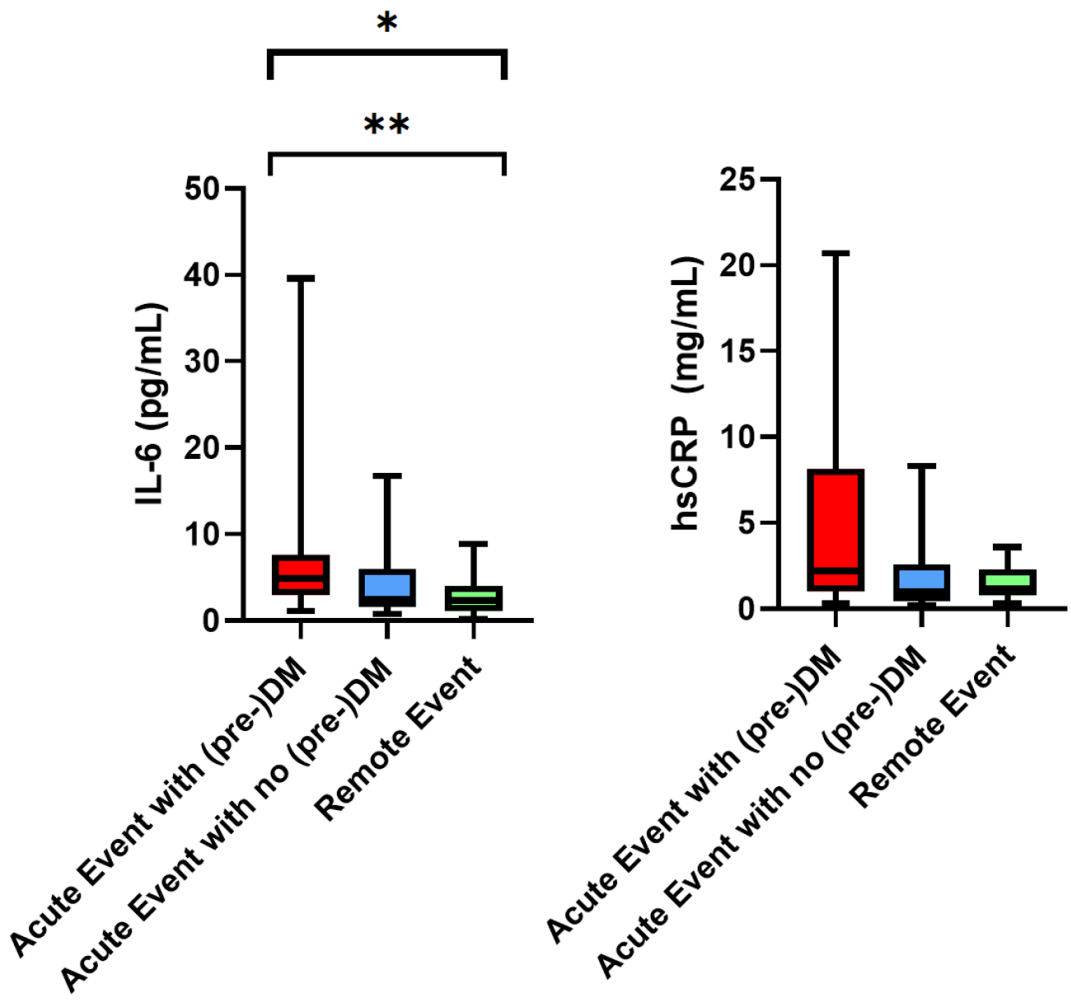

Figure 1. hsCRP and IL-6 values in patients with cardiovascular events.

Abbreviations: DM: diabetes mellitus, hsCRP: high-sensitivity C-reactive protein. IL-6: interleukin-6.

* Kruskal-Wallis testing revealed IL-6 levels differed significantly across the three groups (P<0.05).

**p=0.009 with post-hoc Mann-Whitney testing between Group 1 (acute event with (pre-)DM and Group 3 (remote event).

The subset of patients with prediabetes/diabetes and a recent cardiovascular event (group 1) had a numerical, but non-significant trend towards higher median IL-6 values (4.84pg/ml (IQR: 3.27, 7.45)) compared to the subset of patients with prediabetes/diabetes who experienced remote events (2.77pg/ml (IQR: 1.50, 4.00)). hsCRP levels were also numerically higher in patients with prediabetes/diabetes who suffered a recent cardiovascular event (1.95mg/ml (IQR: 1.09, 7.80)) compared to those with prediabetes/diabetes with remote events (1.75mg/ml (IQR: 1.20, 2.70)) (p=0.815).

Similarly, the subset with no prediabetes/diabetes and a recent cardiovascular event had a trend towards higher IL-6 values (2.47pg/ml (IQR: 1.65, 5.61)) compared to the subset of patients with prediabetes/diabetes who had remote events (IL-6: 1.57pg/ml (IQR: 0.84, 4.26)) (p=152). hsCRP did not differ significantly between the groups with median levels of 1.00mg/ml (IQR: 0.48, 2.50) in the subset with no prediabetes/diabetes and a recent cardiovascular event compared to levels of 1.00mg/ml (IQR: 0.70, 2.45) in patients without prediabetes/diabetes and remote events (p=0.815).

In looking at the group of patients with remote events, IL-6 levels were numerically (but not statistically higher) in patients with prediabetes/diabetes (2.77pg/ml (IQR: 1.50, 4.00)) compared to those without (1.57pg/ml (IQR: 0.84, 4.26)) (p=0.347). Similarly, hsCRP levels were numerically (but not statistically higher) in patients with prediabetes/diabetes (1.75mg/ml (IQR:

1.20, 2.70) compared to those without (1.00mg/ml (IQR: 0.70, 2.45) (p=0.271). Data available upon request.

Conclusions:

In this observational cohort study, IL-6 values in patients with prediabetes/diabetes who had experienced recent CV events, compared to patients with remote CV events, and there was a numerical but non-statistically significant trend of higher median values of hsCRP in patients with prediabetes/diabetes with recent CV vents compared to patients with remote CV events. There was a trend for higher IL-6 and hsCRP levels in patients with prediabetes/diabetes and recent CV events compared to those without dysglycemia who also experienced recent CV events. There was also a trend for higher IL-6 and hsCRP levels in patients with prediabetes/diabetes and a recent CV event compared to the subgroup of patients with prediabetes/diabetes and a remote CV, although this did not reach statistical significance. Finally, there was also a numerical, but non-significant trend for higher IL-6 and hsCRP levels in patients without prediabetes/diabetes and a recent CV event compared to the subgroup of patients with no prediabetes/diabetes and a remote CV.

There is a large body of evidence that supports the notion that atherosclerosis is an inflammatory process.[8-10] Cardiovascular events occur more frequently in persons with higher burdens of circulating inflammatory markers, and treating patients based on these inflammatory markers has been shown to reduce adverse outcomes.[3, 4] Inflammation promotes atherosclerotic cardiovascular disease through multiple mechanisms. It may play a role in the evolution of plaque biology and contribute to acute plaque rupture.[11] It has an overall negative impact on vessel anatomy and function after the acute cardiovascular insults.[12] The inflammatory activity is also the highest

immediately following a CV event, indicating tissue repair pathways being coordinated by inflammatory activation.

The results of our study also support the notion that patients with diabetes or prediabetes and who have had recent CV events[13], have a higher inflammatory burden than patients without recent CV events. There was also a trend to suggest that in patients who have suffered a recent CV event, there is a higher inflammatory burden in patients with dysglycemia than in those without. This is congruent with other literature that supports the notion that diabetes is linked to inflammation.[14-16] There is a growing body of literature that suggests that diabetes is a chronic inflammatory condition.[17] Numerous epidemiologic studies have demonstrated associations between plasma markers of inflammation and diabetes.[18] [19-21] In a nested case-control study of over 32,826 women, baseline levels of the inflammatory markers tumor necrosis factor (TNF)-α receptor 2, interleukin (IL)-6, and C-reactive protein (CRP) were all predictive of the future development of diabetes. In a cohort study of 75 patients undergoing coronary stenting, it was found that patients with diabetes had higher pre-procedural levels of IL-6, IL-1 receptor antagonist, and soluble CD40 ligand than those without diabetes, thereby suggesting a higher inflammatory burden in these patients.[22] There are many proposed mechanisms linking inflammation to diabetes, including pancreatic beta cell inflammation[23], inflammation generated by adipocytes[17], and even contributions related to the gut microbiome[24]. While we utilized IL-6 and hsCRP to monitor inflammation in our patient population, recent literature has suggested that other markers such as platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio may also accurately reflect inflammatory levels and have prognostic value in other inflammatory conditions.[25,26] While our study was not powered to answer these questions in a CV disease population, more research in this area will certainly be needed to

further elucidate the role that these biomarkers could potentially play in coronary artery disease prognostication and management in the future.

Utilizing anti-inflammatory therapies for secondary cardiovascular prevention is a concept that has gained much traction in recent years. In the CANTOS trial, Canakinumab treatment was particularly effective in patients who achieved a significant high-sensitivity C-Reactive Protein (hs-CRP) reduction, but was associated with a significant increase in fatal sepsis, with no impact on de-novo diabetes mellitus or worsening of diabetes control.[3] This is important since approximately 40% of the patients enrolled in the CANTOS trial were patients with diabetes. This notion suggests the importance to identifying among patients with diabetes or prediabetes, those with the most potential for benefit, as opposed to universal treatment with the risk of potential harm. Further support for the concept of anti-inflammatory therapies for reduction of CV events was noted in the LoDoCo study, in which colchicine led to a 67% reduction in the composite of ACS, arrest and ischemic stroke, double the reduction that occurs with statins.[5] LoDoCo prompted the larger randomized trial of Colchicine in 4500 patients, COLCOT, which demonstrated that low-dose Colchicine (0.5mg PO daily), reduces the rates of ischemic CV events in patients who were recently (within 30 days) post-myocardial infarction (MI).[6] Finally the recent LoDoCo 2 study demonstrated outcome benefit in 5522 patients with chronic coronary disease who were treated with Colchicine (0.5mg PO daily).[27]

There are important limitations to note in our study. This was an observational, cross-sectional study. Thus, while we observed associations between inflammatory markers and diabetes and cardiovascular events, care should be taken in the interpretation of these relationships, as we cannot

rule out underlying confounding as the explanation for these associations. Thus, these observed associations are merely hypothesis-generating, and should not be taken as causal. Additionally, our sample size was quite small, which limits the power of our study. However, even given this, the observed numerical trends across groups were consistent, lending strength to our observations.

Inflammation is a key component in the progression of atherosclerosis and its sequelae, such as myocardial infarction (MI), stroke and cardiovascular (CV) death.[3, 28] As such, it represents a potentially transformative therapeutic target. Given that patients with prediabetes/diabetes have a higher inflammatory burden, and are already high-risk for recurrent vascular events, this specific population may be an ideal group to target with therapies aimed at the reduction of inflammatory burden. These results set the stage for the evaluation and potential use of inflammation biomarkers to help define specific populations (such as those with diabetes or prediabetes) or even patients within such populations that will most benefit from anti-inflammatory therapies and thereby allow the personalization of therapies for patients at increased risk of subsequent CV events.

Disclosures

RB is the Vered Chair of Cardiology for the University of Ottawa Heart Institute (UOHI) and a uOttawa Distinguished Chair in Cardiovascular Imaging Research. He has received grants and honoraria from GEHC, LMI and JDI not related to this work. DD holds a clinician-scientist award from the Heart & Stroke Foundation of Canada. KEB holds a fellowship supported by CIHR. AYC has received unrestricted grants from Abbott not related to this work. DS is supported with a mid-career award by Heart and Stroke Foundation of Canada (HSFC). He has received unrestricted grant/in-kind support from Eli Lilly Canada, Spartan Biosciences, Aggredyne and Roche Diagnostics, which are not related to the present work. He has received honoraria for advisory boards from AstraZeneca Canada, Bayer Canada and Servier, Canada. PPL is Vice President of Research at the UOHI, and has received grants from CIHR, Genome Canada/Roche Diagnostics and HSFC.

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Chapter 5: Evaluating the mechanism of action of colchicine

CHAPTER OUTLINE

Chapter 5 addresses the third research question: *What is the mechanism of action of colchicine in the reduction of ASCVD?*

Colchicine has been found to be effective at reducing cardiovascular events as was outlined in **Chapter 3**. However, there are side effects associated with its use as well as the added burden of an additional medication for patients already potentially taking numerous medications for cardiovascular risk reduction. Thus, the ability to define its mechanism of action and then identify a subset of patients that may derive the most benefit from its use is highly desirable. Patients with diabetes are at increased risk of suffering early atherosclerotic disease related cardiovascular and have an increased risk of recurrent events above that seen in the general population as was seen in **Chapter 4**. Thus, this population represents an ideal subset to try to determine if the underlying mechanism of action of colchicine for cardiovascular risk reduction is on plaque inflammation or rather on systemic inflammation.

The protocol for a randomized controlled trial was developed to determine the underlying mechanism of action of colchicine (**Section 5.1**).

Chapter 5 contains one manuscript (Section 5.1) which has been published. The manuscript is described briefly below, and a more in-depth description is found in the first page of the section.

CHAPTER CONTENTS

Section 5.1 describes a protocol of a randomized controlled trial that investigates the underlying mechanism of action of colchicine. This study will be a multicentre, prospective, randomized, double-blinded, placebo-controlled study to determine the effect of colchicine on arterial inflammation as assessed with imaging and circulatory biomarkers, specifically carotid arteries and aortic FDG uptake as well as hs-CRP and IL-6 among others. Patients with T2DM or pre-diabetes who have recently experienced a CV event (within 30 to 120 days after an ACS (i.e. ST-elevation MI (STEMI) or non-STEMI) or TIA/stroke with documented large vessel atherosclerotic disease, will be randomized to treatment with either colchicine 0.6 mg oral daily or placebo. Participants will undergo baseline evaluation including an FDG PET/CT scan of the ascending aorta and left and right carotid arteries. Patients will then undergo treatment with

placebo or colchicine for 6 months and have repeat evaluation with an FDG PET/CT scan at the conclusion of the study. The primary outcome will be the change in the maximum target to background ratio (TBR_{max}) in the ascending aorta (or carotid arteries) (a surrogate for vascular/plaque inflammation) from baseline to follow-up on FDG PET/CT imaging.

Section 5.1 The Canadian Study of Arterial Inflammation in Patients with Diabetes and Recent Vascular Events, Evaluation of Colchicine Effectiveness (CADENCE): protocol for a randomised, double-blind, placebo-controlled trial.

SECTION OVERVIEW

This section builds upon the contents of **Chapter 3**, where colchicine was shown to be an effective treatment in the prevention of cardiovascular events, as well as **Chapter 4**, which showed that patients with dysglycemia have elevated levels of systemic inflammation. Thus, this section presents a manuscript of a protocol for a randomized controlled trial that investigates the underlying mechanism of action of colchicine in a high-risk inflammatory subgroup.

MANUSCRIPT STATUS

This manuscript has been published.

Boczar KE, Shin S, deKemp RA, Dowlatshahi D, Tavoosi A, Wiefels C, Liu P, Lochnan H, MacPherson PA, Chong AY, Torres C, Leung E, Tawakol A, Ahmadi A, Garrard L, Lefebvre C, Kelly C, MacPhee P, Tilokee E, Raggi P, Wells GA, Beanlands R. The Canadian Study of Arterial Inflammation in Patients with Diabetes and Recent Vascular Events, Evaluation of Colchicine Effectiveness (CADENCE): protocol for a randomised, double-blind, placebo-controlled trial. BMJ Open. 2023 Nov 10;13(11):e074463. doi: 10.1136/bmjopen-2023-074463. PMID: 37949621; PMCID: PMC10649523.

Supporting material

Supplementary file 1: SPIRIT checklist

Supplementary file 2: CADENCE event definitions

Supplementary file 3: CADENCE inclusion and exclusion criteria

Supplementary file 4: Administration of CADENCE study

Author roles and contributions

KEB, GW, and RB designed the study. KEB drafted the manuscript, which was revised by all authors. All authors approved the version of the manuscript submitted for publication.

RELATED THESIS APPENDICES

Appendix G: Published manuscripts. The PDF of published manuscript is presented in Appendix G.

The Canadian Study of Arterial Inflammation in Patients with Diabetes and Recent Vascular Events, Evaluation of Colchicine Effectiveness (CADENCE): protocol for a randomised, double-blind, placebo-controlled trial.

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Protocol version 1.0 (March 27, 2023)

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Abstract

Background

Inflammation is a key mediator in the development and progression of the atherosclerotic disease process as well as its resultant complications, like myocardial infarction (MI), stroke, and cardiovascular (CV) death, and is emerging as a novel treatment target. Trials involving anti-inflammatory medications have demonstrated outcome benefit in patients with known CV disease. In this regard, colchicine appears to hold great promise. However, there are potential drawbacks to colchicine use, as some studies have identified an increased risk of infection, and a non-significant trend for increased all-cause mortality. Thus, a more thorough understanding of the underlying mechanism of action of colchicine is needed to enable a better patient selection for this novel CV therapy.

Objective

The primary objective of the CADENCE trial is to assess the effect of colchicine on vascular inflammation in the carotid arteries and ascending aorta measured with ^{18}F-fluorodeoxyglucose (FDG)-positron emission tomography (PET)/computed tomography (CT) in patients with type 2 diabetes mellitus (T2DM) or pre-diabetes who have experienced a recent vascular event (acute coronary syndrome (ACS)/MI, transient ischemic attack (TIA), or stroke). Secondary objectives include determining colchicine's effect on inflammatory biomarkers (high-sensitivity C reactive protein (hsCRP) and interleukin-6 (IL-6). Additionally, we will assess if baseline inflammation imaging or biomarkers are associated with a treatment response to colchicine determined by imaging. Exploratory objectives will look at: i) the difference in the inflammatory response to colchicine in patients with coronary events compared to patients with cerebral events, and ii) the difference in the inflammatory response to colchicine in different vascular beds; iii) the relationship of FDG-PET imaging markers with serum biomarkers; and iv) assessment of quality-of-life changes.

Methods and Design

CADENCE is a multicentre, prospective, randomized, double-blinded, placebo-controlled study to determine the effect of colchicine on arterial inflammation as assessed with imaging and circulatory biomarkers, specifically carotid arteries and aortic FDG uptake as well as hs-CRP and IL-6 among others. Patients with T2DM or pre-diabetes who have recently experienced a CV event (within 30 to 120 days after an ACS (i.e. ST-elevation MI (STEMI) or non-STEMI) or TIA/stroke with documented large vessel atherosclerotic disease, will be randomized to treatment with either colchicine 0.6 mg oral daily or placebo. Participants will undergo baseline clinical evaluation including EQ5D assessment, blood work for inflammatory markers, and FDG PET/CT scan of the ascending aorta and left and right carotid arteries. Patients will undergo treatment for 6 months and have repeat clinical evaluation including EQ5D assessment, blood work for inflammatory markers, and FDG PET/CT scan at the conclusion of the study. The primary outcome will be the change in the maximum target to background ratio (TBR_{max}) in the ascending aorta (or carotid arteries) from baseline to follow-up on FDG PET/CT imaging.

Discussion

Colchicine is an exciting potential new therapy for CV risk reduction. However, its use is associated with side-effects and greater understanding of its underlying mechanism of action is needed. Importantly, the current study will determine whether its anti-inflammatory action is an indirect systemic effect, or a more local plaque action that decreases inflammation. The results will also help identify patients who will benefit most from such therapy.

STRENGTHS & LIMITATIONS OF THIS STUDY

- This study examines the mechanistic effect of colchicine on inflammation within atherosclerotic plaque of high-risk patients with diabetes or pre-diabetes who have suffered recent vascular events (ACS/MI, TIA, stroke).
- Using ^{18}F-fluordeoxyglucose (FDG) PET/CT at two timepoints, this study will evaluate changes in the carotid arteries and thoracic aorta to determine local mechanisms of colchicine.
- The response to colchicine and clinical outcomes will be explored with systemic inflammatory biomarkers (e.g, interleukin-6, interleukin-1β, tumor necrosis factor-α, monocyte chemoattractive protein-1, and others), major adverse cardiac events, all-cause mortality, and quality of life outcomes.
- As this is a mechanistic study, it is underpowered to investigate whether colchicine in high-risk patients would lead to improvements in clinical endpoints but will help justify future studies with such high-risk populations.

Trial Registration

ClinicalTrials.gov Identifier: NCT04181996

Keywords

Atherosclerotic cardiovascular disease, inflammation, colchicine, cardiac imaging, FDG-PET imaging

Introduction

Inflammation plays a crucial role in the development and progression of atherosclerosis and its complications, like myocardial infarction (MI), stroke, and cardiovascular (CV) death.[1-3] Given its etiologic role, inflammation is now emerging as an important treatment target for CV risk reduction.[2-4] A number of trials have demonstrated that anti-inflammatory therapies may improve outcomes in patients with CV disease. The CANTOS Trial[3] demonstrated CV benefit in patients treated with canakinumab, a monoclonal antibody directed against interleukin 1-beta. The intervention was more effective in patients who achieved a greater reduction in the inflammation biomarker high-sensitivity CRP (hs-CRP). However, two factors severely limit the use canakinumab to a large number of patients: its cost and the associated increased risk of sepsis. Indeed, canakinumab has been demonstrated to not be cost-effective for a cardiovascular risk-reduction indication.[5] Thus, the search for a cost-effective anti-inflammatory therapy to reduce CV events continues. Studies including the LoDoCo2 trial[6] and COLCOT[7] have also demonstrated CV outcome benefit in patients with known atherosclerotic coronary disease who are treated with colchicine. Despite its proven efficacy, the mechanism whereby this drug reduces cardiovascular events remains unknown. The true anti-inflammatory properties of colchicine are also largely unknown, although they are believed to include inhibition of neutrophil function via an antitubulin effect, and inhibition of the $NLRP_3$ inflammasone .[8] Clinical efficacy has been demonstrated for several inflammatory conditions including pericarditis[9], gout[10], and familial Mediterranean fever[11]. However, colchicine has several side effects, predominantly gastrointestinal upset, that limit its tolerability and reduce patients' compliance. Additionally, recent cardiovascular trials showed a non-significant trend toward an increase in all-cause mortality with colchicine therapy.[6, 7, 12] Thus, further investigations into colchicine mechanisms of actions are warranted. Although the recent trials showed a reduction in cardiovascular events with colchicine, there was no correlation with baseline and follow-up serum markers of inflammation. Hence, it is possible that colchicine may be acting to reduce inflammation within the atherosclerotic plaque, rather than at a systemic level.

The primary objective of CADENCE is to investigate the biological effect of the medication colchicine, compared to placebo, on the inflammatory activity of atherosclerotic plaque (measured by ^{18}F-fluorodeoxyglucose (FDG)- positron emission tomography (PET)/computed tomography (CT) at baseline and 6 months) in the carotid arteries and ascending aorta in high-risk patients with type 2 diabetes mellitus (T2DM) or pre-diabetes and recent vascular events (acute coronary syndrome (ACS)/MI, transient ischemic attack (TIA), or stroke). Specifically, we will look at whether colchicine acts in a systemic anti-inflammatory fashion or whether its mechanism of action is more of a local effect at the plaque level. FDG PET/CT has demonstrated to be an efficient and practical proxy method to assess inflammation in the aorta.[13] Prior studies have demonstrated that therapies that reduce aortic FDG uptake also predict clinical efficacy.[14-16]

The secondary objectives of this study are to explore **a)** whether baseline inflammation imaging (FDG PET/CT) and/or inflammatory biomarker (hs-CRP and IL-6) levels can identify which patients are most likely to have a positive response to colchicine, as indicated by the reduction in FDG uptake over a period of 6 months; and **b)** the correlation between response to colchicine as measured by reduction of inflammation as proxied by imaging with FDG PET/CT and hs-CRP at baseline and during a 6-month follow-up.

Our exploratory objectives are to investigate **a)** if there are local differences (i.e. in the carotid arteries versus aorta, respectively) in the anti-inflammatory effect of colchicine therapy in patients who have suffered recent coronary events (ACS/MI) compared to those with recent cerebrovascular events (TIA, stroke); **b)** if other systemic inflammatory biomarkers (e.g, interleukin (IL)-6, IL-1β, tumor necrosis factor (TNF)-α, monocyte chemoattractive protein (MCP)-1, others) predict a response to therapy; **c)** the relationship of the inflammatory response as proxied by imaging with FDG PET/CT to: i) systemic inflammation biomarkers; ii) monocyte activation; **d)** the treatment effect of colchicine on clinical outcomes

such as major adverse CV events and all-cause mortality; and **e)** the effect of colchicine on patient quality of life measures.

Materials and Methods

CADENCE is a multicentre, prospective, randomized, double-blind placebo-controlled study designed to assess the effect of anti-inflammation therapy using colchicine, on arterial inflammation using imaging and circulatory biomarkers, specifically carotid arteries and aortic FDG uptake and hs-CRP. This protocol was completed in accordance with the SPIRIT reporting guidelines (see Supplementary Material 1).[17]

Study Population

At the University of Ottawa Heart Institute (UOHI) and the Mazankowski Alberta Heart Institute (MAHI), patients with Type 2 diabetes mellitus (T2DM) or pre-diabetes with a recent ACS in the last 30 to 120 days prior to recruitment (i.e. ST-elevation myocardial infarction (STEMI) or non-ST elevation myocardial infarction (NSTEMI)), stroke, or transient ischemic attack (TIA) will be recruited. We will use standard definitions for ACS events including chest pain, EKG changes and troponin rise. For cerebrovascular events, non-embolic ischemic stroke will be confirmed by CT imaging or magnetic resonance imaging (MRI), and TIA events will be assessed and determined by a neurologist. The criteria for exclusion from the study include patients who have a coronary revascularization procedure planned over 120 days after the qualifying event, decompensated heart failure, severe left ventricular (LV) systolic dysfunction (LV ejection fraction<30%), severe valvular disease that requires intervention, and other active diseases. A detailed list of inclusion

and exclusion criteria are available in Supplementary Material 2. The study start date is August 1, 2020 and the planned completion date is December 31, 2023.

Enrollment and Baseline Assessment

Patients who meet inclusion/exclusion criteria will undergo clinical evaluation, FDG PET imaging with CT angiography (CTA), and blood sample collection within 30 to 120 days of cardiovascular event. HbA1C, Fasting Blood Sugar (FBS), lipids, C-reactive protein (CRP), creatinine (CR), glomerular filtration rate (GFR), complete blood count (CBC), hemoglobin, liver function tests (i.e. AST, ALT), creatine kinase (CK), as well as blood samples for biomarkers and immunological studies will be collected from all the patients at baseline.

Patients recruited into the CADENCE study will be randomized to receive either an oral colchicine 0.6 mg tablet within a capsule or identical placebo capsule administered once daily for 6 months. The placebo capsule will consist of an opaque gelatin capsule shell and a microcrystalline cellulose filler.

Randomization

Eligible and consenting patients (Supplementary material 3) will be randomized 1:1 to the treatment or placebo arm. The Cardiovascular Research Methods Centre (CRMC) at the uOttawa Heart Institute (UOHI) will create a computer-generated randomization schedule with randomly varying block sizes (predefined and masked to study investigators) stratified according to centre,

and whether the recent vascular event was cardiac or cerebral. Randomization will be done centrally through a web-based system.

Follow-up

Clinical evaluation and blood collection of biomarkers, CR, CRP, AST, ALT and CK will be repeated at 3 months and 6 months. FDG PET imaging with CTA will be repeated at 6 months. Clinical outcomes will be determined at each time point (Figure 1). Patients will also be followed up by phone at 1-week and 1-month post-initiation of the study drug, to assess for side effects, safety, and adherence to treatment. Study participants will be instructed to return all unused drug to each follow-up study visit to assist in determining treatment adherence.

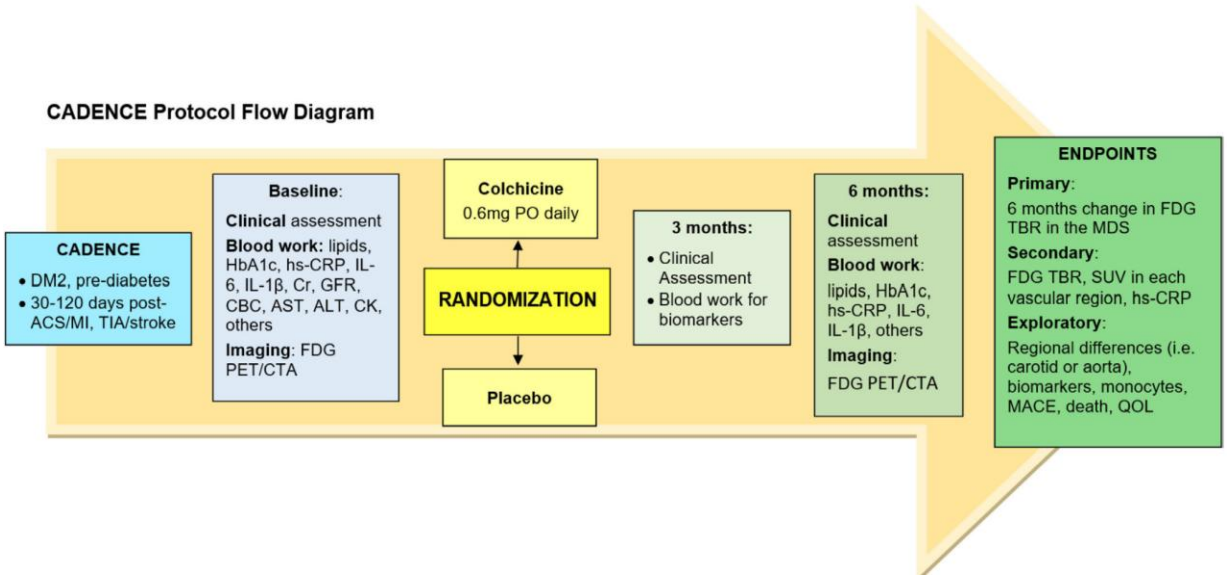

Figure 1. Figure 1 – Flow chart. ACS, acute coronary syndrome; CBC, complete blood count; CK, creatinine kinase; CTA, CT angiography; FDG, ^{18}F-fluorodeoxyglucose; GFR, glomerular filtration rate; hsCRP, high-sensitivity C reactive protein; IL, interleukin; MACE, major adverse cardiac events; MDS: most diseased segment; MI, myocardial

infarction, PET, positron emission tomography; QOL, Quality of Life; SUV, Standardized Uptake Value; TBR, Tissue to Blood Ratio; TIA, transient ischemic attack.

Blinding

This trial will be conducted in a double-blind fashion. Patients will be blinded to their treatment status, and those randomized to the placebo arm will receive a placebo pill that is identical in colour and consistency to the colchicine arm. Physicians and any study personnel having contact with the patients will also be blinded with regards to the patient allocation. However, in the event of an emergency, in which knowledge of the study treatment allocation is critical, the blinding may be broken by the treating physician.

Measures

a) ***FDG imaging of carotid arteries and aorta:*** All patients will be advised to limit strenuous exercise and avoid using any cannabis products for 24 hours before the scan. The advice given to the recruited patients for PET imaging preparation will depend on the anti-diabetic medications that are prescribed to them by their respective treating physicians.

The evening before the PET imaging:

- Low-carbohydrate, high fatty meals (keto diet) for patients on oral anti-diabetic medications (except for insulin secretagogues).
- Regular meal for patients on insulin or insulin secretagogues; such patients also receive personalized advice about their meal to maintain an optimum blood glucose.
- Usual anti-diabetic medications for all patients.
- Fasting overnight for all patients.

On the morning of PET imaging:

- Half of the usual oral anti-diabetic medications (except for insulin secretagogues).
- No insulin or insulin secretagogues.
- A standardized protocol is in place for managing hyperglycemia on the day of the scan. The scan will be postponed if the fasting blood glucose is ≥11 mmol/L until the blood glucose is better controlled.

Upon arrival an IV will be inserted in the arm. FDG (2-3 MBq/kg) will be injected intravenously. After the FDG injection patients will have a 2-3 hour wait period. During the first hour the patient will be asked to sit quietly followed by a 1-2 hour wait period when the patient can move about, talk, and read. The waiting period will allow the uptake of FDG by normal tissues and/or areas of inflammation, while there is a decrease in the remainder of the background activity (i.e. blood pool) creating an ideal target-to-background ratio.

After scout imaging to check proper positioning, a low-dose CT scan will be done for attenuation correction (AC) of the PET data. A respiratory-gated PET scan will be acquired in 3D mode over 3 bed positions to span the common and internal carotid arteries and the thoracic ascending aorta. High-resolution (3-5 mm) images of FDG uptake (Bq/mL) will be reconstructed using iterative statistical reconstruction with no post-filtering. The prep at baseline will be replicated at follow-up including the time of scan whenever possible.

b) *CTA:* Following FDG image acquisition, a non-contrast enhanced helical CT scan will be used to define the limits of the CTA study. CTA will be performed on a multidetector computed tomography scanner with a minimum of 64-slices: 60 ml of contrast will be injected at a rate of 3.2 ml·s-1, 120-180 minutes after FDG injection. Similar to the scanning protocol used in our prior

studies, using smart prep bolus-tracking, the CTA study will be triggered at 80 HU in the ascending aorta.[18-20] The study will be acquired with the following settings: 20 mm detector coverage, 120 kVp, mA dose-modulation between 150-550 mA, 0.5 s per rotation, noise index=16, 0.625 mm thick slices reconstructed at 0.625 mm intervals, table speed 19.37 mm/per rotation to cover the limits determined from the initial helical CT scan in 3.6 s.[18] Anonymized images will be transferred to the UOHI Core Laboratory via a password protected network interface.

c) *PET/CT Image Analysis* will be performed in a standardized fashion by two readers *blinded* to patient data and treatment arm. As described previously,[18, 21, 22] the bifurcation location of the common carotid artery will be identified on CTA. If a CTA cannot be performed, then a non-contrast CT scan will be used instead of the CTA.[18, 21] To quantify FDG uptake, a circular region of interest will be positioned on consecutive transaxial PET images. For the aorta, this begins 1 cm above the aortic valve plane and continues to the base of the aortic arch. For the carotid arteries, it starts 2 cm below the bifurcation up to 2 cm above it in the internal carotid artery.[21] Maximum FDG uptake (Bq/ml) is then measured and will be normalized to the injected activity and body-weight in order to determine the standardized uptake value (SUV g/ml).[18] The Target-to-Blood Ratio (TBR) is calculated by taking the SUVs of the artery wall, and normalizing the values to the background venous activity (SVC or internal jugular for aorta or carotid arteries respectively). Finally, the TBR in the area of maximal FDG uptake (otherwise known as the most diseased segment (MDS)) will be recorded. This approach has excellent intra- and inter-observer variability.[21, 23] The TBR in the MDS from the baseline scan will be compared to the follow-up images at 6 months.

d) *Biomarkers:* At baseline, 3- and 6-month follow-up, soluble markers of inflammation (e.g. hs-CRP, IL-6, IL-1β, TNF-α, MCP-1) will be assessed in cryopreserved plasma samples kept frozen at -80ºC, using individually validated human cytokine ELISA assays (Roche Diagnostics, R&D Systems and Abcam)[24] with coefficient of variation <20% (<10% in most cases).[24] Since other novel biomarkers are regularly being discovered by our group, all blood samples will be stored (with patient consent) to ensure the ability to assess future promising biomarkers.[25-33] Measurements will be made in duplicate.

e) *Immunologic Studies:* We will collect whole blood at baseline and at 6 months into heparinized tubes. Peripheral blood mononuclear cells will then be isolated by Ficoll-Paque density centrifugation. Monocyte sub-populations, as well as their relative proportions, will be detected by flow cytometry using fluorochrome-labeled antibodies to CD14, and CD16.[19] Similar to other literature and our prior study, classical monocytes will be defined as CD14+CD16-, non-classical as CD14+CD16++, and intermediate as CD14++CD16+.[19, 34, 35] Monocyte subsets will be correlated with plaque imaging studies. They will also be compared to normal monocyte datasets derived from previous studies.

f) *Quality of life*: At baseline and at 6 months, the EQ5D will be administered to all participants.[36]

Study Outcomes

The primary endpoint of this study is the change in the FDG TBR at 6 months as a proxy measure of arterial plaque inflammation in the maximally diseased segment (MDS) (the arterial segment with the highest TBR at baseline) in any vascular district imaged (either the left or right carotid artery or ascending aorta).

Secondary endpoints include a) FDG TBR, FDG standard uptake value (SUV) and their change in the MDS of each vascular region: ascending aorta, left and right carotid artery; b) hs-CRP and its change.

Exploratory endpoints include a) plasma levels of other cytokines and inflammation biomarkers; b) levels of activated monocytes; c) major adverse cardiac events (MACE) (defined as the composite of ACS/MI, TIA, stroke, or CV death)); d) non-cardiovascular death (all events will be confirmed by an independent adjudication committee that is blinded to the treatment arm); 3) quality-of-life outcomes measured on the EQ5D[36].

Sample Size

There are no previously published studies evaluating FDG over time in patients with T2DM or pre-diabetes who have suffered recent CV events (our study population) upon which to base a definitive sample size calculation. We evaluated the ascending aorta as a non-culprit region in patients from our prior study[18] with diabetes and recent events where the aorta was in the field of view (n=6): TBR_{max}=3.38±0.60. In the culprit carotid arteries in patients with diabetes (n=12) TBR_{max} was 3.58±0.0.88.

Based on the literature and feedback from experts, the minimal clinically important difference (MCID) in TBR_{max} reduction is 10-15%. This is feasible since FDG differences between treatments in prior studies was also 10-14%.[21, 22] Taking a conservative approach based on our TBR_{max} values in our patients with T2DM with recent events, for a 10% reduction compared to placebo with a power of 80%, alpha 0.05, we will need 50 patients per group. Considering 10% withdrawal, and 2% of patients who complete baseline measures but do not start study drug, 115 patients will be recruited (12/115 (10.4%) withdrawal and 3/115 (2.6%) not starting drug), both similar to the LoDoCo trial[37]. Prior studies have included 21-34 patients/group.[21, 22, 38, 39] Thus, our sample size is expected to enable us to address our hypothesis.

Statistical Analysis

Patient data will be entered and managed using REDcap[40] hosted at UOHI. Descriptive statistics (proportions, means, medians, standard deviations, interquartile ranges) will provide summaries of study characteristics and insight into their distributional properties to determine if underlying assumptions of the

planned analysis are satisfied. Additionally, we will create diagnostic plots and both graphically, as well as numerically, check to ensure that the assumptions of our regression model are satisfied. Our data will be analyzed with an intention-to-treat analysis. Additionally, a per protocol analysis will be conducted as a key secondary analysis. No interim analyses are planned.

The ***primary outcome*** (change in FDG TBR) will be compared between the colchicine and placebo arms within a multiple regression model that includes type of vascular event (cardiac or cerebral) as a covariate.

To evaluate whether inflammation imaging or biomarker levels predict the inflammation response measured as the change in TBR_{max} on FDG PET/CT (as a ***secondary objective***), we will conduct a regression analysis with baseline vascular TBR_{max}, baseline vascular SUV, and inflammatory biomarkers included as covariates. The regression coefficient will be tested using the Wald test and 95% CI of the coefficient will be calculated. A Pearson correlation analysis to evaluate the association of the inflammatory response, as proxied on FDG PET/CT, to the change in hs-CRP levels from baseline to 6 months after treatment with colchicine will be conducted.

Several sources of bias and confounding exist within the trial. As a sensitivity analysis, for assessing the robustness of the primary analysis, if clinically significant differences between arms in pertinent baseline prognostic variables (e.g., hypertension, HbA1C, diabetes vs pre-diabetes) are identified, multiple regression analysis will be conducted to compare FDG TBR between arms adjusting for these variables. As an exploratory analysis, we will look for interactions between treatment assignment, age, sex, vascular bed (carotid arteries versus aorta), time from cardiovascular event, type of vascular event (coronary (ACS/MI) versus cerebrovascular events (TIA, stroke)), and the medication classes of sodium-glucose Cotransporter-2 (SGLT-2) inhibitors and glucagon-like peptide 1 (GLP-1) receptor agonists as covariates within the model both to assess for meaningful interactions of these variables with the primary outcome, but also to ensure the results remain stable after adjustment and ensure our results remain stable after adjustment for these potential confounders. Similar regression analysis strategies will be considered if other biomarkers are also associated with an imaging response to colchicine. We will assess the relationship between the

inflammatory response to colchicine on FDG/PET imaging with MACE by conducting a logistic regression analysis with MACE as the outcome variable and the change in vascular TBR_{max} as a covariate. We will also explore patient-centered quality of life outcomes between colchicine and placebo groups with student t-tests. Any missing data will be considered missing at random.

Patient and Public Involvement

A patient partner with lived experience was actively involved in the design and the ongoing conduct of the study. They currently serve on the steering committee of the study and are involved with regular meetings with respect to study conduct. Study results will be directly communicated to study participants. As part of our outcomes, we are assessing quality of life metrics, as advised by our patient partner.

Discussion

Vascular inflammation is an important contributor to the progression of atherosclerosis and its resultant vascular sequelae, like MI, stroke and CV death.[1, 3, 41, 42] An increasing evidence base has demonstrated that it is a novel therapeutic target for cardiovascular risk reduction. Colchicine has emerged as a novel anti-inflammatory agent for cardiovascular risk reduction. However, concerns have recently been raised regarding the adverse side effects of colchicine beyond its known gastrointestinal side effects, including a non-significant increase in the rate of infections and all-cause mortality. Thus, further research regarding the underlying mode of action will hopefully elucidate the role of colchicine and which patients may be best suited to receive this anti-inflammatory agent. Hence, our goal is to help clarify the underlying mechanism of action of colchicine, utilizing a proxy of inflammation such as FDG-PET imaging as well as levels of circulating biomarkers, to assess local vascular anti-inflammatory versus systemic anti-inflammatory activity.

Our study design has several strengths. First, we will perform the study in a methodologically rigorous fashion by utilizing a prospective, randomized, double-blinded, placebo-controlled design to answer our research question. Second, we will utilize advanced cardiovascular imaging methods, FDG-PET imaging,

to investigate the underlying mechanism of action of colchicine. Trial results looking at the pharmacological impact on arterial inflammation as proxied by imaging with FDG PET/CT have mirrored the results of clinical CV outcome trials.[21, 22] Therefore, FDG PET/CT inflammation imaging represents a practical method to define the mechanistic targets of a medication's effect in smaller patient cohorts over a short time period.[42] It may also be a useful tool to assist in identifying higher risk patients to improve patient selection for novel therapies. Third, as an exploratory outcome, we will look at the effect of colchicine therapy on quality-of-life outcomes. Finally, this is one of the first studies to utilize advanced imaging techniques to evaluate the underlying mechanism of action of colchicine for CV indications.

It is noteworthy that pharmaceutical agents which have shown outcome benefits have demonstrated reductions in inflammation measured by FDG PET including statins, pioglitazone and canakinumab, whereas as drugs which have not shown to reduce cardiovascular events have not shown such local anti-inflammatory effects. We anticipate in this high-risk group of patients in CADENCE, inflammation reduction will also be demonstrated. An added benefit of CADENCE and other studies is that they will support the utilization of inflammation vascular imaging such as FDG PET to enable better selection of patients who benefit from inflammation targeted therapies. Further studies would be needed to support this concept.

Our study also has important limitations. Firstly, as this is a mechanistic study, it is underpowered to evaluate for meaningful differences in clinical endpoints, and will be underpowered to correlate imaging and clinical endpoints. This may limit the clinical validity of our results. However, evaluation of pharmacological impact on arterial inflammation as proxied by imaging with FDG PET/CT have mirrored the results of clinical CV outcome trials in the past.[21, 22, 38] Thus, FDG indices of vascular inflammation serve as a good surrogate marker for important clinical endpoints, without necessitating the large size and scale of a trial looking at clinical endpoints. Secondly, our study recruits patients with both cardiac and neurologic vascular events; thus, there is the potential that any overall effect seen is confounded or mediated by the vascular bed treated (neurologic versus cardiac). To address this, as mentioned, we will perform

interaction t-tests to compare the overall results of our regression analysis in the subgroup of patients with recent coronary (ACS/MI) versus cerebral vascular events (TIA, stroke). Additionally, interaction t-tests will be conducted to evaluate differences in the responses of each of the vascular beds separately (carotid versus aorta).

With a large portion of the population suffering from cardiovascular disease, discovering novel methods of treatment is of high clinical value. Anti-inflammatory therapies such as colchicine may fill this role, however for widespread adoption a thorough understanding of their underlying mechanism of action is needed. Furthermore, with an increasing recognition that many of these therapies, including colchicine, carry some increased non-cardiovascular risk, targeting higher-risk populations may tip the risk-benefit scale in favour of treatment with these agents. The results obtained from this study will provide essential information with respect to the underlying mechanism of action as well as clinical benefit of colchicine therapy in patients with high cardiovascular risk. We believe our findings will bridge an important knowledge gap in this area and will spark new research in cardiac imaging and anti-inflammatory therapies for CV disease.

Conclusion

Colchicine warrants further study to confirm the safety and benefit for patients with CV disease, as well to learn more regarding its mechanism of action. The results of this randomized control study will contribute to our understanding of the mechanism of action of colchicine by measuring the impact on plaque size, biomarkers and MACE outcomes. Results of this study will inform us as to whether additional studies with colchicine should be pursued.

Author contributions

Authors KE Boczar, R Beanlands, P Raggi, and R deKemp were involved with project conceptualization. Authors KE Boczar, GA Wells, R Beanlands, R deKemp, A Tawakol were involved with the design of the methodology. Authors KE Boczar, R Beanlands, P Raggi, P.

MacPhee, L. Garrard, A. Ahmadi, C. Lefebvre, C. Kelly, and R. deKemp are involved in project administration. Trainee involvement in the project has been supervised by authors GA Wells and R Beanlands. Writing of the original protocol was performed by KE Boczar, R Beanlands, G Wells, P. Raggi, and R deKemp. Writing of the original manuscript draft was conducted by authors: KE Boczar and S Shin. Review and editing of the manuscript was conducted by KE Boczar; S Shin; P Raggi, R. deKemp, D Dowlatshahi, A Tavoosi, A Tawakol, A Tavoosi, P Liu; H Lochnan, AY Chong, C Torres, E Leung, C Wiefels, A Ahmadi, C Lefebvre, L Garrard, C Kelly, P MacPherson, P MacPhee, E Tilokee, GA Wells, and R Beanlands.

Competing interests

RB receives or has received honoraria from, and leads grants supported by GE Healthcare, Jubilant DraxImage and Lantheus Medical Grants. These are not related to the current work. RdK receives royalties from rubidium-82 PET technologies licensed to Jubilant Radiopharma and to INVIA Medical Solutions. He has also received research funding and honoraria from Jubilant Radiopharma and IONETIX. Otherwise, the authors declare that they have no competing interest.

Availability of data and material

After the publication, data will be available upon reasonable request.

Acknowledgements

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Ethical and Regulatory Standards

This trial will be conducted in compliance with the protocol, current version of the Declaration of Helsinki, Good Clinical Practice (GCP), as defined by the International Conference on Harmonization

(ICH) where applicable. If revised versions of these documents are published during the course of the trial, the new version will be adopted.

Ethics approval and consent to participate

Before study initiation, this study was approved by the Ottawa Health Science Network Research Ethics Board and University of Alberta Health Research Ethics Board- Biomedical Panel), for the protocol, consent, study materials, study process. All participants are required to give informed written consent to participate in the trial. Consent will be obtained by CADENCE trial personnel.

Consent for publication

Not applicable

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Supplementary file 1.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	4
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	19
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	19
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	2

Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	24
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	SM
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6-7
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	7
Objectives	#7	Specific objectives or hypotheses	7-8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be	8

		collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8, SM
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	SM
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	9-10
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	SM
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	13-14
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	21

Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	14
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10

Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10-12
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	SM
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	SM
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14-15
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	15

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	SM
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	14
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	SM
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	SM
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	19
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	24

Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	24
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	SM
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	24
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	24
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	9-10
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	SM
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	24-25
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	24

Appendices

Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	SM
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

Supplementary file 2:

CADENCE – Event Definitions

EVENT TERMS	DEFINITIONS
Acute Coronary Syndrome (ACS): (1)	ACS would include ST-elevation myocardial infarction, Non-ST-elevation myocardial infarction and unstable angina. For the purposes of this study, ACS should be adjudicated as an MI or Unstable Angina based on the definitions provided below. ACS itself should not be chosen as an event.
Myocardial Infarction: (1)	Based on source documentation provided i.e. admission or discharge summary +/- ECG, labs etc.
STEMI	Would be ST-elevation in 2 contiguous leads PLUS criteria below (Type 1 MI)
NSTEMI	Would be definition below (Type 1 MI) but without ST-elevation
Type 1 MI	Detection of a rise and/or fall of cTn values with at least one value above the 99th percentile URL and with at least one of the following: • Symptoms of acute myocardial ischemia • New ischemic ECG changes • Development of pathological Q waves • Imaging evidence of new loss of viable myocardium or new regional wall motion abnormalities in a pattern consistent with an ischemic etiology. Identification of a coronary thrombus by angiography including intracoronary imaging or by autopsy
Cardiac procedural myocardial injury: PCI procedural MI (1)	Occurring < 48 hours after index procedure with: • Elevation of cTN levels more than 5x the 99th percentile in patients with normal baseline levels • If levels elevated before procedure, post procedure enzyme should rise>20%. Absolute level still needs to be 5 x the 99th percentile URL PLUS ONE OF: • New ECG changes • Development of new Q waves • Imaging evidence of new loss of viable myocardium or new WMA on echo • Cath evidence of new occlusion, dissection, distal embolization
Cardiac procedural myocardial injury: CABG	Occurring < 48 hours after index procedure with: • Elevation of cTN levels >10 x 99th percentile URL in patients with normal baseline

procedural MI (1)	• If baseline abnormal they must rise>20% and still be >10 x URL PLUS ONE OF: • Development of new Q waves • Cath evidence of new graft occlusion or new native artery occlusion • Imaging evidence of new loss of viability or new WMA consistent with an ischemic etiology
Unstable angina: (1)	[New or worsening] symptoms of myocardial ischemia with or without ECG changes, but no elevation of cTn leading to hospital admission.
TYPE II MI *	Detection of a rise and/or fall of cTn values with at least 1 value above the 99th percentile URL, and evidence of an imbalance between myocardial oxygen supply and demand unrelated to acute coronary atherothrombosis, requiring at least 1 of the following: • Symptoms of acute myocardial ischemia • New ischemic ECG changes; • Development of pathological Q waves; • Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischemic etiology
Arrhythmia:	• Documented cardiac arrest • Multiple ICD shocks requiring admission • Documented ventricular tachycardia/fibrillation. • Documented new onset or worsening atrial fibrillation (or flutter) requiring IV therapy or increase in oral rate control medications. *Type of Arrythmia noted will be indicated as Cardiac Arrest, VT, VF, VT with ICD shock, VF with ICD shock, new AF, worsening AF*
Worsening heart failure: (2)	Hospital admission requiring IV diuretics plus at least one sign **AND** one symptom of worsening fluid overload. **Signs:** • Pulmonary rales • Elevated jugular venous pressure > 5 cm above sternal angle • Lower extremity edema • Chest x-ray demonstrating pleural effusion, pulmonary congestion, or cardiomegaly **Symptoms:** • Paroxysmal nocturnal dyspnea • Orthopnea • Dyspnea on mild or moderate exertion
Stroke: (3)	Ischemic stroke confirmed by CT or MRI imaging. Neurological deficit attributed to an acute focal injury of the central nervous system (CNS). All strokes will be considered events. The cause of stroke will be indicated using TOAST and categorized as follows: • Large vessel atheroscerosis • Small vessel disease • Cardioembolism • Other (ie dissections, malignancy, etc)

	• Undetermined
Transient Ischemic Attack:	TIA confirmed as a definite by a neurologist. Transient episode of neurologic dysfunction due to focal brain ischemia, without acute infarction or tissue injury.
Cardiovascular Death: (4)	Directly related to myocardial infarction, presumed arrhythmia, heart failure, or acute coronary syndrome, or related to stroke. Cardiac procedure related deaths will be captured as Cardiac-Other. For the purposes of this study such deaths will be defined as an in-hospital death during index admission for elective cardiac procedure. The death is due to complications resulting directly or indirectly from the cardiac procedure. **For the purposes of this study a death that occurs unexpectedly, out of hospital (at home) without prodrome of, or subacute symptoms of heart failure will be defined as a Cardiac Death-Presumed Arrhythmic.** **Specific CV cause will be defined – MI, ACS, Arrythmia, Presumed Arrythmia, Heart Failure, Stroke.** **Non-Cardiovascular Death – death not attributable to a Cardiovascular cause noted above.**

Supporting documentation required for review of cardiovascular clinical endpoints may include the following:

- Hospital Discharge or Death summary note
- Lab reports, ECGs
- Clinical consultations
- Death certificate
- Narrative summary from family, physicians for events occurring outside of the hospital

Drug related AE's: Any other events deemed related to study drug based on the chart below (possibly, likely, and definitely)

Relationship between use of study drug and AE (Causality)					
AE (is)	**None**	**Unlikely**	**Possibly**	**Likely**	**Definitely**
Clearly the result of an external factor	Yes	No	No	No	No
Probably/possibly the result of another factor	No	Yes	Yes	No	No
Has a chronological relationship with the time of study drug administration and/or represents a known reaction to study drug	No	No	Yes	Yes	Yes

Disappears or decreases after discontinuation of the study drug	NA	NA	NA	Yes	Yes
Recurs on renewed administration (re-challenge)	No	No	NA	NA	Yes or NA*

AE=adverse event; NA=not applicable

* A rechallenge is not required; if done, rechallenge would be expected to be positive

REFERENCES

1. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, White HD and Executive Group on behalf of the Joint European Society of Cardiology /American College of Cardiology /American Heart Association /World Heart Federation Task Force for the Universal Definition of Myocardial I. Fourth Universal Definition of Myocardial Infarction (2018). *Circulation*. 2018;138:e618-e651.
2. Felker GM, Adams KF Jr., Konstam MA, et al**. The problem of decompensated heart failure: nomenclature, classification, and risk stratification**. *Am Heart J* 2003;145(2 Suppl):S18-25.
3. Sacco RL, Kasner SE, Broderick JP, Caplan LR, Connors JJ, Culebras A, Elkind MS, George MG, Hamdan AD, Higashida RT, Hoh BL, Janis LS, Kase CS, Kleindorfer DO, Lee JM, Moseley ME, Peterson ED, Turan TN, Valderrama AL, Vinters HV, American Heart Association Stroke Council CoCS, Anesthesia, Council on Cardiovascular R, Intervention, Council on C, Stroke N, Council on E, Prevention, Council on Peripheral Vascular D, Council on Nutrition PA and Metabolism. An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2013;44:2064-89.
4. Type 2 diabetes in adults: management: Evidence reviews for SGLT-2 inhibitors and GLP-1 mimetics. London: National Institute for Health and Care Excellence (NICE); 2018 Mar. (NICE Guideline, No. 28.) Appendix K, Cardiovascular definitions from CANVAS and CANVAS-R trial. Available from: https://www.ncbi.nlm.nih.gov/books/NBK550011/

Supplementary file 3: CADENCE Inclusion and Exclusion Criteria

Inclusion Criteria	1. Type 2 Diabetes (on diet, oral hypo-glycemic agents and/or insulin) or pre-diabetes (defined by Diabetes Canada as HbA1C=6.0-6.45% or increased fasting blood sugar (FBS) (6.1-6.9 mmol/L) or impaired glucose tolerance (2hPG in a 75g OGTT(mmol/L0=7.8-11);(16) 2. Suffered a recent cardiovascular event (≤ 120 days post ACS (i.e. STEMI or nonSTEMI) or TIA/stroke with associated large vessel atherosclerotic disease confirmed on US, CT or MRI; 3. Stable symptoms and hemodynamics; 4. Aged ≥18 years; 5. Given informed consent. Standard definitions will be used for STEMI, NSTEMI,(17) and for ischemic stroke confirmed by CT or MRI and TIA confirmed by a neurologist.(18)
Exclusion Criteria	1. Planned revascularization of infarct or stroke related artery more than 120 days after the qualifying/index event; 2. Recent CV event likely to have been cardioembolic in the opinion of the neurologist or cardiologist; 3. Recent CV event likely to have been secondary to myocardial infarction with non-obstructive coronary arteries (MINOCA) in the opinion of the cardiologist; 4. Severe LV dysfunction (EF<30%); 5. Severe valve disease requiring intervention; 6. Decompensated heart failure; 7. Active infection (e.g. pneumonia, active skin infections, and on antibiotics); 8. Chronic diarrhea; 9. Immune compromise (e.g. recurrent infection); 10. History of cancer within the last 3 years (other than a successfully treated cutaneous squamous cell or basal cell carcinoma or localized carcinoma in situ of the cervix). 11. Active inflammatory conditions (e.g. rheumatoid arthritis, chronic inflammatory bowel disease, SLE, systemic anti-inflammatory therapy (e.g. prednisone, methotrexate)); 12. Pregnancy (all women of child bearing potential will have a negative BHCG test; 13. Breastfeeding; 14. Women of childbearing potential who refuse to use two forms of contraception (this includes at least one form of highly effective and one effective method of contraception) throughout the study OR men capable of fathering a child who refuse to use contraception. 15. Glomerular filtration rate (GFR) <50 ml/min/1.72m;(3) 16. Use of potent p-glycoprotein inhibitors (i.e. systemic cyclosporine, clarithromycin, or systemic ketoconazole) or a strong CYP3A4 inhibitor (i.e. ritonavir, clarithromycin, or systemic ketoconazole); 17. Hemoglobin < 105(women) <110 (men) g/L; WBC < 3.0x 10(9)/L, platelet count< 110x 10(9)/L;

	18. History of cirrhosis, chronic active hepatitis or severe hepatic disease or with alanine aminotransferase (ALT) levels greater than 3 times the upper limit of normal. 19. Unable to give informed consent. <u>Exclusion for CTA portion of the protocol:</u> Patients with dye allergy will not undergo CTA but will have PET/CT.

Supplementary file 4: Administration of CADENCE study

1.0 Data Safety and Monitoring Board (DSMB) for CADENCE study

The DSMB is responsible for assuring that study participants in "The **C**anadian Study of **A**rterial Inflammation in Patients with **D**iabetes and Recent Vascular Events: **E**valuatio**N** of **C**olchicine **E**ffectiveness (**CADENCE**)" are not exposed to unnecessary or unreasonable risks and that the study is being conducted according to the highest scientific and ethical standards. In order to carry out this responsibility, the DSMB will:

- Familiarize themselves with the Executive Committee's Terms of Reference (if applicable), study protocol (Appendix A), Investigator Brochure/Product Monograph, and manuals prior to the initiation of the study.
- Review descriptive reports at predetermined timelines (typically blinded data).
- Request unblinded data if there are any safety concerns.
- Provide recommendations for or comments regarding change(s) to the design or methodology of the clinical trial.
- Provide feedback on ancillary studies proposed by the Executive Committee /Investigators.
- Alert investigators regarding emerging procedural or ethical issues.
- Comment on the relevancy of new external published data from other trials that may impact on patient safety or efficacy of the study treatments.

At the request of the Study Chair (or Principal Investigator if no formal Study Chair), the DSMB may be called upon to:

- Provide recommendations to the Study Chair/PI and Executive Committee in order to facilitate the timely completion of the trial.
- Advise the Study Chair/PI of any and all factors (e.g., subject recruitment, protocol adherence and adverse events) that could potentially threaten the inferences that may be drawn from the final results of the trial.

This independent data and safety monitoring board will review safety data from the trial, and all Serious Adverse Events (SAE) and non-serious Adverse Events (AE) will be collected and reported in accordance with GCP.

2.0 Trial Steering Committee (TSC) for CADENCE study

The TSC for the CADENCE study is the executive decision-making group that considers the recommendations from the DSMB. Members of the TSC has both independent members as well as

members who are responsible for the day-to-day conduct of the trial. Additionally, a community member/former cardiac patient will serve on the Steering Committee to guide relevance and study conduct from the patients' perspective.

3.0 Adjudication Committee

An adjudication committee of experts who are not participating investigators/collaborators will independently review and adjudicate each clinical event blinded to treatment randomization. The committee will also review events to ensure they have been appropriately captured in terms of diagnosis, seriousness, expectedness, and the relatedness to the study drug. Adjudication Committee will be provided with definitions for adjudication of study events.

4.0 Patient Engagement

As mentioned, a patient will serve on the Steering Committee to guide relevance and study conduct from the patients' perspective.

Supplementary 4: Additional protocol information

Study Drug Interruptions and Discontinuations

Participants may choose to stop the study medication at any time. The site investigator will discuss this decision with the patient and study personnel will continue to follow these patients, in the same manner as the other patients in the trial. Interruptions and discontinuations of study drug will be documented, along with the reason. Restarting medications will be discussed at follow-up visits and noted accordingly. Patients will continue to be followed and data collected accordingly. The below lists the circumstances where the study medication may be discontinued or subject withdrawn from the study:

- Patient is unable to tolerate study intervention
- Pregnancy
- Use of potent p-glycoprotein inhibitors or strong CYP3A4 inhibitors
- Impaired renal or liver function

Concomitant treatments

The following medications should not be used concomitantly with study drug:

- Strong inducers of either CYP3A4 inhibitors (e.g. Clarithromycin, etc) or potent P-glycoprotein inhibitors (e.g. Cyclosporin, etc).
- It is advised to avoid grapefruit and grapefruit products.

Safety and Adverse Events

Adverse events will be captured from when the study intervention is started, to study exit. Serious adverse events will be reported to the Data Safety Monitoring Board, the Ethics Board, and Health Canada in accordance with good clinical practice (GCP).

Data Collection and Storage

Medical history and inclusion/exclusion criteria will be determined using hospital records and patient reported history. Clinical data relevant to the study objectives will be included as part of the study analysis.

All participant information and data will be collected respecting participant confidentiality and privacy and following written informed consent. Personal health information including medical history, medications, lab results, procedures, and test results will be collected for the purposes of this study.

Once we have obtained informed consent from a participant, they will receive a unique study identification (ID) number. This number will be the only identifier used on all study-related blood samples and patient study documents. Hospital unit numbers, Social Security numbers, phone numbers and addresses will not be used as coding mechanisms. A master list containing participants' full name and contact information will be kept by and stored in a separate file from the coded study dataset on a secured computer. Any Personal Health Information (PHI) or Personal Identifying Information (PII) that is collected will be kept in a confidential manner. Any subsequent release of PHI/PII information will only occur if it is required by law.

The research staff will collect data on case report forms (CRFs). The study data will be stored in a secure location accessible only by the investigators and/or study research staff. Any hard copies of CRFs and other paper records will remain on site and stored in a secured manner. Data captured on CRFs will be electronically entered into a RedCap database managed by UOHI research team. This database will be password protected and maintained on a secure internal server. No identifying information will be kept on any mobile devices, and no information will leave the hospital premises. As is required by law, the

research files will be securely stored for 25 years after the study has been completed. At the end of this time, all records will be securely destroyed.

Coordinating Centre

The Ottawa Heart Institute Research Corporation will be the coordinating centre for this trial. The UOHI Cardiovascular Research Methods Centre (CRMC) will be responsible for randomization and for data management and analysis, and storage of data. The Centre will implement and maintain quality assurance procedures related to data generation. The UOHI Cardiac Imaging Research Core Lab will be the core lab for image analyses.

Protocol amendments

Any planned major protocol amendments will be communicated to relevant key stakeholders (including trial steering committee (TSC), Research Ethics Board (REB), Data Safety Monitoring Board, adjudication committee, etc).

Dissemination policy

Full trial results will be submitted for publication upon final follow-up and analysis. Those who have made substantive contributions to the design, conduct, interpretation, and reporting of CADENCE will be recognized through authorship on the final trial report.

Chapter 6: Evaluating the cost-effectiveness of colchicine for the reduction of atherosclerotic cardiovascular disease events

CHAPTER OUTLINE

Chapter 6 addresses the fourth research question: *Is colchicine a cost-effective treatment for reduction of ASCVD compared to current therapeutic options?*

Again, in **Chapter 3** colchicine has been identified to be effective at reducing cardiovascular events. But this efficacy must be balanced with the potential side effects and downstream negative repercussions of its use. Thus, a cost-utility analysis was performed in order to compare the cost-effectiveness of colchicine use with our current standard of care (which does not include anti-inflammatory use) in patients with acute coronary syndromes as well as in patients with stable coronary artery disease.

Chapter 6 contains one manuscript (Section 6.1) which has been published. The manuscript is described briefly below, and a more in-depth description is found in the first page of the section.

CHAPTER CONTENTS

Section 6.1 contains a cost-effectiveness analysis of colchicine for the reduction of recurrent cardiovascular events in patients with acute coronary syndromes as well as in patients with stable coronary artery disease. This manuscript was published in the *Canadian Journal of Cardiology Open*.

Section 6.1 Cost-effectiveness of colchicine for recurrent cardiovascular events.

SECTION OVERVIEW

This section further explores the findings of **Chapter 3**, where colchicine was shown to be an effective treatment in the prevention of cardiovascular events. Thus, this section presents a manuscript of a cost-effectiveness analysis of colchicine for the reduction of cardiovascular events in the Canadian healthcare context.

MANUSCRIPT STATUS

This manuscript has been published.

Boczar KE, Beanlands R, Wells G, Coyle D. Cost-Effectiveness of Colchicine for Recurrent Cardiovascular Events. CJC Open. 2023 Feb 24;5(5):348-356. doi: 10.1016/j.cjco.2023.02.005. PMID: 37377518; PMCID: PMC10290950.

Supporting Material

Supplemental file 1: Impact inventory supplemental table

Author roles and contributions

KEB, GW, RB, and DC designed the study. KEB drafted the manuscript, which was revised by all authors. All authors approved the version of the manuscript submitted for publication.

RELATED THESIS APPENDICES

Appendix G: Published manuscripts. The PDF of published manuscript is presented in Appendix G.

