The Low Dose Colchicine after Myocardial Infarction (LoDoCo-MI) study: A pilot randomized placebo-controlled trial of colchicine following acute myocardial infarction

Thomas Patrick Hennessy MB Bch BAO MRCPI

UWA Student ID:22017667

This thesis is presented for the degree of Master of Clinical Research (MClinRes) degree of The
University of Western Australia
School of Medicine & Pharmacology
2021

ACKNOWLEDGEMENTS

This research was supported by an Australian Government Training Program (RTP) Scholarship for which I am very thankful. This work was funded by the National Heart Foundation of Australia Grant Number 100616. Technical assistance was kindly provided by our colleagues in the Medical Research Foundation (MRF), Royal Perth Laboratory regarding blood specimen procurement, preparation, and analysis. Royal Perth Pharmacy were instrumental in orchestrating the blinding, randomisation process and collation of trial drug data.

Colchicine and matched placebo were provided free of charge by Aspen Pharmaceuticals. I am grateful to the research nurses Department of Cardiology and to the nursing and medical staff of the Coronary Care Unit and Ward 4F, Royal Perth Hospital for their help and support with this work. I would like to sincerely thank the many contributors and mentors who have assisted with the development, performance and completion of this research project and thesis. My supervisor Prof Graham Hillis in association with Prof Carl Schultz who were instrumental in the development of the study and my own development as an early career Investigator. I would also like to thank the staff and contributors to the Master of Clinical Research degree (MClRes) UWA, who are involved in an extremely enjoyable, enriching program. I will also be forever grateful to the independent examiners of this thesis for taking the time to formally assess and critique this research project.

Dr Thomas Hennessy

01/08/2021

ABSTRACT

The Low Dose Colchicine after Myocardial Infarction (LoDoCo-MI) study: A pilot randomised placebo-controlled trial of colchicine following acute myocardial infarction.

Background: Following acute myocardial infarction (MI), patients with persistently elevated high-sensitivity C-reactive protein (hs-CRP), are at increased risk of recurrent cardiovascular events. Colchicine is a unique anti-inflammatory medication that might reduce this risk. This study tested the ability of low-dose colchicine to reduce hs-CRP levels at 30-days after acute MI, a key marker of outcome, and its safety and tolerability in this setting.

Methods: A randomized, double-blind, trial of colchicine 0.5mg daily or placebo in 237 patients admitted with acute MI. The primary endpoint was the proportion of patients with hs-CRP levels $\geq 2\text{mg/L}$ after 30 days of treatment, a threshold associated with a worse prognosis.

Results: At 30-days, 44% of patients treated with colchicine had hs-CRP levels $\geq 2\text{mg/L}$ compared to 50% of those randomized to placebo ($p=0.35$). The median hs-CRP in patients randomized to colchicine was 1.6mg/L (interquartile range [IQR] $0.7\text{-}3.5$) versus 2.0mg/L (IQR $0.9\text{-}4.0$) in patients randomized to placebo ($p=0.11$). The median absolute reduction in hs-CRP levels was -4.3mg/L (IQR -1.1 to -14.1) among colchicine-treated patients and -3.3mg/L (IQR -0.9 to -14.4 , $p=0.44$) in placebo-treated patients. The relative reduction was 78% versus a fall of 64% ($p=0.09$). Low-dose colchicine was well-tolerated and did not reduce compliance with other medications.

Conclusion: Low-dose colchicine was not significantly associated with achieving a hs-CRP level $< 2\text{mg/L}$ or lower levels 30 days after acute MI; beneficial trends were, however, observed and treatment was safe and well-tolerated. A large-scale outcome trial is required, and these data suggest this will be safe and feasible.

TABLE OF CONTENTS

1. Thesis & Authorship Declarations.....	vi to viii
2. List of Figures and Tables.....	ix to xi
3. Abbreviations	xi to xiii
4. Introduction & Background	1
5. Hypothesis & Aims	3
5.1 Overview.....	3
5.2 Trial Aims	4
5.3 Trial Hypothesis	4
6. Burden of cardiovascular disease.....	5
7. Risk factors for acute coronary syndrome	6
7.1 Overview	6
8. Prevalence of coronary atherosclerosis	7
9. Risk Factor Stratification Models in ACS.....	7
9.1 Overview of Commonly used Risk Stratification tools	7
9.2 Future Directions for Cardiovascular Risk models	9
10. Inflammatory conditions and cardiovascular disease.....	10
11. Coronary artery disease & Inflammation	11
11.1 An Overview.....	11
11.2 Inflammation in the Pathophysiology of Atherosclerosis.....	13
11.3 Plaque Rupture and Thrombosis.....	22
12. A Focus on the Inflammasome in CVD	26
13. Diagnosis of Coronary Heart Disease/Acute Coronary Syndrome	28
14. Inflammation and relevance of LoDoCo-MI inflammatory markers	
14.1 Overview	30
15. CV Risk Biomarkers	
15.1 Overview	33
15.2 High Sensitivity C Reactive Protein (Hs-CRP)	35
15.3 Interleukin-6 (IL-6)	40
15.4 Myeloperoxidase (MPO).....	41
15.5 Interleukin-1 β	42
16.Targeting Inflammation in CV Prevention	43

16.1 The Pleiotropic Effect of Statins	43
16.2. Anti-Inflammatory effect of existing standard secondary CV preventative medication ...	44
16.2.1 Notes on the Anti-Inflammatory effects of anti-platelets, ACE-inhibitors, Angiotensin II Receptor Blockers & B-Blockers	45
16.2.2 Other Anti-inflammatory targets that have been trialled in secondary prevention CV disease	45
16.3 Studies prior to LoDoCo-MI Using Colchicine to Target CV Inflammation with Clinical, Cytokine or Radiological Outcome Assessment	47
17. Colchicine: Overview of Its' background, therapeutic profile & potential in cardiovascular prevention through inflammatory suppression	51
17.1 Overview & Background	51
17.2 Pharmacodynamics.....	51
17.3 Mechanism of action	52
17.4 Colchicine's role in non-atherosclerotic cardiac issues	52
17.5 Colchicine & Percutaneous Coronary intervention (PCI) related complications of In-stent Restenosis and stent thrombosis.....	53
18 Antiplatelet resistance	56
19 Flow cytometry analysis of Progenitor blood cells	56
20 LoDoCo-MI Methods	
20.1 Study design & Procedures	57
20.2 Outcomes/End Points and definitions.....	59
20.3 Statistical analysis	60
20.4 Ethics Safety and Adjudication of outcomes.....	61
21. Results.....	61
21.1 Clinical Outcomes	66
21.2 Flow Sub-study results	67
21.3 Tolerability and safety	69
22. Discussion	69
22.1 Overview.....	69
22.2 Strengths and Limitations.....	70
22.3 Implications of Findings.....	72
23. Major Trials published since LoDoCo-MI	73
23.1 Overview.....	73

23.2 Future studies in this Field 76

23.3 Potential Future Trials for Contemplation 77

24 Concluding remarks 80

25 References..... 81

26 Appendices 115

1. Thesis & Authorship Declarations

I, Thomas Hennessy, certify that: This thesis has been substantially accomplished during enrolment in this degree. This thesis does not contain material which has been submitted for the award of any other degree or diploma in my name, in any university or other tertiary institution. In the future, no part of this thesis will be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of The University of Western Australia and where applicable, any partner institution responsible for the joint award of this degree.

This thesis does not contain any material previously published or written by another person, except where due reference has been made in the text and, where relevant, in the Authorship Declaration that follows. This thesis does not violate or infringe any copyright, trademark, patent, or other rights whatsoever of any person. The research involving human data reported in this thesis approved by The Royal Perth Hospital Human Research Ethics Committee (reference 15-090) and The University of Western Australia Human Research Ethics Committee.

Written patient consent has been received and archived for the research involving patient data reported in this thesis. The following approvals were obtained prior to commencing the relevant work described in this thesis: The trial was registered and approved by the Australian and New Zealand Clinical Trials Registry-ACTRN12615001194550.

This thesis contains published work and/or work prepared for publication, some of which has been co-authored. The LoDoCo-MI manuscript was published in the American Heart Journal (AHJ) in September 2019 with 44 PubMed citations to date.

Signature:

A solid black rectangular box used to redact the author's signature.

Date: 10/08/2021

AUTHORSHIP DECLARATION: CO-AUTHORED PUBLICATIONS

This thesis contains work that has been published.

LoDoCo-MI study order of authorship as published in the American Heart Journal (AHJ)

September 2019

Thomas Hennessy,¹ Linda Soh,¹ * Mitchell Bowman,¹ * Rahul Kurup,² Carl Schultz,¹ Sanjay Patel,² Graham S. Hillis.¹

¹ Department of Cardiology, Royal Perth Hospital, Perth and Faculty of Health and Medical Sciences, University of Western Australia.

² Department of Cardiology, Royal Prince Alfred Hospital, Sydney
and Faculty of Medicine, University of Sydney.

* Contributed equally to this work

Dr Thomas Hennessy-Student ID:22017667 contribution to work:

1. Patient baseline screening, Consent & enrolment, randomisation and follow up examination at 1 month as per the protocol outlined.
2. Trial Drug dispensing and pill count follow up collection.
3. Writing up of ethics revisions for sub-studies that were added and providing adequate clarity regarding Colchicine's drug interaction profile in conjunction with Pharmacy. Frequent meetings with trial collaborators auditing the trial process.
4. Implementation of the Trial protocol procedures on the Coronary Care Unit in RPH, ensuring that the necessary structure was in place to easily enrol and ascertain blood samples. Data entry of the above and lab data in Excel and Access sheets.
5. Revisions/Amendments of Trial Patient Consent Form for ethics.
6. Ensuring blood samples were analysed appropriately in the Lab and well as samples stored correctly for future analysis. This also included the Flow Cytometry sub-study which involved collaboration with the Medical Research Foundation (MRF).
7. Collaboration with the Health Research Institute (HRI) in Sydney regarding specimen logistical procurement and analysis.
8. Eventual Collation of data and analysis assisted by my supervisor.
9. Aided in Manuscript preparation & revisions for the AHJ.
10. Frequent Examining of Literature available on the subject by regular literature searches on this topic on PubMed/Medline/Embase. Post-hoc Analysis. A Mega-analysis is currently ongoing between the Canadian COLCOT study research group and RPH.
11. Design and writing of UWA thesis having completed the necessary university modules on Epidemiology, Statistics and Development & Communication of Research.

12. Subsequent Presentation of the Trial Data after publication in the AHJ at the Irish Cardiac Society Young Investigator Award Ceremony winning the 1st Prize of the Brian Maurer Young Investigator 2019.

Student signature:

Date: 10/08/2021

I, Professor Graham S. Hillis certify that the student's statements regarding their contribution to each of the works listed above are correct.

As all co-authors' signatures could not be obtained, I hereby authorise inclusion of the co-authored work in the thesis.

Coordinating supervisor signature:

Date: 1st September 2021

2. List of Figures & Tables

Figure	Table
<ol style="list-style-type: none"> 1. CV risk in chronic inflammatory conditions such as RA arises from a combination of ‘traditional’ RFs and chronic inflammatory milieu. (3) 2. Inflammation in Coronary Plaque Formation. Endothelium disruption followed by fatty streak formation. An Advanced complex lesion forms which becomes more vulnerable and unstable (left to right, top to bottom) (4) 3. Overview of key Inflammatory components of atherosclerosis (5) 4. Activating effect of LDL Infiltration on inflammation of the artery (6) 5. Role of Macrophage Inflammation of the Artery (6) 6. Effects of T-Cell Activation on Plaque Inflammation. (6) 7. Imbalance in adaptive immune pathways between pro-inflammatory M1 macrophages versus anti-inflammatory M2 macrophages which ultimately modulate atherosclerotic plaque activity. (7) 8. The Cytokine Cascade. (6) 9. Superficial erosion versus fibrous cap rupture (8) 10. Consequences of the activation of immune cells in a coronary plaque (6) 11. Destabilising elements of the inflammatory 	<ol style="list-style-type: none"> 1. Variables in TIMI, GRACE, Cadillac, Syntax, Reynold’s risk scores 2. Mediators of the CV Inflammation 3. Vascular effects of Cytokines 4. Trials Preceding LoDoCo-MI examining Colchicine post ACS 5. Neutral RCTs of other Anti-Inflammatories seeking MACE by various cytokine pathway inhibition 6. Trials evaluating Colchicine in reducing Restenosis & In-stent Restenosis 7. LoDoCo-MI Baseline trial Data (18) 8. LoDoCo-MI hs-CRP & Laboratory data at follow-up (18) 9. LoDoCo-MI Inflammatory Biomarker substudy IL-1 β, IL-6, MPO 10. Supplementary MPO table 11. LoDoCo-MI Clinical Outcomes & Compliance (18) 12. Trials in CAD and ACS completed and On-going since the publication of LoDoCo-MI 13. Progress Summary of Translational Research in Atherosclerosis with Future Directions in Mind (‘from bench to clinic’) (19) <p><u>Please Note that numbers in Brackets are relevant citations</u></p>

cascade eventually overwhelm protective factors provoking plaque rupture and exposure plaque components to pro-coagulant serum products. (7)

12. a & b Pathways implicated in arterial thrombosis caused by superficial erosion (7) (6)
13. Certain progenitor cell lineages are pro-inflammatory driving atherosclerosis, while anti-inflammatory cytokines act antagonistically against this process. (9)
14. Mice whose bone marrow-derived cells lacked NLRP3 inflammasome components or IL-1 cytokines were markedly resistant to developing atherosclerosis (10)
15. After intricate priming and activation steps the inflammasome further perpetuates key pro-inflammatory cytokines in the atherosclerotic cascade (7)
16. NLRP3 Inflammasome is an integral piece of patho-physiological machinery in atherosclerosis (11)
17. The predominant pathologies in ACS sub-types (7)
18. Cumulative rates of recurrent myocardial infarction or cardiovascular death among statin-treated patients according to achieved levels of LDL cholesterol in mg/dl and achieved levels of hsCRP in mg/l (12)
19. Markers of inflammation and plaque instability: from foam cell to plaque rupture (13)
20. Additional insight into CVD risk prediction

<p>when hs-CRP is integrated with ‘traditional’ Risk factors (14)</p> <p>21. Atherosclerotic progression is based on the interplay & balance between pro-inflammatory and anti-inflammatory agents (15)</p> <p>22. ‘Pleiotropic’ properties of Statins (15)</p> <p>23. Colchicine chemical structure, mechanism of action & metabolism (16)</p> <p>24. Pathologic Mechanisms of Restenosis (17)</p> <p>25. CONSORT diagram (18)</p> <p>26. hs-CRP and IL-6 levels (18)</p> <p>27. Monocyte Activation & Flow Cytometry novel biomarker sub-study</p>	
--	--

3. Abbreviations

<ul style="list-style-type: none"> ● Ab Antibody ● ACE Angiotensin converting enzyme ● ACS Acute coronary syndrome ● ADP Adenosine diphosphate ● AHJ American Heart Journal ● Apo Apolipoprotein ● ARB Angiotensin receptor blockers ● BMI Body mass index ● BNP B-type natriuretic peptide ● CANTOS Canakinumab Anti-Inflammatory Thrombosis ● CCR CC chemokine receptor ● CD Cluster of differentiation ● CHD Coronary heart disease ● ChE Cholesterol Ester ● CI Confidence interval 	<ul style="list-style-type: none"> ● MHC Major Histocompatibility complex ● MI Myocardial infarction ● MIF Macrophage migration inhibitory factor ● MMP Matrix metalloproteinases ● MPO Myeloperoxidase ● MRI Magnetic resonance imaging ● N cohort number ● NF-kB nuclear factor-kappa B ● NK Natural killer ● NLRP3 Nucleotide-binding leucine-rich repeat pyrin receptor 3 ● NO Nitric oxide ● NSTEMI Non-ST elevation myocardial infarction
--	---

- CK creatine kinase
- COACS Colchicine for Acute Coronary Syndromes
- COLCOT Colchicine Cardiovascular Outcomes Trial
- COX-1 Cyclooxygenase-1
- CRP C-reactive protein
- CTL cytotoxic lymphocyte
- CV Cardiovascular
- CVD Cardiovascular disease
- CXCR CXC chemokine receptor
- CYP Cytochrome P
- DALYs Disability Adjusted Life Years
- DB-RCT Double Blind Randomised Control Trial
- DC Dendritic cell
- EC Endothelial cell
- EDTA Ethylene diamine tetra-acetic acid
- EHJ European Heart journal
- ELISA- Enzyme-linked immunosorbent assay
- eNOS Endothelial nitric oxide synthase
- FGF Fibroblast growth factor
- FoxP3 forkhead box P3
- GLUT4 glucose transporter isoform 4 protein
- GM-CSF Granulocyte-macrophage colony
- GRACE Global Registry of Acute Coronary Events
- HDL high-density lipoprotein
- HMG-CoA 3-hydroxy-3-methylglutaryl

- OR Odds ratio
- Ox-LDL Oxidised LDL
- PAF Platelet activating factor
- PAI-1 Plasminogen activator inhibitor-1
- PAMPs Pathogen associated molecular patterns
- PAPP-A pregnancy associated plasma protein A
- PCI Percutaneous coronary intervention
- PCR Polymerase chain reaction
- PDGF Platelet derived growth factor
- PECAM Platelet/endothelial-cell adhesion molecule
- PGE2 Prostaglandin E2
- PIGF Placental growth factor
- PRRs Pattern Recognition Receptors
- PTCA Percutaneous transluminal coronary angioplasty
- RA Rheumatoid arthritis
- RCT randomized controlled trial
- RNA ribonucleic acid
- ROS Reactive oxygen species
- RR Relative risk
- SAA serum Amyloid A
- sCD40L Soluble CD40 ligand
- SD Standard deviation
- SMC Smooth muscle cell
- sPLA2 Secretory Phospholipase A2
- STEMI ST-elevation myocardial infarction
- TC Total cholesterol
- TG Triglyceride
- TG2 Transglutaminase 2

coenzyme <ul style="list-style-type: none"> ● Hs-CRP high-sensitivity C-reactive protein ● ICAM Intercellular adhesion molecule ● IFN Interferon ● Ig Immunoglobulin ● IGF-1 Insulin-like growth factor ● IL- interleukin ● IL1-RA Interleukin 1 receptor antagonist ● KO Knockout ● LAPV Low attenuation plaque volume ● LDL low-density lipoprotein ● LO Lipoxygenase ● Lp(a) lipoprotein (a) ● LPS Lipopolysaccharide ● MACE Major Adverse Cardiovascular Outcome ● MCP-1 Monocyte chemoattractant protein-1 ● M-CSF Macrophage colony stimulating factor 	<ul style="list-style-type: none"> ● TGFβ Transforming growth factor β ● Th T helper ● TLR Toll Like Receptor ● TNF Tumour necrosis factor ● TNF tumour necrosis factor ● TnT Troponin T ● t-PA tissue plasminogen activator ● TxA2 Thromboxane A2 ● US Ultrasound ● VCAM Vascular cell adhesion molecule ● VCU-ART Virginia Commonwealth University Anakinra Remodeling Trial ● VEGF Vascular endothelial growth factor ● VLDL Very low-density lipoprotein ● vSMC Vascular smooth muscle cells
--	--

4. Introduction & Background

A causal role for inflammation in atherosclerosis was first postulated by Virchow in the 19th Century. (20, 21, 22) Yet up until recent decades the therapeutic strategy was focused solely upon curtailing lipid synthesis. Mounting evidence has sparked immense intrigue on the pivotal role orchestrated by inflammation in atherosclerosis and the potential of yielding novel therapeutics.(4) Consistent observational data from rheumatological conditions conveys a strong interplay between inflammation and atherosclerotic plaque development.(23) The ‘pleiotropic’ benefit of statins by inflammation attenuation in addition to lipid suppression is well described.(24, 25) Studies focusing directly on anti-inflammatory armamentarium in negating vascular risk burden have been under the spotlight in recent years.(26)

Colchicine has emerged as an eligible candidate in secondary cardiovascular prevention. (27) It is anti-inflammatory drug used to treat gout and other inflammatory disorders, such as familial Mediterranean fever (FMF) and recurrent pericarditis. (28, 29) Colchicine binds to microtubule ends and inhibits cytoskeletal microtubule assembly (Figure 23). (30) Colchicine acts on several microtubule-dependent processes such as neutrophil chemotaxis, phagocytosis, and protein excretion as well as inhibiting expression of adhesion molecules on leukocytes and endothelial cells. (31)

Monosodium urate (MSU) or calcium pyrophosphate dihydrate (CPPD) crystals prime the caspase-1-activating NOD-like receptor protein 3 (NLRP3) inflammasome producing interleukin (IL)-1beta and IL-18. (32) Macrophages in mice deficient in the machinery of the inflammasome such as caspase-1 and NLRP3 are incapable of activating IL-1beta. (32) Speculation has arisen that colchicine can also mitigate cholesterol crystal-induced inflammation via NLRP3 attenuation within atherosclerotic plaque. (26) Since Food and Drug Administration (FDA) approval in 2009 for use in gout and FMF, numerous studies have reported that colchicine may be beneficial in different cardiovascular disease states. (33) This is consistent with retrospective observations that found lower rates of myocardial infarction (MI) in gout and FMF with colchicine. (34) Taking this concept forward, the LoDoCo (Low Dose Colchicine for Secondary Prevention of Cardiovascular Disease) study was a prospective randomized trial of 532 patients with stable atherosclerosis to receive either 0.5 mg/day of colchicine or placebo in addition to standard care. After 3 years, the colchicine arm had a marked decrease in the composite endpoint- acute coronary syndrome, out-of-hospital cardiac arrest, or non-cardioembolic ischemic stroke (HR 0.33; 95% CI 0.18–0.59; $p < 0.001$). (35)

Colchicine acutely blunts the local production of NLRP3 inflammasome-related cytokines, IL-1 β , IL-18 and IL-6, in the coronaries of those with acute coronary syndrome. (36) An imaging study using coronary computed tomography (CT) angiography found that regular minimum dose colchicine positively altered the composition of plaque lesions by decreasing low attenuation plaque volume, a proposed surrogate of plaque instability and future major adverse cardiac events (MACE). (37) As with the monoclonal antibody directed at IL-1 β canakinumab, colchicine decreases high sensitivity-CRP (hs-CRP) very significantly in patients who have stable coronary artery disease (CAD) with elevated levels of inflammation (hs-CRP $\geq$ 2 mg/L). (35, 38) The low cost of colchicine in most regions of the world (including the United States where the cost is higher than elsewhere) renders it an attractive agent for repurposing. The cost of colchicine annually is immensely less than that of the human monoclonal, canakinumab. Repurposing colchicine for treating atherosclerosis poses potential obstacles regarding cost in certain parts of the World. The well-documented unfavourable gastrointestinal side-effect profile of colchicine is also an issue. (39, 40) However, colchicine's gastrointestinal side effect profile is <10% which is comparable to current standard therapy like statins.

Thus, overwhelming proof of benefit, cost effectiveness and further insight as to the mechanistic means by which colchicine reduces secondary cardiovascular (CV) risk may further foster universal therapeutic assurance in this ancient drug. As well as examining colchicine as a novel medication in coronary artery disease, this thesis will explore all anti-inflammatory based agent's past, present and future in this setting. There is public health demand to forge novel therapeutics to curtail the foreboding 10% composite MACE that still exists a year post MI. (41) In conjunction with incessant modifications and variations in pharmacological therapy, colchicine may present as a note-worthy auxiliary agent with the potential to work synergistically with regimes such 'triple-therapy post ACS with concomitant atrial fibrillation', prolonged dual anti-platelet therapy (DAPT) post MI and emerging antiplatelet monotherapy strategies where the management dilemma between bleeding versus ischaemia is rife.(42-44)

5. Hypothesis and Aims of LoDoCo-MI

5.1 Overview

Inflammation is central to the pathogenesis of atherosclerosis and subsequent clinical sequelae.(9) Higher than normal residual inflammatory biomarker hs-CRP levels 30 days ($\text{hs-CRP} \geq 2 \text{ mg/L}$) after acute myocardial infarction (MI) signifies increased risk of morbidity and mortality from coronary artery disease (CAD).(45) (46) Acute MI patients remain at increased risk of further vascular events due to this ongoing vascular inflammation.(47) Research in the 1990s hypothesised that therapies specifically targeting inflammatory pathways might reduce recurrent vascular events post MI.(48) One approach is to target specific pro-inflammatory cytokines.(19) Canakinumab Anti-Inflammatory Thrombosis Outcome Study (CANTOS) followed this approach using a monoclonal antibody targeting interleukin- 1β . It significantly reduced MACE in survivors of an acute MI with persistently elevated hs-CRP levels ($\geq 2 \text{ mg/L}$). (49, 50) Canakinumab is subcutaneously administered, very costly and associated with a significant increase in death from sepsis. (49) Thus, there is need for cheaper and safer alternatives.

Colchicine is one of the oldest anti-inflammatory agents available, with a robust track record treating gout, FMF, and pericarditis. (51) Its mechanisms of action are complex. Colchicine attenuates neutrophil response to inflammatory signals and suppresses activation of the NLRP3 inflammasome, a pathway also inhibited by canakinumab.(52) (49) Initial colchicine studies CAD were promising but its' ability to reduce inflammation after acute-MI had not been scrutinized in a large international cohort prior to the subsequent Colchicine Cardiovascular Outcomes trial (COLCOT).(53) Before embarking on a large, expensive end-point trial, extensive interrogation as to the safety, feasibility and tolerability of the drug warrants ascertainment. This is particularly important as the commonest side effect of colchicine is gastrointestinal upset, which led to drug withdrawal in 10% of patients in prior trials and might result in reduced adherence to pertinent post infarct 'mortality-benefit proven' medications. (54) The combination of colchicine and high-dose statins has been rarely associated with myotoxicity and there is a theoretical interaction with ticagrelor, a weak p-glycoprotein and CYP3A4 inhibitor though this issue was not evident in subsequent larger trials (55) LoDoCo-2 and COLCOT subsequently did not demonstrate increased incidence of myotoxicity or drug-interaction issues. The utility of the hs-CRP biomarker as a proposed surrogate of the effectiveness of anti-inflammatory

therapy post MI is also being sought in this study. Additional small exploratory mechanistic sub-study analysis using flow cytometry was also conducted titled ‘Monocyte activation & novel biomarker sub-study of LoDoCo-MI’. Inflammatory monocytes of innate immunity, especially intermediate and non-classical sub-types are believed to play key roles in triggering acute MI with such ‘activated’ monocytes implicated in recurrent events. (57, 58)

5.2 Trial Aims

The primary objective of LoDoCo-MI was to;

- 1) examine the hypothesis that low dose colchicine(0.5mg) after acute MI would significantly lower levels of hs-CRP (a potentially useful marker of vascular inflammation) at 30 days, implying a lower risk from recurrent vascular events.
- 2) Secondary endpoints assessing the effects of low dose colchicine on IL-6 a modulator of inflammation and hepatic CRP production along with IL-1 beta and myeloperoxidase (MPO). (56)
- 3) test the safety, tolerability, and compliance of colchicine with other evidence-based medications when initiated post-acute MI.

An exploratory flow cytometry (FC) sub-study analysed pathological mechanisms at play immediately post-MI. The specific aims of this mechanistic sub-study are to explore the markers of monocyte activation by FC and novel cytokine biomarkers (ILs)in survivors of acute MI at 30 days.

5.3 Trial Hypothesis

We hypothesise that colchicine in addition to standard secondary prevention (such lipid lowering therapy and anti-platelets) post-acute MI will decrease in hs-CRP & IL-6 biomarkers.

Furthermore, we hypothesise that 0.5mg colchicine daily will be well tolerated and not adversely affect compliance with important secondary prevention medication. The benefit of this study will be to assess colchicine as an adjunct to the current treatment options to manage acute MI by lowering inflammation and by assessing hs-CRP as a potential surrogate marker of such anti-inflammatory therapy efficacy in this setting. Additionally, to assess hs-CRP & IL-6 as novel CV biomarkers. In the sub-study, we hypothesise low-dose colchicine will attenuate monocyte activation on flow

cytometry and novel cytokine biomarkers (IL-6, IL-1 beta & MPO) following acute MI and depict potential actionable inflammatory mechanisms by which colchicine may reduce MACE in this high-risk population.

6. Burden of Cardiovascular Disease

CAD persists as the number one cause of morbidity and mortality in Australia and worldwide. (63) (64) (65) CAD causes over one-third of global deaths. (66) Ischaemia culminating from coronary artery disease (CAD) is the predominant aetiology for heart failure (HF)/ischaemic cardiomyopathy (ICM).(67) HF constitutes vast disability morbidity burden globally accounting for at least 5% of all medical admissions.(68) (69) CAD causes 17 million deaths yearly and this toll is predicted to pass 23 million by 2030 due to a growing aging population.(70) (71) Despite progress made in the prevention and treatment of CAD, it remains the leading cause of death in the Western world and the second most common cause worldwide.(72) By the year 2025, it is estimated that nearly 40% of all deaths worldwide will be due to CAD, more than twice the percentage of deaths from cancer. (73)

Mortality from CAD has declined substantially in recent decades due to contemporary management particularly in high-income countries.(72) Two thirds of all CAD disability adjusted life years (DALYs) however, and more than 50% of deaths happen in the Developing World.(74) The epidemiological transition in developing countries (75) (76) has led to more advanced medical therapy yielding an aging population, subjected less to malnutrition and communicable disease and to increased chronic degenerative diseases like CAD.(77)

Thus, further cost appropriate therapies like colchicine are required. The aging, growing population has culminated in increased cardiovascular deaths worldwide from 1990 to 2013. Only a few wealthy European countries have sufficient resources to mitigate this demographic trend. This contrasts with less advantaged health care systems where the implementation of effective percutaneous coronary intervention and post infarct medical therapy is an economic challenge. (78) (64)

7 Risk Factors for Acute Coronary Syndrome

7.1 Overview

The hypothesis generating Framingham studies captured early insight as to the magnitude of risk posed from ‘traditional’ cardiovascular risk factors. (79) (80) The same seminal observational study series portrays the actionable pharmacological targets which may be antagonised in primary and secondary prevention. (81) The homogeneous Framingham population cohort was almost exclusively Caucasian in one localised region. At that time, several risk factors for the condition were identified, labelled ‘classical’ cardiovascular risk factors. Non-modifiable risk factors include family history and increasing age; modifiable factors include smoking, hypertension, hyperlipidaemia, diabetes mellitus and obesity.

Conventional prevention strategies for CAD focus on standard ‘classical’ cardiac risk factors: diabetes mellitus, hypercholesterolemia, hypertension, smoking, obesity (82) with smoking or dyslipidaemia present in two-thirds of patients admitted with acute MIs. (83, 84) The INTERHEART study identified lack of regular physical activity, low vegetable intake and psychosocial factors like depression, as additional equally important modifiable risk factors. (84) Hyperlipidaemia has been highlighted as a principal modifiable risk factor for CV death, yet half of all patients hospitalised with MIs do not have overt hyperlipidaemia by criteria. (85) Hypertension, diabetes, smoking and dyslipidaemia act in part upon the atherothrombotic process by rendering the endothelium dysfunctional. These risk factors are accelerators of atherosclerosis through the crystallisation of cholesterol monohydrate in the vascular bed. (86)

While the established cardiovascular risk factors are integrated into cardiovascular risk assessment models and algorithms, they do not fully explain vulnerability to cardiovascular events possibly by failing to account for the inflammatory processes instrumental in driving atherosclerosis. (87-90) Therefore, additional novel biomarkers have been pursued to augment acumen, including: CRP, ILs, fibrinogen, MPO, homocysteine, and soluble CD40 ligand. (91) Such markers help assess the added overall cardiovascular risk contributed by less traditional cardio-metabolic and inflammatory risk factors along with observational data. Such cytokines are potential markers of ongoing risk of CV events.

8. Prevalence of coronary atherosclerosis

Atherosclerotic plaque forms during indolent sub-clinical stages which may appear clinically quiescent until overt manifestation of angina or MI. Therefore, accounting for simply clinical manifestations alone will not accurately gauge the exact burden of one's 'anatomical' coronary disease. Screening patients without clinical symptoms of CAD for quantification of coronary calcium burden (by CT imaging) illustrated tremendous variation in coronary calcium scores with age discrepancy and the presence or absence of 'traditional' risk factors. (92, 93)

The main message from these studies in the 1990s was age significantly predicted atherosclerosis and subsequent progression with clinical manifestations evident in approximately 50% of males and only 1/3 of females with women developing coronary disease generally 10 years later than men. Women also tend to harbour more hypertension and 'non-traditional' risk factors. (94)

9. Risk Factor Stratification Models in Acute Coronary Syndromes

9.1 Overview of Commonly used Risk Stratification tools

The 'Thrombolysis in Myocardial infarction' (TIMI) score was derived over 25 years ago in the thrombolytic era when defining a MI did not rely on troponins. It related solely to STEMIs aiming to predict 1-month mortality rates. Primary PCI has now vastly become the mainstay treatment over thrombolysis in the Developed World. The TIMI score (table 1) integrates primarily clinical as well as ECG parameters which renders this model as rather dated but is deemed to be an acceptable tool in primary PCI risk prediction. (95, 96) The GRACE score contains a wider expanse of data with clinical, laboratory marker, demographic, ECG, vital sign information to estimate 6-month mortality. (97) It has proved useful in directing appropriate timing of revascularization. Neither of these commonly used risk tools harness the risk prediction potential of imaging techniques that can quantify myocardial scar burden (which is arrhythmia-prone) and extent of CAD post MI.