Cost-effectiveness of colchicine for recurrent cardiovascular events

Short title: Colchicine cost-effectiveness

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Word Count: 5288

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Brief Summary

Colchicine is an anti-inflammatory therapy with a low associated cost that has been shown to reduce cardiovascular events but is associated with side effects. A decision model was developed to estimate the direct costs and outcomes amongst patients who have suffered a myocardial infarction and are treated with both short- and long-term colchicine. In the current analysis, it demonstrated cost-effectiveness in the Canadian public healthcare context with both short-term and long-term use.

Abstract

Background:

Colchicine is an anti-inflammatory therapy with a low associated cost that has been shown in two large studies to reduce cardiovascular (CV) events but is associated with side effects. The main objective for this analysis is to determine whether colchicine is cost effective for the prevention of recurrent-CV events in patients who have suffered a myocardial infarction (MI).

Methods:

A decision model was developed to estimate the healthcare costs in Canadian dollars and clinical outcomes amongst patients who have suffered a MI and are treated with colchicine. Probabilistic Markov modeling was used in combination with Monte Carlo simulation to derive expected lifetime costs and quality-adjusted life years (QALYs), which permitted the calculation of incremental cost-effectiveness ratios (ICERs). Models were derived for both short-term (20-months) as well as long-term (lifelong) colchicine use in this population.

Results:

Long-term colchicine use was dominant over standard of care with lower average lifetime costs per patient ($91,552.80 versus $97,085.84) and higher average QALYs per patient (19.92 versus 19.80). Short-term colchicine use also dominated over standard of care. Results were consistent over a range of scenario analyses.

Conclusion:

Based on two large randomized controlled trials, treatment of patients post MI with colchicine appears cost-effective when compared to standard of care at the current price. Based on these studies and currently accepted willingness to pay thresholds in Canada, health-care payers could consider funding long-term colchicine therapy for CV secondary prevention while we await results from ongoing trials.

Introduction

Atherosclerotic cardiovascular disease is a leading cause of morbidity and mortality around the world.[43, 44] Inflammation has long been known to play a key role in the progression of atherosclerosis and its complications, such as myocardial infarction (MI), stroke and cardiovascular (CV) death; however, until recently effective inflammatory treatment targets have remained elusive. Large trials in the area have been disappointing and have failed to show CV outcome benefits from therapies that target specific inflammation pathways: including low-density lipoprotein (LDL) oxidation;[45] secretory and lipoprotein-associated phospholipase A2 (sPLA2 and LpPLA2);[46-48] P38 mitogen-activated protein (MAP) Kinase inhibitors,[38, 39] methotrexate,[49] and P-selectin.[50]

Colchicine is an anti-inflammatory agent that has been around for centuries and is used for inflammatory conditions like gout, pericarditis, and juvenile idiopathic arthritis. It has been shown in several large trials to have CV outcome benefit.[6, 7] However, its use also has potential negative effects, with trials showing a higher risk of infection in patients taking colchicine.[7]

Thus, while colchicine represents a promising novel therapeutic agent in the treatment of atherosclerotic cardiovascular disease, its use does have potential negative consequences including increased overall cost to the healthcare system. Decision analysis combined with economic analysis allows us to determine the net benefit of a new technology by weighing the potential benefits of a novel therapy (in terms of improved health outcomes) against some of the drawbacks associated with its use (including potential side effects or increased health care system costs). Furthermore, it allows for the determination of whether a new technology will

lead to an improvement in the overall health of the wider population (i.e., whether it is cost-effective). The main objective of this study was to assess the cost-effectiveness associated with using colchicine for the prevention of recurrent-CV events.

Methods

Decision Problem

The specific decision problem that this study addresses is whether a Canadian health care payer should reimburse treatment with colchicine for the reduction of recurrent cardiovascular events in adult patients in Canada with a prior MI. To address this decision problem, a cost effectiveness analysis was performed to compare colchicine to current standard of care therapies (i.e. which do not include anti-inflammatory therapies), from the perspective of the Canadian public healthcare payer over a lifetime horizon (specifically from the perspective of the Ontario Ministry of Health & Long-Term Care). Both long-term (lifelong) and short-term (20-months) treatment options with colchicine were considered.

Model Overview

A probabilistic Markov cohort model was used to derive the estimated direct costs (i.e. healthcare-related costs) and health outcomes (life years and quality adjusted life years (QALYs)) amongst patients who have suffered a MI and are treated with colchicine. The dosing of colchicine 0.5mg orally taken daily was used as the treatment dose of choice, as per the results of the COLCOT and LoDoCo 2 studies[6, 7], and we created and compared models with both short-term colchicine use (20 months), and long-term (lifelong) colchicine.[3] We used a lifetime study horizon (400 cycles of 1-month duration, equating to 33.33 years) to analyze the base-case costs

(in Canadian dollars (CAD)) and utility values from the perspective of the Canadian publicly funded health care system, and we incorporated certain model assumptions that were based on previous published literature.[51] The net present value of future costs (in CAD) and QALYs was determined using a 1.5% discount rate as per guidelines outlined by the Canadian Agency for Drugs and Technologies in Health (CADTH) .[52] A Markov model combined with probabilistic analysis allowed estimation of costs (2022 CAD) and quality-adjusted life years (QALYs) which then allowed for the calculation of incremental cost-effectiveness ratios (ICERs). In the reference case, uncertainty regarding the value of each parameter was incorporated within the probabilistic analysis. Methodological uncertainty was explored by comparing the reference case results to those from non-reference case analyses using discount rates of 0% and 3% as per CADTH directives.[52]

The model was developed in Microsoft Excel and we completed the Impact Inventory from the Second Panel on Cost-effectiveness in Health and Medicine (Supplemental Table S1).[53]

Decision model

We developed a Markov model to follow a hypothetical cohort of patients who had suffered a MI (Figure 1). Base states in the model included a stable state, a stable after recurrent MI state, a stable after stroke state, and a death state. With each cycle, patients entered the event model which included the event states of: stable, recurrent MI, stroke, non-MI revascularization, pneumonia (potential side-effect of colchicine), gout flare, and death. From all events except gout, patients could either recover or experience death, and patients would then return to the appropriate base state following the event. Patients experiencing a gout flare all returned to their

appropriate base state following the event. The model generated cycle specific estimates of costs and QALYs thereby allowing for the calculation of lifetime costs and QALYs.

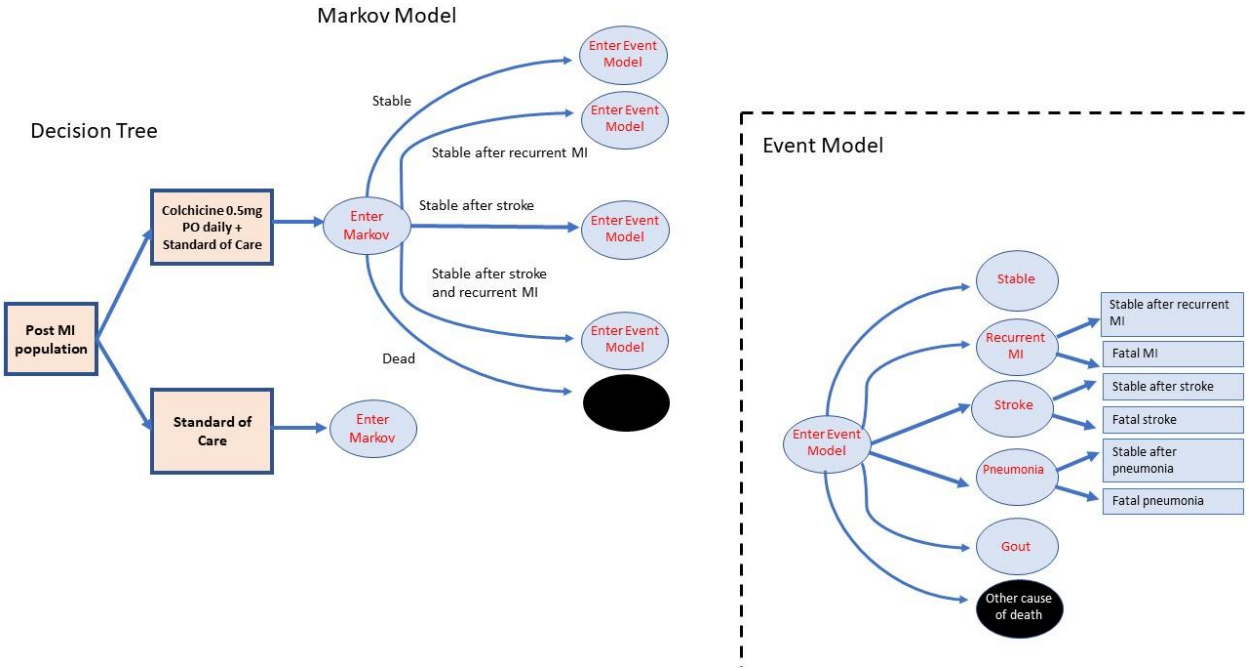

Figure 1. Markov Model. A survivor of myocardial infarction can be assigned to treatment with colchicine 0.5mg oral daily and standard of care, or standard of care alone (Decision Tree). Within each cycle, a survivor of myocardial infarction can then each cycle either remain stable, suffer a recurrent myocardial infarction, suffer a stroke, have a coronary revascularization procedure performed, suffer a pneumonia infection, suffer a gout attack, or remain in the death state. Following non-fatal recurrent MI, stroke, or pneumonia events, patients then cycle back to their stable states. Standard of care therapies following a myocardial infarction include antiplatelet agents, statins, and a beta-blocker.

Abbreviations: MI: myocardial infarction, PO: oral

Patient Population

The cohort of patients were assumed to mirror the patient population within the COLCOT and LoDoCo 2 trials evaluating the impact of colchicine on cardiovascular outcomes.[6, 7] The mean age of participants was 62, and patients had a history of MI with treatment including antiplatelet agents, statins, beta-blockers, and angiotensin-converting enzyme inhibitors. This is similar to the treatment regimens in the COLCOT and LoDoCo2 trials and what is done in clinical practice.

Model inputs

Short- and long-term colchicine was evaluated against current standard of care therapies post MI. There are several perceivable disadvantages to colchicine therapy including the upfront cost, an increased risk of pneumonia for patients, and a potential signal for increased mortality. As per the drug-regimens used in the COLCOT and LoDoCo2 trials, all patients received standard of care therapies, with patients receiving colchicine being administered 0.5mg orally every day. Strategies of both short-term (20 months) as well as long-term colchicine therapy were considered, separately, in the model.

Survival probabilities associated with standard of care therapy as well as colchicine treatment are summarized in Table 1, and were derived from the COLCOT and LoDoCo 2 studies where a total of 10,267 patients were randomly assigned to either placebo or colchicine. The uncertainty around these probabilities were incorporated into the probabilistic analysis.

Table 1- Distributions for probabilistic analysis

	Expected Value[Reference]	Probability Distribution
Event Probabilities		
Probability of recurrent MI in acute phase (Standard Care)	0.041[7]	Beta (98, 2281)
Probability of recurrent MI in chronic phase (Standard Care)	0.042[6]	Beta (116, 2644)
Probability of non-MI revascularization in acute phase (Standard Care)	0.021[7]	Beta (50, 2329)
Probability of non-MI revascularization in chronic phase (Standard Care)	0.064[6]	Beta (177, 2583)
Probability of pneumonia in acute phase (Standard Care)	0.004[7]	Beta (9, 2337)
Probability of pneumonia in chronic phase (Standard Care)	0.022[6]	Beta (55, 2705)
Probability of non cardiovascular death in acute phase (Standard Care)	0.008[7]	Beta (20, 2359)
Probability of non cardiovascular death in chronic phase (Standard Care)	0.007[6]	Beta (23, 207)
Probability of pneumonia in acute phase (Colchicine)	0.009[7]	Beta (21, 2309)
Probability of pneumonia in chronic phase (Colchicine)	0.017[6]	Beta (46, 2716)
Probability of death post revascularization	0.01[66]	Beta (840, 128422)
Probability of MI being fatal	0.04[67]	Beta (31, 759)
Probability of infection being fatal	0.07[3]	Beta (23, 207)
Probability of stroke being fatal	0.18[68]	Beta (20, 0.1)
Hazard Ratios (Colchicine versus Standard Care)		
Hazard ratio of recurrent MI in acute phase (Colchicine)	0.77[7]	Lognormal (0.77, 0.61)

Hazard ratio of recurrent MI in chronic phase (Colchicine)	0.7[6]	Lognormal (0.7, 0.53)
Hazard ratio of non-MI revascularization in acute phase (Colchicine)	0.5[7]	Lognormal (0.5, 0.31)
Hazard ratio of non-MI revascularization in chronic phase (Colchicine)	0.75[6]	Lognormal (0.75, 0.6)
Hazard ratio of non cardiovascular death in acute phase (Colchicine)	0.98[7]	Lognormal (0.98, 0.64)
Hazard ratio of non cardiovascular death in chronic phase (Colchicine)	1.21[6]	Lognormal (1.21, 0.86)

In the base case analysis we assume that patients continue treatment beyond the 5 year time horizon of the clinical trials and the benefit of treatment is maintained long-term. This decision was made as within the LoDoCo2 trial, the survival benefit was stable at 5 years and showed no evidence of treatment waning.[6] Scenario analyses addressed the impact of these assumptions. In a scenario analysis, we adopted a 20-month time horizon, which allowed estimation of the proportion of the forecasted QALY gains for colchicine which were generated after the period covered by the COLCOT trial (short-term colchicine use model) as well as a 49-month time horizon, approximately covering the follow-up period of the COLCOT and LoDoCo2 trials combined. These analyses which examine the impact of extrapolation are recommended within the CADTH guidelines.[52] As per CADTH guidelines, in a further scenario analysis, we assessed the impact of treatment discontinuation by assuming that after both 20 months and 49 months,

respectively, patients would discontinue treatment with colchicine with no continued effect after discontinuation.

The costs (in CAD) associated with each treatment strategy are summarized in Table 2. The monthly costs associated with colchicine administration strategies and the costs of cardiovascular events, complications, and admissions were obtained from the literature and converted to 2022 CAD based on the Bank of Canada inflation calculator.[54-57] Similarly, we applied costs for standard of care treatment for coronary heart disease from published literature[58] and again converted to 2022 CAD based on the Bank of Canada inflation calculator.

Table 2- Cost and Utility Data

	Base-case estimate[Reference]	**Probability Distribution**
Monthly Costs		
Standard care	$238[21]	Gamma (18221.67, 0.01)
Colchicine	$9	Fixed
Stroke	$130[30]	Gamma (61.38, 2.11)
Cost for Event		
Myocardial infarction	$20,410[17]	Gamma (10652.67, 1.92)
Revascularization	$31,771[18]	Gamma (16, 1985.66)
Stroke	$10,003[30]	Gamma (7668, 12668)
Non-fatal pneumonia admission	$10,585[20]	Gamma (16, 2646.25)

Fatal-pneumonia admission	$30,773[20]	Gamma (7261.44, 4.24)
Gout flare	$172[31]	Gamma (16, 10.76)
Utilities		
Stable standard of care	0.868[22]	Beta (3531.89, 537.11)
Stable recurrent MI	0.734[23]	Beta (14.68, 5.32)
Utility toll for stroke	-0.178[32]	Beta (0.134, 0.223)
Utility toll for MI/revascularization/ pneumonia admission	-0.0986[expert opinion]	Beta (1385, 12657)

Utility values were derived from a variety of sources. Utility values associated with the base state of post-MI status were taken from an analysis and comparison of the MONICA/KORA registry to the general population.[59] Utility values for the recurrent MI state were obtained from literature which investigated the stability of time-tradeoff utilities in survivors of MI.[60] Utility values for the recurrent MI and post-stroke state were applied to patients indefinitely following their respective event (i.e. stroke or recurrent MI). Finally, disutility values associated with adverse events were determined from expert opinion, and were applied only to the specific cycle during which the adverse event occurred.[61]

Model outputs and analysis

Markov modeling was used to calculate mean total health care costs and total QALYs gained for each strategy. Cycle specific estimates were summed and discounted over the lifetime horizon to provide estimates of total costs and QALYs. Probabilistic analysis using Monte Carlo simulation

with 5000 replications was performed to account for uncertainty in the input values providing an estimate of the expected values for costs and QALYs. Cost effectiveness was assessed by estimating the incremental cost per QALY gained against a base willingness-to-pay threshold of $50,000 CAD/QALY. This threshold was chosen based on a previous statement by the Assistant Deputy Minister for Ontario.[62] The threshold value for a QALY should reflect the marginal efficiency of the health care system that the result is related to. Empirical work related to this for Canada has not been conducted, although an analysis for the UK health care system suggests an appropriate threshold may be between $20,000 to $30,000 per QALY.[63] A higher cost per QALY threshold would be suggestive of the Canadian system either being less efficient than the UK system, or better funded.

Model Validity

Face validity was confirmed with experts in the field. Internal validation was conducted by the senior health economist reviewing the model structure and coding. Validity of our model was tested by comparing event rates from the COLCOT and LoDoCo2 trials with the risk from our simulated colchicine and standard of care cohorts at 23 and 49 months of elapsed follow-up time, respectively. To assess the external validity of our model, we compared events in the standard of care arm from the CANTOS trial (a similar study population) to results obtained from our standard of care model cohort at 22.6 months of follow-up (which was the median follow-up time from the CANTOS trial).

Results

With respect to validity of our model, cumulative mortality estimates from our model were similar to the results seen in both the COLCOT and LoDoCo2 trials results.

Results of our probabilistic analysis found that compared to standard of care, long-term colchicine was dominant. (Table 3, Figure 2) This strategy was associated with a slightly lower discounted life expectancy (23.62 years versus 23.75 years), but with a reduced lifetime incidence of MIs, revascularisation, stroke, and gout flares (Table 4). Long-term colchicine was associated with lower health care costs than the standard of care group $91,552.80 versus $97,085.84 (Table 3). The incremental reduction in costs with long-term colchicine use ($5,533.04) were almost exclusively due to the lifetime costs of colchicine ($2,640.36), being more than offset by the reduction in MI and revascularization events. Cumulative QALYs were 19.92 in the long-term colchicine group, and 19.80 in the standard of care group, respectively.

Table 3 – Cost Effectiveness Results for Base Analysis and Scenario Analyses

	Lifetime costs	Lifetime QALYs	ICER: Colchicine versus Standard of Care
Base Case			
Standard of care	$97,086	19.80	
Long-term colchicine	$91,553	19.92	Dominant
Short-term colchicine	$96,636	19.86	Dominant
Discount rate 0%			
Standard of care	$121,938	24.83	

Long-term colchicine	$115,082	24.96	Dominant
Short-term colchicine	$113,736	24.91	Dominant
Discount rate 3%			
Standard of care	$78,389	16.16	
Long-term colchicine	$74,171	16.24	Dominant
Short-term colchicine	$73,173	16.21	Dominant
48-month time horizon			
Standard of care	$15,274	3.36	
Long-term colchicine	$14,582	3.37	Dominant
Short-term colchicine	$14,952	3.37	Dominant
20-month time horizon			
Standard of care	$6,121.38	1.41	
Colchicine	$5,865.58	1.42	Dominant
Allowance for discontinuation with treatment			
Standard of care	$97,087	19.79	
Long-term colchicine	$96,161	19.86	Dominant
Short-term colchicine	$96,394	19.84	Dominant

ICER – incremental cost per QALY gained; MI - = myocardial infarction; QALY – quality adjusted life year

Figure 2. Scatterplot of incremental costs against incremental QALYs gained. Standard of care therapies following a myocardial infarction include antiplatelet agents, statins, and a beta-blocker. Dashed line represents a cost-effectiveness threshold of $50,000/QALY.

Abbreviations: CAD: Canadian dollar, QALY: Quality-adjusted life years

Table 4 – Disaggregated Results

	Long-term colchicine	Short-term colchicine	Standard of Care

Incidence	Recurrent MI - non fatal	0.325	0.448	0.457
	Recurrent MI - fatal	0.013	0.018	0.019
	Revascularization - non fatal	0.503	0.667	0.677
	Revascularization - fatal	0.003	0.003	0.004
	Pneumonia - non fatal	0.158	0.193	0.188
	Pneumonia - fatal	0.017	0.021	0.021
	Stroke – non fatal	0.053	0.076	0.079
	Stroke – fatal	0.002	0.004	0.004
	Gout	0.222	0.531	0.551
Costs	No new event	$68,522	$69,334	$69,434
	Colchicine	$2,640	$183	N/A
	Recurrent MI - non fatal	$5,323	$7,292	$7,468
	Recurrent MI - fatal	$217	$297	$304
	Revascularization - non fatal	$12,536	$16,587	$16,894
	Revascularization - fatal	$82	$83	$111
	Pneumonia - non fatal	$1,328	$1,621	$1605
	Pneumonia – fatal	$426	$520	$503
	Stroke – non-fatal	$432	$619	$659
	Stroke - fatal	$16	$30	$32
	Gout	$30	$72	$75
	Total Costs	$91,553	$96,636	$97,086
QALY		19.92	19.86	19.80

Life years	23.62	23.77	23.75

In our probabilistic analysis we found that compared to standard of care, short-term colchicine was also dominant (Table 3, Figure 2). It was associated with slightly higher discounted life expectancy (23.77 years versus 23.75 years). Short-term colchicine was associated with lower health care costs compared to the long-term colchicine group $96,636.33 versus $97,085.84 (Table 3). Cumulative QALYs were 19.86 in the short-term colchicine group, and 19.80 in the standard of care group, respectively.(Table 3).

In a separate comparison, long-term colchicine therapy dominated over short-term use with lower lifetime costs and higher lifetime QALY gains, as above.

At a willingness-to pay threshold for a QALY of $50,000, long-term colchicine had a 72.2% probability of being cost-effective (i.e. the ICER was greater than $50,000 in 72.2% of the 5,000 simulations). Short-term colchicine had a 25.8% probability of being the most cost-effective strategy (Figure 3).

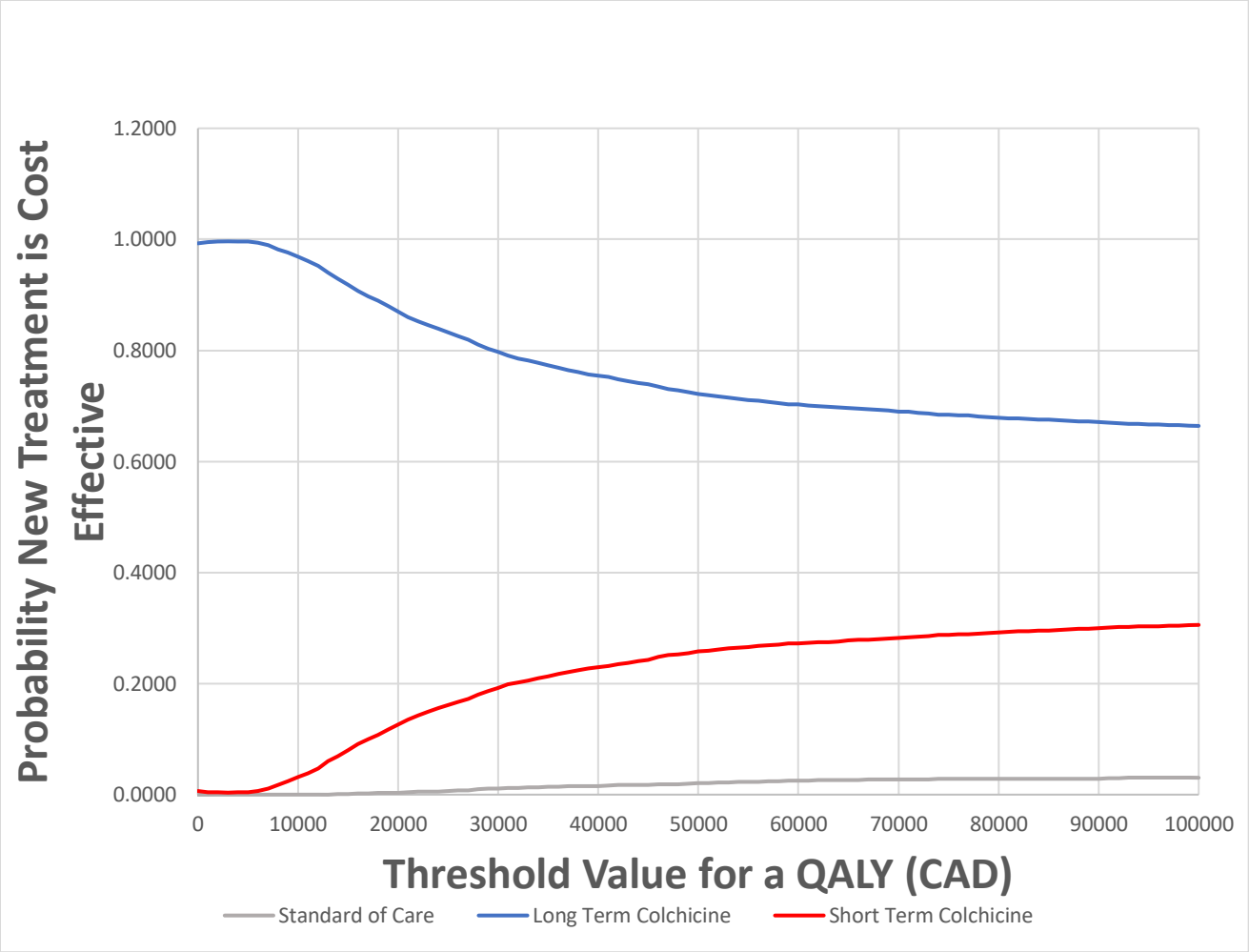

Figure 3. Cost-effectiveness acceptability curve for the probability that each treatment strategy is cost effective against the threshold value for a QALY. Standard of care therapies following a myocardial infarction include antiplatelet agents, statins, and a beta-blocker.

Abbreviations: CAD: Canadian dollar, QALY: Quality-adjusted life years

Scenario analyses relating to discount rates demonstrated consistency with our primary analysis (Table 4). Our results remained stable when using alternative discount rates of 0% and 3%. In the scenario analysis with reduced time horizons of 20 and 49 months, estimated incremental QALY gains were only 0.01, suggesting that less than 17% of the forecasted QALY gains from

colchicine occur during the initial time periods representative of the COLCOT and LoDoCo2 trials, respectively. In a scenario analysis where patients stopped colchicine at 20 or 49 months, respectively, results remained unchanged with both short-term and long-term colchicine therapy continuing to show dominance over standard of care.

Discussion

In this cost-effectiveness analysis, we evaluated the cost-effectiveness of short-term and long-term colchicine against the current standard of care therapies for the secondary prevention of CV events, from the perspective of the Ontario public healthcare system. Our results demonstrate that in comparison, both short-term and long-term colchicine usage are cost-effective strategies in this area, as both were dominant over current standard of care. The predominant driver of lower costs with colchicine therapy was the reduction in CV events, which made up for the marginal increase in costs associated with the drug itself. In our pre-specified willingness to pay threshold of $50,000/QALY, long-term colchicine also emerged as dominant option over short-term use, with lower overall costs and higher QALY gains.

In recent years, targeting inflammation for the reduction of atherosclerotic cardiovascular disease has become a topic of great interest.[3, 4, 41] As inflammation is recognized to play an important role in the pathogenesis of atherosclerotic cardiovascular events,[64, 65] much attention has been focused, with varying success, on trying to identify potential anti-inflammatory agents that could be utilized to reduce CV risk for patients. The anti-inflammatory medication canakinumab represented a breakthrough in this domain, as the CANTOS trial demonstrated benefit for the reduction of CV events in patients who had suffered a previous MI and who had a high residual

inflammatory burden.[3] However, due to the high medication cost, it did not demonstrate cost-effectiveness in multiple analyses.[5, 61] This was largely due to the high cost associated with the medication. Following this, much attention focused around trying to find alternative anti-inflammatory agents that could fill the void. Colchicine, a medication with a low-associated cost and long track-record as a commonly used anti-inflammatory agent, seemed to hold promise in this regard.[6, 7]

Several pivotal trials released following CANTOS demonstrated clinical benefit for colchicine in reducing CV events. In the COLCOT trial, colchicine use in patients with recent MI led to a significantly lower risk of ischemic cardiovascular events than placebo.[7] Separately, the LoDoCo2 study showed that in patients with chronic coronary disease, the risk of cardiovascular events was significantly lower among those who received 0.5 mg of colchicine once daily than among those who received placebo.[6]

Colchicine is not without side effects, however. Some studies, including LoDoCo2 have found a small and potentially non-statistically significant increase in all-cause mortality.[6, 12] Additionally, one smaller study found a statistically significant signal for increased total mortality with colchicine use.[12] There is also a signal for a potential increase in pneumonia events with colchicine use.[7] Thus, while the current medications used for secondary prevention in patients who have suffered a MI is well-established, whether or not colchicine should enter this domain is certainly not. Additionally, if colchicine were to enter guideline directed medical therapy, it is not clear whether colchicine should be used for a short-term course after a CV event, or for long-term use. The cost-effectiveness of the drug will certainly need to be considered in this clinical

decision making, particularly in publicly funded health care systems. Further research is currently being conducted (NCT03048825) looking at colchicine for cardiovascular indications, and will further explore the potential increase in all-cause mortality associated with colchicine use. This will certainly help to shed further light on this area to determine if it truly remains a dominant treatment option in this domain.

By conducting probabilistic analysis, we were able to incorporate a wide range of uncertainty into our model, thereby improving the robustness of our results. Our analyses incorporated the potential increase in all cause mortality but still concluded that the use of colchicine was worthwhile. As evidenced by our study, there is an overwhelming probability that colchicine is cost-effective over standard care using our pre-specified willingness to pay threshold of $50,000/QALY, and this conclusion remains consistent with much lower willingness-to-pay thresholds. However, whether a short-term or long-term strategy of colchicine should be employed is a much more nuanced decision-problem. Long-term colchicine provided slightly higher cumulative QALYs with a higher cost, largely related to the cost of long-term use of the medication. However, long-term use was still deemed to be a cost-effective strategy at the accepted willingness to pay threshold of $50,000/QALY.

Limitations

Our model had several important limitations, and our results should therefore be interpreted within the context of the data inputs and modelling assumptions. First, the efficacy of colchicine was based on two single randomized controlled trials, which limits the generalizability of our results. However, at this point in time, these trials encompass the largest body of Phase 3

evidence on the subject, and are currently what is available for decision-makers. The results of this analysis strongly suggest that given the current data on relative effectiveness, colchicine is highly likely to be cost effective in this context at its current price. However, with the concerning signal regarding mortality associated with colchicine, the results of ongoing randomized-controlled trials are needed to help to clarify this issue further before adopting colchicine into guidelines. The uncertainty regarding the potential signal for mortality is reflected within the scatterplot of incremental costs against incremental QALYs gained shown in Figure 2. As further data emerges, this will clarify the risks (or lack thereof) associated with colchicine-use, and will subsequently reduce the amount of uncertainty within the model. Our scenario analyses adopted assumptions related to discontinuance of treatment and of waning treatment effect which were not favourable towards colchicine, and despite this, colchicine remained a cost-effective option for this indication. The median follow-up for COLCOT and LoDoCo2 studies were 23 and 29 months, respectively, but we assumed therapy would be effective for a much longer period. This was a potentially optimistic estimate of the long-term effects of colchicine therapy. We addressed this by performing a scenario analysis looking at waning of treatment effect as well as discontinuation of therapy, and our results remained consistent. Finally, as the trials did not present sex-stratified results, we were unable to conduct sex-stratified models within our analysis. However, given the small sex differences seen in the incidence of cardiovascular events we expect that our conclusions would not change with the addition of sex-stratified analyses.

Conclusions

Treatment with colchicine for secondary prevention of CV events is likely cost-effective in the Canadian healthcare system. Both short- and long-term therapy with colchicine was dominant over standard care in the reduction of CV events. In our analysis, long-term colchicine therapy emerged as a cost-effective option over short-term use, with an ICER of $46,241.17. Ongoing trials (NCT03048825, NCT04848857, NCT04181996) are certainly needed and will shed additional light on the potential harms associated with colchicine use. Given this uncertainty, it is currently difficult to strongly recommend colchicine use for cardiovascular indications at present until further data are available. Future investigations of anti-inflammatory drugs targeted at atherosclerosis must continue to consider the balance of effectiveness and economic impact on patients and the health-care system.

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GW and DC – nothing to declare

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Appendix 1: Impact inventory

Sector	Type of Impact	Included in analysis healthcare sector perspective?
Formal healthcare sector		
Health	Health outcomes (effects):	
	Longevity effects	x
	Health related quality of life effects	x
	Effects of adverse events: Negative: infections Positive: lung cancer, arthritis	x
	Medical costs	
	Paid for by third-party payers	x
	Paid for by patient out-of-pocket	x
	Future related medical costs	x
	Future unrelated medical costs	x
Informal healthcare sector		
Health	Patient time-costs	NA
	Unpaid caregiver time costs	NA
	Transportation costs	NA
Non-healthcare sector		
Productivity	Labor market earnings lost	NA
	Cost of unpaid lost productivity due to illness	NA
	Cost of uncompensated household production	NA
Consumption	None	NA
Social services	None	NA
Criminal justice	None	NA
Education	None	NA
Housing	None	NA
Environment	None	NA

Supplemental Table S1. Impact inventory

Chapter 7: Biologic therapy to treat vascular inflammation in patients with psoriasis or psoriatic arthritis

CHAPTER OUTLINE

Chapter 7 addresses the fifth question: "*Could biologic therapy be an effective option to reduce vascular inflammation in patients with psoriasis or psoriatic arthritis?"*

Chapters 3 and 6, respectively, identified that anti-inflammatory therapies are effective for the reduction of major cardiovascular events and they are cost-effective in the Canadian context. However, the burden of an additional therapy in a group of patients that are already commonly on three or more preventative medications is a large one to bridge. Thus, perhaps identifying high risk populations that may derive even more cardiovascular benefit from anti-inflammatory therapies may be the right direction to take. Psoriasis and psoriatic arthritis are chronic inflammatory conditions of the skin and joints, respectively. These conditions are associated with and increased risk of cardiovascular disease and therefore may represent an ideal population to target with anti-inflammatory therapies.

Chapter 7 contains one manuscript (**Section 7.1**) which has been published. This manuscript is described below, and a more thorough description is found in the first page of the section.

CHAPTER CONTENTS

Section 7.1 describes the results of a prospective cohort study to evaluate the effect of biologic therapy on vascular inflammation in patients with psoriasis and psoriatic arthritis compared to these patients treated with non-biologic therapy as well as a group of non-inflammatory arthritis patients. This manuscript was published in the *Journal of Nuclear Cardiology*.

Section 7.1 Anti-inflammatory effect of biologic therapies in patients with psoriatic disease

SECTION OVERVIEW

This section further explores the effect of biologic therapies in a high-risk population of patients with psoriatic disease. This section presents a manuscript of a prospective cohort where vascular inflammation is measured in patients with psoriatic arthritis before and after the initiation of biologic therapy and is compared with patients with psoriatic disease who were treated with non-biologic therapy as well as a group of patients with non-inflammatory arthritis.

MANUSCRIPT STATUS

This manuscript has been published.

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Supplemental file 1: Impact inventory supplemental table

Author roles and contributions

KEB and GD designed the study. KEB drafted the manuscript, which was revised by all authors. All authors approved the version of the manuscript submitted for publication.

RELATED THESIS APPENDICES

Appendix G: Published manuscripts. The PDF of published manuscript is presented in Appendix G.

Anti-Inflammatory effect of biologic therapy in patients with psoriatic disease: a prospective cohort FDG-PET study

Short title: Vascular inflammation in patients with psoriatic disease on biologic agent

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Abstract

Aim

The aim of the study was to evaluate the changes in central vascular inflammation measured by FDG PET and myocardial blood flow reserve (MFR) determined by ^{82}Rb PET following therapy with biologic agents for 6 months in patients with psoriatic arthritis (PsA) and/or cutaneous psoriasis (PsO) (group 1) and compare with PsO subjects receiving non-biologic therapy (group 2) and controls (group 3).

Methods and Results:

Target-to-background ratio (TBR) by FDG PET in the most diseased segment of the ascending aorta (TBR_{max}) was measured to assess vascular inflammation. ^{82}Rb PET studies were used to assess changes in left ventricular MFR. A total of 34 participants were enrolled in the study (11 in group 1, 13 in group 2, and 10 controls). A significant drop in the thoracic aorta uptake was observed in the biologic-treated group (ΔTBR_{max}: -0.46±0.55) compared to the PsO group treated with non-biologic therapy (ΔTBR_{max}: 0.23±0.67). Those showing response to biologic agents maintained MFR compared to who showed no response.

Conclusions

In a cohort of psoriasis patients treated with biologics, FDG uptake in the thoracic aorta decreased over the study period. Patients who demonstrated a significant anti-inflammatory response on FDG PET imaging maintained their MFR compared to non-responders.

Keywords: *psoriatic arthritis, inflammation, PET imaging, cardiovascular disease*

Introduction

Psoriatic arthritis (PsA) is a disease of chronic inflammation, and occurs in 14-30% of patients with skin or nail psoriasis (PsO).[123, 124] Similar to other diseases of chronic inflammation, patients with PsA or PsO have higher than expected rates of cardiovascular (CV) disease and an increased risk of major adverse cardiovascular events (MACE).[125, 126] Indeed, CV disease is the single leading cause of death in patients with PsA and dermatologic psoriasis and may account for >50% of all deaths in these populations.[127]

Systemic inflammation is thought to be the key mediator in the link between psoriasis and CV disease.[128] Patients with psoriasis are in a state of chronic immune activation and have increased levels of inflammatory cytokines such as tumor necrosis factor (TNF)-α.[129] Interestingly, data has linked psoriasis severity with the degree of systemic inflammation.[130] Chronic inflammation has a negative effect on the vasculature, and plays a pivotal role in the mediation of the peripheral microvascular dysfunction that is seen in patients with psoriasis.[131] A better understanding of the pathophysiology and potential treatment options behind the accelerated atherosclerosis and inflammation that is seen in patients with psoriasis remains a priority.

F-18-fluorodeoxyglucose positron emission tomography (FDG PET) provides an attractive and pragmatic means to measure and follow vascular inflammation in these patients, as it is the most widely validated and applied imaging probe in this regard.[18, 132, 133] Previous studies have shown that its uptake predicts MACE;[13, 134, 135] and it is also highly sensitive to short-term treatment effects.[21, 22] Furthermore, PET myocardial perfusion imaging in conjunction with rubidium-82 (^{82}Rb) affords the ability to assess regional myocardial blood flow to the left ventricle in absolute terms (ml/min/g).[136] Rubidium-82 PET has been established as a nuclear imaging method for

accurate, reproducible and routine quantification of myocardial blood flow and myocardial blood flow reserve (MFR (stress/rest perfusion)) in humans and in animal models of disease.[137-140]

Patients with psoriasis, especially those requiring systemic biologic therapy, have increased vascular inflammation.[130] We hypothesized that it is increased central vascular inflammation in these patients that is contributing to the development of accelerated atherosclerosis. We sought to use FDG PET to quantify vascular inflammation in the ascending aorta in patients with PsA and PsO and hypothesized that thoracic aortic vascular inflammation would improve in these patients following therapy with biologic agents compared to psoriasis patients receiving non-systemic therapies and control patients with non-inflammatory skin/joint disease. Secondly, we sought to explore changes in MFR over time after treatment with biologic agents, and explore the relationship between changes in vascular inflammation, as measured by FDG PET, to changes in MFR determined by ^{82}Rb PET.

Methods

This was a prospective cohort clinical study designed to determine the effect of biologic therapy on vascular inflammation and MFR in patients with PsA compared to cohorts not treated with biologic therapies. Study approval was obtained from the University of Ottawa Heart Institute's Research Ethics Board in accordance with the principles of Declaration of Helsinki. All study participants provided written informed consent.

We studied 3 patient groups which were consecutively enrolled: i) patients with PsA and/or PsO who were to be started on anti-TNF-α, anti- interleukin (IL) 17 or anti-IL 12/23 therapy biologic agents, ii) patients with psoriasis managed on non-biologic therapies, and iii) control patients with non-inflammatory skin or joint conditions. Participants were enrolled from the Rheumatology and Dermatology Clinics at the Ottawa Hospital, Ottawa, Ontario, Canada. A convenience sample was used, and all participants underwent ^{82}Rb PET and FDG PET examinations at baseline and at 6 months. Demographic and anthropometric data including age, sex, ethnicity, height, weight, waist circumference and blood pressure were recorded as well as current medications, medical history, smoking status, and family history of CV disease.

Group 1 consisted of subjects with PsA and/or PsO scheduled for initiation of anti-TNF-α therapy, anti-IL 17 or anti-IL 12/23 therapy with prior failed response to traditional disease-modifying anti-rheumatic drugs (DMARDs). After inclusion in the study, subjects in Group 1 received 6 months treatment with a biologic therapy following the baseline study investigations. Patients with PsA were included if they met the classification criteria for PsA[139] and had persistent joint inflammation measured as 5 or more swollen joints, despite therapy for at least three months, each with 2 or 3 conventional DMARDs (methotrexate, leflunomide, or sulfasalazine). To minimize the effect that duration of the disease could play on the results, only patients with an recent diagnosis of PsA

were included (i.e. patients that had a diagnosis of PsA for > 3 months and < 2 years).[141] The treatment method chosen for patients was decided by the patient's treating rheumatologist.

Group 2 included subjects with psoriasis on non-biologic treatment (i.e. topical medications, acitretin or phototherapy only). Psoriasis Area and Severity Index (PASI) score was used for the assessment of severity of dermatological psoriasis.[142]

Group 3 included patients with an established diagnosis of non-inflammatory joint diseases such as osteoarthritis. These control patients were identified by a detailed history and physical examination, with normal baseline blood tests (full blood count, renal function, fasting glucose and lipid profile).

Primary Outcome: FDG PET imaging of ascending aortic inflammation

The primary outcome of our study was the change in vascular inflammation in the ascending aorta, which was measured according to standardized methods[21] as the target-to-background ratio (TBR) in the most diseased segment of the ascending aorta at baseline compared to 6-month follow-up (TBR_{max}). All patients underwent FDG PET with low dose computed tomography at baseline and then following 6 months follow-up. Patients in group 1 started on biologic agents following their baseline FDG PET. FDG PET images were analyzed to determine TBR_{max} values by utilizing previously published and validated methodology.[21] In short, we first obtained the maximum standardized uptake value (SUV) for the most diseased segment of the ascending aorta by averaging the SUV for 3 consecutive axial slices centered on the highest uptake slice and the adjacent slices superior and inferior to it, providing approximately 1 cm of the most inflamed section of the aortic wall. We then calculated the TBR_{max} by determining the ratio of this SUV_{max} for the most inflamed region of the ascending aorta to background venous activity, derived from

an image region in the superior vena cava. On the follow-up scan in the treatment group, the same 3 slice locations were used to calculate the follow-up TBR_{max}. Of note, the FDG PET analysis was performed by an independent reader blinded to clinical details.

Secondary Outcome: FDG PET imaging of other aortic inflammation

As a secondary analysis, we also evaluated the change in vascular inflammation in other areas of the aorta, including the aortic arch and descending aorta. Similar to the methods outlined above for the ascending aorta, we measured this as the target-to-background ratio (TBR) in the most diseased segment of the respective portion of the aorta (arch or descending aorta) at baseline compared to 6-month follow-up (TBR_{max}).

Secondary Outcome: 82Rb PET for MFR

Dynamic ^{82}Rb PET was performed according to previously published and validated methodology.[143, 144] In short, absolute myocardial blood flow was calculated at rest and during dipyridamole stress using our FlowQuant© analysis software. The ^{82}Rb analysis was performed by an independent reader blinded to clinical details.

Post-hoc Analysis: MFR

To better understand the relationship of changes in vascular inflammation to microvascular function, in a post-hoc analysis we evaluated the change in MFR values in the subgroup of PsA patients treated with biologic agents. We compared the MFR of patients in the biologic group that had a response in their TBR_{max} (that was greater or equal to the median delta TBR_{max} of this group),

to those that had a response that was less than the median (i.e. post-hoc analysis for effect modification).

Statistical Methods

Descriptive statistics (such as median with interquartile range (IQR) for continuous variables, frequencies with percentages for categorical variables) are used to summarize the three study groups baseline demographic and clinical variables. Continuous variables are presented as medians with interquartile range (IQR) and categorical variables are presented as frequencies and percentages (unless otherwise stated). Demographic characteristics are presented as median (IQR) or percentage as appropriate. Differences between patient groups were evaluated by the Fisher exact test for discrete clinical variables and by the Kruskal-Wallis test for continuous variables. Follow-up data were collected as scheduled. All the tests were two-sided, and a P value of <0.05 was considered statistically significant. Within-group testing of changes in baseline and 6-month TBR_{max} and left ventricle (LV) MFR values were conducted with Wilcoxon Signed Rank testing. The relative changes in TBR_{max} and LV MFR over the study period were compared between groups using a generalized linear mixed effects model (GLMM) for repeated measures analysis. Variables included in the model were age, body mass index, sex, and baseline aortic TBR_{max}. Missing data were considered missing at random and complete case analysis was used to handle the missingness. We also performed multiple imputation as a sensitivity analysis to assess the effect of missingness on our results by using a GLMM with baseline and follow-up TBR_{max} included in the model (thereby adjusting for differences in baseline aortic inflammation across groups). Statistical analyses were performed using MedCalc for Windows version 12.0 (MedCalc Software, Ostend, Belgium) and Stata/IC for Windows version 16.1 (StataCorp LLC, Texas, USA).

Results

A total of 42 participants were enrolled in the study (12 PsA and/or PsO patients who were started on biologic agents, 18 PsO patients treated with non-biologic therapies, and 12 patients in the non-inflammatory control group with osteoarthritis). In the biologics group, 5 patients were started on an anti-TNF-α agent (4 adalimumab and 1 etanercept), 4 on an IL-17 inhibitor (secukinumab) and 2 on an IL-12/23 inhibitor (ustekinumab). One patient in the biologic group dropped out after having an abnormal imaging study that required further intervention, 5 patients in the PsO group not on biologic therapies withdrew after baseline imaging studies and 2 patients in the control group withdrew after baseline imaging studies. This resulted in a total of 34 patients having complete baseline and follow-up imaging data for analysis: 11 PsA and/or PsO patients started on biologic agents, 13 PsO patients treated with non-biologic therapies, and 10 patients in the non-inflammatory control group with osteoarthritis. Additionally, all patients (including patients that withdrew/were lost to follow-up) were included in the sensitivity analysis with multiple imputation.

Participant characteristics are described in Table 1. In summary, 64.7% percent of participants were men, and the median age was 62 years (IQR: 48, 69), which was similar between the three groups. Median BMI was 28.7kg/m^2 (IQR: 27.1, 36.3). More patients in Group 1 had diabetes ($p=0.032$), were more commonly treated with Methotrexate ($p=0.002$), or on medications such as oral steroids ($p<0.001$) and non-steroidal anti-inflammatory drugs (NSAIDs)($p=0.002$).

Table 1. Baseline characteristics of population

Variable	PsA and/or PsO on Biologics (n=11)	PsO on non-biologics (n=13)	Control (n=10)	P value
	Median (IQR) or n (%)			
Age, years	62.0 (44.0, 64.5)	57.0 (47.5, 64.5)	63.5 (55.0, 73.0)	0.218
Male sex, n (%)	5 (45%)	8 (62%)	7 (70%)	0.525
BMI, kg/m^2	35.3 (28.7, 41.4)	29.6 (27.3, 33.6)	28.2 (25.0, 29.4)	0.067
Hypertension, n (%)	5 (45%)	5 (38%)	1 (10%)	0.186
Diabetes, n (%)	3 (27%)	0 (0%)	0 (0%)	0.032**
Dyslipidemia, n (%)	3 (27%)	5 (38%)	4 (40%)	0.793
Current smoking, n (%)	9 (91%)	8 (62%)	5 (50%)	0.299
PASI of patients with PsO	(n=2) 20.0 (10.4, 29.6)	7.0 (5.7, 10.2)	N/A	0.132*
CRP, mg/L	4.10 (1.33, 4.98)	1.90 (0.95, 5.38)	1.80 (1.10, 3.20)	0.634
Other non-biologic medications/treatments				
NSAIDs, n (%)	5 (45%)	0 (0%)	0 (0%)	0.002**
Steroids, n (%)	7 (64%)	0 (0%)	0 (0%)	0.0001**

Methotrexate, n (%)	5 (45%)	0 (0%)	0 (0%)	0.002**
Topical agents, n (%)	7 (64%)	10 (77%)	5 (50%)	0.406
Acitretin, n (%)	1 (9%)	0 (0%)	0 (0%)	0.341
Phototherapy, n (%)	2 (18%)	8 (62%)	0 (0%)	0.004**

BMI, body mass index; CRP, c-reactive protein; NSAIDs, non-steroidal anti-inflammatory drugs; PASI, psoriasis area sensitivity index; PsA, psoriatic arthritis; PsO, cutaneous psoriasis; TBR, target-to-background ratio.

*Mann Whitney test used to compare PASI values of groups

** Statistically significant findings across groups on Kruskal-Wallis or Fisher exact testing.

Inflammation Imaging Results

Baseline ascending aortic TBR_{max} was not statistically different between the three groups: 2.84 (IQR 2.51, 3.37), 2.73 (IQR 2.54, 3.28), versus 2.70 (IQR 2.68, 2.76) in the biologics, non-biologic and control groups, respectively (P=0.725). Similarly, baseline aortic arch TBR_{max} did not differ across the groups (P=0.565), and nor did baseline descending aortic TBR_{max} (P=0.190). For the primary outcome, analysis within groups with Wilcoxon Rank Sum testing demonstrated a statistically significant reduction in vascular inflammation measured as FDG TBR_{max} within the ascending aorta, in the biologic group only [baseline 2.84 (IQR 2.51, 3.37), follow-up 2.50 (IQR 2.27, 2.94) (P=0.033)]. There were no significant changes in either the non-biologic [baseline 2.73 (IQR 2.54, 3.28), follow-up 2.95 (IQR 2.64, 3.63) (P=0.279)], or control groups [baseline 2.70 (IQR 2.68, 2.76), follow-up 2.94 (IQR 2.67, 3.28) (P=0.114)] (Table 2 and Figure 1). Similarly,

there were statistically significant descreases in measured FDG TBR_{max} within the aortic arch (P=0.002) and descending aorta (P=0.007) within the biologic group, but not the other groups (P>0.05). Table 2 illustrates the change in aortic TBR uptake in the ascending aorta as well as change in MFR of the three groups. GLMM for repeated measures analysis revealed the change in FDG TBR_{max} over the study period in Group 1 differed significantly from Group 2 (β±SE: 0.697±0.245, P=0.004) and Group 3 (β±SE: 0.670±0.259, P=0.010). This was true for the aortic arch and descending aorta as well. Sensitivity analysis with multiple imputation for missing FDG TBR_{max} values showed the results remained significant after imputation (biologic group versus non-biologic group: β±SE: 0.651±0.230, P=0.005); biologic group versus control group: β±SE: 0.631±0.261, P=0.016). The change in FDG uptake from baseline to follow-up is shown in Figure 2.

Table 2. Changes in TBR_{max} and LV MFR by Treatment Group During Trial Period

	PsA and/or PsO on Biologics (n=11)	**PsO on non-biologics (n=13)**	**Control (n=10)**	**P value**
Median (IQR) or n (%)				
Baseline ascending aorta TBR_{max}	2.84 (2.51, 3.37)	2.73 (2.54, 3.28)	2.70 (2.68, 2.76)	0.725
Follow-up ascending aorta TBR_{max}	2.50 (2.27, 2.94)	2.95 (2.64, 3.63)	2.94 (2.67, 3.28)	0.102

Delta ascending aorta TBR_{max}	-0.19 (-0.92, 0.11)*,***	0.32 (-0.26, 0.75)*	0.21 (-0.18, 0.58)*	0.021**
Baseline ascending aorta SUV_{max}	3.51 (3.08, 4.16)	3.63 (3.02, 4.72)	3.61 (3.00, 3.96)	0.803
Follow-up ascending aorta SUV_{max}	3.26 (2.67, 3.47)	3.86 (3.02, 4.37)	3.51 (3.33, 3.82)	0.102
Delta ascending aorta SUV_{max}	-0.42 (-0.83, -0.07)***	0.23 (-0.62, 0.57)	0.08 (-0.61, 0.74)	0.103
Baseline aortic arch TBR_{max}	2.76 (2.66, 3.06)	2.62 (2.43, 3.28)	2.87 (2.68, 3.12)	0.565
Follow-up aortic arch TBR_{max}	2.47 (2.12, 2.85)	2.89 (2.51, 3.19)	2.97 (2.44, 3.24)	0.079
Delta aortic arch TBR_{max}	-0.43 (-0.63, -0.29)*,***	0.16 (-0.47, 0.52)*	0.20 (-0.31, 0.48)*	0.029**
Baseline aortic arch SUV_{max}	3.60 (3.07, 4.08)	3.13 (2.79, 4.10)	3.59 (3.54, 4.15)	0.640
Follow-up aortic arch SUV_{max}	3.04 (2.46, 3.33)	3.57 (3.05, 4.01)	3.50 (3.10, 4.12)	0.068
Delta aortic arch SUV_{max}	-0.53 (-0.91, -0.24)***	0.29 (-0.17, 0.73)	-0.31 (-0.46, 0.46)	0.045**

Baseline descending aorta TBR_{max}	2.73 (2.47, 3.46)	2.71 (2.39, 3.14)	3.25 (2.84, 3.35)	0.190
Follow-up descending aorta TBR_{max}	2.25 (2.01, 2.54)	2.78 (2.59, 3.15)	3.22 (2.77, 3.66)	0.002**
Delta descending aorta TBR_{max}	-0.57 (-0.79, -0.17)*,***	0.07 (-0.24, 0.24)*	0.12 (-0.47, 0.50)*	0.011**
Baseline descending aorta SUV_{max}	3.70 (2.83, 3.98)	3.41 (3.01, 3.80)	3.82 (3.66, 4.33)	0.099
Follow-up descending aorta SUV_{max}	2.85 (2.46, 3.04)	3.46 (3.20, 4.00)	3.64 (3.34, 3.96)	0.002**
Delta descending aorta SUV_{max}	-0.74 (-0.95, -0.27)***	0.20 (-0.18, 0.52)	-0.29 (-0.50, 0.02)	0.012**
Flow Data				
Baseline LV MFR	2.96 (2.38, 3.46)	3.14 (2.54, 3.95)	3.43 (2.84, 4.34)	0.528
Follow-up LV MFR	2.85 (2.20, 3.06)	3.58 (3.08, 3.94)	3.92 (2.97, 4.38)	0.035**
Delta LV MFR	-0.24 (-0.46, -0.02)***	0.13 (-0.38, 0.73)	0.30 (-0.83, 0.96)	0.246
Baseline rest SBP	127 (104, 140)	134 (117, 142)	110 (102, 127)	0.098

Baseline rest HR	68 (58, 75)	72 (66, 82)	63 (55, 67)	0.023**
Baseline rest flow	0.80 (0.58, 0.80)	0.89 (0.72, 1.0)	0.62 (0.52, 0.71)	0.061
Baseline stress flow	1.79 (1.64, 2.71)	2.66 (2.40, 2.92)	2.12 (1.89, 2.62)	0.127
Baseline stress SBP	135 (123, 141)	141 (125, 145)	117 (110, 140)	0.049***
Baseline stress HR	90 (83, 95)	96 (87, 102)	80 (77, 87)	0.020***
Follow-up rest SBP	133 (111, 145)	123 (117, 140)	117 (102, 135)	0.400
Follow-up rest HR	67 (63, 79)	67 (64, 73)	60 (58, 61)	0.003***
Follow-up rest flow	0.79 (0.67, 1.01)	0.74 (0.66, 0.87)	0.66 (0.55, 0.88)	0.200
Follow-up stress SBP	142 (126, 159)	130 (124, 141)	118 (107, 144)	0.172
Follow-up stress HR	85 (76, 95)	93 (87, 97)	83 (75, 88)	0.067
Follow-up stress flow	2.15 (1.60, 3.08)	2.60 (2.28, 2.94)	2.36 (2.20, 2.72)	0.528

* Statistically significant findings on GLMM for repeated measures analysis revealed the change in FDG TBRmax over the study period in Group 1 differed significantly ($p<0.05$)

** Statistically significant findings across groups on Kruskal-Wallis testing.

***Within-group testing of changes in baseline and 6-month values were statistically significant with Wilcoxon Signed Rank testing

HT, heart rate; LV MFR, left ventricular myocardial blood flow reserve; MFR, myocardial blood flow reserve; PsA, psoriatic arthritis; PsO, cutaneous arthritis; SBP, systolic blood pressure; TBR, target-to-background ratio.

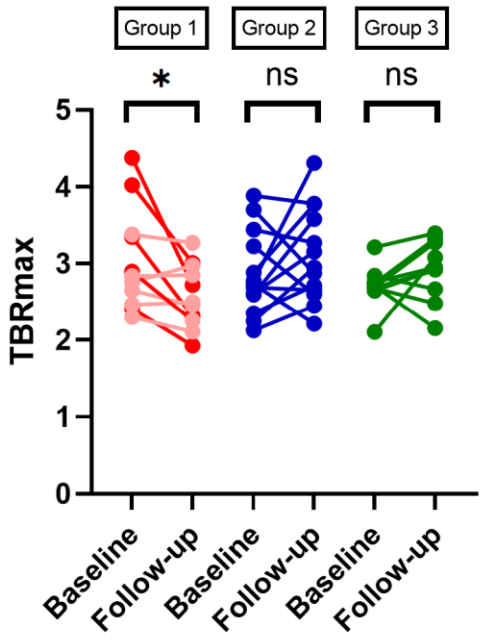

Figure 1: Change in ascending aorta FDG uptake from baseline to 6-month follow-up in Group 1 (patients with PsA and/or PsO on biologics), Group 2 (patients with PsO on non-biologics), and group 3 (control group).

FDG, F-18-fluorodeoxyglucose; PsA, psoriatic arthritis; PsO, psoriatic disease; TBR, target-to-background ratio

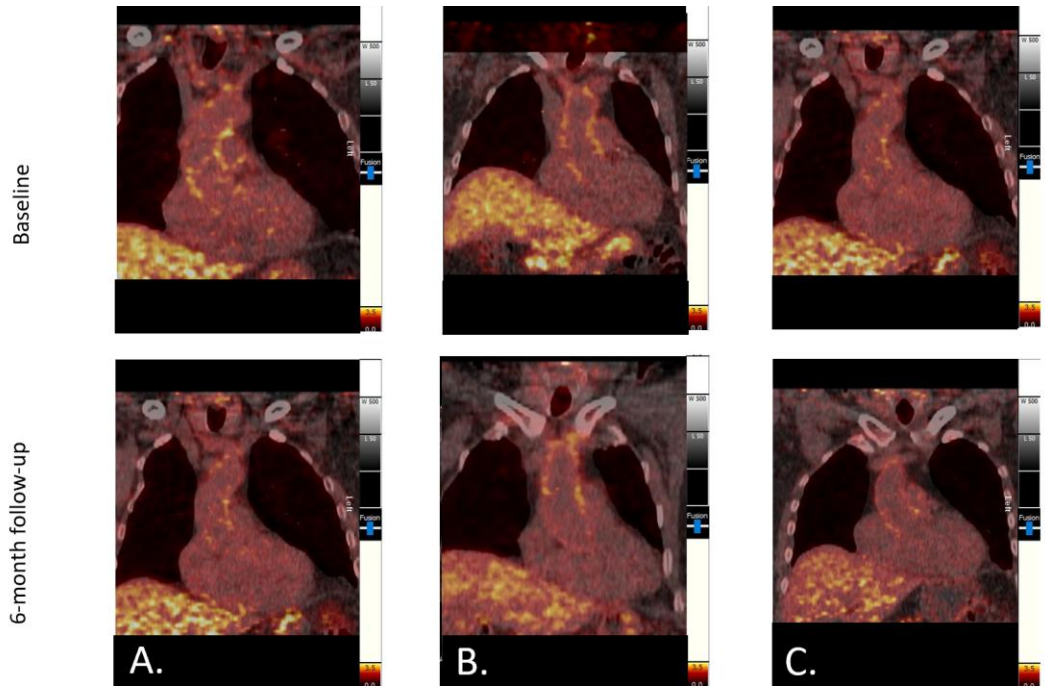

Figure 2: FDG images showing change in ascending aorta FDG uptake from baseline to 6-month follow-up in: A) patients with PsA and/or PsO on biologic agents, B) patients with PsO on non-biologic agents, and C) control patients with non-inflammatory arthritis

FDG, F-18-fluorodeoxyglucose; PsA, psoriatic arthritis; PsO, psoriatic disease

Regarding the exploratory outcome, baseline MFR was not statistically different between the three groups: Baseline MFR was 2.96 (IQR 2.38, 3.46), 3.14 (IQR 2.54, 3.95), and 3.43 (IQR 2.84, 4.34)

in the biologic, non-biologic and control groups, respectively (P=0.528). Analysis within groups with Wilcoxon Rank Sum testing demonstrated a statistically significant reduction in MFR in the biologic group only [baseline 2.96 (IQR 2.38, 3.46), follow-up 2.85 (IQR 2.20, 3.06) (p=0.037)]. There were no significant changes in either the non-biologic [baseline: 3.14 (IQR 2.54, 3.95), follow-up: 3.58 (IQR 3.08, 3.94) (P=0.279)], or control groups [baseline: 3.43 (IQR 2.84, 4.34), follow-up: 3.92 (IQR 2.97, 4.38) (P=0.114)] (Table 2). GLMM for repeated measures analysis revealed no statistically significant differences in the change in MFR over the study period between the biologic group and non-biologic group (β±SE: 0.477±0.370, P=0.198) and control group (β±SE: 0.467±0.395, P=0.237), respectively.

Sensitivity analysis with multiple imputation for missing ascending aortic FDG TBR_{max} values showed results remained non-significant even after imputation (biologic group versus non-biologic group: β±SE: 0.522±0.407, P=0.201; biologic group versus control group: β±SE: 0.384±0.456, P=0.400).

While GLMM for repeated measures analysis revealed no significant difference in the change in MFR between the three groups over the study period, to better understand the relationship of changes in vascular inflammation to microvascular function, we decided to further evaluate the change in MFR in the subgroup of PsA patients treated with biologic agents in a post-hoc analysis. We compared the MFR of patients in the biologic group who had a response in their TBR_{max} that was greater or equal to the median of this group, to those who had a response that was less than the median. We found a significant difference in these groups of patients. The group with a clinical response to biologic agents as measured by a change in TBR_{max} that was greater or equal to the median change (7%) maintained MFR (3.40±1.23 MFR to 3.5±1.2 MFR over 6 months) when compared to the group with a below median response on TBR_{max} which had a drop in MFR

(2.9±0.8 MFR to 2.2±0.6 over 6 months; P=0.03). Spearman's coefficient of rank correlation (rho) between change in TBR_{max} and change in MFR was 0.709 (P=0.0146) (Figure 3). Finally, as an additional exploratory analysis we looked at whether there were any differences in vascular inflammation change between patients in Group 1 on different types of biologics (TNF inhibitor, IL-17 inhibitor, and IL12/23 inhibitor). We found no difference, likely due to the small number of patients.

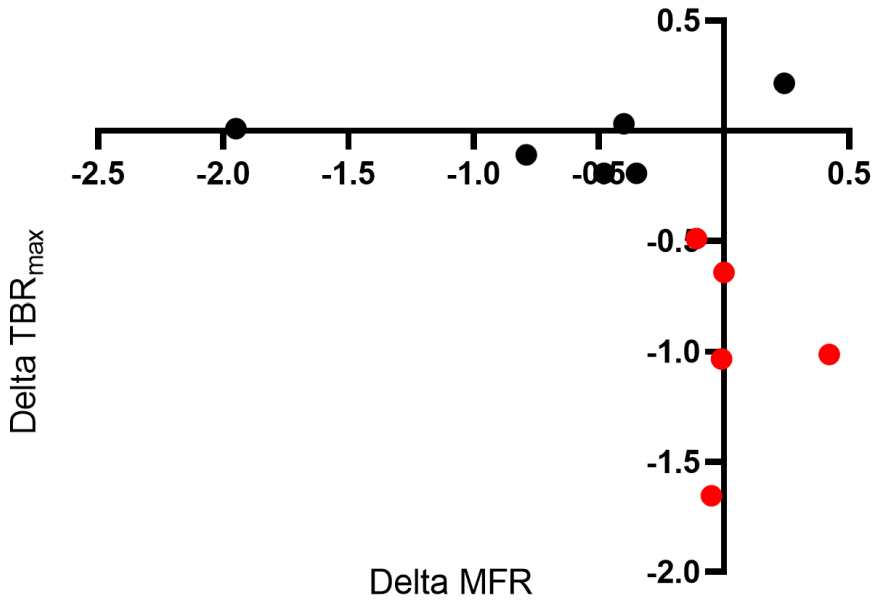

Figure 3: Correlation between change in TBR_{max} and MFR in patients with PsA and/or PsO on biologic agents. Patients with a clinically significant response to biologic agents are coloured red.

Delta TBR_{max}, target-to-background ratio in the most diseased segment of the ascending aorta at baseline compared to 6-month follow-up; Delta MFR, myocardial blood flow reserve at baseline compared to 6-month follow-up

Discussion

We conducted a prospective cohort study to determine the impact of systemic anti-cytokine treatment on vascular inflammation and MFR in patients with psoriasis, an inflammatory disease that is well known to be associated with vascular inflammation.[130] In a cohort of psoriasis patients that were started on biologic therapy, representative of clinical practice, we found that FDG uptake in the thoracic aorta decreased over the 6 month study period compared to no change in psoriasis patients treated with non-systemic therapies or in a cohort of control patients with non-inflammatory joint and skin disease. Additionally, we found that in those psoriasis patients treated with biologics who had imaging evidence of a significant anti-inflammatory response to the biologics, MFR was maintained compared to the group that did not have a significant response to the biologic therapy. The present study helps fill a knowledge gap by suggesting that anti-cytokine therapy may have vascular anti-inflammatory effects. These changes may be linked to changes in the coronary microvasculature in patients with psoriasis and support the notion that anti-inflammatory biologics may have a role in mitigating CV disease in this patient population.

Psoriasis disease is a chronic inflammatory skin condition that affects over 125 million people worldwide.[145] Patients with psoriasis have been found to have an increased incidence of CV disease and increased risk of MACE.[125, 146-148] A key link between psoriasis and heart disease is mediated through inflammation. Patients with psoriasis have increased vascular inflammation measured by FDG PET, and it has been shown that increasing severity of skin disease is associated with increased vascular inflammation.[130, 149] However, at this point it is not yet known whether targeting inflammatory pathways could lead to downstream effects in reducing CV morbidity and mortality in these patients.

In our jurisdiction, PsA patients progressing to a biologic agent need to have ongoing arthritis (5 or more swollen joints) despite treatment with methotrexate for 3 months (usually at doses between 20 and 25 mg/week) and an additional 3 months on leflunomide (20 mg/day) or sulfasalazine. (2000 mg/day). Patients with skin disease need to demonstrate at least 3% of total body skin involvement despite 3 months of treatment with systemic therapy, usually methotrexate or cyclosporine. In the present study, treatment with anti-cytokine therapy was associated with a clinically significant reduction in aortic inflammation both when compared within the biologic group, and also when compared to the non-biologic and control groups. Our data provides insights into the potential linkage of atherosclerosis with inflammation and further suggests that treatment of psoriasis patients with biologic agents may help to mitigate vascular inflammation. Additionally, while the overall change in MFR did not differ between the psoriasis group treated with anti-cytokine therapy and the other groups, when we looked specifically at patients in the psoriasis group that were treated with biologic agents who had a response in their TBR_{max} that was greater than the median (compared to the patients treated with biologic agents with a response less than the median), MFR was maintained in the responders compared to the non-responders (where it significantly dropped).

Our results are consistent with a noncontrolled study in rheumatoid arthritis of anti-TNF-α therapy, which demonstrated a reduction in vascular inflammation on FDG PET imaging after 8 weeks of treatment[150]. They are also consistent with an observational cohort from Kim et. al evaluating the anti-inflammatory effect of 25 patients with PsO treated with Ustekinumab[151]. Furthermore, an observational study from Dey et. al that evaluated a cohort of psoriasis patients being treated systemically had similar findings.[152]

Our results however, are not consistent with a randomized placebo-controlled trial from Mehta et. al, which showed no change in vascular inflammation on FDG PET in patients randomized to adalimumab, phototherapy or placebo for a period of 12 weeks, with an open-label extension of the adalimumab arm for a period of 52 weeks.[153] There are several possible reasons for the discrepancy of our results with that observed by Mehta et. al. Firstly, our study had 64% of the PsA/PsO population with PsA (while only 10% in the Mehta et. al study had PsA). Thus, there are underlying differences in the composition of the study populations which could theoretically influence the vascular response to biologic therapy. Secondly, while there was no difference in baseline inflammatory levels between our three study groups, it should be noted that our biologic therapy group had a higher proportion of patients pretreated with NSAIDS, methotrexate, and steroids. This difference in anti-inflammatory pretreatment across study groups could again theoretically alter the vascular response to biologic agents. Thirdly, our sample size was very small, increasing the risk for our results to be influenced by statistical chance. However, the consistent response of different anatomical sections of the aorta to the biologic agents certainly argues against this explanation. Lastly, ours was a non-randomized study, which could result in potential unmeasured confounding of our results. It should be noted, however, that these possible explanations are purely speculative.

Our study has several important limitations. Firstly, this was a non-randomized prospective observational study with a modest sample size, and limited clinical follow-up. Thus, our results should be interpreted with caution, and while they are largely hypothesis-generating, they should certainly fuel further randomized clinical trials to investigate these findings. Conversely, we employed rigorous methodological analyses as well as a sensitivity analysis with multiple imputation to attempt to overcome some of these limitations and strengthen our results. Secondly,

we utilized imaging outcomes to measure inflammatory response to biologic therapy rather than clinical outcomes. While clinical assessment of joint response to biologic therapy involves subjective measures, the imaging data of vascular inflammation that we utilized for our primary outcome is a completely objective measure of treatment response. Both vascular inflammation and MFR have been shown to be robust indices, which can give important insights into disease activity over shorter periods of clinical observation. Therefore, both vascular inflammation and MFR represent important surrogate markers for imaging trials that allow for the assessment of therapeutic benefit of interventions in a timely manner. In this regard, while FDG PET has been evaluated utilized extensively for the non-invasive detection of inflammation related to atherosclerosis, it does have several limitations which should be highlighted. While FDG PET imaging targets activated macrophages, it is still a non-specific probe that can accumulate in other metabolically active tissues which can thereby introduce interfering background signal.[154, 155] Imaging of the ascending aorta requires strict dietary preparation to suppress FDG-myocardium uptake (with a high-fat, low carbohydrate diet prior to PET imaging), given the significant background myocardial FDG uptake which can make it more difficult to differentiate vascular inflammation from background uptake.[156, 157] FDG PET imaging is also affected by glucose levels, so particular caution in imaging patients with diabetes and hyperglycemia must be employed.[158] From a technical perspective, the partial volume effect and motion artifacts from cardiac and organ motion during imaging all limit the spatial resolution and specificity of the test.[159] Additionally, given the limited spatial resolution of current PET imaging systems, we are currently unable to directly quantify "vulnerable plaque" in smaller sized vessels, such as the coronary arteries meaning we are limited to assessing larger caliber vessels, such as the aorta.[160] Finally, while baseline demographic factors were not statistically different between groups, it is possible that the

numeric difference in the proportion of participants with cardiac risk factors like hypertension, diabetes or current smoking, or differences in baseline BMI could have influenced the differences we saw in baseline TBR_{max} and response to biologics between the groups.

In conclusion, our study suggests that anti-cytokine therapy is associated with decreasing vascular inflammation in patients with PsA as assessed by FDG PET in contrast to non-biologics as well as a non-inflammatory control group. While our study should be interpreted with caution given the small number of participants and limited generalizability, our results do suggest an association between these biologic agents and preserved MFR in patients who respond favourably in terms of their vascular inflammation. This study supports the notion that there is a positive impact of immune therapies on vascular inflammation and microvascular disease in patients with chronic inflammation, however larger prospective clinical outcome trials investigating this are warranted.

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Chapter 8: Low-dose statin therapy in persons living with HIV

CHAPTER OUTLINE

Chapter 8 addresses the sixth question: *"Could low-dose statin therapy be an effective option to reduce vascular inflammation in patients with HIV?"*

Chapter 7 reviewed the effect of biologic therapy in the high-risk population of patients with psoriatic arthritis. Similarly, persons living with HIV represent a high-risk population that warrants investigation to determine if targeting inflammation for this group may lower cardiovascular risk.

Chapter 8 contains one manuscript (**Section 8.1**) which has been published. This manuscript is described below, and a more thorough description is found in the first page of the section.

CHAPTER CONTENTS

Section 8.1 describes the results of a randomized controlled trial to evaluate the effect of low-dose statin therapy on vascular inflammation in patients living with HIV. This manuscript was published in the *Journal of Nuclear Cardiology*.

Section 8.1 Anti-Inflammatory effect of rosuvastatin in patients with HIV infection: an FDG-PET pilot study

SECTION OVERVIEW

This section explores the effect of low-dose statin therapy in persons living with HIV. A manuscript of a small randomized controlled trial is presented which evaluates the effectiveness of low-dose statin therapy (compared with standard of care) in persons with well-controlled HIV infection.

MANUSCRIPT STATUS

This manuscript has been published.

Boczar KE, Faller E, Zeng W, Wang J, Small GR, Corrales-Medina VF, deKemp RA, Ward NC, Beanlands RSB, MacPherson P, Dwivedi G. Anti-inflammatory effect of rosuvastatin in patients with HIV infection: An FDG-PET pilot study. J Nucl Cardiol. 2022 Dec;29(6):3057-3068. doi: 10.1007/s12350-021-02830-4. Epub 2021 Nov 24. PMID: 34820771.

Author roles and contributions

KEB and GD designed the study. KEB drafted the manuscript, which was revised by all authors. All authors approved the version of the manuscript submitted for publication.

RELATED THESIS APPENDICES

Appendix G: Published manuscripts. The PDF of published manuscript is presented in Appendix G.

Anti-Inflammatory effect of rosuvastatin in patients with HIV infection: an FDG-PET pilot study

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Abstract

Aims: This study aimed to evaluate markers of systemic as well as imaging markers of inflammation in the ascending aorta, bone marrow, and spleen measured by 18F-FDG PET/CT, in HIV+ patients at baseline and following therapy with rosuvastatin.

Methods and results: Of the 35 HIV+ patients enrolled, 17 were randomized to treatment with 10mg/day rosuvastatin and 18 to usual care for 6 months. An HIV- control cohort was selected for baseline comparison of serum inflammatory markers and monocyte markers of inflammation. 18F-FDG-PET/CT imaging of bone marrow, spleen, and thoracic aorta was performed in the HIV+ cohort at baseline and 6 months. While CD14++CD16- and CCR2 expressions were reduced, serum levels of IL-7, IL-8, and MCP-1 were elevated in the HIV+ population compared to the controls. There was a significant drop in FDG uptake in the bone marrow (TBR_{max}), spleen (SUV_{max}) and thoracic aortic (TBR_{max}) in the statin-treated group compared to the control group (bone marrow: -10.3±16.9% versus 5.0±18.9%, p=0.0262; spleen: -9.8±20.3% versus 11.3±28.8%, p=0.0497; thoracic aorta: -19.1±24.2% versus 4.3±15.4%, p=0.003).

Conclusions: HIV+ patients had significantly markers of systemic inflammation including monocyte activation. Treatment with low-dose rosuvastatin in the HIV+ cohort significantly reduced bone marrow, spleen and thoracic aortic FDG uptake.

Keywords*: HIV, cardiovascular risk, rosuvastatin, inflammation, atherosclerosis, FDG-PET/CT.*

Abbreviations:

18-FDG: 18F-fluorodeoxyglucose

ART: anti-retroviral therapy

BMI: body mass index

CCR2: C-C chemokine receptor type 2

CVD: cardiovascular disease

HbA1c: hemoglobin A1c

HDL: high-density lipoprotein

HIV: human immunodeficiency virus

hsCRP: high-sensitivity C-reactive protein MI: myocardial infarction

IL: interleukin

LDL: low-density lipoprotein

MCP: monocyte chemoattractant protein

NNRTI: non-nucleoside reverse-transcriptase inhibitors

PET/CT: positron emission tomography/computed tomography

SUV_{max}: maximum standardized uptake value

TBR_{max}: target to background ratio

Introduction

Patients with human immunodeficiency virus (HIV) have a significantly increased risk of cardiovascular disease (CVD).[69] HIV+ individuals are 2-4 times more likely to suffer a myocardial infarction (MI),[70, 71] and CVD now accounts for 10% of all deaths in this population.[69] HIV infection is associated with accelerated progression of atherosclerosis, an association that appears to be independent of traditional CVD risk factors.[72]

Atherosclerosis is a multi-faceted disease with inflammation playing a central role in atherosclerosis initiation and progression, and the triggering of events such as MI and stroke. HIV infection has been associated with persistently increased systemic inflammation, immune activation and pro-coagulant changes despite effective antiretroviral therapy.[73] These effects are thought to drive endothelial dysfunction, monocyte/macrophage activation, and accelerated atherosclerosis, as well as MI and post-MI fibrosis and reduced left ventricular function.[74] Monocyte-derived macrophages are a principal mediator of inflammation in atherosclerotic plaques.[75, 76] Activation of monocyte-derived macrophages infiltrating atherosclerotic plaques appears to occur at early stages in the bone marrow and spleen.[77, 78]

Statins are a widely prescribed lipid-lowering drug-class that reduces cardiovascular morbidity and mortality in both primary and secondary prevention.[79] In addition to their lipid-lowering effects, statins may have additional pleiotropic effects, including improvements in endothelial function, stabilization of atherosclerotic plaques, anti-inflammatory, immune-modulatory and anti-thrombotic effects.[80] Tissue uptake of 18F-fluorodeoxyglucose (18F-FDG) as measured by 18F-FDG positron emission tomography/computed tomography (PET/CT) scanning is a widely employed and sensitive technique for directly assessing inflammation in arterial walls and other organs such as the spleen and bone marrow. This is because inflammatory cells (mainly macrophages) have higher metabolic activity (i.e. higher rate of

glycolysis) than other organ specific cell types (i.e. smooth muscle cells, endothelial cells, fibroblasts, osteoblasts, T-lymphocytes, etc.).[81] Given the potential link between inflammation and accelerated atherosclerosis in HIV+ patients, in this study we use 18F-FDG PET/CT to quantify regional haemopoietic activity[82] and inflammation as measured by FDG bone marrow, spleen and ascending aorta uptake in HIV+ patients with moderate CVD risk, and also measured markers of monocyte activation in this group. We hypothesized that monocyte markers of inflammation would be elevated in an HIV+ cohort compared to an HIV- cohort. Furthermore, in our HIV+ cohort, we hypothesized that imaging markers of inflammation in the ascending aorta as well as bone marrow and spleen as measured by 18F-FDG PET/CT, as well as markers of systemic inflammation including monocyte activation and markers of monocyte activation would improve in HIV+ patients with moderate CVD risk randomized to therapy with rosuvastatin compared to HIV+ patients randomized to standard of care.

Methods

Participants were enrolled from the Immunodeficiency Clinic at the Ottawa Hospital General Campus, Ottawa, Ontario, Canada. Eligible participants were those with documented serologic evidence of HIV infection; $\geq$ 40 years of age; receiving standard ART for >2 years with a viral load below the limits of detection while on ART, a current CD4 count >350 cells/μL; and with a baseline Framingham risk score of 10-20%. Nine HIV- controls with no known cardiovascular disease were selected as age matched volunteers for immunological studies only (no imaging studies). The study was approved by University of Ottawa Heart Institute's Research Ethics Board. All participants provided a written informed consent.

Demographic data including age, sex, ethnicity, height, weight, waist circumference and blood pressure were recorded as well as current medication use, medical history, smoking status, and family history of CVD. Medical records were accessed to ascertain CD4 count, HIV viral load, creatinine, hemoglobin A1c (HbA1C), and total, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, with all tests performed within 1 month prior to the study visit. Participants were randomized to receive rosuvastatin (10mg daily) for 6 months in addition to their current medication or to continue with their current medical therapy only.

Immunological Studies

At baseline and 6 months, whole blood was drawn from study participants into heparinized tubes and plasma was stored at -80°C for later analysis. Monocytes were stained directly in blood using anti CCR2-APC antibodies from R & D systems. Anti CD14-FITC anti CD16-PC7 and anti CD127-PE antibodies were purchased from Beckman Coulter. Blood was fixed and RBC lysed using BD Biosciences FACS Lysing solution and analyzed by Beckman Coulter FC500 MPL machine with CXP software. The presence and proportions of monocyte sub-populations was detected by flow cytometry using fluorochrome-labeled antibodies to CD14 and CD16 listed above. Classical monocytes were defined as CD14+CD16-, non-classical as CD14+CD16++CCR2-, and intermediate as CD14++CD16+CCR2+.[34, 35] CD127 and CCR2 expression were also measured on bulk CD14+ cells. Levels of IL-7, IL-8, MCP-1, were also measured by Luminex in patient plasma from both time points. Plasma samples were analyzed using custom multiplex MAGPIX kit from R&D Systems, with a Luminex MAGPIX Analyzer with xPONENT 4.2 software. Monocyte subsets and plasma cytokine concentrations were also measured in 9 HIV- age matched controls for a baseline inflammatory comparison (of HIV+ individuals to HIV- controls).

FDG-PET/CT imaging

FDG-PET/CT was performed at the University of Ottawa Heart Institute. Participants fasted overnight and had a fasting blood sugar <11 mmol/L. 18F-FDG was produced by the onsite cyclotron in Ottawa using standard production methods and modules.[18] 18F-FDG (5MBq/kg) was injected intravenously and patients were rested for 2 hours to allow for uptake before being imaged on a hybrid PET/CT scanner (Discovery 690 PET/CT 64 slice GE Healthcare). Following an unenhanced CT scan for attenuation correction and anatomic co-registration, PET data was acquired in 3-dimensional mode. Images were reconstructed as 4 mm slices using high-resolution reconstruction after correction for attenuation, dead time, random coincidences and scatter. PET and CT data were fused and analyzed by using the open-source DICOM viewer Hermes (Version 4.0, OsiriX Imaging Software, Geneva, Switzerland).

Bone marrow activity was measured according to previously validated methods.[83] In short, bone marrow 18F-FDG uptake was determine by placing ROIs in axial sections of individual vertebrae from T1 to L5. The maximum standardized uptake value (SUV_{max}) was recorded for each vertebra, and bone marrow activity was calculated as the average of the registered SUV_{max} of all measured vertebrae. We then calculated the maximum FDG target to background ratio (TBR_{max}) by determining the ratio of the SUV_{max} value from the bone marrow to background venous activity, derived from the superior vena cava at the level of the aortic arch. Splenic FDG uptake was assessed by placing regions of interest in 3 orthogonal planes (axial, sagittal, and coronal planes). SUV_{max} was recorded in each plane, and splenic activity was calculated as the mean of SUV_{max} values of the 3 planes. Aortic FDG uptake was evaluated according to previously published and validated methodology.[21] In short, we first obtained SUV_{max} values in the most diseased segment of the ascending aorta of the ascending aorta by averaging the SUV_{max} for 3 consecutive axial slices centered on the hottest slice and the adjacent slices superior and inferior to it, providing approximately 3 cm of the most inflamed section of the aortic wall. We then calculated the maximum FDG target to background ratio (TBR_{max}) by determining the ratio of the SUV_{max} value from the aorta to background venous activity, derived from the superior vena cava at the level of the aortic arch.

Data Analysis

Statistical analyses were performed using MedCalc for Windows version 12.0 (MedCalc Software, Ostend, Belgium). Continuous variables are presented as means with standard deviations (SD) and categorical variables are presented as frequencies and percentages. Demographic characteristics are presented as mean ± SD or percentage as appropriate. Differences between patient groups were evaluated by the χ^2 test or Fisher exact test for discrete clinical variables and by the Students' t-test or Mann-Whitney U test for continuous variables. Follow-up data were collected as scheduled, and dropouts were excluded. All the tests were two-sided, and a P value of <0.05 was considered statistically significant. Shapiro-Wilk testing was used to test the statistical normality of the data.

Results

Baseline Participant Characteristics

Thirty-five HIV+ patients were recruited, 86% of whom were men. Average age was 51.5±6.9 years and the average BMI was 25.7±3.8kg/m^2. Participants were well-matched between the treatment groups, with regards to CVD risk factors, with the exception of BMI, which was higher in the patients receiving statin treatment as well as the proportion of male sex, which was higher in the control group. Additionally, groups were matched with regards to age, sex, smoking, Framingham risk, duration of ART and protease inhibitor (PI) versus non-nucleoside reverse-transcriptase inhibitors (NNRTI) based therapy. Average CD4 count was 653±206 cells/μL and a similar number of participants within the groups were taking protease inhibitors. Average bone marrow SUV in the entire HIV+ study population was 2.99±0.54, average splenic SUV_{max} was 4.11±1.04, and average TBR_{max} in the ascending aorta was 2.99±0.38. These were similar between the rosuvastatin-treated HIV+ group and the standard-of-care HIV+ group (Table 1).

Table 1. Baseline Characteristics of the HIV+ Participants

Variable	Statin treated (n=17)	Control (n=18)	P value
Age, years	50.5±5.6	52.3±7.9	0.445
Male sex, n (%)	12 (71%)	18 (100%)	0.017*
BMI, kg/m^2	27.0±4.0	24.4±3.2	0.040*
Hypertension, n (%)	4 (24%)	1 (6%)	0.721
Diabetes, n (%)	0 (0%)	0 (0%)	1.000
Dyslipidemia, n (%)	1 (6%)	0 (0%)	1.000
Current smoking, n (%)	4 (24%)	3 (17%)	0.835
Any history of Smoking, n (%)	10 (59%)	9 (50%)	0.702
Framingham Risk Score	9.3±3.6	9.6±3.4	0.797
CD4 Count	624±171	680±236	0.423
LDL	2.51±0.74	2.71±0.69	0.403
Protease Inhibitor Use	4 (24%)	2 (11%)	0.730
Baseline bone marrow TBRmax	3.14±0.52	2.82±0.37	0.058
Baseline spleen SUVmax	4.27±1.20	4.25±0.90	0.778
Baseline aortic TBRmax	2.99±0.45	2.99±0.32	0.988

Baseline SVC_{max}	1.67±0.48	1.46±0.30	0.458

Mean±SD or n (%)

* indicates $p<0.05$ between groups.

BMI, body mass index; LDL, low density lipoprotein.

Comparison of monocyte inflammation in the HIV+ population (at baseline) to thenine HIV- controls revealed that CD14++CD16- (64.7±14.5 % positive cells versus 84.5±3.2 % positive cells; p=0.0002) and CCR2 expression (25.36±22.97 % positive cells versus 86.48±4.67 % positive cells; $p<0.0001$) was significantly less in the HIV+ cohort than in the HIV- control cohort. Expression of CD14+ CD16++ (15.20±14.0 % positive cells versus 2.00±1.92 % positive cells; p=0.008), CD14+CD16- (11.24±5.28 % positive cells versus 5.76±1.84 % positive cells; p=0.008), CD127 (32.45±14.74 % positive cells versus 10.76±2.47 % positive cells; p=0.0001) and CD16 (24.03±14.61 % positive cells versus 7.72±3.04 % positive cells; p=0.002) were all significantly higher in HIV+ patients (Table 2).

Table 2. Baseline monocyte markers

Monocyte Marker (% positive cells)	**Healthy Control**	**HIV+ patients**	**P value**
CD14++ CD16 -	84.5±3.2	64.7±14.5	0.0002
CD14++ CD16+	1.86±0.69	1.55±0.89	0.35
CD14+ CD16++	2.00±1.92	15.20±14.0	0.008

CD14+ CD16+	5.52±2.70	6.98±5.48	0.45
CD14+ CD16-	5.76±1.84	11.24±5.28	0.004
CD127	10.76±2.47	32.45±14.74	0.0001
CCR2	86.48±4.67	25.36±22.97	<0.0001
CD16	7.72±3.04	24.03±14.61	0.002

Mean±SD.

Relationship between FDG Data and Serum Inflammatory and Monocyte Markers

The correlation between baseline serum inflammatory and monocyte markers with baseline FDG markers is shown in Table 3.

Table 3. Correlation between in serum inflammatory markers/monocyte markers and FDG markers

	Bone Marrow Uptake (TBR_{max})		Spleen Uptake (SUV_{max})		Thoracic Aorta Uptake (TBR_{max})	
Inflammatory marker/Monocyte marker	Correlation Coefficient	P value	Correlation Coefficient	P value	Correlation Coefficient	P value
IL6	0.226	0.222	0.139	0.462	0.112	0.541
IL7	-0.308	0.092	-0.085	0.655	0.100	0.586
IL8	0.0283	0.880	0.133	0.485	0.458	0.008
MCP	-0.096	0.606	0.336	0.070	0.265	0.144

CD14++ CD16-	0.125	0.504	0.426	0.019	0.201	0.277
CD14++ CD16+	0.426	0.018	0.201	0.277	0.082	0.657
CD14+ CD16++	-0.267	0.151	-0.149	0.425	0.097	0.598
CD14+ CD16+	-0.270	0.142	-0.285	0.127	-0.428	0.015
CD14+ CD16-	-0.285	0.127	-0.270	0.142	-0.428	0.015
CD127	-0.089	0.642	-0.345	0.058	-0.042	0.818
CCR2	0.108	0.570	0.107	0.566	-0.099	0.591

IL, interleukin; MCP, monocyte chemoattractant protein.

Changes over 6 month study period

FDG PET Imaging

There was a significant reduction in bone marrow, spleen and thoracic aorta FDG uptake in the statin-treated group compared with the usual care control group. The statin group demonstrated an average -9.40±16.81% reduction in bone marrow TBR_{max}, compared to a 5.05±18.88% change in the control group (p=0.035) at six months. There was an average -9.75±20.32% change in the spleen SUV_{max} of the statin group compared to 11.28±28.82% change in the control group. Finally, there was an average -11.88±18.18% change in thoracic aorta TBR_{max} in the statin group compared to 10.06±11.78% in the control group. (Figure 1). These changes were independent of the correction for background FDG uptake (SVC SUV_{max}).

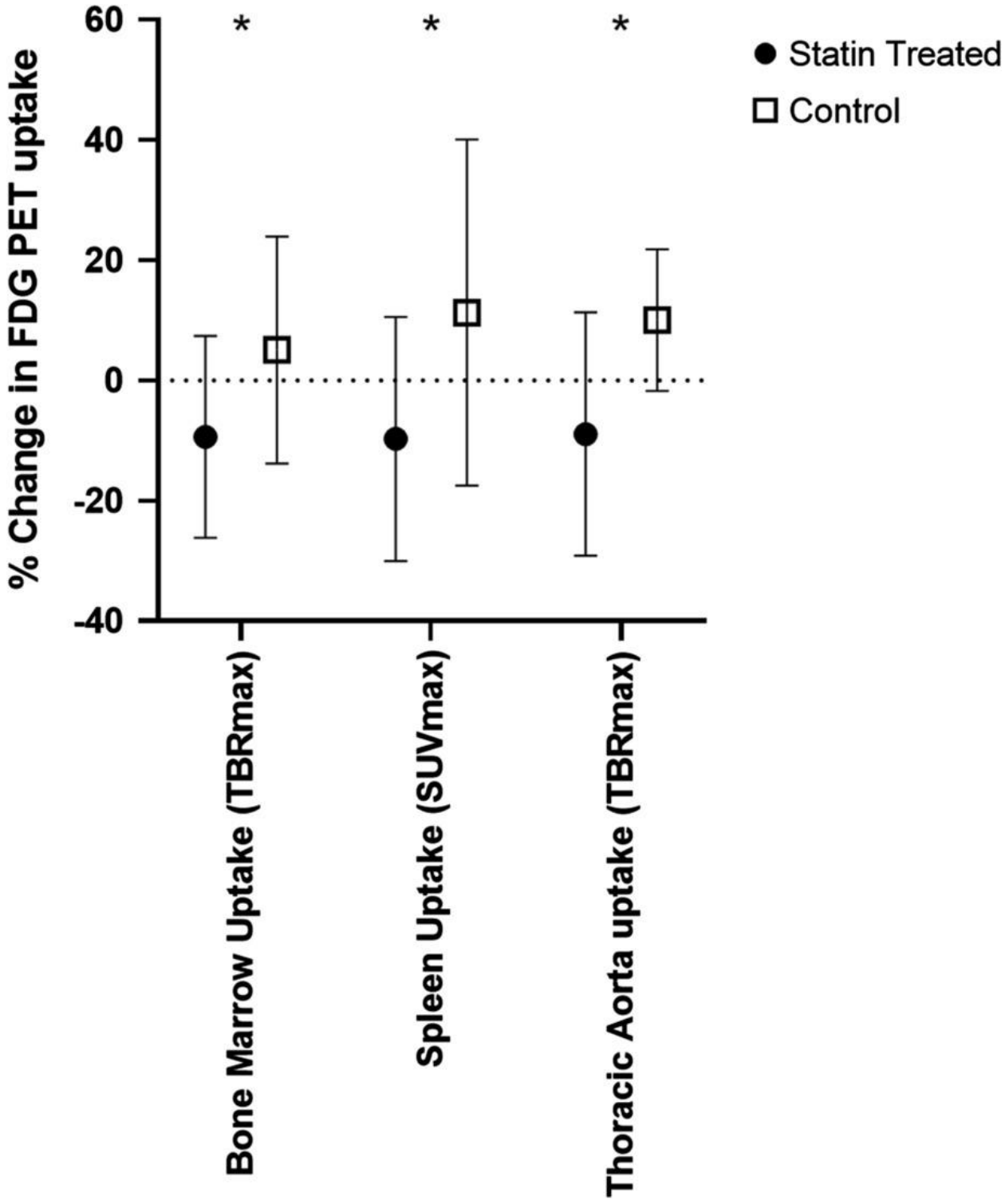

Figure 1. Change in % FDG PET uptake in bone marrow, spleen and thoracic aorta over study period.

*p<.05 for difference in % change between statin and control group

Immunohistochemistry Data

Despite the elevated baseline levels of monocyte inflammation in the HIV+ patients, there were no changes in monocyte sub-populations in either group (rosuvastatin treated or standard of care) over the 6-month treatment period (Table 4).

Table 4. Changes in monocyte markers over the 6-month treatment period

Monocyte Marker (% positive cells)	Statin treated			Control			p value*
	Baseline	Follow-up	Delta	Baseline	Follow-up	Delta	
CD14++ CD16-	58.8±15.6	61.1±16.1	2.3±13.0	69.99±11.31	70.78±10.51	0.8±9.9	0.704
CD14++ CD16+	1.6±1.0	2.0±1.3	0.4±1.1	1.48±0.85	1.47±0.95	0.0±1.1	0.335
CD14+ CD16++	20.0±17.6	17.4±16.5	-2.6±10.2	10.98±8.25	10.42±12.17	-0.6±9.3	0.562
CD14+ CD16+	7.2±4.5	6.6±4.4	-0.6±1.4	6.76±6.34	5.42±2.34	-1.3±6.3	0.652
CD14+ CD16-	11.7±6.3	13.4±7.4	1.7±6.4	10.86±4.30	11.90±6.68	1.0±6.5	0.771
CD127	36.6±17.4	41.7±17.4	5.1±13.2	28.77±11.09	31.19±14.51	2.4±15.0	0.598
CCR2	24.1±20.8	23.9±21.1	-4.6±14.6	19.46±10.92	17.52±11.56	5.7±37.9	0.331

Mean±SD.

*P value between Delta

Serum Inflammatory Marker Data

Serum levels of IL-6, IL-7, IL-8, and MCP were elevated in the HIV+ population compared to a group of healthy controls without HIV infection (IL-6: 0.73±0.45pg/ml versus 0.46±0.25pg/ml for HIV+ population versus HIV- controls, respectively; p=0.09, IL-7: 3.04±0.81pg/ml versus 2.07±0.74pg/ml; p=0.002, IL-8: 4.30±2.74pg/ml versus 0.66±0.58pg/ml; p=0.0003, and MCP: 273.35±91.10pg/ml versus 118.72±24.45pg/ml; p<0.0001) (Table 5). Again, there were no significant changes in inflammatory markers within the statin treated or usual standard of care groups over the 6-month treatment period (Table 6).

Table 5. Baseline Inflammatory markers

Inflammatory Markers (pg/ml)	HIV- Control	HIV+ Patients	P value
IL6	0.46±0.25	0.73±0.45	0.09
IL7	2.07±0.74	3.04±0.81	0.002
IL8	0.66±0.58	4.30±2.74	0.0003
MCP	118.72±24.45	273.35±91.10	<0.0001

Mean±SD. IL, interleukin; MCP, monocyte chemoattractant protein.

Table 6. Changes in serum inflammatory markers of the 6-month treatment period.

Inflammatory Marker (pg/ml)	Statin treated			Control			p value *
	Baseline	Follow-up	Delta Change	Baseline	Follow-up	Delta Change	
IL6	0.74±0.30	0.55±0.26	-0.19±0.37	0.72±0.56	0.57±49	-0.14±0.72	0.813
IL7	2.99±0.90	2.78±1.10	-0.25±0.73	3.10±0.74	3.17±0.98	0.09±0.96	0.274
IL8	4.68±3.57	4.86±3.52	0.125±2.39	3.94±1.65	5.33±3.84	1.38±3.60	0.259
MCP	259.16±83.94	228.30±59.69	-28.34±55.71	286.74±97.86	276.23±88.85	-10.51±75.71	0.458

Mean±SD

IL, interleukin

*P value comparison between Delta Change values

Relationship between FDG Data and Serum Inflammatory Markers

Within the statin treated HIV+ patients, those that had a clinically significant drop in FDG bone marrow uptake (which we defined as a change that was greater than the group average of12% decrease) over the 6 months also had a trend towards a reduction in the IL-7, IL-8 and MCP inflammatory markers compared to those with no reduced in FDG bone marrow uptake (IL-7 19% reduction in responders versus 4% reduction in non-responders; p=0.08, IL-8 27% reduction in responders versus 9% increase in non-responders; p=0.24, MCP 21% reduction in responders versus 11% reduction in non-responders; p=0.008).

Discussion

The present pilot study demonstrated that, in a cohort of HIV+ patients with moderate cardiovascular risk and on optimal medical therapy, there were elevated levels of baseline inflammation as measured by monocyte expression, serum inflammatory markers and FDG-PET markers of inflammation in the bone marrow, spleen and ascending aorta. In addition, treatment with low-dose rosuvastatin for 6 months resulted in significantly decreased FDG uptake in the bone marrow, spleen and thoracic aorta in the HIV+ patients compared to those receiving usual standard care. Although treatment with low-dose rosuvastatin did not change monocyte expression or serum levels of inflammatory markers in the HIV+ patients, in a subpopulation of patients that had a clinically significant drop in FDG bone marrow uptake over the treatment period (>12% decrease), there was a trend towards a reduction in IL-7 and IL-8 markers and a significant reduction in MCP inflammatory markers. In addition, when HIV+ patients were compared with a group of otherwise healthy HIV- controls, the former had significantly increased inflammatory monocyte markers as well as inflammatory cytokine levels.

HIV and inflammation: effect of statin therapy

CVD has replaced acquired immunodeficiency disorder (AIDS) as the leading cause of morbidity and mortality in HIV+ patients. [84, 72, 85] The mechanisms driving the increased CVD risk in HIV+ individuals are not clear but may include direct effects of the virus itself, effects of anti-retroviral therapy (ART) on lipids, insulin resistance and body composition[86, 87], as well as the increased burden of nontraditional risk factors such hepatitis C, other co-infections and substance abuse.[86, 88] The timing of ART initiation may also play a role.[86] Although a number of mechanisms have been proposed for the increased CVD risk in HIV+ patients, the presence of chronic systemic inflammation is thought to play a major role.[89, 90] HIV infection is recognized as a chronic inflammatory disease, driven by viral replication[91] and dysregulated cytokine production.[92] Activity of monocyte-derived macrophages appears to also contribute to the progression of atherosclerosis in HIV+ patients. In a study of 571 HIV+ patients and 207 HIV- controls, soluble CD163, CD14, galectin-3 binding protein and galectin-3, markers of macrophage activity, were significantly elevated in the HIV+ group. Following adjustment for risk factors, including markers of systemic inflammation (high-sensitivity C-reactive protein (hsCRP) and IL-6), soluble CD14 was associated with a significantly increased risk of new plaque formation.[93] In another study of 27 HIV+ patients and 54 HIV- controls with similar cardiovascular risk profile, the presence of arterial inflammation was significantly higher and associated with markers of monocyte and macrophage activation in the HIV+ group.[90]

The benefits of statins now appear to extend beyond lipid metabolism and also include immunomodulatory effects.[94] Indeed statins have been shown to reduce the risk of cardiovascular events and all-cause mortality in patients with elevated hsCRP but normal levels of LDL cholesterol.[95-97] Statins may also have a direct effect on reducing aortic inflammation, with patients on high-dose atorvastatin therapy for 12 weeks having significantly reduced thoracic aorta and carotid artery inflammation compared to a low dose atorvastatin group.[21] In a cohort of HIV+ patients receiving rosuvastatin, atorvastatin or pravastatin, a significant reduction in serum inflammatory markers including hs-CRP and TNF-α was observed.[98] The Stopping

Atherosclerosis and Treating Unhealthy Bone with Rosuvastatin in HIV (SATURN-HIV) trial investigated the effects of rosuvastatin in HIV+ patients on ART. After 24 weeks, patients on rosuvastatin experienced significant decreases in sCD14 and the proportion of circulating CD14+CD16+ monocytes compared to controls.[99] A 24.2% decrease in serum IL-6 levels was also observed compared to a 2.1% drop in controls, although this difference did not reach statistical significance.[100] Notably rosuvastatin has been shown to reduce atherosclerosis in HIV+ patients with a significant decrease in carotid artery intima-media thickness following treatment for 24 months.[101] Finally, statin treatment was shown to reduce non-calcified plaque burden and high risk plaque features in HIV+ patients, despite no effect on aortic arterial inflammation.[102] Interestingly a recent pilot study looking at canakinumab, an IL-1β inhibitor, in HIV+ patients, the majority of whom were already on statin therapy, found significant reductions in circulating inflammatory markers as well as FDG inflammation, which was accompanied by reductions in leukopoietic activity, monocyte cytokine production and arterial inflammation.[103] In contrast, a study looking at low-dose methotrexate in patients with HIV found no significant effect on endothelial function or inflammatory biomarkers, but was associated with a reduction in CD8+ T cells.[104] Thus, evidence continues to mount for the potential anti-inflammatory benefit of statin therapy on cardiovascular risk reduction in HIV+ patients. Our novel findings support the anti-inflammatory role of statin therapy in moderate-risk HIV+ patients, and suggest a potential role for the use of statins for primary prevention in this patient demographic.

The role of the bone marrow and spleen in the inflammatory process

The atherogenic response to dyslipidemia is thought to be driven from the bone marrow and spleen.[77, 78] In response to hyperlipidemia, the bone marrow overproduces inflammatory monocytes that enter the circulation, accumulate in atherosclerotic lesions, and differentiate into macrophages.[105] Work by Tawakol et al. showed that the link between psychological stress and CVD may also be mediated by up-regulated bone marrow activity,[82] while animal models have demonstrated that stress leads to mobilization and release of neutrophils and monocytes, and increased activity of haemopoietic progenitor cells in the bone

marrow.[106] In addition to the link between bone marrow and arterial inflammation, animal models of heart failure have demonstrated migration of pro-inflammatory monocytes from the spleen to the heart,[107] while increased splenic FDG uptake, which was correlated with upregulated inflammatory markers, has been observed in patients suffering acute coronary syndromes.[83] Of significance was the association between increased splenic FDG uptake and an increased risk of cardiovascular events. Taken together, there is a growing body of evidence supporting the presence of a bone-marrow-cardio-splenic axis. Although the present study did not examine the bone marrow, spleen or aortic FDG uptake between HIV+ patients and healthy controls, we did observe significant reductions in FDG uptake following 6 months treatment with a low dose statin (Figures 2 and 3). Specifically, HIV+ individuals treated with low-dose rosuvastatin had a 9.40% reduction in their bone marrow, 9.75% reduction in their splenic, and 11.88% reduction in their thoracic aortic FDG-PET uptake. Our novel findings not only support the link between the bone-marrow-cardio-splenic axis previously mentioned, but they also provide evidence for an anti-inflammatory effect of statin therapy in a HIV+ population.

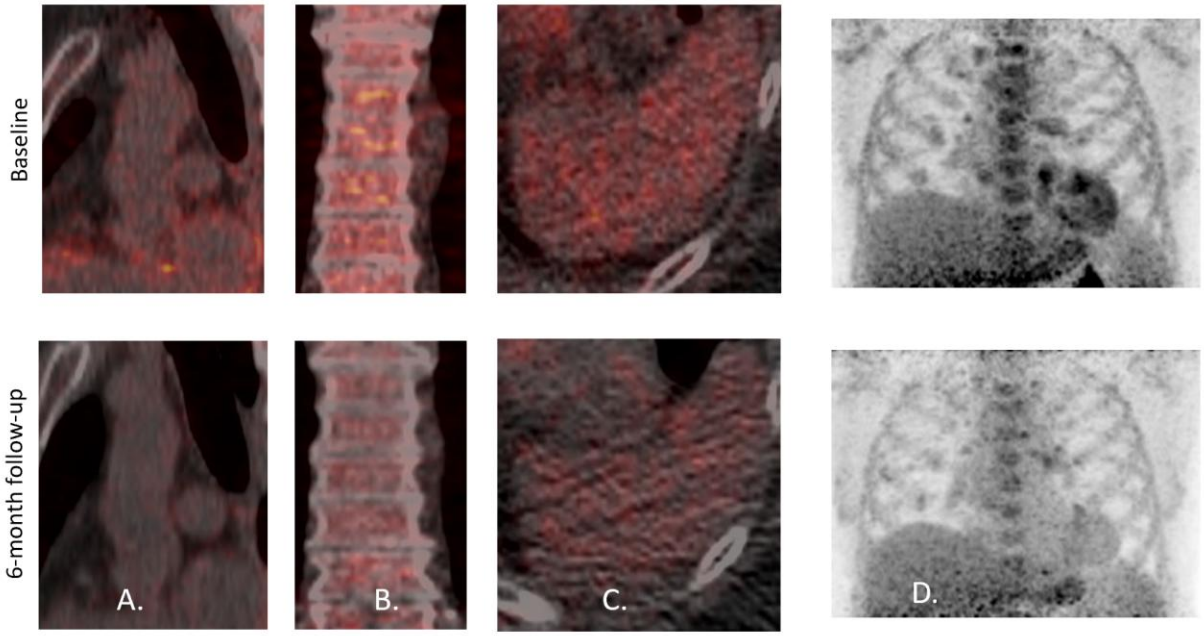

Figure 2. Change in FDG uptake in HIV+ patient over 6 months with treatment of rosuvastatin in (A) the ascending aorta (TBRmax: 2.80 at baseline and 1.39 after), (B) the bone marrow (SUVmax: 3.35 at baseline and 2.26 after), (C) the spleen (SUVmax: 3.49 at baseline and 1.70 after), and (D) anterior planar image.

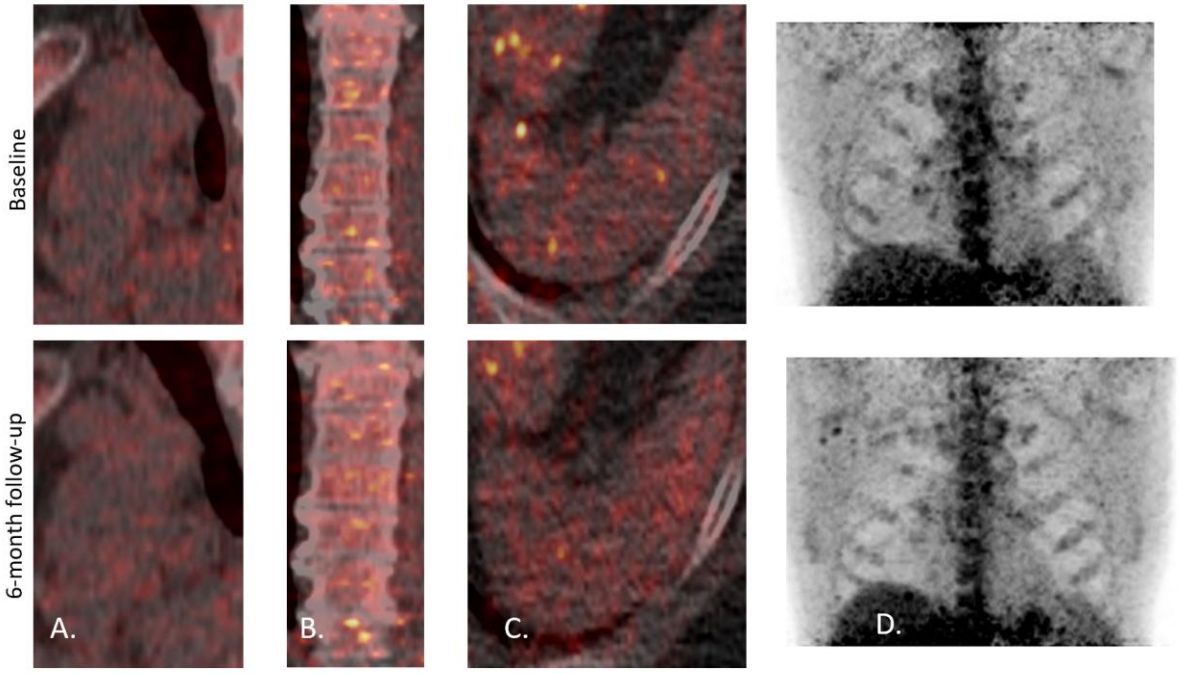

Figure 3. Change in FDG uptake in HIV+ patient over 6 months with standard therapy (control group) in (A) the ascending aorta (TBRmax: 2.85 at baseline and 3.19 after), (B) the bone marrow (SUVmax: 3.35 at baseline and 3.13 after), (C) the spleen (SUVmax: 4.02 at baseline and 3.18 after), and (D) anterior planar image.

Inflammatory biomarkers in HIV

Studies examining HIV associated chronic inflammation have increasingly focused on blood monocytes, a population of cells with inflammatory properties known to be involved in the promotion of

atherosclerosis.[108] Monocytes represent an important source of cytokines and chemokines and may be major contributors to the systemic inflammation in HIV disease. HIV+ individuals have increased numbers of circulating CD16+ monocytes,[109, 110] and CD16+ monocytes have been shown to be independently associated with coronary artery calcification in HIV+ individuals.[111] Supporting this, in the present study we observed elevated levels of CD16+ monocytes in the HIV+ population compared to a HIV- control population. Additionally, expression was significantly higher for CD14+ CD16++ and CD14+CD16- monocyte subpopulations in the HIV+ cohort, as well as monocytes expressing CD127. Previous studies have demonstrated that regulatory T-cells, which are responsible for immune modulation and regulating chronic inflammatory reactions, upregulate CD127 when activated, and this is due to IL-7 and was more prevalent in the bone marrow tissue.[112]

IL-6 is typically elevated in the plasma of HIV-infected individuals compared to uninfected controls,[92, 113] with increased levels of IL-6 independently associated with carotid artery intima-media thickness and stenosis in the general population.[31, 32] Although a relative decrease in IL-6 is seen with the initiation of ART, plasma levels do not normalize in HIV+ patients on treatment and remain significantly higher than levels seen healthy HIV-negative individuals.[108, 114] IL-8 is a cytokine that has been linked to a number of inflammatory diseases that involve neutrophil activation, including CVD.[115, 116] Produced predominately by monocytes and neutrophils, its primary responsibility is to recruit other monocytes and neutrophils, the principal cells involved in the acute inflammatory process.[116] IL-7 has also been proposed to play a role in the development of CVD, with increased levels observed in patients with CVD compared to healthy controls and correlated with serum levels of high-sensitivity C-reactive protein (hs-CRP).[117, 118] HIV infection disrupts IL-7 signaling, including via cytokine production and receptor expression. Studies have shown dysregulated IL-7 signaling in HIV+ individuals, with reduced expression of the IL-7 receptor α-chain (CD127) on CD8 T-cells in HIV+ patients with uncontrolled viral replication, and partial recovery seen in those on ART with sustained viral suppression.[119] Numerous studies have demonstrated increased

plasma concentrations of IL-7 in HIV infected individuals compared to HIV- controls.[120, 121] Although there is a decline following initiation of ART, serum IL-7 levels remain elevated in HIV+ patients despite suppression of HIV replication.[121, 122] Supporting these observations, in the present study, we observed significantly elevated levels of serum IL-7, IL-8 and MCP in the HIV+ cohort compared to a HIV- control population. IL-6 levels were also elevated, although levels did not quite reach statistical significance. Overall, there were no temporal changes in monocyte expression and levels of serum inflammatory markers with rosuvastatin treatment. In the subset of HIV+ patients that achieved a clinically significant response to rosuvastatin (>12% reduction in FDG-PET bone marrow uptake), there was a trend towards a temporal reduction in inflammatory markers.

Limitations

Although this was a prospective randomized imaging trial looking at the efficacy of statin therapy in HIV+ individuals, it was a relatively small sample size. Because of the small cohort, the study was not powered to detect differences in treatment outcomes in terms of monocyte expression or inflammatory markers, although it was powered to detect differences with regards to FDG-PET uptake. Despite the low power, although we did not see an overall trend in a reduction in monocyte expression or inflammatory markers in the rosuvastatin treated group, we did see a trend towards reduction in serum inflammatory markers (IL-7, IL-8, and MCP) in patients that had a clinical response to statins as measured by FDG-PET uptake (namely, those that had >12% reduction in bone marrow FDG-PET uptake).

Conclusions

In conclusion, the present study observed that monocyte markers of inflammation were elevated in HIV+ patients compared to HIV- controls. Additionally, there was a significant decrease in FDG-PET uptake in

the bone marrow, spleen, and thoracic aorta following treatment with rosuvastatin for 6 months in HIV+ patients compared to HIV+ patients receiving usual care. These data suggest wide-range anti-inflammatory effects of rosuvastatin in HIV+ individuals with well-controlled infection on ART, that ultimately resulted in decreased inflammatory activity in the arterial walls of these patients and raises the possibility that such therapeutic approaches can reduce the burden of CVD in this population. This needs to be confirmed in larger studies.

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Chapter 9. Discussion and Conclusion

Section 9.1 Overview of Chapter 9

This thesis explored the use of inflammation in the pathogenesis of atherosclerotic cardiovascular disease, and the utilization of anti-inflammatory therapies to help mitigate this risk. The relative efficacy of anti-inflammatory therapies to reduce adverse cardiac events were evaluated in patients with acute coronary syndromes and patients with stable coronary artery disease (Chapter 3), the systemic inflammatory levels were evaluated in patients with diabetes (Chapter 4), the protocol for a stud to evaluate the mechanism of action of colchicine was outlined (Chapter 5), the cost-efficacy of colchicine as an effective treatment for coronary artery disease is evaluated (Chapter 6), the efficacy of biologic therapy to reduce vascular inflammation in patients with psoriasis or psoriatic arthritis was explored (Chapter 7), the efficacy of low-dose statin therapy to reduce vascular inflammation in patients with human-immunodeficiency virus (HIV) was tested (Chapter 8). Six manuscripts were produced as part of this thesis; of these, five have been published and one has been submitted to peer-reviewed journals.

This last chapter serves as a final discussion and conclusion for the thesis as a whole. Each individual manuscript within Chapters 5 to 7 includes a self-contained discussion pertinent to that study individually. Thus, this chapter serves to:

- Summarize the rational and objective for the thesis (Section 9.2)
- Consider the implications of this thesis for clinical practice and health care policy (Section 9.3)
- Highlight the novelty of this thesis (Section 8.4) and highlight potential strengths and weaknesses (Section 9.4)
- Provide a brief overview of potential ethical issues related to utilizing anti-inflammatory therapies for CAD (Section 9.5)

- Outline suggestions for future research in this field (Section 9.7)

Section 9.2 Summary of thesis rationale

Cardiovascular disease is a leading cause of mortality and morbidity worldwide.[1] Despite focused primary and secondary prevention efforts, its burden continues to increase globally. Atherosclerosis, or the deposition of cholesterol deposits in the blood vessels that can eventually impede blood flow, is the main driver of the progression of CVD globally.[2 3] Currently, the standard of care for reducing the risk of CV events includes intensive modification of risk factors like controlling blood pressure and cholesterol levels. However, recently our understanding of the underlying drivers of atherosclerosis has shifted to focus on the inflammatory hypothesis.

Colchicine is an ancient medication with potent anti-inflammatory effects.[4] The use of colchicine as an anti-inflammatory treatment dates back over a millennium.[5] It is one of the oldest remedies that is still in use today and is derived from the *Colchicum autumnale* plant (or autumn crocus).[5] Its first recorded use dates back to 1500 BCE as an ancient herbal remedy for joint pain.[5] The active ingredient, colchicine, was first isolated in the 1800s and amazingly still remains in medical use today.[5 6] Given it's long history of use in the medial community, it is perhaps somewhat surprising that it took until 2009 for colchicine to be approved for use by the U.S. Federal Drug Administration (FDA). More recently, colchicine use for cardiovascular indications have been approved by the FDA for the secondary prevention of cardiovascular events.[7] This is quite remarkable, as it is the first anti-inflammatory therapy approved for this indication, and it was given the same recommendation by the FDA as that given to the statin class of medications. This highlights the profound change the evidence base has undergone in the last several years in this area. Prior to this thesis, the evidence to support the use of anti-inflammatory therapies to reduce CV events was marginal at best. Thus, this thesis set out to provide concrete evidence for clinicians, patients, and policy makers to inform their decisions about the use of anti-inflammatory therapies for the reduction of CV events. To meet this goal, several research questions were addressed.

Question 1: What are the available anti-inflammatory therapies for ASCVD risk reduction and what is the relative efficacy of these therapies?

Question 2: Do patients with diabetes have elevated systemic inflammatory levels?

Question 3: What is the mechanism of action of colchicine in the reduction of ASCVD?

Question 4: Is colchicine a cost-effective treatment for reduction of ASCVD compared to current therapeutic options?

Question 5: Could biologic therapy be an effective option to reduce vascular inflammation in patients with psoriasis or psoriatic arthritis?

Question 6: Could low-dose statin therapy be an effective option to reduce vascular inflammation in patients with HIV?

Section 9.3 Discussion of findings

The six manuscripts that are included in this thesis included one protocol and five result manuscripts distributed across seven chapters (Chapters 3-7). The main findings from each chapter as they pertain to each research question are summarized in Table 1.

Table 1: Summary of core findings from the thesis

Research Question	Chapter	Key findings
What are the available anti-inflammatory therapies for ASCVD risk reduction and what is the relative efficacy of these therapies?	Chapter 3	35 studies have evaluated anti-inflammatory therapies for ASCVD risk reduction. This includes 29,487 patients evaluated in an ACS context and 41,647 in a stable CAD context

		For ACS, eight different anti-inflammatory therapies were tested. Non-steroidal anti-inflammatory therapies and colchicine were associated with a reduction in MACE For stable CAD, eleven anti-inflammatory therapies were tested. Corticosteroids and colchicine were associated with a reduction in MACE
Do patients with diabetes have elevated systemic inflammatory levels?	Chapter 4	Found hsCRP and IL-6 were elevated in patients with prediabetes/diabetes and a recent cardiovascular event compared to patients with remote events suggesting these patients have a higher inflammatory burden
What is the mechanism of action of colchicine in the reduction of ASCVD?	Chapter 5	***CADENCE*** is a multicentre, prospective, randomized, double-blinded, placebo-controlled study to determine the effect of colchicine on arterial inflammation as

		assessed with imaging and circulatory biomarkers, specifically carotid arteries and aortic FDG uptake as well as hs-CRP and IL-6 among others. Patients with T2DM or pre-diabetes who have recently experienced a CV event (within 30 to 120 days after an ACS (i.e. ST-elevation MI (STEMI) or non-STEMI) or TIA/stroke with documented large vessel atherosclerotic disease, will be randomized to treatment with either colchicine 0.6 mg oral daily or placebo. Participants will undergo baseline clinical evaluation including EQ5D assessment, blood work for inflammatory markers, and FDG PET/CT scan of the ascending aorta and left and right carotid arteries. Patients will undergo treatment for 6 months and have repeat clinical evaluation

		including EQ5D assessment, blood work for inflammatory markers, and FDG PET/CT scan at the conclusion of the study. The primary outcome will be the change in the maximum target to background ratio (TBR_{max}) in the ascending aorta (or carotid arteries) from baseline to follow-up on FDG PET/CT imaging.
Is colchicine a cost-effective treatment for reduction of ASCVD compared to current therapeutic options?	Chapter 6	A decision model was developed to estimate the healthcare costs in Canadian dollars and clinical outcomes amongst patients who have suffered a MI and are treated with colchicine. Long-term colchicine use was dominant over standard of care with lower average lifetime costs per patient ($91,552.80 versus $97,085.84) and higher average QALYs per patient (19.92 versus 19.80). Short-

		term colchicine use also dominated over standard of care.
Could biologic therapy be an effective option to reduce vascular inflammation in patients with psoriasis or psoriatic arthritis?	Chapter 7	Target-to-background ratio (TBR) by FDG PET in the most diseased segment of the ascending aorta (TBRmax) was measured to assess vascular inflammation. 82Rb PET studies were used to assess changes in left ventricular MFR. A total of 34 participants were enrolled in the study (11 in group 1, 13 in group 2, and 10 controls). A significant drop in the thoracic aorta uptake was observed in the biologic-treated group (ΔTBRmax: -0.46±0.55) compared to the PsO group treated with non-biologic therapy (ΔTBRmax: 0.23±0.67). Those showing response to biologic agents maintained MFR compared to who showed no response.

Could low-dose statin therapy be an effective option to reduce vascular inflammation in patients with HIV?	Chapter 8	While CD14++CD16- and CCR2 expressions were reduced, serum levels of IL-7, IL-8, and MCP-1 were elevated in the HIV+ population compared to the controls. There was a significant drop in FDG uptake in the bone marrow (TBRmax), spleen (SUVmax) and thoracic aortic (TBRmax) in the statin-treated group compared to the control group (bone marrow: -10.3±16.9% versus 5.0±18.9%, p=0.0262; spleen: -9.8±20.3% versus 11.3±28.8%, p=0.0497; thoracic aorta: -19.1±24.2% versus 4.3±15.4%, p=0.003).

Section 9.4 Implications for clinical practice

The contemporaneous care of the patient with atherosclerosis includes lipid-lowering therapies to reduce the risk of recurrent CV events. This has been well established, not only in guidelines, but in routine clinical practice. The same cannot be said for the treatment of inflammatory risk in these patients. To this day, residual inflammatory risk in these patients largely remains an untreated target.

In a contemporary analysis of over 31000 patients with atherosclerosis receiving guideline-directed medical care including statins, the residual inflammatory risk (defined by high sensitivity C-reactive protein) was actually more strongly associated with recurrent cardiovascular events, cardiovascular death, and all-cause mortality over and above a patient's residual cholesterol risk (measured by serum levels of low-density lipoprotein cholesterol).[8] These data may begin to influence clinical practice. Recently regulatory bodies have approved low-dose colchicine (0.5mg PO daily) as the first anti-inflammatory treatment to be used to reduce the risk of myocardial infarction, stroke, coronary revascularization, and cardiovascular death. Guidelines in both Europe as well as the United States have also recently included the use of low-dose colchicine for the secondary prevention of atherosclerotic events. However, clinical practice has not caught on. Perhaps this is due to conflicting trial results on the topic.

For physicians considering preventative therapies for their patients with atherosclerosis utilizing statins is an obvious and important choice. However, from a clinical perspective, the next step beyond this remains much more uncertain. It is therefore worthwhile comparing the evidence base evaluating the additional use of secondary LDL cholesterol lowering agents compared to the additional use of anti-inflammatory treatments. Up until 2024, there were 7 large randomized trials with similar baseline risk that formally addressed this question.[9] 1study used adjunctive ezetimibe, 3 used PCSK9 inhibitors, and 3 used adjunctive anti-inflammatory therapies. As illustrated in Figure 1, although all of these therapies did demonstrate incremental benefit in the reduction of major adverse cardiovascular events (MACE), the effects were greater for the anti-inflammatory therapies than the therapies targeted at additional LDL cholesterol lowering. An additional eighth RCT looking at icosapent ethyl for the reduction of MACE reported a 25% relative reduction of this outcome, but the interpretation of the results has been muddied by the use of a comparator that actually increased the risk of adverse events.[10]

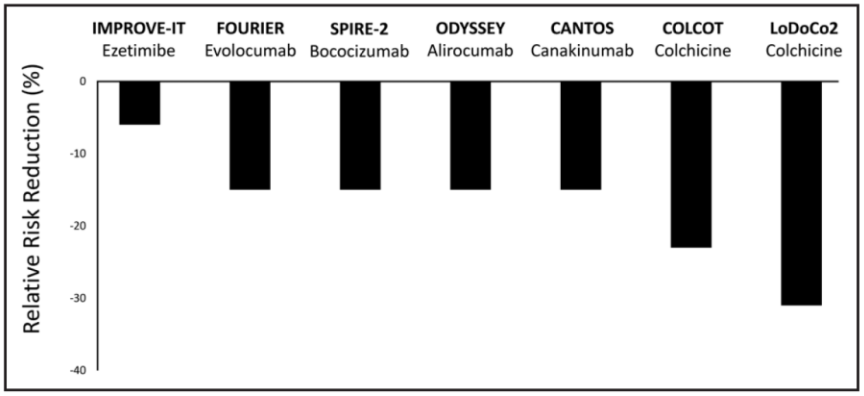

Figure 1. Relative risk reduction reported in randomized controlled trials of ezetimibe, evolocumab, bococizumab, alirocumab, canakinumab, and colchicine, respectively on the incidence of major adverse cardiovascular events when each agent is added as adjunct to statin therapy. Image reproduced with permission from Ridker 2023[9].

It was on the basis of the LoDoCo2 (Low Dose Colchicine -2) and COLCOT (Colchicine Cardiovascular Outcomes Trial) trials that low-dose colchicine was approved by the major regulatory bodies and even found its way into guideline practice. In 2024, the European Society of Cardiology upgrade its recommendation for colchicine from IIb to IIa for patients with ASCVD.[11] Similarly, the U.S. Food and Drug Administration approved colchicine to treat coronary artery disease [12], as did Health Canada. Long term colchicine reduced the risk of major adverse cardiovascular events by 31% among those with stable atherosclerosis in the LoDoCo2 study (hazard ratio (HR) 0.69 (95% CI 0.57-0.83; P<0.001) and by 23% after a recent myocardial infarction in the COLCOT study (HR 0.77 (95% CI 0.61-0.96; P=0.02). Importantly, no adverse reactions with statin therapy were observed (which remains a Class 1 recommendation for these patients). Interestingly, the benefits of colchicine therapy were still present even when the medication was started several years after an initial diagnosis of systemic atherosclerosis was made.[13]

However, clinical adoption has been sluggish. Before 2024, many clinicians found the nonsignificant trend of higher noncardiac deaths in patients on colchicine to be concerning. In a combined analysis of all

low-dose colchicine trials performed prior to 2024, there was a nonsignificant increase in non-cardiovascular deaths seen (HR 1.38 (95% CI 0.99-1.92).[14] While not statistically significant, this trend caused clinicians to take great pause in adopting this therapy in a patient that is already potentially prescribed five or more medications as baseline therapy. Thus, while not explicitly stated, the "bar" has been set higher for prescribers to consider new medications for their patients with atherosclerosis, and to that point, many felt that colchicine had not yet reached the mark. Colchicine was dealt another blow in 2024. In November 2024, the CLEAR-SYNERGY trial results were published. This study was a multicenter trial with a 2 by 2 factorial design which randomly assigned patients who had a myocardial infarction to receive either colchicine or placebo and either spironolactone or placebo. A total of 7062 patients underwent randomization and surprisingly colchicine shows no effect on the primary outcome of MACE (HR 0.99, (95% CI 0.85 to 1.16; P=0.93). The reasons for the divergence between the results of the CLEAR SYNERGY study and prior trials with colchicine in this patient population are not clear and further research is needed. Certainly the CADENCE study, the protocol of which is included in this thesis, will help to further elucidate the effect of colchicine on the cardiovascular system.

Our understanding of the role in the pathogenesis of ASCVD has greatly evolved over the past decade. Importantly, the potential role for both lipid lowering as well as inhibiting inflammation are much better understood now. Specifically, we now understand that these two treatment targets are not mutually exclusive but are rather synergistic – patients can be treated for both cholesterol as well as residual inflammatory risk. One could envisage a future where clinicians are able to treat both cholesterol and inflammation with a combined polypill to aggressively lower a patient's ASCVD risk profile.

In addition to CADENCE, there are other ongoing trials targeting inflammation in ASCVD. Building on the CANTOS trial (Canakinumab Anti-inflammatory Thrombosis Outcomes Study) which first demonstrated that biological therapy that targeted the inflammatory mediator, interleukin-1β, could improve cardiovascular outcomes, ongoing trials of ziltivekimab and clazakizumab will address whether inhibition of the downstream interleukin-6 can also lower CV events. These trials will specfially look at

special populations – notably chronic kidney disease, heart failure with preserved ejection fraction, acute coronary ischemia, and finally amongst patients undergoing dialysis.

8.3.2 Considerations for Canadian policy makers

Chapter 6 of this thesis presented a cost-effectiveness analysis of colchicine for this use in both patients who have suffered an acute myocardial infarction as well as patients with stable coronary artery disease. In this analysis, performed from the perspective of the Canadian Healthcare system, both short-term and long-term colchicine use was dominant over standard of care – indicating that this therapy is a cost-effective option for the reduction of CV events in these patients in Canada. As of August 2021, colchicine extended-release 0.5mg tablets (MYINFLA^{TM}) have been approved by Health Canada for the reduction of cardiovascular events in adult patients with established coronary artery disease, in addition to standard therapy (including LDL-C lowering therapy and antithrombotic therapies

Given the flurry of novel research in this area, an equal amount of attention will need to be spent on ensuring any novel therapies that demonstrate clinical efficacy meet the same bar of cost-efficacy in the Canadian context. In this respect, it is interesting to consider the case of canakinumab, a therapeutic monoclonal IL-1β antibody. The clinical efficacy of canakinumab was evaluated in the CANTOS trial, a large 10,061 patient randomized controlled trial that evaluated canakinumab for the reduction of MACE in patients with prior myocardial infarction and elevated C-reactive protein. In this study, canakinumab use at a dose of 150mg every 3 months led to a significant modest relative reduction of 15% in the rate of recurrent cardiovascular events when compared to placebo. Importantly, this effect was independent of lipid lowering – thereby emphasizing the important role that targeting inflammation can play on the reduction of MACE. However, from a Canadian-perspective, at a cost of $5333 CAD every 3 months, the corresponding incremental cost-effectiveness ratio (ICER) for canakinumab therapy was $535,365 CAD.[15] At a willingness to pay threshold for a QALY of $50,000, canakinumab had a 0% probability of being cost-effective. The small incremental benefit of canakinumab and its associated high sticker tag left many concerns regarding the practicality of this medication for the treatment of atherosclerosis. Indeed, in

2018 the Food and Drug Administration (FDA) rejected Novartis' application to expand the indication for canakinumab to include cardiovascular risk reduction. This should serve as a warning for the manufacturers of other anti-inflammatory therapies that are currently under investigation or development. Novel treatments have a double bar to pass: yes, they must demonstrate clinical efficacy, but ***cost*** efficacy is an equally important consideration. The writing is now on the wall: novel therapies that come with astronomical costs will not be considered in this clinical context

Section 9.5 Guideline for professional associations

In clinical practice, there is still uncertainty regarding the role that anti-inflammatory therapies should play in the treatment of ASCVD. Currently, there are no approved indications of the use of any anti-inflammatory treatments for the reduction of MACE in patients with CAD in Canadian guidelines. The most recent Canadian guidelines for the management of stable CAD, published in 2014, have no mention of the word "anti-inflammatory" or "inflammation", thereby highlighting how far we have come within the last 10 years in terms of our understanding of the pathogenic role of inflammation in the progression of CAD. Given the results of the systematic review and network meta-analysis that was performed as part of this thesis, as well as the cost-effectiveness analysis performed from the Canadian healthcare perspective, colchicine certainly warrants consideration for Canadian guidelines for both acute and chronic forms of coronary artery disease. It offers a cost-effective method to reduce the burden of MACE in these populations as demonstrated in the work of this thesis. Although some uncertainty regarding its side-effects persists, the CADENCE study (again, part of this thesis) is set to finish recruitment shortly and will shed further light on this topic as well as whether there may be incrementally increased benefit in patients with diabetes (a high-risk subpopulation).