The Cadillac risk score (table 1) builds on the TIMI & GRACE tools (both widely accepted in clinical practice in Australia) by incorporating ejection fraction (EF%) on echo with pivotal angiographic (looking for triple vessel disease-TVD and functional indices by assessing TIMI flow post PCI) data as well as pertinent laboratory and clinical parameters (such as very prognostically significant decompensated heart failure by Killip class). (98)

Table 1 Variables in TIMI, GRACE, Cadillac, Syntax, Reynold's risk scores

TIMI	GRACE	Cadillac	Syntax	Reynolds
Age (>65)	Age	Age	Dominance	Age
Low BP	Low BP	Creatinine	Vessel segment	Low BP
ASA use	HR	Heart failure	CTO	Gender
CAD >50%	Creatinine	Final TIMI flow	Trifurcation	Cholesterol
angina	Killip class	TVD	Bifurcation	HDL
+ biomarkers	+biomarkers	EF %	AO lesions	smoking
ST changes	ST changes	Anaemia	Tortuosity	Family Hx
>3 RFs	Cardiac arrest		Length (>20cm)	HbA1C
Weight			Calcification	Hs-CRP
			thrombus	
			Diffuse disease	

- BP-Blood pressure, ASA-Aspirin, RFs-Risk factors, TVD-triple vessel disease, EF-ejection fraction, CTO-chronic total occlusion, AO-Aorto ostial, HR-heart rate. HDL-high density lipoprotein

The enhanced C-statistic (over TIMI and GRACE) speaks to the additional key non-invasive and invasive metrics that the Cadillac score harnesses. What renders the Cadillac rather outdated is the bare metal stent premise leading to imprecise estimates with latest generation drug eluting stents (DES). The GRACE and TIMI have both undergone extensive up-to-date remodifications (e.g., 'dynamic TIMI'), maintaining their relevance in contemporary secondary CV risk prediction. (98) However, the advent of more precise readily accessible imaging technologies warrants exploration and integration into such models. Relevant to the LoDoCo-MI, the Reynolds risk score recognises the contribution exerted by the inflammatory cascade by placing hs-CRP alongside widely accepted risk model components. (99)

The Reynolds augments risk prediction on plaque progression over the Framingham model. (100) These scoring systems remain for the most part mere research reference tools despite formidable validation in ACS prognosis. The chief parameters that guide treatment are the symptoms, ECG changes and typical MI trend in troponin. Scoring systems (like the SYNTAX) that estimate the potential danger conferred by PCI eclipse models lacking such data and tend to have great impact on decision making strategies in heart team meetings between PCI, coronary artery bypass grafting (CABG) or sole medical therapy when combined with other factors like patient preference etc.

Coronary lesion traits help direct the method of revascularisation. The SYNTAX (Synergy between PCI with Taxus and Cardiac Surgery) incorporates length, lesion number, bifurcation lesions with tortuosity, thrombus, and calcium burden in its framework with the presence and degree of these angiographic factors governing prognosis. (101) There is inherent variability in such a lone angiographic estimation (though less so in core lab analysis), however the SYNTAX score remains a vital component in the aiding definitive decisions along with pressure wire technology i.e., Fractional Flow reserve (FFR).

9.2 Future Directions for Cardiovascular Risk models

Current cardiac risk models fall short being somewhat antiquated in stratifying risk in complex heterogenous cohorts. (102) Revolutionary advancements in radiological (cMRI, CT-CA, nuclear medicine) and laboratory biomarker (including genomic/proteomic) investigations warrant prompt integration into risk stratification models to reflect current clinical practice trends. (103)

It is hoped that gene studies will have immeasurable potential in unravelling and enumerating a given individual's inherent propensity towards a cardiac event with in-depth phenotypic foresight possibly overshadowing anything a purely clinical model may estimate. (104) (107) Mendelian randomisation utilises genetic variants as pivotal variables to deduce whether a given risk factor causally influences a health outcome such as CAD. Mendelian (gene) randomisation studies are integral in defining the intrinsic genetic association/causality for biomarkers (e.g., hs-CRP, Lp-PLA2 etc.) (105, 106)

With the re-classifying capacity of hs-CRP & NT-pro BNP (as marker of heart failure severity), further increases in the precision of the TIMI and GRACE risk prediction models in STEMI are possible. (108)

Time will tell whether there is indeed any significant benefit to risk stratifying patients if the treatment options are relatively limited and universal for all patients post MI. (109)

10 Inflammatory conditions and cardiovascular disease

Chronic inflammatory diseases endure a heavy burden of CAD with rheumatological conditions typifying this trend. (34, 71) Rheumatoid arthritis (RA) has a 50% increased risk of CV events with current standard CV risk assessment models like Reynold's index (which includes hs-CRP) and Framingham underestimating the same risk. (110) Disease activity and antibody titres are intimately correlated to cardiac risk with inflammatory effectors including cytokines and T cells causing vasculature injury. (111) Mendelian (gene) randomisation studies in patients with RA have underscored the pertinence of IL-6 signalling on the causative pathway in atherosclerosis (contrasting with hs-CRP) presenting a potentially actionable therapeutic target. (112) Reducing IL-1 with a recombinant agent suppresses deleterious inflammation after MI, abating negative left ventricular remodelling which would lead to heart failure. (113)

Rheumatoid arthritis patients have a significantly increased risk of cardiac mortality with cardiovascular disease accounting for over a third of deaths. (48) The increased incidence of CAD in RA is independent of 'traditional risk' factors. (82) Chronic inflammation is the proposed culprit for this finding in RA (Figure 1). (34) Imaging data confirms a greater burden of atherosclerosis relative to population-based controls after adjusting and stratifying for demographics and risk factors. (114, 115) RA sufferers with more active and progressive disease possessed grossly elevated Coronary CT Calcium score levels with higher plaque risk features more abundant. (116, 117) Inflammatory burden is connected to subsequent evolution of atherosclerosis on serial imaging. (118) The theory implicates chronic and/or high level of systemic inflammation, and several studies of CAD in RA propose those with the highest inflammatory burden as being at highest risk of CV events. (117) This is corroborated with the neutrophil/lymphocyte ratio (NLR) showing higher incidence and prevalence of MACE with higher NLR. (423) More relevant to the LoDoCo-MI study, in retrospective observational studies patients on colchicine for gout had lower incidence of MACE and hs-CRP instating gout as a 'non-traditional' independent RF. (34, 119) Inflammatory monocyte activation is at the core of CV risk in Human Immune Virus (HIV). CD 16 monocytes correlated with coronary artery calcium (CAC) in this population. (120) As a potentially

modifiable risk factor, assertive inflammatory control in such highly active inflammatory conditions is imperative to mitigate against cardiovascular sequelae.

Figure 1 CV risk in chronic inflammatory conditions such as RA arises from a combination of ‘traditional’ RFs and chronic inflammatory milieu. (3)

11. coronary artery disease (CAD) & Inflammation

11.1 An Overview

CAD results from accumulation of cholesterol-rich plaques in the walls of the coronary arteries. (121) Inflammation is central to the atherosclerotic process. (122) Inflammation is triggered by plaque trauma induced by cholesterol crystallisation in plaque. Unregulated inflammation induces plaque instability paving a pathway to erosion and rupture, leading to thrombosis and myocardial infarction (MI). (26) Numerous inflamed vulnerable plaques can co-exist increasing the probability of early post MI events, with rates of 12% at one year and 20% at three years. (26) The reality is current therapeutic armamentarium with statins, do not sufficiently modulate plaque inflammation. (26) Novel agents are warranted, especially those directed specifically at inflammation.

The CANTOS trial utilising an anti-interleukin-1-beta (IL-1 β) monoclonal antibody has stimulated interest in this area. (50) It highlights that not only is inflammation instrumental from a pathogenic and

prognosis perspective in CAD, but that directly suppressing inflammation reaps benefit in absolute reduction in CV events. This was driven by a comparable reduction in non-fatal MI. Methotrexate in established CAD did not reduce levels of interleukin-1 β , interleukin-6, or C-reactive protein and did not result in fewer cardiovascular events than placebo. (123) The fact that methotrexate did not reduce these markers reflects the fact that it may have other anti-inflammatory effects that are more relevant in RA than atherosclerosis. The challenge is to find therapeutic strategies which are practical and amenable universally. One of the greatest triumphs of preventative cardiology has been the identification and subsequent aggressive control of 'traditional' risk factors for atherosclerosis, such as hypertension, diabetes, smoking, and elevated low-density lipoprotein cholesterol (LDL-C). Consequently, we have seen a dramatic decrease in the incidence of MI by up to 40% in many Western nations that have embraced a preventative approach to CAD. (124) One important example of this is the use of cholesterol-lowering statins, that reduce the incidence of MACE by 25% to 40% in individuals with atherosclerosis.

However, despite our best attempts to manage traditional risk factors with contemporary approaches, MACE continue to occur at significant rates in patients with established CAD, with event rates of 10–12% at one year and 18–20% at three years after an index MI. (125) This is largely because atherosclerosis is a complex disease process with a pathogenic basis reaching far beyond intimal infiltration of cholesterol. In particular, numerous converging lines of clinical and experimental evidence show that inflammation is a key player in its initiation, progression, and eventual thrombotic manifestations. (48)

Inflammation is induced by a range of various insults.(126-128) Risk factors including; hypertension, diabetes, and smoking, are perpetuated by catalytic oxidised LDL-C, initiating a chronic inflammatory reaction, the result of which is a vulnerable plaque, prone to rupture and thrombosis.(126) Epidemiological and clinical studies depict robust and coherent correlation between inflammation markers and subsequent CV risk.(129) Inflammation can be detected locally by various emerging imaging techniques and systemically by inflammatory markers. (126) Of these, the most reliable and accessible is at present hs-CRP. (126) Merging several methods may yield more precise risk stratification models enhancing therapeutic accuracy. (130) Statins have been shown to provide effective therapy for lowering hs-CRP concomitant with their lipid-lowering effects.(131) The search to better understand the disease process has led researchers to an enhanced understanding of inflammation in atherogenesis and thrombosis, and to several novel methods for risk reduction and treatment.(71) Hypertension, diabetes and smoking as well as elevated lipids have been shown to play

a role in the atherothrombotic process by making the endothelium dysfunctional. (86) Elevated LDL is particularly implicated, becoming oxidised by reactive oxygen species in the subendothelial space, where (along with other factors) triggers leukocyte adherence, the first key step in the genesis of atherosclerosis (Figure 2 & 3 below) (132)

11.2 Inflammation in the Pathophysiology of Atherosclerosis

Figure 2 Inflammation in Coronary Plaque Formation. Endothelium disruption followed by fatty streak formation. An Advanced complex lesion forms which becomes more vulnerable and unstable (left to right, top to bottom) (4)

Inflammation is ubiquitous in coronary heart disease, (4) central to the pathogenesis of atherosclerosis, initiating and potentiating the growth of atherosclerotic plaques (Figure 2).(4, 133) Inflammatory cells (especially neutrophils) play a part in inciting the erosion and rupture of plaques

culminating in acute myocardial infarction (MI), (4, 133, 134) which is the underlying cause of approximately 80% of all sudden cardiac death (SCD). (126)

Leukocyte recruitment with neutrophils is an early lesion feature (Figure 2). A non-disrupted vascular layer opposes adhesive factors and leukocyte connection. Upon deposition of cholesterol fragments into the vascular bed, neutrophils bind to the endothelium and traverse junctions to the intima where lipid accretes in foam cells. (135, 136) Through ingesting lipids and lipoproteins into a disrupted vascular wall, monocytes transition to macrophages which further create foam cells which go on to propagate atheroma (Figure 2). (137) (138) (139)

Figure 3 Overview of key Inflammatory components of atherosclerosis (5)

Th-T helper, TLR-toll like receptor, MPO myeloperoxidase, MMP-matrix metalloproteinase, IFN- γ interferon, SMC-smooth muscle cell

Lymphocytes and monocytes under the influence of adhesion molecule expression become bound to the vascular wall (figure 3). (140) A cell surface sialo glycoprotein vascular cell adhesion molecule-1 VCAM-1 (an adhesion molecule) intercalates with integrins early in atherosclerosis predominantly in atheroma prone leukocytes. (141) Selectins are also adhesion molecules (outlined in Figure 3 above). E-selectin is less prolific in early angiogenesis and attracts polymorphonuclear leukocytes in the acute inflammatory process. (142) P-selectins play a more influential role in leukocyte

(neutrophil) recruitment in early plaque with greater manifestation in endothelial cells (ECs) causing leukocyte 'rolling'. (143)

Figure 4 Activating effect of LDL Infiltration on inflammation of the artery (6)

Once oxidised, lipoprotein-associated phospholipase A2(Lp-PLA2) synthesises pro-inflammatory lipids. (135) Lp-PLA2 will be discussed in a separate section (regarding novel therapeutic targets). Leukocyte recruitment ensues once the oxidative inflammatory platform is ignited (Figure 3 & 4). In an activated form vascular wall cells increase their expression of leukocyte adhesion molecules, such as VCAM-1 and intercellular adhesion molecule-1 (ICAM-1) (Figure 4 to 6). (26, 144)

The predominant cell type at plaque site rupture is the neutrophil of the innate immune system (Figure 3 & 4). Activated T cells and mast cells, also attach themselves to the vascular wall and this confluence of inflammatory cellular tissue forms the atheromatous lesion, containing a lipid pool guarded by a fibrous cap.(145) The monocyte-macrophage complexes sequester metalloproteinases which catalyse the enzymatic degradation of the collagenous fibrous cap (Figure 5).(146) Production of matrix metalloproteinase 9 (MMP-9) and other proteases orchestrates smooth muscle cell (SMC) migration through lamina and subsequent proliferation under the influence of fibroblast growth factor (FGF) and platelet derived growth factor (PDGF). (147) This cap is thus susceptible to rupture subjecting procoagulant tissue factor (TF) to blood triggering thrombosis.

Figure 5 Role of Macrophage Inflammation of the Artery (6)

Table 2 Mediators of the CV Inflammation

Proinflammatory Cytokines	Immune Cell surface molecules	Eicosanoids
IL-1beta, Il-18, TNF, IFN-gamma	CD40-CD40L	Prostaglandins, leukotrienes

Table 3 Vascular effects of Cytokines

IFN gamma	TNF Cytokine actions
Endothelial cell (EC) activation	Activation of ECs
Smooth muscle cell & collagen inhibition	MMP & NOS promotion
MMP & NOS promotion	Mineralisation regulation-RANKL
	Regulate Lipid metabolism

Proliferation of SMCs in the vascular wall follows the inflammatory phase causing a healing response (figure 6). (146) Cap erosion is mediated by IFN gamma (table 2 & 3) from the adaptive immune system suppressing protective collagen with CD40L and IL-1 producing collagen denudation enzymes (MMP-1/8 and 13). (148) Lipoproteins, such as LDL permeate through the endothelial layer into the intima where a chemical modification results in the oxidation LDL. This oxidised LDL, serves as a pro-inflammatory mediator stimulating vascular wall cells to produce pro-inflammatory molecules and increases the expression of adhesion factors on their cell surface such as ICAM and VCAM-1 to attract monocytes and lymphocytes to the vascular wall. (149,150) Altered monocytes (attracted by chemokines like chemokine C-C motif ligand 2-CCL2) transmigrate into the intima, differentiating into macrophages and phagocytosed oxLDL as well as cholesterol crystals, thereby forming foam cells. (137) The inflammasome is then activated culminating in Pyroptosis (a more highly inflammatory form of cell death) further initiating innate immune response.

Nitric oxide (NO) helps maintain vascular tone by vasodilation and is downregulated at the site of vascular injury allowing platelet aggregation and subsequent thrombotic sequelae to ensue. Leukocytes become tethered to the vascular wall under the influence of adhesion molecules (selectins), (153) further solidified by VLA-4 and the ligands VCAM-1 and ICAM-1 allowing leukocyte attachment to ICAM-1 (expression stimulated by IL-1, TNF alpha, and IFN gamma) and VCAM 1 with subsequent transmigration of leukocytes across the vascular wall. (154)

The continuous accumulation of oxLDL particles and cholesterol crystals which undergo phagocytosis (by macrophages) through scavenger receptors like CD36 leads to the formation of an early atheromatous plaque. (135) From this stage onwards, the plaque progression intensifies, as more immune cells infiltrate, SMCs start to proliferate, and calcium deposition appears. (151) Cholesterol crystallisation within lysosomes causes reactive oxygen species (ROS) to be released from endoplasmic reticulum ending in apoptosis (programmed cell death). An inflammatory cascade of IL-1 β , IL-6, tumour necrosis factor-alpha (TNF- α) and MMPs further perpetuates atherosclerosis by signalling SMC activation. Further key mediators including adhesion molecules, cytokines, growth factors are precipitated, of which IL-6 is a prominent procoagulant & Inflammatory cytokine. (135) IL-6 can increase plasminogen activator inhibitor type 1, and acute phase reactant CRP levels perpetuating pro-coagulation and inflammation, respectively (Figure 8).

In the intima SMCs secrete key plaque remodelling extracellular matrix proteins which cultivate the fibrous cap surrounding the plaque. Thinning of the collagenous cap through denudation is critical in eventual plaque rupture. CD4+ T cells activated by MHC class 2 antigen presenting cells are present in the lesion and contribute to inflammation propagation by producing IFN gamma and CD40L (Figure 7 & 11). (152) Cholesterol crystallisation in the plaque core is pivotal in subsequent rupture. (135) (121)

Figure 6 Effects of T-Cell Activation on Plaque Inflammation. (6)

Figure 7 Imbalance in adaptive immune pathways between pro-inflammatory M1 macrophages versus anti-inflammatory M2 macrophages which ultimately modulate atherosclerotic plaque activity. (7)

FoxP3+ Forkhead Box P3, TGF-β-transforming growth factor beta, CX3CR1 C-X3-C Motif Chemokine Receptor 1, PTPN22-Protein Tyrosine Phosphatase Non-Receptor Type 22

Relevant to the LoDoCo-MI flow cytometry sub-study, circulating monocyte subsets are defined by CD14 and CD16 surface protein expression into classical (CD 14⁺⁺, CD16⁻), intermediate (CD14⁺, CD16⁺) and non-classical (CD14⁺, CD16⁺⁺). Intermediate (CD14⁺ and CD16⁺) and non-classical (CD14⁺ and CD16⁺⁺) are pro-inflammatory being associated closely with ACS. (59, 60) non-classical monocytes are more abundant in atherosclerosis with this monocyte sub-type having a higher potential to migrate towards the chemokine CXCL16 released from macrophage-laden plaques. We hypothesised (in the sub-study) that treatment with low-dose colchicine may reduce residual inflammatory burden (and thus recurrent cardiovascular events) by multiple mechanistic channels in particular the suppression of monocyte and neutrophil activation in patients following an acute MI (as well as aiming to depict an extra mechanistic dimension in the post ischaemic-reperfusion setting). The benefits of colchicine in atherosclerosis are in part mediated by inhibition of inflammasome activation (particularly NLRP3) thereby reducing the release of pro-inflammatory cytokines IL-1β and IL-18. (61) A reduction in NLRP3 activation is reflected in an overall drop in monocyte activation and monocyte-platelet aggregation. (62)

Figure 8 The Cytokine Cascade. (6)

CRP with IL-1 and TNF incite adhesion molecules to facilitate leukocyte adherence to the vascular wall thus setting in motion a vicious pathological cycle (figure 8). CRP is postulated to have its own inflammatory effects and to be directly involved in atherothrombosis. (155) Inhibition of key points of the inflammatory cascade (figure 8) results in a reduction of atherothrombotic cardiovascular events and disability burden. (26)

Superoxide dismutase (SOD) deters oxidative stress by breaking down reactive and superoxide anions. (156) Vascular wall NOS releases the vasodilator nitric oxide (NO) which can mitigate inflammatory disruption of the vascular wall.(157, 158) NO expresses an anti-inflammatory effect by interfering with regulator nuclear factor kappa B (NF-κB).(159) NF-κB modulates a multitude of genes in inflammation and atherosclerosis.(160) Krüppel-like factor 2 (KLF2) can provoke NOS expression suppressing NF-κB function.(161) Scavenger receptors potentiate excessive lipid uptake in foam cells by ‘internalising’ modified lipoproteins.(162) Angiogenic proteins cause plaques to form an intrinsic microvascular circulatory supply.(163) Such microvasculature allows leukocyte trafficking with adhesive molecules more prevalent than in the macro-vasculature.(164) This intrinsic supply allows for autonomous lesion growth even with a paucity of systemic oxygen.(164)

Figure 9 Thrombosis due to erosion versus plaque rupture Libby et al. 2017(8)

PMN-polymorphonuclear, NETs-Neutrophil extracellular traps

Figure 10 Consequences of the activation of immune cells in a coronary plaque (6)

11.3 Plaque Rupture and Thrombosis

The chain of events that lead to a life threatening intra luminal clot centre around the compromise of the vascular membrane. (Figure 10) (139) The endothelial layer of the blood vessel is therefore an integral barrier preventing pro-thrombotic elements beneath from coalescing with serum. The predominant pathological perception of acute coronary syndrome is that of plaque rupture with thrombotic debris obstructing distal vessel flow incited by a myriad of immunological milieu and/or haemodynamic shear stresses. Chronic negative vessel remodelling involves plaques expanding inwards upon the lumen causing blood flow hindrance. Rupture of the fibrous cap is due to imbalance between protective elements and factors that damage cap integrity (Figure 11). (165) Collagen components confer tensile strength in the framework of the fibrous cap. (166) Degradation of this adhesive collagen and its sequestration governs risk of plaque rupture. (167) IFN- γ , originating from adaptive immunity T cells negates collagen production from SMCs (Figure 11). (168) Counteracting such forces are TGF- β & PDGF from platelet progenitor lineages augmenting collagen levels and fortifying plaque architecture (Figure 13). (169) Matrix derived denudation enzymes also deteriorate cap strength. (170)

Figure 11 Destabilising elements of the inflammatory cascade eventually overwhelm protective factors provoking plaque rupture and exposure plaque components to pro-coagulant serum products.

(7)

TXA₂-Thromboxane A₂, PGI₂-prostaglandin I₂

Advanced atheromatous plaques have activated macrophages exuding catalytic MMPs which thin the cap causing rupture (Figure 11).(171) Inflammatory regulators instigate the programmed cell death of SMCs with a paucity of SMCs at inflammatory rich areas and rupture sites. (172, 173) A large lipid pool and macrophage concentration in plaque shoulder areas are biomechanically susceptible to rupture (Figure 9).(174) Tissue Factor (TF) which incites thrombosis is secreted by macrophages and SMCs (Figure 12 a).(175) Lesions associated with superficial erosion harbour high concentrates of proto aminoglycans, granulocytes and a low chronic inflammatory burden.(176) The Toll like receptor of innate immunity modifies endothelial signalling further causing erosion (Figure 12 a). This contrasts with rupture plaques which greatly lack collagen and are inflammatory prone (Figure 12). Granulocytes in an active form release neutrophil extracellular traps (NETs). (177) DNA fragments coalesce with neutrophil constituents triggering oxidative, inflammatory, and thrombotic milieu. (Figure 12 b)

Figure 12 a & b Pathways implicated in arterial thrombosis caused by superficial erosion (7) (6)
HOCL-Hypochlorous acid, PAD4-protein arginine deiminases

Recurring cycles of plaque disturbance, thrombosis and healing culminate in sequential plaque enlargement. (146) Disruption of plaque and subsequent healing chronologically precede the terminal thrombi which causes SCD. Healing forces are driven by platelet derived TGF- β and platelet derived

growth factor (PDGF) as well as thrombin which recruit SMCs and thus synthesising protective collagen (Figure 13 below). (178) In its most advanced stage due to programmed cell death, the atheroma is heavily calcific, lipid poor, less cellular and less vulnerable to rupture (Figure 13).(179) Recognition of high-risk plaque features has stimulated a deluge of imaging evaluation to elucidate morphological traits associated with high propensity for ACS.(180, 181) The incorporation of such radiological imaging data with biomarkers of inflammation which are proven to correlate with ACS may further generate prognostic models and therapeutic targets as well as tailoring specific therapies to the individual.(103)

Figure 13 Adaptive Immunity in Atherosclerosis (9)

Ab-antibody, CTL-cytotoxic T cell

12. Focus on the Inflammasome in CVD

Inflammasomes are intracellular protein complexes essential in innate immunity governing the activation of inflammatory mediators. They are activated by danger associated molecular patterns (DAMPs) along with the NLRP3 inflammasome involved in atherosclerosis (Figure 14 & 15). (182) (183) (184) (185) Inflammasomes are pattern recognition receptors (PRRs) that are formed in response to a multitude of stimuli (Figure 15 & 16). (186) Inflammasome activation is involved in the pathogenesis of various disease entities including MI and HF. (187-189) Inflammasome-based signalling plays a crucial role during the development of atherosclerosis and acute infarction. (190, 191)

Figure 14 Mice whose bone marrow-derived cells lacked (KO: Gene Knock-Out) NLRP3 inflammasome components or IL-1 cytokines were markedly resistant to developing atherosclerosis (10)

After priming and activation stages, inflammasomes create pro-inflammatory cytokines IL-1 β and IL-18, inducing a signal transduction immune cascade culminating in atherosclerotic disease progression (Figure 15 & 16). (183, 192) Heightened interest has arisen recently regarding the inflammasomes in

CAD as targeted interference with inflammasome-mediated signalling could reduce inflammation and attenuate the severity of disease. (188)

Figure 15 After intricate priming and activation steps the inflammasome further perpetuates key pro-inflammatory cytokines in the atherosclerotic cascade (7)

The most frequently studied inflammasome in CAD is the NLRP3 inflammasome, composed of NLRP3 an adaptor protein ASC, and the protease caspase-1. (193) The activation of this multimeric complex initiates downstream responses including the maturation of the pro-inflammatory cytokines IL-1 β and IL-18 and inflammation related cell death named Pyroptosis (Figure 15). (194, 195)

NLRP3 inflammasome activity is increased in atherosclerosis and the level of activation correlates with the severity of disease.(196)The inflammasome components (NLRP3, caspase-1) and its downstream interleukin molecules (IL-1 β and IL-18) are upregulated in human atherosclerotic lesions.(183) Vulnerable plaques also show increased expression of the inflammasome compared to morphologically stable plaques.(197) Unstable Plaques with higher IL-18 & messenger ribonucleic acid (mRNA) levels have elevated inflammasome expression versus stable lesions with IL-1 β , IL-18 and the IL-18 receptors more frequent in macrophage rich areas.(198, 199) NLRP3 levels correlate with the severity of coronary artery disease (on coronary angiography by anatomical SYNTAX scores) and with clinical risk scores (e.g. GRACE score).(200) Levels of NLRP3, IL-1 β and IL-18 in subcutaneous adipose tissue are independent predictors of coronary atherosclerotic severity.(196) CANTOS was the first landmark trial to target IL-1 β just downstream of the inflammasome to attain a 0.6% absolute reduction in CV events. (201) Regarding Colchicine's action upon the

Inflammasome, a 1.5mg ('high dose') of colchicine attenuated IL-1B, IL-18 and caspase-1 with higher trans-coronary gradients of the same cytokines/biomarkers portrayed in a different study using high dose colchicine in ACS. (202, 203)

INFLAMMATION IN ATHEROSCLEROSIS

Figure 16 NLRP3 Inflammasome is an integral piece of patho-physiological machinery in atherosclerosis (11)

13. Diagnosis of Coronary Heart Disease/Acute Coronary Syndrome

Coronary atherosclerosis may be quiescent for a prolonged period until manifestation of symptomatic ischaemia. Exertional chest pain is the usual symptom that comes to mind but is far from ubiquitous. About 1/3 of women do not demonstrate the 'classic' symptoms often with deleterious consequences. There are three types of ACS defined by using ECG and troponins: ST elevation (formerly Q-wave) MI (STEMI), non-ST elevation (formerly non-Q wave) MI (NSTEMI), Unstable angina (UA). STEMI and NSTEMI have a typical rise and/or fall in biomarkers. (204) The third universal definition of MI (2012) was the standard definition in LoDoCo-MI. (204) The common diagnostic dilemma is to delineate between type 1 vs type 2 MI: Type 1 (spontaneous MI): MI consequent to a pathologic process in the wall of the coronary artery (e.g., plaque erosion/rupture, fissuring, or dissection), resulting in intraluminal thrombus. (204) It was type 1 MI that was included in the LoDoCo-MI inclusion criteria as with similar trials in this field (Figure 17 A). Type 2 (MI secondary to an ischemic imbalance): MI consequent to increased oxygen demand or decreased supply with presence of coronary artery disease as shown in Figure 17 (D) (e.g., coronary artery spasm, coronary artery embolus, tachyarrhythmias, anaemia, respiratory failure, hypotension etc). Additional subtypes exist including type 3, sudden unexpected cardiac death with evidence of new coronary thrombus on

autopsy. Type 4A which is deemed to be PCI-related and type 4B being stent thrombosis. Types 3, 4A and 4B are of particular interest and relevance to Colchicine studies in the domains of colchicine inhibiting in-stent restenosis, stent thrombosis and the potential for colchicine to shield against the threat of slow clopidogrel metabolism in certain individuals/populations. (205, 206, 420) Type 5 pertains to graft loss from CABG. (204)

Of the gamut of cardiac biomarkers that exist for diagnosis, risk stratification and prognosis in MI. (207) Troponin T is currently the preferred marker for the diagnosis of myocardial injury because of increased specificity and better sensitivity over predecessors. (204) Larger troponin peaks are associated with a poorer prognosis (in spontaneous MI without reperfusion therapy) and help estimate infarct size in combination with left ventricular function on imaging. In acute MI, the extent of myocardial damage is closely linked to prognosis. (208) Infarct size is the strongest determinant of post-MI term left ventricular function. (209) Early determination of infarct size post AMI is key to assessing prognosis and guiding appropriate therapeutic strategies, minimising irretrievable myocyte tissue loss to scarring. (210)

Figure 17 The predominant pathologies in ACS sub-types (7)

14. Inflammation and relevance of LoDoCo-MI inflammatory markers

14.1 Overview

Despite keen insight into the importance of inflammation in the progression of CAD and ACS, attempts to find effective modulators of the inflammatory process have yielded limited success. Until LoDoCo, no specific anti-inflammatory treatments had been shown to reduce CV events. This trial spurred on CANTOS (50) and COLCOT (211), LoDoCo-2, LoDoCo-MI.

Efforts to stabilise atherosclerotic plaque and reduce post-MI complications have relied mainly on a combination of antithrombotic and antiplatelet agents, revascularisation and lipid lowering medications. (212) Statins, in addition to lowering LDL-cholesterol, possess well-documented anti-inflammatory properties bearing in mind the anti-inflammatory effect may accrue from lipid lowering. (213) Statin therapy is associated with a reduction in the hs-CRP biomarker (213) and, following an acute coronary syndrome, there is a correlation between the fall in hs-CRP observed when on statin therapy and the risk of further clinical events. (47) It is a challenge to unravel the quantity of positive effect from specific anti-inflammatory action versus solely LDL-lowering. The benefits of statin therapy after acute MI are clear but limitations exist. In the PROVE-IT trial, patients receiving high-dose atorvastatin still had a 22% incidence of recurrent CV events over the next 2 years, (214) with a significantly higher event rate observed in patients with hs-CRP levels above the median $\geq 2\text{mg/L}$ versus non-elevated residual levels ($< 2\text{mg/L}$). This has led to the concept that ongoing low-grade inflammation, as reflected by chronic mildly elevated levels of hs-CRP, as a pertinent determinant of cardiovascular risk universally in both primary and secondary prevention. (129, 215) It is thus not unreasonable to think that therapies that lower hs-CRP may lead to improved outcomes given the strong correlation with risk. (216)

The introduction of cholesterol lowering statins to lower LDL has led to significant improvement in cardiovascular outcomes and hs-CRP is an independent and as good predictor of risk as LDL. (217, 218) It is known that aggressive statin therapy improves cardiovascular outcomes lowering hs-CRP and LDL-C. (47) The mechanism of lowered hs-CRP is additional to the LDL effect (Figure 18). (213, 219) Other potential targets proposed are the inhibition of IL-6 and TNF alpha which are involved in stimulating hepatocyte CRP production. (220) (221) The benefit of pravastatin was greater in patients with higher hs-CRP (CARE trial). (222) The Women's Health Study demonstrated

a significant cardiovascular benefit among healthy women at 8 years when comparing the highest to lowest quintile of hs-CRP, independent of LDL-C. (155) The PROVE IT-TIMI 22 trial confirmed that using statins lowered LDL and hs-CRP as well as improving CV outcomes. (223) The magnitude of risk reduction with lowering hs-CRP exceeds the expected benefit of the lowered LDL with statins. (223)

Figure 18 Cumulative rates of recurrent myocardial infarction or cardiovascular death among statin-treated patients according to achieved levels of LDL cholesterol in mg/dl and achieved levels of hsCRP in mg/l (12)

Although the PROVE IT – TIMI 22 trial demonstrated that pravastatin lowered LDL and hs-CRP and that patients who achieved a hs-CRP of <1 had the best outcomes there was no demonstrable link between LDL-C and hs-CRP (Figure 18). (223) (12) It also showed that if therapy did both actions (i.e., reduce LDL and hs-CRP) that it was better than when it achieved only one or neither affect with the cut points illustrated in figure 18 reflecting that therapy did not always do both actions. The Jupiter trial recruited 17,802 healthy men and women without prior symptomatic cardiovascular disease and with normal LDL but elevated hs-CRP (>2 mg/L) and randomised patients to 20mg Rosuvastatin or placebo. (213) The trial demonstrated a survival benefit at one year and decreased rates of acute myocardial infarction (AMI) and stroke in the statin arm. (213)

Despite the clear association of lowered hs-CRP and cardiovascular risk reduction it may not be possible to delineate a direct anti-inflammatory effect of statins as distinct from their lipid lowering effect. (224) Earlier work sought a safe, effective agent that targeted inflammation and was devoid of effect on lipid profile, BP, platelet function. (225) The main requirement for such a trial is to identify an agent that inhibits the inflammatory process including hs-CRP, that has no significant direct effect on traditional cardiovascular risk factors with an acceptable safety profile. LoDoCo (2013) had previously documented that colchicine will lower hs-CRP in a stable CAD population along with standard therapy. (226) The concept was broadened to a 'dual instigator' model which puts forth dyslipidaemia as well concurrent inflammation (124) as the predominant inciters for atherosclerosis. From a therapeutic viewpoint, this has laid the basis for the use of pharmacological agents with an anti-inflammatory component in their modality.(56) There are several steps in atherosclerotic genesis with the pathological intercalation of dyslipidaemia and inflammation which coalesce to sequester the fundamental cytokinetic elements the chain of atherosclerosis.(134) In the past, the spotlight was on disruption of the vascular wall due to plaque rupture or erosion. Recently, more gravity has been shone upon the inflammatory monocytes which sequester the pivotal tissue factor precipitants for coronary thrombi genesis. (19) Post-mortem studies have shown that coronary thrombi superimposed on eroded plaques contain a higher density of MPO positive cells than thrombus superimposed on ruptured plaques. (227) This posits biomarkers as 'fingerprints' or 'signatures' for unique pathological processes at play as distinct from others. Whether it is indeed worth-while to routinely utilise such inflammatory markers in clinical practice is being explored in this thesis given the relatively fixed, universal pharmacological agents that all ACS patients are treated with globally.