Section 9.6 Novelty of the thesis

With novel anti-inflammatory medications currently being developed to reduce the risk of major adverse cardiac events, we need a method to efficiently test the effectiveness of these new medications.

Throughout this thesis, the utility of FDG PET imaging as a tool for this task was consistently demonstrated. Both circulating and imaging biomarkers offer a window inside the underlying disease mechanisms of inflammation and atherogenesis. They enable observation of changes in the systemic state of inflammation in the case of blood biomarkers and local atherosclerotic plaque inflammation in the case of imaging. As inflammation has a key role in atherosclerosis, identification of both generalized and more specifically plaque inflammation may become paramount for patient risk stratification to optimize therapy selection. A variety of inflammatory blood and imaging biomarkers have been studied. They represent different inflammatory pathways implicated in initiation and progression of atherosclerosis.

Section 9.6.1. Biomarker Analysis: Numerous biomarkers have been studied and implicated in the progression of atherosclerosis. The most widely studied and most clinically used is CRP.[16] Increased hs-CRP has been linked with CV outcomes.[17-21] In an exploratory analysis of CANTOS, patients with a greater reduction in hs-CRP had greater reduction in CV events.[22] In a recent meta-analysis of 29 RCTs, statin use resulted in a significant reduction in hs-CRP levels,[23] which aligns with the well-establish dose-dependent inflammatory reduction benefit of statin intervention measured using FDG-PET.[24] However, it has also been shown that hs-CRP has a poor specificity for atherosclerosis-related inflammation, as it does not necessarily link to plaque biology.[25-29] In secondary prevention, its prognostic value for predicting future CV events in patients who are already on optimal medications is considered weak at best.[16 30] Other inflammatory cytokines have also been studied as biomarkers. Interleukin (IL)-6 signaling has been shown to be related to plaque destabilization, microvascular dysfunction and adverse outcomes in the setting of acute CV ischemia.[16 31 32] IL-1β is the primary circulating form of IL-1, which is a potent inducer of innate immunity, and has been shown to play a central role in the inflammatory response and the IL-6 signaling pathway.[22] Furthermore, Mendelian randomization studies show that IL-6 has a causal link to CVD55 (whereas hs-CRP is simply a risk marker).[33 34] IL-1β plays multiple roles in atherothrombotic plaque development, including induction of procoagulant activity, monocyte adhesion to endothelial cells, and the growth of vascular smooth muscle.[35-37] A host of other biomarkers hold promise

to track inflammation.[16 38-48] While blood biomarkers including hs-CRP, IL-6 and others hold promise to track inflammation[16] their use has been restricted by poor specificity and weak prognostic value.

Section 9.6.2. **FDG as a marker of plaque inflammation**: While carotid 3D ultrasound, magnetic resonance imaging (MRI) and CT angiography (CTA) can each define plaque volume, vessel lumen and characterize different aspects of plaque composition and morphology,[49-54] none directly measure plaque inflammation.[55] Active inflammation within plaque is considered a major criterion to identify its vulnerability to rupture.[56] Many imaging probes have been validated by our group and others as markers of the inflammation cascade. FDG is the most widely validated and applied in this regard.[57-62] Several studies including our own support the validity of FDG uptake in plaque and its relationship to CD68 immunohistology, a marker of inflammatory cells.[57 59-62] While the spatial resolution of FDG-PET imaging precludes assessment of the coronary vessels themselves, the ascending aorta (i.e. the arterial vessel most proximal to the heart) has become a well-established surrogate of cardiac inflammation.

Section 9.6.3. **FDG PET for Evaluation of Therapy**: Joseph & Tawakol noted: "since atherosclerosis is an active inflammatory process, measuring changes in arterial inflammation can provide insight into how therapeutic agents impact atherosclerosis development."[58] Noteworthy is that seven drug classes have been evaluated with FDG PET/CT inflammation imaging and also have been assessed in clinical outcome trials.(Table 2)

Table 2: Drug classes on vascular inflammation and clinical outcomes

Drug	Reduced Inflammation (FDG PET/CT)	Reduced Circulating Inflammatory Biomarkers	Outcome Benefits
Statins	Positive	Positive	Positive
Pioglitazone	Positive	Negative	Positive
Alirocumab	Positive	Negative	Positive
Canakinumab	Not Measured	Positive	Positive
CETP inhibitor	Negative	Positive	Negative
Lp-PLA2 inhibitor	Negative	Not Measured	Negative
MAP Kinase Inhibitor	Negative	Negative	Negative

Evaluation of pharmacological impact on arterial inflammation measured using FDG PET/CT (but not other approaches) has uniquely demonstrated concordance with clinical CV outcome trials: The dose-dependent outcome benefit of statin therapy is well established.87 Dose-dependent reduction in FDG uptake in the arterial vasculature, supporting the local anti-inflammatory effect on the plaque has also been observed. Tawakol studied 67 patients with atherosclerosis randomized to high- vs low-dose atorvastatin. The high-dose significantly reduced 12-week FDG uptake compared to a low-dose. The authors note that the study supports the use of FDG PET as a tool for treatment assessment.46 Mizoguchi et al. studied patients with diabetes or pre-diabetes randomized to 4 months of pioglitazone versus glimepiride. Pioglitazone significantly reduced arterial inflammation measured using FDG uptake versus glimepiride.88 As with the statin studies above, the plaque inflammation findings parallel the clinical trials which demonstrate significant CV outcome benefits.89 In contrast, several studies with different agents did not yield any demonstrated reduction in arterial inflammation (using FDG PET/CT). These include a CETP inhibitor, dalcetrapib; Lp-PLA2 inhibitors (rilapladib, darapladib) and inhibitors of MAP Kinase.90-92 Consistently, large clinical trials of the same drugs do not show CV outcome benefits.93-96 Evaluation of pharmacological impact on arterial inflammation measured using FDG PET/CT (but not other approaches) has uniquely demonstrated concordance with clinical CV outcome trials.

Section 9.6.4. **FDG PET for Prediction of Adverse Events**: Among inflammation imaging probes, only FDG uptake on PET/CT has been shown to predict progression of atherosclerosis41,81,85,97 as well as CV events independent of risk factors.9,98,99 In 513 patients, Figueroa et al. showed that increased aorta FDG uptake is a strong independent predictor of CV events (HR: 4.71; 95%CI:1.98-11.2; $p<0.001$).9 In a prospective study of 60 patients with recent stroke or TIA, Marnane et al. showed that carotid FDG uptake was a strong predictor of recurrent stroke within 90 days which occurred in 22% of patients.99 These data support the predictive value of FDG PET/CT- to be used in high-risk patients in the current proposal. Thus FDG PET/CT inflammation imaging is an attractive pragmatic means to define the mechanistic targets of a drug's effect in small cohorts over short periods of time.

Section 9.7 Strengths and limitations

This thesis explored the relationship between inflammation and the cardiovascular system, potential treatments available to help mitigate this risk, and also explored several subpopulations that may be at increased cardiovascular risk as a result of an increased inflammatory burden. Six manuscripts were prepared as part of this thesis (presented in Chapters 3-8), all of which have been published or submitted for publication. In this section, the strengths of each study are described (Table 3) followed by a discussion of the limitations that are common across the included studies.

Table 3: Strengths of the thesis

Chapter	**Strengths**
Chapter 3 (one published manuscript, one submitted manuscript)	• Comprehensive systematic review of the published and grey literature • Published protocol[63] with an extensive search strategy developed in conjunction with an experienced • Network meta-analysis allowed direct and indirect comparisons of efficacy between different anti-inflammatory treatments
Chapter 4 (one published manuscript)	• Cross-sectional study to identify the prevalence of elevated inflammatory blood biomarkers in patients with diabetes who have suffered a recent myocardial infarction (MI)

	• The study (published) supported the notion that patients with prediabetes/diabetes and recent CV events have higher inflammatory burdens than patients without recent CV events or dysglycemia[64]
Chapter 5 (one published manuscript)	• Study protocol for randomized controlled trial to assess the effect of colchicine on vascular inflammation in the carotid arteries and ascending aorta measured with 18F-fluorodeoxyglucose (FDG)-positron emission tomography (PET)/computed tomography (CT) in patients with type 2 diabetes mellitus (T2DM) or pre-diabetes who have experienced a recent vascular event (acute coronary syndrome (ACS)/MI, transient ischemic attack (TIA), or stroke). • Efficient study methodology to test the effect of a medication with a limited sample size • The study protocol was published[65]

Chapter 6 (one published manuscript)	• Decision model was developed to estimate the healthcare costs in Canadian dollars and clinical outcomes amongst patients who have suffered a MI and are treated with colchicine. Probabilistic Markov modeling was used in combination with Monte Carlo simulation to derive expected lifetime costs and quality-adjusted life years (QALYs), which permitted the calculation of incremental cost-effectiveness ratios (ICERs). • Models were derived for both short-term (20-months) as well as long-term (lifelong) colchicine use in this population. • The study was published.[15]
Chapter 7 (one published manuscript)	• Prospective cohort clinical study undertaken to determine the effect of biologic therapy on vascular inflammation and myocardial flow reserve (MFR) in patients with psoriatic arthritis compared to cohorts not treated with biologic therapies.

	• Study was published.[66]
Chapter 8 (one published study)	• Randomized controlled trial to evaluate markers of systemic as well as imaging markers of inflammation in the ascending aorta, bone marrow, and spleen measured by 18F-FDG PET/CT, in HIV+ patients at baseline and following therapy with rosuvastatin. • Study was published.[67]

Despite the comprehensive nature of this thesis work, there are important limitations that need to be considered. This section briefly discusses these limitations. Notably, for Chapters 3-8, the limitations relevant to each individual study are discussed, however the focus in this sectin is on the limitations that apply to the thesis as a whole.

Firstly, while part of the intent of this thesis was to provide information regarding some subpopulations that may benefit the effects of anti-inflammatory therapies to reduce cardiovascular events, the number of subpopulations that may benefit is extremely large and far beyond what could realistically be explored in the thesis. While this may limit the scope and generalizability of the findings, the thesis certainly opens the door for further exploration in this area.

Secondly, much of the work in this thesis, including Chapters 5, 7, and 8 involved the use of a surrogate imaging marker as a primary endpoint with FDG-PET. The use of FDG PET has several advantages. Firstly, it allows for the evaluation of drug mechanism of action, as it allows the direct visualization of metabolic activity of tissue and plaque. Additionally, it allows for the efficient use of resources to test efficacy of therapies in a smaller sample size. This theoretically allows for a better utilization of precious

research resources by allowing for the avoidance of large costly randomized controlled trials for drugs that are shown to have no efficacy in surrogate FDG PET trials. While FDG PET is a well-established and accepted surrogate marker, it is still a surrogate marker, and the results of these efficacy trials are still hypothesis generating and will need to be tested in a true clinical RCT. The rationale for selecting FDG PET as a surrogate marker is discussed further in the Appendix.

Next, three chapters of this thesis (Chapters 3, 5, and 6) were evaluating low-dose colchicine for the role of cardiovascular risk reduction. Notably, two different doses of low-dose colchicine are available in Canada. The most available low dose of colchicine is a 0.6mg tablet. This is the dose of colchicine that is used for gout, familial mediterranean fever as well as pericarditis. The cost of this medication is currently ~0.78 CAD$/tablet in 2025. The other dose of colchicine is 0.5mg and was the dose tested in the COLCOT and LoDoCo2 studies.[68 69] The cost of this dose in Canada is currently ~1.30 CAD$/tablet in 2025. While this thesis treats these two doses as equivalent, it is currently unknown whether there is any clinical difference in effectiveness between the 0.5mg and 0.6mg tablets of colchicine. This will need to be tested going forward.

Several studies in this thesis were observational and therefore prone to bias by confounding factors. Chapter 4 described a cross-sectional study that evaluated and compared inflammatory markers (high-sensitivity CRP (hs-CRP) and interleukin-6 (IL-6) in patients with and without dysglycemia and a recent cardiovascular (CV) event as well as with patients with remote CV events. Given the observational nature of this study, there are concerns regarding confounding variables influencing the assessment of the relationship between glycemic status and inflammatory markers. We elected not to use more sophisticated analytic approaches in our analysis and adjust for these possible confounding factors as our sample size was modest, and the primary objective of this analysis was descriptive and hypothesis generating rather than causal inference. The presence of confounding is an issue, and is discussed in more depth in the Appendix D. In short, our results remained stable when we performed a sensitivity analysis using regression modeling and controlled for relevant covariates.

Chapter 7 described a prospective cohort study that compared a cohort of patients with psoriasis and psoriatic arthritis who were started on biologic agents to a cohort of patients with psoriasis who were maintained on non-biologic disease modifying anti-rheumatic drugs (DMARDs) and a group of patients with non-inflammatory arthritis. In a prospective cohort study, a potential source of bias that can arise is immortal time bias. Immortal time bias occurs in an observational study when a period of follow-up during which the outcome cannot occur is incorrectly classified as exposure time. Classically this occurs when treatment or exposure begins after cohort entry (and individuals must remain event free in order to be defined as exposed). However, in the present study, immortal time bias was highly unlikely to play a meaningful effect in the observed results. All participants started their biologic therapy as soon as the baseline imaging was performed (i.e. time zero of the cohort). Additionally, the results were based on changes in the imaging biomarkers at fixed time periods; this was not a time-to-event analysis. This is discussed in more detail within the Appendix.

Section 9.8 Directions for further research

Certainly, the results from the CLEAR SYNERGY trial have tempered the excitement surrounding colchicine for the reduction of ASCVD. With conflicting results, studies such as CADENCE will help to shed more light on this important topic.

However, there are many additional questions in the field yet to be answered. Currently, there are multiple novel anti-inflammatory therapies that are currently undergoing investigation. Ongoing trials of ziltivekimab and clazakizumab are exploring whether interleukin-6 inhbition can lower cardiovascular events in specific populations.[70] The ZEUS trial (Ziltivekimab Cardiovascular Outcomes Study; Unique Identifier: NCT05021835) will invesgiate whether patients specifically with significant chronic kidney disease will benefit from IL-6 inhibition, and the phase 3 trial of clazakizumab will investigate IL-6 inhibition in patients on dialysis. These are important subpopulations as colchicine-use is generally contra-indicated in these patients.

Additionally, how early could the benefit of anti-inflammatory treatments be extended? Is there a possibility for consideration of anti-inflammatory treatments for primary prevention in high risk subpopulations? Could it prove useful in patients with large-vessel peripheral arterial disease? Is there a possibility that some of the benefits that we have seen in a population of patients with ASCVD could extend to those with chronic carotid disease? Perhaps there may even be benefits in patients with acute coronary ischemia.

Section 9.9 Conclusion

This thesis explored the current and future role of anti-inflammatory treatments for the reduction of cardiovascular events in high-risk patients with the aim of providing useful information to clinicians, researchers, and policy makers. Given the increasing awareness of the role of inflammation in the pathogenesis of atherosclerosis, as well as the increasing attention being given to potential treatments in this domain, this thesis makes and important contribution to the literature by offering insights into the efficacy of the available products and populations that may benefit from them. This thesis also investigated the cost-effectiveness of both short- and long-term colchicine use for the reduction of cardiovascular events in patients with atherosclerotic cardiovascular disease, finding that colchicine may be a cost-effective treatment for CV disease compared with the current standard of care. There still remains uncertainty regarding the utility of colchicine for the reduction of major adverse cardiovascular events (MACE) given the conflicting evidence base.[12 68 69] However, the network meta-analysis performed as part of this thesis incorporated all available evidence and concluded that colchicine remains superior to standard of care for the reduction of MACE. These findings will be useful in the development of policies and guidelines related to the use of anti-inflammatory therapies for atherosclerosis, to clinicians making treatment decisions about the use of anti-inflammatory drugs, and for researchers in the field looking at where the field is moving.

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APPENDICES

Appendix A. Abstracts accepted for poster presentation

Abstract 14289: Anti-Inflammatory Therapies to Prevent Cardiovascular Events: A Systematic Review and Network Meta-Analysis of Randomized Controlled Trials

Kevin E Boczar, Sheojung Shin, Alexander Pearson, Saba Shahab, Aishwarya Geejo, Sarah Visintini, Christophe Fehlmann, Kathryn Bezzina, Rob S Beanlands, and George Wells

PRESENTED AT: American Heart Association Scientific Sessions November 2023 (Philadelphia)

Introduction: Anti-inflammatory therapies have been increasingly investigated for the reduction of cardiovascular (CV) events. The goal of this study was to summarize and compare the relative effectiveness of anti-inflammatory medications for the reduction of CV events in a systematic review and network-meta-analysis.

Methods: In our systematic review, we included studies from Medline, Embase, the Cochrane Central Register of Controlled Trials, as well as clinical trial registry websites, Europe PMC, and conference abstract hand-searching. Randomized controlled trials were selected for inclusion if they included at least one anti-inflammatory treatment and involved patients with CAD. Bayesian network meta-analyses was performed to calculate risk estimates using random effects analyses in patients with ACS as well as stable CAD for the reduction of MACE.

Results: A total of 14,699 studies were screened; sixty-one articles, all randomized control trials, met eligibility criteria for inclusion. A total of 41,647 patients were included in the stable CAD network analysis and 29,388 patients were included in the ACS network analysis. In the ACS analysis, both non-steroidal anti-inflammatory (NSAID) use (OR: 0.30, 95% Credible Limits [CrI]: 0.11-0.74) and colchicine use were associated with a significant reduction in MACE compared to control (OR: 0.71, 95% Credible Limits [CrI]: 0.58-0.88). In the stable CAD analysis,

both corticosteroids (OR: 0.44, 95% CrI: 0.26-0.73) and colchicine (OR: 0.65, 95% Credible Limits [CrI]: 0.54-0.77) were associated with a significant reduction in MACE compared to control.

Conclusions: In patients with acute coronary syndromes, NSAIDs and colchicine were associated with a reduction in the risk of major adverse cardiac events. In patients with stable coronary artery disease, both colchicine and steroids were associated with a reduction in the risk of major adverse cardiac events. For a comprehensive analysis of benefits and harms of anti-inflammatory therapies, large comparative studies or network meta-regression analyses of patient-level data will be required. Systematic review registration number: PROSPERO registry—CRD4202230328

Abstract 13835: Anti-inflammatory Effect of Rosuvastatin in Patients With HIV Infection: A Randomized Controlled FDG-PET Study

Kevin E Boczar, Elliott Faller, Jerry Wang, Wanzhen Zeng, Gary R Small, Vicente Corrales-Medina, Robert deKemp, Natalie Ward, Rob S Beanlands, Paul MacPherson, and Girish Dwivedi

PRESENTED AT: American Heart Association Scientific Sessions November 2020 (virtual)

Introduction: Cardiovascular disease (CVD) is a major source of morbidity and mortality in HIV+ patients, which is thought to be in part due to a state of persistent systemic inflammation that results in accelerated atherosclerosis.

Hypothesis: We hypothesized that inflammation, as measured by 18F-fluorodeoxyglucose (18F-FDG) uptake measured by 18F-FDG positron emission tomography/computed tomography (18F-FDG-PET/CT) in the bone marrow, spleen and thoracic aorta would improve in HIV+ patients following therapy with rosuvastatin compared to HIV+ patients not receiving a statin.

Methods: HIV+ patients with a moderate Framingham risk score were enrolled in the study and randomized to treatment with rosuvastatin or usual treatment for 6 months. Patients were matched for age, sex, smoking status, Framingham risk score, duration of anti-retroviral therapy (ART) and type of ART. Fasting blood was collected and 18F-FDG-PET/CT imaging of bone marrow, spleen, and thoracic aorta was performed at baseline and 6 months.

Results: Thirty-five HIV+ patients were enrolled in the study; 17 were randomized to treatment with rosuvastatin and 18 were randomized to the control group. There was a significant drop in the 18F-FDG bone marrow, spleen and thoracic aortic uptake in the statin-treated group compared to the control group (bone marrow: -10.3±16.9% versus 5.0±18.9%, p=0.0262; spleen: -9.8±20.3%

versus 11.3±28.8%, p=0.0497; thoracic aorta: -8.6±24.5% versus 12.6±29.5%, p=0.0343). 18F-FDG changes over the study period are shown in Figure 1.

Conclusions: The present study observed a significant decrease in 18F-FDG-PET uptake in the bone marrow, spleen, and thoracic aorta following treatment with rosuvastatin for 6 months in HIV+ patients. These data suggest a wide-range anti-inflammatory effect of rosuvastatin in HIV+ individuals with well-controlled infection on ART, ultimately resulting in decreased inflammatory activity in the arterial walls of these patients.

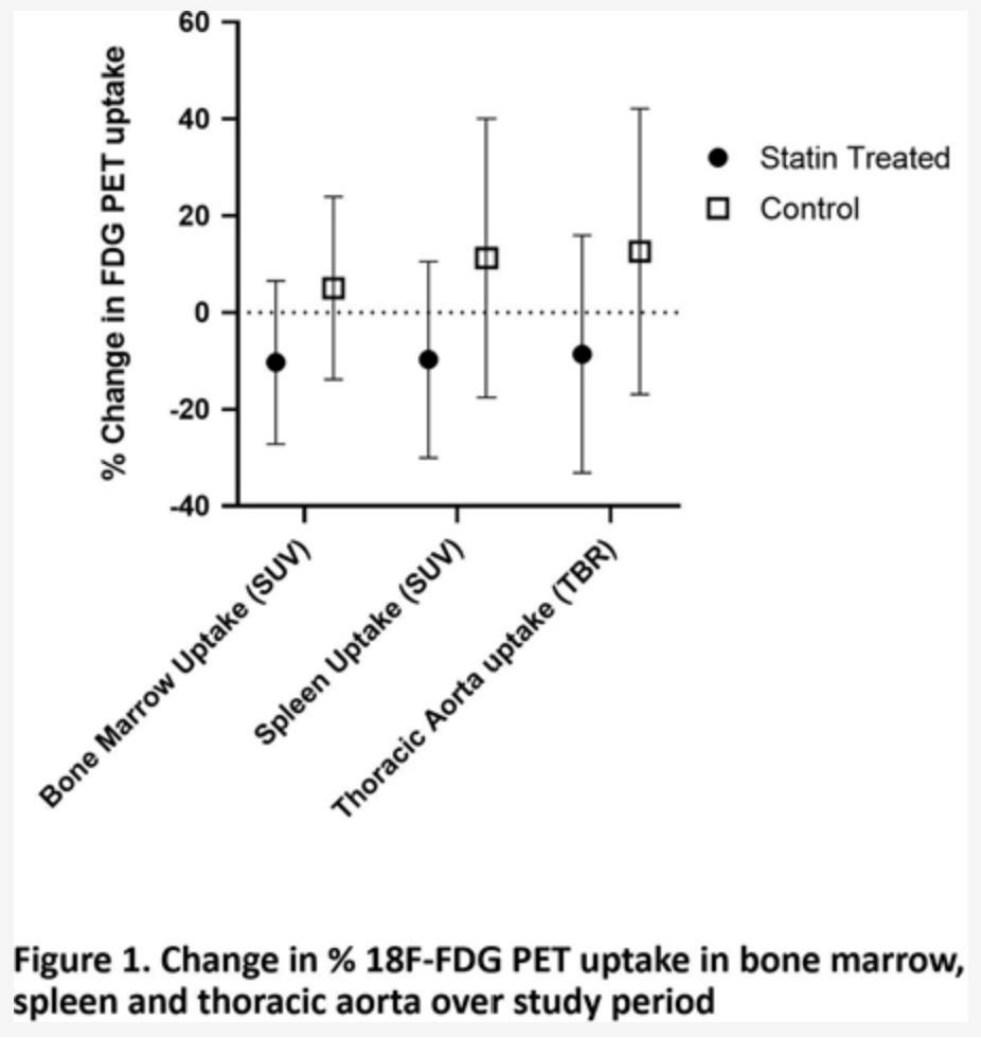

Figure 1. Change in % 18F-FDG PET uptake in bone marrow, spleen and thoracic aorta over study period

Abstract 9057: Anti-inflammatory Effect of Biologic Therapy in Patients With Psoriatic Disease: A Prospective Cohort Fdg-pet Study

Kevin E Boczar, Steven Glassman, Jerry Wang, Wanzhen Zeng, Robert deKemp, Natalie Ward, Rob S Beanlands, Christophe A Fehlmann, George Wells, Jacob Karsh, and Girish Dwivedi

PRESENTED AT: American Heart Association Scientific Sessions November 2021 (virtual)

Introduction: Psoriatic arthritis (PsA) and cutaneous psoriasis (PsO) are linked with an increased risk of adverse cardiovascular events.

Hypothesis: Central vascular inflammation (VI) as measured on F-18-fluorodeoxyglucose (FDG) positron emission tomography (PET) in the ascending aorta will improve in patients following therapy with biologic agents compared to PsO patients receiving non-biologic therapy and controls. We also postulate that in patients responding to biologics, there will be an improvement in coronary microvascular dysfunction as measured by myocardial blood flow reserve (MFR) determined by rubidium-82 (82Rb) PET.

Methods: We studied 3 patient cohorts (patients with PsA and/or PsO started on biologic agents, patients with PsO managed on non-biologic therapy, and patients with non-inflammatory arthritis). The primary outcome of our study was the change in VI, estimated as the target-to-background ratio by FDG PET in the most diseased segment of the ascending aorta at baseline compared to 6-month follow-up (TBRmax). We also performed 82Rb PET studies at baseline and 6-month follow-up to determine any changes on left ventricular MFR.

Results: 34 participants were enrolled in the study (11 PsA and/or PsO patients on biologic agents, 13 patients in PsO group managed on non-biologic therapy, and 10 in the non-inflammatory control

group). A significant drop in the thoracic aorta uptake was observed in the biologic-treated group (change in TBRmax: -0.46±0.55) when compared to the the PsO group treated with non-biologic therapy (change in TBRmax: 0.23±0.67). The group with imaging evidence of a response to biologic agents (i.e. TBRmax drop greater than median value) maintained MFR (change in MFR of 0.05±0.19) when compared to the group with a response on TBRmax that was below the median, which had a drop in MFR (-2.9±0.8) (P=0.03).

Conclusions: VI in the thoracic aorta decreased compared to psoriasis patients treated with non-systemic therapies and controls. Psoriasis patients treated with biologics who demonstrated a significant response on FDG PET imaging, maintained their MFR compared to non-responders. There is a positive impact of immune therapies on VI and microvascular disease in patients with chronic inflammation.

ANTI-INFLAMMATORY EFFECT OF BIOLOGIC THERAPY IN PATIENTS WITH PSORIATIC DISEASE COMPARED WITH TREATMENT WITH NON-BIOLOGIC DMARDS: A PROSPECTIVE COHORT FDG-PET STUDY

K Boczar, S Glassman, J Wang, W Zeng, R de Kemp, N Ward, R Beanlands, C Fehlmann, G Wells, J Karsh, G Dwivedi

PRESENTED AT: Canadian Cardiovascular Congress, October 2021 (virtual)

BACKGROUND: Psoriatic arthritis (PsA) and cutaneous psoriasis (PsO) are linked with an increased risk of adverse cardiovascular events. We hypothesized that central vascular inflammation on F-18-fluorodeoxyglucose (FDG) positron emission tomography (PET) in the ascending aorta would improve in these patients following therapy with biologic agents compared to PsO patients receiving non-biologic therapy and non-inflammatory control patients. We also postulated that in patients responding to biologics, there would an improvement in coronary microvascular dysfunction as measured by myocardial blood flow reserve (MFR) determined by rubidium-82 (82Rb) PET.

METHODS AND RESULTS: We studied 3 patient cohorts (patients with PsA and/or PsO started on biologic agents, patients with PsO managed on non-biologic therapy, and control patients with non-inflammatory arthritis). The primary outcome of our study was the change in vascular inflammation, estimated as the target-to-background ratio (TBR) by FDG PET in the most diseased segment of the ascending aorta at baseline compared to 6-month follow-up (TBRmax). We also performed 82Rb PET studies at baseline and 6-month follow-up to determine any changes on left ventricular MFR. A total of 34 participants were enrolled in the study (11 PsA and/or PsO patients

on biologic agents, 13 patients in PsO group managed on non-biologic therapy, and 10 in the non-inflammatory control group). The majority (64.7%) of participants were men and the median age was 62 years (IQR: 48, 69). A significant drop in the thoracic aorta uptake was observed in the biologic-treated group (thoracic aorta change in TBRmax: -0.46±0.55) when compared to the change in the thoracic aorta FDG uptake of the PsO group treated with non-biologic therapy (thoracic aorta change in TBRmax: 0.23±0.67). Furthermore, the group with imaging evidence of a response to biologic agents (i.e. TBRmax drop > median value of 7%) maintained MFR (3.40±1.23 MFR to 3.5±1.2 MFR over 6 months) when compared to the group with a below median response on TBRmax which had a drop in MFR (2.9±0.8 MFR to 2.2±0.6 over 6 months) (P=0.03).

CONCLUSION: In a cohort of psoriasis patients treated with biologics, the FDG uptake in the thoracic aorta decreased over the study period compared to psoriasis patients treated with non-systemic therapies as well as controls. Additionally, psoriasis patients treated with biologics who demonstrated a significant anti-inflammatory response on FDG PET imaging, maintained their MFR compared to non-responders. This study supports the notion that there is a positive impact of immune therapies on vascular inflammation and microvascular disease in patients with chronic inflammation.

OTTAWA CRP STUDY AFTER TIA AND VASCULAR EVENTS (OCTAVE)

K Boczar, D Dowlatshahi, P Liu, R DeKemp, C Kelly, L Garrard, C Lefebvre, L Zhang, A Guo, A Chong, D So, R Beanlands

PRESENTED AT: Canadian Cardiovascular Congress, October 2019 (Montreal)

Background: Atherosclerosis with its associated cardiovascular (CV) complications, is a lead cause of morbidity and mortality. Inflammation is a key process in the progression of atherosclerosis and represents a potentially transformative therapeutic target. Recent trials have revealed outcome benefit in patients with cardiovascular disease who are treated with anti-inflammatory therapies but these therapies have potential risks. Measurement of elevated inflammatory markers post-cardiovascular event may enable identification of those at highest risk for subsequent CV events and permit personalization of imaging and inflammation targeted therapies. We hypothesized that the prevalence of vascular inflammation in patients with diabetes or pre-diabetes (as a potential pro-inflammation state) following recent cardiovascular (CV) events (ACS, TIA, CVA) as reflected by elevated the inflammatory mediators hsCRP and IL-6, would be greater than in patients without recent acute CV events.

Methods and Results: An observational cohort study was conducted in patients who had suffered a recent CV event (ACS, TIA or CVA within 30-60 days) and were symptomatically and hemodynamically stable and in patients with remote prior events (>1 year). IL-6 and hsCRP were measured within 30-60 days following their vascular event. Patients were divided into 3 groups: Group 1: recent CV event with (pre-)diabetes (n=19); group 2: recent CV event without (pre-)diabetes (n=20); group 3: remote CV event (> 1 year) (n=25). Table shows baseline characteristics. IL-6 values for groups 1, 2 and 3 were 6.578.81pg/ml, 4.483.89pg/ml,

3.002.34pg/ml, respectively (p=0.048, group 1 vs 3). hsCRP values were 4.535.51mg/ml, 2.593.51mg/ml, 1.761.15mg/ml, respectively (p=0.075 group 1 vs 3) (Figure 1).

Conclusion: In this observational cohort study, IL-6 was significantly higher in patients with (pre-)diabetes who had a recent CV event compared to patients with remote CV events. There was a trend for higher hsCRP levels in patients with (pre-)diabetes and a recent CV event compared to those with a remote CV event. The results support the notion that patients who are at high risk for CV events (i.e. diabetes or pre-diabetes with recent CV events) have higher inflammatory burden than patients without recent CV events. These results set the stage for the use of inflammation biomarkers to define populations where approaches such as selective plaque imaging may be used to personalize novel anti-inflammatory therapy for the prevention of subsequent CV events. This will be tested in a prospective randomized trial.

Appendix B. Economic evaluation of canakinumab for cardiovascular events – a cost-utility analysis.

APPENDIX OVERVIEW and CONNECTION TO THE THESIS

This appendix presents a cost-utility analysis that was done as part of the candidate's PhD coursework and therefore does not represent work done as part of the candidate's thesis objectives.

The manuscript contained within this appendix describes a *de novo* model developed by Kevin Emery Boczar and Doug Coyle, with input from the other co-authors, to evaluate the cost-effectiveness of canakinumab for the reduction of recurrent cardiovascular events. This model was subsequently updated and adapted for use in **Chapter 6.1** to evaluate the cost-effectiveness of colchicine for reduction of recurrent cardiovascular events.

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KEB, GW, RB, and DC designed the study. KEB drafted the manuscript, which was revised by all authors. All authors approved the version of the manuscript submitted for publication.

Cost-effectiveness of Canakinumab from a Canadian perspective for recurrent cardiovascular events

Short title: Canakinumab cost-effectiveness for cardiovascular benefit

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Brief Summary

Canakinumab is a novel monoclonal antibody (biologic) therapy that has been shown to reduce cardiovascular events but is associated with side effects and has a high associated cost. A decision model was developed to estimate the direct costs and outcomes amongst patients who have suffered a myocardial infarction and are treated with Canakinumab. In the current analysis, it did not demonstrate cost-effectiveness in the Canadian public healthcare context largely due to the high medication cost.

Abstract

Background:

Cardiovascular disease is a condition with high morbidity and mortality. Canakinumab is a novel monoclonal antibody therapy that has been shown to reduce CV events but is associated with side effects and has a high associated cost. The main objective for this analysis is to determine whether Canakinumab is cost effective for the prevention of recurrent-CV events.

Methods:

A decision model was developed to estimate the direct costs and outcomes amongst patients who have suffered a myocardial infarction and are treated with Canakinumab. A lifetime study horizon was used to analyze the base-case costs and utilities from the perspective of the Canadian publicly funded health care system. Markov modeling was used in combination with Monte Carlo simulation to derive expected values for costs and quality-adjusted life years (QALYs), permitting the calculation of incremental cost-effectiveness ratios (ICERs).

Results:

Canakinumab was associated with higher average lifetime costs per patient ($457,982 versus $82,565) and higher average QALYs per patient (14.90 versus 14.20) when compared to standard of care. Thus, the incremental cost per QALY gained for Canakinumab versus standard of care was $535,365. The probability that Canakinumab is cost effective was 0%. Results were consistent over a range of scenario analyses.

Conclusions:

Treatment of patients post myocardial infarction with Canakinumab is not cost-effective when compared to standard of care at the current price. Based on currently accepted willingness to pay thresholds in

Canada, a reduction in price of 91% is required to yield a cost per patient that would be considered appropriate.

Introduction

The atherosclerotic disease process is responsible for a whole host of vascular complications – and is a leading cause of morbidity and mortality for Canadians and worldwide.[1, 2] Inflammation is a key mediator which contributes to the progression of atherosclerosis and its complications, such as myocardial infarction (MI), stroke and cardiovascular (CV) death; it also represents a potentially transformative therapeutic target.[3, 4] While the compelling evidence on the role of inflammation in atherosclerosis continues to mount, until recently, defining effective anti-inflammatory treatments to reduce events has been elusive. Glucocorticoids used in inflammatory diseases increase CV events.[5] Large trials in the area have been disappointing and have failed to show CV outcome benefits from therapies that target specific inflammation pathways: including LDL oxidation;[6] secretory and lipoprotein-associated phospholipase A2 (sPLA2 and LpPLA2);[7-9] P38 mitogen-activated protein (MAP) Kinase inhibitors,[10, 11] Methotrexate,[12] and P-selectin.[13]

The recent Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) trial demonstrated that targeted inflammation therapy can impact CV outcomes.[4] In 10,061 patients who were more than 30 days post-MI with elevated blood inflammatory markers (high-sensitivity C-Reactive Protein (hs-CRP) >2 mg/L), the IL-1β monoclonal antibody canakinumab reduced the primary composite end point of nonfatal MI, nonfatal stroke, or CV death (HR 0.85; 95% CI : 0.74-0.98; P=0.021 for the 150 mg dose) and the secondary composite end point which included hospitalization for unstable angina (HR 0.83; 95%CI: 0.73-0.95; p=0.005).[4]

Canakinumab represents a potential novel therapeutic agent in the treatment of atherosclerotic cardiovascular disease and its devastating sequelae. However, canakinumab is certainly not a silver bullet; its use was associated with an increased risk for potentially lethal adverse events (i.e. fatal sepsis) (0.31 vs

0.18 events x100 person years; p=0.02).[4] Further, as a biologic therapy approved for use in juvenile arthritis, it carries high costs to both patients as well as the healthcare system. The main objective for this analysis is to assess the cost-effectiveness associated with using Canakinumab for the prevention of recurrent-CV events.

Methods

Decision Problem

The specific decision problem that this study addresses is whether a Canadian health care payer should reimburse treatment with Canakinumab for the reduction of recurrent cardiovascular events in adult patients in Canada with a prior myocardial infarction and high residual inflammatory burden. To address this decision problem, a cost utility analysis (CUA) was performed to compare Canakinumab to current standard of care therapies (i.e. which do not include anti-inflammatory therapy), from the perspective of the Canadian public healthcare payer over a lifetime horizon (specifically from the perspective of the Ontario Ministry of Health & Long-Term Care).

Model Overview

A probabilistic Markov cohort model was used to derive the estimated direct costs and health outcomes (life years and quality adjusted life years (QALYs)) amongst patients who have suffered a myocardial infarction and have a residual high-inflammatory burden, who are treated with Canakinumab. The dosing of Canakinumab 150mg subcutaneously administered every 3 months was used as the treatment dose of choice, as per the results of the CANTOS study.[4] We used a lifetime study horizon (400 cycles equating to 33.33 years) to analyze the base-case costs and utilities from the perspective of the Canadian publicly funded health care system, and we incorporated certain model assumptions that were based on previous published literature.[14] The net present value of future costs and QALYs was determined using a 1.5% discount rate as per guidelines outlined by the Canadian Agency for Drugs and Technologies in Health (CADTH) .[15] A Markov model combined with probabilistic analysis allowed estimation of costs (2021

CAD) and quality-adjusted life years (QALYs) which then allowed for the calculation of incremental cost-effectiveness ratios (ICERs). In the reference case, uncertainty regarding the value of each parameter was incorporated within the probabilistic analysis. Methodological uncertainty was explored by comparing the reference case results to those from non-reference case analyses using discount rates of 0% and 3% as per CADTH directives.[15] The impact on cost effectiveness of uncertainty on the estimated parameters for costs and outcomes for the intervention, was assessed by estimating the probability that Canakinumab was cost effective for alternative threshold values for a QALY.

Decision model

We developed a Markov model to follow a hypothetical cohort of patients who had suffered a myocardial infarction and had residual inflammation (Figure 1). Base states in the model included a stable state, a stable after recurrent myocardial infarction state, and a death state. With each cycle, patients entered the event model which included the event states of: stable, recurrent myocardial infarction, non-myocardial infarction revascularization, infection, and death. We did not include stroke as a state in our model, as there was no reduction of stroke in the CANTOS trial.[16] Patients could either recover or experience death with each of these events, and patients would then return to the appropriate base state following the event. The model allowed estimation of cycle specific estimates of costs and QALYs allowing for estimation of life time costs and QALYs.

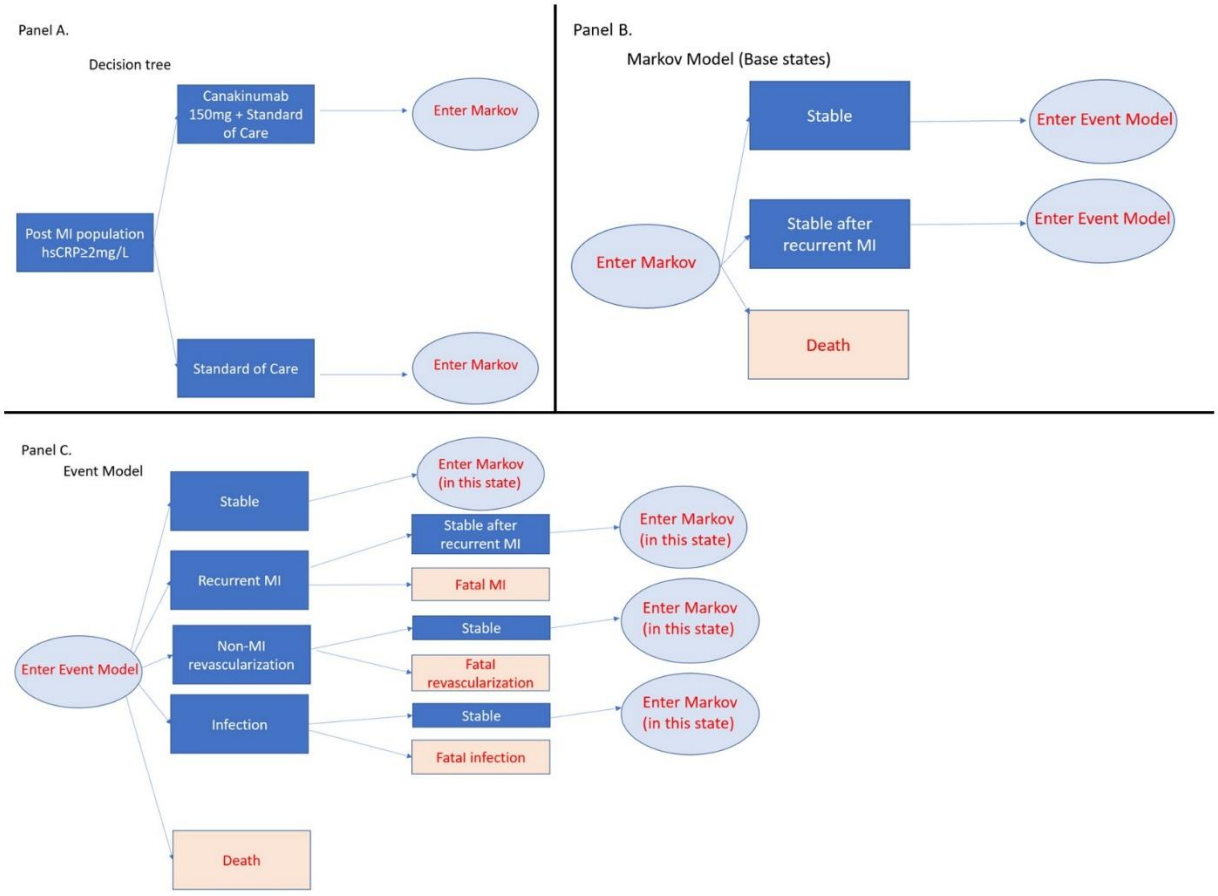

Figure 1 –Markov Model. A survivor of myocardial infarction can be assigned to treatment with Canakinumab 150mg subcutaneously daily and standard of care, or standard of care alone (Panel A). Within each cycle, a survivor of myocardial infarction can then each either remain stable, suffer a recurrent myocardial infarction (in which case they then enter the recurrent MI state), have a coronary revascularization procedure performed, suffer an infection, or remain in the death state (Panels B and C).

Patient Population

The cohort of patients were assumed to mirror the patient population within the CANTOS trail evaluating the impact of Canakinumab on cardiovascular outcomes.[4] The mean age of participants was 60, and patients had a history of myocardial infarction and a blood level of high-sensitivity C-reactive protein of 2mg or more per litre despite the use of aggressive secondary prevention strategies (i.e. standard of care

which did not include anti-inflammatory therapy, but did include treatment with antiplatelet agents, statins, beta-blockers, and angiotensin-converting enzyme inhibitors; this is similar to the treatment regimen in the CANTOS trial and what is done in clinical practice). Patients were excluded if they had a history of chronic or recurrent infections, previous cancer (other than basal-cell skin carcinoma), suspected or known immunocompromised state, history of tuberculosis, or ongoing use of alternative anti-inflammatory treatments.

Model inputs

Canakinumab was evaluated against current standard of care therapies post myocardial infarction. In current practice, post-MI care does not include anti-inflammatory therapies to reduce the risk of recurrent CV events. However, the recent CANTOS trial did indeed reveal outcome benefit to patients treated with Canakinumab following a MI. There are several perceivable disadvantages to this therapy however, in particular, an increased risk of infection. As per the drug-regimen used in this trial, all patients received standard of care therapies, and the patients receiving Canakinumab received 150mg subcutaneously every 3 months.

Survival probabilities associated with standard of care therapy as well as Canakinumab treatment are summarized in Table 1, and were derived from the CANTOS study where 10,061 patients were randomly assigned to either placebo or Canakinumab. The uncertainty around these probabilities were incorporated into the probabilistic analysis.

Table 1- Distributions for probabilistic analysis

	Expected Value	**Probability Distribution**
Event Probabilities		
Probability of recurrent MI (Standard Care)	0.087	Beta (292, 3052)
Probability of Non-MI revascularization (Standard Care)	0.126	Beta (421, 2923)
Probability of Infection (Standard Care)	0.102	Beta (342, 3002)
Probability of Infection (Canakinumab)	0.113	Beta (258, 2026)
Probability of death post revascularization	0.01	Beta (840, 128422)
Probability of MI being fatal	0.04	Beta (31, 759)
Probability of infection being fatal (Standard Care)	0.07	Beta (23, 207)

Probability of infection being fatal (Canakinumab)	0.09	Beta (24, 241)
Probability of non cardiovascular death (Standard Care)	0.042	Beta (140, 3204)
Hazard Ratios (Canakinumab versus Standard Care)		
Hazard ratio of recurrent MI (Canakinumab)	0.76	Lognormal (0.76, 0.62)
Hazard ratio of Non-MI revascularization (Canakinumab)	0.68	Lognormal (0.68, 0.58)
Hazard ratio of non cardiovascular death (Canakinumab)	0.97	Lognormal (0.97, 0.74)
Derived Monthly Transition Probabilities		
Standard Care		
Probability of recurrent MI	0.00202	
Probability of Non-MI revascularization	0.00300	
Probability of Infection	0.00238	
Probability of non cardiovascular death	0.00092	
Canakinumab		
Probability of recurrent MI	0.00154	
Probability of Non-MI revascularization	0.00204	
Probability of Infection	0.00260	
Probability of non cardiovascular death	0.00090	

In the base case analysis we assume that patients continue treatment beyond the four year time horizon of the clinical trial and the, benefit of treatment is maintained long term. Scenario analysis addressed the impact of these assumptions. In a scenario analysis, we adopted a 48 month time horizon, which allowed estimation of the proportion of the forecasted QALY gains for Canakinumab which are generated after the period covered by the CANTOS trial. This analysis which examines the impact of extrapolation is recommended within the CADTH guidelines.[15] As per CADTH guidelines, in a further scenario analysis, we assumed a decline in treatment effect with the effect demonstrated within the clinical trial being maintained for the first 48 months followed by a linear decline in treatment effect until at 96 months there was no continued effect. Additionally, we assessed the impact of treatment discontinuation by assuming that after 48 months, patients would discontinue treatment with Canakinumab at a rate of 10% per annum with no continued effect after discontinuation. Finally, we assessed the cost effectiveness under a scenario whereby Canakinumab was 100% effective at reducing MIs and revascularizations and with no risk of infections.

The costs associated with each treatment strategy are summarized in Table 2. The monthly costs associated with Canakinumab administration strategies and the costs of cardiovascular events, complications, and admissions were obtained from the literature and converted to 2021 CAD based on the Bank of Canada inflation calculator.[17-20] Similarly, we applied costs for standard of care treatment for coronary heart disease from published literature[21] and again converted to 2021 CAD based on the Bank of Canada inflation calculator. Given the lack of available contemporaneous Canadian cost data for fatal myocardial infarction and revascularization admissions, we elected to utilize the conservative estimate of costing them the same as a non fatal myocardial infarction or revascularization procedure.

Table 2- Cost and Utility Data

	Base-case estimate[Reference]	**Probability Distribution**
Monthly Costs		
Standard care	$238[21]	Gamma (18221.67, 0.01)
Canakinumab	$5,333	Fixed
Cost for Event		
Myocardial infarction	$25,028[17]	Gamma (10652.67, 1.92)
Revascularization	$40,100[18]	Gamma (16, 1985.66)
Non-fatal infection admission	$14,715[20]	Gamma (16, 661.56)
Fatal-infection admission	$49,158[20]	Gamma (7261.44, 4.24)
Utilities		
Stable standard of care	0.868[22]	Beta (3531.89, 537.11)
Stable recurrent MI	0.734[23]	Beta (14.68, 5.32)
Utility toll for MI/revascularization/ infection admission	-0.0986[expert opinion]	Beta (1385, 12657)

Utility values were derived from a variety of sources. Utility values associated with the base state of post-MI status was taken from an analysis and comparison of the MONICA/KORA registry to the general population.[22] Utility values for the recurrent MI state were obtained from literature which investigated the stability of time-tradeoff utilities in survivors of myocardial infarction.[23] Finally, disutility values associated with adverse outcomes were determined from expert opinion.[24]

There were several additional model assumptions that were included. First, it was assumed that the consequences, not the probabilities, of adverse events in terms of costs and quality life effects were consistent across the treatments. This could certainly lead to an underestimation of the cost estimates, as well as potential underestimation (or overestimation) of the disutility associated with these adverse events. However, we felt it was a reasonable assumption to make in order to reflect the average cost and disutility associated with the adverse events in our model. Second, based on literature from the CANTOS trial, we assumed that the 150mg dose of Canakinumab was the optimal dose for all patients.[4] A final limitation is the lack of contemporaneous costs data.

Model outputs and analysis

Markov modeling was used to calculate mean total health care costs and total QALYs gained for each strategy. Mean costs and utility values for each strategy for each cycle were estimated based on a probability weighted sum for the weights for each state within the model. Cycle specific estimates were then summed and discounted over the lifetime horizon to provide estimates of total costs and QALYs. Probabilistic analysis using Monte Carlos simulation with 5000 replications was performed to account for uncertainty in the input values providing an estimate of the expected values for costs and QALYs. Cost effectiveness was assessed by estimating the incremental cost per QALY gained against a base willingness-to-pay threshold of $50,000/QALY, with scenario analysis considering thresholds of $30,000 and $100,000.

Probabilistic scenario analyses were conducted to assess the impact of alternative modelling assumptions on the results of our analysis. Analyses related to alternative discount rates (0% and 3%) and alternative assumptions with respect to the continuance of treatment effect beyond the trial time horizon and discontinuation with treatment were also performed.

Model Validity

Face validity was confirmed with experts in the field. Internal validation was conducted by the senior health economist reviewing the model structure and coding. Validity of our model was tested by comparing event rates from the CANTOS trial with the risk from our simulated Canakinumab and standard of care cohorts at 3.7 years of elapsed follow-up time (the median follow-up time from the CANTOS trial). To assess the external validity of our model, we compared events in the standard of care arm from the COLCOT trial (a similar study population, albeit with slightly different study primary outcomes of cardiovascular death, spontaneous myocardial infarction, or ischemic stroke) to results obtained from our standard of care model cohort (with outcomes of death or myocardial infarction) at 22.6 months of follow-up (which was the median follow-up time from this trial).

Results

With respect to validity of our model, cumulative mortality estimates from our model were within the 95% confidence intervals of the CANTOS trial estimates at 3.7 years of follow-up for both the standard of care and canakinumab groups. The mortality estimate for the canakinumab cohort was 9.5% in our model vs 9.6% (95% CI, 8.4-10.8) in the CANTOS trial. Event estimates from the standard of care group in our model were also comparable to those from the COLCOT standard of care group at 22.6 months of follow-up. The standard of care group total event estimate was 9.6% in our model, and was 9.6% in the COLCOT trial.

Results of our probabilistic analysis found that compared to standard of care Canakinumab was associated with greater discounted life expectancy (17.72 years versus 16.99 years), reduced lifetime incidence of MIs and revascularisation, but with an increased incidence of infections (Table 3). Canakinumab was associated with higher health care costs than standard of care group $457,982 versus $82,565 (Table 4). The incremental costs of Canakinumab ($375,417) are almost exclusively due to the lifetime costs of Canakinumab ($379,943), partially offset by the reductions in MIs and revascularizations. Cumulative

QALYs were 14.90 in the Canakinumab group, and 14.20 in the standard of care group, respectively. The corresponding ICER for Canakinumab therapy was $535,365. (Table 4).

Table 3 – Disaggregated Results

		Canakinumab	Standard of Care	Difference
Incidence	Recurrent MI - non fatal	0.387	0.484	-0.097 (-0.173, -0.008)
	Recurrent MI - fatal	0.016	0.020	-0.004 (-0.008, 0)
	Revascularization - non fatal	0.531	0.717	-0.187 (-0.283, -0.082)
	Revascularization - fatal	0.003	0.029	-0.026 (-0.037, -0.016)
	Infection - non fatal	0.616	0.533	0.083 (-0.019, 0.186)
	Infection - fatal	0.061	0.059	0.003 (-0.032, 0.038)
Costs	No new event	$50,572	$48,533	$2,039 (-$1,176, $5,083)
	Canakinumab	$379,943	N/A	$379,943 ($351,874, $407,106)
	Recurrent MI - non fatal	$6,455	$8,115	-$1,659 (-$2,913, -$180)
	Recurrent MI - fatal	$262	$330	-$67 (-$130, -$7)
	Revascularization - non fatal	$13,794	$18,730	-$4,935 (-$8,958, -$1,937)
	Revascularization - fatal	$90	$759	-$669 (-$1,190, -$322)
	Infection - non fatal	$5,318	$4,617	$701 (-$158, $1,783)
	Infection – fatal	$1,547	$1,482	$65 (-$819, $941)
	Total Costs	$457,982	$82,565	$375,417 ($342,599, $406,810)
QALY		14.90	14.20	0.701 (-0.247, 1.593)
Life years		17.70	16.99	0.712 (-0.408, 1.771)

Figures in parenthesis are the 95% credible interval for the differences

Table 4 – Cost Effectiveness Results for Base Analysis and Scenario Analyses

	Lifetime costs	Lifetime QALYs	ICER: Canakinumab versus Standard of Care
Base Case			
Standard of care	$82,565	14.20	
Canakinumab	$457,982	14.90	$535,365
Discount rate 0%			
Standard of care	$100,457	17.22	
Canakinumab	$560,099	18.17	$480,408
Discount rate 3%			
Standard of care	$62,239	11.94	
Canakinumab	$383.057	12.49	$576,118
48 month time horizon			
Standard of care	$17,734	3.14	$9,214,677
Canakinumab	$251,288	3.17	
Gradual decline of treatment effect			
Standard of care	$82,527	14.19	
Canakinumab	$452,108	14.47	$1,334,400
Allowance for discontinuation with treatment			
Standard of care	$82,658	14.20	
Canakinumab	$686,574	14.43	$2,553,491
Canakinumab is 100% effective at reducing MIs, revascularization and infection			
Standard of care	$82,566	14,19	
Canakinumab	$434,807	15.52	$265,622

ICER – incremental cost per QALY gained; MI - = myocardial infarction; QALY – quality adjusted life year

At a willingness-to pay threshold for a QALY of $50,000, Canakinumab had a 0% probability of being cost-effective (i.e. the ICER was greater than $50,000 in all 5,000 simulations), with this finding holding for threshold values up to $179,000. To achieve an ICER of $50,000 per QALY, the costs of Canakinumab has to be reduced by 91% to $1,477 per 150 mg injection. For willingness-to-pay thresholds of $30,000 and $100,000 per QALY, the necessary price reductions were 94% and 82% respectively.

Scenario analyses relating to discount rates demonstrated consistency with our primary analysis. (Table 4) In the scenario analysis with a reduced time horizon of 48 months, estimated incremental QALY gains were only 0.03, suggesting that less than 4% of the forecasted QALY gains from Canakinumab occur during the initial time period representative of the CANTOS trial. Analyses incorporating waning of

treatment effect with Canakinumab and discontinuation of treatment with Canakinumab reported much higher ICERS than in the base case (Table 4). Of note, in the scenario analysis whereby Canakinumab was assumed 100% effective, the estimated ICER was still $265,622.

Discussion

As inflammation is now recognized to play an important role in the pathogenesis of atherosclerotic cardiovascular events,[25, 26] much attention has been focused in recent years in trying to identify potential anti-inflammatory agents that could be used to target this pathway to reduce CV risk for patients. A number of prospective randomized studies have been performed in recent years, with varying results. The monoclonal antibody Canakinumab recently demonstrated benefit for the reduction of CV events in patients who have suffered a previous myocardial infarction and who have a high residual inflammatory burden in the CANTOS trial.[4] However, in the current analysis, it did not demonstrate cost-effectiveness in the Canadian public healthcare context. This was largely due to the high cost associated with the medication.

In the CANTOS trial, Canakinumab was the first drug to show benefit from a CV event reduction perspective by solely acting on an inflammatory pathway.[4] Canakinumab is not without side effects, however. Its beneficial actions are a direct result of its ability to modulate the immune system. However, this immune system modulation also has negative consequences too. While Il-1β is not an immunosuppressant, and does not increase the risk of opportunistic infections or malignancy, it has bene shown to increase the risk of fatal infections.[27] This is thought to likely be the result of a blunting of the inflammatory cascade that is a normal party of the body's reaction to infection.[27] Another major drawback to Canakinumab use is its cost. The cost of Canakinumab is $16,000 per 150mg vial – this works out to an annual cost of approximately $64,000 per patient assuming a dosing regimen similar to what was done in the CANTOS trial.[4] Thus, while the current medications used for secondary prevention in patients who have suffered a MI is well-established, whether or not Canakinumab should enter this

domain is certainly not. The cost-effectiveness of the drug will certainly need to be considered in this clinical decision making, particularly in publicly funded health care systems. In this cost-effectiveness analysis, we evaluated the cost-effectiveness of Canakinumab against the current standard of care therapies for the secondary prevention of CV events, from the perspective of the Ontario public healthcare system. Our results demonstrate that in comparison, Canakinumab is currently not a cost-effective strategy in this area.

The predominant driver of higher costs was the drug costs. In one scenario analysis where we assumed Canakinumab was 100% effective at reducing events, the ICER was still in excess of $250,000, suggesting that regardless of the assumption of effectiveness Canakinumab would not be cost effective at the current price.

By conducting probabilistic analysis, we were able to incorporate a wide range of uncertainty into our model, thereby improving the robustness of our results. As evidenced by our study, there is negligible probability that Canakinumab is cost-effective using our pre-specified willingness to pay threshold of $50,000/QALY, and this conclusion remains consistent with much higher willingness-to-pay thresholds.

The major limitation of our study is that the efficacy of Canakinumab was based on a single randomized controlled trial. However, at this point in time, this single trial encompasses almost the entire body of Phase 3 evidence on the subject, and is currently what is available for decision-makers. The results of this analysis strongly suggest that regardless of relative effectiveness, Canakinumab is unlikely to be cost effective in this context at its current price. Our base case analysis adopted assumptions related to continuance with treatment and of treatment effect which were favourable towards Canakinumab. Given the lack of available contemporaneous Canadian cost data for fatal myocardial infarction and revascularization admissions, we elected to utilize the conservative estimate of costing them the same as a non fatal myocardial infarction or revascularization procedure admissions. Again, these assumptions were

favourable towards Canakinumab and would not alter the final conclusions of our analyses. Scenario analyses suggest that Canakinumab may be significantly less cost effective than in our base case. The patients in the standard of care arm in CANTOS were indeed receiving excellent care with close to 80% or more of patients receiving anti-lipid, antithrombotic, antianginal, and ace-inhibitor therapies. It is a common misbelief that patients being followed closely in a trial setting generally receive superior care, however, research has shown that this is in fact not the case.[28] However, if we were to assume that this assumption was indeed true, and that patients in a real-world setting receiving standard of care actually receive inferior care compared to the standard of care arm in the CANTOS trial, our model is overly conservative with respect to the estimate of benefit for canakinumab. Excellent care compared to poorer care is likely cost-effective (as it is reimbursed within the Canadian healthcare system), and therefore our model is possibly underestimating the cost effectiveness of canakinumab versus current care; however, this being said, it is unlikely to change the conclusion of our study given the high proposed cost of the drug is driving the results.

Anti-inflammatory medications for the reduction of CV events in high-risk patients seems to be a promising future direction in secondary prevention. Several medications in the area have been investigated in this regard, with some showing promise. However, as evidenced by this study, in future research, considerations will need to be given towards therapeutics that are effective both from clinical as well as economic perspectives. In general, there are occasions when exceptions are made to reimburse medications and therapeutic options in the Canadian health care system that do not meet a WTP threshold. Certainly, weighing the safety, efficacy, and efficiency of a drug is important, but care and consideration can also be given towards whether a novel therapy fills an unmet need for a patient demographic. In the case of Canakinumab, while it is the first anti-inflammatory therapy to show benefit in this patient population, it is certainly not the last, and there are already other anti-inflammatory drugs that in the time following CANTOS, have since additionally shown CV benefit at a fraction of the cost.[29]

Conclusion

Treatment with Canakinumab for secondary prevention of CV events is not cost-effective in the Canadian healthcare system. A substantial price reduction of 91% would need to occur before it could be considered a potentially cost-effective use of scarce health care resources. Future investigations of anti-inflammatory drugs such as biologics, targeted at atherosclerosis must consider the balance of effectiveness and economic impact on patients and the health-care system.

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GW and DC – nothing to declare

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Appendix C. Commentary on inflammation in cardiovascular disease.

This appendix presents a commentary based on the contents of this PhD thesis and that was done during the course of the candidate's PhD coursework but was not a formal part of the candidate's thesis and therefore does not represent work done as part of the candidate's thesis objectives.

The manuscript contained within this appendix describes a *commentary* written by Kevin Emery Boczar and Rob Beanlands, about the pathogenic role of inflammation in atherosclerotic cardiovascular disease.

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Hearts on fire: the role of inflammation in the pathogenesis of atherosclerotic cardiovascular disease and how we can tend to the flames

Short title: Inflammation and cardiovascular disease

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Brief Summary

Inflammation is being increasingly recognized as a major driver, and potential treatment target, of the atherosclerotic disease process. However, while great strides have been made in this area, more research is needed before anti-inflammatory medications become standard of care for patients with CAD. This paper reviews the current literature on this topic.

Despite our best attempts to manage traditional cardiovascular (CV) risk factors with contemporary approaches, major adverse cardiac events continue to occur at high rates in patients with established atherosclerotic coronary artery disease (CAD). Inflammation is being increasingly recognized as a major driver, and potential treatment target, of the atherosclerotic disease process. Many physicians are now pondering the question of whether anti-inflammatory agents should enter their toolkit for treating patients with CAD. However, while great strides have been made in the area, more research is needed before anti-inflammatory medications become standard of care for patients with CAD.

Anichkov observed that atherosclerosis contained macrophages and foam cells more than 100 years ago; yet it is only recently we have been able to demonstrate that therapy targeting inflammation can reduce CV events. In recent years there has been an explosion of interest in the area to discover an effective and economical option to target the inflammatory pathway to reduce CV events. Several agents have even shown CV benefit in well conducted clinical trials.

Inflammation: a novel CAD treatment target

The Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial was groundbreaking, in that it demonstrated that amongst patients with normal LDL but elevated high-sensitivity C-reactive protein (hsCRP), the medication rosuvastatin reduced the incidence of CV events, thereby fanning the flames of the inflammatory hypothesis of CV disease and showing that inflammation could be a potential future therapeutic target.

Canakinumab: targeted Il-1 inhibition

In the CANTOS trial, canakinumab (a monoclonal antibody that targets the inflammatory cytokine lL-1 beta) was found to prevent recurrent cardiovascular events in patients that had a persistently high inflammatory response after their first cardiovascular event.(1) It was the first trial to show that a medication purely targeting inflammation could alter cardiovascular outcomes.(1) However, a high price-

point as well as an increase in the rate of fatal infections and sepsis have prevented its wide-spread adoption for this indication. Thus, while the CANTOS study further supported the inflammatory hypothesis and fuelled the search for a cost-effective means to target inflammation to reduce CV events, canakinumab itself has not become standard of care for CV risk reduction.

Colchicine

Colchicine is a potent anti-inflammatory medication that has been used for centuries in a variety of inflammatory conditions like gout, pericarditis, and juvenile idiopathic arthritis.(2) In the 2020 LoDoCo 2 study, just over 5500 patients were randomized to either Colchicine 0.5mg/day or placebo.(3) The primary end point composite of cardiovascular death, spontaneous (nonprocedural) myocardial infarction (MI), ischemic stroke, or ischemia-driven coronary revascularization was reduced in the colchicine group, yielding a hazard ratio of 0.69. While the LoDoCo 2 trial targeted patients with stable CAD, the COLCOT study looked to establish the role of colchicine in treatment of CAD in the setting of higher-risk patients who were post-MI.(2) In COLCOT, colchicine reduced the incidence of the composite endpoint of cardiac death, MI, resuscitated cardiac arrest, stroke, urgent hospitalization for angina leading to coronary revascularization, for a hazard ratio of 0.77.

Colchicine seems to be a very promising agent which has demonstrated cardiovascular outcome benefit in several trials. However, these exciting results have also been tempered by a possible signal for increased harm. Colchicine has shown a signal for increased risk of non-CV death in a number of studies, such as in LoDoCo 2 where there was a trend for increased all cause mortality in the colchicine arm with a hazard ratio of 1.21.(3) However, this trend has not been consistent in all studies. In COLCOT, the incidence of all-cause mortality was similar in placebo and colchicine arms.(2) A Cochrane systematic review and meta-analysis by Hemkens et al. found no effect of colchicine on all-cause mortality or any adverse events, but the quality of evidence for adverse events was low.(4) The authors conclude that insufficient evidence currently exists regarding both the benefits and harms associated with colchicine use for the reduction of cardiovascular events.(4) Thus, it is currently difficult to determine where the scale should

settle out with respect to risk/benefit from colchicine. Future and current trials will hopefully shed more light on this.