Systemic MPO (released from activated inflammatory cells/neutrophils) levels are also elevated in patients with ACS presenting with eroded plaque as compared to those presenting with rupture, suggesting that elevations in selective inflammatory biomarkers may reflect specific acute coronary events.(228) Hs-CRP level is dramatically elevated in patients with sudden cardiac death (SCD) from severe coronary artery disease.(229) Serum level of hs-CRP correlates positively with its presence in plaques and is also a predictor of increase in the number of thin-cap fibroatheroma. (230) The goal is to identify biomarkers of vascular risk, as well as mediators of vascular inflammation that would be actionable targets for inhibition with acceptable feasibility and practicality.

While traditionally established risk factors like smoking, diabetes mellitus, and hypertension are used in cardiovascular risk assessment, they fall short in estimating measurable susceptibility to cardiovascular events.(87-90) Clinicians have sought additional biomarkers to augment clinical decision making precision, including: hs-CRP, MPO, IL-6.(91) It is reasonable that several biomarkers could be measured simultaneously in relationship to each other to improve risk stratification in patients.(231) There is the potential to derive novel risk stratification and prognostic indices from integrating such biomarkers.(232) Biomarkers that surge just prior to plaque rupture possess the largest impact potential in forewarning of an event.(9) More advanced downstream markers like ST2 and NT-proBNP are indicators of detrimental myocardial strain and dysfunction.(233, 234) In LoDoCo-MI only the two most thoroughly investigated biomarkers (C-Reactive Protein and IL-6) were addressed to exemplify their common link to inflammation and strong correlation with vulnerable atherosclerotic plaque burden. (235)

15 CV Risk Biomarkers

15.1 Overview

Biomarkers of prognosis with reliable assays have been plentiful with highly sensitive Troponin T (TnT) the accepted gold standard. Assay precision may be measured using a value known as the coefficient of variation (the ratio of the standard deviation to the mean value) for a molecular concentration of biomarker at the level of 99th centile of the normal range of a healthy population. The Universal Definition of MI document recommends the use of assays with a coefficient of variation of less than 10% at the 99th centile. (236)

The most extensively examined markers in CV prevention are fibrinogen, hs-CRP, white cell count (WCC), and IL-1 act in acute phase response reflecting the current inflammatory state. (237) Figure 19 depicts the spectrum of biomarkers instigated in certain steps of atherosclerosis, their expression providing a unique signature of the specific pathological process that is most active at a given time and propensity to the predilection towards a given downstream complication. These biomarkers were used in epidemiological studies to examine the hypothesis “elevated baseline inflammation predicts risk of CAD”.

Figure 19 Markers of inflammation and plaque instability: from foam cell to plaque rupture (13)

**Please ignore the Asterix (*) above, nil relevance to the explanation of the above text/figure for the purpose of this thesis.*

Logic and intuition would suggest a panel of pertinent biomarkers measured simultaneously may synergistically enhance CV risk stratification. (231) There is the potential to derive novel risk stratification and prognostic indices as well as modifying existing models from amalgamating a multitude of biomarkers. Biomarkers that surge just prior to coronary plaque rupture may harbour the largest potential. (9) LoDoCo-MI only the two most thoroughly investigated biomarkers (hs-CRP and IL-6) were included in the final trial manuscript paper with both addressed to exemplify their common link as close-by mediators in the inflammatory pathway. High CRP levels strongly correlate with vulnerable atherosclerotic plaque burden. (235)

Meta-analyses convey hs-CRP is a risk predictor for CAD. (238) The Atherosclerosis Risk in Communities study (ARIC) examined incident CAD in over 15,000 people for 20 years and examined 19 novel as well as all conventional risk factors.(239) Including hs-CRP only added very little to the overall figure nor did other novel markers.(239) These results were confirmed in the Framingham study, showing that traditional risk factors had a high C-statistic of 0.78 for CVD events, a value not influenced by hs-CRP addition. (240) The Women's Health Study (WHS) argues that CRP adds accuracy to classifying women by stratification of risk (i.e. 5%-10%, or 10-20% risk over 10 years) reclassifying approx. 20% of women to more accurate categories.(241) Cook et al (2006) points out that when using the improvement in C-statistic as the criteria for use as a clinical prognostic marker, cholesterol measurements (LDL) add similarly little value to the Framingham assessment as hs-

CRP.(242) This incites the query as to why include cholesterol levels and not hs-CRP as the mainstay marker of atherosclerosis? (243)

A Multiple biomarker approach has been shown to predict plaque destabilization and rupture and subsequent clinical complications. (13) Such cytokines measure the substantial relative risk of cardiovascular events on the order of 2-3-fold, and therefore may not yet be sufficient in clinical decision-making with regards to early interventions. This is exemplified by exhaustive clinical hs-CRP studies which fail to gauge hs-CRPs additional incremental value in risk stratification. Supplementary biomarkers which stratify and predict further clinical events are greatly needed to diminish the current event rate after MI. (244) If discovered, these biomarkers must credibly demonstrate the necessary specificity, sensitivity, practical feasibility, and economic effectiveness to ultimately be implemented into guidelines. (245) The time intensive nature of such biomarker pursuit (microRNA as an example) in a pragmatic coronary care environment has proven the largest barrier to practical implementation. (246) This is analogous to the well described challenges faced by conventional flow cytometry techniques in current practice.

15.2 High Sensitivity C Reactive Protein (Hs-CRP)

CRP was first identified in 1930 as an inflammatory marker and was more recently proposed as an aetiologic agent in cardiovascular disease. (265) CRP has undergone scrutiny over the years to determine if it is merely a bystander marker of risk or plays a role in the development and progression of coronary atherothrombosis. (266) CRP is primarily produced from hepatocytes under the influence of IL-6 and TNF alpha as part of the acute phase reaction. (265) CRP is also produced from adipose tissue, and this contributes significantly to CRP levels in the setting of the metabolic syndrome. (267) CRP opsonises these particles through activation of the classical complement pathway and hence mediate innate defence and help prevent autoimmunity. (244) It promotes the development of foam cells through the opsonisation of modified LDLs in the vascular wall. (272) CRP has cytokine and procoagulant properties to mediate vascular wall dysfunction. (273) There is high prevalence of inflammatory conditions in individuals with high CRP concentrations. (274) CRP plays a role in the innate immune system but is also proposed to be involved at the atherosclerotic plaque. (265) Despite CRPs presence in unstable atherosclerotic plaque, this observation does not mean it is a culprit cytokine. (268) There are multiple studies investigating links between CRP genotype and expression

and cardiovascular risk. Genetically elevated hs-CRP levels did not result in an increase in CAD. (269) Hs-CRP adds a net reclassification improvement when added to traditional risk factors at a value greater than LDL or blood pressure in the Framingham, which certainly makes a case for it to be routinely measured alongside such risk factors. (270)

Hs-CRP has been shown in over 30 large studies to be an effective risk marker in CAD. (247) The question of the role that hs-CRP plays in the development and progression of the atherothrombotic process is far less transparent. (248) An enzyme-linked immunosorbent assay (ELISA) based assay is available that allows a high sensitivity measurement of hs-CRP to be performed (249) which are widely available, cheap, and reliable methods of quantifying hs- CRP. (250) In the absence of an inflammatory condition which mandates repeat measurement, the cut-offs for low, moderate, and high risk for cardiovascular events are <1mg/L, 1-3mg/L and >3mg/L respectively. (251) Lowered hs-CRP with treatment has been shown to correlate with improved outcomes. (216) However, whether hs-CRP itself is a causative factor or simply a bystander biomarker remains a debate with genetic analysis implying as much. (252, 253)

Figure 20 Additional insight into cardiovascular disease risk prediction when hs-CRP is integrated with ‘traditional’ Risk factors (14)

The evidence for hs-CRP as a predictor of cardiovascular risk is well documented. (254) The Physicians Health Study demonstrated a relative risk of 2.9 ($p < 0.001$) for myocardial infarction when comparing the highest quartile of hs-CRP ($> 2.11 \text{ mg/L}$) with the lowest quartile ($< 0.55 \text{ mg/L}$) even after adjustment for smoking and lipid status. (255) The Women’s Health Study found elevated levels of hs-CRP, IL-6, serum amyloid A (SAA), and sICAM- 1 all correlated with increased risk of CV

events. (256) An elevated hs-CRP as well as elevated SAA predicted cardiovascular outcome when measured in 700 women undergoing coronary angiography. (257) A similar pattern was corroborated in the Framingham cohort with an increased estimate of 10-year risk with increasing hs-CRP levels. (258) In fact, hs-CRP is a stronger predictor of cardiovascular events than LDL cholesterol and adds prognostic information for all levels of calculated Framingham risk (Figure 20 exemplifying the benefit of hs-CRP addition to a 'traditional RF like DM in enhancing risk assessment precision). (259) Following an acute coronary syndrome hs-CRP level is prognostic. (260) Patients with a persistent hs-CRP elevation at discharge following an acute myocardial infarction are at higher risk for subsequent AMI. (261) However, does it provide additional information beyond standard risk models after multivariate analysis? Receiver operator curves are largely unchanged when hs-CRP is added to Framingham 10-year data and despite some data that demonstrates hs-CRP can significantly reclassify patients' risk when added to Framingham or other risk scores the effect is small. Circulating levels of hs-CRP help to estimate risk for initial cardiovascular events and may be used most effectively in persons at intermediate risk for vascular events, offering moderate improvement in reclassification of risk. (262) The benefit is perhaps far too minuscule to make a case for its' implementation into routine clinical practice.

Measurement of hs-CRP as a predictor of risk is recommended by the American Heart Association (AHA) as part of the global cardiovascular risk assessment. (263) The Reynolds Risk score which incorporates hs-CRP has been shown to be better calibrated than the Framingham model with improved discrimination yet is side-lined as a research tool in current practice. (271)

Hs-CRP is a nonspecific marker of inflammation and a predictor of incident coronary heart disease, CV disease, and subclinical vascular disease. (274) Hs-CRP provides prognostic information but may not have direct causality in the atherosclerotic pathway. (275)

High-sensitivity CRP has been proposed for use in cardiac risk stratification and preventive decision-making. (276) Meta-analyses of genetic mendelian randomised trials have so far failed to implicate hs-CRP as being causal in increasing the risk of CAD. (277-279)

Much has recently been published about the pleiotropic effects of statins, particularly their seeming ability to dampen inflammation but this effect may accrue from its influence on lipid lowering, blood pressure, platelets etc.(280) Some authors have ascribed this alternative function to being a "CRP lowering" function, and speculate that this hs-CRP lowering provides some evidence of a causal role

of CRP in CAD risk.(281) While it is true that statins lower hs-CRP levels along with cholesterol,(282) this is largely due to inhibition of activation of nuclear transcription factor kappa b (NFκB), and activator protein-1 (AP-1) which are responsible for aiding transcription of many pro-inflammatory cytokines including CRP.(282, 283) Since this protective effect is both immunosuppressive and immunomodulatory, lower incidences of acute CAD cannot be attributed to CRP level reduction alone.(284) Hence attempts to unravel influences of statins on hs-CRP and CAD outcomes in the PROVE-IT (Pravastatin Or Atorvastatin Evaluation and Infection Therapy) trial are essentially impossible.(285) The literature therefore appears to confirm that both hs-CRP and fibrinogen are markers of CAD risk but has not as yet convincingly causally implicated either marker with mendelian (genetic) randomisation studies suggesting no role in causation.(235, 277, 278) Since both of these inflammatory markers are proteins of the acute phase response, it stands to empirical reason that stimulators of the acute phase response may also be risk markers (at the very least due to indirect associations with acute phase reactants).(286)

Stable plaques showed a modest elevation, erosive plaques a greater elevation, and marked elevations in hs-CRP were seen in ruptured plaques.(287) Plasma concentrations in the highest quartile were associated with a 1.5- to 7-fold increase in relative risk (RR) of symptomatic atherosclerosis.(71) In all cases of SCD, some elevation of hs-CRP was seen, regardless of the presence or absence of thrombosis, suggesting that hs-CRP identifies lesions rich in lipids and macrophages, and that it is associated with a risk of a vascular event, even in patients with clinically stable CAD.(71) Particularly applicable to LoDoCo-MI and associated colchicine & ACS family of trials, higher hs-CRP levels at the time of the PCI was predictive for 10-year mortality and MI. (288) emphasising the call to universally raise the status of this biomarker in CV prevention beyond an ancillary research resource.

Johnson et al confirmed an elevated hs-CRP as well as elevated SAA predicted cardiovascular outcome when measured in 700 women undergoing coronary angiography. (257) A similar pattern was corroborated in the Framingham cohort with an increased estimate of 10-year risk with increasing hs-CRP levels. (258) In fact, hs-CRP is a stronger predictor of cardiovascular events than LDL cholesterol and adds prognostic information for all levels of calculated Framingham risk (Figure 20 exemplifying the benefit of hs-CRP addition to a 'traditional RF like DM in enhancing risk assessment precision). (259) Following an acute coronary syndrome hs-CRP level is prognostic. (260) Patients with a persistent CRP elevation at discharge following an acute myocardial infarction are at higher risk for subsequent AMI. (261) Hs-CRP is a marker of risk in healthy populations, stable CAD, and ACS populations. (248) However, does it provide additional information beyond standard risk

models after multivariate analysis that is practical to clinical practice? Receiver operator curves are largely unchanged when hs-CRP is added to Framingham 10-year data and despite some data that demonstrates hs-CRP can significantly reclassify patients' risk when added to Framingham or other risk scores the effect is small. (262)

CRP correlates with many risk factors and additional biomarkers, and it is difficult to identify possible confounding or the direction of causality in these biological relationships, so it remains unclear if CRP is merely a formidable marker of risk or integral link in the causative pathway of atherosclerosis. (264) Higher CRP concentrations should be interpreted with caution in cardiovascular disease risk assessment. (274) C-reactive protein (CRP) is a nonspecific marker of inflammation and a predictor of incident CAD, CV disease, and subclinical vascular disease. (274) Markedly high CRP concentrations (>10 mg/L) also have been shown to predict cardiovascular risk. (274) High sensitivity C-Reactive Protein (hs-CRP) levels add prognostic information to 10-year Framingham Risk assessments, and this occurs for all levels of Framingham risk. (48) Hs-CRP provides prognostic information but may not have direct causality in the atherosclerotic pathway. (275)

High-sensitivity CRP has been proposed for use in cardiac risk stratification and preventive decision-making. (276) Meta-analyses of genetic randomised trials have so far failed to implicate either CRP as being causal agents in increasing the risk of CAD. (277-279)

Much has recently been published about the pleiotropic effects of statins, particularly their seeming ability to dampen inflammation but this may be as a result of the lipid lowering effect as well as influences on BP and platelets. (280) Some authors have ascribed this alternative function to being a "CRP lowering" function, and speculate that this CRP lowering provides some evidence of a causal role of CRP in CAD risk. (281) While it is true that statins lower CRP levels concomitantly with cholesterol, (282) this is largely due to inhibition of activation of nuclear transcription factor kappa b (NFκB), and activator protein-1 (AP-1) which are responsible for aiding transcription of many pro-inflammatory cytokines including CRP. (282, 283) Since this protective effect is both immunosuppressive and immunomodulatory in a multi-factorial manner however, lower incidences of acute CHD cannot be attributed to CRP level reduction. (284) Hence attempts to unravel influences of statins on hs-CRP and CAD outcomes in the PROVE-IT (Pravastatin Or Atorvastatin Evaluation and Infection Therapy) trial are essentially impossible. (285) CRP and fibrinogen are markers of CAD risk but has not as yet convincingly causally implicated either marker with mendelian (genetic) randomisation studies suggesting no role in causation. (235, 277, 278) Since both of these

inflammatory markers are proteins of the acute phase response, it stands to empirical reason that stimulators of the acute phase response may also be risk markers (at the very least due to indirect associations with acute phase reactants).(286)

Stable plaques showed a modest elevation, erosive plaques a greater elevation, and marked elevations in hs-CRP were seen in ruptured plaques.(287) Plasma concentrations in the highest quartile were associated with a 1.5- to 7-fold increase in relative risk (RR) of symptomatic atherosclerosis.(71) In all cases of SCD, some elevation of hs-CRP was seen, regardless of the presence or absence of thrombosis, suggesting that hs-CRP identifies lesions rich in lipids and macrophages, and that it is associated with a risk of a vascular event, even in patients with clinically stable CAD.(71) Particularly applicable to LoDoCo-MI and associated colchicine & ACS family of trials, higher hs-CRP levels at the time of the PCI was predictive for 10-year mortality and MI. (288) emphasizing the call to universally raise the status of this biomarker in CV prevention beyond an ancillary research resource.

15.3 Interleukin-6 (IL-6)

IL-6 is a cytokine expressed in activated monocytes/macrophages and endothelial cells. IL-6 is a pleiotropic cytokine produced by endothelial cells, macrophages, adipocytes, and lymphocytes. It influences T- and B-cell differentiation and endothelial cells to secrete chemokines and plays a role in the transition between acute and chronic inflammatory responses. (291) IL-6 positively correlated with hs-CRP levels increasing its production from hepatocytes. (289) IL-6 was an independent predictor of cardiovascular events in the WISE study. (290)

Exogenous IL-6 exacerbates atherogenesis in pre-clinical models. Major correlates of IL-6 are age, smoking and acute phase response markers.(292) A meta-analysis of non-fatal MI or CAD death, demonstrated that men in the top third of the population for IL-6 expression had an OR 1.67 independent of conventional risk markers.(293) Hence IL-6, like CRP is associated with atherogenesis but more importantly resides on the causative pathway which differentiates it from its' close signal partner CRP.(279, 289) IL-6 accentuates free radical oxygen production and endothelial dysfunction in unstable plaques. (294) IL-6 amplifies plaque inflammation being a potent pro-coagulant cytokine involved in atherothrombosis (Figure 21). Indeed, several studies have demonstrated a predictive value of circulating IL-6 levels for future cardiovascular events. (294) The Framingham and Fast Revascularization During Instability in Coronary Artery Disease II (FRISC II)

studies demonstrate that patients with high IL-6 benefit the most from an early coronary revascularization strategy. (295)

Figure 21 Atherosclerotic progression is based on the interplay & balance between pro-inflammatory and anti-inflammatory agents (15)

15.4 Myeloperoxidase (MPO)

Myeloperoxidase (MPO) is an enzyme stored in neutrophils and macrophages, secreted during the inflammatory process. (296) Myocardial inflammation following acute ischemic injury is linked to negative cardiac remodelling and heart failure development. (297) Myeloperoxidase being involved in oxidative stress and active cardiac inflammation has sparked interest in it as a possible marker of plaque instability and a clinical tool in coronary heart disease evaluation. (296) (298) Statins reduce serum MPO and CRP concentrations in patients with ACS by virtue of limiting LDL supply to the vascular bed. (299) explaining some clinical benefits of statins in this cohort. Baseline MPO levels independently predict the early risk of MI in angina (300) and development of adverse cardiac events up to 6 months after acute coronary syndrome (ACS). (300) MPO levels independently predict MI at

2 years in patients with ACS. (300) High MPO level group showed a poorer outcome than the low MPO level group in a meta-analysis. (301) ACS patients with high MPO levels had a poor long-term prognosis. (302) (298)

Regarding MPOs correlation to traditional cardiac risk factors, hypertension, diabetes mellitus and age do not influence clinical prognostic value of MPO in comparison to female gender and smoking which strongly affect prognostic value of MPO in terms of mortality and recurrent MI. (210)

15.5 Interleukin-1 β

IL-1 β plays a key role in atherosclerosis and post-MI inflammation (Figure 21). (303). IL-1 β reduced cardiac function through directly decreasing cardiac contractility and enhancement of myocardial infarct size. (304). Anakinra has been registered for clinical usage in rheumatoid arteritis, off target testing of these compounds was feasible and clinical evidence on the role of IL-1 β in the healing process of MI is also available. (305) Two studies (VCU-ART and VCU-ART2) included ST-segment elevation MI patients. Patients received Anakinra for 3 months. Although no significant results were seen regarding major adverse cardiac events, the anakinra treated patients did show lower levels of CRP in the immediate aftermath and were less likely to develop new onset HF. A trend towards improved left ventricular parameters was also seen in these patients. (306) Like IL-1 β , IL-18 is also expressed after activation of the (NLRP3) inflammasome from caspase-1 cleavage.(307) The inflammatory cascade caused by IL-1 β and IL-18 (both intimately linked) depends on inflammasome activation and also on the intrinsic balance between these cytokines and their naturally occurring antagonists.(308) IL-1 β was recently conveyed to be an independent MACE predictor in the ‘AtheroGene’ registry with corroborative biomarker and infarct size estimation on echo.(309)

16 Targeting Inflammation in CV Prevention

16.1 The Pleiotropic Effect of Statins

Risk modification with statins is common practice in both the primary and secondary CV prevention. (310) Statins have previously been shown to exhibit anti-inflammatory effects in addition to lowering lipid levels (Figure 22). (311) The ‘pleiotropic’ means by which statins act is through interference with mevalonate and activating Krüppel-like factor 2 as well as endothelial protection by upregulation NO. (282) Trials attempting to quantify overall inflammatory burden utilize high sensitivity C-reactive protein (hs-CRP), as it is increasingly available and cost-effective capturing most of the downstream inflammatory activity. (312) The anti-inflammatory effect of statins was formally tested in the JUPITER (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin) trial. (213) In this study, participants with LDL-C levels < 3.4 mmol/L and elevated hs-CRP > 2 mg/L were randomized to receive either 20 mg/day of rosuvastatin or placebo. Of the 17,803 apparently healthy individuals recruited, those treated with rosuvastatin had a 44% risk reduction in first ever cardiovascular events compared to the placebo group (HR 0.56; 95% CI 0.46–0.69; $p < 0.00001$). Instrumental to the hypothesis, rosuvastatin lowered hs-CRP levels by 47% after 12-months of follow up. This lays a strong argument that patients on standard therapy with statins as part of secondary prevention obtain a sizable degree of anti-inflammatory coverage. (213) Despite this, atherosclerotic events continue to occur at alarming rates, and it is thought that residual inflammatory risk may be an important driving force behind this. (26)

Analysis of the PROVE-IT (Figure 18) trial showed that of the 2099 patients who had a history of preceding acute coronary syndrome and were given 80 mg atorvastatin, 44% achieved reductions to target in both LDL-C (< 1.8 mmol/L) and hsCRP (< 2 mg/L). (47, 214) Therefore, there is a clear need for future novel therapies to address the currently unmet issue of residual inflammatory risk in patients found to have persistent inflammation following standard care. Although CAD and AMI are inflammatory disorders, the only drugs with clear-cut anti-inflammatory effect so far widely used in ischemic heart disease are aspirin and statins with less pronounced indirect auxiliary anti-inflammatory influence conferred from ACEi, ARBs, B Blockers (see 14.3.1). (26)

Figure 22 'Pleiotropic' properties of Statins Tousoulis et al. EHJ 2016(15)

16.2 Anti-Inflammatory effect of existing standard secondary CV preventative medication

16.2.1 Notes on the Anti-Inflammatory effects of anti-platelets, ACE-inhibitors (ACEi), Angiotensin II Receptor Blockers (ARB) & B-Blockers

Early stages of plaque formation are instigated by activated platelets adhering to the endothelium propagating an inflammatory cytokine cascade governed by P-selectin. (325) Anti-platelet therapies precipitate 15-epi-lipoxin A4 further potentiating nitric oxide production abating leukocyte trafficking and the activation of the endothelium. (326) The shoulder region of plaque contains an abundance of ACE & ARB as well as IL-6 dense macrophages. Angiotensin II triggers NF- κ B in smooth muscle cells to perpetuate the cytokine cascade. (327) By inhibiting the angiotensin II receptor, anti-inflammatory T regs are preserved and Th1 and Th17 reduced along with IL-10 from B cells. (328, 329) The suppression of reactive oxygen species (ROS) in neutrophils and circulating monocytes has been observed with B-blockade in ACS. (330) The end result is to attenuate infarct size independent of reperfusion which is monumental in ascertaining mortality benefit for these drugs. (331) Statins have been discussed in chapter 14.1. The mechanisms of proprotein convertase subtilisin/kexin type 9 (PCSK9) antibodies are indirectly intertwined with the anti-inflammatory

cytokine cascade by virtue of downregulation on LDL and PCSK9 receptor expression in macrophages, augmenting NF-kB activity.

16.2.2 Other Anti-inflammatory targets that have been trialled in secondary prevention CV disease.

Lipoprotein-associated phospholipase A2 (Lp-PLA2) levels correlate with CV risk. Lp-PLA2 creates pro-inflammatory cytokines by catalysing oxidised phospholipids which further trigger immune cells and elements of the necrotic core. Recombinant inhibitors of Lp-PLA2 in early trials reduced IL-6 but failed to statistically lower hs-CRP, plaque burden on IVUS. Subsequent studies showed Lp-PLA2 inhibition halted necrotic core formation and enhanced fibrous constituents in plaque. The STABILITY (Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy) and SOLID-TIMI 52 (Stabilization of Plaque Using Darapladib-Thrombolysis in Myocardial Infarction 52) were both had neutral clinical endpoints despite large trial numbers. As with hs-CRP, Lp-PLA2 genetic studies have failed to implicate Lp-PLA2 on the causal pathway of atherosclerosis, thus postulating the role of Lp-PLA2 as another mere biomarker of inflammation as opposed to a causal cytokine.

Secreted phospholipases A2 (sPLA2s) hydrolyse non-oxidised phospholipids in comparison to Lp-PLA2 which act on oxidised phospholipids only. The sPLA2 molecule orchestrates inflammation through fatty acids and phospholipids in combination with modified LDL to accentuate inflammatory cell trafficking further igniting inflammatory cascades. As with Lp-PLA2, sPLA2 activity is linked with ACS and hs-CRP. An inhibitor of sPLA2 was tested in the VISTA-16 but again was a neutral result on MACE endpoints with early cessation due to risk of impending harm (Table 4).

The p38 mitogen-activated protein kinase (MAPK) is triggered by various types of extracellular elements igniting inflammatory cascade aiding lipid retention in white cells and foam cell creation. The inhibitor of MAPK (Losmapimod) displayed promise through CRP reduction and modest FDG-PET CT change in highly inflamed regions in early experimental studies. Echo data was also significant with this agent. Clinical equipoise was observed in the clinical endpoint-driven ACS trial (LATITUDE-TIMI 60). Despite notable drops in hs-CRP and BNP in the same study, further trial phases were abandoned.

Another attempt at repurposing in ACS was the Cardiovascular Inflammation Reduction Trial (CIRT) assessing methotrexate (an oncology & rheumatology drug) which targets dihydrofolate reductase (DHFR) negating DNA production. Analogous to other rheumatological data on CVD, MTX seems to be protective. Moreover, it deters trafficking of monocytes and reactive oxide species leading to T lymphocyte activation. The CIRT was negative, being stopped early at just over 2 years for futility.

16.3 Studies prior to LoDoCo-MI Using Colchicine to Target CV Inflammation with Clinical, Cytokine or Radiological Outcome Assessment

There is enormous intrigue in therapies that target inflammatory mechanisms to reduce the risk and sequelae of atherosclerosis. (26) Monoclonal antibodies against pro-inflammatory cytokines like IL-1 β have been shown to significantly reduce major adverse cardiovascular events post MI and residual hs-CRP levels ≥ 2 mg/L, on conventional secondary prevention. (313) Despite well documented anti-inflammatory actions and supportive observational data the Cardiovascular Inflammation Reduction Trial investigators, reported disappointing results in the post-MI setting with patients randomized to low-dose methotrexate or placebo, not significantly reducing MACE, hs-CRP or ILs 1 β /6 (314) with the trial stopped early due to crossing the pre-specified futility threshold.(314) Colchicine a well profiled, cheaper, once daily, oral alternative that pursues analogous inflammatory targets to canakinumab and is well tolerated long-term. It showcased considerable promise in early studies with CAD. (315)

In the Low Dose Colchicine (LoDoCo) study, 0.5 mg colchicine daily in addition to standard treatment in stable CAD significantly reduced the risk of developing an acute coronary syndrome, an out of hospital cardiac arrest or non-cardioembolic stroke. (35) This study was not, however, placebo controlled, though endpoints were adjudicated by clinicians blinded to what treatment the patients had received. LoDoCo-2, double-blind randomised controlled trial MACE was lower with Colchicine. In acute coronary syndrome colchicine reduces the local production of IL-1 β , IL-18 and IL-6, as measured in coronary sinus blood samples. (203) These reductions in all 3 inflammatory cytokines were marked (with a reduction in IL-6 of 88%) and occurred after only 24 hours of colchicine therapy.

In addition, short term (24 hours) treatment with colchicine also reduces inflammasome-dependent inflammation in patients following an acute coronary syndrome, as measured by in vitro production of IL-1 β and caspase-1 protein by monocytes. (202) Thus, there are plausible biological mechanisms supporting the hypothesis that prompt treatment after acute MI would result in an early reduction in pro-inflammatory pathways and potentially a lower risk of clinical events. (316)

Raju et al. (Table 4) has also assessed the impact of colchicine on hs-CRP levels after ACS or stroke.(317) This randomised 80 patients (over 90% post-acute coronary syndrome) to colchicine 1mg daily or placebo for 30 days at which time colchicine had a median hs-CRP level of 1mg/L

versus 1.5mg/L in placebo($p=0.22$).⁽³¹⁷⁾ Likewise, the percentage of patients with a hs-CRP level $<2\text{mg/l}$ was higher in the colchicine treated group (77%) compared to placebo (62%). Though this did not attain statistical significance ($p=0.18$) this study was very underpowered (hsCRP levels at 30 days were only available in 68 patients, 31 who had received colchicine and 37 who had received placebo). ⁽³¹⁷⁾ The results are consistent with the findings from the COLCOT and suggest that colchicine has modest, but potentially clinically beneficial, effect in the post MI setting with LoDoCo 2 underscoring the benefit in longer term CV prevention. Deftereos et al. (Table 4) showed short term (5 days) treatment with colchicine appears to reduce the extent of myocardial necrosis after ST-elevation MI ⁽³¹⁸⁾ and long-term treatment has been associated with reduced features of plaque instability on coronary artery CT angiography, in particular low intensity plaque volume. ⁽³⁷⁾ In this latter study (CT observer blinded) treatment with colchicine 0.5 mg daily was also associated with a significantly lower level of hs-CRP at 1 year, mean value in colchicine treated patients ($n=40$) 1.85 mg/L (SD 0.9) versus 2.26 mg/L (SD 1.05) in controls ($n=40$), with a 37% reduction observed in colchicine treated patients compared to a 15% reduction in the untreated arm. Likewise, the impact of colchicine on hs-CRP levels was broadly like the current double-blinded placebo-controlled trial.

Table 4 Trials Preceding LoDoCo-MI examining Colchicine post ACS

Trial/Author	Design & Intervention	n	Outcome	Benefit
COLIN trial Akodad et al. (319)	STEMI patients 1mg daily for 1 month	44	Change in CRP	NS
Deftereous et al. (318)	STEMI patients, colchicine 1.5 mg at time of revascularisation, then 0.5 mg 1 hour later, then 0.5 mg BID vs placebo for 5 days. Change in LV Infarct Size & CK-MB AUC on cMRI	151	Change in CK-MB AUC Indexed CMR-defined infarct size in the colchicine group and the relative infarct size as a proportion of LV myocardial volume in the colchicine group compared with placebo.	Positive
Martínez et al. (203)	ACS patients 1 mg colchicine followed by 0.5 mg within 24 hours before coronary sinus sampling vs no treatment	83	Trans coronary gradients for IL-1b, IL-18, and IL-6 in patients with ACS	Positive
COOL trial Raju et al. (317)	ACS or Ischaemic stroke Colchicine 1 mg/d vs placebo for 30 days	80	Absolute reduction of hs-CRP at 30 days or difference in hs-CRP from baseline to 30 days	NS
Vaidya et al. (37)	ACS (< 1 month) 0.5 mg/d vs no treatment with colchicine for 1 year Change in low attenuation plaque (LAP) in Coronary CT and hs-CRP	80	Change/reduction in Low attenuation plaque (LAP) & hs-CRP	Positive

Table 5 Neutral RCTs of other Anti-Inflammatories seeking MACE by various cytokine pathway inhibition

Trial/Author	Drug & pathway	n	Primary outcome	Outcome
STABILITY (320)	Darapladib	15,828	MACE	negative
SOLID-TIMI 52(321)	Lp-PLA2 inhibitor	13,206		
VISTA-16 (322)	Varespladib sPLA2s inhibitor 1	5,145	MACE	Negative/stopped early
LATITUDE-TIMI (323)	Losmapimod p38 MAPK inhibitor 1	3,503	MACE	negative
CIRT (324)	Methotrexate (MTX) MTX Dihydrofolate reductase inhibitor	4786	MACE	negative

17. Colchicine: Overview of Its' background, therapeutic profile & potential in cardiovascular prevention through inflammatory suppression

17.1 Overview & Background

Colchicine is an anti-tubulin preventing microtubule polymerisation, thereby negating inflammatory cell migration (Figure 23). (30) Effects include inhibition of mitosis, gene expression, and signal transduction with a preference on cells with high turnover. (337, 338) Colchicine derived from the Autumn crocus plant (332) boosts 2000 years of benefit in inflammatory conditions, licensed for gout, Familial Mediterranean Fever (FMF), and Behcet's disease (Figure 23). (333) Additionally, it treats pericarditis (334) and Postpericardiotomy syndrome. (335) (336)

17.2 Pharmacodynamics

Colchicine has good bioavailability, is lipophilic with a long half-life, (339) is primarily hepatically excreted with direct gastrointestinal enteric excretion and 10-20% renal excretion. (51) Colchicine is metabolized by CYP3A4 inducing several important drug-drug interactions (Figure 23). (30) In addition to CYP3A4 inhibitors important interactions include HMG CoA reductase inhibitors (myopathy and rhabdomyolysis) when adding one to a stable regimen of the other, and P-gp Inhibitors (Figure 23). (340) Colchicine has unfavourable GI effects particularly at high doses. (333) Common side effects include nausea, diarrhoea (at higher doses), and myopathy/rhabdomyolysis (very rare).

Figure 23 Colchicine chemical structure, mechanism of action, metabolism (16)

17.3 Mechanisms of Action

Colchicine is avidly taken up predilection by leukocytes (341) (342) potentially inducing myeloid suppression in a small percentage of cases in high dose and prolonged exposure. Colchicine's action in gout is to inhibit urate-crystal induced IL-1beta expression (30) and targeting neutrophils (343) Colchicine reduces neutrophil migration by inhibiting leukotriene B4 and IL-8. (344) FMF sufferers on colchicine (in a small retrospective observational study) had less coronary artery disease versus controls and others with chronic inflammation not treated with colchicine. (345) With observational data the hypothesis was tested prospectively in a stable CAD cohort with hs-CRP >2mg/L showing colchicine could lower hs-CRP independent of aspirin and statins. (226)

Colchicine decreases TNF-alpha and IL-2 secretion and alters the distribution of E-selectin, L-selectin and VCAM-1 on endothelial cells further attenuating migrating inflammatory cells. (347) Colchicine acts to inhibit prostanoid synthesis by inhibiting cyclooxygenase 2 in mono-sodium urate induced monocytes. (348) Additionally, colchicine targets gene expression such as caspase-1 involved in the production of IL-1, nitric oxide synthase 3 involved in nitric oxide production, as well as a lowering of hs-CRP, a decrease in MMP 9, TNF alpha and IL-6. (349) Colchicine has also been shown to inhibit phospholipase A2 (PLA2) activation, the expression of cyclooxygenase-2 and the elaboration of PLA2-activating protein (PLAP). (337) PLA2 subgroups have been identified as markers of cardiovascular risk like hs- CRP (129) but mechanistic targeting studies (320) did not show significant reduction in CV events.

17.4 Colchicine's role in non-atherosclerotic cardiac issues

There is an intimate link between inflammatory substrate and clinical atrial fibrillation with the connection underpinned by a plethora of biomarkers. (350) Colchicine influences atrial myocytes, shifting the atrial response to autonomic effects i.e., mitigating against sympathetic drive and augmenting parasympathetic tone. Colchicine started before and continued after catheter ablation for AF is effective in abating early AF recurrence with concomitant CRP & IL-6 reduction.

The biomarker suppression trend coincided with less rate of clinical AF recurrence. (351) A meta-analysis of colchicine (integrating 15 trials) shows it reduced adverse outcomes in cardiac ailments including pericarditis, PPS, peri/post-procedural AF. (33) The Colchicine for the Prevention of the Post

pericardiotomy Syndrome (COPPS) study demonstrated the drugs' benefit in negating post-pericardiotomy syndrome (PPS). (352) COPPS-2 displayed that in cardiac surgery, high dose perioperative colchicine diminished PPS but not of post-op AF. A significant patient number were intolerant to the GI side effects and were thus not able to receive the benefit from the medication. (353)

17.5 Colchicine & Percutaneous Coronary intervention (PCI) related complications of In-stent Restenosis and stent thrombosis

Platelets activation/aggregation with subsequent thrombus formation is the main event in the pathophysiology of acute coronary syndrome.(354) Dual antiplatelet therapy (DAPT) is a necessity post-MI and PCI to circumvent thrombotic sequelae (MI sub-types discussed also in Chapter 13).(355) Ticagrelor (the predominant ADP receptor antagonist in LoDoCo-MI) is the potent second antiplatelet of choice in the guidelines. However given scenarios of higher bleeding risk (often due to concurrent AF necessitating anticoagulation) clopidogrel use is often required. (356) Clopidogrel therapeutic efficacy is compromised in up to a third of the population due to high platelet reactivity (HPR), inciting the foreboding prospect of subsequent CV events (in particular stent thrombosis).(357, 358) Clopidogrel remains the mainstay adjunct antiplatelet in the Developing World due to economic feasibility (359) when pitted against ticagrelor. A very significant percentage of the Asian population are slow metabolisers predisposing to in-stent thrombosis. (206) Colchicine reduces platelet-neutrophil interactions important in atherothrombosis. Colchicine also hampers platelet aggregation (as with clopidogrel) by disturbing cytoskeletal assembly. (360) Colchicine decreases platelet aggregation in both clopidogrel responders and non-responders. Colchicine has also been shown to reduce aggregation of platelets in clopidogrel non responders in which ADP continued to incite activation in the setting of DAPT (although there is no effect on bleeding time).(205) This postulates another novel role for the readily repurposed colchicine as an adjunct in combating a potentially fatal consequence of inadequate clopidogrel metabolism and mitigating against the additional cost in burden in under-privileged regions and is indeed thought provoking with regard to future trial concepts.(361) Those high-risk non-responders in whom DAPT may be imperative in an immediate post-MI setting can attain appropriate platelet aggregation in the face of a high platelet reactivity (prothrombotic) predilection. (205)

Recent years have showcased diminished thrombotic milieu (in-stent thrombosis) with the advent of robust P2Y₁₂ receptor blockers (ticagrelor) and perpetual progress in drug eluting stent (DES)

technology. (362) Conversely a resurgence of bleeding issues has ensued thus instigating a risk-benefit dilemma incorporating a gamut of medico-social considerations. In response to evolving trends in the epidemiological transition (i.e., much larger elderly population living longer with non-communicable disease), long term use of more potent ADP receptor inhibitors like ticagrelor the concept of DAPT ‘de-escalation’ in the pharmacological strategy has surfaced. (354) (363) Problems like long-term affordability, ticagrelor induced dyspnoea and bleeding in more elderly frail patients warrant ticagrelor cessation and clopidogrel re-introduction.

The stents embedded into the vascular wall greatly agitate the this lining unleashing a wave of inflammatory-thrombotic elements such as leukocytes and pro-coagulants that incite neointimal hyperplasia.(364-366) Colchicine by anti-mitotic action induces less in-stent restenosis (ISR) in bare metal stent (BMS) PCI among Diabetics.(367) This inflammatory reduction of in-stent neointima formation(368) observed on IVUS is pertinent in scenarios where drug eluting stenting (DES) is non advantageous.(369) Neointimal hyperplasia invokes in-stent restenosis (ISR), which correlates closely to the level of wall insult from stent insertion and the inflammatory cellular surge that ensues locally which an anti-proliferative agent like colchicine can attenuate. Factors governing why certain stents have greater ISR are; genetic resistance to the eluted cytotoxin, hypersensitivity, diabetes, stent under expansion, stent strut thickness and length and barotrauma to non-stented areas. (370, 420)

Figure 24 Pathologic Mechanisms of Restenosis over time (17)

(Note Abbreviations & Nomenclature in Fig 24 explained in full previously and in Abbreviations section.)

Table 6 Trials evaluating Colchicine in reducing Restenosis & In-stent Restenosis

Trial/author	Design & Intervention	n	Outcome	Benefit observed
O’Keefe et al. (371)	Double-blind, prospective, randomised trial, Colchicine 0.6 mg twice daily for 6 months after elective coronary balloon angioplasty for preventing restenosis.	197	Restenosis	NS
Freed et al. (372)	Open-label pilot trial, plain old balloon angioplasty statin, enalapril, and colchicine 0.6 mg twice daily for at least 4 months after PTCA.	50	Restenosis after PTCA	NS
Deftereos et al. (367)	Double-blind, prospective, randomized trial, Colchicine 0.5 mg twice daily for 6 months after percutaneous coronary intervention with bare-metal stent implantation	196	Neointimal hyperplasia and in-stent restenosis	Benefit in reducing both Neointimal hyperplasia and in-stent restenosis Angiographic ISR rate was 16% in the colchicine group and 33% in the control group (p = 0.007; odds ratio: 0.38, 95% confidence interval: 0.18 to 0.79).

18. Antiplatelet resistance

P2Y₁₂ inhibitors reduce ADP-induced platelet activation and aggregation, attenuating thrombosis. Clopidogrel remains the most widely used agent globally. (374) It has a diverse spectrum of metabolic responses with approximately a 30% partial or complete resistance. Adverse platelet activity has long been known to propagate recurrent MACE. (375) Prasugrel also harbours resistance potential but far less than clopidogrel, with only a few case reports of on treatment ticagrelor resistance and stent thrombosis. (376) (377) Incremental increase in platelet reactivity correlates with enhanced stent thrombosis and MACE. (378)

There is a U-shaped curve regarding clinical outcomes with low and high platelet reactivity in patients on antiplatelet therapy with low platelet activity bearing a propensity to instigate bleeding problems. (379) Ticagrelor is emerging as the essential anti-platelet with trials of ticagrelor monotherapy post ACS in combination with the latest generation DES revealing statistical MACE significance. With the advent of colchicine as an adjunctive secondary CV medication, consideration must be given to a trial using colchicine versus placebo as an ancillary protective agent in antiplatelet monotherapy cohorts post ACS. (380)

19. Flow cytometry analysis of Progenitor blood cells

Platelet aggregation which has been extensively utilised in clopidogrel metabolism in ACS, may also be studied using flow cytometric techniques along with monocyte/macrophage progenitor cells which was explored in LoDoCo-MI sub-analyses. A flow cytometer is a device for studying the various properties of large numbers of small particles. Fluid containing particles flows through a device; the particles are labelled with fluorescent stain, a laser focussing on the flow of constituents producing the signature patterns. Platelets can be studied both for their degree of activation, degree of reactivity and response to other agents. They are labelled using monoclonal antibodies to the various platelet receptors. White blood cells are flagged in a similar fashion. Drawbacks of the technique is that it requires a flow cytometer access, is labour and expertise intensive (skilled specimen preparation) and may not be conducive to routine clinical use in a high-volume coronary care unit resigning it to a research tool rather than a real practical utility like light transmission aggregometry (LTA). These methods are largely superseded by the 'VerifyNow' rapid platelet reactivity test (point of care). (373) Note Platelet reactivity testing was not examined in our study and most patients (>85%) were on

ticagrelor due to practice policy in Perth in a region with a large Asian population who have a higher incidence of slow clopidogrel metabolism.

20. LoDoCo-MI Methods

20.1 Study design & Procedures

This was an investigator initiated randomized, double-blind placebo controlled clinical trial. Patients were randomized in a 1:1 ratio to receive either colchicine 0.5 mg once daily or matched placebo for 30 days. Adult (>18 years old) patients were eligible for enrolment if they had sustained a type 1 acute MI (by the 3rd universal definition) (204) within the prior 7 days. All patients were recruited at Royal Perth Hospital, Perth, Western Australia. All patients presenting to Royal Perth Hospital with an AMI were screened for eligibility.

Patients were invited into the study if they fulfilled the following key inclusion criteria: (1) age $\geq$ 18 years at time of presentation to RPH cardiology ward and, (2) have a confirmed diagnosis of type 1 MI. Key exclusion criteria were: (1) a history of myopathy, leukopenia, or thrombocytopenia; (2) severe renal impairment (< CKD stage 3b/ an estimated glomerular filtration rate <45 mL/min per 1.73m²); (3) severe hepatic dysfunction (alanine aminotransferase-ALT > 3x upper limit of normal (ULN)); (4) undergoing therapy with P-glycoprotein inhibitor(e.g., cyclosporin, verapamil or quinidine) and/or strong CYP3A4 inhibitor(e.g., ritonavir, clarithromycin or ketoconazole); (5) females of child-bearing age who are pregnant, lactating, or not using contraception; and (6) having a pre-existing indication for colchicine therapy/ any active inflammatory or infectious disease process.

The study was registered prospectively with the Australian and New Zealand Clinical Trials Registry (ACTRN12615001194550) and approved by the local human research ethics committee. All participants provided written informed consent. Funding for this research was provided by the National Heart Foundation of Australia (grant number 100616) and the Royal Perth Hospital Medical Research Foundation. Colchicine and placebo were provided free of charge by Aspen Pharmaceuticals. Patients were recruited during their index admission following acute MI. Baseline clinical data, medications, biochemistry, full blood count and creatine kinase (CK) levels were recorded, and blood tests repeated at 30 days. Signed consent forms were required prior to patient participation in the trial. Patients were randomly assigned, in a 1:1 ratio to receive placebo, or

colchicine at a dose of 0.5mg within 7 days after an AMI. Randomisation of participants and dispensing of medication was carried out by the research site's pharmacy. Troponin I level at presentation and the peak levels during hospitalization were recorded using a high sensitivity assay (Abbott Laboratories, Abbott Park, IL).

Left ventricular ejection fraction was assessed using standard echocardiographic methods (biplane Simpson's assessment) by a consultant cardiologist blinded to treatment allocation. Levels of CRP were measured using a high sensitivity assay (Architect c16000 Analyzer, Abbott Laboratories, Abbott Park, IL) at baseline (immediately prior to commencing study treatment) and at 30 days. In addition, EDTA-plasma samples taken at baseline and 30 days were aliquoted and stored at -80°C for subsequent IL-6 level measurement by highly sensitive ELISA (R&D Systems, Minneapolis, MN). The sensitivity of this assay is 0.7 pg./mL with inter- and intra-assay coefficients of variation $<7\%$ and $<5\%$ respectively. At 30 days patients underwent a standardized questionnaire to document the acceptability of the study procedures and any adverse events graded by the patients as mild, moderate, or severe. Patients were also asked to report their compliance with study medication and return their study medication bottles with any remaining tablets for a pill count.

Prior to randomisation, the investigator ensured that all enrolled patients had a baseline clinical questionnaire completed. This included details of current admission, demographics, and cardiovascular risk factors. Study medications were then dispensed by the pharmacy. Participants were considered to have stopped intervention if they took the study medication for < 7 days. Participants were then required to attend a post discharge follow-up appointment at day 25-35 of the trial period in a cardiology outpatient clinic for endpoint assessment. Patients were required to return medication bottles with any unconsumed medication during this visit and remaining pills were counted by the research pharmacists.

A 30-day questionnaire recording adverse events, adherence and acceptability of a longer outcome trial was also given to each participant. Patients unable to attend follow-up appointments were given the option to reschedule their appointment, or to have their bloods taken by regional pathology centres and return medication bottles via mail. An over-the-phone interview was used to complete 30-day questionnaires. Serum samples were obtained at baseline and 30-day assessments and stored for future analysis. During the trial, monitoring and recording of all adverse events were performed. Adverse events were defined as unexpected events that were fatal, life-threatening, or required

hospitalisation, medical or surgical intervention to prevent adverse outcomes. All reported adverse events were reviewed by the investigators during the 30-day follow-up period.

We assessed monocyte activation by performing flow cytometry on fresh blood samples from patients recruited into the LoDoCo-MI study. Specimens were analysed at baseline (within 7 days of the index MI) and at 30 days using a panel of monocyte activation biomarkers.

ELISA was performed for cytokines/biomarkers on bio banked specimens including IL-1 β , IL-6 and MPO in addition to the hs-CRP (the primary biomarker in the primary LoDoCo-MI study) from a portion of the LoDoCo-MI cohort.

Exploratory flow cytometry sub-analysis of 6 patients at baseline and 30 days post-treatment. 1) CD14⁺/MAN1⁺ (activated monocytes or macrophages) 2) CD14⁻/CD41⁺ (platelets) 3) CD14⁻/CD16⁺ (NK cells, neutrophils, or T cells) 4) CD14⁻/CD61⁺ normal and activated platelets).

20.2 Outcomes/End Points and definitions

The trial's primary endpoint is the 30-day follow-up CRP levels of participants in each group (in mg/L). Secondary endpoints include compliance, adverse events, and drug tolerability during the treatment period. Patients who have completed the treatment and successfully attended the 30-day follow-up were included in per-protocol (PP) analysis. Intention to treat analysis included patients who were (a) lost to follow-up; (b) withdrawn from the study; (c) experiencing adverse side effects; (d) non-compliant on treatment regime. Outcomes: The primary endpoint was the proportion of patients with a residual hs-CRP level ≥ 2 mg/L at 30 days. Pre-specified secondary endpoints included the actual levels of hs-CRP at 30 days and the relative and absolute change in CRP levels from baseline to 30 days. Other important pre-specified outcomes included a) the proportion of recruited patients completing the study; b) adverse events; c) participant-reported compliance with study medications; and d) death and major cardiovascular events (further MI or stroke) at 30 days.

20.3 Statistical analysis

This study was to establish the feasibility and safety of a large clinical trial that will assess the effects of colchicine on clinically important outcomes, such as death and re-infarction. . The primary endpoint was analysed as both a qualitative and continuous variable. Qualitative analysis assessed the number of patients in each group with a hs-CRP $<2\text{mg/L}$ as per the cut-off defined in the PROVE IT-TIMI 22 trial. (47) The median hs-CRP levels between the two groups were compared using the Mann Whitney U test. Other continuous variables assumed a normal distribution and their means were tested using a two-sided t-test. Other qualitative values were also compared with chi square analysis.

Based on prior data we estimated that 30 days after an acute MI approximately 42% of patients receiving a potent statin and aspirin will have a hs-CRP level $\geq 2\text{ mg/L}$. On this basis, a study of 210 patients has 90% power (2 sided $\alpha = 0.05$) to detect a 50% reduction in the prevalence of hs-CRP levels above this threshold (i.e., to 21% with CRP $\geq 2\text{ mg/L}$), allowing for 7% data loss (loss to follow-up etc). This reduction in levels of hs-CRP is less than previously reported in patients with stable CAD treated with colchicine but more than what Raju et al. reported post ACS. Data are presented as numbers (%), mean (standard deviation) and median (interquartile range), as appropriate. The normality of the distribution of variables was assessed using the Shapiro–Wilk test. Continuous variables with skewed distributions are compared using the Kruskal-Wallis test and categorical variable using the χ^2 test or Fisher's exact test, as appropriate. All analyses were performed according to the intention to treat principle using SPSS v24. All p-values are 2-sided.

20.4 Ethics Safety and Adjudication of outcomes

An independent Data and Safety Monitoring Committee monitored adverse events and outcomes during follow-up. Outcomes of the trial were determined by an endpoint adjudication committee, of which the members were blinded to the patient treatment allocation.

21 Results of LoDoCo-MI

Figure 25 CONSORT diagram

Two hundred thirty-seven patients were recruited between February 2016 and July 2017 and 224 patients (95%) completed the study (figure 25). Two patients, both of whom were randomized to placebo, were only able to undergo telephone follow up and did not have a follow up blood sample taken. The baseline demographic and clinical characteristics of both study groups were similar. The mean age of participants was 61 years and 182 (77%) were men. Baseline blood samples were taken

with a mean of 1.5 (SD 1.1) days and follow-up samples a mean of 33.3 (SD 5.6) days following the index MI.

The median level of hs-CRP at baseline was 7.4 mg/L (interquartile range 3.0–16.9) and was similar in both groups ($P = 0.77$). At 30-day follow up, 44% (49 out of 111 participants) treated with colchicine had a hs-CRP level ≥ 2 mg/L compared to 50% (56 out of 111 participants) of those randomised to placebo ($P = .35$) and the median hs-CRP in patients randomised to colchicine was 1.6 mg/L (interquartile range 0.7–3.5) compared to 2.0 mg/L (interquartile range 0.9–4.0) in patients randomised to placebo ($P = 0.11$). The median absolute reduction in hs-CRP levels at 30 days among colchicine treated patients was -4.3 mg/L (interquartile range -1.1 to -14.1) and in placebo treated patients it was -3.3 mg/L (interquartile range -0.9 to -14.4 , $P = .44$). The relative reduction was a fall of 78% compared to a fall of 64% ($P = .09$).

Levels of IL-6 at 30 days were similar in both groups both at baseline and follow-up and there were similar absolute and relative decreases in levels between baseline and 30 days. White cell counts, liver and muscle enzymes and other blood tests were also similar.

Table 7 LoDoCo-MI Baseline data

Characteristic	Total (n = 237)	Colchicine (n = 119)	Placebo (n = 118)	P
Age in years, mean (standard deviation)	61 (13.0)	61 (13.6)	61 (12.5)	.77
Male	182 (77%)	89 (75%)	93 (79%)	.46
Diabetes	52 (22%)	27 (23%)	25 (21%)	.78
Hypertension	112 (47%)	64 (54%)	48 (41%)	.04
Prior or current smoking	143 (60%)	77 (65%)	67 (57%)	.21
ST-elevation myocardial infarction	134 (56.5%)	63 (53%)	71 (60%)	.26
Previous myocardial infarction	36 (15%)	18 (15%)	18 (15%)	.98
Prior myocardial revascularization	27 (11%)	13 (11%)	14 (12%)	.60
Coronary angiogram during index admission	236 (99.6%)	118 (99%)	118 (100%)	.32
PCI during index admission	213 (90%)	105 (88%)	108 (91.5%)	.40
Left ventricular ejection fraction	52 (42–60)	55 (42–60)	50 (44–60)	.90
Aspirin	237 (99.6%)	118 (99%)	118 (100%)	.32
Second anti-platelet agent	233 (98%)	115 (97%)	117 (99%)	.18
Beta blocker	220 (93%)	111 (93%)	109 (92%)	.79
ACE inhibitor / Angiotensin receptor blocker	223 (94%)	114 (96%)	109 (92%)	.26
Statin	233 (98%)	115 (97%)	118 (100%)	.05
Body mass index,	28 (26–30)	28 (25–30)	28 (26–30)	.98
Admission high sensitivity troponin I (ng/L)	1345 (221–15,525)	885 (521–14,125)	2220 (298–16,425)	.19
Peak high sensitivity troponin I (ng/L)	5920 (611–30,450)	3250 (358–32,450)	9360 (975–28,875)	.18
Baseline hemoglobin, g/L	141 (15)	141 (15)	141 (16)	.94
Baseline white cell count, $\times 10^9/L$	9.0 (7.4–10.7)	9.0 (7.4–10.7)	9.2 (7.4–10.6)	.88
Baseline neutrophil count, $\times 10^9/L$	6.0 (4.7–7.7)	5.7 (4.7–7.5)	6.2 (4.7–7.8)	.39
Baseline lymphocyte count, $\times 10^9/L$	1.9 (1.5–2.4)	1.8 (1.5–2.4)	1.9 (1.5–2.3)	.46
Baseline creatinine, $\mu\text{mol/L}$	76 (68–87)	75 (68–87)	77 (69–87)	.38
Baseline high sensitivity C-reactive protein, mg/L	7.4 (3.0–16.9)	6.9 (3.1–14.8)	7.7 (3.0–18.9)	.77
Baseline interleukin-6, pg/mL	4.84 (3.10–9.50)	5.09 (3.42–9.99)	4.55 (3.05–8.33)	.15

Categorical data presented as n (%) and continuous data as median (interquartile range), unless otherwise stated.
 PCI, percutaneous coronary intervention; ACE, angiotensin converting enzyme.

Table 8 hs-CRP & Laboratory data at follow-up in LoDoCo-MI

	Colchicine group (n = 111)	Placebo group (n = 111)	P
High sensitivity C-reactive protein ≥ 2 mg/L	49 (44%)	56 (50%)	.35
High sensitivity C-reactive protein, mg/L	1.6 (0.7–3.5)	2.0 (0.9–4.0)	.11
Absolute change in C-reactive protein, mg/L	−4.3 (−1.1 to −14.1)	−3.3 (−0.90 to −14.4)	.44
Relative change in C-reactive protein (%)	−78 (−44 to −92)	−64 (−30 to −88)	.09
White cell count, $\times 10^9$ /L	6.7 (5.8–8.7)	7.2 (6.1–8.8)	.22
Neutrophil count, $\times 10^9$ /L	4.3 (3.4–5.4)	4.3 (3.7–5.8)	.25
Lymphocyte count, $\times 10^9$ /L	1.9 (1.4–2.3)	1.8 (1.5–2.1)	.64
Hemoglobin, g/L	140 (133–151)	142 (132–152)	.36
Creatine kinase, U/L	116 (81–165)	101 (70–138)	.14
Alanine aminotransferase, U/L	33 (25–54)	33 (26–49)	.84
Gamma glutamyl transferase, U/L	30 (20–56)	31 (25–49)	.68
Interleukin-6, pg/mL	2.16 (1.72–2.76)	2.12 (1.66–2.77)	.95
Absolute change in interleukin-6, pg/mL	−2.44 (−0.94 to −7.56)	−2.33 (−0.56 to −5.15)	.21
Relative change in interleukin-6 (%)	−58 (−32 to −79)	−52 (−19 to −66)	.16

Categorical data presented as n (%) and continuous data as median (interquartile range).

Figure 26 hs-CRP and IL-6 levels

ELISA was performed for cytokines/biomarkers on bio banked specimens including IL-1 β (n=193), IL-6 (n=199) and MPO (n=200) in addition to the hs-CRP (the primary biomarker in the primary LoDoCo-MI study) from a portion of the LoDoCo-MI cohort. We have stored EDTA-plasma and serum samples at -80 C. These have been analysed for interleukin-6 (IL-6) and MPO levels. In the placebo group 74% (82/111) of patients had a fall in hs-CRP of $\geq 50\%$ and 81% (90/111) a fall of $\geq 25\%$. In the colchicine group the corresponding figures were 63% (70/111) and 77% (86/111), respectively. As noted above, however, changes in levels following an acute event may be affected by a variety of factors. Unfortunately, we did not assess pharmacokinetic data on colchicine in this trial so exact therapeutic levels in individuals could not be ascertained.

Inflammatory biomarkers at 30 days

	Number of patients	All patients, median (IQR)	Colchicine group	Placebo group	P-value
IL-1 β	193 ¹	0.31 (0.22-0.48)	0.31 (0.21-0.47)	0.31 (0.22-0.52)	0.66
IL-6	199 ²	2.12 (1.71-2.77)	2.16 (1.72-2.76)	2.12 (1.66-2.77)	0.98
MPO	200 ³	1.23 (0.70-2.34)	1.23 (0.66-2.34)	1.21 (0.71-2.33)	0.95

¹ 97 received colchicine and 96 placebo; ² 100 received colchicine and 99 placebo; ³ 100 received colchicine and 100 placebo

Table 9 LoDoCo-MI Inflammatory Biomarker substudy IL-1 β , IL-6, MPO

Myeloperoxidase data

	All patients	Colchicine group	Placebo group	P-value
Baseline MPO levels (ng/mL)	3.43 (1.35-10.60)	3.41 (1.41-9.52)	3.57 (1.28-11.55)	0.95
30 day MPO levels (ng/mL)	1.23 (0.70-2.34)	1.23 (0.66-2.34)	1.21 (0.72-2.33)	0.98

Table 10 (supplementary MPO table)

21.1 Clinical outcomes

No deaths were recorded during the 30 day follow up period, though the vital status of 2 patients who were lost to trial follow-up was uncertain. Two patients suffered a major cardiac event (myocardial infarction) within 30 days; both participants had been randomized to placebo. Overall, 38 patients (17% of the 224 who completed the study protocol) experienced any adverse event during 30 day follow up. Of these patients, 18 (of 111 who completed the study, 16%) had received colchicine and 20 (of the 113 who completed the study, 18%) placebo ($P = .859$). Fifteen patients were readmitted to hospital within 30 days of their index MI (1 due to recurrent non-ST-elevation myocardial infarction, 1 due to stent thrombosis, 7 due to non-cardiac chest pain, 2 due to pericarditis, 1 due to breathlessness, 1 due to vasovagal syncope, 1 for insertion of an implantable defibrillator and 1 due to haematuria). Of these 3 were in the colchicine treated group (out of 111, 3%) and 12 were in the placebo group (out 113, 11%; $P = .029$).

Gastrointestinal symptoms occurred in 18 patients (8%), 12 who received colchicine (11% of the 111 patients in this group who completed the study) and 6 who received placebo (5% of the 113 patients in this group who completed the study; $P = .147$). Nine patients rated these to be moderate or severe (4 who received colchicine and 5 who received placebo). Adherence with study and other secondary prevention medications was generally good. Although the readmission rate was statistically higher in the placebo group the numbers are small, the events themselves were diverse and the majority were for benign reasons and/or events that seem unlikely related to the trial medications. Thus, the observed differences seem very likely to be due to the 'play of chance' and the null hypothesis ought to be heeded.

Table 11 LoDoCo-MI Clinical Outcomes & Compliance

	Colchicine group (n = 111)	Placebo group (n = 113)	P
Any adverse event	18 (16%)	20 (18%)	.859
Re-admission to hospital	3 (3%)	12 (11%)	.029
Adverse event not requiring hospitalisation ¹	16 (14%) ¹	8 (7%)	.087
Gastrointestinal	12 (11%)	6 (5%)	.147
Other ²	4 (4%)	2 (2%)	.440
Discontinued study treatment due to adverse event	2 (2%)	4 (4%)	.439
Took ≥25 days of study treatment ³	102 (92%)	103 (93%) ³	1.000
Other medications being taken at follow up ³			
Aspirin	110 (99%)	111 (100%) ³	1.000
Second anti-platelet agent	106 (95%)	110 (99%) ³	.212
Statin	107 (96%)	109 (98%) ³	.683
Beta-blocker	105 (95%)	109 (98%) ³	.280
ACE inhibitor or angiotensin receptor blocker	104 (94%)	106 (95%) ³	.768

ACE, angiotensin converting enzyme.

¹ One patient had readmission to hospital with chest pain (non-cardiac) and also independently experienced an adverse event not requiring hospitalization (gastrointestinal symptoms); ² other adverse events consisted of cough (x2), rash, palpitation, breathlessness and bruising; ³ in 2 patients (both in the placebo group) these data were not recorded.

21.2 Flow Sub-study results

No significant difference between treatment and control arm for IL cytokine biomarkers IL 1beta, IL-6, MPO (tables 8, 9, 10). On FC, non-classical inflammatory monocytes CD14+ CD16++ were markedly elevated (versus classical monocytes, ‘less inflammatory’ CD14++ CD16-) in all acute MI patients(n=6) with abrupt suppression of the same following treatment. The platelet % (CD41+ and CD61+) appear to always be lower after treatment initiation (dual anti-platelet therapy and other standard secondary prevention drugs-figure 27 below) The CD61+ results are variable after treatment.

Figure 27 Monocyte Activation & Flow Cytometry novel biomarker sub-study

CD14⁺/MAN1⁺ (activated monocytes or macrophages)
 CD14⁺/CD41⁺ (platelets)
 CD14⁺/CD16⁺ (NK cells, neutrophils or T cells)
 CD14⁺/CD61⁺ (normal and activated platelets)

21.3 Tolerability and safety

Overall, 38 patients (17% of the 224 who completed the study protocol) experienced an adverse event during 30 day follow up. Of these patients, 17 had received colchicine and 21 placebo. Fifteen patients were readmitted to hospital (1 due to recurrent non-ST-elevation myocardial infarction, 1 due to stent thrombosis, 7 due to non-cardiac chest pain, 2 due to pericarditis, 1 due to breathlessness, 1 due to vasovagal syncope, 1 for insertion of an implantable defibrillator and 1 due to haematuria).

Gastrointestinal symptoms occurred in 18 patients (8%), 12 who received colchicine (11% of the 113 patients in this group who completed the study) and 6 who received placebo (5% of the 111 patients in this group who completed the study). Nine patients rated these to be moderate or severe (4 who received colchicine and 5 who received placebo). Adherence with study and other secondary prevention medications was generally good (table 11). Eight patients discontinued their study medication entirely (5 who were receiving colchicine and 3 who were receiving placebo) but 207 (out of the 224 patients who completed the study, 92%) took at least 25 days of study treatment.