Ongoing colchicine trials

Other RCTs involving colchicine currently underway include the CONVINCE trial, which will look at colchicine in patients with ischemic stroke (NCT02898610), the COACS trial which is looking at the effect of low-dose colchicine on all-cause mortality, MI and ischemic stroke in patients with ACS (NCT01906749), the LEADER-PAD (Low Dose ColchicinE in pAtients With Peripheral Artery DiseasE to Address Residual Vascular Risk) study which is looking at the effect of low-dose colchicine on vascular events in patients with symptomatic peripheral artery disease (NCT04774159), and the CLEAR SYNERGY (Organization to Assess Strategies for Ischemic Syndromes OASIS 9) randomized controlled trial which will look at the long-term effects of treatment with colchicine (as well as Spironolactone) following PCI to treat ST elevation MI (NCT03048825). These trials should provide a lot more clarity on the issue of all-cause mortality and colchicine. A key take-away is that while there is a lot of promise in the field, there are still a lot of questions to be answered.

Perhaps a future solution will be to "stack the deck in our favour". Instead of treating all patients with colchicine (or other anti-inflammatory agents), targeting high-risk inflammatory subgroups may yield incrementally more benefit with these agents. An important step in this realm of research is to identify patient subgroups that we feel will benefit most from novel anti-inflammatory therapies. Literature supports several high-risk subgroups, such as patients with rheumatoid arthritis or psoriasis(5), where inflammation is likely contributing to an increased incidence of atherosclerotic cardiovascular disease. Targeting these subgroups with anti-inflammatory therapies may yield a higher incremental CV benefit. The CIRT trial (NCT01594333) was not able to show outcome benefit of methotrexate in patients with CAD likely at least in part due to a lack of pre-selection for patients with elevated inflammation as demonstrated by elevated hsCRP.

Clarity regarding colchicine's mechanism of action may also allow better patient selection; currently, the mechanism of action of colchicine's CV benefit is not known. In this regard, the CADENCE study is a multi-centre RCT evaluating the efficacy and underlying mechanism of action of colchicine in the reduction of CV events by utilizing FDG-PET imaging in patients with (pre-)diabetes (NCT04181996). It will look at the change in vascular inflammation measured by FDG-PET in these patients to determine if colchicine reduces vascular inflammation or if it acts to reduce systemic inflammation.

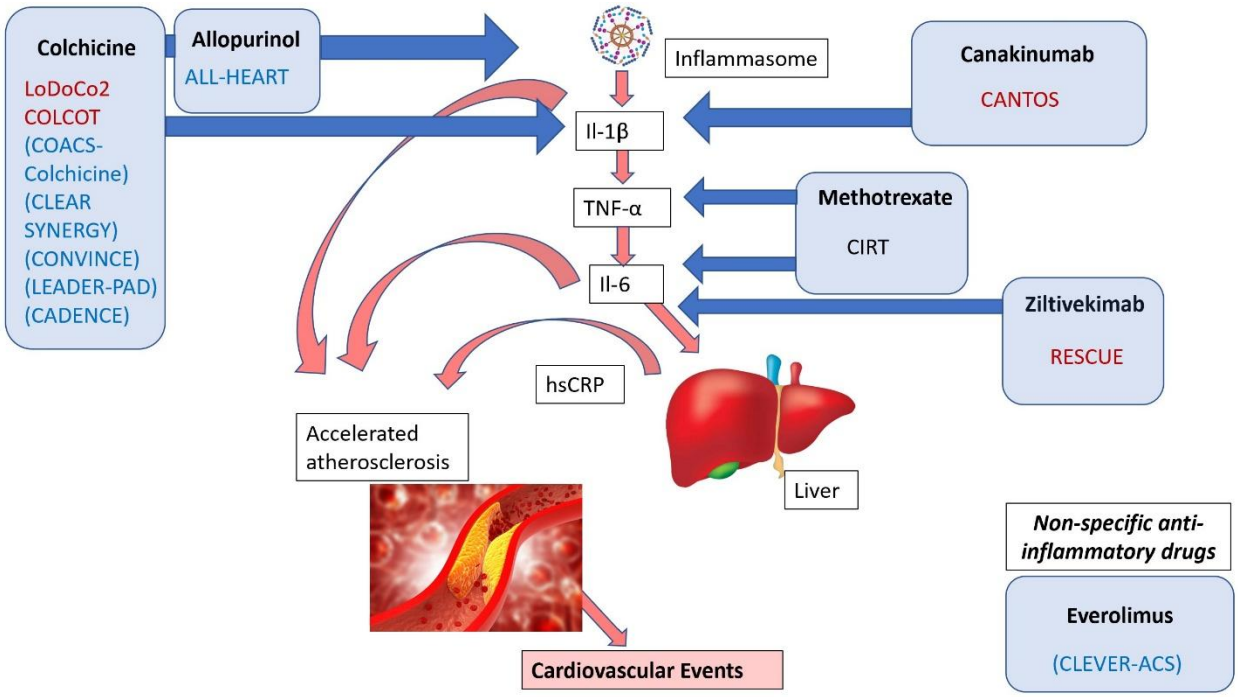

Figure 1. Mechanistic overview outlining the inflammatory pathway contributing to cardiovascular events, as well as the inflammatory interleukins that are potential targets for cardiovascular benefit. Multiple inflammatory signals involved in the inflammatory cascade provide various targets for novel agents aimed at reducing cardiovascular events. Anti-inflammation therapies, sites of action and related trials (primary outcome positive in **red**; primary outcome not significant in **black**; ongoing or pending results trials in **blue**).

hsCRP: high-sensitivity C-reactive protein, IL: interleukin, TNF: tumor necrosis factor

Other anti-inflammatory agents under study

Another trial that recently targeted a high-risk patient subgroup with anti-inflammatory therapy was the Phase 2 trial, RESCUE (NCT03926117). This study found that ziltivekimab, a monoclonal antibody directed against the IL-6 ligand, safely and effectively reduced biomarkers of inflammation among patients with high cardiovascular risk. Based on these data, a large-scale cardiovascular outcomes trial will investigate the effect of ziltivekimab in patients with chronic kidney disease, increased hsCRP, and established cardiovascular disease (NCT05021835). Several other studies involving various anti-inflammatory agents are planned or underway. The CLEVER ACS trial will look at infarct size in patients with ST-elevation MI administered Everolimus (NCT01529554). The ALL HEART study is a multi-center prospective randomized open label study looking at the effect of Allopurinol on a composite of nonfatal MI, nonfatal stroke or CV death in patients with known ischemic heart disease (ISRCTN32017426).

Certainly, the future is bright in the field of potential novel anti-inflammatory therapies for the treatment of cardiovascular disease. Inflammation plays a crucial role in the pathogenesis of atherosclerotic cardiovascular disease; this includes the induction of procoagulant activity, promotion of monocyte and leukocyte adhesion to vascular endothelial cells, and the promotion of the growth of vascular smooth-muscle cells.(1) Evidence continues to mount regarding novel anti-inflammatory medications for CV disease and more trials will continue to shed light on this area. While there is data to support use and some clinicians may have started to add this to their armamentarium for patients with CAD, concerns for adverse outcomes yield continued equipoise. As such, current guidelines do not currently recommend use (including the 2012 CCF/AHA/ACP/AATS/PCNA/SCAI/STS Guideline for the Diagnosis and Management of Patients With Stable Ischemic Heart Disease, 2019 ESC Guidelines on Chronic Coronary Syndromes, and 2014 Canadian Cardiovascular Society Guidelines for the Diagnosis and Management of Stable Ischemic Heart Disease) possibly due to concerns over mortality – instead, clinicians and patients are encouraged to enroll in ongoing RCTs to enable us to determine ‘how we can tend the flames’ of

inflammation. Finally, perhaps in the future, we will preferentially target high-risk inflammatory subgroups with these therapies.

Table 1. Summary of anti-inflammatory trials for the reduction of cardiovascular events.

Trial Name	Study design	Study population	Estimated enrollment	Estimated completion date	Intervention	Primary outcomes	Trial registry identifier:	Findings
Completed Trials								
RESCUE	Phase IIb, Randomized, Double-Blind, Placebo-Controlled Trial	Patients older than 18 years of age who have a history of Stage 3-5 CKD, hs-CRP>2mg/L	264	June 26, 2020	Ziltivekimab 7.5mg, 15mg, and 30mg subcutaneous monthly vs. placebo	Percentage change from baseline in hs-CRP	NCT03926117	Median high-sensitivity CRP levels were reduced by 77% for the 7.5 mg group, 88% for the 15 mg group, and 92% for the 30 mg group compared with 4% for the placebo group
CIRT	Phase III multicenter, double blind, randomized placebo-controlled	Patients older than 18 years of age who have a history of myocardial infarction or multivessel coronary disease and either DM2 or metabolic syndrome	4786	April 2, 2018	Methotrexate	Composite of nonfatal MI, nonfatal stroke, or CV death	NCT01594333	No difference in primary endpoint but higher incidence non-basal-cell skin cancer with methotrexate
COLCOT	Phase III multicenter, double blind, randomized placebo-controlled	Patients older than 18 years of age who have suffered an acute myocardial infarction within the last 30 days	4745	July 17, 2019	Colchicine 0.5mg/day vs. placebo	CV death, resuscitated Cardiac Arrest, acute MI, stroke, or urgent hospitalization for angina requiring coronary revascularization	NCT02551094	Colchicine reduced primary endpoint but increased pneumonia

LoDoCo 2	Phase III multicenter, double blind, randomized placebo-controlled	Patients with chronic coronary disease	5522	December 3, 2018	Colchicine 0.5mg/day vs. placebo	Composite of CV death, spontaneous (nonprocedural) MI, ischemic stroke, or ischemia-driven coronary revascularization	ACTRN12614000093684	Colchicine reduced primary endpoint but trend for increased incidence of non-CV death
CANTOS	Phase III multicenter, double blind, randomized placebo-controlled	History of myocardial infarction and had a blood level of high-sensitivity C-reactive protein of 2 mg or more per liter despite the use of aggressive secondary prevention strategies	10,061	March 2014	Canakinumab 50mg, 150mg, 300mg subcut q3monthly vs. placebo	First occurrence of nonfatal MI, any nonfatal stroke, or CV death	NCT01327846	Canakinumab 150mg subcut q3monthly reduced primary endpoint but increased incidence of fatal infection
CLEVER-ACS trial	Phase I-II randomized, paralleled placebo-controlled trial	Patients with STEMI	150	December 31, 2020	Everolimus (7.5mg for 3d followed by 5mg for 2d)	MI size	NCT01529554	No results posted
Ongoing Trials								
ALL-HEART study	Multi-center, controlled, prospective, randomized, open-label blinded	Patients aged 60 years and older with ischemic heart disease	5,215	Unknown	Allopurinol (up to 600mg PO daily)	Composite of nonfatal MI, nonfatal stroke, or CV death	ISRCTN32017426	N/A
COACS: Colchicine for Acute Coronary Syndromes	Phase III multicenter, double blind, randomized placebo-controlled	Patients with ACS (unstable angina or acute myocardial infarction)	500	Unknown	Colchicine 0.5mg/day vs. placebo	Overall mortality, new acute coronary syndrome, and ischemic stroke	NCT01960749	N/A

CLEAR SYNERGY	Phase III randomized placebo-controlled 4 study arms, 2 x 2 factorial design	Patients with MI/SYNERGY Stent Registry	7,000	March 30, 2025	Colchicine 1mg/day and/or spironolactone 25mg/day and/or placebo and/or synergy stent	Major adverse cardiac events for SYNERGY Stent (defined as the composite of death, recurrent target vessel MI, stroke or ischemia drive target vessel revascularizatio n)	NCT03048825	N/A
CONVINCE	Phase III Multicenter, open-label, placebo controlled	Patients older than 40 years of age who have suffered an ischemic stroke or transient ischemic attack not caused by cardiac embolism or other defined causes	2,623	October 2021	Colchicine 0.5mg/day vs. placebo	Nonfatal recurrent ischemic stroke and coronary events and vascular death	NCT02898610	N/A
LEADER-PAD	Randomized, double blind, multicenter pilot/vanguard trial	Patients older than 18 years of age who have a history of symptomatic lower extremity PAD with one or more high risk features	150	Septemb er 2023	Colchicine 0.5mg/day vs. placebo	Mean number of patients recruited per center per month	NCT04774159	N/A
CADENCE	Phase III multicenter, double blind, randomized placebo-controlled	Patients older than 18 years of age who have a history of (pre-)diabetes and have suffered a recent cardiovascular event	115	June 2024	Colchicine 0.6mg/day vs. placebo	Change in vascular inflammation measured by FDG-PET	NCT04181996	N/A

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Appendix D. Methodological Clarifications and Sensitivity Analyses Arising from Examiner Review

Confounding:

Several studies in this thesis are observational in nature and are therefore prone to risk of bias by confounding. In particular, Chapter 4 involves a cross-sectional study that evaluated and compared inflammatory markers (high-sensitivity CRP (hs-CRP) and interleukin-6 (IL-6) in patients with and without dysglycemia and a recent cardiovascular (CV) event as well as with patients with remote CV events. A major challenge of addressing this question with a cross-sectional design is the fact that levels of the inflammatory markers hs-CRP and IL-6 are time-varying and can also be confounded by other baseline variables. They vary not only with recency to a CV event, but they also vary with age and other factors. This is a valid concern with this study design. However, it should be emphasized that the goal of this project was purely descriptive and observational; it was not intended to draw causal influences. The results of this study were utilized to support a hypothesis to drive Chapter 5 (the CADENCE study).

However, we also agree with the premise that levels of inflammatory markers can be influenced by many factors including proximity to CV event, patient age, sex, and medication use. All of these factors may act as potential confounders

With the limited sample size, the Kruskall-Wallis test was used to determine if levels of inflammatory markers differed across the groups. Notably, this does not adjust for covariates and important potential confounders of the relationship between dysglycemia and circulating levels of inflammatory markers were not considered.

With a larger sample size there are several analytic strategies that could be employed to more formally address confounding while preserving interpretability.

Regression models are a common approach that include key covariates directly. With respect to the model structure with this approach:

Outcome:

- Outcome variable would be the hsCRP or IL-6 value, respectively (a continuous variable)
- Primary exposure: group (dysglycemia, no dysglycemia, remote event control group)
- Covariates: age, sex, body mass index, medications

Procedure:

- Utilize a generalized linear model
- Covariates selected a priori based on biological plausibility
- Check distributional assumptions underlying the model

Propensity score methods offer an alternative framework that separates confounder adjustment from outcome modeling. With respect to the model structure with this approach:

Propensity score with inverse probability weighting (IPW):

- A regression model would be fit to estimate the probability of belonging to the dysglycemia/recent event group based on relevant baseline demographic/clinical covariates (age, sex, medication use, and cardiovascular risk factors).

- Each participant would then be weighted by the inverse of their probability of being in their observed group. This creates a weighted pseudo-population in which measured covariates are balanced across groups.

Outcome

- The inflammatory biomarkers could then be compared between groups in the weighted sample using regression or rank-based methodology

Propensity score with overlap weighting (OW):

- This is a more recent and (oftentimes) more stable alternative to traditional IPW. With this methodology, participants are weighted in proportion to their probability of belonging in the opposite group. The benefit of this is that it emphasizes participants that have substantial overlap in their covariates and conversely downweights those with extreme propensity scores/mismatched covariates. In theory, this allows for improved covariate balance while reducing the influence of extreme weights.

Procedure:

- Similar to IPW, propensity scores would be generated
- Instead of applying inverse probability weights, overlap weights would be applied instead
- One would then assess covariate balance using standardized differences.

With the limited samples size, a regression model sensitivity analysis approach was considered to assess the robustness of the results in the analysis of the OCTAVE study. Specifically, separate regression

models for hsCRP and IL-6 were fit as outcomes. In each model, study group was entered as the primary exposure (defined as dysglycemia with recent cardiovascular event, no dysglycemia with recent event, and remote cardiovascular event). Given the limited sample size and the risk of model overfitting, only one covariate was considered at a time rather than simultaneously. Age, sex, and ACE inhibitor were selected for use a priori as clinically and biologically plausible confounders, reflecting demographic influences, known sex differences in inflammatory biomarkers, and medication effects with potential anti-inflammatory properties. Separate models were therefore constructed adjusting individually for age, sex, or ACE inhibitor use.

Across these sensitivity analyses, the association between study group and both hsCRP and interleukin 6 remained statistically significant, and dysglycemic status in the context of a recent cardiovascular event continued to be associated with higher inflammatory biomarker levels. These results are reported in Tables 1 and 2, respectively. The consistency of these findings across multiple adjusted models supports the robustness of the primary results and suggests that the observed associations were not driven solely by these individual sources of confounding.

Table 1: Linear regression model of association between dysglycemia and CRP in the OCTAVE study

Variable	**β±SE**	***P*-value**
Group	**-1.79 ±0.73**	**0.02**
Male sex	-1.02 ±1.23	0.41
Age	-0.006 ±0.06	0.91
ACEi use	-0.91 ±1.3	0.51

Table 2: Linear regression model of association between dysglycemia and IL-6 in the OCTAVE study

Variable	β±SE	*P*-value
Group	**-2.22 ±0.85**	**0.01**
Male sex	2.20 ±1.68	0.19
Age	0.10 ±0.08	0.21
ACEi use	0.27 ±1.7	0.88

These sensitivity analyses were intended to assess the stability of the findings rather than to provide definitive adjusted effect estimates. They reinforce the interpretation of this chapter as demonstrating a signal of heightened inflammatory burden in patients with dysglycemia following recent cardiovascular events.

With respect to the temporal variation of inflammatory markers following a CV event, we attempted to address this at the design stage through the inclusion of a comparator group with remote CV events. This allowed us to compare not only patients with dysglycemia to those without, but it also allowed us to compare patients with recent CV events to those with stable CV disease. We acknowledge that there are alternative methods for addressing temporal variations in biomarkers in the analysis phase. Notably, time varying covariates could also be used in the aforementioned regression models, however this would require a longitudinal study design, as repeated measures of the varying covariate would be required for the model. Thus we felt that for the objectives of our study, as well as the constraints that existed with respect to time and resources, that a cross-sectional design with inclusion of a "remote CV event" group would suffice.

Chapter 7 described a prospective cohort study that compared a cohort of patients with psoriasis and psoriatic arthritis who were started on biologic agents to a cohort of patients with psoriasis who were maintained on non-biologic disease modifying anti-rheumatic drugs (DMARDs) and a group of patients

with non-inflammatory arthritis. Given its observational nature, concerns regarding residual confounding may be an issue.

With the design of the study separating three separate cohorts with potentially quite different baseline inflammatory burden, concern regarding being subject to confounded by indication. Confounding by indication arises when the clinical indication for prescribing a therapy is associated with the outcome. In this case, the impetus for starting patients with psoriasis/psoriatic arthritis on a biologic therapy would inherently be assumed to be a worse disease state or more active inflammatory disease. Therefore, these patients would likely have a higher baseline level of inflammation than either of the other two groups included. We certainly took this into account in the analysis phase of our study. We utilized generalized linear mixed models which focused on the follow-up level of aortic inflammation (TBR_{max}), and importantly we included the baseline level of inflammation for each participant. This allowed us to account and control for baseline differences in inflammatory burden for each individual patient. This approach reduces the risk of confounding by indication, though residual confounding cannot completely be fully excluded. Thus, our results should be interpreted as adjusted associations and hypothesis-generating rather than causal estimates.

The issue of immortal time bias may be an issue with this study design. Immortal time bias occurs in an observational study when a period of follow-up during which the outcome cannot occur is incorrectly classified as exposure time. Most classically this arises when treatment begins after cohort entry (and individuals must remain event free in order to be defined as exposed). Classifying this pre-treatment period as “exposed time” can lead to spuriously protective treatment effects. The classic scenario in which this can arise is a survival analysis with delayed treatment initiation.

In the present study, immortal time bias was highly unlikely to meaningfully affect the results. Firstly, outcomes were based on changes in imaging biomarkers at fixed time points rather than time dependent clinical events. Secondly, all participants had identical follow-up periods, and there was no time between the baseline scan and treatment initiation for patienst in group 1 (i.e. no period of time that this group was

classified as “exposed” to biologic medications but were actually not). For these reasons, it was felt that immortal time bias was not relevant in this study.

Suitable surrogate marker for measuring cardiac vascular inflammation:

Blood biomarkers including hs-CRP, IL-6 and others hold promise to track inflammation[1] but use has been restricted by poor specificity and weak prognostic value. As such, we elected to use FDG-PET/CT, an inflammatory imaging modality, to more specifically measure inflammation within the atherosclerotic plaque.

FDG as a marker of plaque inflammation: While carotid 3D ultrasound, magnetic resonance imaging (MRI) and CT angiography (CTA) can each define plaque volume, vessel lumen and characterize different aspects of plaque composition and morphology,[2-8] none directly measure plaque inflammation.[9] Active inflammation within plaque is considered a major criterion to identify its vulnerability to rupture.[10] The key mediators of inflammation within plaque are macrophages. Many imaging probes have been validated by our group and others as markers of the inflammation cascade. FDG is the most widely validated and applied in this regard.[11-16] Several studies including our own support the validity of FDG uptake in plaque and its relationship to CD68 immunohistology, a marker of inflammatory cells.[11, 13-17] While the spatial resolution of FDG-PET imaging precludes assessment of the coronary vessels themselves, the ascending aorta (i.e. the arterial vessel most proximal to the heart) has become a well-established surrogate of cardiac inflammation.

Evaluation of Therapy: Joseph & Tawakol[12] noted: “since atherosclerosis is an active inflammatory process, measuring changes in arterial inflammation can provide insight into how therapeutic agents impact atherosclerosis development.” Noteworthy is that seven drug classes

have been evaluated with FDG PET/CT inflammation imaging and also have been assessed in clinical outcome trials.(**Table 2**)

The dose-dependent outcome benefit of statin therapy is well established.[18] Dose-dependent reduction in FDG uptake in the arterial vasculature, supporting the *local anti-inflammatory effect* on the plaque has also been observed. Tawakol studied 67 patients with atherosclerosis randomized to high- vs low-dose atorvastatin. The high-dose significantly reduced 12-week FDG uptake compared to a low-dose. The authors note that the study supports the ***use of FDG PET as a tool for treatment assessment.[19]***

Mizoguchi et al. studied patients with diabetes or pre-diabetes randomized to 4 months of pioglitazone versus glimepiride. Pioglitazone significantly reduced arterial inflammation measured using FDG uptake versus glimepiride.[20] As with the statin studies above, the plaque inflammation findings parallel the clinical trials which demonstrate significant CV outcome benefits.[21]

In contrast, several studies with different agents did not yield any demonstrated reduction in arterial inflammation (using FDG PET/CT). These include a CETP inhibitor, dalcetrapib; Lp-PLA2 inhibitors (rilapladib, darapladib) and inhibitors of MAP Kinase.[22-24] These drugs did <u>not</u> yield improvement in inflammation measured using FDG.[7] Consistently, large clinical trials of the same drugs do <u>not</u> show CV outcome benefits.[25-28]

Evaluation of pharmacological impact on *arterial inflammation* measured using FDG PET/CT (but not other approaches) has uniquely demonstrated concordance with clinical CV outcome trials. ***Thus FDG PET/CT inflammation imaging is an attractive pragmatic means to define the mechanistic targets of a drug's effect in small cohorts over short periods of time. It may also***

become beneficial to define at-risk patients and optimize patient selection for therapies. ***FDG PET/CT will be applied in CADENCE to evaluate the mechanism of colchicine's effect in high-risk patients.***

Prediction of Adverse Events: Among inflammation imaging probes, only FDG uptake on PET/CT has been shown to predict progression of atherosclerosis[2, 12, 17] as well as CV events independent of risk factors.[29-31] In 513 patients, Figueroa et al. showed that increased aorta FDG uptake is a strong independent predictor of CV events (HR:4.71; 95%CI:1.98-11.2; p<0.001).[30] In a prospective study of 60 patients with recent stroke or TIA, Marnane et al. showed that carotid FDG uptake was a strong predictor of recurrent stroke within 90 days which occurred in 22% of patients.[31] ***These data support the predictive value of FDG PET/CT- to be used in high-risk patients in the current proposal.*** **Thus, findings from our and others' data validate FDG uptake as an accurate and reproducible non-invasive surrogate marker of inflammatory cell burden within human atherosclerotic plaque that indicates higher levels of inflammatory activation and CV risk (Table 3). [11, 32-36]** Evaluation of pharmacological impact on *arterial inflammation* measured using FDG PET/CT (but not other approaches) has uniquely demonstrated concordance with clinical CV outcome trials.

TABLE 3	**Clinical studies supporting the use of ^{18}FDG PET imaging of the aorta as a surrogate of inflammatory activity in coronaries**		
Element evaluated	**Study**	**Population**	**Finding**
Accuracy	Reeps et al.[32]	25 patients with undergoing elective abdominal aneurysm repair	^{18}FDG uptake strongly correlated with macrophage infiltration in aortic wall biopsies (r = 0.81, p <0.01)

A) ^{18}FDG uptake in the ascending aorta does correlate with vascular inflammation in the context of atherosclerotic disease	Rudd et al.[33]	33 patients with vascular disease or multiple risk factors	^{18}FDG uptake in the ascending aorta strongly correlated with serum levels of matrix metalloproteinase-3 (r = 0.49, p= 0.02)
B) ^{18}FDG uptake in the ascending aorta correlates with inflammatory activity in the coronaries and risk of AMI	Rogers et al.[34]	25 patients with coronary heart disease (10 with acute coronary syndrome [ACS], 15 with stable angina)	^{18}FDG uptake was higher in the ACS group versus the stable angina group both in the ascending aorta (TBR 3.30 [IQR: 2.69 to 4.12] vs. 2.43 [IQR: 2.00 to 2.86], p = 0.02) and the left main coronary artery (2.48 [IQR: 2.30 to 2.93] vs. 2.00 [IQR: 1.71 to 2.44], p = 0.03). 18FDG uptake was also greater for culprit lesions associated with ACS than for lesions stented for stable coronary syndromes (2.61 vs. 1.74, p = 0.02)
Reproducibility	Rudd et al.[36]	11 subjects with established vascular disease or elevated Framingham risk scores	Minimal variability in the quantification of TBR of the ascending aorta over 2-week period (mean TBR at weeks one and two, 1.39 [SD ±0.18] and 1.38 [SD ±0.17], respectively; difference 0.01 (SD ±0.07). Intraclass correlation coefficients (ICCs) for intraobserver, interobserver, and interscan variability were 0.98 (CI: 0.94–1.00), 0.92 (CI: 0.70–0.98), and 0.92 (CI 0.75–0.98), respectively (values ≥0.8 imply excellent reproducibility).
Safety	Silberstein et al.[35]	33,925 radiopharmaceutical doses of positron emitting tracers (including 18FDG) and nonradioactive drugs used in PET studies	No adverse reactions reported

In addition, a recent pilot study demonstrated that high-dose omega 3 fatty acids reduce arterial inflammation on FDG-PET in patients with elevated Lp(a), showing suitability to assess treatment response for this specific class of therapy.[37]

Thus FDG PET/CT inflammation imaging is an attractive pragmatic means to define the mechanistic targets of a drug's effect in small cohorts over short periods of time. It may also become beneficial to define at-risk patients and optimize patient selection for therapies. FDG PET/CT will be applied in CAPTAIN to evaluate the mechanism of IPE's effect in high-risk patients.

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Appendix E. Published Manucripts

OVERVIEW

This appendix includes the PDFs of manuscripts published as part of this thesis. The citation for each PDF, as well as the permission obtained, are included below:

CHAPTER 3

Section 3.1

Boczar KE, Shin S, Bezzina KA, Geejo A, Pearson AL, Shahab S, Fehlmann CA, Visintini S, Beanlands R, Wells GA. Examining anti-inflammatory therapies in the prevention of cardiovascular events: protocol for a systematic review and network meta-analysis of randomised controlled trials. BMJ Open. 2022 Jun 27;12(6):e062702. doi: 10.1136/bmjopen-2022-062702. PMID: 35760536; PMCID: PMC9237867.

CHAPTER 4

Section 4.1

Boczar KE, Liu P, Chong AY, So D, Dowlatshahi D, Rayner K, Beanlands R. Observational Cross-Sectional Study of Inflammatory Markers After Transient Ischemic Attacks, Acute

Coronary Syndromes, and Vascular Stroke Events. CJC Open. 2020 Dec 31;3(5):675-679. doi: 10.1016/j.cjco.2020.12.020. PMID: 34027372; PMCID: PMC8134934.

CHAPTER 5

Section 5.1

Boczar KE, Shin S, deKemp RA, Dowlatshahi D, Tavoosi A, Wiefels C, Liu P, Lochnan H, MacPherson PA, Chong AY, Torres C, Leung E, Tawakol A, Ahmadi A, Garrard L, Lefebvre C, Kelly C, MacPhee P, Tilokee E, Raggi P, Wells GA, Beanlands R. The Canadian Study of Arterial Inflammation in Patients with Diabetes and Recent Vascular Events, Evaluation of Colchicine Effectiveness (CADENCE): protocol for a randomised, double-blind, placebo-controlled trial. BMJ Open. 2023 Nov 10;13(11):e074463. doi: 10.1136/bmjopen-2023-074463. PMID: 37949621; PMCID: PMC10649523.

CHAPTER 6

Section 6.1

Boczar KE, Beanlands R, Wells G, Coyle D. Cost-Effectiveness of Colchicine for Recurrent Cardiovascular Events. CJC Open. 2023 Feb 24;5(5):348-356. doi: 10.1016/j.cjco.2023.02.005. PMID: 37377518; PMCID: PMC10290950.

CHAPTER 7

Section 7.1

Boczar KE, Beanlands RS, Glassman SJ, Wang J, Zeng W, deKemp RA, Ward NC, Fehlmann CA, Wells GA, Karsh J, Dwivedi G. Anti-inflammatory effect of biologic therapy in patients with psoriatic disease: A prospective cohort FDG PET study. J Nucl Cardiol. 2023 Aug;30(4):1642-1652. doi: 10.1007/s12350-023-03204-8. Epub 2023 Feb 8. PMID: 36754934; PMCID: PMC10372102.Supporting Material

CHAPTER 8

Section 8.1

Boczar KE, Faller E, Zeng W, Wang J, Small GR, Corrales-Medina VF, deKemp RA, Ward NC, Beanlands RSB, MacPherson P, Dwivedi G. Anti-inflammatory effect of rosuvastatin in patients with HIV infection: An FDG-PET pilot study. J Nucl Cardiol. 2022 Dec;29(6):3057-3068. doi: 10.1007/s12350-021-02830-4. Epub 2021 Nov 24. PMID: 34820771.

BMJ Open

Examining anti-inflammatory therapies in the prevention of cardiovascular events: protocol for a systematic review and network meta-analysis of randomised controlled trials

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ABSTRACT

Introduction Inflammation is emerging as an important risk factor for atherosclerotic cardiovascular disease and has been a recent target for many novel therapeutic agents. However, comparative evidence regarding efficacy of these anti-inflammatory treatment options is currently lacking.

Methods and analysis This systematic review will include randomised controlled trials evaluating the effect of anti-inflammatory agents on cardiovascular outcomes in patients with known cardiovascular disease. Studies will be retrieved from Medline, Embase, the Cochrane Central Register of Controlled Trials, as well as clinical trial registry websites, Europe PMC and conference abstract handsearching. No publication date or language restrictions will be imposed. Eligible interventions must have some component of anti-inflammatory agent. These include (but are not limited to): non-steroidal anti-inflammatory drugs (NSAIDs), colchicine, prednisone, methotrexate, canakinumab, pexelizumab, anakinra, succinobucol, losmapimod, inclacumab, atreleuton, LP-PLA$_2$ (darapladib) and sPLA$_2$ (varespladib). The primary outcomes will include major adverse cardiac events (MACE), and each individual component of MACE (myocardial infarction, stroke and cardiovascular death). Key secondary outcomes will include unstable angina, heart failure, all-cause mortality, cardiac arrest and revascularisation. Screening, inclusion, data extraction and quality assessment will be performed independently by two reviewers. Network meta-analysis based on the random effects model will be conducted to compare treatment effects both directly and indirectly. The quality of the evidence will be assessed with appropriate tools including the Grading of Recommendations, Assessment, Development and Evaluation profiler or Confidence in Network Meta-Analysis tool.

Ethics and dissemination Ethics approval is not required for this systematic review. The findings will be disseminated through a peer-reviewed journal.

PROSPERO registration number CRD42022303289.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Analysis of data within the structure of a network meta-analysis allows for the direct and indirect comparison of anti-inflammatory medications for atherosclerotic cardiovascular disease.
⇒ A rigorous search of published and unpublished data will be conducted.
⇒ Article selection process, data extraction and risk of bias will all be performed by two reviewers in parallel.
⇒ Quality of evidence across the included studies will be assessed and summarised.
⇒ Potential limitations include residual confounding factors biasing results.

INTRODUCTION

Atherosclerotic cardiovascular disease (ASCVD) is a leading cause of mortality and morbidity around the world.[1 2] The incidence of myocardial infarctions (MI) has been dramatically lowered in populations that have pursued a strategy of aggressive detection and control of traditional cardiovascular risk factors for coronary artery disease (CAD), like hypertension, diabetes, cigarette smoking and elevated low-density lipoprotein cholesterol (LDL-C).[2]

Despite adopting a strategy of aggressively controlling traditional risk factors for CVD, major adverse cardiovascular events (MACE) unfortunately continue to occur at high rates.[3] Thus, much attention is now focused on other potentially modifiable risk factors that can be targeted to further reduce the burden of ASCVD.

The pathogenic basis of atherosclerosis is a complex process; we now know that its biological basis is more intricate than simply attributing it to intimal infiltration of LDL-C. Thus, we are beginning to acknowledge that our therapies will need to extend beyond treatment for the traditional risk factors. In

particular, recent clinical and experimental evidence has supported inflammation as playing a key role in the initiation, progression and eventual overt clinical manifestations of ASCVD.[4]

Contribution of inflammation in the pathophysiology of atherosclerosis

ASCVD is now thought of as a chronic inflammatory disease of the coronary vasculature which is initially triggered by intimal LDL-C infiltration.[2] An early mechanism in the development of clinically manifest CAD is the exposure of the intimal endothelium to harmful stimuli, like hypercholesterolaemia, elevated blood pressure and importantly, inflammation.[2] This impairs its ability to act as a functional barrier and leads to its 'activation'. After their activation, vessel endothelial cells increase their expression of leucocyte adhesion molecules.[2 5 6] This increased expression allows the migration of neutrophils and monocytes into the subendothelial space from the circulating blood.[2] Once inside the vessel wall, these monocytes then differentiate into macrophages and begin to ingest modified LDL-C particles, eventually becoming lipid-laden foam cells.[2] The aggregation of foam cells results in a yellow coloured 'fatty streak' within the arterial wall and thereby the first overt sign of ASCVD.[2]

From a clinical perspective, inflammatory mediators have shown to play a crucial role in mediating thrombotic complications of atherosclerosis, namely MI and ischaemic stroke.[7 8] This fact has spurred the clinical evaluation of inflammation as a therapeutic target in an attempt to further reduce the burden of CVD.[9–12]

Interventions

The encouraging results obtained from basic CVD research endorsed the early translation of anti-inflammatory agents into the clinical setting, which unfortunately failed on several early investigations.[13 14] However, since these early clinical trials, there have also been a multitude of successes, and there is a plethora of new research looking at various anti-inflammatory therapies for the mitigation of cardiovascular events.

While these randomised controlled trials (RCTs) have primarily compared these novel anti-inflammatory agents to placebo (and background of statin therapy), few, if any, have been compared with other anti-inflammatory therapies. Thus, there is currently a paucity of literature regarding the relative effectiveness of these therapies.

This review will provide a contemporary investigation of the relative efficacy of various anti-inflammatory medications for the prevention of MACE. The study is unique in that it will compare a comprehensive list of anti-inflammatory therapies not just with placebo, but with other anti-inflammatory interventions using network meta-analysis (NMA). Additionally, our review will be conducted in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) recommendations.[15]

Objectives

The primary objective of our systematic review and NMA is to assess the relative effectiveness of anti-inflammatory therapies in cardiac disease, examined in RCTs. Our results will strengthen the understanding of the benefit of each individual anti-inflammatory therapy, and will also allow the comparison of the relative effects of each intervention.

METHODS AND ANALYSIS

This protocol was developed according to the PRISMA-P 2015 checklist[15] (see online supplemental file 1). Important amendments made to the protocol will be documented and published alongside the results of the systematic review.

Types of studies

RCTs will be included in this study.

Population

Our systematic review will include all patients with known CAD, regardless of age or sex. Additionally, participants with both acute coronary syndromes (ACS) as well as stable CAD will be included. However, if significant subgroup differences are discovered between interventions used to treat those with ACS versus those with stable CAD, we will conduct a subgroup analysis to further explore this heterogeneity.

Intervention

Eligible interventions must have some component of anti-inflammatory agent. We will include any anti-inflammatory medication, including (but not limited to): non-steroidal anti-inflammatory drugs (NSAIDs), colchicine, prednisone, methotrexate, canakinumab, pexelizumab, anakinra, succinobucol, losmapimod, inclacumab, atreleuton, LP-PLA_2 (darapladib) and $sPLA_2$ (Varespladib). We will not consider any medication which does not have a primary mechanism of action via the inhibition of inflammation (eg, statins or allopurinol).

Comparisons

All medications with a primary mechanism of action that targets the inflammatory pathway will be included in this systematic review. Treatment arms will be considered regardless of whether they received any other type of control or experimental intervention.

Primary outcome

The following primary outcome will be extracted:

- MACE and each individual component of MACE:
  - MI.
  - Stroke.
  - Cardiovascular death.

We will extract secondary outcomes and adverse outcomes from the studies that meet the inclusion criteria.

Key secondary outcomes

- Unstable angina.
- Heart failure.
- All-cause mortality.
- Cardiac arrest.
- Revascularisation.

Key adverse outcomes

These relate to adverse events suggested in previous trials and include, but are not limited to:

- Infection/pneumonia.
- Diarrhoea/GI upset.
- Malignancy.

Years of publication considered

There will be no limitations on the year of publication of studies.

Language

There will be no restriction based on language of the publication. If a potential study is identified that is not written in English, we will use translational services if possible.

Study publication status

We will include both published and unpublished studies in our systematic review. We will search for ongoing studies in the Clinicaltrials.gov and WHO's International Clinical Trials Registry Platform (ICTRP) and will consider these for inclusion when relevant.

Search strategy

The search strategy will be developed by a medical librarian (SV) in collaboration with team members using a combination of subject headings and keywords in Medline; it will then be peer-reviewed by a second librarian as per PRESS guidelines.[16] It will then be run in the various databases listed below from inception. Search results will be exported to Covidence and duplicates will be eliminated using the platform's duplication identification feature. We will rerun our search prior to the final analysis. A draft of the Medline search strategy is included in online supplemental file 2.

Filters

Cochrane RCT search filters will be employed for both Medline and Embase.[17]

Information sources

We will conduct searches of MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials (CENTRAL).

If there is missing information that is not reported, we will contact study authors by email to obtain more information. If no replies from authors are received, we will send two subsequent emails at 2 and 4 weeks.

We will search reference lists of identified studies by hand to identify additional possible relevant literature.

Grey literature will be searched to identify potential relevant research that has not been published. These sources include:

- Clinical trial registries:
  - ClinicalTrials.gov
  - WHO's ICTRP.
- Preprints from Europe PubMed Central (PMC).
- Conference abstracts will be included as part of the Embase database search. Gaps in Embase indexation will be addressed with hand searching of select relevant conferences:
  - American Cardiology Conference.
  - American Heart Association Conference.
  - European Society of Cardiology Conference.
  - Canadian Cardiovascular Congress.

If RCTs are registered but have not been published at the time of our search, they will be screened and will be included in the analysis if they are eligible and sufficient information is available.

Selection process

COVIDENCE software will be used for study screening. Following duplication removal, study screening and selection will be conducted by two independent reviewers in parallel (KEB, KAB, ShS, ALP, AG and SaS), based on the prespecified inclusion and exclusion criteria.

The first stage of identifying potentially eligible studies will be conducted by screening titles and abstracts in COVIDENCE. When disagreements between two reviewers occur, discussion and consensus will be used to resolve the conflict. When agreement cannot be reached, a third reviewer (KAB) will ultimately resolve the disagreement. Once the first round of screening by titles and abstracts is completed, eligible studies will undergo a full text review by the two reviewers independently (KEB, KAB, ShS, ALP, AG and SaS) according to the process outlined above.

We will track and report reasons for exclusion in a PRISMA flow diagram. If there are multiple reports of the same study that are identified, we will be consider them together.

Data extraction and management

For data collection, a predesigned, standardised data extraction sheet will be used. The reviewers will first test the extraction sheet on five studies. We will then discuss and make amendments to the extraction sheet as necessary. Finally, the data extraction process as well as risk of bias assessment of all included studies will be performed in parallel by two independent reviewers (KEB, KAB, ShS, ALP, AG and SaS). Information pertaining to anti-inflammatory medication characteristics (type of anti-inflammatory, length of therapy, dose), participant characteristics, comparators, setting, lost to follow-up and clinical outcomes will be included in the extraction process.

We will preferentially extract unadjusted results over adjusted results, if available, to improve consistency.

Data items

Participants

Participant characteristics that are deemed to potentially modify treatment effects from the anti-inflammatory agents will be recorded. Patient characteristics of interest include age, sex, comorbidities (including presence of other inflammatory conditions) and concomitant alternative anti-inflammatory medication use. We will also record the number of participants that were included at baseline in each study, and the number of participants lost to follow-up.

Intervention

We will extract information such as treatment length, length of follow-up, and length of time from acute coronary syndrome (ACS) (if relevant), which could potentially modify the anti-inflammatory treatment effect.

Comparator

We will extract data on the type of comparator used, including dose and duration, as well as baseline demographic data regarding the comparator group participants.

Risk of bias in individual studies

The risk of bias of each included study will be assessed independently by two reviewers (KEB, ShS, ALP, AG and SaS) using the updated Cochrane Collaboration's Risk of Bias (RoB 2) Assessment Tool.[18] Disagreements will be resolved through discussion, and if needed a third reviewer (KAB) will settle any disputes.

The RoB 2 Assessment Tool assesses potential sources of bias in five domains including the randomisation process, deviations from the intended interventions, missing outcome data, measurement of the outcome and selective reporting. We will evaluate each category as being at 'low risk' of bias, 'high risk' of bias or having 'some concerns' for bias. Finally, we will then give an overall judgement of the trial regarding the risk of bias, with studies again being scored as being at 'low risk' of bias, 'high risk' of bias or having 'some concerns' regarding risk of bias.

Randomisation process

We will assess the randomisation and allocation methods to determine the potential for bias to be introduced due to the creation of groups with important underlying baseline differences.

Deviations from the intended interventions

We will assess whether the effect of assignment to intervention and the effect of adherence to intervention could act as potential sources of bias. This includes assessing whether the participant allocation process was concealed, whether both participants and personnel were blinded when participants were allocated to treatment groups, and whether outcome assessors were also blinded to participant assignment. We will also assess whether deviations from the intended intervention were balanced between groups, and whether failure to adhere to the intended intervention could influence the outcomes.

Missing outcome data

We will assess whether outcome data was available for all randomised participants, and if not, whether the missingness could influence the results.

Measurement of the outcome

We will assess whether the method of measuring the outcome was appropriate, and whether the ascertainment of the outcome could have differed between intervention groups.

Selective outcome reporting

We will look for evidence that the authors omitted reporting relevant outcomes, or that data were not evaluated in accordance with a prespecified analysis plan.

Summary measures of treatment effect

Dichotomous outcomes will be presented as either ORs or risk ratios and reported with 95% CIs.

Data synthesis

Clinical heterogeneity will be explored by examining the variation in several patient and study characteristics including population baseline participant demographic variables, the use and composition of 'optimal medical therapy', study outcome definitions and other relevant study characteristics.

Network meta-analysis

If we identify that at least two studies are clinically homogeneous, then we will perform a meta-analysis. Our results will be analysed using an NMA.[19] An NMA uses an interconnected network of treatments, which thereby allows for the assessment of the relative efficacy of these treatments for a particular medical indication.[20] Both direct and indirect comparisons can be made within the network, so long as all trials included in the analysis are contained within the network.[21–23] For our NMA, we will create a model which compares anti-inflammatory interventions for ASCVD.

Data analysis

We will perform our statistical analyses in a Bayesian framework using the OpenBUGS software.[24] To address statistical heterogeneity, we will use random effects models. We will assess the fit of each model to the data by using the posterior mean residual deviance. We will then compare the models by using the Deviance information criterion.[25] Satisfying the consistency assumption is critical in the validation of an NMA, in part to ensure that included studies are comparable within the network. We will assess the validity of this assumption by reviewing the patient inclusion and exclusion criteria for each study included in the summary analysis, to ensure that the patient and study characteristics are sufficiently similar.

If quantitative data analysis is not appropriate, a qualitative description and table of the included studies and data will be performed and displayed.

Subgroup analysis

Several prespecified subgroup analyses will be conducted if data permits. These include:

- Sex.
- Setting of ASCVD (ACS vs non-ACS setting).
- Time after index event for initiation of anti-inflammatory agent.
- Published vs unpublished literature.

Assessment of reporting biases

To assess for small-study effects we will include the total number of patients in the study as a covariate in our meta-regression analysis. We will also create funnel plots[26] to evaluate for potential reporting bias.

Sensitivity analyses

We will perform an additional analysis whereby we exclude studies which are deemed to be at either 'high risk' or to have 'some concerns' of bias on the Cochrane RoB 2 Assessment Tool.[18]

We will also conduct additional analyses with fixed-effects models for the pairwise and NMA.

Confidence in cumulative evidence

The quality of the evidence across included studies will be summarised with an appropriate tool,[27]with possible tools including the Grading of Recommendations, Assessment, Development,and Evaluation profiler[28] or Confidence in Network Meta-Analysis tool.[29]

Patient and public involvement

There is no patient or public involvement in this study.

ETHICS AND DISSEMINATION

We did not require ethics approval for this systematic review and NMA. The findings will be disseminated through a peer-reviewed journal.

DISCUSSION

This systematic review and NMA will address questions regarding the comparative effectiveness of anti-inflammatory therapies for the treatment of ASCVD. This topic is important for several reasons. As mentioned, ASCVD is an extremely common problem, and is a leading cause of morbidity and mortality.[1 2] With a growing number of therapeutic agents being developed to target the inflammation pathway, it is extremely useful for practitioners to have knowledge regarding the relative comparison of these novel drugs. Thus, this study could be important for clinical practice, providing information regarding relative benefits and harms from these anti-inflammatory agents. Furthermore, with the burden of the potential financial cost of these drugs for healthcare payers, this review will be potentially useful regarding funding decisions.

The major strength of this systematic review and NMA is that it will comprehensively summarise the evidence regarding anti-inflammatory benefit and harm for the treatment of ASCVD. Moreover, this NMA will allow both direct and indirect comparison between these novel anti-inflammatory medications. Additionally, our comprehensive search strategy will attempt to discover both published and unpublished (grey) literature in the field. Potential limitations include the possibility of residual confounding influencing our results. The strength of our review will be dependent on the existing evidence base for this topic area. If only one or two RCTs exist for a given comparison in the network, then the strength of the analysis will reflect that. The sample size and the number of included studies may be small due to the novelty and resource-intensive nature of conducting an RCT of this nature on this topic.

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Contributors Conceptualisation: KEB and GAW; Methodology: KEB, SV, RB and GAW; Project administration: KEB; supervision: GAW and RB; writing—original draft: KEB; writing—review and editing: KEB, ShS, ALP, AG, SaS, KAB, CAF, SV, RB and GAW. KEB is the guarantor of the review.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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Protocol for a systematic review and network meta-analysis of randomised controlled trials examining anti-inflammatory therapies in the prevention of cardiovascular events

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Online Supplemental: PRISMA-P 2015 Checklist

This checklist has been adapted from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

Section/topic	#	Checklist item	Information reported		Section
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	X		Title
Update	1b	If the protocol is for an update of a previous systematic review, identify as such		X	
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	X		Abstract
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	X		Title
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	X		Contributors
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments		X	
Support					
Sources	5a	Indicate sources of financial or other support for the review	X		Funding
Sponsor	5b	Provide name for the review funder and/or sponsor	X		Funding
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	X		Contributors, Funding
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	X		Introduction

Section/topic	#	Checklist item	Information reported		Section
			Yes	No	
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	X		Methods
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	X		Methods
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	X		Methods
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	X		Appendix
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	X		Methods
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	X		Methods
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	X		Methods
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	X		Methods
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	X		Methods
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	X		Methods
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	X		Methods
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	X		Methods
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	X		Methods
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	X		Methods

Section/topic	#	Checklist item	Information reported		Section
			Yes	No	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	X		Methods
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	X		Methods

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Online Supplemental: Search Strategies

Table 1. Medline Search

#	Searches
1	exp Arteriosclerosis/ or Plaque, Atherosclerotic/
2	(atherosclero* or athero-sclero* or arteriosclero* or arterial-sclero* or arteriolosclero* or arteriolo-sclero* or vascular sclero* or ASCVD or ASCAD or intima plaque).ti,ab,kf,kw.
3	Acute Coronary Syndrome/ or exp Coronary Disease/
4	((coronary adj3 (arter* or stenos* or disease? or disorder? or syndrom?)) or CAD or SCAD).ti,ab,kf,kw.
5	exp Percutaneous Coronary Intervention/ or exp Myocardial Revascularization/
6	(((aortocoronary or aorto-coronary or coronary) adj3 bypass*) or CABG).ti,ab,kf,kw.
7	exp endarterectomy/ or exp thrombectomy/ or exp Atherectomy/ or exp Embolectomy/
8	(angioplast* or atherectom* or endarterectom* or thrombectom* or thromboendarterectom* or thrombo-endarterectom* or PCI or PTCA or (Percutaneous adj3 (intervent* or revascular*))).ti,ab,kf,kw.
9	or/1-8
10	exp Anti-Inflammatory Agents, Non-Steroidal/
11	(((non-steroidal or nonsteroidal) adj2 (anti-inflammatory or antiinflammatory or analg?esic*)) or NSAID*).ti,ab,kf,kw.
12	(acalabrutinib* or aceclofenac* or acemetacin* or acetaminosalol* or acetylsalicylate* or acetylsalicylic* or aclantate* or actarit* or adalimumab* or afasevikumab* or afimetoran* or alclofenac* or aldafermin* or alminoprofen* or aloxiprin* or amfenac* or aminophenazone* or aminosalicylic* or amlitelimab* or amlodipine* or ampiroxicam* or amtolmetin guacil* or anirolac* or antiflammin* or apadenoson* or apremilast* or araprofen* or ascription* or asivatrep* or astegolimab* or atibuclimab* or atliprofen* or aviptadil* or azathioprine* or azelaic acid* or bakeprofen* or balsalazide* or bardoxolone* or bardoxolone methyl* or belumosudil* or bendazac* or benorilate* or benoxaprofen* or bermoprofen* or bimosiamose* or brazikumab* or brensocatib* or brimonidine* or bromfenac* or broperamole* or bucloxic acid* or bucolome* or bufexamac* or butibufen* or camobucol* or carbasalate* or carotegrast* or carprofen* or cedirogant* or celecoxib* or cibinetide* or cicloprofen* or cimicoxib* or cinmetacin* or cinnoxicam* or clidanac* or clofezone* or clonixin* or clonixin lysine* or cloximate* or crisaborole*).ti,ab,kf,kw.

13	(dagrocorat* or danicopan* or dapansutrile* or dapatifagene navolactibac* or darbufelone* or daxdilimab* or dazodalibep* or dehydrozingerone* or demethoxycurcumin* or deracoxib* or deucravacitinib* or dexibuprofen* or dexketoprofen* or dexpemedolac* or diclofenac* or didemethoxycurcumin* or diflunisal* or diftalone* or dimethyl fumarate* or dimethyl sulfoxide* or diphenpyramide* or ditazole* or droxicam* or duometacin* or ebdarokimab* or ebselen* or edasalonexent* or efruxifermin* or elsibucol* or emavusertib* or emorfazone* or emvododstat* or endolac* or enfenamic acid* or enflicoxib* or enpatoran* or epirizole* or etodolac* or etofenamate* or etoricoxib* or evobrutinib* or felbinac* or fenamic acid* or fenbufen* or fenclofenac* or fenclozic acid* or fendosal* or fenflumizole* or fenoprofen* or fentiazac* or fepradinol* or feprazone* or firategrast* or firocoxib* or flobufen* or flosulide* or flufenamate aluminum* or flufenamic acid* or flunixin* or flunoxaprofen* or fluproquazone* or flurbiprofen* or flutiazin* or fosdagrocorat* or fosfosal* or furaprofen* or furcloprofen* or furobufen* or furofenac* or fuzapladib*).ti,ab,kf,kw.
14	(glucametacin* or gluconate zinc* or guacetisal* or guaimesal* or gusacitinib* or hydroxychloroquine* or ibrigampar* or ibufenac* or ibuprofen* or ibuproxam* or icoduline* or icosapentaenoic acid* or iguratimod* or ilonidap* or imidazole salicylate* or imisopasem manganese* or imsidolimab* or incyclinide* or indameth* or indometacin* or indoprofen* or ipsalazide* or iptacopan* or isecarosmab* or isofezolac* or isonixin* or isoxepac* or isoxicam* or itepekimab* or kebuzone* or ketoprofen* or ketoprofen lysine* or ketorolac* or lazertinib* or lazucirnon* or leflunomide* or lenabasum* or licofelone* or lifitegrast* or lirentelimab* or lobuprofen* or lonazolac* or lorecivivint* or lornoxicam* or losmiprofen* or loxoprofen* or lumiracoxib* or lusvertikimab* or lyprinol* or lysine acetylsalicylate*).ti,ab,kf,kw.
15	(mabuprofen* or magnesium salicylate* or manoalide* or mapracorat* or mavacoxib* or meclofenam* or mefenamic acid* or meloxicam* or melrilimab* or mesalazine* or methotrexate* or metiazinic acid* or metoxibutropate* or milategrast* or mipragoside* or mirococept* or miroprofen* or mivavotinib* or mofebutazone* or mofezolac* or mongersen* or morazone* or morniflumate* or mosedipimod* or nabumetone* or nangibotide* or naproxcinod* or naproxen* or navamepent* or nepafenac* or neurofenac* or neurotropin* or nictindole* or niflumic acid* or nimesulide* or ocarocoxib* or odalprofen* or olsalazine* or ordesekimab* or ormeloxifene* or orpanoxin* or otenaproxesul* or oxaceprol* or oxametacin* or oxaprazine* or oxaprozin* or oxicam derivative* or oxindanac* or oxyphenbutazone*).ti,ab,kf,kw.
16	(palifermin* or parcetasal* or parecoxib* or pelubiprofen* or pemedolac* or perisoxal* or phenylbutazone* or phenylbutazone megallate* or picolamine salicylate* or piketoprofen* or pimeprofen* or pipebuzone* or piproxen* or pirazolac* or pirfenidone* or piroxicam* or piroxicam beta cyclodextrin* or pirprofen* or plonmarlimab* or plozalizumab* or polmacoxib* or pralnacasan* or pranoprofen* or prinomide* or prinomide triethanolamine* or proglumetacin* or proquazone* or pyrazinobutazone* or quellor* or rapamycin* or rasagiline* or ravagalimab* or relfovetmab* or reltecimod* or remestemcel L* or resatorvid* or rimacalib* or rimazolium* or risankizumab* or robenacoxib* or rofecoxib* or romazarit* or rosiptor* or rosmarinic acid* or rovazolac* or rozibafusp alfa* or ruxolitinib* or salazosulfapyridine* or salicylic acid* or salnacedin* or salsalate* or satralizumab* or scalaradial* or semapimod* or semorinemab* or sibofimloc* or simufilam* or sudoxicam* or sulfosalicylate samarium* or sulindac* or suprofen* or suxibuzone*).ti,ab,kf,kw.
17	(talniflumate* or tapinarof* or tazofelone* or telazorlimab* or tenidap* or tenosal* or tenosiprol* or tenoxicam* or tepoxalin* or teriflunomide* or tesnatilimab* or tiaprofenic acid* or tiaramide* or tilmacoxib* or tilnoprofen arbamel* or tilomisole* or timegadine* or tioxamast* or

	tioxaprofen* or tirnovetmab* or tolebrutinib* or tolfenamic acid* or tolmetin* or tomaralimab* or tomicorat* or torudokimab* or tozorakimab* or tralokinumab* or tribuzone* or triethanolamine salicylate* or tropesin* or tryptamide* or ufenamate* or valategrast* or valdecoxib* or valerylsalicylic acid* or vasoactive intestinal polypeptide* or vedaprofen* or velsecorat* or vemircopan* or verramed* or vilobelimab* or vixarelimab* or ximoprofen* or zabedosertib* or zaloglanstat* or zaltoprofen* or zaurategrast* or zidometacin* or zinc salicylate* or zoliprofen* or zomepirac*).ti,ab,kf,kw.
18	exp Colchicine/
19	(Colbenemid* or Colchichin* or colchicum* or colchily* or colchineos* or colchimedio* or colchiquim* or colchisol* or colchysat* or colcin* or colcrys* or colctab* or colgout* or colrefuz* or colsaloid* or Condylon* or gloperba* or goutichine* or goutnil* or kolkicin* or kolkisin* or mitigare* or tolchicine*).ti,ab,kf,kw.
20	(64-86-8* or SML2Y3J35T*).rn.
21	Prednisone/
22	(adasone* or acsis* or ancortone* apo-prednisone* or bicortone* or biocortone* or cartancyl* or colisone* or Cortan* or cortancyl* or cortidelt* or cortiprex* or cotone* or cutason* or dacorten* or dacortin* or decortancyl* or decortin* or de-cortisyl* or decortisyl* or dihydrocortisone* or dihydrocortisone* or dekortin or dellacort* or deltacorten* or delta-cortelan* or delta-cortisone* or deltacortisone* or deltacortone* or delta-dome* or delta-prenovis* or deltasone* or delitisone* or deltison* or deltra* or diadreson* or di-adreson* or drazone* or econosone* or encorton* or encortone* or enkorton* or enkortolon* or fernisone* or fiasone* or hostacortin* or insone* or incocortyl* or juvason* or kortancyl* or liquid pred* or lodotra* or lodtra* or lisacort* or me-korti* or meprison* or metacortandracin* or Meticorten* or meticortine* or nisona* or nizon* or novoprednisone* or nurison* or Orasone* or orisane* or panafcort* or paracort* or panasol* or parmenison* or pehacort* or predeltin* or precort* or precortal* or prednicen* or prednicorm* or prednicort* or prednicot* or predni tablinen* or prednidib* or prednilonga* or predniment* or prednison* or prednitone* or prednizon* or prednovister* or presone* or pronison* or pronizon* or pulmison* or rayos* or rectodelt* or rectrocortine* or servisone* or sone$2 or steerometz* or sterapred* or supercortil* or ultracorten* or urtilone* or winpred* or wojtab* or zenadrid*).ti,ab,kf,kw.
23	(53-03-2 or VB0R961HZT).rn.
24	Methotrexate/
25	(abitextrate* or abitrexate* or amethopterin* or amethopterine* or ametopterine* or antifolan* or biotrexate* or brimexate* or canceren* or emtexate* or emthexat* or emthexate* or emtrexate* or enthexate* or farmitrexat* or farmotrex* or fauldexato* or folex* or ifamet* or imeth$2 or intradose MTX or jylamvo* or lantarel* or ledertrexate* or lumexon* or maxtrex* or medsatrexate* or metatrexan* or metex* or methoblastin* or methohexate* or methotrate* or methotrexat* or methylaminopterin* or metical* or metoject* or metotressato* or metothrexate* or metotrexat* or metotrexin* or metrex* or metrotex* or mexate* or neotrexate* or nordimet* or novatrex* or otrexup* or rasuvo* or reditrex* or reumatrex* or rheumatrex* or texate* or texorate* or tremetex* or trexall* or trexeron* or trixilem* or xaken* or xatmep* or zexate*).ti,ab,kf,kw.
26	(59-05-2 or YL5FZ2Y5U1).rn.
27	Interleukin-1beta/
28	(ACZ-885* or ACZ885* or Canakinumab* or ilaris*).ti,ab,kf,kw.
29	(914613-48-2 or 37CQ2C7X93).rn.

30	Antibodies, Monoclonal, Humanized/
31	(pexelizumab or "h5G1.1-SC*").ti,ab,kf,kw.
32	(219685-93-5 or CHZ6OLQ3UU).rn.
33	Interleukin 1 Receptor Antagonist Protein/
34	(Anakinra* or Antril* or Kineret* or il-1ra* or (interleukin 1 receptor adj1 (antagonist or block* or inhibit*))).ti,ab,kf,kw.
35	(143090-92-0 or 9013DUQ28K).rn.
36	(agi-1067* or agi1067* or agz-1067* or agz1067* or probucol succinate* or Succinobucol*).ti,ab,kf,kw.
37	(216167-82-7 or J1J54V24R4).rn.
38	(ftx-1821* or ftx1821* or gsk-856553* or gsk856553* or gw-856553* or gw856553* or Losmapimod* or sb-856553* or sb856553*).ti,ab,kf,kw.
39	(F2DQF16BXE or 585543-15-3).rn.
40	(lc1004* or lc-1004* or inclacumab* or ro4905417* or ro-4905417*).ti,ab,kf,kw.
41	(1256258-86-2 or A6734I702L).rn.
42	(Atreleuton* or a85761* or a-85761* or abt761* or abt-761* or via-2291* or via2291*).ti,ab,kf,kw.
43	(U301T88E1M or 154355-76-7).rn.
44	(darapladib* or sb-480848* or sb480848*).ti,ab,kf,kw.
45	(356057-34-6 or UI1U1MYH09).rn.
46	(Varespladib* or Varepladib* or ly315920* or ly-315920* or s-5920* or s5920*).ti,ab,kf,kw.
47	(2Q3P98DATH or 172732-68-2).rn.
48	or/10-47
49	randomized controlled trial.pt.
50	controlled clinical trial.pt.
51	randomized.ab.
52	placebo.ab.
53	clinical trials as topic.sh.
54	randomly.ab.
55	trial.ti.
56	49 or 50 or 51 or 52 or 53 or 54 or 55
57	exp animals/ not humans.sh.
58	56 not 57
59	9 and 48 and 58

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Emerging Evidence

Observational Cross-Sectional Study of Inflammatory Markers After Transient Ischemic Attacks, Acute Coronary Syndromes, and Vascular Stroke Events

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ABSTRACT

We identified the prevalence of elevated high-sensitivity C-reactive protein and interleukin-6 in patients with recent cardiovascular (CV) events with or without prediabetes/diabetes, and in a control group of patients with remote CV events. Interleukin-6 was elevated in patients with prediabetes/diabetes and recent CV events (median, 4.84 pg/mL; interquartile range, 3.27-7.45) compared with patients with remote events (2.36 pg/mL; interquartile range, 1.09-4.00). There was a trend for elevated high-sensitivity C-reactive protein in patients with acute events and prediabetes/diabetes ($P = 0.147$). This supports the notion that patients with prediabetes/diabetes and recent CV events have higher inflammatory burdens than patients without recent CV events or dysglycemia.

RÉSUMÉ

Nous avons défini la prévalence de l'augmentation du taux de protéine C réactive à haute sensibilité et d'interleukine-6 chez des patients ayant récemment subi des événements cardiovasculaires (CV), atteints ou non de prédiabète ou de diabète, et dans un groupe témoin de patients ayant subi des événements CV antérieurement. Le taux d'interleukine-6 était élevé chez les patients atteints de prédiabète ou de diabète ayant récemment subi des événements CV (médiane de 4,84 pg/ml; écart interquartile de 3,27 à 7,45) par rapport aux patients ayant subi des événements antérieurement (2,36 pg/ml; écart interquartile de 1,09 à 4,00). Le taux de protéine C réactive à haute sensibilité avait tendance à être élevé chez les patients atteints de prédiabète ou de diabète ayant subi des événements aigus ($p = 0,147$). Ces données appuient la notion selon laquelle les patients atteints de prédiabète ou de diabète qui ont récemment subi des événements CV présentent des fardeaux inflammatoires supérieurs à ceux des patients qui n'ont pas récemment subi d'événements CV ou présenté de dysglycémie.

Patients with diabetes are at increased risk of suffering premature atherosclerotic disease-related cardiovascular (CV) events (eg, acute coronary syndrome [ACS], stroke, transient ischemic attacks [TIAs]), and have a higher recurrent event rate than the general population.[1] Systemic and vascular inflammation has been extensively investigated as a promoter of atherosclerosis development and destabilization.[2] Furthermore, several recent trials have shown that therapies focused at blocking the inflammatory cascade directly reduced vascular events.[3-6] The results from these trials raise an intriguing question of how to identify the patients who might benefit the most from these innovative therapies. By examining biomarkers of inflammation, we might be able to identify patients who are at particularly high risk of vascular events, and might benefit the most from inflammation-targeted therapies to reduce subsequent CV events.

Currently the prevalence of elevated serum high-sensitivity (hs-) C-reactive protein (CRP) levels after a vascular event has not been widely studied. Identification of the prevalence of patients with elevated inflammatory markers post event might enable the prospective evaluation of inflammation-targeted therapies and the identification of patients at high risk for CV events.

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Ethics Statement: This study was approved by University of Ottawa Heart Institute's Research Ethics Board. All participants gave written informed consent.

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See page 678 for disclosure information.

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We hypothesized that because of the link between inflammation and CV events, as well as the higher prevalence of CV events in patients with diabetes, these diabetic or prediabetic patients might have a higher burden of inflammation than a general population of patients with CV disease. We posited that the prevalence of increased hs-CRP and interleukin (IL)-6 as markers of vascular inflammation in patients with diabetes or prediabetes after recent CV events (ACS, TIA, stroke), would be greater than in a control group of patients without recent acute CV events.

Methods

We conducted an observational cross-sectional study in 2 cohorts of patients: (1) those with a recent CV event (ACS, TIA, or stroke) within 30-60 days; and (2) those with remote previous CV events (> 1 year). Patients were divided into 3 groups. Group 1 comprised patients with a recent CV event with prediabetes/diabetes (as defined by the Canadian Diabetes Association Guidelines)[7]; group 2, those with a recent CV event without prediabetes/diabetes, and group 3, patients who suffered a remote CV event (> 1 year).

Participants were enrolled from either the University of Ottawa Heart Institute or the Stroke Prevention Clinic at the Ottawa Hospital. Enrolled participants for group 1 and 2 had to: have suffered a recent CV event (ACS, TIA, or stroke) within 30-60 days, be 18 years of age or older, and be symptomatically and hemodynamically stable. Group 1 patients had prediabetes/diabetes, group 3 (control group) patients had a remote CV event (> 1 year earlier) and met the other inclusion criteria. All patients provided written informed consent. Ethics approval was obtained from the Ottawa Health Science Network Research Ethics Board and the study was conducted in accordance with the Declaration of Helsinki.

For patients in group 1 and 2, IL-6 and hs-CRP were measured within 30-60 days after their CV event. For those in group 3, IL-6 and hs-CRP were measured > 1 year after their CV event. hs-CRP was measured with a commercially available kit available on a high-throughput diagnostic platform (ROCHE sro, Diagnostics Division, Praha, Czech Republic). IL-6 was assayed using a commercially available enzyme-linked immunosorbent assay kit (R&D Systems Inc, Minneapolis, MN).

Demographic data including age, sex, ethnicity, height, weight, waist circumference, and blood pressure were recorded as well as data on current medications, medical history, smoking status, and family history of CV disease. IL-6 and hs-CRP were measured within 30-60 days after their vascular event in those in groups 1 and 2 and at time of enrollment for those in group 3.

Demographic characteristics are presented as median (interquartile range [IQR]). Differences between patient groups were evaluated using the Fisher exact test for discrete clinical variables and the Mann-Whitney U test for continuous variables. Follow-up data were collected as scheduled. All tests were 2-sided, and a P value of < 0.05 was considered statistically significant. For comparisons of hs-CRP and IL-6 values between groups, Kruskal-Wallis tests were conducted to compare the medians between groups. Missing data were considered missing completely at random and pairwise deletion was used to handle the missingness. Statistical analyses were done using MedCalc Statistical Software version 19.5.3 (MedCalc Software Ltd, Ostend, Belgium).

Results

Participant characteristics are described in Table 1. In summary, 24% of participants were women, with a median age of 64.0 (IQR, 57.0-71.0) years—these were similar between the 3 groups. Median body mass index was 28.6 (IQR, 25.0-33.0) and participants in the 3 groups had a similar proportion of cardiac risk factors except for diabetes (as expected).

IL-6 median values for groups 1, 2, and 3 were 4.84 (IQR, 3.27-7.45) pg/mL, 2.47 (IQR, 1.65-5.61) pg/mL, and 2.36 (IQR, 1.09-4.00) pg/mL, respectively, with a significant difference between IL-6 levels across the 3 groups ($P = 0.034$). A post hoc Mann-Whitney test revealed the median between group 1 and 3 was significantly different ($P = 0.009$). hs-CRP median values were 2.20 (IQR, 1.20-7.98) mg/mL, 1.00 (IQR, 0.48-2.50) mg/mL, and 1.20 (IQR, 0.78-2.10) mg/mL, respectively, and showed a trend toward significant differences across the 3 groups ($P = 0.147$). Results shown in Figure 1.

The subset of patients with prediabetes/diabetes and a recent CV event (group 1) had a numerical, but nonsignificant trend toward higher median IL-6 values (4.84; IQR, 3.27-7.45 pg/mL) compared with the subset of patients with prediabetes/diabetes who experienced remote events (2.77; IQR, 1.50-4.00 pg/mL). hs-CRP levels were also numerically higher in patients with prediabetes/diabetes who suffered a recent CV event (1.95; IQR, 1.09-7.80 mg/mL) compared with those with prediabetes/diabetes with remote events (1.75; IQR, 1.20-2.70 mg/mL; $P = 0.815$).

Similarly, the subset with no prediabetes/diabetes and a recent CV event had a trend toward higher IL-6 values (2.47; IQR, 1.65-5.61 pg/mL) compared with the subset of patients with prediabetes/diabetes who had remote events (IL-6: 1.57; IQR, 0.84-4.26 pg/mL; $P = 152$). hs-CRP did not differ significantly between the groups with median levels of 1.00 (IQR, 0.48-2.50) mg/mL in the subset with no prediabetes/diabetes and a recent CV event compared with levels of 1.00 (IQR, 0.70-2.45) mg/mL in patients without prediabetes/diabetes and remote events ($P = 0.815$).

In investigating the group of patients with remote events, IL-6 levels were numerically (but not statistically) higher in patients with prediabetes/diabetes (2.77; IQR, 1.50-4.00 pg/mL) compared with those without (1.57; IQR, 0.84-4.26 pg/mL; $P = 0.347$). Similarly, hs-CRP levels were numerically (but not statistically) higher in patients with prediabetes/diabetes (1.75; IQR, 1.20-2.70 mg/mL) compared with those without (1.00; IQR, 0.70-2.45 mg/mL; $P = 0.271$). Data are available upon request.

Discussion

In this observational cohort study, we compared IL-6 values in patients with prediabetes/diabetes who had experienced recent CV events, with those in patients with remote CV events, and there was a numerical but not statistically significant trend of higher median values of hs-CRP in

Table 1. Baseline characteristics of the participants

Variable	Acute events with (pre-)DM (n = 18)	Acute events without (pre-)DM (n = 17)	Remote events (n = 24)
Age, years	60.5 (57.0-71.0)	64.0 (55.5-70.25)	67.0 (60.5-71.0)
Male sex	13 (72)	13 (76)	19 (79)
BMI	28.8 (25.0-33.3)	26.6 (24.6-28.8)	29.1 (25.8-34.2)
Hypertension	13 (72)	8 (47)	15 (62)
Dyslipidemia	13 (72)	8 (47)	23 (96)
Smoking	10 (56)	6 (32)	14 (58)
Diabetes/pre-diabetes	18 (100)*	0 (0)*	12 (50)*
Baseline medical therapy			
ECASA	18 (95)	16 (94)	21 (88)
P2Y12 inhibitors	18 (95)*	15 (88)*	12 (50)*
Anticoagulant	1 (5)	1 (6)	4 (17)
β-Blocker	15 (79)	14 (82)	18 (75)
ACEi	16 (84)	15 (88)	15 (63)
CCB	2 (11)	2 (12)	0 (0)
Statin	19 (100)	15 (88)	23 (96)
Metformin	6 (32)	0 (0)	3 (13)
Insulin	3 (16)	0 (0)	2 (8)
Steroids	0 (0)	0 (0)	2 (8)
Any other anti-inflammatory medication	0 (0)	0 (0)	1 (4)
Cell count and differential			
Leukocytes × 10^9/L	8.3 (6.9-9.6)	6.9 (5.2-8.1)	8.0 (7.0-9.3)
Platelets × 10^9/L	234.5 (177.0-271.0)	220.2 (170.3-271.3)	220.0 (181.5-254.0)
Neutrophils × 10^9/L	5.2 (4.5-6.0)	3.9 (2.9-5.3)	4.8 (4.1-5.8)
Lymphocytes × 10^9/L	2.0 (1.7-2.3)	1.7 (1.2-2.1)	2.1 (1.6-2.4)
Monocytes × 10^9/L	0.6 (0.6-0.7)	0.6 (0.4-0.7)	0.6 (0.5-0.7)
Eosinophils × 10^9/L	0.3 (0.2-0.4)	0.2 (0.1-0.3)	0.2 (0.1-0.3)
Biomarkers/biochemistry			
IL-6, pg/mL	4.83 (3.27-7.45)†	2.47 (1.65-5.61)†	2.36 (1.09-4.00)†
hs-CRP, mg/mL	2.20 (1.20-7.98) (n = 17)	1.00 (0.48-2.50) (n = 17)	1.20 (0.78-2.10) (n = 13)
Random glucose level, mmol/L	6.10 (5.38-7.53)	5.30 (5.03-5.53)	5.50 (4.88-7.18)
Creatinine, μmol/L	82.0 (73.0-97.0)	79.0 (73.0-92.5)	81.50 (73.0-93.5)
HbA1c, %	6.25 (5.90-7.20)	5.50 (5.40-5.70)	5.95 (5.50-6.65)

Data are median (IQR) or n (%).

ACEi, angiotensin converting enzyme inhibitor; BMI, body mass index; CCB, calcium channel blocker; DM, diabetes mellitus; ECASA, ; HbA1c, hemoglobin A1c; hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; IQR, interquartile range; P2Y12, purinergic signalling receptor Y_{12}.

*Distribution of variables differed significantly across groups on Fisher exact testing ($P < 0.05$).

†Median values differed across groups during Kruskal-Wallis testing.

patients with prediabetes/diabetes with recent CV events compared with patients with remote CV events. There was a trend for higher IL-6 and hs-CRP levels in patients with prediabetes/diabetes and recent CV events compared with those without dysglycemia who also experienced recent CV events. There was also a trend for higher IL-6 and hs-CRP levels in patients with prediabetes/diabetes and a recent CV event compared with the subgroup of patients with prediabetes/diabetes and a remote CV, although this did not reach statistical significance. Finally, there was also a numerical, but not significant trend for higher IL-6 and hs-CRP levels in patients without prediabetes/diabetes and a recent CV event compared with the subgroup of patients with no prediabetes/diabetes and a remote CV.

There is a large body of evidence that supports the notion that atherosclerosis is an inflammatory process.[8-10] CV events occur more frequently in persons with higher burdens of circulating inflammatory markers, and treating patients on the basis of these inflammatory markers has been shown to reduce adverse outcomes.[3,4] Inflammation promotes atherosclerotic CV disease through multiple mechanisms. It might play a role in the evolution of plaque biology and contribute to acute plaque rupture.[11] It has an overall negative effect on vessel anatomy and function after the acute CV insults.[12] The inflammatory activity is also the highest immediately after a CV event, indicating tissue repair pathways being coordinated by inflammatory activation.

The results of our study also support the notion that patients with diabetes or prediabetes and who have had recent CV events[13] have a higher inflammatory burden than patients without recent CV events. There was also a trend to suggest that in patients who have suffered a recent CV event, there is a higher inflammatory burden in patients with dysglycemia than in those without. This is congruent with other literature that supports the notion that diabetes is linked to inflammation.[14-16] There is a growing body of literature that suggests that diabetes is a chronic inflammatory condition.[17] Numerous epidemiologic studies have shown associations between plasma markers of inflammation and diabetes.[18-21] In a nested case-control study of > 32,826 women, baseline levels of the inflammatory markers, tumour necrosis factor-α receptor 2, IL-6, and CRP were all predictive of the future development of diabetes. In a cohort study of 75 patients who underwent coronary stenting, it was reported that patients with diabetes had higher preprocedural levels of IL-6, IL-1 receptor antagonist, and soluble CD40 ligand than those without diabetes, thereby suggesting a higher inflammatory burden in these patients.[22] There are many proposed

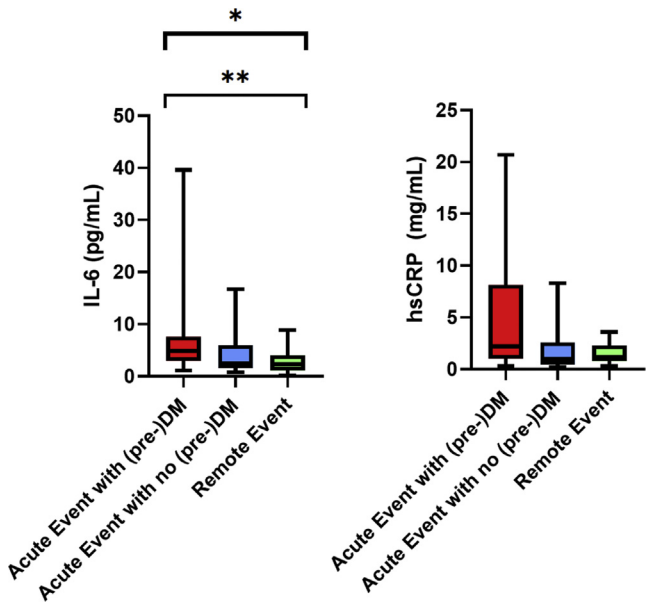

Figure 1. High-sensitivity C-reactive protein (hsCRP) and interleukin (IL)-6 values in patients with cardiovascular events. DM, diabetes mellitus. * Kruskal-Wallis testing revealed IL-6 levels differed significantly across the 3 groups ($P < 0.05$). ** $P = 0.009$ with post hoc Mann-Whitney testing between group 1 (acute event with (pre-)DM and group 3 (remote event).

mechanisms linking inflammation to diabetes, including pancreatic β-cell inflammation,[23] inflammation generated by adipocytes,[17] and even contributions related to the gut microbiome.[24] Although we used IL-6 and hs-CRP to monitor inflammation in our patient population, recent literature has suggested that other markers such as platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio might also accurately reflect inflammatory levels and have prognostic value in other inflammatory conditions.[25,26] Although our study was not powered to answer these questions in a CV disease population, more research in this area will certainly be needed to further elucidate the role that these biomarkers could potentially play in coronary artery disease prognostication and management in the future.

Using anti-inflammatory therapies for secondary CV prevention is a concept that has gained much traction in recent years. In the **C**anakinumab **An**ti-inflammatory **T**hrombosis **O**utcomes **S**tudy (CANTOS) trial, canakinumab treatment was particularly effective in patients who achieved a significant hs-CRP reduction, but was associated with a significant increase in fatal sepsis, with no effect on de novo diabetes mellitus or worsening of diabetes control.[3] This is important because approximately 40% of the patients enrolled in the CANTOS trial were patients with diabetes. This notion suggests the importance of identifying among patients with diabetes or prediabetes, those with the most potential for benefit, as opposed to universal treatment with the risk of potential harm. Further support for the concept of anti-inflammatory therapies for reduction of CV events was noted in the **Lo**w-**Do**se **Col**chicine for Secondary Prevention of Cardiovascular Disease (LoDoCo) study, in which colchicine led to a 67% reduction in the composite of ACS, arrest, and ischemic stroke, double the reduction that occurs with statins.[5] LoDoCo prompted the larger randomized trial of colchicine in 4500 patients, **Col**chicine **C**ardiovascular **O**utcomes **T**rial (COLCOT), which showed that low-dose colchicine (0.5 mg orally daily), reduces the rates of ischemic CV events in patients who recently (within 30 days) had myocardial infarction.[6] Finally, the recent LoDoCo 2 study showed outcome benefit in 5522 patients with chronic coronary disease who were treated with colchicine (0.5 mg orally daily).[27]

There are important limitations to note in our study. This was an observational, cross-sectional study. Thus, although we observed associations between inflammatory markers and diabetes and CV events, care should be taken in the interpretation of these relationships, because we cannot rule out underlying confounding as the explanation for these associations. Thus, these observed associations are merely hypothesis-generating, and should not be taken as causal. Additionally, our sample size was quite small, which limits the power of our study. However, even with these limitations, the observed numerical trends across groups were consistent, lending strength to our observations.

Inflammation is a key component in the progression of atherosclerosis and its sequelae, such as myocardial infarction, stroke, and CV death.[3,28] As such, it represents a potentially transformative therapeutic target. Because patients with prediabetes/diabetes have a higher inflammatory burden, and are already at high risk for recurrent vascular events, this specific population might be an ideal group to target with therapies aimed at the reduction of inflammatory burden. These results set the stage for the evaluation and potential use of inflammation biomarkers to help define specific populations (such as those with diabetes or prediabetes) or even patients within such populations that will most benefit from anti-inflammatory therapies and thereby allow the personalization of therapies for patients at increased risk of subsequent CV events.

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The Canadian Study of Arterial Inflammation in Patients with Diabetes and Recent Vascular Events, Evaluation of Colchicine Effectiveness (CADENCE): protocol for a randomised, double-blind, placebo-controlled trial

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ABSTRACT

Background Inflammation is a key mediator in the development and progression of the atherosclerotic disease process as well as its resultant complications, like myocardial infarction (MI), stroke and cardiovascular (CV) death, and is emerging as a novel treatment target. Trials involving anti-inflammatory medications have demonstrated outcome benefit in patients with known CV disease. In this regard, colchicine appears to hold great promise. However, there are potential drawbacks to colchicine use, as some studies have identified an increased risk of infection, and a non-significant trend for increased all-cause mortality. Thus, a more thorough understanding of the underlying mechanism of action of colchicine is needed to enable a better patient selection for this novel CV therapy.

Objective The primary objective of the Canadian Study of Arterial Inflammation in Patients with Diabetes and Recent Vascular Events, Evaluation of Colchicine Effectiveness (CADENCE) trial is to assess the effect of colchicine on vascular inflammation in the carotid arteries and ascending aorta measured with ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET)/CT in patients with type 2 diabetes mellitus (T2DM) or pre-diabetes who have experienced a recent vascular event (acute coronary syndrome (ACS)/MI, transient ischaemic attack (TIA) or stroke). Secondary objectives include determining colchicine's effect on inflammatory biomarkers (high-sensitivity C reactive protein (hs-CRP) and interleukin-6 (IL-6)). Additionally, we will assess if baseline inflammation imaging or biomarkers are associated with a treatment response to colchicine determined by imaging. Exploratory objectives will look at: (1) the difference in the inflammatory response to colchicine in patients with coronary events compared with patients with cerebral events; (2) the difference in the inflammatory response to colchicine in different vascular beds; (3) the relationship of FDG-PET imaging markers with serum biomarkers and (4) assessment of quality-of-life changes.

Methods and design CADENCE is a multicentre, prospective, randomised, double-blinded, placebo-controlled study to determine the effect of colchicine on arterial inflammation as assessed with imaging and circulatory biomarkers, specifically carotid arteries and aortic FDG uptake as well as hs-CRP and IL-6 among others. Patients with T2DM or pre-diabetes who have recently experienced a CV event (within 30–120 days after an ACS (ie, ST-elevation MI (STEMI) or non-STEMI))

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study examines the mechanistic effect of colchicine on inflammation within atherosclerotic plaque of high-risk patients with diabetes or pre-diabetes who have suffered recent vascular events (acute coronary syndrome/myocardial infarction, transient ischaemic attack, stroke).
⇒ Using ^{18}F-fluordeoxyglucose positron emission tomography/CT at two timepoints, this study will evaluate changes in the carotid arteries and thoracic aorta to determine local mechanisms of colchicine.
⇒ The response to colchicine and clinical outcomes will be explored with systemic inflammatory biomarkers (eg, interleukin-6, interleukin-1β, tumour necrosis factor-α, monocyte chemoattractive protein-1 and others), major adverse cardiac events, all-cause mortality and quality of life outcomes.
⇒ As this is a mechanistic study, it is underpowered to investigate whether colchicine in high-risk patients would lead to improvements in clinical endpoints but will help justify future studies with such high-risk populations.

or TIA/stroke with documented large vessel atherosclerotic disease will be randomised to treatment with either colchicine 0.6 mg oral daily or placebo. Participants will undergo baseline clinical evaluation including EQ5D assessment, blood work for inflammatory markers and FDG PET/CT scan of the ascending aorta and left and right carotid arteries. Patients will undergo treatment for 6 months and have repeat clinical evaluation including EQ5D assessment, blood work for inflammatory markers and FDG PET/CT scan at the conclusion of the study. The primary outcome will be the change in the maximum target to background ratio (TBR_{max}) in the ascending aorta (or carotid arteries) from baseline to follow-up on FDG PET/CT imaging.

Discussion Colchicine is an exciting potential new therapy for CV risk reduction. However, its use is associated with side effects and greater understanding of its underlying mechanism of action is needed. Importantly, the current study will determine whether its anti-inflammatory action is an indirect systemic effect, or a more local plaque action that decreases inflammation. The results will also help identify patients who will benefit most from such therapy.

Trial registration number NCT04181996.

INTRODUCTION

Inflammation plays a crucial role in the development and progression of atherosclerosis and its complications, like myocardial infarction (MI), stroke and cardiovascular (CV) death.[1–3] Given its aetiologic role, inflammation is now emerging as an important treatment target for CV risk reduction.[1–3] A number of trials have demonstrated that anti-inflammatory therapies may improve outcomes in patients with CV disease. The CANTOS Trial[3] demonstrated CV benefit in patients treated with canakinumab, a monoclonal antibody directed against interleukin (IL) 1-beta. The intervention was more effective in patients who achieved a greater reduction in the inflammation biomarker high-sensitivity C reactive protein (hs-CRP). However, two factors severely limit the use canakinumab to a large number of patients: its cost and the associated increased risk of sepsis. Indeed, canakinumab has been demonstrated to not be cost-effective for a CV risk-reduction indication.[4] Thus, the search for a cost-effective anti-inflammatory therapy to reduce CV events continues. Studies including the LoDoCo2 trial[5] and COLCOT[6] have also demonstrated CV outcome benefit in patients with known atherosclerotic coronary disease who are treated with colchicine. Despite its proven efficacy, the mechanism whereby this drug reduces CV events remains unknown. The true anti-inflammatory properties of colchicine are also largely unknown, although they are believed to include inhibition of neutrophil function via an antitubulin effect, and inhibition of the $NLRP_3$ inflammasone.[7] Clinical efficacy has been demonstrated for several inflammatory conditions including pericarditis,[8] gout[9] and familial Mediterranean fever.[10] However, colchicine has several side effects, predominantly gastrointestinal upset, that limit its tolerability and reduce patients' compliance. Additionally, recent CV trials showed a non-significant trend toward an increase in all-cause mortality with colchicine therapy.[5 6 11] Thus, further investigations into colchicine mechanisms of actions are warranted. Although the recent trials showed a reduction in CV events with colchicine, there was no correlation with baseline and follow-up serum markers of inflammation. Hence, it is possible that colchicine may be acting to reduce inflammation within the atherosclerotic plaque, rather than at a systemic level.

The primary objective of Canadian Study of Arterial Inflammation in Patients with Diabetes and Recent Vascular Events, Evaluation of Colchicine Effectiveness (CADENCE) is to investigate the biological effect of the medication colchicine, compared with placebo, on the inflammatory activity of atherosclerotic plaque (measured by ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET)/CT at baseline and 6 months) in the carotid arteries and ascending aorta in high-risk patients with type 2 diabetes mellitus (T2DM) or pre-diabetes and recent vascular events (acute coronary syndrome (ACS)/MI, transient ischaemic attack (TIA) or stroke). Specifically, we will look at whether colchicine acts in a systemic anti-inflammatory fashion or whether its mechanism of action is more of a local effect at the plaque level. FDG PET/CT has demonstrated to be an efficient and practical proxy method to assess inflammation in the aorta.[12] Prior studies have demonstrated that therapies that reduce aortic FDG uptake also predict clinical efficacy.[13–15]

The secondary objectives of this study are to explore (a) whether baseline inflammation imaging (FDG PET/CT) and/or inflammatory biomarker (hs-CRP and IL-6) levels can identify which patients are most likely to have a positive response to colchicine, as indicated by the reduction in FDG uptake over a period of 6 months and (b) the correlation between response to colchicine as measured by reduction of inflammation as proxied by imaging with FDG PET/CT and hs-CRP at baseline and during a 6-month follow-up.

Our exploratory objectives are to investigate (a) if there are local differences (ie, in the carotid arteries vs aorta, respectively) in the anti-inflammatory effect of colchicine therapy in patients who have suffered recent coronary events (ACS/MI) compared with those with recent cerebrovascular events (TIA, stroke); (b) if other systemic inflammatory biomarkers (eg, interleukin (IL)-6, IL-1β, tumour necrosis factor (TNF)-α, monocyte chemoattractive protein (MCP)-1, others) predict a response to therapy; (c) the relationship of the inflammatory response as proxied by imaging with FDG PET/CT to: (1) systemic inflammation biomarkers; (2) monocyte activation; (d) the treatment effect of colchicine on clinical outcomes such as major adverse CV events and all-cause mortality and (e) the effect of colchicine on patient quality of life measures.

MATERIALS AND METHODS

CADENCE is a multicentre, prospective, randomised, double-blind placebo-controlled study designed to assess the effect of anti-inflammation therapy using colchicine, on arterial inflammation using imaging and circulatory biomarkers, specifically carotid arteries and aortic FDG

uptake and hs-CRP. This protocol was completed in accordance with the Standard Protocol Items: Recommendations for Interventional Trials reporting guidelines (see online supplemental material 1).[16]

Study population

At the University of Ottawa Heart Institute (UOHI) and the Mazankowski Alberta Heart Institute, patients with T2DM or pre-diabetes with a recent ACS in the last 30–120 days prior to recruitment (ie, ST-elevation myocardial infarction (STEMI) or non-ST elevation myocardial infarction (NSTEMI)), stroke or TIA will be recruited. We will use standard definitions for ACS events including chest pain, electrocardiogram changes and troponin rise. For cerebrovascular events, non-embolic ischaemic stroke will be confirmed by CT imaging or MRI, and TIA events will be assessed and determined by a neurologist. The criteria for exclusion from the study include patients who have a coronary revascularisation procedure planned over 120 days after the qualifying event, decompensated heart failure, severe left ventricular (LV) systolic dysfunction (LV ejection fraction <30%), severe valvular disease that requires intervention, and other active diseases. A detailed list of inclusion and exclusion criteria is available in online supplemental material 2. The study start date is 1 August 2020 and the planned completion date is 31 December 2023.

Enrolment and baseline assessment

Patients who meet inclusion/exclusion criteria will undergo clinical evaluation, FDG PET imaging with CT angiography (CTA), and blood sample collection within 30–120 days of CV event. HbA1C, fasting blood sugar, lipids, C reactive protein (CRP), creatinine (CR), glomerular filtration rate, complete blood count, haemoglobin, liver function tests (ie, AST, ALT), creatine kinase (CK), as well as blood samples for biomarkers and immunological studies will be collected from all the patients at baseline.

Patients recruited into the CADENCE study will be randomised to receive either an oral colchicine 0.6 mg tablet within a capsule or identical placebo capsule administered once daily for 6 months. The placebo capsule will consist of an opaque gelatin capsule shell and a microcrystalline cellulose filler.

Randomisation

Eligible and consenting patients (online supplemental material 3) will be randomised 1:1 to the treatment or placebo arm. The Cardiovascular Research Methods Centre at the UOHI will create a computer-generated randomisation schedule with randomly varying block sizes (predefined and masked to study investigators) stratified according to centre, and whether the recent vascular event was cardiac or cerebral. Randomisation will be done centrally through a web-based system.

Follow-up

Clinical evaluation and blood collection of biomarkers, CR, CRP, AST, ALT and CK will be repeated at 3 months and 6 months. FDG PET imaging with CTA will be repeated at 6 months. Clinical outcomes will be determined at each time point (figure 1). Patients will also be followed up by phone at 1-week and 1-month post-initiation of the study drug, to assess for side effects, safety and adherence to treatment. Study participants will be instructed to return

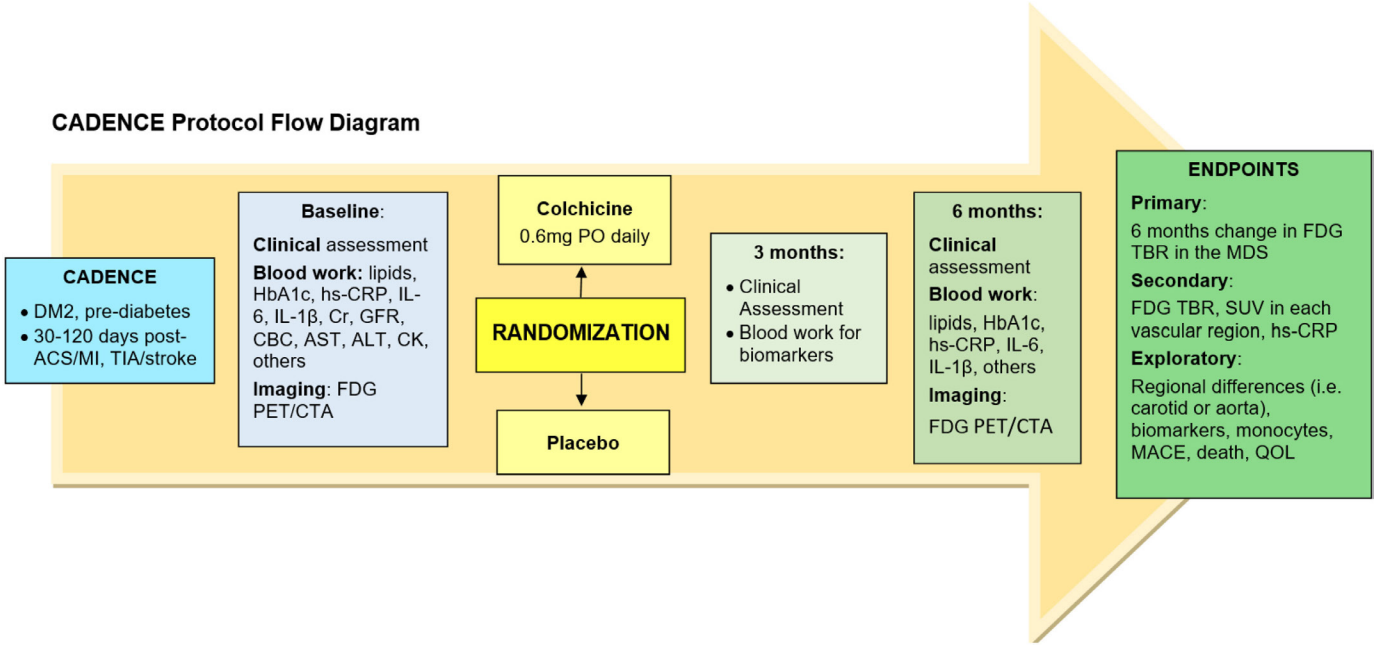

Figure 1 Flow chart. ACS, acute coronary syndrome; CBC, complete blood count; CK, creatine kinase; CTA, CT angiography; FDG, ^{18}F-fluorodeoxyglucose; GFR, glomerular filtration rate; hsCRP, high-sensitivity C reactive protein; IL, interleukin; MACE, major adverse cardiac events; MDS, most diseased segment; MI, myocardial infarction; PET, positron emission tomography; QOL, quality of life; SUV, standardised uptake value; TBR, target to blood ratio; TIA, transient ischaemic attack.

all unused drug to each follow-up study visit to assist in determining treatment adherence.

Blinding

This trial will be conducted in a double-blind fashion. Patients will be blinded to their treatment status, and those randomised to the placebo arm will receive a placebo pill that is identical in colour and consistency to the colchicine arm. Physicians and any study personnel having contact with the patients will also be blinded with regards to the patient allocation. However, in the event of an emergency, in which knowledge of the study treatment allocation is critical, the blinding may be broken by the treating physician.

Measures

FDG imaging of carotid arteries and aorta

All patients will be advised to limit strenuous exercise and avoid using any cannabis products for 24 hours before the scan. The advice given to the recruited patients for PET imaging preparation will depend on the anti-diabetic medications that are prescribed to them by their respective treating physicians.

The evening before the PET imaging:

- Low-carbohydrate, high fatty meals (keto diet) for patients on oral anti-diabetic medications (except for insulin secretagogues).
- Regular meal for patients on insulin or insulin secretagogues; such patients also receive personalised advice about their meal to maintain an optimum blood glucose.
- Usual anti-diabetic medications for all patients.
- Fasting overnight for all patients.

On the morning of PET imaging:

- Half of the usual oral anti-diabetic medications (except for insulin secretagogues).
- No insulin or insulin secretagogues.
- A standardised protocol is in place for managing hyperglycaemia on the day of the scan. The scan will be postponed if the fasting blood glucose is ≥11 mmol/L until the blood glucose is better controlled.

On arrival an IV will be inserted in the arm. FDG (2–3 MBq/kg) will be injected intravenously. After the FDG injection patients will have a 2–3 hour wait period. During the first hour, the patient will be asked to sit quietly followed by a 1–2 hour wait period when the patient can move about, talk and read. The waiting period will allow the uptake of FDG by normal tissues and/or areas of inflammation, while there is a decrease in the remainder of the background activity (ie, blood pool) creating an ideal target-to-background ratio.

After scout imaging to check proper positioning, a low-dose CT scan will be done for attenuation correction (AC) of the PET data. A respiratory-gated PET scan will be acquired in 3D mode over three bed positions to span the common and internal carotid arteries and the thoracic ascending aorta. High-resolution (3–5 mm) images of FDG uptake (Bq/mL) will be reconstructed using iterative statistical reconstruction with no post-filtering. The prep at baseline will be replicated at follow-up including the time of scan whenever possible.

CT angiography

Following FDG image acquisition, a non-contrast enhanced helical CT scan will be used to define the limits of the CTA study. CTA will be performed on a multidetector CT scanner with a minimum of 64-slices: 60 mL of contrast will be injected at a rate of 3.2 mL/s, 120–180 min after FDG injection. Similar to the scanning protocol used in our prior studies, using smart prep bolus-tracking, the CTA study will be triggered at 80 HU in the ascending aorta.[17–19] The study will be acquired with the following settings: 20 mm detector coverage, 120 kVp, mA dose-modulation between 150 and 550 mA, 0.5 s per rotation, noise index=16, 0.625 mm thick slices reconstructed at 0.625 mm intervals, table speed 19.37 mm/per rotation to cover the limits determined from the initial helical CT scan in 3.6 s.[17] Anonymised images will be transferred to the UOHI Core Laboratory via a password protected network interface.

PET/CT image analysis

PET/CT image analysis will be performed in a standardised fashion by two readers blinded to patient data and treatment arm. As described previously,[17 20 21] the bifurcation location of the common carotid artery will be identified on CTA. If a CTA cannot be performed, then a non-contrast CT scan will be used instead of the CTA.[17 20] To quantify FDG uptake, a circular region of interest will be positioned on consecutive transaxial PET images. For the aorta, this begins 1 cm above the aortic valve plane and continues to the base of the aortic arch. For the carotid arteries, it starts 2 cm below the bifurcation up to 2 cm above it in the internal carotid artery.[20] Maximum FDG uptake (Bq/mL) is then measured and will be normalised to the injected activity and body-weight in order to determine the standardised uptake value (SUV, g/mL).[17] The target-to-blood ratio (TBR) is calculated by taking the SUVs of the artery wall, and normalising the values to the background venous activity (superior vena cava or internal jugular for aorta or carotid arteries, respectively). Finally, the TBR in the area of maximal FDG uptake (otherwise known as the most diseased segment (MDS)) will be recorded. This approach has excellent intra-observer and inter-observer variability.[20 22] The TBR in the MDS from the baseline scan will be compared with the follow-up images at 6 months.

Biomarkers

At baseline, 3-month and 6-month follow-up, soluble markers of inflammation (eg, hs-CRP, IL-6, IL-1β, TNF-α, MCP-1) will be assessed in cryopreserved plasma samples kept frozen at −80°C, using individually validated human cytokine ELISA (Roche Diagnostics, R&D Systems and Abcam)[23] with coefficient of variation <20% (<10% in most cases).[23] Since other novel biomarkers are regularly

being discovered by our group, all blood samples will be stored (with patient consent) to ensure the ability to assess future promising biomarkers.[24–32] Measurements will be made in duplicate.

Immunologic studies

We will collect whole blood at baseline and at 6 months into heparinised tubes. Peripheral blood mononuclear cells will then be isolated by Ficoll-Paque density centrifugation. Monocyte subpopulations, as well as their relative proportions, will be detected by flow cytometry using fluorochrome-labelled antibodies to CD14, and CD16.[18] Similar to other literature and our prior study, classical monocytes will be defined as CD14+CD16-, non-classical as CD14+CD16++ and intermediate as CD14++CD16+.[18 33 34] Monocyte subsets will be correlated with plaque imaging studies. They will also be compared with normal monocyte datasets derived from previous studies.

Quality of life

At baseline and at 6 months, the EQ5D will be administered to all participants.[35]

Study outcomes

The primary endpoint of this study is the change in the FDG TBR at 6 months as a proxy measure of arterial plaque inflammation in the MDS (the arterial segment with the highest TBR at baseline) in any vascular district imaged (either the left or right carotid artery or ascending aorta).

Secondary endpoints include (a) FDG TBR, FDG SUV and their change in the MDS of each vascular region: ascending aorta, left and right carotid artery; (b) hs-CRP and its change.

Exploratory endpoints include (a) plasma levels of other cytokines and inflammation biomarkers; (b) levels of activated monocytes; (c) major adverse cardiac events (MACE) (defined as the composite of ACS/MI, TIA, stroke or CV death)); (d) non-CV death (all events will be confirmed by an independent adjudication committee that is blinded to the treatment arm) and (e) quality-of-life outcomes measured on the EQ5D.[35]

Sample size

There are no previously published studies evaluating FDG over time in patients with T2DM or pre-diabetes who have suffered recent CV events (our study population) on which to base a definitive sample size calculation. We evaluated the ascending aorta as a non-culprit region in patients from our prior study[17] with diabetes and recent events where the aorta was in the field of view (n=6): TBR_{max}=3.38±0.60. In the culprit carotid arteries in patients with diabetes (n=12) TBR_{max} was 3.58±0.00.88.

Based on the literature and feedback from experts, the minimal clinically important difference (MCID) in TBR_{max} reduction is 10%–15%. This is feasible since FDG differences between treatments in prior studies was also 10%–14%.[20 21] Taking a conservative approach based on our TBR_{max} values in our patients with T2DM with recent events, for a 10% reduction compared with placebo with a power of 80%, alpha 0.05, we will need 50 patients per group. Considering 10% withdrawal, and 2% of patients who complete baseline measures but do not start study drug, 115 patients will be recruited (12/115 (10.4%) withdrawal and 3/115 (2.6%) not starting drug), both similar to the LoDoCo trial.[36] Prior studies have included 21–34 patients/group.[20 21 37 38] Thus, our sample size is expected to enable us to address our hypothesis.

Statistical analysis

Patient data will be entered and managed using REDcap[39] hosted at UOHI. Descriptive statistics (proportions, means, medians, SD, IQR) will provide summaries of study characteristics and insight into their distributional properties to determine if underlying assumptions of the planned analysis are satisfied. Additionally, we will create diagnostic plots and both graphically, as well as numerically, check to ensure that the assumptions of our regression model are satisfied. Our data will be analysed with an intention-to-treat analysis. Additionally, a per protocol analysis will be conducted as a key secondary analysis. No interim analyses are planned.

The primary outcome (change in FDG TBR) will be compared between the colchicine and placebo arms within a multiple regression model that includes type of vascular event (cardiac or cerebral) as a covariate.

To evaluate whether inflammation imaging or biomarker levels predict the inflammation response measured as the change in TBR_{max} on FDG PET/CT (as a secondary objective), we will conduct a regression analysis with baseline vascular TBR_{max}, baseline vascular SUV and inflammatory biomarkers included as covariates. The regression coefficient will be tested using the Wald test and 95% CI of the coefficient will be calculated. A Pearson correlation analysis to evaluate the association of the inflammatory response, as proxied on FDG PET/CT, to the change in hs-CRP levels from baseline to 6 months after treatment with colchicine will be conducted.

Several sources of bias and confounding exist within the trial. As a sensitivity analysis, for assessing the robustness of the primary analysis, if clinically significant differences between arms in pertinent baseline prognostic variables (eg, hypertension, HbA1C, diabetes vs pre-diabetes) are identified, multiple regression analysis will be conducted to compare FDG TBR between arms adjusting for these variables. As an exploratory analysis, we will look for interactions between treatment assignment, age, sex, vascular bed (carotid arteries vs aorta), time from CV event, type of vascular event (coronary (ACS/MI) vs cerebrovascular events (TIA, stroke)) and the medication classes of sodium-glucose Cotransporter-2 inhibitors and glucagon-like peptide 1 receptor agonists as covariates within the model both to assess for meaningful interactions of these variables with the primary outcome, but also to ensure the results remain stable after adjustment and ensure our results remain stable after adjustment for these potential confounders. Similar regression analysis strategies will be considered if other biomarkers are

also associated with an imaging response to colchicine. We will assess the relationship between the inflammatory response to colchicine on FDG/PET imaging with MACE by conducting a logistic regression analysis with MACE as the outcome variable and the change in vascular TBR_{max} as a covariate. We will also explore patient-centred quality of life outcomes between colchicine and placebo groups with Student's t-tests. Any missing data will be considered missing at random.

Patient and public involvement

A patient partner with lived experience was actively involved in the design and the ongoing conduct of the study. They currently serve on the steering committee of the study and are involved with regular meetings with respect to study conduct. Study results will be directly communicated to study participants. As part of our outcomes, we are assessing quality of life metrics, as advised by our patient partner.

DISCUSSION

Vascular inflammation is an important contributor to the progression of atherosclerosis and its resultant vascular sequelae, like MI, stroke and CV death.[1 3 40 41] An increasing evidence base has demonstrated that it is a novel therapeutic target for CV risk reduction. Colchicine has emerged as a novel anti-inflammatory agent for CV risk reduction. However, concerns have recently been raised regarding the adverse side effects of colchicine beyond its known gastrointestinal side effects, including a non-significant increase in the rate of infections and all-cause mortality. Thus, further research regarding the underlying mode of action will hopefully elucidate the role of colchicine and which patients may be best suited to receive this anti-inflammatory agent. Hence, our goal is to help clarify the underlying mechanism of action of colchicine, using a proxy of inflammation such as FDG-PET imaging as well as levels of circulating biomarkers, to assess local vascular anti-inflammatory vs systemic anti-inflammatory activity.

Our study design has several strengths. First, we will perform the study in a methodologically rigorous fashion by using a prospective, randomised, double-blinded, placebo-controlled design to answer our research question. Second, we will use advanced CV imaging methods, FDG-PET imaging, to investigate the underlying mechanism of action of colchicine. Trial results looking at the pharmacological impact on arterial inflammation as proxied by imaging with FDG PET/CT have mirrored the results of clinical CV outcome trials.[20 21] Therefore, FDG PET/CT inflammation imaging represents a practical method to define the mechanistic targets of a medication's effect in smaller patient cohorts over a short time period.[41] It may also be a useful tool to assist in identifying higher risk patients to improve patient selection for novel therapies. Third, as an exploratory outcome, we will look at the effect of colchicine therapy on quality-of-life outcomes. Finally, this is one of the first studies to use advanced imaging techniques to evaluate the underlying mechanism of action of colchicine for CV indications.

It is noteworthy that pharmaceutical agents which have shown outcome benefits have demonstrated reductions in inflammation measured by FDG PET including statins, pioglitazone and canakinumab, whereas as drugs which have not shown to reduce CV events have not shown such local anti-inflammatory effects. We anticipate in this high-risk group of patients in CADENCE, inflammation reduction will also be demonstrated. An added benefit of CADENCE and other studies is that they will support the utilisation of inflammation vascular imaging such as FDG PET to enable better selection of patients who benefit from inflammation targeted therapies. Further studies would be needed to support this concept.

Our study also has important limitations. First, as this is a mechanistic study, it is underpowered to evaluate for meaningful differences in clinical endpoints, and will be underpowered to correlate imaging and clinical endpoints. This may limit the clinical validity of our results. However, evaluation of pharmacological impact on arterial inflammation as proxied by imaging with FDG PET/CT have mirrored the results of clinical CV outcome trials in the past.[20 21 37] Thus, FDG indices of vascular inflammation serve as a good surrogate marker for important clinical endpoints, without necessitating the large size and scale of a trial looking at clinical endpoints. Second, our study recruits patients with both cardiac and neurologic vascular events; thus, there is the potential that any overall effect seen is confounded or mediated by the vascular bed treated (neurologic vs cardiac). To address this, as mentioned, we will perform interaction t-tests to compare the overall results of our regression analysis in the subgroup of patients with recent coronary (ACS/MI) vs cerebral vascular events (TIA, stroke). Additionally, interaction t-tests will be conducted to evaluate differences in the responses of each of the vascular beds separately (carotid vs aorta).

With a large portion of the population suffering from CV disease, discovering novel methods of treatment is of high clinical value. Anti-inflammatory therapies such as colchicine may fill this role, however for widespread adoption a thorough understanding of their underlying mechanism of action is needed. Furthermore, with an increasing recognition that many of these therapies, including colchicine, carry some increased non-CV risk, targeting higher-risk populations may tip the risk-benefit scale in favour of treatment with these agents. The results obtained from this study will provide essential information with respect to the underlying mechanism of action as well as clinical benefit of colchicine therapy in patients with high CV risk. We believe our findings will bridge an important knowledge gap in this area and will spark new research in cardiac imaging and anti-inflammatory therapies for CV disease.

CONCLUSION

Colchicine warrants further study to confirm the safety and benefit for patients with CV disease, as well to learn more regarding its mechanism of action. The results of this randomised control study will contribute to our understanding of the mechanism of action of colchicine by measuring the impact on plaque size, biomarkers and MACE outcomes. Results of this study will inform us as to whether additional studies with colchicine should be pursued.

Ethical and regulatory standards

This trial will be conducted in compliance with the protocol, current version of the Declaration of Helsinki, Good Clinical Practice, as defined by the International Conference on Harmonization where applicable. If revised versions of these documents are published during the course of the trial, the new version will be adopted.

Ethics approval and consent to participate

Before study initiation, this study was approved by the Ottawa Health Science Network Research Ethics Board and University of Alberta Health Research Ethics Board-Biomedical Panel), for the protocol, consent, study materials, study process. All participants are required to give informed written consent to participate in the trial. Consent will be obtained by CADENCE trial personnel.

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Contributors KEB, RB, PR and RAdK were involved with project conceptualisation. KEB, GAW, RB, RAdK, AT were involved with the design of the methodology. KEB, RB, PR, PMP, LG, AA, CL, CK and RAdK are involved in project administration. Trainee involvement in the project has been supervised by authors GAW and RB. Writing of the original protocol was performed by KEB, RB, GW, PR and RdK. Writing of the original manuscript draft was conducted by authors KEB and SS. Review and editing of the manuscript was conducted by KEB, SS, PR, RAdK, DD, AT, AT, AT, PL, HL, AYC, CT, EL, CW, AA, CL, LG, CK, PaMP, PoMP, ET, GAW and RB.

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Competing interests RB receives or has received honoraria from, and leads grants supported by GE Healthcare, Jubilant DraxImage and Lantheus Medical Grants. These are not related to the current work. RdK receives royalties from rubidium-82 PET technologies licensed to Jubilant Radiopharma and to INVIA Medical Solutions. He has also received research funding and honoraria from Jubilant Radiopharma and IONETIX. Otherwise, the authors declare that they have no competing interest.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	4
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	19
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	19
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	2
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	24

Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	SM
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6-7
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	7
Objectives	#7	Specific objectives or hypotheses	7-8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8, SM
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9

Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	SM
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	9-10
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	SM
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	13-14
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	21
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	14
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document	9

		that is unavailable to those who enrol participants or assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10-12
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	SM
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values).	SM

		Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14-15
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	15
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	SM
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	14
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	SM
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	SM
Ethics and dissemination			

Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	19
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	24
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	24
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	SM
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	24
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	24
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	9-10
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	SM
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	24-25
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	24

Appendices

Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	SM
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

Supplementary Material

Part 1:

CADENCE – Event Definitions

EVENT TERMS	DEFINITIONS
Acute Coronary Syndrome (ACS): (1)	ACS would include ST-elevation myocardial infarction, Non-ST-elevation myocardial infarction and unstable angina. For the purposes of this study, ACS should be adjudicated as an MI or Unstable Angina based on the definitions provided below. ACS itself should not be chosen as an event.
Myocardial Infarction: (1) **STEMI** **NSTEMI** **Type 1 MI**	Based on source documentation provided i.e. admission or discharge summary +/- ECG, labs etc. Would be ST-elevation in 2 contiguous leads PLUS criteria below (Type 1 MI) Would be definition below (Type 1 MI) but without ST-elevation Detection of a rise and/or fall of cTn values with at least one value above the 99th percentile URL and with at least one of the following: • Symptoms of acute myocardial ischemia • New ischemic ECG changes • Development of pathological Q waves • Imaging evidence of new loss of viable myocardium or new regional wall motion abnormalities in a pattern consistent with an ischemic etiology. Identification of a coronary thrombus by angiography including intracoronary imaging or by autopsy
Cardiac procedural myocardial injury: PCI procedural MI (1)	Occurring < 48 hours after index procedure with: • Elevation of cTN levels more than 5x the 99th percentile in patients with normal baseline levels • If levels elevated before procedure, post procedure enzyme should rise>20%. Absolute level still needs to be 5 x the 99th percentile URL PLUS ONE OF: • New ECG changes • Development of new Q waves • Imaging evidence of new loss of viable myocardium or new WMA on echo • Cath evidence of new occlusion, dissection, distal embolization
Cardiac procedural	Occurring < 48 hours after index procedure with:

myocardial injury: CABG procedural MI (1)	• Elevation of cTN levels >10 x 99th percentile URL in patients with normal baseline • If baseline abnormal they must rise>20% and still be >10 x URL PLUS ONE OF: • Development of new Q waves • Cath evidence of new graft occlusion or new native artery occlusion • Imaging evidence of new loss of viability or new WMA consistent with an ischemic etiology
Unstable angina: (1)	[New or worsening] symptoms of myocardial ischemia with or without ECG changes, but no elevation of cTn leading to hospital admission.
TYPE II MI *	Detection of a rise and/or fall of cTn values with at least 1 value above the 99th percentile URL, and evidence of an imbalance between myocardial oxygen supply and demand unrelated to acute coronary atherothrombosis, requiring at least 1 of the following: • Symptoms of acute myocardial ischemia • New ischemic ECG changes; • Development of pathological Q waves; • Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischemic etiology
Arrhythmia:	• Documented cardiac arrest • Multiple ICD shocks requiring admission • Documented ventricular tachycardia/fibrillation. • Documented new onset or worsening atrial fibrillation (or flutter) requiring IV therapy or increase in oral rate control medications. *Type of Arrythmia noted will be indicated as Cardiac Arrest, VT, VF, VT with ICD shock, VF with ICD shock, new AF, worsening AF*
Worsening heart failure: (2)	Hospital admission requiring IV diuretics plus at least one sign **AND** one symptom of worsening fluid overload. **Signs:** • Pulmonary rales • Elevated jugular venous pressure > 5 cm above sternal angle • Lower extremity edema • Chest x-ray demonstrating pleural effusion, pulmonary congestion, or cardiomegaly **Symptoms:** • Paroxysmal nocturnal dyspnea • Orthopnea • Dyspnea on mild or moderate exertion

Stroke: (3)	Ischemic stroke confirmed by CT or MRI imaging. Neurological deficit attributed to an acute focal injury of the central nervous system (CNS). All strokes will be considered events. The cause of stroke will be indicated using TOAST and categorized as follows: • Large vessel atheroscerosis • Small vessel disease • Cardioembolism • Other (ie dissections, malignancy, etc) • Undetermined
Transient Ischemic Attack:	TIA confirmed as a definite by a neurologist. Transient episode of neurologic dysfunction due to focal brain ischemia, without acute infarction or tissue injury.
Cardiovascular Death: (4)	Directly related to myocardial infarction, presumed arrhythmia, heart failure, or acute coronary syndrome, or related to stroke. Cardiac procedure related deaths will be captured as Cardiac-Other. For the purposes of this study such deaths will be defined as an in-hospital death during index admission for elective cardiac procedure. The death is due to complications resulting directly or indirectly from the cardiac procedure. **For the purposes of this study a death that occurs unexpectedly, out of hospital (at home) without prodrome of, or subacute symptoms of heart failure will be defined as a Cardiac Death-Presumed Arrhythmic.** **Specific CV cause will be defined – MI, ACS, Arrythmia, Presumed Arrythmia, Heart Failure, Stroke.** **Non-Cardiovascular Death – death not attributable to a Cardiovascular cause noted above.**

Supporting documentation required for review of cardiovascular clinical endpoints may include the following:

- Hospital Discharge or Death summary note
- Lab reports, ECGs
- Clinical consultations
- Death certificate
- Narrative summary from family, physicians for events occurring outside of the hospital

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Drug related AE's: Any other events deemed related to study drug based on the chart below (possibly, likely, and definitely)

Relationship between use of study drug and AE (Causality)					
AE (is)	**None**	**Unlikely**	**Possibly**	**Likely**	**Definitely**
Clearly the result of an external factor	Yes	No	No	No	No
Probably/possibly the result of another factor	No	Yes	Yes	No	No
Has a chronological relationship with the time of study drug administration and/or represents a known reaction to study drug	No	No	Yes	Yes	Yes
Disappears or decreases after discontinuation of the study drug	NA	NA	NA	Yes	Yes
Recurs on renewed administration (re-challenge)	No	No	NA	NA	Yes or NA*

AE=adverse event; NA=not applicable

* A rechallenge is not required; if done, rechallenge would be expected to be positive

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1. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, White HD and Executive Group on behalf of the Joint European Society of Cardiology /American College of Cardiology /American Heart Association /World Heart Federation Task Force for the Universal Definition of Myocardial I. Fourth Universal Definition of Myocardial Infarction (2018). *Circulation*. 2018;138:e618-e651.
2. Felker GM, Adams KF Jr., Konstam MA, et al**. The problem of decompensated heart failure: nomenclature, classification, and risk stratification**. *Am Heart J* 2003;145(2 Suppl):S18-25.
3. Sacco RL, Kasner SE, Broderick JP, Caplan LR, Connors JJ, Culebras A, Elkind MS, George MG, Hamdan AD, Higashida RT, Hoh BL, Janis LS, Kase CS, Kleindorfer DO, Lee JM, Moseley ME, Peterson ED, Turan TN, Valderrama AL, Vinters HV, American Heart Association Stroke Council CoCS, Anesthesia, Council on Cardiovascular R, Intervention, Council on C, Stroke N, Council on E, Prevention, Council on Peripheral Vascular D, Council on Nutrition PA and Metabolism. An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2013;44:2064-89.
4. Type 2 diabetes in adults: management: Evidence reviews for SGLT-2 inhibitors and GLP-1 mimetics. London: National Institute for Health and Care Excellence (NICE); 2018 Mar. (NICE Guideline, No. 28.) Appendix K, Cardiovascular definitions from CANVAS and CANVAS-R trial. Available from: https://www.ncbi.nlm.nih.gov/books/NBK550011/

Part 2:

CADENCE Inclusion and Exclusion Criteria

Inclusion Criteria	1. Type 2 Diabetes (on diet, oral hypo-glycemic agents and/or insulin) or pre-diabetes (defined by Diabetes Canada as HbA1C=6.0-6.45% or increased fasting blood sugar (FBS) (6.1-6.9 mmol/L) or impaired glucose tolerance (2hPG in a 75g OGTT(mmol/L0=7.8-11);(16) 2. Suffered a recent cardiovascular event (≤ 120 days post ACS (i.e. STEMI or nonSTEMI) or TIA/stroke with associated large vessel atherosclerotic disease confirmed on US, CT or MRI; 3. Stable symptoms and hemodynamics; 4. Aged ≥18 years; 5. Given informed consent. Standard definitions will be used for STEMI, NSTEMI,(17) and for ischemic stroke confirmed by CT or MRI and TIA confirmed by a neurologist.(18)
Exclusion Criteria	1. Planned revascularization of infarct or stroke related artery more than 120 days after the qualifying/index event; 2. Recent CV event likely to have been cardioembolic in the opinion of the neurologist or cardiologist; 3. Recent CV event likely to have been secondary to myocardial infarction with non-obstructive coronary arteries (MINOCA) in the opinion of the cardiologist; 4. Severe LV dysfunction (EF<30%); 5. Severe valve disease requiring intervention; 6. Decompensated heart failure; 7. Active infection (e.g. pneumonia, active skin infections, and on antibiotics); 8. Chronic diarrhea;

	9. Immune compromise (e.g. recurrent infection); 10. History of cancer within the last 3 years (other than a successfully treated cutaneous squamous cell or basal cell carcinoma or localized carcinoma in situ of the cervix). 11. Active inflammatory conditions (e.g. rheumatoid arthritis, chronic inflammatory bowel disease, SLE, systemic anti-inflammatory therapy (e.g. prednisone, methotrexate)); 12. Pregnancy (all women of child bearing potential will have a negative BHCG test; 13. Breastfeeding; 14. Women of childbearing potential who refuse to use two forms of contraception (this includes at least one form of highly effective and one effective method of contraception) throughout the study OR men capable of fathering a child who refuse to use contraception. 15. Glomerular filtration rate (GFR) <50 ml/min/1.72m;(3) 16. Use of potent p-glycoprotein inhibitors (i.e. systemic cyclosporine, clarithromycin, or systemic ketoconazole) or a strong CYP3A4 inhibitor (i.e. ritonavir, clarithromycin, or systemic ketoconazole); 17. Hemoglobin < 105(women) <110 (men) g/L; WBC < 3.0x 10(9)/L, platelet count< 110x 10(9)/L; 18. History of cirrhosis, chronic active hepatitis or severe hepatic disease or with alanine aminotransferase (ALT) levels greater than 3 times the upper limit of normal. 19. Unable to give informed consent. <u>Exclusion for CTA portion of the protocol:</u> Patients with dye allergy will not undergo CTA but will have PET/CT.

Part 3: Administration of CADENCE study

1.0 Data Safety and Monitoring Board (DSMB) for CADENCE study

The DSMB is responsible for assuring that study participants in "The **C**anadian Study of **A**rterial Inflammation in Patients with **D**iabetes and Recent Vascular Events: **E**valuatio**N** of **C**olchicine **E**ffectiveness (**CADENCE**)" are not exposed to unnecessary or unreasonable risks and that the study is being conducted according to the highest scientific and ethical standards. In order to carry out this responsibility, the DSMB will:

- Familiarize themselves with the Executive Committee's Terms of Reference (if applicable), study protocol (Appendix A), Investigator Brochure/Product Monograph, and manuals prior to the initiation of the study.
- Review descriptive reports at predetermined timelines (typically blinded data).
- Request unblinded data if there are any safety concerns.
- Provide recommendations for or comments regarding change(s) to the design or methodology of the clinical trial.
- Provide feedback on ancillary studies proposed by the Executive Committee /Investigators.
- Alert investigators regarding emerging procedural or ethical issues.
- Comment on the relevancy of new external published data from other trials that may impact on patient safety or efficacy of the study treatments.

At the request of the Study Chair (or Principal Investigator if no formal Study Chair), the DSMB may be called upon to:

- Provide recommendations to the Study Chair/PI and Executive Committee in order to facilitate the timely completion of the trial.
- Advise the Study Chair/PI of any and all factors (e.g., subject recruitment, protocol adherence and adverse events) that could potentially threaten the inferences that may be drawn from the final results of the trial.

This independent data and safety monitoring board will review safety data from the trial, and all Serious Adverse Events (SAE) and non-serious Adverse Events (AE) will be collected and reported in accordance with GCP.

2.0 Trial Steering Committee (TSC) for CADENCE study

The TSC for the CADENCE study is the executive decision-making group that considers the recommendations from the DSMB. Members of the TSC has both independent members as well as members who are responsible for the day-to-day conduct of the trial. Additionally, a community

member/former cardiac patient will serve on the Steering Committee to guide relevance and study conduct from the patients' perspective.

3.0 Adjudication Committee

An adjudication committee of experts who are not participating investigators/collaborators will independently review and adjudicate each clinical event blinded to treatment randomization. The committee will also review events to ensure they have been appropriately captured in terms of diagnosis, seriousness, expectedness, and the relatedness to the study drug. Adjudication Committee will be provided with definitions for adjudication of study events.

4.0 Patient Engagement

As mentioned, a patient will serve on the Steering Committee to guide relevance and study conduct from the patients' perspective.

Part 4: Additional protocol information

Study Drug Interruptions and Discontinuations

Participants may choose to stop the study medication at any time. The site investigator will discuss this decision with the patient and study personnel will continue to follow these patients, in the same manner as the other patients in the trial. Interruptions and discontinuations of study drug will be documented, along with the reason. Restarting medications will be discussed at follow-up visits and noted accordingly. Patients will continue to be followed and data collected accordingly. The below lists the circumstances where the study medication may be discontinued or subject withdrawn from the study:

- Patient is unable to tolerate study intervention
- Pregnancy
- Use of potent p-glycoprotein inhibitors or strong CYP3A4 inhibitors
- Impaired renal or liver function

Concomitant treatments

The following medications should not be used concomitantly with study drug:

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- Strong inducers of either CYP3A4 inhibitors (e.g. Clarithromycin, etc) or potent P-glycoprotein inhibitors (e.g. Cyclosporin, etc).
- It is advised to avoid grapefruit and grapefruit products.

Safety and Adverse Events

Adverse events will be captured from when the study intervention is started, to study exit. Serious adverse events will be reported to the Data Safety Monitoring Board, the Ethics Board, and Health Canada in accordance with good clinical practice (GCP).

Data Collection and Storage

Medical history and inclusion/exclusion criteria will be determined using hospital records and patient reported history. Clinical data relevant to the study objectives will be included as part of the study analysis.

All participant information and data will be collected respecting participant confidentiality and privacy and following written informed consent. Personal health information including medical history, medications, lab results, procedures, and test results will be collected for the purposes of this study.

Once we have obtained informed consent from a participant, they will receive a unique study identification (ID) number. This number will be the only identifier used on all study-related blood samples and patient study documents. Hospital unit numbers, Social Security numbers, phone numbers and addresses will not be used as coding mechanisms. A master list containing participants' full name and contact information will be kept by and stored in a separate file from the coded study dataset on a secured computer. Any Personal Health Information (PHI) or Personal Identifying Information (PII) that is collected will be kept in a confidential manner. Any subsequent release of PHI/PII information will only occur if it is required by law.

The research staff will collect data on case report forms (CRFs). The study data will be stored in a secure location accessible only by the investigators and/or study research staff. Any hard copies of CRFs and other paper records will remain on site and stored in a secured manner. Data captured on CRFs will be electronically entered into a RedCap database managed by UOHI research team. This database will be password protected and maintained on a secure internal server. No identifying information will be kept on any mobile devices, and no information will leave the hospital premises. As is required by law, the research files will be securely stored for 25 years after the study has been completed. At the end of this time, all records will be securely destroyed.

Coordinating Centre

The Ottawa Heart Institute Research Corporation will be the coordinating centre for this trial. The UOHI Cardiovascular Research Methods Centre (CRMC) will be responsible for randomization and for data

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management and analysis, and storage of data. The Centre will implement and maintain quality assurance procedures related to data generation. The UOHI Cardiac Imaging Research Core Lab will be the core lab for image analyses.

Protocol amendments

Any planned major protocol amendments will be communicated to relevant key stakeholders (including trial steering committee (TSC), Research Ethics Board (REB), Data Safety Monitoring Board, adjudication committee, etc).

Dissemination policy

Full trial results will be submitted for publication upon final follow-up and analysis. Those who have made substantive contributions to the design, conduct, interpretation, and reporting of CADENCE will be recognized through authorship on the final trial report.

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UNIVERSITY OF OTTAWA HEART INSTITUTE
INSTITUT DE CARDIOLOGIE DE L'UNIVERSITÉ D'OTTAWA

Main Informed Consent Form for Participation in a Research Study

Study Title: The **C**anadian Study of **A**rterial Inflammation in Patients with **D**iabetes and Recent Vascular Events: **E**valuatio**N** of **C**olchicine **E**ffectiveness (CADENCE)

OHSN-REB Number: 20190355-01H

Study Doctor: Dr. Rob Beanlands, University of Ottawa Heart Institute

Study Sponsor: Ottawa Hearth Institute Research Corporation (OHIRC)

Study Funder: Canadian Institute for Health Research (CIHR)

Emergency Contact Number (24 hours / 7 days a week): XXX

Non-Emergency contact numbers are noted at the end of this document under the section heading "Contacts".

INTRODUCTION

You are being invited to participate in a clinical trial (a type of trial that involves research). You are invited to participate in this study because you have diabetes or pre-diabetes and your doctor has diagnosed you with a recent vascular event. A vascular event may affect the heart or the brain. In the heart, blockages of the arteries may cause a condition called acute coronary syndrome (ACS). This is a sudden, reduced blood flow to the heart that may cause a heart attack or chest pain. In the brain, blockages in the arteries may cause temporary or permanent signs and symptoms of a stroke.

This consent form provides you with information to help you make an informed choice. Please read this document carefully and ask any questions you may have. All your questions should be answered to your satisfaction before you decide whether to participate in this research study.

The study staff will tell you about the study timelines for making your decision.

Taking part in this study is voluntary. You have the option to not participate at all or you may choose to leave the study at any time. Whatever you choose, it will not affect the usual medical care that you receive outside the study.

IS THERE A CONFLICT OF INTEREST?

The Study Doctor, Dr. Rob Beanlands, is receiving financial payment from the Canadian Institute of Health Research to cover the cost of conducting this study.

WHAT IS THE BACKGROUND INFORMATION FOR THIS STUDY?

Inflammation plays a role in the development of blockages in our arteries. Patients with diabetes are at a higher risk of developing these blockages. Inflammation can be measured with a simple blood test (markers) and an FDG PET/CTA scan (Fluorodeoxyglucose positron emission tomography with computed tomography angiography) which is a common nuclear imaging test. There are medications called anti-inflammatories, such as Colchicine, that may reduce

inflammation in the arteries. The usual treatment after a vascular event is to control the progression of disease. Drugs used to control risk factors and imaging to determine the extent of disease may be part of this treatment.

Colchicine is an approved anti-inflammatory drug used to treat types of arthritis or gout. It is not approved for treatment of inflammation of the arteries. FDG is an approved radioactive tracer, but is not routinely used for this purpose. Health Canada, the regulatory body that oversees the use of drugs in Canada, has approved the use of Colchicine and FDG in this study.

WHY IS THIS STUDY BEING DONE?
The purpose of this study is to find out if treatment with an anti-inflammatory drug affects inflammation markers in the blood or arteries. To do this, some of the participants in this study will get Colchicine and others will receive a placebo (a substance that looks like the study drug but does not have any active or medicinal ingredients). The placebo in this study is not intended to have any effect on your condition. A placebo is used to make the results of the study more reliable.