22 Discussion

22.1 Overview

The main findings of this study are that treatment with low dose colchicine is not associated with a significantly increased likelihood of achieving a CRP level <2 mg/L or lower absolute levels of CRP 30 days after an acute MI. Nevertheless, a trend in this direction was observed and, importantly, treatment with colchicine in this setting was safe and well tolerated. Readmission rates were significantly lower in the colchicine treated patients, but the numbers are small, the events were diverse, and the majority were for relatively benign reasons and/or events that seem unlikely related to the trial medication. Thus, the observed differences seem likely to be due to chance as this trial was not powered for clinical endpoints. This significant finding in the clinical readmission rate reduction has been remarked upon and cited by numerous review articles (44 PubMed citations to date). (1) (2) The relevance of hs-CRP as a prognostic marker remains an area of ongoing debate and the effect of colchicine requires establishment by robust adequately powered clinical trials evaluating key clinical outcomes.

Indeed, after the publication of LoDoCo-MI, the COLCOT trial depicted that colchicine in this exact setting 30 days post MI had a 1.6% absolute reduction in primary CV composite endpoints at a median follow-up of 22.6 months as well as being favourable regarding safety, compliance. (381) Looking at the results LoDoCo-MI in the context of current clinical practice, there is likely very limited value in routinely measuring inflammatory markers after MI. Based on current evidence, they are a poor guide as to the selection or effectiveness of current standard therapy.

22.2 Strengths and limitations

This study was the largest to date to assess hs-CRP post ACS assessing the tolerability of colchicine following acute MI and its effects on markers of inflammation in patients receiving contemporary treatment, including a range of evidence based secondary preventative medications. (381) McKnight et al. depicts the status of LoDoCo-MI as scoring full marks score of 5/5 on the ‘Jadad scale’ harnessing the largest degree of evidence in this setting at time of publication. (2, 382) Nonetheless, it has several limitations. It is a small, single centre study which was not powered to demonstrate any impact of colchicine on adverse cardiovascular events. Likewise, it was powered to demonstrate a significant reduction in levels of CRP, which was not observed in the current study, though larger reductions in CRP levels have been reported in patients with stable CHD treated with colchicine (albeit at a higher dose of 0.5 mg twice daily). (226) It is possible that a larger cohort size in LoDoCo-MI would have shown a significant reduction in such levels assuming a smaller, but still clinically relevant, effect size. Thus, there was potentially a type 2 error in our group (as well as the Raju et al. study which exhibited similar findings). The other main drawback of the current trial is that it only tested the tolerability of low dose colchicine over just 30 days, with subsequent trials eclipsing LoDoCo-MI regarding follow up longevity (Table 11).

LoDoCo-2 shows colchicine is well tolerated and clinically advantageous at a dosage of 0.5 mg daily in patients with stable CAD at 28.6 months median follow-up. (38) Fiolet et al. (2020) sub-analysed the 30 day effect of colchicine assessing the relative degree of hs-CRP change for each CV risk factor but showed nil statistical significance.(383) LoDoCo-MI provides robust reassurance with respect to the tolerability in patients receiving many other medications, some of which have theoretical or known interactions with colchicine, often for the first time after an acute MI: a setting where the adverse effects of one medication may have implications for compliance with other treatments that are known to have crucial benefits (prevention of stent thrombosis, left ventricular remodelling etc).

On-going Trials unravelling colchicine's influence on cardiac outcomes in COVID-19 cohorts will further extend the therapeutic boundaries of this multi-mechanistic, hepatic metabolised agent in a more systemically unwell, potentially multi-organ affected patient profile.

The delay from chest pain onset until hs-CRP sampling may be a significant confounder. Samples were taken a mean of 1.46 (SD1.07) days and a median of 1 (IQR 1-2) day after the index event, with 85% (201 out of 237 patients) taken within 48 hours and 96% (228 out of 237) within 72 hours. Of the remaining 9 patients 7 had their samples taken on day 4, 1 on day 5 and 1 on day 6. Insightful sub-analysis from the COLCOT trial underscored the importance of early induction of colchicine post MI to deter myocardial necrosis. (384) We found no significant reduction in the median level of hs-CRP, the absolute or relative reduction in levels or the percentage of patients who had levels of hs-CRP below 2 mg/L after 30-days. All were, however, lower in colchicine treated patients which is consistent with prior data. The totality of evidence supports the need for large-scale outcome trials in patients with recent MI. Importantly in this regard the current study demonstrates the safety and tolerability of low dose colchicine after acute MI. Our findings coupled with the promise from COLCOT and Martinez et al. lay a strong case for the use of colchicine post MI. (211)

The findings fit with and add to the current paradigm. There remains significant debate over the role of hs-CRP as a mere biomarker or mechanistically involvement in atherothrombosis. (155) The results of this study do not provide mechanistic support and the final analysis of colchicine effect on hs-CRP and IL-6 were statistically insignificant.. This draws into question the utility of measuring hs-CRP at all in routine clinical practice post-MI. The lowered hs-CRP and IL-6 levels with colchicine while statistically not significant on analysis are in line with the findings as demonstrated by Nidorf et al. 2011. This was the first double-blind randomised study for its time to examine colchicine in inflammatory suppression in ACS. We demonstrated that IL-6 and hs-CRP baseline values as well as the change in IL-6 and hs-CRP were correlated with colchicine treatment. This finding corroborates the suggestion that the action of colchicine to lower hs-CRP and IL-6 may occur at the similar point in the inflammatory cascade. The final analysis did not however demonstrate a statistically significant difference in any biomarker with colchicine treatment. (18)

A 'biologically plausible' elevation in CD14+ and CD16+ inflammatory 'non-classical' monocytes was observed immediately after type 1 MI (plaque rupture) and a sharp drop with treatment consistent with prior pre-clinical and clinical studies. (385, 386, 387) Study numbers in this mechanistic

biomarker substudy were far too small and thus under-powered to reveal any significant trend in contrast to the main study which showed trend towards hs-CRP attenuation with colchicine. It did, however, portray the flare of immune-inflammatory milieu in the peri-infarct period. This same substudy did underline the previously described logistical obstacles that are encountered by attempting to explore flow cytometry in this setting. There is a very limited time window to attain samples with specimen preparation being very labour and time intensive, incompatible with the challenging CCU management environment. Thus, it is perhaps not a practical avenue to pursue on an extensive scale with alternative options perhaps offering far more feasibility. It offers pilot data to help guide and design a larger scale future study and is thus hypothesis-generating. The consistent eruption in 'non-classical' inflammatory monocytes at the time of the clinical infarct is consistent with prior data and portrays the flare in inflammation at the time of infarct with resolution with therapy. (388)

22.3 Implications of findings

LoDoCo-MI did not show statistical support for a pathogenic role of hs-CRP in MI, but the totality of evidence suggests a consistent impact on inflammatory attenuation. The flow cytometry sub-analysis depicts the mechanism of inflammation flare at the time of ACS. This speaks to the view of hs-CRP as a bystander molecule, a marker of cardiovascular risk but not a target in the causative pathway for inhibition. (155) The positive clinical outcomes from COLCOT underscores the significance of larger trial numbers and longer follow up of endpoints perhaps causing a type 2 error in LoDoCo-MI informing future power estimates. (381) LoDoCo-MI study provides insight into the possible mechanism of action of colchicine on lowering hs- CRP and IL-6 in the immediate aftermath of an MI and the COLCOT builds further on these findings meeting statistical significance with far greater sample size. (381)

23. Major Trials published since LoDoCo-MI

23.1 An Overview

Among patients with a recent myocardial infarction, colchicine at a dose of 0.5 mg daily led to a significantly lower risk of ischemic cardiovascular events than placebo. (211) Acute preprocedural administration of colchicine attenuated the increase in IL-6 and hs-CRP after PCI when compared with placebo but did not lower the risk of PCI-related myocardial injury. (389) In a randomised trial involving 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. (38) In 138 consecutive patients with chronic CAD and hs-CRP >2 mg/L, other novel inflammatory markers, lipids, haematologic parameters and renal function were measured at baseline and after 30 days exposure to colchicine 0.5mg once daily. (383) In patients with chronic coronary artery disease and elevated hs-CRP, one-month exposure to colchicine 0.5 mg once daily was associated with a reduction of inflammatory markers. A small effect was seen on white blood cell count and platelet count, as well as a small decrease in estimated glomerular filtration rate. (383)

In the proteomic sub-study of LoDoCo-2, 30-day treatment with colchicine resulted in a significant median reduction in serum expression of 37 proteins. (422) Attenuation of the NLRP3 inflammasome pathway was supported by a reduction of IL-18, IL-1 receptor antagonist, and IL-6. The NLRP3 inflammasome cleaves pro-IL-18 and pro-IL-1 β into their active form, and attenuation of IL-1 β is reflected by attenuated levels of IL-1 receptor antagonist and IL-6. These effects may be partially upstream of the NLRP3 inflammasome inhibition, as suggested by a 10.7% reduction of NF- κ B (nuclear factor- κ B) essential modulator (P=0.008), an activator of NF- κ B, which is necessary for NLRP3 inflammasome activation. (422)

Table 12 Trials in CAD completed and On-going since the publication of LoDoCo-MI

Trial	Design & Intervention	n	Outcome	Benefit Observed
LoDoCo 2(38)	DB-RCT	5500	MACE	Yes
COLCOT (211)	DB-RCT	4745	MACE	Yes
COPS (390)	DB-RCT Colchicine 0.5mg BD for 1 month then 0.5mg od until 1-year vs Placebo	795	MACE	Nil
COLCHICINE-PCI Randomized Trial (389)	In a prospective, single-site trial, subjects referred for possible PCI (n=714) were randomised to acute preprocedural oral administration of colchicine 1.8 mg or placebo.	400	-Primary outcome of PCI-related myocardial injury/MACE -Attenuation of increase in interleukin-6 and high-sensitivity C-reactive protein concentrations after PCI when compared with placebo	Positive for Biomarker suppression but not for PCI related myocardial injury
COACS (NCT01906749)	RCT, double blind, randomised trial to assess the effect of low-dose colchicine (0.5mg/day) on MACE. The study hypothesis is that colchicine may reduce the specified combined endpoint from 16% to 7-8% at a 2-years follow-up.	500	MACE	On-going
CLEAR-SYNERGY	A 2x2 Factorial Randomised Controlled Trial of Colchicine	7000	MACE with sub-study on Neutrophil activation	On-going

(NCT03048825)	and Spironolactone in Patients with Myocardial Infarction / SYNERGY Stent Registry		assessing change in L-selectin	
COVERT-MI (NCT03156816)	Randomised parallel assignment Colchicine or Placebo. 3-month follow-up	194	% Change in LVEDV on cMRI at 3 months. Evaluate adverse LV remodelling, infarct size & microvascular obstruction reduction	Enrolling
COLCARDIO-ACS (ACTRN12616000400460)	RCT double-blind, Colchicine or Placebo. 3 year follow up	3000	MACE	Enrolling
COCOMO-ACS OCT imaging study-The Colchicine for coronary plaque modification in Acute Coronary Syndrome Study (ACTRN12618000809235)	Participants are randomly assigned to treatment, Colchicine 0.5 mg or control group for 12 months.	64	To compare the percentage change in coronary plaque minimum fibrous cap thickness (FCT) as determined by OCT. To compare the change in plaque macrophage content as determined by OCT.	Finished recruiting

23.2 Future studies in this Field

Further research in this area (summarised in Table 12) is abundant and may further decipher the role of hs-CRP in the CAD shining further light on the bystander vs pathologic role debacle. While this study did not provide any statistically significant benefit for colchicine suppression of hs-CRP, the lack of effect may in part be due to the identified study design issues. On-going studies investigating the effect of colchicine on coronary artery disease are recruiting much larger cohorts with higher CV risk (hs-CRP>2) for longer follow up periods having been thoroughly informed from preceding studies.

Clear-Synergy (OASIS 9) trial involves the randomisation of STEMI patients to either 0.5 mg of colchicine, 25 mg of spironolactone, or placebo in a 2 x 2 design for 1 year looking at inflammatory markers and MACE as well as factors influencing colchicine responders from non-responders (Table 11). Exploratory analysis into the derivation of a risk score from the mentioned outcomes will be performed. The COACs (Colchicine for ACS-NCT01906749) is also ongoing in Italy. The Colchicine for Left Ventricular Remodeling Treatment in Acute myocardial Infarction (COVERT-MI) will analyse ventricular remodeling by cMRI in 194 STEMI patients who will receive a loading dose of 2mg at time of revascularization followed by 0.5mg for 5 days thereafter. (319) Optical coherence technology (OCT) focussed imaging studies like COCOMO-ACS (ACTRN12618000809235) will be intriguing, assessing colchicine's effect on plaque burden.

Future years may show enhanced molecular insight into the microenvironment of plaque instability utilising numerous integrated modalities with the dawn of the nanotechnology era with the potential to locally administer the agent in question minimising collateral systemic impact.(391,392) Previously unfavourable systemically delivered medications may reach positive endpoints by direct local delivery to the plaque by nano-medicine.(393, 394) Delving further into this frontier should see the formation of novel biomarker models (assisted by artificial intelligence) accurately pin-pointing unfavourable plaque evolution further before clinical presentation. Such a vividly detailed depiction of the natural history of plaque evolution through emerging technologies may pave the way for more tailored individualised therapeutic interventions being eternally cognisant of cost-effectiveness margins. (395, 396)

It is worth remarking in this COVID-19 pandemic era that colchicine is also being examined in a remedy against the cardiovascular sequelae of COVID-19. (397) This is indeed intriguing as such patients are likely to have a lower general performance status than the cohorts enrolled in the trials mentioned above. Relationships between biomarkers like IL-6 and integrated serial imaging should be fascinating as well as broadening colchicine's experimental repertoire with more systemically compromised patients selected where previously more stringent exclusion criteria would have resulted in exclusion.

23.3 Potential Future Trials for Contemplation

With the advent of antiplatelet monotherapy post ACS especially in patients with high risk for bleeding and/or other contraindications to DAPT, (380) using colchicine as an extra protective adjunct (against in-stent thrombosis and restenosis) in unison with polymer free stent technology is worth exploring given its' emerging status as a resolute secondary preventative. Conversely, patients in whom triple-therapy and/or longer term DAPT cover may have been advantageous (with PIONEER AF PCI and PEGASUS-TIMI 54 studies in mind) but is ceased for various reasons may benefit from extra colchicine cover in keeping with lessons from the colchicine trials in chronic coronary syndrome. (401-403)

An arrhythmia study using Implantable Loop Recorders (ILRs) exploring sympathetic system & inflammatory influence on arrhythmia burden post myocardial infarction. Initially the LoDoCo-MI proposed a small 'Arrhythmia sub-study' drawn up which did gain RPH ethics clearance which was intended to involve implanting roughly 50 implantable Loop Recorders (ILRs) in the treatment and placebo arms of the trial. However, this sub-analysis did not materialize due to logistical obstacles and early observations of patient refusal to consent to this component of the trial despite tremendous patient interest in the main trial which is informative from a Trial design and feasibility perspective. The average ejection of the LoDoCo-MI cohort was almost 48%, a low level of LV dysfunction which is not greatest coordinated to adverse post-MI arrhythmia burden.

Such a study would provide considerable insight as to patients at highest risk of a fatal arrhythmia especially in patients with significant myocardial scar burden and EF <40% which is a huge nidus for arrhythmia with significant influence from sympathetic dysregulation. It would have the capacity to guide further clinical decision processes around the need for further revascularization or an intra

cardiac defibrillator (ICD). Correlations and trends would be interrogated between integrated data on arrhythmia burden with Monocyte Activation & a panel of athero-inflammatory biomarkers. In addition to ILRs, telemetry/cardiac monitoring/prolonged Holter readings could be analysed with the serum biomarkers. Occult arrhythmias like AF for example (if noted on loop recordings) would prompt the need to revise a patient's antiplatelet regimen given the consequential necessity for anticoagulation. Therefore, offering a more thoroughly inciteful therapeutic opinion.

Future Colchicine in ACS trials should focus on procedure related parameters such as lesion site, number of stents, total stent length, stent diameter, clinical ISR, target vessel revascularization and stent thrombosis with large trials numbers and extensive follow longevity building upon the premise of smaller preceding studies examining the same. (404) Lesion morphology on IVUS and OCT would be intriguing in a colchicine versus non-colchicine arm.

Colchicine's role as a plausible antianginal against chronic small vessel ischaemia adjunct needs examining, especially in those vulnerable multi-morbid patients where lowering of the systolic and diastolic blood pressure further may compromise cerebral and coronary perfusion, respectively. It may be a 'sparing agent', mitigating against further titration of nitrates, beta blocker, calcium channel blocker in whom have a contra indication to such agents or have refractory angina symptoms despite the aforementioned.

Larger Prospective multi-modality incorporated studies with CT-coronary angiogram, cMRI and PET-CT are being conducted in the post infarct setting. (405) Such investigations are becoming more increasingly accessible even in regional facilities in the Developed World. Trials with PET-CT may be more cumbersome to coordinate given the greater logistic hazards in relation to PET-CT availability. However, PET-CT can with great effectiveness estimate vascular macrophage activity and coronary thrombotic rupture/MACE prediction beyond the Framingham model. (406-408) Current clinical/symptoms driven risk factor models like the GRACE/TIMI/FRISC/Reynolds's index may be greatly modified, and novel models forged give the potential of the modalities above.

Within the speciality of cMRI, novel assessment indices like 'myocardial salvage index' have been coined and validated along with microvascular obstruction which can now be elicited and trials in the realm (e.g., ASSAIL-MI trial) have been drawn up. (409) (410) Colchicine's effect on these parameters warrants investigation. Studies regarding Colchicine's influence on coronary

microvascular obstruction (MVO) on cMRI should be performed, digressing beyond its effect on large vessel atherosclerosis requiring PCI which is currently very much under the microscope along with infarct attenuation on cMRI.

Genetic exploration with proteomic and genomic strategies harbours infinite potential in primary and secondary CV prevention. (411) The large gaping void in current more 'traditional' Framingham CV risk models may be bridged and revolutionised by extensive forthcoming purported gene analysis. (412) Recognising the complex intricacies between genotype and phenotype correlation, gene profiling will continue to unravel an individual's or ethnicities susceptibility and individualised bespoke response to therapeutics curtailing inevitable side effects inherent to broader spectrum therapies.

Certain genomic information may transcend the cardiovascular insight sought i.e. gene segments single nucleotide polymorphisms (SNPs) in CDKN2BAS harbouring capacity to invoke a plethora of multi system issues beyond vascular/cardiac system physiology.(413) Genome-wide association studies (GWAS) and polymorphism analysis on genes (like CDKN2A, CDKN2B, and MTAP to mention just a few) which are linked to plaque vulnerability, rupture and adverse remodelling may be ascertained at the time of primary infarct informing of potential future recurrent and de novo coronary sequelae with higher risk gene positive patients followed with more scrutiny with imaging and functional investigations.(414)

Micro RNAs (micro nucleic acids) which are non-coding RNAs are causally involved throughout athero-thrombosis. (415) Micro RNA (miRNA 93-5 p as an example) independently predicts in-stent restenosis (ISR) and CAD outcomes after adjusting for more 'traditional' RFs. (416) Further larger studies in this field are warranted to evaluate mi-RNAs putative role in ACS causation and colchicine's influence on suppressing this potential novel biomarker (Table 13). (417)

'Anti-inflammatory' (M2 phenotype) macrophage subtypes possess cardioprotective effects sequestering IL-10 in contrast to the M1 pro-inflammatory subpopulation (Figure 7). (418) The protective effects of MSCs on infarct repair are mediated by macrophages (M2), which are essential for early healing and repair. Thus, selectively targeting macrophages could be a novel strategy (using stem cells therapeutic means) to improve infarct healing and regeneration minimising adverse heart

failure sequelae. (419) Revolutionary PET tracers can locate negative coronary macrophage activity augmenting therapeutic precision.

<u>Table 13 Progress Summary of Translational Research in Atherosclerosis with Future Directions in Mind ('from bench to clinic')</u>
Humanization of animal bench models
Model plaque degradation & rupture
Enhancement of Biomarkers with spotlight on proximal upstream immune regulators and plaque architecture constituents
DNA & miRNA interrogation of Bio banked specimens
<i>Adapted from Libby, Ridker, Hansson-Nature 2011(19)</i>

24. Concluding remarks

In conclusion, LoDoCo-MI displayed no significant reduction in the median level of hs-CRP, the absolute or relative reduction in levels or the percentage of patients who had levels of hs-CRP below 2mg/L after 30-days. All were, however, lower in colchicine treated patients which is consistent with prior data. We believe that the totality of findings from this trial are thought-provoking, providing fertile-ground for larger-scale colchicine outcome trials in patients with recent MI. Such trials have since followed proving statistical significance and there is an incessant procession of similar trials in this field. LoDoCo-MI demonstrates the safety and tolerability of low dose colchicine after acute MI. This trial validates colchicine along with standard post-MI therapy regarding all realms of feasibility, tolerability, safety, and compliance cultivating a pivotal platform upon which to further build on. There is a trend towards benefit with colchicine in attenuating hs-CRP immediately after AMI but not significant in the LoDoCo-MI pilot cohort. These findings were subsequently corroborated by similar non-significant hs-CRP & IL-6 trends in the acute phase of the COLCOT study. (381) This negative result in both studies is likely a type two error due to a combination of low trial numbers and relatively short follow up period. It also raises further significant doubt about the futility of routinely assessing inflammatory biomarkers in this setting. The results suggest that colchicine acts by suppressing CRP and near-by affiliated signal markers in the innate immune pathway. (30)

The general opinion at the time of publication of LoDoCo-MI was that a larger study would have a high probability of a significant MACE benefit, should such a positive result truly exist. This indeed has been proven in the COLCOT study. (381) LoDoCo-2 has gone on to solidify Colchicine's status as a worthy post-MI therapy long term. A recent meta-analysis of 12 trials (including our LoDoCo-MI) proposes that colchicine may be beneficial for both acute and chronic CAD patients. (421) Of particular interest, in the post-ACS setting, adjuvant colchicine of 0.5 mg daily significantly reduced the rate of ischemic cardiovascular events: HR 0.77 (95% CI 0.61-0.96), p=0.02. Upcoming trials in this field will further elucidate Colchicine's role in the post MI setting.

25. References

1. Cao D, Chiarito M, Mehran R. Treating Inflammation Prior to Percutaneous Coronary Intervention. *Circulation: Cardiovascular Interventions*. 2020;13(4): e009127.
2. McKnight AH, Katzenberger DR, Britnell SR. Colchicine in Acute Coronary Syndrome: A Systematic Review. *Annals of Pharmacotherapy*. 0(0):1060028020942144.
3. DeMizio DJ, Geraldino-Pardilla LB. Autoimmunity and Inflammation Link to Cardiovascular Disease Risk in Rheumatoid Arthritis. *Rheumatology and Therapy*. 2020;7(1):19-33.
4. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med*. 1999;340(2):115-26.
5. Raggi P, Genest J, Giles JT, Rayner KJ, Dwivedi G, Beanlands RS, et al. Role of inflammation in the pathogenesis of atherosclerosis and therapeutic interventions. *Atherosclerosis*. 2018; 276:98-108.
6. Hansson GK. Inflammation, Atherosclerosis, and coronary artery disease. *New England Journal of Medicine*. 2005;352(16):1685-95.
7. Crea F, Libby P. Acute Coronary Syndromes: The Way Forward from Mechanisms to Precision Treatment. *Circulation*. 2017;136(12):1155-66.
8. Libby P. Superficial erosion and the precision management of acute coronary syndromes: not one-size-fits-all. *Eur Heart J*. 2017;38(11):801-3.
9. Libby P, Ridker PM, Hansson GK, Leducq Transatlantic Network on A. Inflammation in atherosclerosis: from pathophysiology to practice. *Journal of the American College of Cardiology*. 2009;54(23):2129-38.
10. Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature*. 2010;464(7293):1357-61.

11. Fioranelli M, Bottaccioli AG, Bottaccioli F, Bianchi M, Rovesti M, Roccia MG. Stress, and Inflammation in Coronary Artery Disease: A Review Psychoneuroendocrineimmunology-Based. *Front Immunol*. 2018; 9:2031.
12. Ridker PM. C-Reactive Protein and the Prediction of Cardiovascular Events Among Those at Intermediate Risk: Moving an Inflammatory Hypothesis Toward Consensus. *Journal of the American College of Cardiology*. 2007;49(21):2129-38.
13. Koenig W, Khuseyinova N. Biomarkers of Atherosclerotic Plaque Instability and Rupture. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2007;27(1):15-26.
14. Kholeif MA. C-Reactive Protein for Cardiovascular Risk Assessment. *Diabetes Care*. 2005;28(7):1833-4.
15. Tousoulis D, Oikonomou E, Economou EK, Crea F, Kaski JC. Inflammatory cytokines in atherosclerosis: current therapeutic approaches. *Eur Heart J*. 2016;37(22):1723-32.
16. Yan BP, Tan G-M. What's Old is New Again - A Review of the Current Evidence of Colchicine in Cardiovascular Medicine. *Curr Cardiol Rev*. 2017;13(2):130-8.
17. Bauriedel G, Schluckebier S, Welsch U, Klingel K, Kandolf R, Steinbeck G. [Incidence and localization of apoptosis bodies in human arteriosclerosis lesions]. *Z Kardiol*. 1996;85(7):509-18.
18. Hennessy T, Soh L, Bowman M, Kurup R, Schultz C, Patel S, et al. The low dose colchicine after myocardial infarction (LoDoCo-MI) study: A pilot randomized placebo-controlled trial of colchicine following acute myocardial infarction 2019.
19. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. *Nature*. 2011;473(7347):317-25.
20. Virchow R. *Cellular Pathology as Based Upon Physiological and Pathological Histology*: JB Lippincott; 1863.
21. Libby P. Inflammation in atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology*. 2012;32(9):2045-51.
22. Mayerl C, Lukasser M, Sedivy R, Niederegger H, Seiler R, Wick G. Atherosclerosis research from past to present--on the track of two pathologists with opposing views, Carl von Rokitansky and Rudolf Virchow. *Virchows Arch*. 2006;449(1):96-103.
23. England BR, Sayles H, Michaud K, Caplan L, Davis LA, Cannon GW, et al. Cause-specific mortality in male US veterans with rheumatoid arthritis. *Arthritis care & research*. 2016;68(1):36-45.
24. van de Ree MA, Huisman MV, Princen HM, Meinders AE, Kluft C. Strong decrease of high sensitivity C-reactive protein with high-dose atorvastatin in patients with type 2 diabetes mellitus. *Atherosclerosis*. 2003;166(1):129-35.

25. Heeschen C, Hamm CW, Laufs U, Snapinn S, Böhm M, White HD. Withdrawal of statins increases event rates in patients with acute coronary syndromes. *Circulation*. 2002;105(12):1446-52.
26. Nguyen MT, Fernando S, Schwarz N, Tan JT, Bursill CA, Psaltis PJ. Inflammation as a Therapeutic Target in Atherosclerosis. *J Clin Med*. 2019;8(8):1109.
27. Gelosa P, Castiglioni L, Camera M, Sironi L. Drug repurposing in cardiovascular diseases: Opportunity or hopeless dream? *Biochemical Pharmacology*. 2020; 177:113894.
28. Dinarello CA, Wolff SM, Goldfinger SE, Dale DC, Alling DW. Colchicine therapy for familial mediterranean fever. A double-blind trial. *N Engl J Med*. 1974;291(18):934-7.
29. Imazio M, Bobbio M, Cecchi E, Demarie D, Demichelis B, Pomari F, et al. Colchicine in addition to conventional therapy for acute pericarditis: results of the COLchicine for acute PERicarditis (COPE) trial. *Circulation*. 2005;112(13):2012-6.
30. Leung YY, Yao Hui LL, Kraus VB. Colchicine--Update on mechanisms of action and therapeutic uses. *Semin Arthritis Rheum*. 2015;45(3):341-50.
31. Demidowich AP, Davis AI, Dedhia N, Yanovski JA. Colchicine to decrease NLRP3-activated inflammation and improve obesity-related metabolic dysregulation. *Med Hypotheses*. 2016; 92:67-73.
32. Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature*. 2006;440(7081):237-41.
33. Verma S, Eikelboom JW, Nidorf SM, Al-Omran M, Gupta N, Teoh H, et al. Colchicine in cardiac disease: a systematic review and meta-analysis of randomized controlled trials. *BMC Cardiovasc Disord*. 2015; 15:96.
34. Crittenden DB, Lehmann RA, Schneck L, Keenan RT, Shah B, Greenberg JD, et al. Colchicine use is associated with decreased prevalence of myocardial infarction in patients with gout. *J Rheumatol*. 2012;39(7):1458-64.
35. Nidorf SM, Eikelboom JW, Budgeon CA, Thompson PL. Low-dose colchicine for secondary prevention of cardiovascular disease. *J Am Coll Cardiol*. 2013;61(4):404-10.
36. Martínez GJ, Robertson S, Barraclough J, Xia Q, Mallat Z, Bursill C, et al. Colchicine acutely suppresses local cardiac production of inflammatory cytokines in patients with an acute coronary syndrome. *Journal of the American Heart Association*. 2015;4(8): e002128.
37. Vaidya K, Arnott C, Martinez GJ, Ng B, McCormack S, Sullivan DR, et al. Colchicine Therapy and Plaque Stabilization in Patients with Acute Coronary Syndrome: A CT Coronary Angiography Study. *JACC Cardiovasc Imaging*. 2018;11(2 Pt 2):305-16.

38. Nidorf SM, Fiolet ATL, Mosterd A, Eikelboom JW, Schut A, Opstal TSJ, et al. Colchicine in Patients with Chronic Coronary Disease. *New England Journal of Medicine*. 2020.
39. Hemkens LG, Ewald H, Gloy VL, Arpagaus A, Olu KK, Nidorf M, et al. Colchicine for prevention of cardiovascular events. *Cochrane Database Syst Rev*. 2016;2016(1): Cd011047.
40. Kesselheim AS, Solomon DH. Incentives for drug development--the curious case of colchicine. *N Engl J Med*. 2010;362(22):2045-7.
41. Santos IS, Goulart AC, Brandão RM, Santos RCdO, Bittencourt MS, Sitnik D, et al. One-year Mortality after an Acute Coronary Event and its Clinical Predictors: The ERICO Study. *Arquivos brasileiros de cardiologia*. 2015;105(1):53-64.
42. Fake A, Ranchord A, Harding S, Larsen P. Triple Therapy Versus Dual Antiplatelet Therapy for Patients with Atrial Fibrillation and Acute Coronary Syndromes: A Systematic Literature Review. *Curr Cardiol Rev*. 2017;13(4):325-33.
43. Bonaca MP, Bhatt DL, Cohen M, Steg PG, Storey RF, Jensen EC, et al. Long-term use of ticagrelor in patients with prior myocardial infarction. *New England Journal of Medicine*. 2015;372(19):1791-800.
44. Angiolillo DJ, Baber U, Mehran R. Ticagrelor monotherapy in patients with diabetes mellitus undergoing percutaneous coronary interventions: insights from the TWILIGHT trial. *Cardiovascular Research*. 2020;116(7): e70-e2.
45. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation*. 2003;107(3):363-9.
46. Morrow DA, Rifai N, Antman EM, Weiner DL, McCabe CH, Cannon CP, et al. C-reactive protein is a potent predictor of mortality independently of and in combination with troponin T in acute coronary syndromes: a TIMI 11A substudy. *Thrombolysis in Myocardial Infarction. J Am Coll Cardiol*. 1998;31(7):1460-5.
47. Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, et al. C-reactive protein levels and outcomes after statin therapy. *N Engl J Med*. 2005;352(1):20-8.
48. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med*. 1997;336(14):973-9.
49. Ridker PM. Clinician's Guide to Reducing Inflammation to Reduce Atherothrombotic Risk: JACC Review Topic of the Week. *J Am Coll Cardiol*. 2018;72(25):3320-31.
50. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Engl J Med*. 2017;377(12):1119-31.

51. Ben-Chetrit E, Levy M. Colchicine: 1998 update. *Semin Arthritis Rheum*. 1998;28(1):48-59.
52. Robertson S, Martínez GJ, Payet CA, Barraclough JY, Celermajer DS, Bursill C, et al. Colchicine therapy in acute coronary syndrome patients acts on caspase-1 to suppress NLRP3 inflammasome monocyte activation. *Clinical science*. 2016;130(14):1237-46.
53. Waters DD. Inflammation in Cardiovascular Disease: From Basic Concepts to Clinical Application. *International Journal of Cardiovascular Sciences*. 2020.
54. Finkelstein Y, Aks SE, Hutson JR, Juurlink DN, Nguyen P, Dubnov-Raz G, et al. Colchicine poisoning: the dark side of an ancient drug. *Clinical toxicology*. 2010;48(5):407-14.
55. Danielak D, Karażniewicz-Łada M, Głowska F. Assessment of the Risk of Rhabdomyolysis and Myopathy During Concomitant Treatment with Ticagrelor and Statins. *Drugs*. 2018;78(11):1105-12.
56. Ridker PM. From C-Reactive Protein to Interleukin-6 to Interleukin-1: Moving Upstream to Identify Novel Targets for Atheroprotection. *Circ Res*. 2016;118(1):145-56.
57. Groh L, Keating ST, Joosten LAB, Netea MG, Riksen NP. Monocyte and macrophage immunometabolism in atherosclerosis. *Semin Immunopathol*. 2018;40(2):203-14.
58. Bekkering S, van den Munckhof I, Nielen T, Lamfers E, Dinarello C, Rutten J, et al. Innate immune cell activation and epigenetic remodeling in symptomatic and asymptomatic atherosclerosis in humans in vivo. *Atherosclerosis*. 2016; 254:228-36.
59. Kapellos TS, Bonaguro L, Gemünd I, Reusch N, Saglam A, Hinkley ER, et al. Human Monocyte Subsets and Phenotypes in Major Chronic Inflammatory Diseases. *Frontiers in Immunology*. 2019;10(2035).
60. Zeng S, Zhou X, Ge L, Ji WJ, Shi R, Lu RY, et al. Monocyte subsets and monocyte-platelet aggregates in patients with unstable angina. *J Thromb Thrombolysis*. 2014;38(4):439-46.
61. Yang M, Lv H, Liu Q, Zhang L, Zhang R, Huang X, et al. Colchicine Alleviates Cholesterol Crystal-Induced Endothelial Cell Pyroptosis through Activating AMPK/SIRT1 Pathway. *Oxidative Medicine and Cellular Longevity*. 2020; 2020:9173530.
62. Rolfes V, Ribeiro LS, Hawwari I, Böttcher L, Rosero N, Maasewerd S, et al. Platelets Fuel the Inflammasome Activation of Innate Immune Cells. *Cell Rep*. 2020;31(6):107615-.
63. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2095-128.

64. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. *Journal of the American College of Cardiology*. 2017;70(1):1-25.
65. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation*. 2015;131(4): e29-322.
66. Barquera S, Pedroza-Tobias A, Medina C, Hernandez-Barrera L, Bibbins-Domingo K, Lozano R, et al. Global Overview of the Epidemiology of Atherosclerotic Cardiovascular Disease. *Arch Med Res*. 2015;46(5):328-38.
67. Ziaeian B, Fonarow GC. Epidemiology and aetiology of heart failure. *Nat Rev Cardiol*. 2016;13(6):368-78.
68. Savarese G, Lund LH. Global Public Health Burden of Heart Failure. *Card Fail Rev*. 2017;3(1):7-11.
69. Kyu HH, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, et al. Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*. 2018;392(10159):1859-922.
70. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, et al. heart disease and stroke statistics--2014 update: a report from the American Heart Association. *Circulation*. 2014;129(3): e28-e292.
71. Veitenhansl M, Stegner K, Hierl FX, Dieterle C, Feldmeier H, Gutt B, et al. 40(th) EASD Annual Meeting of the European Association for the Study of Diabetes: Munich, Germany, 5-9 September 2004. *Diabetologia*. 2004;47(Suppl 1): A1-A464.
72. Mensah GA, Wei GS, Sorlie PD, Fine LJ, Rosenberg Y, Kaufmann PG, et al. Decline in Cardiovascular Mortality: Possible Causes and Implications. *Circ Res*. 2017;120(2):366-80.
73. Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, et al. heart disease and Stroke Statistics—2020 Update: A Report from the American Heart Association. *Circulation*. 2020;141(9): e139-e596.
74. Vedanthan R, Seligman B, Fuster V. Global perspective on acute coronary syndrome: a burden on the young and poor. *Circ Res*. 2014;114(12):1959-75.
75. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: part I: general considerations, the epidemiologic transition, risk factors, and impact of urbanization. *Circulation*. 2001;104(22):2746-53.

76. Celermajer DS, Chow CK, Marijon E, Anstey NM, Woo KS. Cardiovascular Disease in the Developing World: Prevalences, Patterns, and the Potential of Early Disease Detection. *Journal of the American College of Cardiology*. 2012;60(14):1207-16.
77. Bennett JE, Stevens GA, Mathers CD, Bonita R, Rehm J, Kruk ME, et al. NCD Countdown 2030: worldwide trends in non-communicable disease mortality and progress towards Sustainable Development Goal target 3.4. *The Lancet*. 2018;392(10152):1072-88.
78. Horton R. Offline: Chronic diseases—the social justice issue of our time. *The Lancet*. 2015;386(10011):2378.
79. D'Agostino RB, Sr., Grundy S, Sullivan LM, Wilson P, Group CHDRP. Validation of the Framingham coronary heart disease prediction scores: results of a multiple ethnic groups investigation. *JAMA*. 2001;286(2):180-7.
80. Hajar R. Framingham Contribution to Cardiovascular Disease. *Heart Views*. 2016;17(2):78-81.
81. D'Agostino RB, Sr., Pencina MJ, Massaro JM, Coady S. Cardiovascular Disease Risk Assessment: Insights from Framingham. *Glob Heart*. 2013;8(1):11-23.
82. Libby P. Changing concepts of atherogenesis. *J Intern Med*. 2000;247(3):349-58.
83. Gaziano T, Reddy KS, Paccaud F, Horton S, Chaturvedi V. Cardiovascular Disease. In: Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M, Evans DB, et al., editors. *Disease Control Priorities in Developing Countries*. Washington (DC) New York: The International Bank for Reconstruction and Development / The World Bank Oxford University Press
Copyright © 2006, The International Bank for Reconstruction and Development/The World Bank Group.; 2006.
84. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet*. 2004;364(9438):937-52.
85. Nelson RH. Hyperlipidemia as a risk factor for cardiovascular disease. *Prim Care*. 2013;40(1):195-211.
86. Petrie JR, Guzik TJ, Touyz RM. Diabetes, Hypertension, and cardiovascular disease: Clinical Insights and Vascular Mechanisms. *Can J Cardiol*. 2018;34(5):575-84.
87. Hozawa A, Folsom AR, Sharrett AR, Chambless LE. Absolute and attributable risks of cardiovascular disease incidence in relation to optimal and borderline risk factors: comparison of

African American with white subjects--Atherosclerosis Risk in Communities Study. *Arch Intern Med.* 2007;167(6):573-9.

88. Ezzati M, Obermeyer Z, Tzoulaki I, Mayosi BM, Elliott P, Leon DA. Contributions of risk factors and medical care to cardiovascular mortality trends. *Nat Rev Cardiol.* 2015;12(9):508-30.

89. Wilson PW. Established risk factors and coronary artery disease: the Framingham Study. *American journal of hypertension.* 1994;7(7_Pt_2):7S-12S.

90. Payne RA. Cardiovascular risk. *Br J Clin Pharmacol.* 2012;74(3):396-410.

91. Ridker PM. Clinical Application of C-Reactive Protein for Cardiovascular Disease Detection and Prevention. *Circulation.* 2003;107(3):363-9.

92. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *Journal of the American College of Cardiology.* 1990;15(4):827-32.

93. Janowitz WR, Agatston AS, Kaplan G, Viamonte Jr M. Differences in prevalence and extent of coronary artery calcium detected by ultrafast computed tomography in asymptomatic men and women. *The American journal of cardiology.* 1993;72(3):247-54.

94. Milner KA, Funk M, Richards S, Wilmes RM, Vaccarino V, Krumholz HM. Gender differences in symptom presentation associated with coronary heart disease. *Am J Cardiol.* 1999;84(4):396-9.

95. Ryan TJ. The thrombolysis in myocardial infarction risk index: a formula with a future. *J Am Coll Cardiol.* 2004;44(4):790-2.

96. Bradshaw PJ, Ko DT, Newman AM, Donovan LR, Tu JV. Validation of the Thrombolysis in Myocardial Infarction (TIMI) risk index for predicting early mortality in a population-based cohort of STEMI and non-STEMI patients. *The Canadian journal of cardiology.* 2007;23(1):51-6.

97. Eagle KA, Lim MJ, Dabbous OH, Pieper KS, Goldberg RJ, Van de Werf F, et al. A validated prediction model for all forms of acute coronary syndrome: estimating the risk of 6-month postdischarge death in an international registry. *Jama.* 2004;291(22):2727-33.

98. Littnerova S, Kala P, Jarkovsky J, Kubkova L, Prymusova K, Kubena P, et al. GRACE Score among Six Risk Scoring Systems (CADILLAC, PAMI, TIMI, Dynamic TIMI, Zwolle) Demonstrated the Best Predictive Value for Prediction of Long-Term Mortality in Patients with ST-Elevation Myocardial Infarction. *PloS one.* 2015;10(4): e0123215-e.

99. Ridker PM, Buring JE, Rifai N, Cook NR. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. *Jama.* 2007;297(6):611-9.

100. DeFilippis AP, Blaha MJ, Ndumele CE, Budoff MJ, Lloyd-Jones DM, McClelland RL, et al. The association of Framingham and Reynolds risk scores with incidence and progression of coronary artery calcification in MESA (Multi-Ethnic Study of Atherosclerosis). *Journal of the American College of Cardiology*. 2011;58(20):2076-83.
101. Kim YH, Park DW, Kim WJ, Lee JY, Yun SC, Kang SJ, et al. Validation of SYNTAX (Synergy between PCI with Taxus and Cardiac Surgery) score for prediction of outcomes after unprotected left main coronary revascularization. *JACC Cardiovasc Interv*. 2010;3(6):612-23.
102. Iyngkaran P, Noaman S, Chan W, Mahadavan G, Thomas MC, Rajendran S. Non-invasive Risk Stratification for Coronary Artery Disease: Is It Time for Subclassifications? *Current Cardiology Reports*. 2019;21(8):87.
103. Tsaknis G, Tsangaris I, Ikonomidis I, Tsantes A. Clinical Usefulness of Novel Serum, and Imaging Biomarkers in Risk Stratification of Patients with Stable Angina. *Dis Markers*. 2014; 2014:831364.
104. Lamounier Júnior A, Ferrari F, Max R, Ritt LEF, Stein R. Importance of Genetic Testing in Dilated Cardiomyopathy: Applications and Challenges in Clinical Practice. *Arquivos Brasileiros de Cardiologia*. 2019; 113:274-81.
105. Wensley F, Gao P, Burgess S, Kaptoge S, Di Angelantonio E, Shah T, et al. Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data. *Bmj*. 2011;342: d548.
106. Polfus LM, Gibbs RA, Boerwinkle E. Coronary Heart Disease and Genetic Variants with Low Phospholipase A2 Activity. *New England Journal of Medicine*. 2015;372(3):295-6.
107. Mebazaa A, Tolppanen H, Mueller C, Lassus J, DiSomma S, Baksys G, et al. Acute heart failure and cardiogenic shock: a multidisciplinary practical guidance. *Intensive care medicine*. 2016;42(2):147-63.
108. Klingenberg R, Aghlmandi S, Räber L, Gencer B, Nanchen D, Heg D, et al. Improved risk stratification of patients with acute coronary syndromes using a combination of hsTnT, NT-proBNP and hsCRP with the GRACE score. *European heart journal Acute cardiovascular care*. 2016; 7:2048872616684678.
109. Gelijns AC, Rosenberg N. Sources of medical technology: Universities and industry: National Academies Press; 1900.
110. Boyer J-F, Gourraud P-A, Cantagrel A, Davignon J-L, Constantin A. Traditional cardiovascular risk factors in rheumatoid arthritis: a meta-analysis. *Joint Bone Spine*. 2011;78(2):179-83.

111. López-Longo FJ, Oliver-Miñarro D, de la Torre I, González-Díaz de Rábago E, Sánchez-Ramón S, Rodríguez-Mahou M, et al. Association between anti-cyclic citrullinated peptide antibodies and ischemic heart disease in patients with rheumatoid arthritis. *Arthritis Care & Research: Official Journal of the American College of Rheumatology*. 2009;61(4):419-24.
112. Swerdlow DI, Holmes MV, Kuchenbaecker KB, Engmann JE, Shah T, Sofat R, et al. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. *Lancet*. 2012;379(9822):1214-24.
113. Ikonomidis I, Lekakis JP, Nikolaou M, Paraskevaidis I, Andreadou I, Kaplanoglou T, et al. Inhibition of interleukin-1 by anakinra improves vascular and left ventricular function in patients with rheumatoid arthritis. *Circulation*. 2008;117(20):2662-9.
114. Mohan A, Sada S, Kumar BS, Sarma KVS, Devi BV, Rao PVLNS, et al. Subclinical atherosclerosis in patients with rheumatoid arthritis by utilizing carotid intima-media thickness as a surrogate marker. *Indian J Med Res*. 2014;140(3):379-86.
115. Cheng IT, Wong KT, Li EK, Wong PCH, Lai BT, Yim IC, et al. Comparison of carotid artery ultrasound and Framingham risk score for discriminating coronary artery disease in patients with psoriatic arthritis. *RMD Open*. 2020;6(3): e001364.
116. Giles JT, Szklo M, Post W, Petri M, Blumenthal RS, Lam G, et al. Coronary arterial calcification in rheumatoid arthritis: comparison with the Multi-Ethnic Study of Atherosclerosis. *Arthritis research & therapy*. 2009;11(2): R36-R.
117. Karpouzas GA, Malpeso J, Choi TY, Li D, Munoz S, Budoff MJ. Prevalence, extent, and composition of coronary plaque in patients with rheumatoid arthritis without symptoms or prior diagnosis of coronary artery disease. *Ann Rheum Dis*. 2014;73(10):1797-804.
118. Hong J, Maron DJ, Shirai T, Weyand CM. Accelerated atherosclerosis in patients with chronic inflammatory rheumatologic conditions. *Int J Clin Rheumtol*. 2015;10(5):365-81.
119. Choi HK, Curhan G. Independent impact of gout on mortality and risk for coronary heart disease. *Circulation*. 2007;116(8):894-900.
120. Baker JV, Hullsiek KH, Singh A, Wilson E, Henry K, Lichtenstein K, et al. Immunologic predictors of coronary artery calcium progression in a contemporary HIV cohort. *Aids*. 2014;28(6):831-40.
121. Lusis AJ. Atherosclerosis. *Nature*. 2000;407(6801):233-41.
122. Galkina E, Ley K. Immune, and inflammatory mechanisms of atherosclerosis (*). *Annu Rev Immunol*. 2009; 27:165-97.

123. Ridker PM, Everett BM, Pradhan A, MacFadyen JG, Solomon DH, Zaharris E, et al. Low-Dose Methotrexate for the Prevention of Atherosclerotic Events. *New England Journal of Medicine*. 2018;380(8):752-62.
124. Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1 β inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). *Am Heart J*. 2011;162(4):597-605.
125. Jernberg T, Hasvold P, Henriksson M, Hjelm H, Thuresson M, Janzon M. Cardiovascular risk in post-myocardial infarction patients: nationwide real-world data demonstrate the importance of a long-term perspective. *Eur Heart J*. 2015;36(19):1163-70.
126. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation*. 2004;109(21 Suppl 1): Ii2-10.
127. Luscinskas FW, Gimbrone MA, Jr. Endothelial-dependent mechanisms in chronic inflammatory leukocyte recruitment. *Annu Rev Med*. 1996; 47:413-21.
128. Gimbrone MA, Jr., García-Cardena G. Vascular endothelium, hemodynamics, and the pathobiology of atherosclerosis. *Cardiovasc Pathol*. 2013;22(1):9-15.
129. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, 3rd, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*. 2003;107(3):499-511.
130. Eagle KA, Ginsburg GS, Musunuru K, Aird WC, Balaban RS, Bennett SK, et al. Identifying patients at high risk of a cardiovascular event soon: status and future directions: report of a national heart, lung, and blood institute working group. *Circulation*. 2010;121(12):1447-54.
131. Liao JK. Effects of statins on 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibition beyond low-density lipoprotein cholesterol. *The American journal of cardiology*. 2005;96(5A):24F-33F.
132. Wu MY, Li CJ, Hou MF, Chu PY. New Insights into the Role of Inflammation in the Pathogenesis of Atherosclerosis. *Int J Mol Sci*. 2017;18(10):2034.
133. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. 2002;105(9):1135-43.
134. Libby P. Mechanisms of acute coronary syndromes and their implications for therapy. *N Engl J Med*. 2013;368(21):2004-13.

135. Linton MRF, Yancey PG, Davies SS, Jerome WG, Linton EF, Song WL, et al. The Role of Lipids and Lipoproteins in Atherosclerosis. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, Dungan K, Grossman A, et al., editors. Endotext. South Dartmouth (MA): MDTText.com, Inc.; 2000.
136. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J*. 2017;38(32):2459-72.
137. Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. *Nat Rev Immunol*. 2013;13(10):709-21.
138. Gibson MS, Domingues N, Vieira OV. Lipid and Non-lipid Factors Affecting Macrophage Dysfunction and Inflammation in Atherosclerosis. *Front Physiol*. 2018; 9:654.
139. Libby P. Coronary artery injury and the biology of atherosclerosis: inflammation, thrombosis, and stabilization. *Am J Cardiol*. 2000;86(8B):3J-8J; discussion J-9J.
140. Granger DN, Senchenkova E, editors. Inflammation and the Microcirculation. Colloquium series on integrated systems physiology: from molecule to function; 2010: Morgan & Claypool Life Sciences.
141. Chen C, Mobley JL, Dwir O, Shimron F, Grabovsky V, Lobb RR, et al. High affinity very late antigen-4 subsets expressed on T cells are mandatory for spontaneous adhesion strengthening but not for rolling on VCAM-1 in shear flow. *J Immunol*. 1999;162(2):1084-95.
142. Kelly M, Hwang JM, Kubes P. Modulating leukocyte recruitment in inflammation. *J Allergy Clin Immunol*. 2007;120(1):3-10.
143. Lawrence MB, Springer TA. Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. *Cell*. 1991;65(5):859-73.
144. Baatar D, Siddiqi MZ, Im WT, Ul Khaliq N, Hwang SG. Anti-Inflammatory Effect of Ginsenoside Rh2-Mix on Lipopolysaccharide-Stimulated RAW 264.7 Murine Macrophage Cells. *J Med Food*. 2018;21(10):951-60.
145. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med*. 2005;352(16):1685-95.
146. Ross R. Atherosclerosis—an inflammatory disease. *New England journal of medicine*. 1999;340(2):115-26.
147. Wang X, Khalil RA. Matrix Metalloproteinases, Vascular Remodeling, and vascular disease. *Adv Pharmacol*. 2018; 81:241-330.

148. Packard RR, Libby P. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. *Clin Chem*. 2008;54(1):24-38.
149. Heinecke JW. Lipoprotein oxidation in cardiovascular disease: chief culprit or innocent bystander? *J Exp Med*. 2006;203(4):813-6.
150. Wu M-Y, Li C-J, Hou M-F, Chu P-Y. New Insights into the Role of Inflammation in the Pathogenesis of Atherosclerosis. *Int J Mol Sci*. 2017;18(10):2034.
151. Rafieian-Kopaei M, Setorki M, Douadi M, Baradaran A, Nasri H. Atherosclerosis: process, indicators, risk factors and new hopes. *Int J Prev Med*. 2014;5(8):927-46.
152. Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol*. 2006;6(7):508-19.
153. Langer HF, Chavakis T. Leukocyte-endothelial interactions in inflammation. *J Cell Mol Med*. 2009;13(7):1211-20.
154. Čejková S, Králová Lesná I, Poledne R. Monocyte adhesion to the endothelium is an initial stage of atherosclerosis development. *Cor et Vasa*. 2016;58(4): e419-e25.
155. Yousuf O, Mohanty BD, Martin SS, Joshi PH, Blaha MJ, Nasir K, et al. High-sensitivity C-reactive protein and cardiovascular disease: a resolute belief or an elusive link? *J Am Coll Cardiol*. 2013;62(5):397-408.
156. Yasui K, Baba A. Therapeutic potential of superoxide dismutase (SOD) for resolution of inflammation. *Inflamm Res*. 2006;55(9):359-63.
157. Lamas S, Marsden PA, Li GK, Tempst P, Michel T. Endothelial nitric oxide synthase: molecular cloning and characterization of a distinct constitutive enzyme isoform. *Proc Natl Acad Sci U S A*. 1992;89(14):6348-52.
158. Peng HB, Spiecker M, Liao JK. Inducible nitric oxide: an autoregulatory feedback inhibitor of vascular inflammation. *J Immunol*. 1998;161(4):1970-6.
159. van Hinsbergh VW. Endothelium--role in regulation of coagulation and inflammation. *Semin Immunopathol*. 2012;34(1):93-106.
160. Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene*. 1999;18(49):6853-66.
161. Das H, Kumar A, Lin Z, Patino WD, Hwang PM, Feinberg MW, et al. Kruppel-like factor 2 (KLF2) regulates proinflammatory activation of monocytes. *Proc Natl Acad Sci U S A*. 2006;103(17):6653-8.

162. Yamada Y, Doi T, Hamakubo T, Kodama T. Scavenger receptor family proteins: roles for atherosclerosis, host defence and disorders of the central nervous system. *Cell Mol Life Sci*. 1998;54(7):628-40.
163. Khurana R, Simons M, Martin JF, Zachary IC. Role of Angiogenesis in Cardiovascular Disease. *Circulation*. 2005;112(12):1813-24.
164. Scalia R. The microcirculation in adipose tissue inflammation. *Reviews in Endocrine and Metabolic Disorders*. 2013;14(1):69-76.
165. Davies MJ, Thomas A. Thrombosis, and acute coronary-artery lesions in sudden cardiac ischemic death. *N Engl J Med*. 1984;310(18):1137-40.
166. Shekhonin BV, Domogatsky SP, Idelson GL, Koteliansky VE, Rukosuev VS. Relative distribution of fibronectin and type I, III, IV, V collagens in normal and atherosclerotic intima of human arteries. *Atherosclerosis*. 1987;67(1):9-16.
167. Davies MJ. Stability and instability: two faces of coronary atherosclerosis. The Paul Dudley White Lecture 1995. *Circulation*. 1996;94(8):2013-20.
168. Warner SJ, Friedman GB, Libby P. Immune interferon inhibits proliferation and induces 2'-5'-oligoadenylate synthetase gene expression in human vascular smooth muscle cells. *J Clin Invest*. 1989;83(4):1174-82.
169. Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. *Clin Dermatol*. 2007;25(1):9-18.
170. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *The Journal of clinical investigation*. 1994;94(6):2493-503.
171. Newby AC. Matrix metalloproteinases regulate migration, proliferation, and death of vascular smooth muscle cells by degrading matrix and non-matrix substrates. *Cardiovasc Res*. 2006;69(3):614-24.
172. Kolodgie FD, Burke AP, Farb A, Weber DK, Kutys R, Wight TN, et al. Differential accumulation of proteoglycans and hyaluronan in culprit lesions: insights into plaque erosion. *Arterioscler Thromb Vasc Biol*. 2002;22(10):1642-8.
173. Libby P, Tabas I, Fredman G, Fisher EA. Inflammation, and its resolution as determinants of acute coronary syndromes. *Circ Res*. 2014;114(12):1867-79.
174. Falk E. Unstable angina with fatal outcome: dynamic coronary thrombosis leading to infarction and/or sudden death. Autopsy evidence of recurrent mural thrombosis with peripheral embolization culminating in total vascular occlusion. *Circulation*. 1985;71(4):699-708.

175. Edgington TS, Mackman N, Brand K, Ruf W. The structural biology of expression and function of tissue factor. *Thromb Haemost.* 1991;66(1):67-79.
176. Quillard T, Franck G, Mawson T, Folco E, Libby P. Mechanisms of erosion of atherosclerotic plaques. *Current opinion in lipidology.* 2017;28(5):434-41.
177. Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol.* 2011;11(8):519-31.
178. Andrae J, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. *Genes Dev.* 2008;22(10):1276-312.
179. Kolodgie FD, Burke AP, Farb A, Gold HK, Yuan J, Narula J, et al. The thin cap fibroatheroma: a type of vulnerable plaque: the major precursor lesion to acute coronary syndromes. *Curr Opin Cardiol.* 2001;16(5):285-92.
180. Nadkarni SK, Pierce MC, Park BH, de Boer JF, Whittaker P, Bouma BE, et al. Measurement of collagen and smooth muscle cell content in atherosclerotic plaques using polarization-sensitive optical coherence tomography. *J Am Coll Cardiol.* 2007;49(13):1474-81.
181. Motoyama S, Sarai M, Harigaya H, Anno H, Inoue K, Hara T, et al. Computed tomographic angiography characteristics of atherosclerotic plaques subsequently resulting in acute coronary syndrome. *J Am Coll Cardiol.* 2009;54(1):49-57.
182. Amin J, Boche D, Rakic S. What do we know about the inflammasome in humans? *Brain Pathology.* 2017;27(2):192-204.
183. Franchi L, Eigenbrod T, Muñoz-Planillo R, Nuñez G. The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. *Nat Immunol.* 2009;10(3):241-7.
184. Schroder K, Tschopp J. The inflammasomes. *Cell.* 2010;140(6):821-32.
185. Silvis MJM, Demkes EJ, Fiolet ATL, Dekker M, Bosch L, van Hout GPJ, et al. Immunomodulation of the NLRP3 Inflammasome in Atherosclerosis, Coronary Artery Disease, and Acute Myocardial Infarction. *Journal of Cardiovascular Translational Research.* 2020.
186. Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. *Annu Rev Immunol.* 2009; 27:229-65.
187. Bando S, Fukuda D, Soeki T, Nishimoto S, Uematsu E, Matsuura T, et al. Expression of NLRP3 in subcutaneous adipose tissue is associated with coronary atherosclerosis. *Atherosclerosis.* 2015;242(2):407-14.
188. van Hout GPJ, Bosch L. The Inflammasomes in Cardiovascular Disease. *Experientia supplementum* (2012). 2018; 108:9-40.

189. Zhou W, Chen C, Chen Z, Liu L, Jiang J, Wu Z, et al. NLRP3: A Novel Mediator in Cardiovascular Disease. *Journal of Immunology Research*. 2018; 2018:5702103.
190. Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature*. 2010;464(7293):1357-61.
191. Grebe A, Hoss F, Latz E. NLRP3 Inflammasome and the IL-1 Pathway in Atherosclerosis. *Circ Res*. 2018;122(12):1722-40.
192. Bauernfeind FG, Horvath G, Stutz A, Alnemri ES, MacDonald K, Speert D, et al. Cutting edge: NF- κ B activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *The Journal of Immunology*. 2009;183(2):787-91.
193. An N, Gao Y, Si Z, Zhang H, Wang L, Tian C, et al. Regulatory Mechanisms of the NLRP3 Inflammasome, a Novel Immune-Inflammatory Marker in Cardiovascular Diseases. *Frontiers in immunology*. 2019; 10:1592-.
194. Bergsbaken T, Fink SL, Cookson BT. Pyroptosis: host cell death and inflammation. *Nature Reviews Microbiology*. 2009;7(2):99-109.
195. Yang Y, Wang H, Kouadir M, Song H, Shi F. Recent advances in the mechanisms of NLRP3 inflammasome activation and its inhibitors. *Cell death & disease*. 2019;10(2):128-.
196. Afrasyab A, Qu P, Zhao Y, Peng K, Wang H, Lou D, et al. Correlation of NLRP3 with severity and prognosis of coronary atherosclerosis in acute coronary syndrome patients. *Heart and vessels*. 2016;31(8):1218-29.
197. Tabas I. Consequences and therapeutic implications of macrophage apoptosis in atherosclerosis: the importance of lesion stage and phagocytic efficiency. *Arterioscler Thromb Vasc Biol*. 2005;25(11):2255-64.
198. Mallat Z, Corbaz A, Scoazec A, Besnard S, Lesèche G, Chvatchko Y, et al. Expression of Interleukin-18 in Human Atherosclerotic Plaques and Relation to Plaque Instability. *Circulation*. 2001;104(14):1598-603.
199. Liuzzo G, Kopecky SL, Frye RL, Fallon WMO, Maseri A, Goronzy JJ, et al. Perturbation of the T-cell repertoire in patients with unstable angina. *Circulation*. 1999;100(21):2135-9.
200. Altaf A, Qu P, Zhao Y, Peng K, Wang H, Lou D, et al. Correlation of NLRP3 with severity and prognosis of coronary atherosclerosis in acute coronary syndrome patients. *Heart and vessels*. 2015;31.
201. Verma S, Leiter LA, Bhatt DL. CANTOS Ushers in a New Calculus of Inflammasome Targeting for Vascular Protection-and Maybe More. *Cell Metab*. 2017;26(5):703-5.

202. Robertson S, Martinez GJ, Payet CA, Barraclough JY, Celermajer DS, Bursill C, et al. Colchicine therapy in acute coronary syndrome patients acts on caspase-1 to suppress NLRP3 inflammasome monocyte activation. *Clin Sci (Lond)*. 2016;130(14):1237-46.
203. Martinez GJ, Robertson S, Barraclough J, Xia Q, Mallat Z, Bursill C, et al. Colchicine Acutely Suppresses Local Cardiac Production of Inflammatory Cytokines in Patients with an Acute Coronary Syndrome. *J Am Heart Assoc*. 2015;4(8): e002128.
204. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third universal definition of myocardial infarction. *Circulation*. 2012;126(16):2020-35.
205. Cirillo P, Taglialatela V, Pellegrino G, Morello A, Conte S, Di Serafino L, et al. Effects of colchicine on platelet aggregation in patients on dual antiplatelet therapy with aspirin and clopidogrel. *Journal of thrombosis and thrombolysis*. 2020;50(2):468-72.
206. Scott SA, Sangkuhl K, Stein CM, Hulot JS, Mega JL, Roden DM, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. *Clin Pharmacol Ther*. 2013;94(3):317-23.
207. Trepels T, Zeiher AM, Fichtlscherer S. [Acute coronary syndrome and inflammation. Biomarkers for diagnostics and risk stratification]. *Herz*. 2004;29(8):769-76.
208. Burns RJ, Gibbons RJ, Yi Q, Roberts RS, Miller TD, Schaer GL, et al. The relationships of left ventricular ejection fraction, end-systolic volume index and infarct size to six-month mortality after hospital discharge following myocardial infarction treated by thrombolysis. *J Am Coll Cardiol*. 2002;39(1):30-6.
209. Wu E, Ortiz JT, Tejedor P, Lee DC, Bucciarelli-Ducci C, Kansal P, et al. Infarct size by contrast enhanced cardiac magnetic resonance is a stronger predictor of outcomes than left ventricular ejection fraction or end-systolic volume index: prospective cohort study. *Heart*. 2008;94(6):730-6.
210. Kumar A, Cannon CP. Acute coronary syndromes: diagnosis and management, part I. *Mayo Clin Proc*. 2009;84(10):917-38.
211. Tardif J-C, Kouz S, Waters DD, Bertrand OF, Diaz R, Maggioni AP, et al. Efficacy and Safety of Low-Dose Colchicine after Myocardial Infarction. *New England Journal of Medicine*. 2019;381(26):2497-505.
212. Spratt JC, Camenzind E. Plaque stabilisation by systemic and local drug administration. *Heart*. 2004;90(12):1392-4.
213. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008;359(21):2195-207.

214. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, et al. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N Engl J Med*. 2004;350(15):1495-504.
215. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, et al. C-reactive protein, and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med*. 2004;350(14):1387-97.
216. Savoia C, Schiffrin EL. Reduction of C-reactive protein, and the use of anti-hypertensives. *Vasc Health Risk Manag*. 2007;3(6):975-83.
217. Topol EJ. Intensive statin therapy--a sea change in cardiovascular prevention. *N Engl J Med*. 2004;350(15):1562-4.
218. Datta S, Iqbal Z, Prasad KR. Comparison Between Serum hsCRP and LDL Cholesterol for Search of a Better Predictor for Ischemic Heart Disease. *Indian J Clin Biochem*. 2011;26(2):210-3.
219. Kamath DY, Xavier D, Sigamani A, Pais P. High sensitivity C-reactive protein (hsCRP) & cardiovascular disease: An Indian perspective. *Indian J Med Res*. 2015;142(3):261-8.
220. Nishimoto N, Terao K, Mima T, Nakahara H, Takagi N, Kakehi T. Mechanisms, and pathologic significances in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti-IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease. *Blood*. 2008;112(10):3959-64.
221. Loppnow H, Zhang L, Buerke M, Lautenschlager M, Chen L, Frister A, et al. Statins potently reduce the cytokine-mediated IL-6 release in SMC/MNC cocultures. *J Cell Mol Med*. 2011;15(4):994-1004.
222. Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, et al. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. *N Engl J Med*. 1996;335(14):1001-9.
223. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, et al. Intensive versus Moderate Lipid Lowering with Statins after Acute Coronary Syndromes. *New England Journal of Medicine*. 2004;350(15):1495-504.
224. Blake GJ, Ridker PM. Are statins anti-inflammatory? *Curr Control Trials Cardiovasc Med*. 2000;1(3):161-5.
225. Ridker PM. A Test in Context: High-Sensitivity C-Reactive Protein. *J Am Coll Cardiol*. 2016;67(6):712-23.