WHAT OTHER CHOICES ARE THERE?
You do not have to take part in this study in order to receive treatment or care. Please talk to your usual doctor or the study doctor about the known benefits and risks of these other options before you decide to take part in this study. Other options include drugs to slow the progression of the artery disease or having imaging tests to monitor the extent of your disease. Your usual doctor can also discuss with you what will happen if you decide not to undertake any treatment at this time.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?
It is anticipated that about 115 people will take part in this study, from research sites located in Canada. We expect approximately 85 people will participate from the University of Ottawa Heart Institute.

This study should take 3.5 years to complete and the results should be known in about 5 years.

WHAT WILL HAPPEN DURING THIS STUDY?

ASSIGNMENT TO A GROUP
If you decide to participate you will be "randomized" into one of the groups described below. Randomization means that you are put into a group by chance (like flipping a coin). There is no way to predict which group you will be assigned to. You will have an equal chance of being placed in either group. Neither you, the study staff, nor the study doctors can choose what group you will be in.

This is a double-blind study, which means that neither you, the study doctors, the study staff, nor your usual health care providers will know which group you are in. Your group assignment can be identified if medically necessary. Requests to reveal your assignment for your information or participation in other research studies will not be considered until this study has been completed and the results are known.

WHAT IS THE STUDY INTERVENTION?

Investigational/ Study Specific Intervention
If you agree to take part in this study you will be randomized to one of 2 groups:

Group 1: Standard care plus Colchicine 0.6 mg dose (active medicine).

Group 2: Standard care plus placebo (inactive medicine).

Regardless of the group to which you are assigned, you will take 1 tablet of the study drug (Colchicine or placebo) by mouth once a day for 6 months.

WHAT ELSE DO I NEED TO KNOW ABOUT THE STUDY INTERVENTION?
If you have side effects while you are on this study, the study doctor may make changes to the intervention. You will be asked to keep a diary of when you take the study drug each day and if you have had any side effects.

WHAT ARE THE STUDY PROCEDURES?

VISITS	**Screening Visit (if needed)**	**Baseline Visit (within 120 days of vascular event)**	**Phone Call 1 Week after Baseline Visit**	**Phone Call 1 Month after Baseline Visit**	**3 Month Visit**	**6 Month Visit**	**Phone Call 1 Week after 6 Month Visit**
Length of Visit	**1 hour**	**4-5 hours**	**15-20 minutes**	**15-20 minutes**	**1 hour**	**4-5 hours**	**5 minutes**
Informed Consent	**X**						
Randomization		**X**					
Medical History	**X**				**x**	**x**	
EQ-5D-5L Questionnaire		**X**				**X**	
Clinical Assessment	**X**				**X**	**X**	
Blood Tests	**X**	**X**			**X**	**X**	
PET Scan		**X**				**X**	
Checking Your Health and Medications			**X**	**X**			**X**

If a blood test is needed to ensure you are eligible for the study a screening visit will be arranged. Otherwise all screening procedures noted above will be completed at the baseline visit (start of the study).

Questionnaires

You will be provided with a questionnaire which will assess your quality of life at the baseline visit and the 6-month visit. The purpose of the questionnaire is to provide a profile of your health on that specific date and to measure changes in health status over time. Each questionnaire will take about only a few minutes to complete.

The information you provide is for research purposes only. Some of the questions are personal. You can choose not to answer questions if you wish.

Even though you may have provided information on a questionnaire, these responses will not be reviewed promptly by your health care team. If you wish them to know this information, please bring it to their attention.

Investigational Procedures
The following tests are considered investigational and will only be done for participants in this study:

FDG PET/CT with CTA Scan at baseline and 6 months (and if a repeat is necessary):
This scan will be used to detect inflammation in the blood vessels. A urine pregnancy test will be carried out on all females of child-bearing potential, on the morning of the scan to rule out pregnancy.

Preparation:
- On the day before the scan, you will be asked to avoid strenuous activity.
- You will receive specific instructions about your diet for the night before the scan based on your current diabetes medications.
- Then you will not eat or drink anything except for water, for 12 hours prior to the scan. You will be able to take your usual medication except for some diabetic medications.
- Your blood sugar will be monitored on the day of the scan. If your fasting blood sugar is too high, the scan will be postponed until your blood sugar is better controlled. You will be asked to hold your diabetic medication including insulin for 12 hours prior to the scan.

When you arrive, an IV (intravenous, small plastic tube), will be inserted into a vein in your arm. The radioactive tracer FDG will be injected through the IV then you will be asked to sit or lie quietly for 60 minutes. After the first 60 minutes you will have an additional 60-120 minutes of free time during which you may move around, read and talk. You will not be able to eat or drink, except for water, until after the scan has been completed. This waiting period will allow the FDG to be absorbed. A total of 2-3 hours after the injection you will be asked to lie still on the scanning bed with your arms by your side while the images are collected. Near the end of the scan an IV contrast or dye (Omniopaque), will be injected and the final set of images will be taken. Dye is routinely used to better highlight the difference between normal and abnormal areas of blood vessels. You will be in the scanner for approximately 45-60 minutes.

All patients undergo standard monitoring, such as blood pressure and heart rate measurements, and monitoring for possible side effects throughout the imaging procedure. All imaging procedures are performed under the supervision of an attending physician. The total time for this scan is approximately 4-5 hours In rare instances, the PET scan may be repeated due to technical issues, which would add another visit (4-5 hours) to the study procedures outlined above.

Blood test:
Blood samples will be collected at baseline, 3 and 6 months to look at inflammation in the blood. Samples at baseline and 6 months will also look at the immune system.

Non-Experimental/Standard Care Procedures
The following tests will be done as part of this study. Some of these tests may be done as part of your standard care, in which case the results may be added to the study data. Some of these tests may be done more frequently than if you were not taking part in this study and some may be done only for the purpose of the study. If the results show that you are not able to continue participating, the study doctor(s) will let you know.

Clinical Assessment:
You will be asked about your medical history and current medications, measurements such as blood pressure, height and weight will be collected.
Participant Diaries
You will be asked to keep a diary of when you take your study medication. Please record the

exact time you take your study drug in your daily diary. You will be asked to bring your diary to each follow up visit.

Blood test:
Blood tests such as kidney and liver function will be collected at baseline, 3 and 6 months to monitor your health. Other standard blood tests such as blood sugar and cholesterol levels will also be collected.

MANDATORY SAMPLE COLLECTION
The researchers doing this study need to do tests on samples to test for inflammation in your blood. The collection of these samples is a necessary part of this study. Samples will be used only for these purposes. The samples will not be sold.

Once these tests have been completed, any leftover samples will be destroyed.

Reports about any investigational research tests done with your samples will not be given to you, your doctor, or other health care provider(s). These reports will not be put in your medical records.

Blood Collection (Required)
Blood samples will be taken by inserting a needle into a vein in your arm. These will be taken at the same time as your standard of care tests whenever possible. At each visit approximately 35 ml (about 2 tablespoons) of blood will be drawn. Part of the blood sample will be sent to The Ottawa Hospital, General campus, Ottawa, Canada where it will be examined. The samples for future testing will be frozen and stored securely in the University of Ottawa Heart Institute, Ottawa, Canada for up to 10 years and used only for the purposes of this study

How will samples be identified?
To protect your identity, your samples will be labeled with a unique study ID number that cannot be traced back to your personal information. This unique study ID is recorded on a Master Participant List. This list is the only location where your personal identification is linked to your unique study ID. Only Dr. Beanlands' research team members would have access to this list.

Can I withdraw these samples?
If you no longer want your samples to be used in this research, you should tell the study doctor, who will ensure the samples are destroyed.

If tests have already been done on your sample(s) it will not be possible to withdraw those results. However, no further testing will be done.

WHAT ARE THE RESPONSIBILITIES OF STUDY PARTICIPANTS?
If you choose to participate in this study, you will be expected to:

- Tell the study doctor about your current medical conditions.
- Tell the study doctor about all prescription and non-prescription medications and supplements, including vitamins and herbals, and check with the study doctor before starting, stopping or changing any of these. This is for your safety as these may interact with the intervention you receive on this study.
- Complete all the required study components.
- Tell the study doctor if you are thinking about participating in another research study
- Return any unused study medication.
- Return any diaries that you take home to complete
- Tell the study doctor if you become pregnant.
- Avoid drinking/eating on the 2 visits where you will have your imaging tests. This is called

fasting. You will fast overnight for 12 hours prior to the scan.
- Avoid taking grapefruit or grapefruit juice for the duration of the study. Grapefruit interacts with the effects of colchicine.
- The study drug is for you alone, and must not be shared with others.

HOW LONG WILL PARTICIPANTS BE IN THE STUDY?
The study intervention will last for about 6 months. You will be seen at baseline, then at 3 month and 6 months after you signed the consent form at the Heart Institute. You will receive a follow-up phone call at one week and one month after your baseline visit. You will be asked about your health and the medications you are taking. You may be seen more often if the study doctor determines that this is necessary. No matter which group you are randomized to, and even if you stop the study intervention early, we would like to keep track of your health for the 6 month duration.

CAN PARTICIPANTS CHOOSE TO LEAVE THE STUDY?
You can choose to end your participation in this research (called withdrawal) at any time without having to provide a reason. If you choose to withdraw from the study, you are encouraged to contact the study doctor or study staff.

You may withdraw your permission to use information that was collected about you for this study at any time by letting the study doctor know. However, this would also mean that you withdraw from the study.

Information that was recorded before you withdrew will be used by the researchers for the purposes of the study, but no information will be collected after you withdraw your permission. We encourage you to continue the follow ups even if your participation from the study must be withdrawn.

CAN PARTICIPATION IN THIS STUDY END EARLY?
The study doctor may stop your participation in the study early, and without your consent, for reasons such as:
- You are unable to tolerate the study intervention
- You are unable to complete all required study procedures
- New information shows that the study intervention is no longer in your best interest
- The study doctor no longer feels this is the best option for you
- The Regulatory Authority/ies (for example, Health Canada) or the Ottawa Health Science Network Research Ethics Board withdraw permission for this study to continue
- If you plan to or become pregnant

If this happens, it may mean that you would not receive the study intervention for the full period described in this consent form. If you are removed from this study, the study doctor will discuss the reasons with you. Plans will be made for your continued care outside of the study.

WHAT ARE THE RISKS OR HARMS OF PARTICIPATING IN THIS STUDY?
You may experience side effects from participating in this study. Some side effects are known and are listed below, but there may be other side effects that are not expected. You should discuss these with the study doctor. The study doctor will watch you closely to see if you have side effects.

Colchicine Side Effects
The side effects reported are generally reversible by temporarily stopping treatment or lowering the dose of colchicine.

Risks and side effects related to Colchicine include:

Categories:
Very likely (21-100%)
- diarrhea

Less likely (5-20%)
- nausea and/or vomiting
- abdominal cramps or pain

Rarely (1-4%)
- fatigue
- headache
- gout (an inflammatory arthritis that causes foot pain)
- throat pain

A similar research study using Colchicine was recently done. It showed 10% of participants had stomach upset or a stomach intolerance to the drug which prompted discontinuation of the drug.

It is possible that other drugs, vitamins or herbals can interact with the study intervention. Other drugs can mean prescription or non-prescription. This can result in either the intervention not working as expected or result in severe side effects. Check with the study doctor before taking any new drugs or supplements. Rarely, Colchicine may increase the risk of muscle pain or muscle damage in people who are also taking a statin (cholesterol lowering) medication. Abnormal kidney function can increase this risk therefore if you have poor kidney function you will not be included in this study.

Colchicine should be taken only as directed. Do not chew, crush, or split the medication and keep it out of reach of children.

Blood Draw Risks
There are minor risks and discomforts related to an IV or blood sampling, including the possibility (5-20%) of bleeding, pain, and inflammation (redness and swelling) at the insertion site or leaking of the study drug from the vein into surrounding tissue after injection. These problems usually resolve easily or may require minor treatments, such as application of an ice-pack or slight pressure for a few minutes. The injection site will be monitored before and after the injection.

FDG PET /CT with CTA Risks
You will be required to lie still on the scanner bed with your arms above your head for each scan. You may develop some aching or soreness in your shoulders or arms which you may find uncomfortable.

Omnipaque Contrast Risks:
When the IV contrast dye is being given you may feel a slight burning at the IV site, a metallic taste in the mouth and a warm flushing throughout the body (very likely, 90 % of the time). This sensation is normal and will last only a few seconds. Mild allergic reactions, such as itching and hives are less likely (13% of the time). These reactions may pass without treatment or respond quickly to medication. Very rarely (less than 0.5%) more severe reactions such as breathing difficulty, swelling of the throat or swelling of other body parts can occur. Should you experience any side effects, you will be appropriately cared for and monitored in the Cardiac PET unit until

all symptoms resolve. If you have previously had a severe reaction or allergy to contrast dye, you will not have the CTA portion of the test.

Risk of Insurability
It is possible that your participation in research may affect your insurability under certain insurance policies.

WHAT ARE THE REPRODUCTIVE RISKS?
It is known that in early pregnancy exposure to radiation may harm the fetus and therefore PET scans are not performed in women who may be pregnant. In the event of pregnancy, or suspected pregnancy at the time of the scan, you must tell the study coordinator immediately and you must agree not to have the scan. Women who are pregnant or nursing are excluded from this study.

The effects of colchicine on an unborn or nursing baby are unknown. Studies in pregnant animals have shown that colchicine can harm an unborn or nursing baby. You must not become pregnant or father a baby while participating in this study. The study doctor/staff will discuss family planning with you to ensure that you do not become pregnant or father a baby during the study. Participants should discuss these risks with sexual partners of the opposite sex. Women should not nurse (breastfeed) a baby during the study because the study drug might be present in breast mild and could be harmful to a baby.

If you become pregnant or father a child during the study, you should immediately notify the study doctor/staff. The study doctor/staff will ask if you/your partner are willing to provide information about the pregnancy as part of this study. If your partner becomes pregnant, she will be given a separate consent document to sign to give permission for the collection of this information. You or your partner may choose not to give consent for the collection of this information or may withdraw consent at any time without giving a reason. This will not impact your participation in the study and will not result in any penalty or affect your or your partner.

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?
If you agree to take part in this study, the investigational intervention may or may not be of direct benefit to you. You will not benefit from the placebo used in this study. We hope the information learned from this study will help other people with the same condition in the future.

HOW WILL PARTICIPANT INFORMATION BE KEPT CONFIDENTIAL?
If you decide to participate in this study, the study doctors and study staff will only collect the information they need for this study. Records identifying you at this centre will be kept confidential and, to the extent permitted by the applicable laws, will not be disclosed or made publicly available, except as described in this consent document.

Authorized representatives of the following organizations may look at your original (identifiable) medical/clinical and study records at the site where these records are held, to check that the information collected for the study is correct and follows proper laws and guidelines.

- The Ottawa Health Science Network Research Ethics Board who oversees the ethical conduct of this study.
- Ottawa Heart Institute Research Corporation, to oversee the conduct of research at this location and as the Sponsor.
- Health Canada (because they oversee the use of drugs and radiopharmaceuticals in Canada)

Information that is collected about you for the study (called study data) may also be sent to the

organizations listed above. Your name, address, email, or other information that may directly identify you will not be used. The records received by these organizations may contain your age, gender, height, weight, postal code, occupation, education, religion and racial/ethnic origin. They may also contain information in your medical history, and clinical data collected about your participation in the study.

Studies involving humans sometimes collect information on race and ethnicity as well as other characteristics of individuals because these characteristics may influence how people respond to different interventions. Providing information on your race or ethnic origin is voluntary.

If the results of this study are published, your identity will remain confidential. It is expected that the information collected during this study will be published/presented to the scientific community at meetings and in journals. This information may also be used as part of a submission to regulatory authorities around the world to support the approval of the study intervention. The study data will be retained for 25 years.

Your de-identified data from this study may be used for other research purposes. If your study data is shared with other researchers, information that links your study data directly to you will not be shared.

Even though the likelihood that someone may identify you from the study data is very small, it can never be completely eliminated.

Blood samples leaving the UOHI will only contain a coded study number.

A copy of the consent form that you sign to enter the study may be included in your health record/hospital chart.

WILL FAMILY DOCTORS/HEALTH CARE PROVIDERS KNOW WHO IS PARTICIPATING IN THIS STUDY?

Your family doctor/health care provider will not be informed by the study team that you are taking part in the study. You can choose to let your family doctor/health care provider know, if you like.

WILL INFORMATION ABOUT THIS STUDY BE AVAILABLE ONLINE?

A description of this clinical trial/study will be available on http://www.clinicaltrials.gov. This website will not include information that can identify you. You can search this website at any time.

This research study can be found on the above listed website by using the clinical trial registration number NCT04181996.

WHAT IS THE COST TO PARTICIPANTS?

The study drug (Colchicine or placebo) will be supplied at no charge while you take part in this study. Participation in this study will not involve any additional costs to you or your private health care insurance. A parking voucher will be provided for any study related visits.

ARE STUDY PARTICIPANTS PAID TO BE IN THIS STUDY?

You will not be paid for taking part in this study. In the case of research-related side effects or injury, medical care will be provided by your doctor or you will be referred for the proper medical care.

WHAT ARE THE RIGHTS OF PARTICIPANTS IN A RESEARCH STUDY?

You will be told, in a timely manner, about new information that may be relevant to your willingness to stay in this study.

You have the right to be informed of the results of this study once the entire study is complete. The results of this study will be available on the clinical trial registry (see the "Will information about this study be available online" section for more details).

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to ensure that your privacy is respected.

By signing this form you do not give up any of your legal rights against the study doctor, sponsor or involved institutions for compensation, nor does this form relieve the study doctor, sponsor or their agents of their legal and professional responsibilities.

You will be given a copy of this signed and dated consent form prior to participating in this study.

WHAT IF RESEARCHERS DISCOVER SOMETHING ABOUT A RESEARCH PARTICIPANT?
If any new clinically important information about your health is obtained as a result of your participation in this study, you will be given the opportunity to decide whether you wish to be made aware of that information.

Incidental Findings
The scans performed as part of this research study are for research purposes only. The scans will not be reviewed by a physician for diagnostic purposes and are not intended to provide health information.

For study purposes, scans are typically read in batches and may not be reviewed until a much later time, even after your participation in the study has ended. Some scans may reveal an incidental finding (IF). An IF is a medical finding that is discovered unintentionally and is beyond the aim of the study. IFs are a risk and benefit of research. If an IF is discovered and you choose to be informed of it, this may make you feel anxious, lead to further tests to confirm or rule out a clinical problem or may turn out to be a false positive or provide uncertain results. However, some IFs may lead to important diagnoses that would have otherwise not been detected which could be a benefit to your health. If necessary, your medical records such as previous scan results may be reviewed in order to determine the importance of the IF.
For this study, only IFs with potential health or reproductive importance will be reported. If you so choose, you and your family or referring physician (when applicable) will be notified of this type of IF. If you choose to have your family or referring physician notified the finding will become a part of your permanent medical record. IFs of minor or uncertain clinical significance that are not anticipated to affect your health will not be reported.

WHOM DO PARTICIPANTS CONTACT FOR QUESTIONS?
If you have questions about taking part in this study, or if you suffer a research-related injury, you can talk to your study doctor, or the doctor who oversees the study at this institution. That person is:

Dr. Rob Beanlands	**XXX**
_____	_____
Principal Investigator Name	Telephone

If you have questions about your rights as a participant or about ethical issues related to this study, you can talk to someone who is not involved in the study at all. Please contact The Ottawa Health Science Network Research Ethics Board, Chairperson at XXX.

UNIVERSITY OF OTTAWA HEART INSTITUTE
INSTITUT DE CARDIOLOGIE DE L'UNIVERSITÉ D'OTTAWA

Study Title: The **C**anadian Study of **A**rterial Inflammation in Patients with **D**iabetes and Recent Vascular Events: **E**valuatio**N** of **C**olchicine **E**ffectiveness (CADENCE)

OHSN-REB Number: 20190355-01H

SIGNATURES

- All my questions have been answered,
- I understand the information within this informed consent form,
- I allow access to medical records and transfer of specimens and related personal health information as explained in this consent form,
- I do not give up any of my legal rights by signing this consent form,
- I agree, to take part in this study.

If an incidental finding in study scans or unexpected, significant abnormalities in blood tests are found, I agree that my family or referring physician can be informed of the result and of my study participation.
Yes_____ No_____ (Initial choice)

Signature of Participant /	Printed Name	Date
Signature of Person Conducting the Consent Discussion	Printed Name and Role	Date

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OHSN-REB Number: 20190355-01H
Participant Assistance

Complete the following declaration only if the participant is unable to read:

☐ The consent form was read to the participant. The person signing below attests that the study as set out in this form was accurately explained to the participant, and any questions have been answered.

Signature of Impartial Witness	Printed Name	Date

_____
Relationship to Participant

Complete the following declaration only if the participant has limited proficiency in the language in which the consent form is written and interpretation was provided as follows:

☐ The person signing below acted as an interpreter, and attests that this study as set out in the consent form is accurately sight translated and/or interpreted, and that interpretation was provided on questions, responses and in additional discussion arising from this process.

Signature of Interpreter	Printed Name	Date

CJC Open 5 (2023) 348–356

Original Article

Cost-Effectiveness of Colchicine for Recurrent Cardiovascular Events

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ABSTRACT

Background: Colchicine is an anti-inflammatory therapy with a low associated cost that has been shown in 2 large studies to reduce cardiovascular (CV) events, but its use is associated with side effects. The main objective for this analysis is to determine whether colchicine therapy is cost-effective for the prevention of recurrent CV events in patients who have suffered a myocardial infarction (MI).

Methods: A decision model was developed to estimate the healthcare costs in Canadian dollars and the clinical outcomes among patients who have suffered an MI and are treated with colchicine. Probabilistic

RÉSUMÉ

Contexte : La colchicine est un traitement anti-inflammatoire peu coûteux qui, selon deux grandes études, réduit le taux d'événements cardiovasculaires (CV), mais son utilisation est également associée à des effets secondaires. L'objectif principal de cette analyse était de déterminer si le traitement par la colchicine présente un rapport coût-efficacité intéressant en prévention des événements CV récurrents chez les patients ayant subi un infarctus du myocarde (IM).

Méthodologie : Un modèle décisionnel a été mis au point pour estimer les coûts des soins de santé en dollars canadiens et les issues cli-

Atherosclerotic cardiovascular (CV) disease is a leading cause of morbidity and mortality around the world.[1,2] Inflammation has long been known to play a key role in the progression of atherosclerosis and its complications, such as myocardial infarction (MI), stroke, and CV death. However, until recently, effective inflammatory treatment targets have remained elusive. Large trials in the area have been disappointing and have failed to show CV outcome benefits from therapies that target specific inflammation pathways, including the following: low-density lipoprotein (LDL) oxidation[3]; secretory and lipoprotein-associated phospholipase A2 (sPLA2 and LpPLA2)[4-6]; P38 mitogen-activated protein (MAP) kinase inhibitors,[7,8] methotrexate,[9] and P-selectin.[10]

Colchicine is an anti-inflammatory agent that has been around for centuries and is used to treat inflammatory conditions, such as gout, pericarditis, and juvenile idiopathic arthritis. In several large trials, colchicine has been shown to have CV outcome benefit.[11,12] However, its use also has potential negative effects, with trials showing a higher risk of infection in patients taking colchicine.[12]

Thus, although colchicine represents a promising novel therapeutic agent in the treatment of atherosclerotic CV disease, its use does have potential negative consequences, including increased overall cost to the healthcare system. Decision analysis combined with economic analysis allows us to determine the net benefit of a new technology by weighing the potential benefits of a novel therapy (in terms of improved health outcomes) against some of the drawbacks associated with its use (including potential side effects or increased healthcare system costs). Furthermore, it allows for the determination of whether a new technology will lead to an improvement in the overall health of the wider population (ie, whether it is cost-effective). The main objective of this study was to assess the cost-effectiveness associated with using colchicine for the prevention of recurrent CV events.

Methods

Decision problem

The specific decision problem that this study addresses is whether a Canadian healthcare payer should reimburse the cost of treatment with colchicine for the reduction of recurrent CV events in adult patients in Canada with a prior MI. To address this decision problem, a cost-effectiveness analysis was performed to compare colchicine to current standard-of-care therapies (ie, those that do not include anti-

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Markov modelling was used in combination with Monte Carlo simulation to derive expected lifetime costs and quality-adjusted life-years, which permitted the calculation of incremental cost-effectiveness ratios. Models were derived for both short-term (20 months) and long-term (lifelong) colchicine use in this population.

Results: **Long-term colchicine use was dominant over standard of care, with lower average lifetime costs per patient (CAD$91,552.80 vs $97,085.84) and a higher average number of quality-adjusted life-years per patient (19.92 vs 19.80). Short-term colchicine use also dominated over standard of care. Results were consistent over a range of scenario analyses.**

Conclusions: **Based on 2 large randomized controlled trials, treatment of patients post-MI with colchicine appears cost-effective, compared to the standard of care at the current price. Based on these studies and currently accepted willingness-to-pay thresholds in Canada, healthcare payers could consider funding long-term colchicine therapy for CV secondary prevention while we await results from ongoing trials.**

niques chez les patients qui ont subi un IM et qui sont traités par la colchicine. Un modèle probabiliste de Markov a été utilisé en association avec une simulation de Monte-Carlo pour obtenir le coût attendu pendant la vie et le nombre d'années de vie ajustées en fonction de la qualité, données qui ont permis de calculer les rapports coût-efficacité différentiels. Les modèles ont été adaptés pour l'utilisation de la colchicine à court terme (20 mois) et à long terme (à vie) dans la même population.

Résultats : **L'utilisation de colchicine à long terme donnait de meilleurs résultats que le traitement de référence, avec un coût moyen à vie par patient plus faible (91 552,80 $ CA c. 97 085,84 $ CA) et un plus grand nombre moyen d'années de vie ajustées selon la qualité (19,92 c. 19,80). L'utilisation à court terme de la colchicine donnait aussi de meilleurs résultats que le traitement de référence. Ces résultats ont été observés pour un éventail de scénarios analysés.**

Conclusions : **Selon deux grands essais randomisés contrôlés, le traitement par la colchicine des patients ayant subi un IM semble avoir un meilleur rapport coût-efficacité que le traitement de référence en fonction des coûts actuels. Selon ces études et les seuils de propension à payer actuellement acceptés au Canada, les payeurs pourraient envisager de financer le traitement à long terme par la colchicine en prévention d'événements CV secondaires, jusqu'à l'obtention des résultats des essais en cours.**

inflammatory therapies), from the perspective of the Canadian public healthcare payer over a lifetime horizon (specifically, from the perspective of the Ontario Ministry of Health and Long-Term Care). Both long-term (lifelong) and short-term (20 months) treatment options with colchicine were considered.

Model overview

A probabilistic Markov cohort model was used to derive the estimated direct costs (ie, healthcare-related costs) and health outcomes (life-years and quality-adjusted life-years [QALYs]) among patients who have suffered an MI and are treated with colchicine. The dose of colchicine of 0.5 mg orally taken daily was used as the treatment dose of choice, per the results of the **Col**chicine **C**ardiovascular **O**utcomes **T**rial (COLCOT) and **Lo**w-**Do**se **Co**lchicine (LoDoCo 2) studies,[11,12] and we created and compared models with both short-term colchicine use (20 months), and long-term (lifelong) colchicine use.[13] We used a lifetime study horizon (400 cycles of 1-month duration each, equating to 33.33 years) to analyze the base-case costs (in Canadian dollars [CAD$]) and utility values from the perspective of the Canadian publicly funded healthcare system, and we incorporated certain model assumptions that were based on previously published literature.[14] The net present value of future costs (in CAD$) and QALYs was determined using a 1.5% discount rate per the guidelines outlined by the Canadian Agency for Drugs and Technologies in Health (CADTH).[15] A Markov model combined with probabilistic analysis allowed estimation of costs (2022 CAD$) and QALYs, which then allowed for the calculation of incremental cost-effectiveness ratios (ICERs). In the reference case, uncertainty regarding the value of each parameter was incorporated within the probabilistic analysis. Methodological uncertainty was explored by comparing the reference case results to those from non-reference-case analyses using discount rates of 0% and 3%, per CADTH directives.[15]

The model was developed in Excel (Microsoft, Redmond, WA), and we completed the Impact Inventory from the Second Panel on Cost-effectiveness in Health and Medicine (Supplemental Table S1).[16]

Decision model

We developed a Markov model to follow a hypothetical cohort of patients who had suffered an MI (Fig. 1). Base states in the model included a stable state, a stable-after-recurrent-MI state, a stable-after-stroke state, and a death state. With each cycle, patients entered the event model, which included the following event states: stable; recurrent MI; stroke; non-MI revascularization; pneumonia (potential side-effect of colchicine); gout flare; and death. From all events except gout, patients could either recover or experience death, and patients would then return to the appropriate base state following the event. Patients experiencing a gout flare all returned to their appropriate base state following the event. The model generated cycle-specific estimates of costs and QALYs, thereby allowing for the calculation of lifetime costs and QALYs.

Patient population

The cohort of patients was assumed to mirror the patient population within the COLCOT and LoDoCo 2 trials evaluating the impact of colchicine on CV outcomes.[11,12] The mean age of participants was 62 years, and patients had a history of MI with treatment including antiplatelet agents, statins, beta-blockers, and angiotensin-converting enzyme inhibitors. This treatment is similar to the treatment regimens in the COLCOT and LoDoCo2 trials and to treatment given in clinical practice.

Model inputs

Short- and long-term colchicine was evaluated against current standard-of-care therapies post-MI. Colchicine therapy has several perceivable disadvantages, including the upfront cost, an increased risk of pneumonia for patients, and a potential signal for increased mortality. Per the drug regimens used in the COLCOT and LoDoCo2 trials, all patients received standard-of-care therapies, with patients receiving colchicine being administered 0.5 mg orally every day. Strategies of both short-term (20 months) and long-term colchicine therapy were considered, separately, in the model.

Survival probabilities associated with standard-of-care therapy, as well as colchicine treatment, are summarized in Table 1, and they were derived from the COLCOT and LoDoCo 2 studies, in which a total of 10,267 patients were randomly assigned to either placebo or colchicine treatment. The uncertainties around these probabilities were incorporated into the probabilistic analysis.

In the base-case analysis, we assume that patients continue treatment beyond the 5-year time horizon of the clinical trials and that the benefit of treatment is maintained long-term. This decision was made based on the fact that within the LoDoCo2 trial, the survival benefit was stable at 5 years and showed no evidence of treatment waning.[11] Scenario analyses addressed the impact of these assumptions. In a scenario analysis, we adopted a 20-month time horizon, which allowed estimation of the proportion of the forecasted QALY gains for colchicine. which were generated after the period covered by the COLCOT trial (short-term colchicine use model), as well as a 49-month time horizon, covering approximately the follow-up period of the COLCOT and LoDoCo2 trials combined. These analyses, which examine the impact of extrapolation, are recommended in the CADTH guidelines.[15] Per the CADTH guidelines, in a further scenario analysis, we assessed the impact of treatment discontinuation by assuming that after both 20 months and 49 months, respectively, patients would discontinue treatment with colchicine with no continued effect after discontinuation.

The costs (in CAD$) associated with each treatment strategy are summarized in Table 2. The monthly costs associated with colchicine administration strategies and the costs of cardiovascular events, complications, and admissions were obtained from the literature and converted to 2022 CAD$ based on the Bank of Canada inflation calculator.[17-20] Similarly, we applied costs for standard-of-care treatment for coronary heart disease from published literature,[21] and we again converted to 2022 CAD$ based on the Bank of Canada inflation calculator.

Utility values were derived from a variety of sources. Utility values associated with the base state of post-MI status were taken from an analysis and comparison of the **Moni**toring Trends and Determinants in **Ca**rdiovascular Disease/Kooperative Gesundheitsforschung in der Region Augsburg (MONICA/KORA) registry to the general population.[22] Utility values for the recurrent MI state were obtained from literature that investigated the stability of time-tradeoff utilities in survivors of MI.[23] Utility values for the recurrent MI and post-stroke state were applied to patients indefinitely following their respective event (ie, stroke or recurrent MI). Finally, disutility values associated with adverse events were determined from expert opinion, and they were applied to only the specific cycle during which the adverse event occurred.[24]

Model outputs and analysis

Markov modeling was used to calculate mean total healthcare costs and total QALYs gained for each strategy. Cycle-specific estimates were summed and discounted over the lifetime horizon, to provide estimates of total costs and QALYs. Probabilistic analysis using Monte Carlo simulation with 5000 replications was performed to account for uncertainty in the input values, providing an estimate of the expected values for costs and QALYs. Cost-effectiveness was assessed by estimating the incremental cost per QALY gained against a base willingness-to-pay threshold of $50,000 CAD/QALY. This threshold was chosen based on a previous statement by the Assistant Deputy Minister for Ontario.[25] The threshold value for a QALY should reflect the marginal efficiency of the healthcare system to which the result is related. Empirical work related to this value for Canada has not been conducted, although an analysis for the United Kingdom healthcare system suggests that an appropriate threshold may be between $20,000 and $30,000 per QALY.[26] A higher cost-per-QALY threshold would suggest that the Canadian system is either less efficient or better funded than the United Kingdom system.

Model validity

Face validity was confirmed with experts in the field. Internal validation was conducted by the senior health economist reviewing the model structure and coding. Validity of our model was tested by comparing event rates from the COLCOT and LoDoCo2 trials with the risk from our simulated colchicine and standard-of-care cohorts at 23 and 49 months of elapsed follow-up time, respectively. To assess the external validity of our model, we compared events in the standard-of-care arm from the **C**anakinumab **An**ti-inflammatory **T**hrombosis **O**utcomes **S**tudy (CANTOS) trial (a similar study population) to results obtained from our standard-of-care model cohort at 22.6 months of follow-up (which was the median follow-up time from the CANTOS trial).

Results

With respect to the validity of our model, cumulative mortality estimates from our model were similar to the results in both the COLCOT and LoDoCo2 trial results.

Results of our probabilistic analysis found that compared to standard of care, long-term colchicine use was dominant (Table 3; Fig. 2). This strategy was associated with a slightly lower discounted life expectancy (23.62 years vs 23.75 years) but a reduced lifetime incidence of MI, revascularization, stroke, and gout flare (Table 4). Long-term colchicine use was associated with lower healthcare costs than those for the standard-of-care group—$91,552.80 vs $97,085.84 (Table 3). The incremental reductions in costs with long-term colchicine use ($5533.04) were almost exclusively due to the lifetime costs of colchicine ($2640.36), which was more than offset by the reduction in MI and revascularization events. Cumulative QALYs were 19.92 in the long-term colchicine

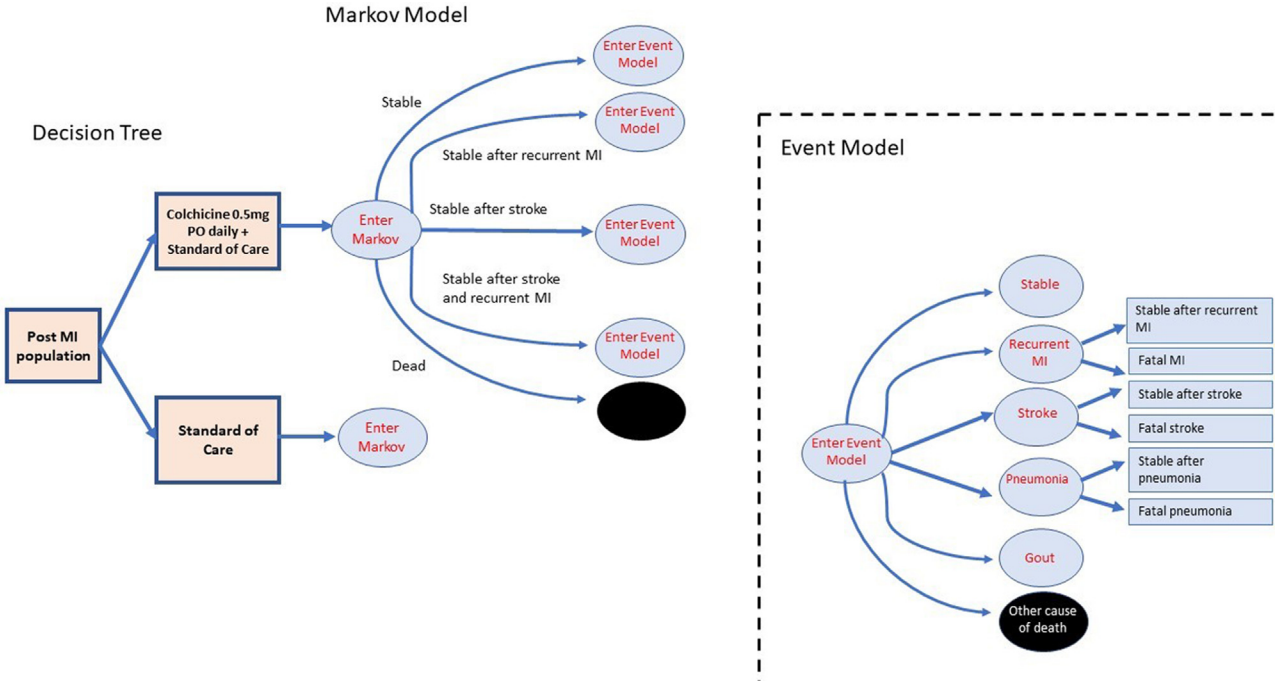

Figure 1. Markov model. A survivor of myocardial infarction (MI) can be assigned to treatment with colchicine at 0.5 mg oral (PO) daily and standard of care, or standard of care alone (decision tree). Within each cycle, a survivor of MI can then either remain stable, suffer a recurrent MI, suffer a stroke, have a coronary revascularization procedure performed, suffer a pneumonia infection, suffer a gout attack, or remain in the death state. Following nonfatal recurrent MI, stroke, or pneumonia events, patients then cycle back to their stable states. Standard-of-care therapies following an MI include antiplatelet agents, statins, and a beta-blocker.

use group, and 19.80 in the standard-of-care group, respectively.

In our probabilistic analysis, we found that, compared to standard-of-care treatment, short-term colchicine treatment was also dominant (Table 3; Fig. 2). It was associated with a slightly higher discounted life-expectancy (23.77 years vs 23.75 years). Short-term colchicine use was associated with lower healthcare costs, compared to those in the long-term

Table 1. Distributions for probabilistic analysis

	Expected value[Reference]	Probability distribution
Event probabilities		
Standard care		
Recurrent MI in acute phase	0.041[12]	Beta (98, 2281)
Recurrent MI in chronic phase	0.042[11]	Beta (116, 2644)
Non-MI revascularization in acute phase	0.021[12]	Beta (50, 2329)
Non-MI revascularization in chronic phase	0.064[11]	Beta (177, 2583)
Pneumonia in acute phase	0.004[12]	Beta (9, 2337)
Pneumonia in chronic phase	0.022[11]	Beta (55, 2705)
Noncardiovascular death in acute phase	0.008[12]	Beta (20, 2359)
Noncardiovascular death in chronic phase	0.007[11]	Beta (23, 207)
Colchicine		
Pneumonia in acute phase	0.009[12]	Beta (21, 2309)
Pneumonia in chronic phase	0.017[11]	Beta (46, 2716)
Death		
Post-revascularization	0.01[33]	Beta (840, 128422)
From MI	0.04[34]	Beta (31, 759)
From infection	0.07[13]	Beta (23, 207)
From stroke	0.18[35]	Beta (20, 0.1)
Hazard ratio (colchicine vs standard care)		
Recurrent MI in acute phase (colchicine)	0.77[12]	Lognormal (0.77, 0.61)
Recurrent MI in chronic phase (colchicine)	0.7[11]	Lognormal (0.7, 0.53)
Non-MI revascularization in acute phase (colchicine)	0.5[12]	Lognormal (0.5, 0.31)
Non-MI revascularization in chronic phase (colchicine)	0.75[11]	Lognormal (0.75, 0.6)
Noncardiovascular death in acute phase (colchicine)	0.98[12]	Lognormal (0.98, 0.64)
Noncardiovascular death in chronic phase (colchicine)	1.21[11]	Lognormal (1.21, 0.86)

MI, myocardial infarction.

Table 2. Cost and utility data

	Base-case estimate[Reference]	Probability distribution
Monthly costs, CAD$		
Standard care	$238[21]	Gamma (18,221.67, 0.01)
Colchicine	$9	Fixed
Stroke	$130[30]	Gamma (61.38, 2.11)
Cost for event, CAD$		
MI	$20,410[17]	Gamma (10,652.67, 1.92)
Revascularization	$31,771[18]	Gamma (16, 1985.66)
Stroke	$10,003[30]	Gamma (7668, 12,668)
Nonfatal pneumonia admission	$10,585[20]	Gamma (16, 2646.25)
Fatal-pneumonia admission	$30,773[20]	Gamma (7261.44, 4.24)
Gout flare	$172[31]	Gamma (16, 10.76)
Utilities		
Stable standard of care	0.868[22]	Beta (3531.89, 537.11)
Stable recurrent MI	0.734[23]	Beta (14.68, 5.32)
Utility toll for stroke	−0.178[32]	Beta (0.134, 0.223)
Utility toll for MI / revascularization / pneumonia admission	−0.0986[expert opinion]	Beta (1385, 12657)

Expert opinion: utility toll from healthcare professional opinion.
CAD$, Canadian dollars; MI, myocardial infarction.

colchicine use group—$96,636.33 vs $97,085.84 (Table 3). Cumulative QALYs were 19.86 in the short-term colchicine group, and 19.80 in the standard-of-care group, respectively (Table 3).

In a separate comparison, long-term colchicine therapy was found to dominate over short-term use, with lower lifetime costs and higher lifetime QALY gains, as above.

At a willingness-to-pay threshold for a QALY of $50,000, long-term colchicine use had a 72.2% probability of being cost-effective (ie, the ICER was greater than $50,000 in 72.2% of the 5000 simulations). Short-term colchicine use had a 25.8% probability of being the most cost-effective strategy (Fig. 3).

Scenario analyses relating to discount rates demonstrated consistency with our primary analysis (Table 4). Our results remained stable when alternative discount rates of 0% and 3% were used. In the scenario analysis with reduced time horizons of 20 and 49 months, estimated incremental QALY gains were only 0.01, suggesting that less than 17% of the forecasted QALY gains from colchicine use occurs during the initial time periods representative of the COLCOT and LoDoCo2 trials, respectively. In a scenario analysis in which patients stopped colchicine therapy at 20 or 49 months, respectively, results remained unchanged, with both short-term and long-term colchicine therapy continuing to show dominance over standard-of-care treatment.

Discussion

In this cost-effectiveness analysis, we evaluated the cost-effectiveness of short-term and long-term colchicine use against the current standard-of-care therapies for the secondary prevention of CV events, within the context of the Ontario public healthcare system. Our comparison results

Table 3. Cost-effectiveness results for base analysis and scenario analyses

Base case and scenario analyses	Lifetime costs, CAD$	Lifetime QALYs	ICER: colchicine vs standard of care
Base case			
Standard of care	$97,086	19.80	
Long-term colchicine	$91,553	19.92	Dominant
Short-term colchicine	$96,636	19.86	Dominant
Discount rate 0%			
Standard of care	$121,938	24.83	
Long-term colchicine	$115,082	24.96	Dominant
Short-term colchicine	$113,736	24.91	Dominant
Discount rate 3%			
Standard of care	$78,389	16.16	
Long-term colchicine	$74,171	16.24	Dominant
Short-term colchicine	$73,173	16.21	Dominant
48-month time horizon			
Standard of care	$15,274	3.36	
Long-term colchicine	$14,582	3.37	Dominant
Short-term colchicine	$14,952	3.37	Dominant
20-month time horizon			
Standard of care	$6121.38	1.41	
Colchicine	$5865.58	1.42	Dominant
Allowance for discontinuation with treatment			
Standard of care	$97,087	19.79	
Long-term colchicine	$96,161	19.86	Dominant
Short-term colchicine	$96,394	19.84	Dominant

CAD$, Canadian dollars; ICER, incremental cost-effectiveness ratio.

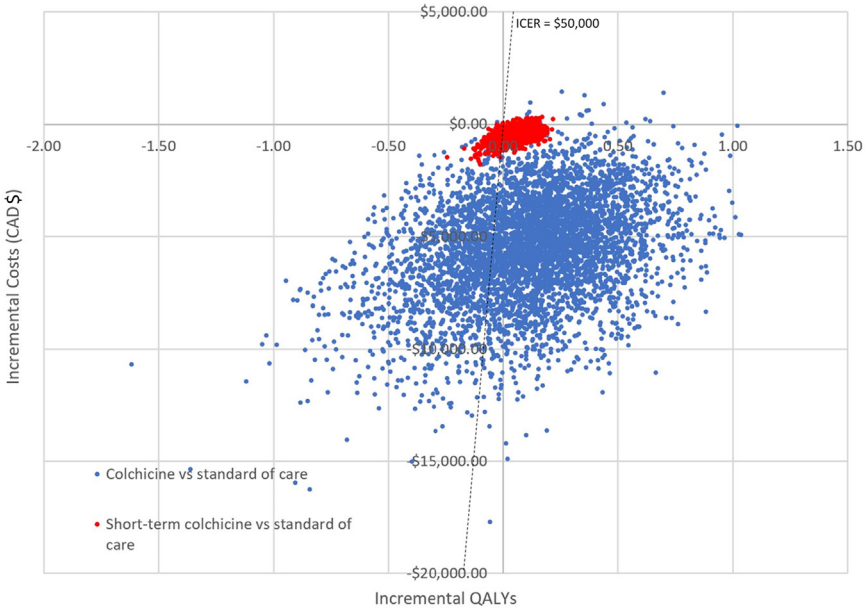

Figure 2. Scatterplot of incremental costs (in Canadian $s [CAD$]) against incremental quality-adjusted life-years (QALYs) gained. Standard-of-care therapies following a myocardial infarction include antiplatelet agents, statins, and a beta-blocker. **Dashed line** represents a cost-effectiveness threshold of $50,000/QALY.

demonstrate that both short-term and long-term colchicine usage are cost-effective strategies in this area, as both were dominant over current standard-of-care treatment. The predominant driver of lower costs with colchicine therapy was the reduction in CV events, which made up for the marginal increase in costs associated with the drug itself. In our prespecified willingness-to-pay threshold of $50,000/QALY, long-term colchicine use also emerged as a dominant option over short-term use, with lower overall costs and higher QALY gains.

In recent years, the targeting of inflammation for reduction of atherosclerotic CV disease has become a topic of great interest.[13,27,28] As inflammation is recognized as playing an important role in the pathogenesis of atherosclerotic CV events,[29,30] much attention has been focused, with varying success, on trying to identify potential anti-inflammatory agents that could be utilized to reduce CV risk for patients. The anti-inflammatory medication canakinumab represented a breakthrough in this domain, as the CANTOS trial demonstrated the benefit of reduction of CV events in patients who had suffered a previous MI and who had a high residual inflammatory burden.[13] However, due to the high medication cost, the trial did not demonstrate cost-effectiveness in multiple analyses,[24,31] largely due to the high cost associated with the medication. Following this trial, much attention was focused around trying to find alternative anti-inflammatory agents that could fill the void. Colchicine, a medication with a low-associated cost and a long track record as a commonly used anti-inflammatory agent, seemed to hold promise in this regard.[11,12]

Several pivotal trials released following the CANTOS trial demonstrated clinical benefit of colchicine in reducing CV events. In the COLCOT trial, colchicine use in patients with recent MI led to a significantly lower risk of ischemic CV events than did placebo.[12] Separately, the LoDoCo2 study showed that in patients with chronic coronary disease, the risk of CV events was significantly lower among those who received 0.5 mg of colchicine once daily than it was among those who received placebo.[11]

Colchicine is not without side effects, however. Some studies, including LoDoCo2 have found a small and potentially non-statistically significant increase in all-cause mortality.[11,32] Additionally, one smaller study found a statistically significant signal for increased total mortality with colchicine use.[32] A signal for a potential increase in pneumonia events with colchicine use also has been found.[12] Thus, although the list of current medications used for secondary prevention in patients who have suffered an MI is well established, whether colchicine should be added to this domain is certainly not. Additionally, if colchicine were to become part of guideline-directed medical therapy, whether it should be used for a short-term course after a CV event, or long-term, is not clear The cost-effectiveness of the drug certainly needs to be considered in this clinical decision-making process, particularly in publicly funded healthcare systems. Further research is currently being conducted (NCT03048825) that evaluates colchicine use for CV indications, and it will further explore the potential associated increase in all-cause mortality. This information certainly will help shed further light on this area, to determine if colchicine truly remains a dominant treatment option in this domain.

By conducting a probabilistic analysis, we were able to incorporate a wide range of uncertainty into our model, thereby improving the robustness of our results. Our analyses incorporated the potential increase in all-cause mortality but still support the conclusion that the use of colchicine was worthwhile. As evidenced by our study, the overwhelming probability is that colchicine is cost-effective over standard care, using our prespecified willingness-to-pay threshold of $50,000/QALY, and this conclusion remains consistent even

Table 4. Disaggregated results

	Long-term colchicine	Short-term colchicine	Standard of care
Incidence			
Recurrent MI—nonfatal	0.325	0.448	0.457
Recurrent MI—fatal	0.013	0.018	0.019
Revascularization—nonfatal	0.503	0.667	0.677
Revascularization—fatal	0.003	0.003	0.004
Pneumonia—nonfatal	0.158	0.193	0.188
Pneumonia—fatal	0.017	0.021	0.021
Stroke—nonfatal	0.053	0.076	0.079
Stroke—fatal	0.002	0.004	0.004
Gout	0.222	0.531	0.551
Costs, CAD$			
No new event	$68,522	$69,334	$69,434
Colchicine	$2640	$183	N/A
Recurrent MI—nonfatal	$5323	$7292	$7468
Recurrent MI—fatal	$217	$297	$304
Revascularization—nonfatal	$12,536	$16,587	$16,894
Revascularization—fatal	$82	$83	$111
Pneumonia—nonfatal	$1328	$1621	$1605
Pneumonia—fatal	$426	$520	$503
Stroke—nonfatal	$32	$619	$659
Stroke—fatal	$16	$30	$32
Gout	$30	$72	$75
Total costs	$91,553	$96,636	$97,086
QALYs	19.92	19.86	19.80
Life-years	23.62	23.77	23.75

CAD$, Canadian dollars; MI, myocardial infarction; N/A, not applicable; QALY, quality-adjusted life-years.

with much lower willingness-to-pay thresholds. However, whether a short-term or long-term strategy of colchicine treatment should be employed is a much more nuanced decision problem. Long-term colchicine therapy provided a slightly higher number of cumulative QALYs, with a higher cost that was largely related to the cost of long-term use of the medication. However, long-term use was still deemed to be a cost-effective strategy at the accepted willingness-to-pay threshold of $50,000/QALY.

Limitations

Our model had several important limitations, and our results should therefore be interpreted within the context of the data inputs and modelling assumptions. First, the efficacy of colchicine was based on only 2 single randomized controlled trials, which limits the generalizability of our results. However, at this point in time, these trials encompass the largest body of phase 3 evidence on the subject, and they currently provide the evidence available for decision-makers. The results

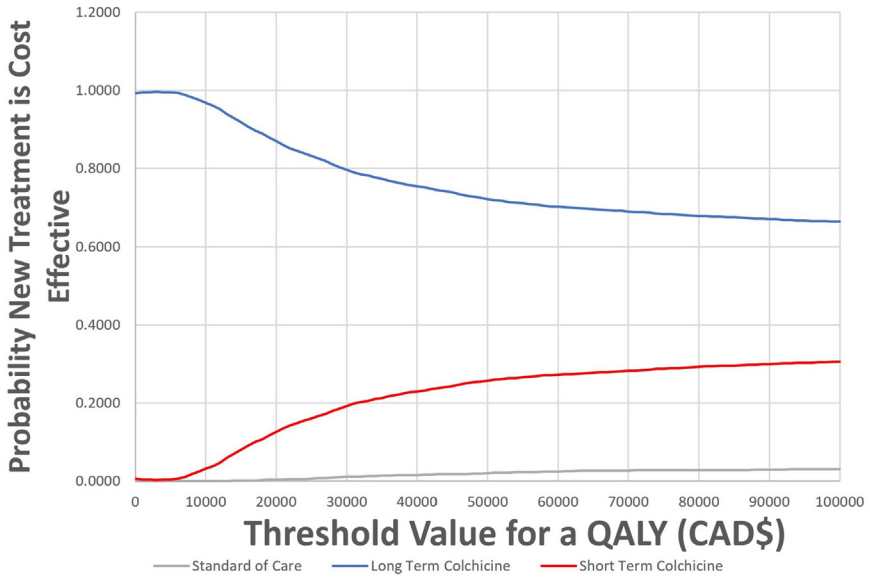

Figure 3. Cost-effectiveness acceptability curve for the probability that each treatment strategy is cost-effective against the threshold value for a quality-adjusted life-year (QALY). Standard-of-care therapies following a myocardial infarction include antiplatelet agents, statins, and a beta-blocker.

of this analysis strongly suggest that, given the current data on relative effectiveness, colchicine therapy is highly likely to be cost-effective in this context, at its current price. However, with the concerning signal regarding mortality associated with colchicine use, the results of ongoing randomized controlled trials are needed to help clarify this issue further before use of colchicine is adopted into guidelines. The uncertainty regarding the potential signal for mortality is reflected within the scatterplot of incremental costs against incremental QALYs gained, shown in Figure 2. As further data emerge, this balance will clarify the risks (or lack thereof) associated with colchicine use, and subsequently reduce the amount of uncertainty within the model. Our scenario analyses adopted assumptions related to discontinuance of treatment, and an assumption of waning treatment effect, that were not favourable toward colchicine use, and despite this, colchicine remained a cost-effective option for this indication. The median follow-up for the COLCOT and LoDoCo2 studies were 23 and 29 months, respectively, but we assumed therapy would be effective for a much longer period. This assumption potentially was an overly optimistic estimate of the long-term effects of colchicine therapy. We addressed this issue by performing a scenario analysis lof a waning of treatment effect, as well as discontinuation of therapy, and our results remained consistent. Finally, as the trials did not present sex-stratified results, we were unable to conduct sex-stratified models within our analysis. However, given the small sex differences seen in the incidence of cardiovascular events, we expect that our conclusions would not change with the addition of sex-stratified analyses.

Conclusions

Treatment with colchicine for secondary prevention of CV events is likely cost-effective in the Canadian healthcare system. Both short-term and long-term therapy with colchicine were dominant over standard care in the reduction of CV events. In our analysis, long-term colchicine therapy emerged as a cost-effective option over short-term use, with an ICER of $46,241.17. Ongoing trials (NCT03048825, NCT048 48857, NCT04181996) are certainly needed and will shed additional light on the potential harms associated with colchicine use. Given this uncertainty, a strong recommendation for colchicine use for CV indications is difficult to make until further data are available. Future investigations of anti-inflammatory drugs targeted at atherosclerosis must continue to consider the balance of effectiveness and economic impact on patients and the healthcare system.

Ethics Statement

The research reported has adhered to the relevant ethical guidelines.

Funding Sources

The authors have no funding sources to declare.

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Supplementary Material

To access the supplementary material accompanying this article, visit *CJC Open* at https://www.cjcopen.ca/ and at https://doi.org/10.1016/j.cjco.2023.02.005.

Anti-inflammatory effect of biologic therapy in patients with psoriatic disease: A prospective cohort FDG PET study

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Aim. **The aim of the study was to evaluate the changes in central vascular inflammation measured by FDG PET and myocardial blood flow reserve (MFR) determined by ^{82}Rb PET following therapy with biologic agents for 6 months in patients with psoriatic arthritis (PsA) and/or cutaneous psoriasis (PsO) (group 1) and compare with PsO subjects receiving non-biologic therapy (group 2) and controls (group 3).**

Methods and Results. **Target-to-background ratio (TBR) by FDG PET in the most diseased segment of the ascending aorta (TBR_{max}) was measured to assess vascular inflammation. ^{82}Rb PET studies were used to assess changes in left ventricular MFR. A total of 34 participants were enrolled in the study (11 in group 1, 13 in group 2, and 10 controls). A significant drop in the thoracic aorta uptake was observed in the biologic-treated group (ΔTBR_{max}: − .46 ± .55)**

Jacob Karsh and Girish Dwivedi—Co-senior authors.

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compared to the PsO group treated with non-biologic therapy (ΔTBR_{max}: .23 ± .67). Those showing response to biologic agents maintained MFR compared to who showed no response.

Conclusion. **In a cohort of psoriasis patients treated with biologics, FDG uptake in the thoracic aorta decreased over the study period. Patients who demonstrated a significant anti-inflammatory response on FDG PET imaging maintained their MFR compared to non-responders. (J Nucl Cardiol 2023;30:1642–52.)**

Key Words: Psoriatic arthritis • inflammation • PET imaging • cardiovascular disease

See related editorial, pp. 1653–1655

INTRODUCTION

Psoriatic arthritis (PsA) is a disease of chronic inflammation and occurs in 14-30% of patients with skin or nail psoriasis (PsO).[1,2] Similar to other diseases of chronic inflammation, patients with PsA or PsO have higher than expected rates of cardiovascular (CV) disease and an increased risk of major adverse cardiovascular events (MACE).[3,4] Indeed, CV disease is the single leading cause of death in patients with PsA and dermatologic psoriasis and may account for > 50% of all deaths in these populations.[5]

Systemic inflammation is thought to be the key mediator in the link between psoriasis and CV disease.[6] Patients with psoriasis are in a state of chronic immune activation and have increased levels of inflammatory cytokines, such as tumor necrosis factor (TNF)-α.[7] Interestingly, data have linked psoriasis severity with the degree of systemic inflammation.[8] Chronic inflammation has a negative effect on the vasculature and plays a pivotal role in the mediation of the peripheral microvascular dysfunction that is seen in patients with psoriasis.[9] A better understanding of the pathophysiology and potential treatment options behind the accelerated atherosclerosis and inflammation that is seen in patients with psoriasis remains a priority.

F-18-fluorodeoxyglucose positron emission tomography (FDG PET) provides an attractive and pragmatic means to measure and follow vascular inflammation in these patients, as it is the most widely validated and applied imaging probe in this regard.[10–12] Previous studies have shown that its uptake predicts MACE;[13–15] and it is also highly sensitive to short-term treatment effects.[16,17] Furthermore, PET myocardial perfusion imaging in conjunction with rubidium-82 (^{82}Rb) affords the ability to assess regional myocardial blood flow to the left ventricle in absolute terms ($mL \cdot min^{-1} \cdot g^{-1}$).[18] Rubidium-82 PET has been established as a nuclear imaging method for accurate, reproducible, and routine quantification of myocardial blood flow and myocardial blood flow reserve [MFR (stress/rest perfusion)] in humans and in animal models of disease.[19–22]

Patients with psoriasis, especially those requiring systemic biologic therapy, have increased vascular inflammation.[8] We hypothesized that it is increased central vascular inflammation in these patients that is contributing to the development of accelerated atherosclerosis. We sought to use FDG PET to quantify vascular inflammation in the ascending aorta in patients with PsA and PsO and hypothesized that thoracic aortic vascular inflammation would improve in these patients following therapy with biologic agents compared to psoriasis patients receiving non-systemic therapies and control patients with non-inflammatory skin/joint disease. Secondly, we sought to explore changes in MFR over time after treatment with biologic agents and explore the relationship between changes in vascular inflammation, as measured by FDG PET, to changes in MFR determined by ^{82}Rb PET.

METHODS

This was a prospective cohort clinical study designed to determine the effect of biologic therapy on vascular inflammation and MFR in patients with PsA compared to cohorts not treated with biologic therapies. Study approval was obtained from the University of Ottawa Heart Institute's Research Ethics Board in accordance with the principles of Declaration of Helsinki. All study participants provided written informed consent.

We studied 3 patient groups which were consecutively enrolled: (i) patients with PsA and/or PsO who were to be started on anti-TNF-α, anti-interleukin (IL)-17, or anti-IL-12/23 therapy biologic agents, (ii) patients with psoriasis managed on non-biologic therapies, and (iii) control patients with non-inflammatory skin or joint conditions. Participants were enrolled from the Rheumatology and Dermatology Clinics at the Ottawa Hospital, Ottawa, Ontario, Canada. A convenience sample was used, and all participants underwent ^{82}Rb PET and FDG PET examinations at baseline and at 6 months. Demographic and anthropometric data including age, sex, ethnicity, height, weight, waist circumference, and blood pressure were recorded as well as current medications, medical history, smoking status, and family history of CV disease.

Group 1 consisted of subjects with PsA and/or PsO scheduled for initiation of anti-TNF-α therapy, anti-IL-17, or anti-IL-12/23 therapy with prior failed response to traditional disease-modifying anti-rheumatic drugs (DMARDs). After inclusion in the study, subjects in

Group 1 received 6-month treatment with a biologic therapy following the baseline study investigations. Patients with PsA were included if they met the classification criteria for PsA[21] and had persistent joint inflammation measured as 5 or more swollen joints, despite therapy for at least three months, each with 2 or 3 conventional DMARDs (methotrexate, leflunomide, or sulfasalazine). To minimize the effect that duration of the disease could play on the results, only patients with a recent diagnosis of PsA were included (i.e., patients that had a diagnosis of PsA for > 3 months and < 2 years).[23] The treatment method chosen for patients was decided by the patient's treating rheumatologist.

Group 2 included subjects with psoriasis on non-biologic treatment (i.e., topical medications, acitretin, or phototherapy only). Psoriasis Area and Severity Index (PASI) score was used for the assessment of severity of dermatological psoriasis.[24]

Group 3 included patients with an established diagnosis of non-inflammatory joint diseases, such as osteoarthritis. These control patients were identified by a detailed history and physical examination, with normal baseline blood tests (full blood count, renal function, fasting glucose, and lipid profile).

Primary outcome: FDG PET imaging of ascending aortic inflammation

The primary outcome of our study was the change in vascular inflammation in the ascending aorta, which was measured according to standardized methods[16] as the target-to-background ratio (TBR) in the most diseased segment of the ascending aorta at baseline compared to 6-month follow-up (TBR_{max}). All patients underwent FDG PET with low-dose computed tomography at baseline and then following 6-month follow-up. Patients in group 1 started on biologic agents following their baseline FDG PET. FDG PET images were analyzed to determine TBR_{max} values by utilizing previously published and validated methodology.[16] In short, we first obtained the maximum standardized uptake value (SUV) for the most diseased segment of the ascending aorta by averaging the SUV for 3 consecutive axial slices centered on the highest uptake slice and the adjacent slices superior and inferior to it, providing approximately 1 cm of the most inflamed section of the aortic wall. We then calculated the TBR_{max} by determining the ratio of this SUV_{max} for the most inflamed region of the ascending aorta to background venous activity, derived from an image region in the superior vena cava. On the follow-up scan in the treatment group, the same 3 slice locations were used to calculate the follow-up TBR_{max}. Of note, the FDG PET analysis was performed by an independent reader blinded to clinical details.

Secondary outcome: FDG PET imaging of other aortic inflammation

As a secondary analysis, we also evaluated the change in vascular inflammation in other areas of the aorta, including the aortic arch and descending aorta. Similar to the methods outlined above for the ascending aorta, we measured this as the TBR in the most diseased segment of the respective portion of the aorta (arch or descending aorta) at baseline compared to 6-month follow-up (TBR_{max}).

Secondary outcome: ^{82}Rb PET for MFR

Dynamic ^{82}Rb PET was performed according to previously published and validated methodology.[25,26] In short, absolute myocardial blood flow was calculated at rest and during dipyridamole stress using our FlowQuant© analysis software. The ^{82}Rb analysis was performed by an independent reader blinded to clinical details.

Post hoc analysis: MFR

To better understand the relationship of changes in vascular inflammation to microvascular function, in a post hoc analysis we evaluated the change in MFR values in the subgroup of PsA patients treated with biologic agents. We compared the MFR of patients in the biologic group that had a response in their TBR_{max} (that was greater or equal to the median delta TBR_{max} of this group) to those that had a response that was less than the median (i.e., post hoc analysis for effect modification).

Statistical methods

Descriptive statistics (such as median with interquartile range (IQR) for continuous variables, frequencies with percentages for categorical variables) are used to summarize the three study groups' baseline demographic and clinical variables. Continuous variables are presented as medians with IQR and categorical variables are presented as frequencies and percentages (unless otherwise stated). Demographic characteristics are presented as median (IQR) or percentage as appropriate. Differences between patient groups were evaluated by the Fisher exact test for discrete clinical variables and by the Kruskal–Wallis test for continuous variables. Follow-up data were collected as scheduled. All the tests were two-sided and a P value of $< .05$ was

considered statistically significant. Within-group testing of changes in baseline and 6-month TBR_{max} and left ventricle (LV) MFR values were conducted with Wilcoxon Signed Rank testing. The relative changes in TBR_{max} and LV MFR over the study period were compared between groups using a generalized linear mixed effects model (GLMM) for repeated measures analysis. Variables included in the model were age, body mass index, sex, and baseline aortic TBR_{max}. Missing data were considered missing at random and complete case analysis was used to handle the missingness. We also performed multiple imputation as a sensitivity analysis to assess the effect of missingness on our results using a GLMM with baseline and follow-up TBR_{max} included in the model (thereby adjusting for differences in baseline aortic inflammation across groups). Statistical analyses were performed using MedCalc for Windows version 12.0 (MedCalc Software, Ostend, Belgium) and Stata/IC for Windows version 16.1 (StataCorp LLC, TX, USA).

RESULTS

A total of 42 participants were enrolled in the study (12 PsA and/or PsO patients who were started on biologic agents, 18 PsO patients treated with non-biologic therapies, and 12 patients in the non-inflammatory control group with osteoarthritis). In the biologics group, 5 patients were started on an anti-TNF-α agent (4 adalimumab and 1 etanercept), 4 on an IL-17 inhibitor (secukinumab), and 2 on an IL-12/23 inhibitor (ustekinumab). One patient in the biologic group dropped out after having an abnormal imaging study that required further intervention, 5 patients in the PsO group not on biologic therapies withdrew after baseline imaging studies, and 2 patients in the control group withdrew after baseline imaging studies. This resulted in a total of 34 patients having complete baseline and follow-up imaging data for analysis: 11 PsA and/or PsO patients started on biologic agents, 13 PsO patients treated with non-biologic therapies, and 10 patients in the non-inflammatory control group with osteoarthritis. Additionally, all patients (including patients that withdrew/were lost to follow-up) were included in the sensitivity analysis with multiple imputation.