226. Nidorf M, Thompson PL. Effect of colchicine (0.5 mg twice daily) on high-sensitivity C-reactive protein independent of aspirin and atorvastatin in patients with stable coronary artery disease. *Am J Cardiol.* 2007;99(6):805-7.
227. Otsuka F, Yasuda S, Noguchi T, Ishibashi-Ueda H. Pathology of coronary atherosclerosis and thrombosis. *Cardiovasc Diagn Ther.* 2016;6(4):396-408.
228. Ferrante G, Nakano M, Prati F, Niccoli G, Mallus MT, Ramazzotti V, et al. High levels of systemic myeloperoxidase are associated with coronary plaque erosion in patients with acute coronary syndromes: a clinicopathological study. *Circulation.* 2010;122(24):2505-13.
229. Kubo T, Matsuo Y, Hayashi Y, Yamano T, Tanimoto T, Ino Y, et al. High-sensitivity C-reactive protein and plaque composition in patients with stable angina pectoris: a virtual histology intravascular ultrasound study. *Coron Artery Dis.* 2009;20(8):531-5.
230. Koyama K, Yoneyama K, Mitarai T, Ishibashi Y, Takahashi E, Kongoji K, et al. Association between inflammatory biomarkers and thin cap fibroatheroma detected by optical coherence tomography in patients with coronary heart disease. *Arch Med Sci.* 2015;11(3):505-12.
231. Brown TM, Bittner V. Biomarkers of atherosclerosis: clinical applications. *Current cardiology reports.* 2008;10(6):497-504.
232. Tsaknis G, Tsangaris I, Ikonomidis I, Tsantes A. Clinical usefulness of novel serum and imaging biomarkers in risk stratification of patients with stable angina. *Dis Markers.* 2014; 2014:831364-.
233. Ky B, French B, McCloskey K, Rame JE, McIntosh E, Shahi P, et al. High-sensitivity ST2 for prediction of adverse outcomes in chronic heart failure. *Circ Heart Fail.* 2011;4(2):180-7.
234. McMurray JJ, Adamopoulos S, Anker SD, Auricchio A, Böhm M, Dickstein K, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. *Eur Heart J.* 2012;33(14):1787-847.
235. Osman R, L'Allier PL, Elgharib N, Tardif J-C. Critical appraisal of C-reactive protein throughout the spectrum of cardiovascular disease. *Vasc Health Risk Manag.* 2006;2(3):221-37.
236. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third universal definition of myocardial infarction. *Eur Heart J.* 2012;33(20):2551-67.
237. Gulhar R, Ashraf MA, Jialal I. Physiology, Acute Phase Reactants. *StatPearls. Treasure Island (FL): StatPearls Publishing Copyright © 2020, StatPearls Publishing LLC.; 2020.*

238. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, et al. C-reactive protein, and other circulating markers of inflammation in the prediction of coronary heart disease. *New England Journal of Medicine*. 2004;350(14):1387-97.
239. Wattanakit K, Folsom AR, Chambless LE, Nieto FJ. Risk factors for cardiovascular event recurrence in the Atherosclerosis Risk in Communities (ARIC) study. *American Heart Journal*. 2005;149(4):606-12.
240. Wilson PWF, Nam B-H, Pencina M, D'Agostino RB, Sr, Benjamin EJ, O'Donnell CJ. C-Reactive Protein and Risk of Cardiovascular Disease in Men and Women from the Framingham Heart Study. *Archives of Internal Medicine*. 2005;165(21):2473-8.
241. Albert MA, Glynn RJ, Buring J, Ridker PM. C-reactive protein levels among women of various ethnic groups living in the United States (from the Women's Health Study). *Am J Cardiol*. 2004;93(10):1238-42.
242. Shah T, Casas JP, Cooper JA, Tzoulaki I, Sofat R, McCormack V, et al. Critical appraisal of CRP measurement for the prediction of coronary heart disease events: new data and systematic review of 31 prospective cohorts. *Int J Epidemiol*. 2009;38(1):217-31.
243. Rietzschel E, Buyzere MD. High-sensitive C-reactive protein: universal prognostic and causative biomarker in heart disease? *Biomarkers in Medicine*. 2012;6(1):19-34.
244. Sproston NR, Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. *Frontiers in immunology*. 2018; 9:754-.
245. Perlis RH. Translating biomarkers to clinical practice. *Mol Psychiatry*. 2011;16(11):1076-87.
246. Wang J, Tan G-J, Han L-N, Bai Y-Y, He M, Liu H-B. Novel biomarkers for cardiovascular risk prediction. *Journal of geriatric cardiology: JGC*. 2017;14(2):135-50.
247. Musunuru K, Kral BG, Blumenthal RS, Fuster V, Campbell CY, Gluckman TJ, et al. The use of high-sensitivity assays for C-reactive protein in clinical practice. *Nat Clin Pract Cardiovasc Med*. 2008;5(10):621-35.
248. Osman R, L'Allier PL, Elgharib N, Tardif JC. Critical appraisal of C-reactive protein throughout the spectrum of cardiovascular disease. *Vasc Health Risk Manag*. 2006;2(3):221-37.
249. Luo Y, Zhang B, Chen M, Jiang T, Zhou D, Huang J, et al. Sensitive and rapid quantification of C-reactive protein using quantum dot-labeled microplate immunoassay. *J Transl Med*. 2012; 10:24.
250. Lv Y, Wu R, Feng K, Li J, Mao Q, Yuan H, et al. Highly sensitive and accurate detection of C-reactive protein by CdSe/ZnS quantum dot-based fluorescence-linked immunosorbent assay. *J Nanobiotechnology*. 2017;15(1):35.

251. Pfutzner A, Forst T. High-sensitivity C-reactive protein as cardiovascular risk marker in patients with diabetes mellitus. *Diabetes Technol Ther*. 2006;8(1):28-36.
252. Oh J, Teoh H, Leiter LA. Should C-reactive protein be a target of therapy? *Diabetes Care*. 2011;34 Suppl 2(Suppl 2): S155-S60.
253. Elliott P, Chambers JC, Zhang W, Clarke R, Hopewell JC, Peden JF, et al. Genetic Loci associated with C-reactive protein levels and risk of coronary heart disease. *JAMA*. 2009;302(1):37-48.
254. Yeh ET. High-sensitivity C-reactive protein as a risk assessment tool for cardiovascular disease. *Clin Cardiol*. 2005;28(9):408-12.
255. Aday AW, Ridker PM. Targeting Residual Inflammatory Risk: A Shifting Paradigm for Atherosclerotic Disease. *Front Cardiovasc Med*. 2019;6(16):16.
256. Bell SP, Giuseffi JL, Forman DE. Cardiovascular biomarkers and their utility in the older adult. *Curr Cardiovasc Risk Rep*. 2012;6(5):397-403.
257. Johnson BD, Kip KE, Marroquin OC, Ridker PM, Kelsey SF, Shaw LJ, et al. Serum amyloid A as a predictor of coronary artery disease and cardiovascular outcome in women: the National Heart, Lung, and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE). *Circulation*. 2004;109(6):726-32.
258. Cushman M, Arnold AM, Psaty BM, Manolio TA, Kuller LH, Burke GL, et al. C-reactive protein and the 10-year incidence of coronary heart disease in older men and women: the cardiovascular health study. *Circulation*. 2005;112(1):25-31.
259. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med*. 2002;347(20):1557-65.
260. Mani P, Puri R, Schwartz GG, Nissen SE, Shao M, Kastelein JJP, et al. Association of Initial and Serial C-Reactive Protein Levels with Adverse Cardiovascular Events and Death After Acute Coronary Syndrome: A Secondary Analysis of the VISTA-16 Trial. *JAMA Cardiol*. 2019;4(4):314-20.
261. Qintar M, Sharma PP, Pokharel Y, Tang Y, Lu Y, Jones P, et al. Prevalence and predictors of elevated high-sensitivity C-reactive protein in post-myocardial infarction patients: Insights from the VIRGO and TRIUMPH registries. *Clin Cardiol*. 2017;40(12):1205-11.
262. Wilson PW, Pencina M, Jacques P, Selhub J, D'Agostino R, Sr., O'Donnell CJ. C-reactive protein and reclassification of cardiovascular risk in the Framingham Heart Study. *Circ Cardiovasc Qual Outcomes*. 2008;1(2):92-7.

263. Rifai N. High-sensitivity C-reactive protein: a useful marker for cardiovascular disease risk prediction and the metabolic syndrome. *Clin Chem*. 2005;51(3):504-5.
264. Montecucco F, Mach F. New evidence for C-reactive protein (CRP) deposits in the arterial intima as a cardiovascular risk factor. *Clin Interv Aging*. 2008;3(2):341-9.
265. Sproston NR, Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. *Front Immunol*. 2018; 9:754.
266. Zhang Z, Yang Y, Hill MA, Wu J. Does C-reactive protein contribute to atherothrombosis via oxidant-mediated release of pro-thrombotic factors and activation of platelets? *Front Physiol*. 2012; 3:433.
267. Devaraj S, Singh U, Jialal I. Human C-reactive protein, and the metabolic syndrome. *Curr Opin Lipidol*. 2009;20(3):182-9.
268. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest*. 2003;111(12):1805-12.
269. Elliott P, Chambers JC, Zhang W, Clarke R, Hopewell JC, Peden JF, et al. Genetic Loci associated with C-reactive protein levels and risk of coronary heart disease. *JAMA*. 2009;302(1):37-48.
270. Cook NR, Buring JE, Ridker PM. The effect of including C-reactive protein in cardiovascular risk prediction models for women. *Ann Intern Med*. 2006;145(1):21-9.
271. Cook NR, Paynter NP, Eaton CB, Manson JE, Martin LW, Robinson JG, et al. Comparison of the Framingham and Reynolds Risk scores for global cardiovascular risk prediction in the multiethnic Women's Health Initiative. *Circulation*. 2012;125(14):1748-S11.
272. Singh SK, Suresh MV, Voleti B, Agrawal A. The connection between C-reactive protein and atherosclerosis. *Ann Med*. 2008;40(2):110-20.
273. Montecucco F, Mach F. New evidence for C-reactive protein (CRP) deposits in the arterial intima as a cardiovascular risk factor. *Clin Interv Aging*. 2008;3(2):341-9.
274. Dhingra R, Gona P, Nam B-H, D'Agostino RB, Sr., Wilson PWF, Benjamin EJ, et al. C-reactive protein, inflammatory conditions, and cardiovascular disease risk. *The American journal of medicine*. 2007;120(12):1054-62.
275. Wang J, Tan GJ, Han LN, Bai YY, He M, Liu HB. Novel biomarkers for cardiovascular risk prediction. *J Geriatr Cardiol*. 2017;14(2):135-50.
276. Musunuru K, Kral BG, Blumenthal RS, Fuster V, Campbell CY, Gluckman TJ, et al. The use of high-sensitivity assays for C-reactive protein in clinical practice. *Nat Clin Pract Cardiovasc Med*. 2008;5(10):621-35.

277. Lawlor DA, Harbord RM, Timpson NJ, Lowe GDO, Rumley A, Gaunt TR, et al. The association of C-reactive protein and CRP genotype with coronary heart disease: findings from five studies with 4,610 cases amongst 18,637 participants. *PloS one*. 2008;3(8): e3011-e.
278. Casas JP, Shah T, Cooper J, Hawe E, McMahon AD, Gaffney D, et al. Insight into the nature of the CRP–coronary event association using Mendelian randomization. *Int J Epidemiol*. 2006;35(4):922-31.
279. Zhuang Q, Shen C, Chen Y, Zhao X, Wei P, Sun J, et al. Association of highly sensitive C-reactive protein with coronary heart disease: a Mendelian randomization study. *BMC Med Genet*. 2019;20(1):170-.
280. Liao JK, Laufs U. Pleiotropic effects of statins. *Annual review of pharmacology and toxicology*. 2005; 45:89-118.
281. Lowe G, Pepys M. C-reactive protein, and cardiovascular disease: Weighing the evidence. *Current atherosclerosis reports*. 2006; 8:421-8.
282. Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. *Nat Rev Drug Discov*. 2005;4(12):977-87.
283. Blanco-Colio LM, Tuñón J, Martín-Ventura JL, Egido J. Anti-inflammatory, and immunomodulatory effects of statins. *Kidney International*. 2003;63(1):12-23.
284. Wolf D, Ley K. Immunity, and Inflammation in Atherosclerosis. *Circ Res*. 2019;124(2):315-27.
285. Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, et al. C-Reactive Protein Levels and Outcomes after Statin Therapy. *New England Journal of Medicine*. 2005;352(1):20-8.
286. Jain S, Gautam V, Naseem S. Acute-phase proteins: As diagnostic tool. *J Pharm Bioallied Sci*. 2011;3(1):118-27.
287. Virmani R, Burke AP, Farb A, Kolodgie FD. Pathology of the unstable plaque. *Prog Cardiovasc Dis*. 2002;44(5):349-56.
288. Oemrawsingh RM, Cheng JM, Akkerhuis KM, Kardys I, Degertekin M, van Geuns RJ, et al. High-sensitivity C-reactive protein predicts 10-year cardiovascular outcome after percutaneous coronary intervention. *EuroIntervention*. 2016;12(3):345-51.
289. Collins R, Reith C, Emberson J, Armitage J, Baigent C, Blackwell L, et al. Interpretation of the evidence for the efficacy and safety of statin therapy. *Lancet*. 2016;388(10059):2532-61.
290. Zakynthinos E, Pappa N. Inflammatory biomarkers in coronary artery disease. *J Cardiol*. 2009;53(3):317-33.

291. Rincon M. Interleukin-6: From an inflammatory marker to a target for inflammatory diseases. *Trends in immunology*. 2012; 33:571-7.
292. Liang B, Gardner DB, Griswold DE, Bugelski PJ, Song XYR. Anti-interleukin-6 monoclonal antibody inhibits autoimmune responses in a murine model of systemic lupus erythematosus. *Immunology*. 2006;119(3):296-305.
293. Welsh PI. Inflammatory markers as novel predictors of cardiovascular disease: University of Glasgow; 2008.
294. Narazaki M, Kishimoto T. The Two-Faced Cytokine IL-6 in Host Defense and Diseases. *Int J Mol Sci*. 2018;19(11):3528.
295. Blake GJ, Ridker PM. Inflammatory biomarkers and cardiovascular risk prediction. *Journal of Internal Medicine*. 2002;252(4):283-94.
296. Loria V, Dato I, Graziani F, Biasucci LM. Myeloperoxidase: a new biomarker of inflammation in ischemic heart disease and acute coronary syndromes. *Mediators Inflamm*. 2008; 2008:135625.
297. Ong S-B, Hernández-Reséndiz S, Crespo-Avilan GE, Mukhametshina RT, Kwek X-Y, Cabrera-Fuentes HA, et al. Inflammation following acute myocardial infarction: Multiple players, dynamic roles, and novel therapeutic opportunities. *Pharmacology & therapeutics*. 2018; 186:73-87.
298. Kolodziej AR, Abo-Aly M, Elsawalhy E, Campbell C, Ziada KM, Abdel-Latif A. Prognostic Role of Elevated Myeloperoxidase in Patients with Acute Coronary Syndrome: A Systemic Review and Meta-Analysis. *Mediators of inflammation*. 2019.
299. Zhou T, Zhou SH, Qi SS, Shen XQ, Zeng GF, Zhou HN. The effect of atorvastatin on serum myeloperoxidase and CRP levels in patients with acute coronary syndrome. *Clin Chim Acta*. 2006;368(1-2):168-72.
300. Cavusoglu E, Ruwende C, Eng C, Chopra V, Yanamadala S, Clark LT, et al. Usefulness of baseline plasma myeloperoxidase levels as an independent predictor of myocardial infarction at two years in patients presenting with acute coronary syndrome. *Am J Cardiol*. 2007;99(10):1364-8.
301. Chen Y, Zhang F, Dong L, Shu X. Long-term Prognostic Value of Myeloperoxidase on Acute Coronary Syndrome: A Meta-analysis. *Archives of Medical Research*. 2011;42(5):368-74.
302. Baldus S, Heeschen C, Meinertz T, Zeiher AM, Eiserich JP, Münzel T, et al. Myeloperoxidase Serum Levels Predict Risk in Patients with Acute Coronary Syndromes. *Circulation*. 2003;108(12):1440-5.
303. Bujak M, Frangogiannis NG. The role of IL-1 in the pathogenesis of heart disease. *Arch Immunol Ther Exp (Warsz)*. 2009;57(3):165-76.

304. Szekeley Y, Arbel Y. A Review of Interleukin-1 in heart disease: Where Do We Stand Today? *Cardiol Ther.* 2018;7(1):25-44.
305. Quan L-D, Thiele GM, Tian J, Wang D. The Development of Novel Therapies for Rheumatoid Arthritis. *Expert Opin Ther Pat.* 2008;18(7):723-38.
306. Abbate A, Trankle CR, Buckley LF, Lipinski MJ, Appleton D, Kadariya D, et al. Interleukin-1 Blockade Inhibits the Acute Inflammatory Response in Patients With ST-Segment Elevation Myocardial Infarction. *Journal of the American Heart Association.* 2020;9(5): e014941.
307. Martinon F, Burns K, Tschopp J. The Inflammasome: A Molecular Platform Triggering Activation of Inflammatory Caspases and Processing of proIL- β . *Molecular Cell.* 2002;10(2):417-26.
308. Latz E, Xiao TS, Stutz A. Activation, and regulation of the inflammasomes. *Nat Rev Immunol.* 2013;13(6):397-411.
309. Rocha B, Aguiar C. Síndrome Coronária Aguda em Mulheres Idosas: A Inflamação Ataca Novamente. *Arquivos Brasileiros de Cardiologia.* 2020; 114:515-7.
310. Cooper A, O'Flynn N. Risk assessment and lipid modification for primary and secondary prevention of cardiovascular disease: summary of NICE guidance. *Bmj.* 2008;336(7655):1246-8.
311. Diamantis E, Kyriakos G, Quiles-Sanchez LV, Farmaki P, Troupis T. The Anti-Inflammatory Effects of Statins on Coronary Artery Disease: An Updated Review of the Literature. *Curr Cardiol Rev.* 2017;13(3):209-16.
312. Ridker PM. A Test in Context: High-Sensitivity C-Reactive Protein. *Journal of the American College of Cardiology.* 2016;67(6):712-23.
313. Lorenzatti A, Servato ML. Role of Anti-inflammatory Interventions in Coronary Artery Disease: Understanding the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). *Eur Cardiol.* 2018;13(1):38-41.
314. Ridker PM, Everett BM, Pradhan A, MacFadyen JG, Solomon DH, Zaharris E, et al. Low-Dose Methotrexate for the Prevention of Atherosclerotic Events. *N Engl J Med.* 2019;380(8):752-62.
315. Thompson PL, Nidorf SM. Anti-inflammatory therapy with canakinumab for atherosclerotic disease: lessons from the CANTOS trial. *J Thorac Dis.* 2018;10(2):695-8.
316. Rymer JA, Newby LK. Failure to Launch: Targeting Inflammation in Acute Coronary Syndromes. *JACC Basic Transl Sci.* 2017;2(4):484-97.
317. Raju NC, Yi Q, Nidorf M, Fagel ND, Hiralal R, Eikelboom JW. Effect of colchicine compared with placebo on high sensitivity C-reactive protein in patients with acute coronary syndrome or acute stroke: a pilot randomized controlled trial. *J Thromb Thrombolysis.* 2012;33(1):88-94.

318. Deftereos S, Giannopoulos G, Angelidis C, Alexopoulos N, Filippatos G, Papoutsidakis N, et al. Anti-Inflammatory Treatment with Colchicine in Acute Myocardial Infarction: A Pilot Study. *Circulation*. 2015;132(15):1395-403.
319. Akodad M, Sicard P, Fauconnier J, Roubille F. Colchicine, and myocardial infarction: A review. *Archives of Cardiovascular Diseases*. 2020.
320. Darapladib for Preventing Ischemic Events in Stable Coronary Heart Disease. *New England Journal of Medicine*. 2014;370(18):1702-11.
321. White HD, Held C, Stewart R, Tarka E, Brown R, Davies RY, et al. Darapladib for preventing ischemic events in stable coronary heart disease. *N Engl J Med*. 2014;370(18):1702-11.
322. Nicholls SJ, Kastelein JJ, Schwartz GG, Bash D, Rosenson RS, Cavender MA, et al. Varespladib and cardiovascular events in patients with an acute coronary syndrome: the VISTA-16 randomized clinical trial. *Jama*. 2014;311(3):252-62.
323. O'Donoghue ML, Glaser R, Cavender MA, Aylward PE, Bonaca MP, Budaj A, et al. Effect of Losmapimod on Cardiovascular Outcomes in Patients Hospitalized with Acute Myocardial Infarction: A Randomized Clinical Trial. *Jama*. 2016;315(15):1591-9.
324. Aday AW, Ridker PM. Antiinflammatory Therapy in Clinical Care: The CANTOS Trial and Beyond. *Front Cardiovasc Med*. 2018; 5:62.
325. Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat Med*. 2003;9(1):61-7.
326. Paul-Clark MJ, Van Cao T, Moradi-Bidhendi N, Cooper D, Gilroy DW. 15-epi-lipoxin A4-mediated induction of nitric oxide explains how aspirin inhibits acute inflammation. *J Exp Med*. 2004;200(1):69-78.
327. Kranzhöfer R, Schmidt J, Pfeiffer CA, Hagl S, Libby P, Kübler W. Angiotensin induces inflammatory activation of human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*. 1999;19(7):1623-9.
328. Platten M, Youssef S, Hur EM, Ho PP, Han MH, Lanz TV, et al. Blocking angiotensin-converting enzyme induces potent regulatory T cells and modulates TH1- and TH17-mediated autoimmunity. *Proc Natl Acad Sci U S A*. 2009;106(35):14948-53.
329. Ponnuswamy P, Joffre J, Herbin O, Esposito B, Laurans L, Binder CJ, et al. Angiotensin II synergizes with BAFF to promote atheroprotective regulatory B cells. *Scientific Reports*. 2017;7(1):4111.

330. García-Prieto J, Villena-Gutiérrez R, Gómez M, Bernardo E, Pun-García A, García-Lunar I, et al. Neutrophil stunning by metoprolol reduces infarct size. *Nature Communications*. 2017;8(1):14780.
331. Pizarro G, Fernández-Friera L, Fuster V, Fernández-Jiménez R, García-Ruiz JM, García-Álvarez A, et al. Long-term benefit of early pre-reperfusion metoprolol administration in patients with acute myocardial infarction: results from the METOCARD-CNIC trial (Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction). *J Am Coll Cardiol*. 2014;63(22):2356-62.
332. Nussinovitch U, Livneh A. Familial Mediterranean Fever. In: Nussinovitch U, editor. *The Heart in Rheumatic, Autoimmune and Inflammatory Diseases*: Academic Press; 2017. p. 577-614.
333. Slobodnick A, Shah B, Krasnokutsky S, Pillinger MH. Update on colchicine, 2017. *Rheumatology (Oxford)*. 2018;57(suppl_1): i4-i11.
334. Yan BP, Tan GM. What's Old is New Again - A Review of the Current Evidence of Colchicine in Cardiovascular Medicine. *Curr Cardiol Rev*. 2017;13(2):130-8.
335. Shah SR, Alweis R, Shah SA, Arshad MH, Manji AA, Arfeen AA, et al. Effects of colchicine on pericardial diseases: a review of the literature and current evidence. *J Community Hosp Intern Med Perspect*. 2016;6(3):31957.
336. Campbell KB, Cicci TA, Vora AK, Burgess LD. Beyond Gout: Colchicine Use in the Cardiovascular Patient. *Hosp Pharm*. 2015;50(10):859-67.
337. Terkeltaub R. 189 - Management of gout and hyperuricemia. In: Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Weisman MH, editors. *Rheumatology (Sixth Edition)*. Philadelphia: Content Repository Only! 2015. p. 1575-82.
338. Ting JY. Acute pancreatitis related to therapeutic dosing with colchicine: a case report. *J Med Case Rep*. 2007; 1:64.
339. Imazio M, Brucato A, Trincheri R, Spodick D, Adler Y. Colchicine for pericarditis: hype or hope? *Eur Heart J*. 2009;30(5):532-9.
340. Neuvonen PJ. Drug interactions with HMG-CoA reductase inhibitors (statins): the importance of CYP enzymes, transporters, and pharmacogenetics. *Curr Opin Investig Drugs*. 2010;11(3):323-32.
341. Roddy E, Mallen CD. Colchicine in overdose. *Br J Gen Pract*. 2017;67(655):61.
342. Ben-Chetrit E. Colchicine. *Textbook of Autoinflammation*. 2018:729-49.
343. Ragab G, Elshahaly M, Bardin T. Gout: An old disease in new perspective - A review. *J Adv Res*. 2017;8(5):495-511.
344. Cronstein BN, Sunkureddi P. Mechanistic aspects of inflammation and clinical management of inflammation in acute gouty arthritis. *J Clin Rheumatol*. 2013;19(1):19-29.

345. Manna R, Rigante D. Familial Mediterranean Fever: Assessing the Overall Clinical Impact and Formulating Treatment Plans. *Mediterr J Hematol Infect Dis*. 2019;11(1): e2019027.
346. Ben-Chetrit E, Bergmann S, Sood R. Mechanism of the anti-inflammatory effect of colchicine in rheumatic diseases: a possible new outlook through microarray analysis. *Rheumatology (Oxford)*. 2006;45(3):274-82.
347. Cronstein BN, Molad Y, Reibman J, Balakhane E, Levin RI, Weissmann G. Colchicine alters the quantitative and qualitative display of selectins on endothelial cells and neutrophils. *J Clin Invest*. 1995;96(2):994-1002.
348. Pouliot M, James MJ, McColl SR, Naccache PH, Cleland LG. Monosodium urate microcrystals induce cyclooxygenase-2 in human monocytes. *Blood*. 1998;91(5):1769-76.
349. Libby P. Interleukin-1 Beta as a Target for Atherosclerosis Therapy: Biological Basis of CANTOS and Beyond. *Journal of the American College of Cardiology*. 2017;70(18):2278-89.
350. Guo Y, Lip GY, Apostolakis S. Inflammation in atrial fibrillation. *J Am Coll Cardiol*. 2012;60(22):2263-70.
351. Deftereos S, Giannopoulos G, Kossyvakis C, Efremidis M, Panagopoulou V, Kaoukis A, et al. Colchicine for Prevention of Early Atrial Fibrillation Recurrence After Pulmonary Vein Isolation: A Randomized Controlled Study. *Journal of the American College of Cardiology*. 2012;60(18):1790-6.
352. Imazio M, Trinchero R, Brucato A, Rovere ME, Gandino A, Cemin R, et al. COLchicine for the Prevention of the Post-pericardiotomy Syndrome (COPPS): a multicentre, randomized, double-blind, placebo-controlled trial. *Eur Heart J*. 2010;31(22):2749-54.
353. Imazio M, Brucato A, Ferrazzi P, Pullara A, Adler Y, Barosi A, et al. Colchicine for Prevention of Postpericardiotomy Syndrome and Postoperative Atrial Fibrillation: The COPPS-2 Randomized Clinical Trial. *JAMA*. 2014;312(10):1016-23.
354. Ambrose JA, Weinrauch M. Thrombosis in ischemic heart disease. *Arch Intern Med*. 1996;156(13):1382-94.
355. McFadden EP, Stabile E, Regar E, Cheneau E, Ong AT, Kinnaird T, et al. Late thrombosis in drug-eluting coronary stents after discontinuation of antiplatelet therapy. *Lancet*. 2004;364(9444):1519-21.
356. Orme RC, Parker WAE, Thomas MR, Judge HM, Baster K, Sumaya W, et al. Study of Two Dose Regimens of Ticagrelor Compared with Clopidogrel in Patients Undergoing Percutaneous Coronary Intervention for Stable Coronary Artery Disease (STEEL-PCI). *Circulation*. 2018;138(13):1290-300.

357. Bonello L, Tantry US, Marcucci R, Blindt R, Angiolillo DJ, Becker R, et al. Consensus and future directions on the definition of high on-treatment platelet reactivity to adenosine diphosphate. *J Am Coll Cardiol*. 2010;56(12):919-33.
358. Collet J-P. Current Concepts in the Clinical Utility of Platelet Reactivity Testing. *Interv Cardiol*. 2013;8(2):100-6.
359. Berg J, Lindgren P, Spiesser J, Parry D, Jönsson B. Cost-effectiveness of clopidogrel in myocardial infarction with ST-segment elevation: a European model based on the CLARITY and COMMIT trials. *Clin Ther*. 2007;29(6):1184-202.
360. Cimmino G, Tarallo R, Conte S, Morello A, Pellegrino G, Loffredo FS, et al. Colchicine reduces platelet aggregation by modulating cytoskeleton rearrangement via inhibition of cofilin and LIM domain kinase 1. *Vascular Pharmacology*. 2018; 111:62-70.
361. Patel A, Vidula M, Kishore SP, Vedanthan R, Huffman MD. Building the Case for Clopidogrel as a World Health Organization Essential Medicine. *Circ Cardiovasc Qual Outcomes*. 2015;8(4):447-51.
362. Magnani G, Valgimigli M. Dual Antiplatelet Therapy After Drug-eluting Stent Implantation. *Interv Cardiol*. 2016;11(1):51-3.
363. Valgimigli M, Bueno H, Byrne RA, Collet JP, Costa F, Jeppsson A, et al. 2017 ESC focused update on dual antiplatelet therapy in coronary artery disease developed in collaboration with EACTS: The Task Force for dual antiplatelet therapy in coronary artery disease of the European Society of Cardiology (ESC) and of the European Association for Cardio-Thoracic Surgery (EACTS). *Eur Heart J*. 2018;39(3):213-60.
364. Caramori PR, Lima VC, Seidelin PH, Newton GE, Parker JD, Adelman AG. Long-term endothelial dysfunction after coronary artery stenting. *J Am Coll Cardiol*. 1999;34(6):1675-9.
365. Mitra AK, Agrawal DK. In stent restenosis: bane of the stent era. *J Clin Pathol*. 2006;59(3):232-9.
366. Farb A, Sangiorgi G, Carter AJ, Walley VM, Edwards WD, Schwartz RS, et al. Pathology of acute and chronic coronary stenting in humans. *Circulation*. 1999;99(1):44-52.
367. Deftereos S, Giannopoulos G, Raisakis K, C K, Kaoukis A, Panagopoulou V, et al. Colchicine treatment for prevention of in-bare-metal-stent restenosis in diabetics. *Journal of the American College of Cardiology*. 2013.
368. Libby P, Schwartz D, Brogi E, Tanaka H, Clinton SK. A cascade model for restenosis. A special case of atherosclerosis progression. *Circulation*. 1992;86(6 Suppl): Iii47-52.

369. Bucerri D, Piraino D, Andolina G, Cortese B. Understanding and managing in-stent restenosis: a review of clinical data, from pathogenesis to treatment. *Journal of thoracic disease*. 2016;8(10): E1150-E62.
370. Borhani S, Hassanajili S, Ahmadi Tafti SH, Rabbani S. Cardiovascular stents: overview, evolution, and next generation. *Prog Biomater*. 2018;7(3):175-205.
371. O'Keefe JH, McCallister BD, Bateman TM, Kuhnlein DL, Ligon RW, Hartzler GO. Ineffectiveness of colchicine for the prevention of restenosis after coronary angioplasty. *Journal of the American College of Cardiology*. 1992;19(7):1597-600.
372. Freed M, Safian RD, O'Neill WW, Safian M, Jones D, Grines CL. Combination of lovastatin, enalapril, and colchicine does not prevent restenosis after percutaneous transluminal coronary angioplasty. *Am J Cardiol*. 1995;76(16):1185-8.
373. Godino C, Mendolicchio L, Figini F, Latib A, Sharp AS, Cosgrave J, et al. Comparison of VerifyNow-P2Y12 test and Flow Cytometry for monitoring individual platelet response to clopidogrel. What is the cut-off value for identifying patients who are low responders to clopidogrel therapy? *Thromb J*. 2009; 7:4.
374. Layne K, Ferro A. Antiplatelet Therapy in Acute Coronary Syndrome. *European cardiology*. 2017;12(1):33-7.
375. Foussas SG, Zairis MN, Tsirimpis VG, Makrygiannis SS, Patsourakos NG, Adamopoulou EN, et al. The impact of aspirin resistance on the long-term cardiovascular mortality in patients with non-ST segment elevation acute coronary syndromes. *Clin Cardiol*. 2009;32(3):142-7.
376. Norgard NB, Abu-Fadel M. Comparison of prasugrel and clopidogrel in patients with acute coronary syndrome undergoing percutaneous coronary intervention. *Vasc Health Risk Manag*. 2009; 5:873-82.
377. Musallam A, Lev EI, Roguin A. Stent thrombosis in a patient with high on-treatment platelet reactivity despite ticagrelor treatment. *Eur Heart J Acute Cardiovasc Care*. 2015;4(1):85-7.
378. Brar SS, ten Berg J, Marcucci R, Price MJ, Valgimigli M, Kim H-S, et al. Impact of Platelet Reactivity on Clinical Outcomes After Percutaneous Coronary Intervention: A Collaborative Meta-Analysis of Individual Participant Data. *Journal of the American College of Cardiology*. 2011;58(19):1945-54.
379. Mangiacapra F, Patti G, Barbato E, Peace AJ, Ricottini E, Vizzi V, et al. A therapeutic window for platelet reactivity for patients undergoing elective percutaneous coronary intervention: results of the ARMYDA-PROVE (Antiplatelet therapy for Reduction of MYocardial Damage during

Angioplasty-Platelet Reactivity for Outcome Validation Effort) study. *JACC Cardiovasc Interv.* 2012;5(3):281-9.

380. Kim B-K, Hong S-J, Cho Y-H, Yun KH, Kim YH, Suh Y, et al. Effect of Ticagrelor Monotherapy vs Ticagrelor with Aspirin on Major Bleeding and Cardiovascular Events in Patients with Acute Coronary Syndrome: The TICO Randomized Clinical Trial. *JAMA.* 2020;323(23):2407-16.

381. Tardif JC, Kouz S, Waters DD, Bertrand OF, Diaz R, Maggioni AP, et al. Efficacy and safety of low-dose colchicine after myocardial infarction. *New Engl J Med.* 2019;381.

382. Clark HD, Wells GA, Huët C, McAlister FA, Salmi LR, Fergusson D, et al. Assessing the Quality of Randomized Trials: Reliability of the Jadad Scale. *Controlled Clinical Trials.* 1999;20(5):448-52.

383. Fiolet ATL, Silvis MJM, Opstal TSJ, Bax WA, van der Horst FAL, Mosterd A, et al. Short-term effect of low-dose colchicine on inflammatory biomarkers, lipids, blood count and renal function in chronic coronary artery disease and elevated high-sensitivity C-reactive protein. *PLoS One.* 2020;15(8): e0237665.

384. Bouabdallaoui N, Tardif J-C, Waters DD, Pinto FJ, Maggioni AP, Diaz R, et al. Time-to-treatment initiation of colchicine and cardiovascular outcomes after myocardial infarction in the Colchicine Cardiovascular Outcomes Trial (COLCOT). *Eur Heart J.* 2020.

385. Tapp LD, Shantsila E, Wrigley BJ, Pamukcu B, Lip GY. The CD14⁺⁺CD16⁺ monocyte subset and monocyte-platelet interactions in patients with ST-elevation myocardial infarction. *J Thromb Haemost.* 2012;10(7):1231-41.

386. Leers MPG, Stockem C, Ackermans D, Loeffen R, ten Cate H, Kragten JA, et al. Intermediate and nonclassical monocytes show heterogeneity in patients with different types of acute coronary syndrome. *Cytometry Part A.* 2017;91(11):1059-67.

387. Layne K, Di Giosia P, Ferro A, Passacuale G. Anti-platelet drugs attenuate the expansion of circulating CD14^{high}CD16⁺ monocytes under pro-inflammatory conditions. *Cardiovasc Res.* 2016;111(1):26-33.

388. Urbanski K, Ludew D, Filip G, Filip M, Sagan A, Szczepaniak P, et al. CD14(+) CD16(++) "nonclassical" monocytes are associated with endothelial dysfunction in patients with coronary artery disease. *Thromb Haemost.* 2017;117(5):971-80.

389. Shah B, Pillinger M, Zhong H, Cronstein B, Xia Y, Lorin JD, et al. Effects of Acute Colchicine Administration Prior to Percutaneous Coronary Intervention: COLCHICINE-PCI Randomized Trial. *Circ Cardiovasc Interv.* 2020;13(4): e008717.

390. Tong DC, Quinn S, Nasis A, Hiew C, Roberts-Thomson P, Adams H, et al. Colchicine in Patients with Acute Coronary Syndrome: The Australian COPS Randomized Clinical Trial. *Circulation*.0(0).
391. Wagner V, Dullaart A, Bock A-K, Zweck A. The emerging nanomedicine landscapes. *Nature biotechnology*. 2006;24(10):1211-7.
392. Zhang L, Gu FX, Chan JM, Wang AZ, Langer RS, Farokhzad OC. Nanoparticles in medicine: therapeutic applications and developments. *Clin Pharmacol Ther*. 2008;83(5):761-9.
393. Wei L, MacDonald TM, Walker BR. Taking glucocorticoids by prescription is associated with subsequent cardiovascular disease. *Ann Intern Med*. 2004;141(10):764-70.
394. Lobatto ME, Fayad ZA, Silvera S, Vucic E, Calcagno C, Mani V, et al. Multimodal clinical imaging to longitudinally assess a nanomedical anti-inflammatory treatment in experimental atherosclerosis. *Mol Pharm*. 2010;7(6):2020-9.
395. Fleg JL, Stone GW, Fayad ZA, Granada JF, Hatsukami TS, Kolodgie FD, et al. Detection of high-risk atherosclerotic plaque: report of the NHLBI Working Group on status and future directions. *JACC Cardiovasc Imaging*. 2012;5(9):941-55.
396. Liu L, Gardecki JA, Nadkarni SK, Toussaint JD, Yagi Y, Bouma BE, et al. Imaging the subcellular structure of human coronary atherosclerosis using micro-optical coherence tomography. *Nat Med*. 2011;17(8):1010-4.
397. Papadopoulos C, Patoulas D, Teperikidis E, Mouselimis D, Tsarouchas A, Toumpourleka M, et al. Colchicine as a Potential Therapeutic Agent Against Cardiovascular Complications of COVID-19: an Exploratory Review. *Sn Comprehensive Clinical Medicine*. 2020:1 - 11.
398. Deftereos S, Giannopoulos G, Raisakis K, Kossyvakis C, Kaoukis A, Panagopoulou V, et al. Colchicine Treatment for the Prevention of Bare-Metal Stent Restenosis in Diabetic Patients. *Journal of the American College of Cardiology*. 2013;61(16):1679-85.
399. Verweij SL, Stiekema LCA, Delewi R, Zheng KH, Bernelot Moens SJ, Kroon J, et al. Prolonged hematopoietic and myeloid cellular response in patients after an acute coronary syndrome measured with 18F-DPA-714 PET/CT. *European Journal of Nuclear Medicine and Molecular Imaging*. 2018;45(11):1956-63.
400. Samuel M, Tardif J-C, Khairy P, Roubille F, Waters DD, Grégoire JC, et al. Cost-effectiveness of low-dose colchicine after myocardial infarction in the Colchicine Cardiovascular Outcomes Trial (COLCOT). *European Heart Journal - Quality of Care and Clinical Outcomes*. 2020.
401. Cannon CP, Bhatt DL, Oldgren J, Lip GYH, Ellis SG, Kimura T, et al. Dual Antithrombotic Therapy with Dabigatran after PCI in Atrial Fibrillation. *N Engl J Med*. 2017;377(16):1513-24.

402. Bonaca MP, Bhatt DL, Cohen M, Steg PG, Storey RF, Jensen EC, et al. Long-Term Use of Ticagrelor in Patients with Prior Myocardial Infarction. *New England Journal of Medicine*. 2015;372(19):1791-800.
403. Nidorf SM, Fiolet ATL, Mosterd A, Eikelboom JW, Schut A, Opstal TSJ, et al. Colchicine in Patients with Chronic Coronary Disease. *New England Journal of Medicine*. 2020;383(19):1838-47.
404. Marino BCA, Nascimento GA, Rabelo W, Marino MA, Marino RL, Ribeiro ALP. Clinical Coronary In-Stent Restenosis Follow-Up after Treatment and Analyses of Clinical Outcomes. *Arquivos Brasileiros de Cardiologia*. 2015; 104:375-86.
405. Dewey M, Siebes M, Kachelrieß M, Kofoed KF, Maurovich-Horvat P, Nikolaou K, et al. Clinical quantitative cardiac imaging for the assessment of myocardial ischaemia. *Nature Reviews Cardiology*. 2020;17(7):427-50.
406. Rominger A, Saam T, Vogl E, Ubleis C, la Fougère C, Förster S, et al. In vivo imaging of macrophage activity in the coronary arteries using ⁶⁸Ga-DOTATATE PET/CT: correlation with coronary calcium burden and risk factors. *J Nucl Med*. 2010;51(2):193-7.
407. Tawakol A, Migrino RQ, Bashian GG, Bedri S, Vermylen D, Cury RC, et al. In vivo ¹⁸F-fluorodeoxyglucose positron emission tomography imaging provides a noninvasive measure of carotid plaque inflammation in patients. *J Am Coll Cardiol*. 2006;48(9):1818-24.
408. Figueroa AL, Abdelbaky A, Truong QA, Corsini E, MacNabb MH, Lavender ZR, et al. Measurement of arterial activity on routine FDG PET/CT images improves prediction of risk of future CV events. *JACC Cardiovasc Imaging*. 2013;6(12):1250-9.
409. Anstensrud AK, Woxholt S, Sharma K, Broch K, Bendz B, Aakhus S, et al. Rationale for the ASSAIL-MI-trial: a randomised controlled trial designed to assess the effect of tocilizumab on myocardial salvage in patients with acute ST-elevation myocardial infarction (STEMI). *Open Heart*. 2019;6(2): e001108.
410. Bouleti C, Mewton N, Germain S. The no-reflow phenomenon: State of the art. *Archives of Cardiovascular Diseases*. 2015;108(12):661-74.
411. Ouzounian M, Lee DS, Gramolini AO, Emili A, Fukuoka M, Liu PP. Predict, prevent, and personalize: Genomic and proteomic approaches to cardiovascular medicine. *The Canadian journal of cardiology*. 2007;23 Suppl A (Suppl A):28A-33A.
412. deGoma EM, Knowles JW, Angeli F, Budoff MJ, Rader DJ. The evolution and refinement of traditional risk factors for cardiovascular disease. *Cardiology in review*. 2012;20(3):118-29.

413. Ounzain S, Micheletti R, Beckmann T, Schroen B, Alexanian M, Pezzuto I, et al. Genome-wide profiling of the cardiac transcriptome after myocardial infarction identifies novel heart-specific long non-coding RNAs. *Eur Heart J*. 2015;36(6):353-68a.
414. Dai X, Wiernek S, Evans JP, Runge MS. Genetics of coronary artery disease and myocardial infarction. *World journal of cardiology*. 2016;8(1):1-23.
415. Birney E, Stamatoyannopoulos JA, Dutta A, Guigó R, Gingeras TR, Margulies EH, et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature*. 2007;447(7146):799-816.
416. O'Sullivan JF, Neylon A, Fahy EF, Yang P, McGorrian C, Blake GJ. MiR-93-5p is a novel predictor of coronary in-stent restenosis. *Heart Asia*. 2019;11(1): e011134.
417. Barraclough J, Joglekar M, Januszewski A, Martínez G, Celermajer D, Keech A, et al. A MicroRNA Signature Modulated by Colchicine in Acute Coronary Syndrome Patients. *Heart, Lung and Circulation*. 2019;28: S280.
418. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest*. 2012;122(3):787-95.
419. Ben-Mordechai T, Holbova R, Landa-Rouben N, Harel-Adar T, Feinberg MS, Abd Elrahman I, et al. Macrophage Subpopulations Are Essential for Infarct Repair with and Without Stem Cell Therapy. *Journal of the American College of Cardiology*. 2013;62(20):1890-901.
420. Cheng G, Chang F-J, Wang Y, You P-H, Chen H-C, Han W-Q, et al. Factors Influencing Stent Restenosis After Percutaneous Coronary Intervention in Patients with Coronary Heart Disease: A Clinical Trial Based on 1-Year Follow-Up. *Med Sci Monit*. 2019; 25:240-7.
421. Andre N, Renata P, Mahruzza MH, Santoso RM. Colchicine as an Adjuvant Therapy for Coronary Artery Disease: A Systematic Review. *Indonesian Journal of Cardiology*.
422. Opstal, T.S.J., Hoogeveen, R.M., Fiolet, A.T.L., Silvis, M.J.M., The, S.H.K., Bax, W.A., De Kleijn, D.P.V., Mosterd, A., Stoes, E.S.G., Cornel, J.H., 2020. Colchicine Attenuates Inflammation Beyond the Inflammasome in Chronic Coronary Artery Disease. *Circulation* 142, 1996–1998. doi:10.1161/circulationaha.120.050560
423. Dentali F, Nigro O, Squizzato A, Gianni M, Zuretti F, Grandi AM, Guasti L. Impact of neutrophils to lymphocytes ratio on major clinical outcomes in patients with acute coronary syndromes: A systematic review and meta-analysis of the literature. *Int J Cardiol*. 2018 Sep 1; 266:31-37. Doi: 10.1016/j.ijcard.2018.02.116. PMID: 29887466.

26 Appendices

(A) LoDoCo-MI cited with other Randomized Placebo-Controlled Trials Evaluating the Effect of Periprocedural Anti-Inflammatory Drugs in Patients Undergoing CA/PCI (1)

Author (Study Name)	Target Population (No. of Patients)	Study Drug (Dose)	Treatment Timing and Duration	Effect on Inflammatory or Myocardial Injury Biomarkers	Clinical Outcomes
Akoddad et al ¹⁰ (COLIN)	STEMI (n=44)	Colchicine (1 mg od)	First dose within 24 h of diagnosis, continued for 30 d	No difference in CRP	No difference in MACE
Martinez et al ⁸	NSTE-ACS (n=40); CCS (n=33); Healthy controls (n=10)	Colchicine (1.5 mg)*	Single dose 6–24 h before CA	↓ IL-1β ↓ IL-6 ↓ IL-18	NA
Deftereos et al ⁹	STEMI (n=151)	Colchicine (loading dose: 2 mg†; maintaining dose: 0.5 mg bid)	Loading dose after CA, maintaining dose continued for 5 d	↓ CK-MB ↓ Troponin ↓ CRP ↓ Neutrophil count	↓ Infarct size on MRI-LGE No difference in MACE
Hennessy et al ⁷ (LoDoCo-MI)	Type 1 MI (n=237)	Colchicine (0.5 mg od)	First dose within 7 d of MI, continued for 30 d	No difference in CRP and IL-6	↓ Re-hospitalization
Abbate et al ¹¹	STEMI (n=20)	Anakinra (100 mg od)	First dose within 24 h of PCI, continued for 14 d	↓ CRP at 72 h No difference in CK-MB	No difference in MACE ↓ Rate of death or new-onset HF‡
Morton et al ¹² (MRC-ILA Heart study)	NSTE-ACS (n=182)	Anakinra (100 mg od)	First dose within 48 h of symptoms onset, continued for 14 d	At 7 and 14 d: ↓ CRP ↓ IL-6 At 30 d (16 d after drug discontinuation): ↑ CRP	No difference in MACE at 30 d and 3 mo ↑ MACE at 1 y (driven by ↑ MI)
Kleveland et al ¹³	NSTE-ACS (n=117)	Tocilizumab (280 mg)	Single dose before CA	↓ CRP ↓ Troponin	No difference in MACE

(B) LoDoCo-MI & all other trials to date examining Colchicine in ACS (2)

Reference	Study design	Duration	Sample size (randomization scheme)	Intervention(s)	Efficacy (colchicine vs control)	Safety (colchicine vs control)	Jadad Scale
Raju et al ³⁶	Double blind	30 days	80 Patients (1:1); 68 with CRP lab at follow-up	Colchicine 1 mg daily vs placebo	Mean Δ CRP: 12.2 vs 20.9 mg/L Mean absolute CRP: 7.2 vs 2.9 mg/L	Diarrhea 38.9% vs 18.4%; no myopathy, neutropenia, or leukopenia	5/5
Hennessy et al ³⁷	Double blind	30 Days	237 Patients (1:1); 222 with labs for analysis	Colchicine 0.5 mg daily + standard of care vs standard of care alone	Proportion of patients with CRP $\geq$ 2 mg/L at 30 days: 44% vs 50% Median CRP: 1.6 vs 2.0 mg/L Relative reduction in CRP: 78% vs 64% Relative reduction in IL-6: 58% vs 52%	Overall per-protocol patient- reported ADR: 16% vs 18% 30-Day readmissions: 3% vs 11% ^a GI ADRs: 11% vs 5%	5/5
Akodad et al ³⁸	Open label	30 Days	44 Patients (1:1)	Colchicine 1 mg daily + standard of care vs standard of care alone	CRP peak during hospitalization 29.03 vs 21.86 mg/L Troponin T peak: 6476.8 vs 4635.6 ng/L 30-Day MACE not significantly different	43.4% (n = 10) GI intolerance; only 13% (n = 3) resulting in discontinuation; no hepatotoxicity or myelotoxicity reported	4/5
Robertson et al ³⁹	Open label	2 Days	21 Patients (1:1); 9 additional controls	Colchicine 1 mg once followed by 0.5 mg 1 hour later or no therapy	Monocyte intracellular IL-1 β reduced 114.5% ^a and secretion 595.7% ^a without change in venous concentrations Monocyte secretion of IL-18 reduced 26.7 pg/mL ^a without change in intracellular or venous concentration Monocyte pro-caspase-1 mRNA reduced 5.73% ^a with 30.2% decrease in monocyte caspase-1 secretion ^a	Not assessed	2/5
Martínez et al ⁴⁰	Open label	1 Day	40 Patients in the ACS group (1:1)	Colchicine 1 mg once then 0.5 mg 1 hour later + standard of care vs standard of care alone	Transcoronary gradient reduced with colchicine for IL-1 β , ^a IL-18, ^a and IL-6 ^a Δ Mean concentration: IL-1 β : -4.47 pg/mL IL-18: nonsignificant IL-6: -64.21 pg/mL	Not assessed	3/5
Tucker et al ⁴¹	Single blind	1 Day	38 Patients (n = 25 presenting with ACS; 1:1)	Colchicine 1 mg once followed by 0.5 mg 1 hour later + standard of care vs standard of care alone	Decreased transcoronary gradient of CCL2 (12.2 vs -12 pg/mL), ^a CCL5 (12.6 ng/mL vs -16.2 pg/mL), ^a and CX3CL1 (73.4 vs -7.8 pg/mL) ^a Relative increase in intracellular monocyte concentrations of CCL2 (0.4 vs 1.3), ^a CCL5 (2.5 vs 9.7), ^a and CX3CL1 (0.9 vs 1.3) ^a	No adverse reactions reported in 12 patients who received colchicine	3/5

Deftereos et al ⁴²	Double blind	5 Days	151 Patients (1:1)	Colchicine 2 mg loading dose then 0.5 mg bid (0.5 mg daily if weight <60 kg) + standard of care vs standard of care alone	Median 72-hour AUC of CK-MB fraction ^a : 3144 vs 6184 ng h mL ⁻¹ Median peak troponin T: 19763 vs 45550 pg/mL ^a Infarct volume on cardiac MRI: 25.1 vs 18.8 mL ^a	26% (n = 20) Discontinued colchicine because of GI ADRs, except 1 patient with ALT 5× the upper limit of normal	5/5
Shah et al ⁴³	Double blind	30 Days; 24 hours for biomarkers	400 Patients with PCI (n = 198; 49.5% presenting with ACS) 280 Patients in nested biomarker substudy, including ACS and non-ACS	Colchicine 1.2 mg 1-2 hours prior to coronary angiography then 0.6 mg 1 hour later	ACS patients 24-hour PCI-related myocardial injury: 55.3% vs 57.6% PCI-related MI: 3.9% vs 3.3% 30-day MACE: 17.5% vs 12.6% Biomarker substudy (ACS and non-ACS) 22 To 24-hour percentage increase in IL-6: 76% vs 338% ^a 22 To 24-hour percentage increase in IL-1β: 0% vs 0% 22 To 24-hour percentage increase in CRP: 11% vs 66% ^a	GI symptoms: 9.3% vs 3.2% ^a Hemodynamic instability: 0% vs 1.4% ^a	5/5
Tardif et al ⁴⁴	Double blind	22.6 Months; 6 and 12 months in biomarker substudy	4745 Patients (1:1); 207 patients in biomarker substudy	Colchicine 0.5 mg daily + standard of care vs standard of care alone	Composite of CV death, resuscitated cardiac arrest, MI, stroke, or urgent hospitalization for angina leading to revascularization 5.5% vs 7.1% (HR = 0.77) ^a Substudy Mean percentage change in CRP at 6 months (-70.0% vs -66.6%) Mean percentage change in leukocyte counts at 12 months (-18.8% vs -19.0%)	Overall ADR: 16% vs 15.8% Increased nausea (1.8% vs 1.0%), ^a flatulence (0.6% vs 0.2%), ^a and pneumonia (0.9% vs 0.4%) ^a	5/5

(C) Morisky Adherence Questionnaire at 30 day follow up

	YES	NO
1. Do you sometimes forget to take your medication?		
2. People sometimes miss taking their medications for reasons other than forgetting. Over the past 2 weeks, were there any days when you did not take your medication?		
3. Have you ever cut back or stopped taking your medication without telling your doctor because you felt worse when you took it?		
4. When you travel or leave home, do you sometimes forget to bring your medication?		
5. Did you take all your medication yesterday?		
6. When you feel like your symptoms are under control, do you sometimes stop taking your medication?		
7. Taking medication every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your treatment plan?		
8. How often do you have difficulty remembering to take all your medication?		
Never/Rarely.....		
Once in a while.....		
Sometimes.....		
Usually.....		
All the time.....		

(D)

Royal Perth Hospital

PARTICIPANT INFORMATION SHEET

Low Dose Colchicine After Acute Myocardial Infarction

The LoDoCo MI Study

Principal Investigator: Professor Graham Hillis, Department of Cardiology RPH

You are invited to participate in a research study because you have been diagnosed as having a myocardial infarction (M.I. – a ‘heart attack’). This information sheet explains the LoDoCo MI study and describes what will be involved should you decide to participate. Please read the information carefully and feel free to ask any questions you might have. You may also wish to discuss the study with a relative or friend or your GP.

Background and aim of the Study:

Despite many improvements in the treatment of MI, it continues to be a major cause of ongoing complications (such as dying or disability) both in Australia and around the world.

One of the main things that causes MI is instability or erosion of atherosclerotic plaque (the fatty deposits that build up on the inside of the artery wall). One of the main triggers for this is inflammation in the vessel wall. People who have been diagnosed with a MI are at increased risk of this happening again, and this is largely in part due to ongoing inflammation.

There are no current treatments that have been proven to reduce this vessel wall (‘vascular’) inflammation specifically and successfully. Recent data suggest that low doses of colchicine, a unique anti-inflammatory drug mainly used to treat gout, may reduce vascular inflammation and, therefore, the risk of heart attacks and other major complications in patients with coronary heart disease.

This study is the first stage in a program of work that aims to test whether colchicine will reduce the risk of recurrent cardiovascular events (repeat heart attacks and strokes etc) in patients following an acute MI (heart attack). The proposed pilot (small) study will assess the feasibility, tolerability, and safety of adding low-dose colchicine to existing therapy after a MI.

The study will also determine how effective the use of colchicine is, in survivors of MI, by exploring the effects of the treatment on a blood test that gives a measure of the amount of inflammation in the body, called C-reactive protein (CRP).

What participation in the study involves?

If you agree to participate, we will ask you about your medical history and current medications. You will be asked to have measurement of your height, weight, blood pressure and pulse rate. We will also take 10 mls of blood (approximately 2 tablespoons) to measure routine tests in addition to the CRP. You will be asked to take one tablet of study medication every day for a total of 30 days. It is important that this therapy is started within 7 days of your MI. This will be in addition to your standard medications.

Both you and the study staff will not know whether the study medication is the colchicine or a matching placebo (pretend medication). This is known as a placebo-controlled trial or a ‘double-blind study’.

A placebo-controlled trial means that you will receive either the active study drug or a tablet with no active ingredient. The randomized design means that the treatment that you are allocated to will be determined by a random process like a roll of the dice. This is to ensure that neither you nor the study staff will know which treatment you will be on to safeguard the scientific interpretation of the study. If it becomes necessary for health reasons it will be possible to determine which concealed treatment you are taking during the study.

The following table is a summary of what is required for the study:

Visit	Location	Tests and procedures	Duration
<i>Baseline</i> Visit 1	Cardiology Ward/CCU RPH.	Short medical history Current medications Blood tests (maximum 15ml) Height, weight, BP, heart rate and temperature (T°).	30 minutes
<i>Randomisation</i>	Once the baseline tests have been done you will be randomly allocated (as if by the roll of a dice) to one of the two study drugs: either colchicine or placebo.		
<i>Treatment until Day 30</i>	Cardiology Department, Level 4, Block A. RPH	Short medical history and review of medication. Return of study drug.	30 minutes

Visit 2		Blood tests (maximum 15ml) Weight, BP, heart rate and T° Questionnaire	
---------	--	--	--

Storage of blood samples for future research

In addition to using your study blood samples for this study, we would like to store them in a freezer for use in further research studies. These studies will investigate the effects of colchicine on markers of inflammation (other than C-reactive protein) and other blood “markers” that may indicate the risk of future cardiovascular events. Samples may potentially be used for genetic analysis. The testing will focus only on assessing the possible role of genetic factors in determining the response to the study medication, the extent of any inflammatory response after acute MI and/or the risk of future cardiovascular events. The samples will be identified only by the study number and your initials and hospital number. We plan to store these samples for up to 10 years.

The use of your samples for future research is optional. You can participate in this study and decide NOT to agree to this. The attached Consent Form gives you the option to opt in or out of this option.

Possible side effects and risks

All medical procedures, whether for diagnosis or treatment involve some risk of injury.

The blood test may cause brief discomfort when the needle is inserted and may result in slight bleeding or a bruise that will resolve within a few days. For this short term study, the most common side effects that may be related to the study medication are gastro-intestinal upset (predominantly loose bowel motions). These symptoms are generally mild, and we hope to minimise these effects by using a very low dose of the study drug (colchicine 0.5mg once daily).

Other side effects that are rarely associated with the use of colchicine and high-dose statin (cholesterol lowering) therapy (which is commonly prescribed after a MI), include major muscle problems. This association occurs mainly primarily in patients with moderate to severe kidney dysfunction receiving high doses of colchicine. Partly for this reason we are using a low dose of colchicine and will not include patients in the study if they have significant kidney problems. Other reported side effects associated with long term use of colchicine are rare but may include: muscle pains (myalgia, 0.9%), reversible inflammation of the nerves in the limbs (peripheral neuritis, <0.5%), inflammation of the muscles (myopathy, <0.5%), Hair loss (alopecia, <0.5%), inhibition of spermatogenesis (but not fertility, <0.5%), vitamin B12 deficiency (<0.5%), and bone marrow suppression (<0.5%), severe inflammation of the muscles (rhabdomyolysis). These risks may be higher in patients with advanced kidney or liver disease and with concomitant use of specific other medications i.e., clarithromycin, cyclosporine erythromycin, ketoconazole, and high dose statins. Please inform the study nurse or doctor if you think that any of these may apply to you. Taking colchicine in doses higher than prescribed for the study may be dangerous and colchicine should be kept out of reach of children.

If you agree to the study your GP will be informed of your participation and of these risks.

Possible benefits

It is not known for certain whether a 1-month course of colchicine will reduce the risk of vascular inflammation. Therefore, the current study is important. Participation in this study may have no direct benefit for you, but may help to develop better treatments for patients following a MI. If colchicine proves to be a simple, safe, cheap, and effective treatment that could reduce vascular inflammation, promote stability of atherosclerotic plaque, and prevent recurrent events in this study the data will be used to help the study group to design a major international outcome trial. This would assess whether colchicine was useful to reduce the risks of heart attacks, strokes, and death in patients after a MI. If successful such a trial would potentially change the way that we treat patients in the future.

Privacy and confidentiality

Information about you may be obtained from your health records held at this institution and may be obtained from other health services (G.P. other hospitals, laboratory results) for the purposes of this research. The information gathered about you by the investigator or obtained during this study will be held by the investigator in strict confidence and all the people who handle your information will comply with the Privacy Act 1988. If the results of the trial are published in a medical journal, as is intended, no reader will be able to identify individual patients.

What if something goes wrong?

If you suffer an expected or unexpected side effect or medical accident during this study that arises from your participation, you will be offered all full and necessary treatment by Royal Perth Hospital.

Costs to participation; There will be no costs incurred because of participation in this study. You will not be charged for any of the blood tests or study medication. You will not be paid for participation, although reimbursement will be available for reasonable travel costs associated with the study-related visits.

Voluntary participation and withdrawal

Participation in this study is entirely voluntary. You do not have to participate if you do not want to and your decision to participate or not will in no way affect your current or future care at Royal Perth Hospital. You are also free to withdraw from the study at any time without reason or justification.

Contact Information; If you have questions about this study, please contact Professor Graham Hillis on (08) 9224 2067 or the study coordinator 08 9224 8069 at Royal Perth Hospital. This study has been approved by the Royal Perth Hospital Human Research Ethics Committee. If you have any concerns about the conduct of the study or your rights as a research participant, please contact Prof Frank van Bockxmeer, Chairman of the RPH Ethics Committee, via (08) 6151 1180 or SMHS.REG@health.wa.gov.au and quote the ethics approval number (15-090).

Royal Perth Hospital

CONSENT FORM

Low Dose Colchicine After Acute Myocardial Infarction
The LoDoCo MI Study

Principal Investigator: Associate Professor Graham Hillis, Department of Cardiology RPH

I, agree to participate in the above study. I have been provided with a copy of the Participant Information Sheet explaining the study which I have read and understood. I have been given the opportunity to ask questions about the study by the Investigator and any questions have been answered to my satisfaction. I understand that I may withdraw from the study at any time without affecting any future medical treatment, or the treatment of the condition which is the subject of the study. I am aware that all research data collected will only be used for the purpose of this study and will be kept confidential and that my participation will not be disclosed without my consent.

I agree to the storage of samples for future research as specified in the Information Sheet (please circle):

YES ☐ NO ☐

Signed _____ Date _____

Signature _____ Date _____
of person obtaining consent

Name _____
of person obtaining consent

PathWest <small>Wellington Street PERTH 6000</small> <small>LABORATORY MEDICINE WA</small> <small>Royal Perth Hospital</small>		RESULTS & ENQUIRIES 9224 2422	PATHOLOGY REQUEST												
Unit No. _____ Medicare Number _____ Surname _____ Given Names _____ Date of Birth _____ Age _____ Sex _____ Address _____		Consultant Prof. G Hillis (H1540) Requesting Doctor _____ Surname _____ Initials _____ Address _____ Page _____ Provider Number _____ Doctors Signature X _____ Request Date _____		Source / Hospital RPH Ward CARD Days For Collection M T W Thu F S Su _____											
TESTS REQUESTED <div style="border: 1px solid black; padding: 5px; text-align: center;"> URGENT SAMPLES MUST BE PROCESSED ASAP </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 45%; border: 1px solid black; padding: 5px;"> RPH CSRA Label samples, spin SST and register form. Send all samples to FSH ASAP on next courier run for specimen processing & storage </div> <div style="width: 45%; border: 1px solid black; padding: 5px;"> FSH CSRA Please notify research contact (22726 / 23190) ASAP for specimen processing and storage </div> </div>		When collecting ANTIBIOTICS and DRUG assays fill in this box: Drug _____ Dosage _____ Date _____ Time _____ Date of Collection _____ Time of Collection _____ <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td>CLOT</td> <td>CIT</td> <td>HEP</td> <td>EDTA</td> </tr> <tr> <td>GLU</td> <td>ESR</td> <td>ABG</td> <td>URINE</td> </tr> <tr> <td>SWAB</td> <td>SLIDE</td> <td colspan="2">Other _____</td> </tr> </table>		CLOT	CIT	HEP	EDTA	GLU	ESR	ABG	URINE	SWAB	SLIDE	Other _____	
CLOT	CIT	HEP	EDTA												
GLU	ESR	ABG	URINE												
SWAB	SLIDE	Other _____													
CLINICAL NOTES LoDoCo MI Study Samples for storage Visit: Baseline , 30 days Trial Coordinator : Michelle Bonner (48069) FSH Lab contact: Linda / Adrienne (6152 2726 / 6152 3190)		Copy Reports to: _____ Specimen Required: 1 x 5mL SST tube (6HSST) 1 x 9mL EDTA tube Fasting Yes ☐ No ☐ Rule 3 Exemption Yes ☐ No ☐ Anticoagulant Therapy Warfarin ☐ Heparin ☐ Self Determine ☐													
RPH Register Using Code: KST Results to be: _____ Faxed _____ Phone _____		Collector's Signature _____ <small>I certify that the blood specimen(s) accompanying this request was drawn from the patient named above and I established the identity of this patient by direct enquiry and/or by inspection of wrist band and immediately upon the blood being drawn I labelled the specimen(s)</small> X Collection Centre: RPH Bill To: RT494 Patient Class: Outpatient (O)													

PathWest <small>Wellington Street PERTH 6000</small> <small>LABORATORY MEDICINE WA</small> <small>Royal Perth Hospital</small>		RESULTS & ENQUIRIES 9224 2422	PATHOLOGY REQUEST												
Unit No. _____ Medicare Number _____ Surname _____ Given Names _____ Date of Birth _____ Age _____ Sex _____ Address _____		Consultant Prof. G Hillis (H1540) Requesting Doctor _____ Surname _____ Initials _____ Address _____ Page _____ Provider Number _____ Doctors Signature X _____ Request Date _____		Source / Hospital RPH Ward CARD Days For Collection M T W Thu F S Su _____											
TESTS REQUESTED hsCRP		When collecting ANTIBIOTICS and DRUG assays fill in this box: Drug _____ Dosage _____ Date _____ Time _____ Date of Collection _____ Time of Collection _____ <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td>CLOT</td> <td>CIT</td> <td>HEP</td> <td>EDTA</td> </tr> <tr> <td>GLU</td> <td>ESR</td> <td>ABG</td> <td>URINE</td> </tr> <tr> <td>SWAB</td> <td>SLIDE</td> <td colspan="2">Other _____</td> </tr> </table>		CLOT	CIT	HEP	EDTA	GLU	ESR	ABG	URINE	SWAB	SLIDE	Other _____	
CLOT	CIT	HEP	EDTA												
GLU	ESR	ABG	URINE												
SWAB	SLIDE	Other _____													
Register Using Codes: HSC, SHF CLINICAL NOTES LoDoCo MI Study Visit: Baseline , 30 days Trial Coordinator : Michelle Bonner (48069)		Copy Reports to: _____ Specimen Required: 1 x 3mL Lith Hep tube (PST) Fasting Yes ☐ No ☐ Rule 3 Exemption Yes ☐ No ☐ Anticoagulant Therapy Warfarin ☐ Heparin ☐ Self Determine ☐													
Results to be: _____ Faxed _____ Phone _____		Collector's Signature _____ <small>I certify that the blood specimen(s) accompanying this request was drawn from the patient named above and I established the identity of this patient by direct enquiry and/or by inspection of wrist band and immediately upon the blood being drawn I labelled the specimen(s)</small> X Collection Centre: RPH Bill To: RT494 Patient Class: Outpatient (O)													

(E)

(F) Colchicine doses and duration of treatment in ACS trials with pre-specified PCI time