Participant characteristics are described in Table 1. In summary, 64.7% of participants were men, and the median age was 62 years (IQR: 48, 69), which was similar between the three groups. Median BMI was 28.7 $kg{\cdot}m^{-2}$ (IQR: 27.1, 36.3). More patients in Group 1 had diabetes ($P = .032$), were more commonly treated with Methotrexate ($P = .002$), or on medications, such as oral steroids ($P < .001$) and non-steroidal anti-inflammatory drugs (NSAIDs) ($P = .002$).

Inflammation imaging results

Baseline ascending aortic TBR_{max} was not statistically different between the three groups: 2.84 (IQR 2.51, 3.37), 2.73 (IQR 2.54, 3.28), versus 2.70 (IQR 2.68, 2.76) in the biologics, non-biologic, and control groups, respectively ($P = .725$). Similarly, baseline aortic arch TBR_{max} did not differ across the groups ($P = .565$) and nor did baseline descending aortic TBR_{max} ($P = .190$). For the primary outcome, analysis within groups with Wilcoxon Rank Sum testing demonstrated a statistically significant reduction in vascular inflammation measured as FDG TBR_{max} within the ascending aorta, in the biologic group only [baseline 2.84 (IQR 2.51, 3.37), follow-up 2.50 (IQR 2.27, 2.94) ($P = .033$)]. There were no significant changes in either the non-biologic [baseline 2.73 (IQR 2.54, 3.28), follow-up 2.95 (IQR 2.64, 3.63) ($P = .279$)] or control groups [baseline 2.70 (IQR 2.68, 2.76), follow-up 2.94 (IQR 2.67, 3.28) ($P = .114$)] (Table 2 and Fig. 1). Similarly, there were statistically significant decreases in measured FDG TBR_{max} within the aortic arch ($P = .002$) and descending aorta ($P = .007$) within the biologic group, but not the other groups ($P > .05$). Table 2 illustrates the change in aortic TBR uptake in the ascending aorta as well as change in MFR of the three groups. GLMM for repeated measures analysis revealed the change in FDG TBR_{max} over the study period in Group 1 differed significantly from Group 2 ($\beta \pm$ SE: .697 ± .245, $P = .004$) and Group 3 ($\beta \pm$ SE: .670 ± .259, $P = .010$). This was true for the aortic arch and descending aorta as well. Sensitivity analysis with multiple imputation for missing FDG TBR_{max} values showed the results remained significant after imputation (biologic group versus non-biologic group: $\beta \pm$ SE: .651 ± .230, $P = .005$); biologic group versus control group: $\beta \pm$ SE: .631 ± .261, $P = .016$). The change in FDG uptake from baseline to follow-up is shown in Fig. 2.

Regarding the exploratory outcome, baseline MFR was not statistically different between the three groups: Baseline MFR was 2.96 (IQR 2.38, 3.46), 3.14 (IQR 2.54, 3.95), and 3.43 (IQR 2.84, 4.34) in the biologic, non-biologic, and control groups, respectively ($P = .528$). Analysis within groups with Wilcoxon Rank Sum testing demonstrated a statistically significant reduction in MFR in the biologic group only [baseline 2.96 (IQR 2.38, 3.46), follow-up 2.85 (IQR 2.20, 3.06) ($P = .037$)]. There were no significant changes in either the non-biologic [baseline: 3.14 (IQR 2.54, 3.95), follow-up: 3.58 (IQR 3.08, 3.94) ($P = .279$)] or control groups [baseline: 3.43 (IQR 2.84, 4.34), follow-up: 3.92

Table 1. Baseline characteristics of population

Variable	PsA and/or PsO on Biologics (n = 11)	PsO on non-biologics (n = 13)	Control (n = 10)	P value
	Median (IQR) or n (%)			
Age, years	62.0 (44.0, 64.5)	57.0 (47.5, 64.5)	63.5 (55.0, 73.0)	.218
Male sex, n (%)	5 (45%)	8 (62%)	7 (70%)	.525
BMI, $kg{\cdot}m^{-2}$	35.3 (28.7, 41.4)	29.6 (27.3, 33.6)	28.2 (25.0, 29.4)	.067
Hypertension, n (%)	5 (45%)	5 (38%)	1 (10%)	.186
Diabetes, n (%)	3 (27%)	0 (0%)	0 (0%)	.032**
Dyslipidemia, n (%)	3 (27%)	5 (38%)	4 (40%)	.793
Current smoking, n (%)	9 (91%)	8 (62%)	5 (50%)	.299
PASI of patients with PsO	(n = 2) 20.0 (10.4, 29.6)	7.0 (5.7, 10.2)	N/A	.132*
CRP, $mg{\cdot}L^{-1}$	4.10 (1.33, 4.98)	1.90 (.95, 5.38)	1.80 (1.10, 3.20)	.634
Other non-biologic medications/treatments				
NSAIDs, n (%)	5 (45%)	0 (0%)	0 (0%)	.002**
Steroids, n (%)	7 (64%)	0 (0%)	0 (0%)	.0001**
Methotrexate, n (%)	5 (45%)	0 (0%)	0 (0%)	.002**
Topical agents, n (%)	7 (64%)	10 (77%)	5 (50%)	.406
Acitretin, n (%)	1 (9%)	0 (0%)	0 (0%)	.341
Phototherapy, n (%)	2 (18%)	8 (62%)	0 (0%)	.004**

BMI, body mass index; *CRP*, c-reactive protein; *NSAIDs*, non-steroidal anti-inflammatory drugs; *PASI*, psoriasis area sensitivity index; *PsA*, psoriatic arthritis; *PsO*, cutaneous psoriasis; *TBR*, target-to-background ratio *Mann–Whitney test used to compare PASI values of groups **Statistically significant findings across groups on Kruskal–Wallis or Fisher exact testing

(IQR 2.97, 4.38) (P = .114)] (Table 2). GLMM for repeated measures analysis revealed no statistically significant differences in the change in MFR over the study period between the biologic group and non-biologic group ($\beta \pm$ SE: .477 ± .370, P = .198) and control group ($\beta \pm$ SE: .467 ± .395, P = .237), respectively.

Sensitivity analysis with multiple imputation for missing ascending aortic FDG TBR_{max} values showed that results remained non-significant even after imputation (biologic group versus non-biologic group: $\beta \pm$ SE: .522 ± .407, P = .201; biologic group versus control group: $\beta \pm$ SE: .384 ± .456, P = .400).

While GLMM for repeated measures analysis revealed no significant difference in the change in MFR between the three groups over the study period, to better understand the relationship of changes in vascular inflammation to microvascular function, we decided to further evaluate the change in MFR in the subgroup of PsA patients treated with biologic agents in a post hoc analysis. We compared the MFR of patients in the biologic group who had a response in their TBR_{max} that was greater or equal to the median of this group to those who had a response that was less than the median. We found a significant difference in these groups of patients. The group with a clinical response to biologic agents as measured by a change in TBR_{max} that was greater or equal to the median change (7%) maintained MFR (3.40 ± 1.23 MFR to 3.5 ± 1.2 MFR over 6 months) when compared to the group with a below median response on TBR_{max} which had a drop in MFR (2.9 ± .8 MFR to 2.2 ± .6 over 6 months; P = .03). Spearman's coefficient of rank correlation (rho) between change in TBR_{max} and change in MFR was .709 (P = .0146)

Table 2. Changes in TBR_{max} and LV MFR by treatment group during trial period

	PsA and/or PsO on biologics (n = 11)	PsO on non-biologics (n = 13)	Control (n = 10)	*P* value
Median (IQR) or n (%)				
Baseline ascending aorta TBR_{max}	2.84 (2.51, 3.37)	2.73 (2.54, 3.28)	2.70 (2.68, 2.76)	.725
Follow-up ascending aorta TBR_{max}	2.50 (2.27, 2.94)	2.95 (2.64, 3.63)	2.94 (2.67, 3.28)	.102
Delta ascending aorta TBR_{max}	− .19 (− .92, .11)*,****	.32 (− .26, .75)*	.21 (− .18, .58)*	.021**
Baseline ascending aorta SUV_{max}	3.51 (3.08, 4.16)	3.63 (3.02, 4.72)	3.61 (3.00, 3.96)	.803
Follow-up ascending aorta SUV_{max}	3.26 (2.67, 3.47)	3.86 (3.02, 4.37)	3.51 (3.33, 3.82)	.102
Delta ascending aorta SUV_{max}	− .42 (− .83, − .07)***	.23 (− .62, .57)	.08 (− .61, .74)	.103
Baseline aortic arch TBR_{max}	2.76 (2.66, 3.06)	2.62 (2.43, 3.28)	2.87 (2.68, 3.12)	.565
Follow-up aortic arch TBR_{max}	2.47 (2.12, 2.85)	2.89 (2.51, 3.19)	2.97 (2.44, 3.24)	.079
Delta aortic arch TBR_{max}	− .43 (− .63, − .29)*,****	.16 (− .47, .52)*	.20 (− .31, .48)*	.029**
Baseline aortic arch SUV_{max}	3.60 (3.07, 4.08)	3.13 (2.79, 4.10)	3.59 (3.54, 4.15)	.640
Follow-up aortic arch SUV_{max}	3.04 (2.46, 3.33)	3.57 (3.05, 4.01)	3.50 (3.10, 4.12)	.068
Delta aortic arch SUV_{max}	− .53 (− .91, − .24)***	.29 (− .17, .73)	− .31 (− .46, .46)	.045**
Baseline descending aorta TBR_{max}	2.73 (2.47, 3.46)	2.71 (2.39, 3.14)	3.25 (2.84, 3.35)	.190
Follow-up descending aorta TBR_{max}	2.25 (2.01, 2.54)	2.78 (2.59, 3.15)	3.22 (2.77, 3.66)	.002**
Delta descending aorta TBR_{max}	− 57 (− .79, − 0.17)*,****	.07 (− .24, .24)*	.12 (− .47, .50)*	.011**
Baseline descending aorta SUV_{max}	3.70 (2.83, 3.98)	3.41 (3.01, 3.80)	3.82 (3.66, 4.33)	.099
Follow-up descending aorta SUV_{max}	2.85 (2.46, 3.04)	3.46 (3.20, 4.00)	3.64 (3.34, 3.96)	.002**
Delta descending aorta SUV_{max}	− .74 (− .95, − .27)***	.20 (− .18, .52)	− .29 (− .50, .02)	.012**
Flow data				
Baseline LV MFR	2.96 (2.38, 3.46)	3.14 (2.54, 3.95)	3.43 (2.84, 4.34)	.528
Follow-up LV MFR	2.85 (2.20, 3.06)	3.58 (3.08, 3.94)	3.92 (2.97, 4.38)	.035**
Delta LV MFR	− .24 (− .46, − .02)***	.13 (− .38, .73)	.30 (− .83, .96)	.246
Baseline rest SBP	127 (104, 140)	134 (117, 142)	110 (102, 127)	.098
Baseline rest HR	68 (58, 75)	72 (66, 82)	63 (55, 67)	.023**
Baseline rest flow	.80 (.58, .80)	.89 (.72, 1.0)	.62 (.52, .71)	.061

Table 2 continued

	PsA and/or PsO on biologics (n = 11)	PsO on non-biologics (n = 13)	Control (n = 10)	*P* value
Baseline stress flow	1.79 (1.64, 2.71)	2.66 (2.40, 2.92)	2.12 (1.89, 2.62)	.127
Baseline stress SBP	135 (123, 141)	141 (125, 145)	117 (110, 140)	.049***
Baseline stress HR	90 (83, 95)	96 (87, 102)	80 (77, 87)	.020***
Follow-up rest SBP	133 (111, 145)	123 (117, 140)	117 (102, 135)	.400
Follow-up rest HR	67 (63, 79)	67 (64, 73)	60 (58, 61)	.003***
Follow-up rest flow	.79 (.67, 1.01)	.74 (.66, .87)	.66 (.55, .88)	.200
Follow-up stress SBP	142 (126, 159)	130 (124, 141)	118 (107, 144)	.172
Follow-up stress HR	85 (76, 95)	93 (87, 97)	83 (75, 88)	.067
Follow-up stress flow	2.15 (1.60, 3.08)	2.60 (2.28, 2.94)	2.36 (2.20, 2.72)	.528

HT, heart rate; *LV MFR*, left ventricular myocardial blood flow reserve; *MFR*, myocardial blood flow reserve; *PsA*, psoriatic arthritis; *PsO*, cutaneous arthritis; *SBP*, systolic blood pressure; *TBR*, target-to-background ratio
*Statistically significant findings on GLMM for repeated measures analysis revealed the change in FDG TBRmax over the study period in Group 1 differed significantly ($P < .05$)
**Statistically significant findings across groups on Kruskal-Wallis testing.
***Within-group testing of changes in baseline and 6-month values were statistically significant with Wilcoxon Signed Rank testing

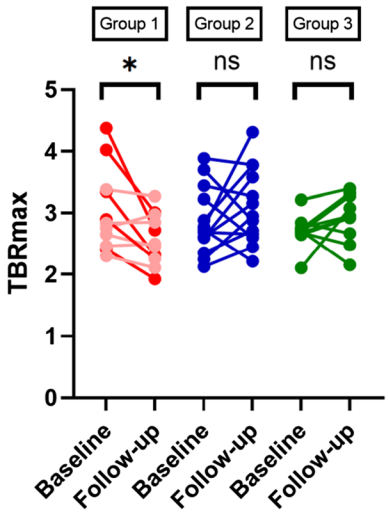

Figure 1. Change in ascending aorta FDG uptake from baseline to 6-month follow-up in Group 1 (patients with PsA and/or PsO on biologics), Group 2 (patients with PsO on non-biologics), and group 3 (control group). *FDG*, F-18-fluorodeoxyglucose; *PsA*, psoriatic arthritis; *PsO*, psoriatic disease; *TBR*, target-to-background ratio.

(Fig. 3). Finally, as an additional exploratory analysis we looked at whether there were any differences in vascular inflammation change between patients in Group 1 on different types of biologics (TNF inhibitor, IL-17 inhibitor, and IL-12/23 inhibitor). We found no difference, likely due to the small number of patients.

DISCUSSION

We conducted a prospective cohort study to determine the impact of systemic anti-cytokine treatment on vascular inflammation and MFR in patients with psoriasis, an inflammatory disease that is well known to be associated with vascular inflammation.[8] In a cohort of psoriasis patients that were started on biologic therapy, representative of clinical practice, we found that FDG uptake in the thoracic aorta decreased over the 6-month study period compared to no change in psoriasis patients treated with non-systemic therapies or in a cohort of control patients with non-inflammatory joint and skin disease. Additionally, we found that in those psoriasis patients treated with biologics who had imaging evidence of a significant anti-inflammatory response to the biologics, MFR was maintained compared to the group that did not have a significant response to the biologic therapy. The present study helps fill a knowledge gap by suggesting that anti-cytokine therapy may have vascular

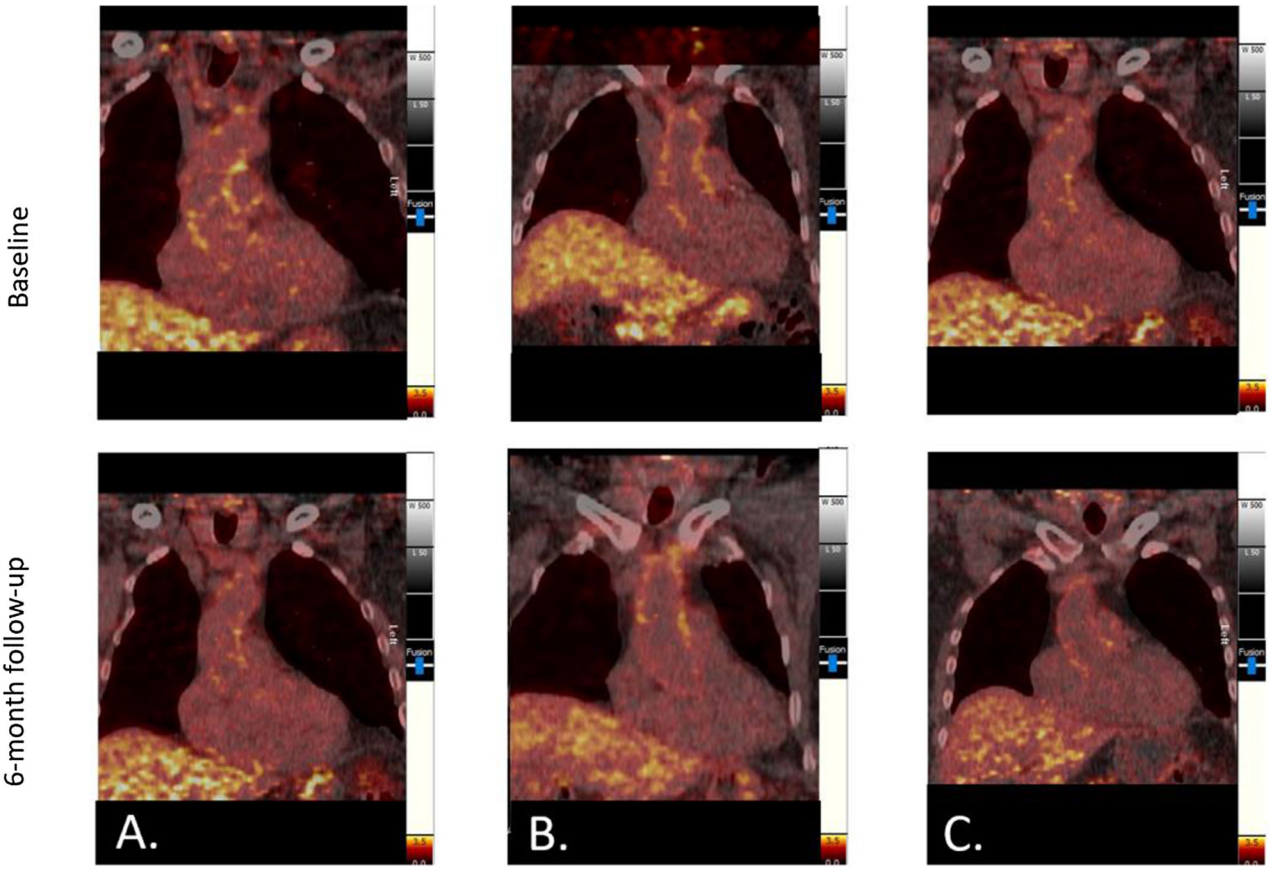

Figure 2. FDG images showing change in ascending aorta FDG uptake from baseline to 6-month follow-up in (**A**) patients with PsA and/or PsO on biologic agents, (**B**) patients with PsO on non-biologic agents, and (**C**) control patients with non-inflammatory arthritis. *FDG*, F-18-fluorodeoxyglucose; *PsA*, psoriatic arthritis; *PsO*, psoriatic disease.

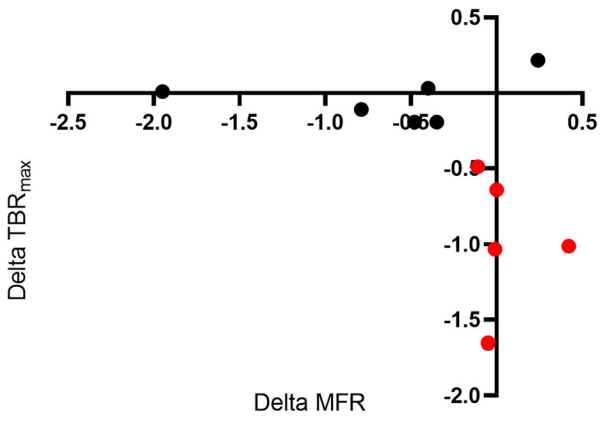

Figure 3. Correlation between change in TBR_{max} and MFR in patients with PsA and/or PsO on biologic agents. Patients with a clinically significant response to biologic agents are colored red. *Delta* TBR_{max}, target-to-background ratio in the most diseased segment of the ascending aorta at baseline compared to 6-month follow-up; *Delta MFR*, myocardial blood flow reserve at baseline compared to 6-month follow-up.

anti-inflammatory effects. These changes may be linked to changes in the coronary microvasculature in patients with psoriasis and support the notion that anti-inflammatory biologics may have a role in mitigating CV disease in this patient population.

Psoriasis disease is a chronic inflammatory skin condition that affects over 125 million people worldwide.[27] Patients with psoriasis have been found to have an increased incidence of CV disease and increased risk of MACE.[3,28–30] A key link between psoriasis and heart disease is mediated through inflammation. Patients with psoriasis have increased vascular inflammation measured by FDG PET, and it has been shown that increasing severity of skin disease is associated with increased vascular inflammation.[8,31] However, at this point it is not yet known whether targeting inflammatory pathways could lead to downstream effects in reducing CV morbidity and mortality in these patients.

In our jurisdiction, PsA patients progressing to a biologic agent need to have ongoing arthritis (5 or more

swollen joints) despite treatment with methotrexate for 3 months (usually at doses between 20 and 25 mg·week^{-1}) and an additional 3 months on leflunomide (20 mg·day^{-1}) or sulfasalazine (2000 mg·day^{-1}). Patients with skin disease need to demonstrate at least 3% of total body skin involvement despite 3 months of treatment with systemic therapy, usually methotrexate or cyclosporine. In the present study, treatment with anti-cytokine therapy was associated with a clinically significant reduction in aortic inflammation both when compared within the biologic group and also when compared to the non-biologic and control groups. Our data provide insights into the potential linkage of atherosclerosis with inflammation and further suggests that treatment of psoriasis patients with biologic agents may help to mitigate vascular inflammation. Additionally, while the overall change in MFR did not differ between the psoriasis group treated with anti-cytokine therapy and the other groups, when we looked specifically at patients in the psoriasis group that were treated with biologic agents who had a response in their TBR_{max} that was greater than the median (compared to the patients treated with biologic agents with a response less than the median), MFR was maintained in the responders compared to the non-responders (where it significantly dropped).

Our results are consistent with a noncontrolled study in rheumatoid arthritis of anti-TNF-α therapy, which demonstrated a reduction in vascular inflammation on FDG PET imaging after 8 weeks of treatment.[32] They are also consistent with an observational cohort from Kim et al. evaluating the anti-inflammatory effect of 25 patients with PsO treated with Ustekinumab.[33] Furthermore, an observational study from Dey et al. that evaluated a cohort of psoriasis patients being treated systemically had similar findings.[34]

Our results, however, are not consistent with a randomized placebo-controlled trial from Mehta et al., which showed no change in vascular inflammation on FDG PET in patients randomized to adalimumab, phototherapy, or placebo for a period of 12 weeks, with an open-label extension of the adalimumab arm for a period of 52 weeks.[35] There are several possible reasons for the discrepancy of our results with that observed by Mehta et al. Firstly, our study had 64% of the PsA/PsO population with PsA (while only 10% in the Mehta et al., study had PsA). Thus, there are underlying differences in the composition of the study populations which could theoretically influence the vascular response to biologic therapy. Secondly, while there was no difference in baseline inflammatory levels between our three study groups, it should be noted that our biologic therapy group had a higher proportion of patients pretreated with NSAIDS, methotrexate, and steroids. This difference in anti-inflammatory pretreatment across study groups could again theoretically alter the vascular response to biologic agents. Thirdly, our sample size was very small, increasing the risk for our results to be influenced by statistical chance. However, the consistent response of different anatomical sections of the aorta to the biologic agents certainly argues against this explanation. Lastly, ours was a non-randomized study, which could result in potential unmeasured confounding of our results. It should be noted, however, that these possible explanations are purely speculative.

Our study has several important limitations. Firstly, this was a non-randomized prospective observational study with a modest sample size, and limited clinical follow-up. Thus, our results should be interpreted with caution, and while they are largely hypothesis generating, they should certainly fuel further randomized clinical trials to investigate these findings. Conversely, we employed rigorous methodological analyses as well as a sensitivity analysis with multiple imputation to attempt to overcome some of these limitations and strengthen our results. Secondly, we utilized imaging outcomes to measure inflammatory response to biologic therapy rather than clinical outcomes. While clinical assessment of joint response to biologic therapy involves subjective measures, the imaging data of vascular inflammation that we utilized for our primary outcome are a completely objective measure of treatment response. Both vascular inflammation and MFR have been shown to be robust indices, which can give important insights into disease activity over shorter periods of clinical observation. Therefore, both vascular inflammation and MFR represent important surrogate markers for imaging trials that allow for the assessment of therapeutic benefit of interventions in a timely manner. In this regard, while FDG PET has been evaluated utilized extensively for the non-invasive detection of inflammation related to atherosclerosis, it does have several limitations which should be highlighted. While FDG PET imaging targets activated macrophages, it is still a non-specific probe that can accumulate in other metabolically active tissues which can therefore introduce interfering background signal.[36,37] Imaging of the ascending aorta requires strict dietary preparation to suppress FDG-myocardium uptake (with a high-fat, low-carbohydrate diet prior to PET imaging), given the significant background myocardial FDG uptake which can make it more difficult to differentiate vascular inflammation from background uptake.[38,39] FDG PET imaging is also affected by glucose levels, so particular caution in imaging patients with diabetes and hyperglycemia must be employed.[40] From a technical perspective, the partial

volume effect and motion artifacts from cardiac and organ motion during imaging all limit the spatial resolution and specificity of the test.[41] Additionally, given the limited spatial resolution of current PET imaging systems, we are currently unable to directly quantify ''vulnerable plaque'' in smaller-sized vessels, such as the coronary arteries meaning we are limited to assessing larger caliber vessels, such as the aorta.[42] Finally, while baseline demographic factors were not statistically different between groups, it is possible that the numeric difference in the proportion of participants with cardiac risk factors like hypertension, diabetes or current smoking, or differences in baseline BMI could have influenced the differences we saw in baseline TBR_{max} and response to biologics between the groups.

In conclusion, our study suggests that anti-cytokine therapy is associated with decreasing vascular inflammation in patients with PsA as assessed by FDG PET in contrast to non-biologics as well as a non-inflammatory control group. While our study should be interpreted with caution given the small number of participants and limited generalizability, our results do suggest an association between these biologic agents and preserved MFR in patients who respond favorably in terms of their vascular inflammation. This study supports the notion that there is a positive impact of immune therapies on vascular inflammation and microvascular disease in patients with chronic inflammation; however, larger prospective clinical outcome trials investigating this are warranted.

NEW KNOWLEDGE GAINED

Anti-cytokine therapy is associated with decreasing vascular inflammation in patients with psoriatic arthritis as assessed by FDG PET.

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Data availability

Data is available upon request.

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Anti-inflammatory effect of rosuvastatin in patients with HIV infection: An FDG-PET pilot study

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Aims. **This study aimed to evaluate markers of systemic as well as imaging markers of inflammation in the ascending aorta, bone marrow, and spleen measured by 18F-FDG PET/CT, in HIV+ patients at baseline and following therapy with rosuvastatin.**

Methods and results. **Of the 35 HIV+ patients enrolled, 17 were randomized to treatment with 10 mg/day rosuvastatin and 18 to usual care for 6 months. An HIV− control cohort was selected for baseline comparison of serum inflammatory markers and monocyte markers of inflammation. 18F-FDG-PET/CT imaging of bone marrow, spleen, and thoracic aorta was performed in the HIV+ cohort at baseline and 6 months. While CD14++CD16− and CCR2 expressions were reduced, serum levels of IL-7, IL-8, and MCP-1 were elevated in the HIV+ population compared to the controls. There was a significant drop in FDG uptake in the bone marrow (TBR_{max}), spleen (SUV_{max}) and thoracic aortic (TBR_{max}) in the statin-treated group compared to the control group (bone marrow: − 10.3 ± 16.9% versus 5.0 ± 18.9%, $p = .0262$; spleen: − 9.8 ± 20.3% versus 11.3 ± 28.8%, $p = .0497$; thoracic aorta: − 19.1 ± 24.2% versus 4.3 ± 15.4%, $p = .003$).**

Conclusions. **HIV+ patients had significantly markers of systemic inflammation including monocyte activation. Treatment with low-dose rosuvastatin in the HIV+ cohort significantly reduced bone marrow, spleen and thoracic aortic FDG uptake. (J Nucl Cardiol 2022;29:3057–68.)**

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Key Words: HIV • Cardiovascular risk • Rosuvastatin • Inflammation • Atherosclerosis • FDG-PET/CT

Abbreviations

18-FDG	18F-fluorodeoxyglucose
ART	Anti-retroviral therapy
CVD	Cardiovascular disease
HIV	Human immunodeficiency virus
MI	Myocardial infarction
IL	Interleukin
LDL	Low-density lipoprotein
PET/CT	Positron emission tomography/computed tomography
SUV_{max}	Maximum standardized uptake value
TBR_{max}	Target to background ratio

See related editorial, pp. 3069–3071

INTRODUCTION

Patients with human immunodeficiency virus (HIV) have a significantly increased risk of cardiovascular disease (CVD).[1] HIV+ individuals are 2-4 times more likely to suffer a myocardial infarction (MI),[2,3] and CVD now accounts for 10% of all deaths in this population.[1] HIV infection is associated with accelerated progression of atherosclerosis, an association that appears to be independent of traditional CVD risk factors.[4]

Atherosclerosis is a multi-faceted disease with inflammation playing a central role in atherosclerosis initiation and progression, and the triggering of events such as MI and stroke. HIV infection has been associated with persistently increased systemic inflammation, immune activation and pro-coagulant changes despite effective antiretroviral therapy.[5] These effects are thought to drive endothelial dysfunction, monocyte/macrophage activation, and accelerated atherosclerosis, as well as MI and post-MI fibrosis and reduced left ventricular function.[6] Monocyte-derived macrophages are a principal mediator of inflammation in atherosclerotic plaques.[7,8] Activation of monocyte-derived macrophages infiltrating atherosclerotic plaques appears to occur at early stages in the bone marrow and spleen.[9,10]

Statins are a widely prescribed lipid-lowering drug-class that reduces cardiovascular morbidity and mortality in both primary and secondary prevention.[11] In addition to their lipid-lowering effects, statins may have additional pleiotropic effects, including improvements in endothelial function, stabilization of atherosclerotic plaques, anti-inflammatory, immune-modulatory and anti-thrombotic effects.[12] Tissue uptake of 18F-fluorodeoxyglucose (18F-FDG) as measured by 18F-FDG positron emission tomography/computed tomography (PET/CT) scanning is a widely employed and sensitive technique for directly assessing inflammation in arterial walls and other organs such as the spleen and bone marrow. This is because inflammatory cells (mainly macrophages) have higher metabolic activity (i.e. higher rate of glycolysis) than other organ specific cell types (i.e. smooth muscle cells, endothelial cells, fibroblasts, osteoblasts, T-lymphocytes, etc.).[13] Given the potential link between inflammation and accelerated atherosclerosis in HIV+ patients, in this study we use 18F-FDG PET/CT to quantify regional haemopoietic activity[14] and inflammation as measured by FDG bone marrow, spleen and ascending aorta uptake in HIV+ patients with moderate CVD risk, and also measured markers of monocyte activation in this group. We hypothesized that monocyte markers of inflammation would be elevated in an HIV+ cohort compared to an HIV− cohort. Furthermore, in our HIV+ cohort, we hypothesized that imaging markers of inflammation in the ascending aorta as well as bone marrow and spleen as measured by 18F-FDG PET/CT, as well as markers of systemic inflammation including monocyte activation and markers of monocyte activation would improve in HIV+ patients with moderate CVD risk randomized to therapy with rosuvastatin compared to HIV+ patients randomized to standard of care.

METHODS

Participants were enrolled from the Immunodeficiency Clinic at the Ottawa Hospital General Campus, Ottawa, Ontario, Canada. Eligible participants were those with documented serologic evidence of HIV infection; ≥ 40 years of age; receiving standard ART for >2 years with a viral load below the limits of detection while on ART, a current CD4 count >350 cells/μL; and with a baseline Framingham risk score of 10-20%. Nine HIV- controls with no known cardiovascular disease were selected as age matched volunteers for immunological studies only (no imaging studies). The study was approved by University of Ottawa Heart Institute's Research Ethics Board. All participants provided a written informed consent.

Demographic data including age, sex, ethnicity, height, weight, waist circumference and blood pressure were recorded as well as current medication use, medical history, smoking status, and family history of CVD. Medical records were accessed to ascertain CD4 count, HIV viral load, creatinine, hemoglobin A1c (HbA1C), and total, high-density lipoprotein (HDL)

and low-density lipoprotein (LDL) cholesterol, with all tests performed within 1 month prior to the study visit. Participants were randomized to receive rosuvastatin (10mg daily) for 6 months in addition to their current medication or to continue with their current medical therapy only.

Immunological studies

At baseline and 6 months, whole blood was drawn from study participants into heparinized tubes and plasma was stored at -80°C for later analysis. Monocytes were stained directly in blood using anti CCR2-APC antibodies from R & D systems. Anti CD14-FITC anti CD16-PC7 and anti CD127-PE antibodies were purchased from Beckman Coulter. Blood was fixed and RBC lysed using BD Biosciences FACS Lysing solution and analyzed by Beckman Coulter FC500 MPL machine with CXP software. The presence and proportions of monocyte sub-populations was detected by flow cytometry using fluorochrome-labeled antibodies to CD14 and CD16 listed above. Classical monocytes were defined as CD14+CD16-, non-classical as CD14+CD16+ +CCR2-, and intermediate as CD14++CD16 +CCR2+.[15,16] CD127 and CCR2 expression were also measured on bulk CD14+ cells. Levels of IL-7, IL-8, MCP-1, were also measured by Luminex in patient plasma from both time points. Plasma samples were analyzed using custom multiplex MAGPIX kit from R&D Systems, with a Luminex MAGPIX Analyzer with xPONENT 4.2 software. Monocyte subsets and plasma cytokine concentrations were also measured in 9 HIV-agematched controls for a baseline inflammatory comparison (of HIV+ individuals to HIV- controls).

FDG-PET/CT imaging

FDG-PET/CT was performed at the University of Ottawa Heart Institute. Participants fasted overnight and had a fasting blood sugar <11 mmol/L. 18F-FDG was produced by the onsite cyclotron in Ottawa using standard production methods and modules.[17] 18F-FDG (5MBq/kg) was injected intravenously and patients were rested for 2 hours to allow for uptake before being imaged on a hybrid PET/CT scanner (Discovery 690 PET/CT 64 slice GE Healthcare). Following an unenhanced CT scan for attenuation correction and anatomic co-registration, PET data were acquired in three-dimensional mode. Images were reconstructed as 4 mm slices using high-resolution reconstruction after correction for attenuation, dead time, random coincidences and scatter. PET and CT data were fused and analyzed using the open-source DICOM viewer Hermes (Version 4.0, OsiriX Imaging Software, Geneva, Switzerland).

Bone marrow activity was measured according to previously validated methods.[18] In short, bone marrow 18F-FDG uptake was determine by placing ROIs in axial sections of individual vertebrae from T1 to L5. The maximum standardized uptake value (SUV_{max}) was recorded for each vertebra, and bone marrow activity was calculated as the average of the registered SUV_{max} of all measured vertebrae. We then calculated the maximum FDG target to background ratio (TBR_{max}) by determining the ratio of the SUV_{max} value from the bone marrow to background venous activity, derived from the superior vena cava at the level of the aortic arch. Splenic FDG uptake was assessed by placing regions of interest in 3 orthogonal planes (axial, sagittal, and coronal planes). SUV_{max} was recorded in each plane, and splenic activity was calculated as the mean of SUV_{max} values of the 3 planes. Aortic FDG uptake was evaluated according to previously published and validated methodology.[19] In short, we first obtained SUV_{max} values in the most diseased segment of the ascending aorta of the ascending aorta by averaging the SUV_{max} for 3 consecutive axial slices centered on the hottest slice and the adjacent slices superior and inferior to it, providing approximately 3 cm of the most inflamed section of the aortic wall. We then calculated the maximum FDG target to background ratio (TBR_{max}) by determining the ratio of the SUV_{max} value from the aorta to background venous activity, derived from the superior vena cava at the level of the aortic arch.

Data analysis

Statistical analyses were performed using MedCalc for Windows version 12.0 (MedCalc Software, Ostend, Belgium). Continuous variables are presented as means with standard deviations (SD) and categorical variables are presented as frequencies and percentages. Demographic characteristics are presented as mean ± SD or percentage as appropriate. Differences between patient groups were evaluated by the χ^2 test or Fisher exact test for discrete clinical variables and by the Students' t test or Mann–Whitney U test for continuous variables. Follow-up data were collected as scheduled, and dropouts were excluded. All the tests were two-sided, and a P value of $< .05$ was considered statistically significant. Shapiro–Wilk testing was used to test the statistical normality of the data.

RESULTS

Baseline participant characteristics

Thirty-five HIV+ patients were recruited, 86% of whom were men. Average age was 51.5 ± 6.9 years and

Table 1. Baseline characteristics of the HIV+ participants

Variable	Statin treated (n = 17)	Control (n = 18)	*p* value
Age, years	50.5 ± 5.6	52.3 ± 7.9	.445
Male sex, n (%)	12 (71%)	18 (100%)	.017*
BMI, kg/m^2	27.0 ± 4.0	24.4 ± 3.2	.040*
Hypertension, n (%)	4 (24%)	1 (6%)	.721
Diabetes, n (%)	0 (0%)	0 (0%)	1.000
Dyslipidemia, n (%)	1 (6%)	0 (0%)	1.000
Current smoking, n (%)	4 (24%)	3 (17%)	.835
Any history of Smoking, n (%)	10 (59%)	9 (50%)	.702
Framingham Risk Score	9.3 ± 3.6	9.6 ± 3.4	.797
CD4 Count	624 ± 171	680 ± 236	.423
LDL	2.51 ± .74	2.71 ± .69	.403
Protease Inhibitor Use	4 (24%)	2 (11%)	.730
Baseline bone marrow TBRmax	3.14 ± .52	2.82 ± .37	.058
Baseline spleen SUVmax	4.27 ± 1.20	4.25 ± .90	.778
Baseline aortic TBRmax	2.99 ± .45	2.99 ± .32	.988
Baseline SVC_{max}	1.67 ± .48	1.46 ± .30	.458

Mean ± SD or n (%)
*Indicates $p < .05$ between groups
BMI, body mass index; LDL, low density lipoprotein

the average BMI was 25.7 ± 3.8 kg/m^2. Participants were well-matched between the treatment groups, with regards to CVD risk factors, with the exception of BMI, which was higher in the patients receiving statin treatment as well as the proportion of male sex, which was higher in the control group. Additionally, groups were matched with regards to age, sex, smoking, Framingham risk, duration of ART and protease inhibitor (PI) versus non-nucleoside reverse-transcriptase inhibitors (NNRTI)-based therapy. Average CD4 count was 653 ± 206 cells/μL and a similar number of participants within the groups were taking protease inhibitors. Average bone marrow SUV in the entire HIV+ study population was 2.99 ± .54, average splenic SUV_{max} was 4.11 ± 1.04, and average TBR_{max} in the ascending aorta was 2.99 ± .38. These were similar between the rosuvastatin-treated HIV+ group and the standard-of-care HIV+ group (Table 1).

Comparison of monocyte inflammation in the HIV+ population (at baseline) to the nine HIV- controls revealed that CD14++CD16- (64.7 ± 14.5% positive cells versus 84.5 ± 3.2% positive cells; $p = .0002$) and CCR2 expression (25.36 ± 22.97% positive cells versus 86.48 ± 4.67% positive cells; $p < .0001$) was significantly less in the HIV+ cohort than in the HIV- control cohort. Expression of CD14+ CD16++ (15.20 ± 14.0% positive cells versus 2.00 ± 1.92% positive cells; $p = .008$), CD14+CD16- (11.24 ± 5.28% positive cells versus 5.76 ± 1.84% positive cells; $p = .008$), CD127 (32.45 ± 14.74% positive cells versus 10.76 ± 2.47% positive cells; $p = .0001$) and CD16 (24.03 ± 14.61% positive cells versus 7.72 ± 3.04% positive cells; $p = .002$) were all significantly higher in HIV+ patients (Table 2). The correlation between baseline serum inflammatory and monocyte markers with baseline FDG markers is shown in Table A (supplementary file). Expression of CD14++ CD16- correlated significantly with the splenic SUV_{max} and expressions of CD14+ CD16+, CD14+ CD16- and IL-8 correlated with TBR_{max} in the ascending aorta.

Changes over 6 month study period

FDG PET Imaging There was a significant reduction in bone marrow, spleen and thoracic aorta FDG uptake in the statin-treated group compared with the usual care control group. The statin group demonstrated an average of − 9.40 ± 16.81% reduction in bone marrow TBR_{max}, compared to a 5.05 ± 18.88% change in the control group ($p = .035$) at six months. There was an average − 9.75 ± 20.32% change in the spleen SUV_{max} of the statin group compared to 11.28 ± 28.82% change in the control group. Finally, there was an average − 11.88 ± 18.18% change in

Table 2. Baseline monocyte markers

Monocyte marker (% positive cells)	Healthy control	HIV+patients	*p* value
CD14++ CD16 -	84.5 ± 3.2	64.7 ± 14.5	.0002
CD14++ CD16+	1.86 ± .69	1.55 ± .89	.35
CD14+ CD16++	2.00 ± 1.92	15.20 ± 14.0	.008
CD14+ CD16+	5.52 ± 2.70	6.98 ± 5.48	.45
CD14+ CD16-	5.76 ± 1.84	11.24 ± 5.28	.004
CD127	10.76 ± 2.47	32.45 ± 14.74	.0001
CCR2	86.48 ± 4.67	25.36 ± 22.97	< .0001
CD16	7.72 ± 3.04	24.03 ± 14.61	.002

Mean ± SD

thoracic aorta TBR_{max} in the statin group compared to 10.06 ± 11.78% in the control group (Figure 1). These changes were independent of the correction for background FDG uptake (superior vena cava SUV_{max}).

Immunohistochemistry data

Despite the elevated baseline levels of monocyte inflammation in the HIV+ patients, there were no changes in monocyte sub-populations in either group (rosuvastatin treated or standard of care) over the 6-month treatment period (Table 3).

Serum inflammatory marker data

Serum levels of IL-6, IL-7, IL-8, and MCP were elevated in the HIV+ population compared to a group of healthy controls without HIV infection (IL-6: .73 ± .45 pg/ml versus .46 ± .25 pg/ml for HIV+ population versus HIV− controls, respectively; $p = .09$, IL-7: 3.04 ± .81 pg/ml versus 2.07 ± .74 pg/ml; $p = .002$, IL-8: 4.30 ± 2.74 pg/ml versus .66 ± .58 pg/ml; $p = .0003$, and MCP: 273.35 ± 91.10 pg/ml versus 118.72 ± 24.45 pg/ml; $p < .0001$) (Table 4). Again, there were no significant changes in inflammatory markers within the statin treated or usual standard of care groups over the 6-month treatment period (Table 5).

Relationship between FDG data and serum inflammatory markers

Within the statin-treated HIV+ patients, those that had a clinically significant drop in FDG bone marrow uptake (which we defined as a change that was greater than the group average of 12% decrease) over the 6 months also had a trend towards a reduction in the IL-7, IL-8 and MCP inflammatory markers compared to those with no reduced in FDG bone marrow uptake (IL-7 19% reduction in responders versus 4% reduction in non-responders; $p = .08$, IL-8 27% reduction in responders versus 9% increase in non-responders; $p = .24$, MCP 21% reduction in responders versus 11% reduction in non-responders; $p = .008$).

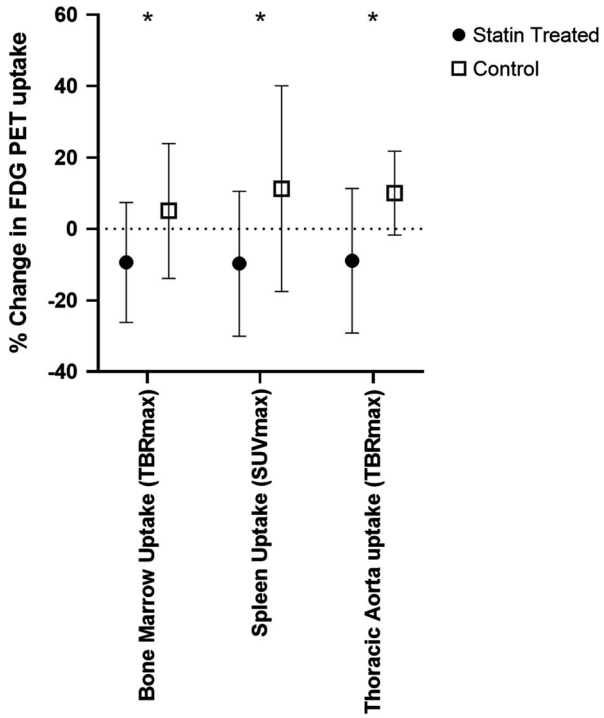

Figure 1. Change in % FDG PET uptake in bone marrow, spleen and thoracic aorta over study period. *$p < .05$ for difference in % change between statin and control group.

DISCUSSION

The present pilot study demonstrated that, in a cohort of HIV+ patients with moderate cardiovascular risk and on optimal medical therapy, there were elevated

Table 3. Changes in monocyte markers over the 6-month treatment period

Monocyte marker (% positive cells)	Statin treated			Control			*p* value*
	Baseline	Follow-up	Delta	Baseline	Follow-up	Delta	
CD14++ CD16−	58.8 ± 15.6	61.1 ± 16.1	2.3 ± 13.0	69.99 ± 11.31	70.78 ± 10.51	.8 ± 9.9	.704
CD14++ CD16+	1.6 ± 1.0	2.0 ± 1.3	.4 ± 1.1	1.48 ± .85	1.47 ± .95	.0 ± 1.1	.335
CD14+ CD16++	20.0 ± 17.6	17.4 ± 16.5	− 2.6 ± 10.2	10.98 ± 8.25	10.42 ± 12.17	− .6 ± 9.3	.562
CD14+ CD16+	7.2 ± 4.5	6.6 ± 4.4	− .6 ± 1.4	6.76 ± 6.34	5.42 ± 2.34	− 1.3 ± 6.3	.652
CD14+ CD16−	11.7 ± 6.3	13.4 ± 7.4	1.7 ± 6.4	10.86 ± 4.30	11.90 ± 6.68	1.0 ± 6.5	.771
CD127	36.6 ± 17.4	41.7 ± 17.4	5.1 ± 13.2	28.77 ± 11.09	31.19 ± 14.51	2.4 ± 15.0	.598
CCR2	24.1 ± 20.8	23.9 ± 21.1	− 4.6 ± 14.6	19.46 ± 10.92	17.52 ± 11.56	5.7 ± 37.9	.331

Mean ± SD
*p value between Delta

levels of baseline inflammation as measured by monocyte expression, serum inflammatory markers and FDG-PET markers of inflammation in the bone marrow, spleen and ascending aorta. In addition, treatment with low-dose rosuvastatin for 6 months resulted in significantly decreased FDG uptake in the bone marrow, spleen and thoracic aorta in the HIV+ patients compared to those receiving usual standard care. Although treatment with low-dose rosuvastatin did not change monocyte expression or serum levels of inflammatory markers in the HIV+ patients, in a subpopulation of patients that had a clinically significant drop in FDG bone marrow uptake over the treatment period (>12% decrease), there was a trend towards a reduction in IL-7 and IL-8 markers and a significant reduction in MCP inflammatory markers. In addition, when HIV+ patients were compared with a group of otherwise healthy HIV- controls, the former had significantly increased inflammatory monocyte markers as well as inflammatory cytokine levels.

HIV and inflammation: effect of statin therapy

CVD has replaced acquired immunodeficiency disorder (AIDS) as the leading cause of morbidity and mortality in HIV+ patients. [4,20,21] The mechanisms driving the increased CVD risk in HIV+ individuals are not clear but may include direct effects of the virus itself, effects of anti-retroviral therapy (ART) on lipids, insulin resistance and body composition[22,23], as well as the increased burden of nontraditional risk factors such hepatitis C, other co-infections and substance abuse.[22,24] The timing of ART initiation may also play a role.[22] Although a number of mechanisms have been proposed for the increased CVD risk in HIV+ patients, the presence of chronic systemic inflammation is thought to play a major role.[25,26] HIV infection is recognized as a chronic inflammatory disease, driven by viral replication[27] and dysregulated cytokine production.[28] Activity of monocyte-derived macrophages appears to also contribute to the progression of atherosclerosis in HIV+ patients. In a study of 571 HIV+ patients and 207 HIV- controls, soluble CD163, CD14, galectin-3 binding protein and galectin-3, markers of macrophage activity, were significantly elevated in the HIV+ group. Following adjustment for risk factors, including markers of systemic inflammation (high-sensitivity C-reactive protein (hsCRP) and IL-6), soluble CD14 was associated with a significantly increased risk of new plaque formation.[29] In another study of 27 HIV+ patients and 54 HIV- controls with similar cardiovascular risk profile, the presence of arterial inflammation was significantly

Table 4. Baseline Inflammatory markers

Inflammatory markers (pg/ml)	HIV-control	HIV+patients	*p* value
IL6	.46 ± .25	.73 ± .45	.09
IL7	2.07 ± .74	3.04 ± .81	.002
IL8	.66 ± .58	4.30 ± 2.74	.0003
MCP	118.72 ± 24.45	273.35 ± 91.10	< .0001

Mean ± SD
IL, interleukin; MCP, monocyte chemoattractant protein

higher and associated with markers of monocyte and macrophage activation in the HIV+ group.[26]

The benefits of statins now appear to extend beyond lipid metabolism and also include immunomodulatory effects.[30] Indeed statins have been shown to reduce the risk of cardiovascular events and all-cause mortality in patients with elevated hsCRP but normal levels of LDL cholesterol.[31-33] Statins may also have a direct and dose dependent effect on reducing aortic inflammation, with patients on high-dose atorvastatin therapy for 12 weeks having significantly reduced thoracic aorta and carotid artery inflammation compared to a low dose atorvastatin group.[19] Our results are in line with the latter observation. In our study, the HIV+ individuals treated with low-dose rosuvastatin (equivalent to 40 mg atorvastatin) demonstrated a modest reduction (11.88%) in inflammation when compared to a previous other statin intervention study in HIV- subjects where patients receiving high dose (80mg) and low dose (10 mg) atorvastatin demonstrated 14.42% and 4.2% reduction in inflammation respectively.[19]

In a cohort of HIV+ patients receiving rosuvastatin, atorvastatin or pravastatin, a significant reduction in serum inflammatory markers including hs-CRP and TNF-α was observed.[34] The Stopping Atherosclerosis and Treating Unhealthy Bone with Rosuvastatin in HIV (SATURN-HIV) trial investigated the effects of rosuvastatin in HIV+ patients on ART. After 24 weeks, patients on rosuvastatin experienced significant decreases in sCD14 and the proportion of circulating CD14+CD16+ monocytes compared to controls.[35] A 24.2% decrease in serum IL-6 levels was also observed compared to a 2.1% drop in controls, although this difference did not reach statistical significance.[36] Notably rosuvastatin has been shown to reduce atherosclerosis in HIV+ patients with a significant decrease in carotid artery intima-media thickness following treatment for 24 months.[37] Finally, statin treatment was shown to reduce non-calcified plaque burden and high risk plaque features in HIV+ patients, despite no effect on aortic arterial inflammation.[38] Interestingly a recent pilot study looking at canakinumab, an IL-1β inhibitor, in HIV+ patients, the majority of whom were already on statin therapy, found significant reductions in circulating inflammatory markers as well as FDG inflammation, which was accompanied by reductions in leukopoietic activity, monocyte cytokine production and arterial inflammation.[39] In contrast, a study looking at low-dose methotrexate in patients with HIV found no significant effect on endothelial function or inflammatory biomarkers, but was associated with a reduction in CD8+ T cells.[40] Thus, evidence continues to mount for the potential anti-inflammatory benefit of statin therapy on cardiovascular risk reduction in HIV+ patients. Our novel findings support the anti-inflammatory role of statin therapy in moderate-risk HIV+ patients, and suggest a potential role for the use of statins for primary prevention in this patient demographic.

The role of the bone marrow and spleen in the inflammatory process

The atherogenic response to dyslipidemia is thought to be driven from the bone marrow and spleen.[9,10] In response to hyperlipidemia, the bone marrow overproduces inflammatory monocytes that enter the circulation, accumulate in atherosclerotic lesions, and differentiate into macrophages.[42] Work by Tawakol et al. showed that the link between psychological stress and CVD may also be mediated by up-regulated bone marrow activity,[14] while animal models have demonstrated that stress leads to mobilization and release of neutrophils and monocytes, and increased activity of haemopoietic progenitor cells in the bone marrow.[43] In addition to the link between bone marrow and arterial inflammation, animal models of heart failure have demonstrated migration of pro-inflammatory monocytes from the spleen to the heart,[44] while increased splenic FDG uptake, which was correlated with upregulated inflammatory markers, has been observed in patients suffering acute coronary syndromes.[18] Of significance was the

Table 5. Changes in serum inflammatory markers of the 6-month treatment period

Inflammatory marker (pg/ml)	Statin treated			Control			*p* value *
	Baseline	Follow-up	Delta Change	Baseline	Follow-up	Delta Change	
IL6	.74 ± .30	.55 ± .26	− .19 ± .37	.72 ± .56	.57 ± 49	− .14 ± .72	.813
IL7	2.99 ± .90	2.78 ± 1.10	− .25 ± .73	3.10 ± .74	3.17 ± .98	.09 ± .96	.274
IL8	4.68 ± 3.57	4.86 ± 3.52	.125 ± 2.39	3.94 ± 1.65	5.33 ± 3.84	1.38 ± 3.60	.259
MCP	259.16 ± 83.94	228.30 ± 59.69	− 28.34 ± 55.71	286.74 ± 97.86	276.23 ± 88.85	− 10.51 ± 75.71	.458

Mean ± SD
IL, interleukin
* *p* value comparison between Delta Change values

association between increased splenic FDG uptake and an increased risk of cardiovascular events. Taken together, there is a growing body of evidence supporting the presence of a bone-marrow-cardio-splenic axis. Although the present study did not examine the bone marrow, spleen or aortic FDG uptake between HIV+ patients and healthy controls, we did observe significant reductions in FDG uptake following 6 months treatment with a low dose statin (Figures 2, 3). Specifically, HIV+ individuals treated with low-dose rosuvastatin had a 9.40% reduction in their bone marrow, 9.75% reduction in their splenic, and 11.88% reduction in their thoracic aortic FDG-PET uptake. Our novel findings not only support the link between the bone-marrow-cardio-splenic axis previously mentioned, but they also provide evidence for an anti-inflammatory effect of statin therapy in a HIV+ population.

Inflammatory biomarkers in HIV

Studies examining HIV associated chronic inflammation have increasingly focused on blood monocytes, a population of cells with inflammatory properties known to be involved in the promotion of atherosclerosis.[45] Monocytes represent an important source of cytokines and chemokines and may be major contributors to the systemic inflammation in HIV disease. HIV+ individuals have increased numbers of circulating CD16+ monocytes,[46,47] and CD16+ monocytes have been shown to be independently associated with coronary artery calcification in HIV+ individuals.[48] Supporting this, in the present study we observed elevated levels of CD16+ monocytes in the HIV+ population compared to a HIV- control population. Additionally, expression was significantly higher for CD14+ CD16++ and CD14+CD16- monocyte subpopulations in the HIV+ cohort, as well as monocytes expressing CD127. Previous studies have demonstrated that regulatory T-cells, which are responsible for immune modulation and regulating chronic inflammatory reactions, upregulate CD127 when activated, and this is due to IL-7 and was more prevalent in the bone marrow tissue.[49]

IL-6 is typically elevated in the plasma of HIV-infected individuals compared to uninfected controls,[28,50] with increased levels of IL-6 independently associated with carotid artery intima-media thickness and stenosis in the general population.[51,52] Although a relative decrease in IL-6 is seen with the initiation of ART, plasma levels do not normalize in HIV+ patients on treatment and remain significantly higher than levels seen healthy HIV-negative individuals.[45,53] IL-8 is a cytokine that has been linked to a number of inflammatory diseases that involve neutrophil activation, including CVD.[54,55] Produced predominately by

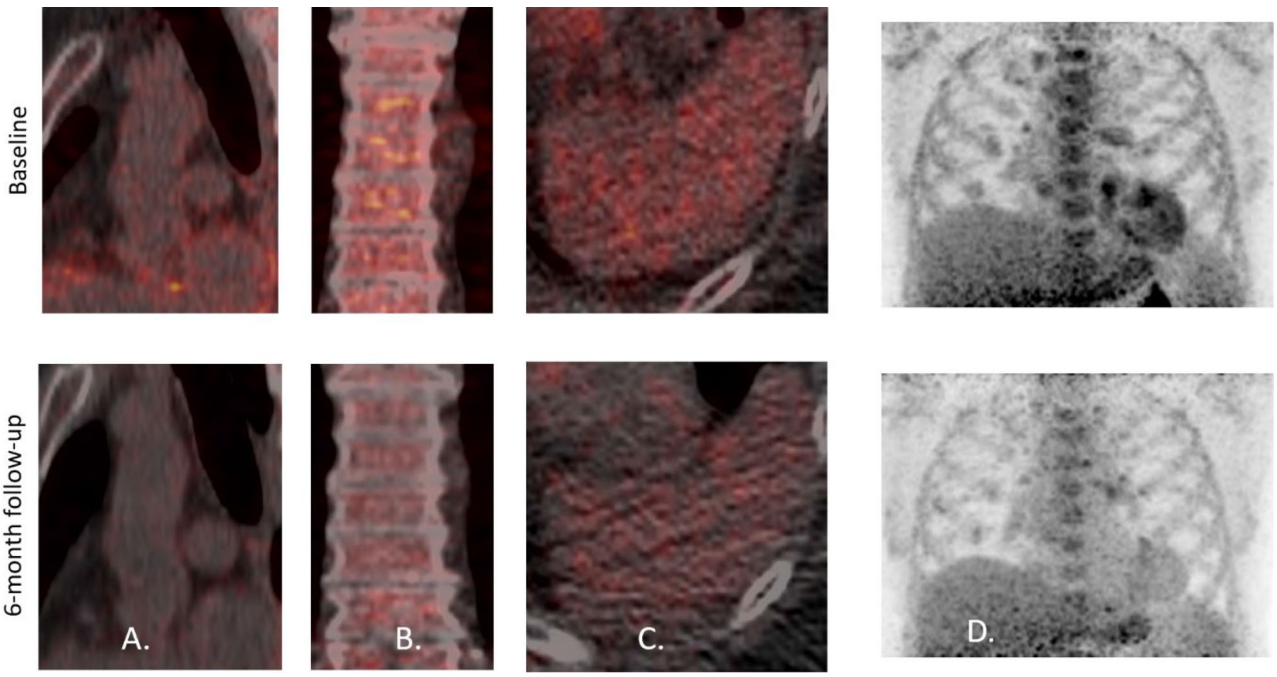

Figure 2. Change in FDG uptake in HIV+ patient over 6 months with treatment of rosuvastatin in (**A**) the ascending aorta (TBR_{max}: 2.80 at baseline and 1.39 after), (**B**) the bone marrow (SUV_{max}: 3.35 at baseline and 2.26 after), (**C**) the spleen (SUV_{max}: 3.49 at baseline and 1.70 after), and (**D**) anterior planar image.

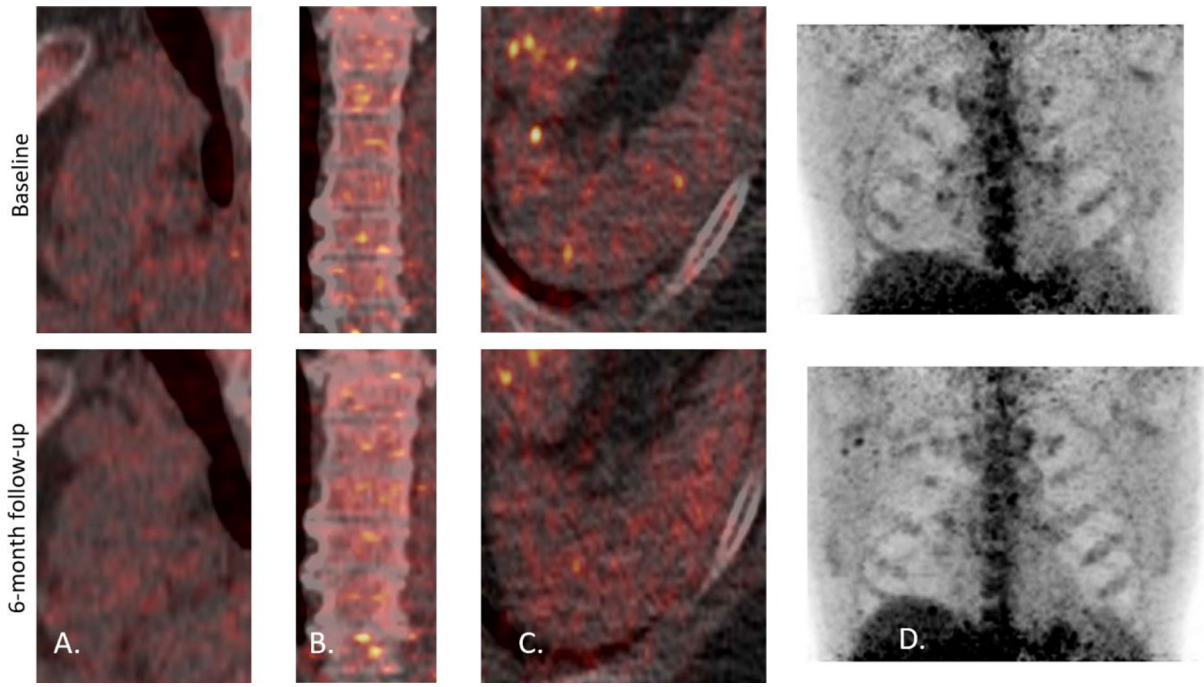

Figure 3. Change in FDG uptake in HIV+ patient over 6 months with standard therapy (control group) in (**A**) the ascending aorta (TBRmax: 2.85 at baseline and 3.19 after), (**B**) the bone marrow (SUVmax: 3.35 at baseline and 3.13 after), (**C**) the spleen (SUVmax: 4.02 at baseline and 3.18 after), and (D) anterior planar image.

monocytes and neutrophils, its primary responsibility is to recruit other monocytes and neutrophils, the principal cells involved in the acute inflammatory process.[55] IL-7 has also been proposed to play a role in the development of CVD, with increased levels observed in patients with CVD compared to healthy controls and correlated with serum levels of high-sensitivity C-reactive protein (hs-CRP).[56,57] HIV infection disrupts IL-7 signaling, including via cytokine production and receptor expression. Studies have shown dysregulated IL-7 signaling in HIV+ individuals, with reduced expression of the IL-7 receptor α-chain (CD127) on CD8 T-cells in HIV+ patients with uncontrolled viral replication, and partial recovery seen in those on ART with sustained viral suppression.[58] Numerous studies have demonstrated increased plasma concentrations of IL-7 in HIV-infected individuals compared to HIV- controls.[59,60] Although there is a decline following initiation of ART, serum IL-7 levels remain elevated in HIV+ patients despite suppression of HIV replication.[60,61] Supporting these observations, in the present study, we observed significantly elevated levels of serum IL-7, IL-8 and MCP in the HIV+ cohort compared to a HIV- control population. IL-6 levels were also elevated, although levels did not quite reach statistical significance. Overall, there were no temporal changes in monocyte expression and levels of serum inflammatory markers with rosuvastatin treatment. In the subset of HIV+ patients that achieved a clinically significant response to rosuvastatin (>12% reduction in FDG-PET bone marrow uptake), there was a trend towards a temporal reduction in inflammatory markers.

Limitations

Although this was a prospective randomized imaging trial looking at the efficacy of statin therapy in HIV+ individuals, it was a relatively small sample size. Because of the small cohort, the study was not powered to detect differences in treatment outcomes in terms of monocyte expression or inflammatory markers, although it was powered to detect differences with regards to FDG-PET uptake. Despite the low power, although we did not see an overall trend in a reduction in monocyte expression or inflammatory markers in the rosuvastatin treated group, we did see a trend towards reduction in serum inflammatory markers (IL-7, IL-8, and MCP) in patients that had a clinical response to statins as measured by FDG-PET uptake (namely, those that had >12% reduction in bone marrow FDG-PET uptake).

CONCLUSIONS

In conclusion, the present study observed that monocyte markers of inflammation were elevated in HIV+ patients compared to HIV- controls. Additionally, there was a significant decrease in FDG-PET uptake in the bone marrow, spleen, and thoracic aorta following treatment with rosuvastatin for 6 months in HIV+ patients compared to HIV+ patients receiving usual care. These data suggest wide-range anti-inflammatory effects of rosuvastatin in HIV+ individuals with well-controlled infection on ART, that ultimately resulted in decreased inflammatory activity in the arterial walls of these patients and raises the possibility that such therapeutic approaches can reduce the burden of CVD in this population. This needs to be confirmed in larger studies.

DISCLOSURES

EF, JW, GRS, VFC, NCW have nothing to disclose. RdK reports grants, consulting fees, and license revenues from Jubilant DraxImage and uOttawa Heart Institute (UOHI), outside the submitted work. RSBB reports grants and honoraria from Lantheus Medical Imaging, grants and honoraria from Jubilant DraxImage, and grants and honoraria from GE Healthcare, outside the submitted work. RSBB was a Career Investigator supported but the Heart and Stroke Foundation of Ontario, the UOHI Vered Chair of Cardiology and a University of Ottawa Distinguished Chair of Cardiovascular Imaging Research. GD was supported by a CIHR new investigator salary support award (supported by OHTN) while at UOHI. Currently he is Wesfarmers Chair in Cardiology at the University of Western Australia with an Adjunct Professor appointment at UOHI. He has received honoraria from Amgen, Pfizer, Artrya Pty Ltd and has an equity interest in Artrya Pty Ltd, all outside the submitted work. PM has received honoraria from Merck and an investigator-driven research grant from Gilead, all outside the submitted work. The study was performed at UOHI.

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