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State-of-the-Art Review

Inflammation and cardiovascular disease: From mechanisms to therapeutics

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A B S T R A C T

Inflammation constitutes a complex, highly conserved cascade of molecular and cellular events. Inflammation has been labeled as "the fire within," is highly regulated, and is critical to host defense and tissue repair. In general, inflammation is beneficial and has evolved to promote survival. However, inflammation can also be maladaptive when chronically activated and sustained, leading to progressive tissue injury and reduced survival. Examples of a maladaptive response include rheumatologic disease and atherosclerosis. Despite evidence gathered by Virchow over 100 years ago showing that inflammatory white cells play a role in atherogenesis, atherosclerosis was until recently viewed as a disease of passive cholesterol accumulation in the subendothelial space. This view has been supplanted by considerable basic scientific and clinical evidence demonstrating that every step of atherogenesis, from the development of endothelial cell dysfunction to foam cell formation, plaque formation and progression, and ultimately plaque rupture stemming from architectural instability, is driven by the cytokines, interleukins, and cellular constituents of the inflammatory response. Herein we provide an overview of the role of inflammation in atherosclerotic cardiovascular disease, discuss the predictive value of various biomarkers involved in inflammation, and summarize recent clinical trials that evaluated the capacity of various pharmacologic interventions to attenuate the intensity of inflammation and impact risk for acute cardiovascular events.

1. Introduction

Despite the significant reduction in cardiovascular events with intensive low-density lipoprotein cholesterol (LDL-C) lowering with statin therapy, patients with cardiovascular disease (CVD) still experience residual cardiovascular (CV) risk [1]. The various contributors to this residual risk are broad ranging and their interactions are complex [2]. Inflammation plays a critical role in the genesis, progression, and manifestation of CVD [3]. While safely modulating inflammation using targeted therapeutics remains a challenge, the results from recent prospective studies demonstrate that targeting inflammation may offer a novel approach to reducing risk for acute CV events.

Data from observational cohorts and clinical trials draw attention to the high prevalence of residual inflammatory risk in patients with CVD despite statin therapy. In the Variation in Recovery - Role of Gender on Outcomes of Young AMI (VIRGO) registry, 60% of young patients with acute myocardial infarction (MI) had elevated high-sensitivity C-reactive protein (hsCRP), a common measure of low-grade inflammation, of ≥ 2 mg/L [4]. Evidence from clinical trials with statins show similar results. In the Pravastatin or Atorvastatin Evaluation and Infection Therapy

(PROVE-IT) trial, 43% of patients on high-intensity atorvastatin had hsCRP levels ≥ 2 mg/L [5]. In the Improved Reduction of Outcomes - Vytorin Efficacy International (IMPROVE-IT) trial, similar results were found with 47% of those randomized to statin plus ezetimibe having on-treatment hsCRP levels ≥ 2 mg/L [6]. In both PROVE-IT and IMPROVE-IT, residual hsCRP elevation was associated with increased risk of events despite achievement of LDL-C control < 70 mg/dL, which has led some experts to advocate for achievement of "dual targets" of both LDL-C < 70 mg/dL and hsCRP < 2 mg/dL [6,7]. A more recent report of patients post percutaneous coronary intervention (PCI) with LDL-C < 70 mg/dL showed that 34% had evidence of residual low-grade inflammation despite aggressive secondary prevention therapies [8]. Clearly, residual heightened systemic inflammation is associated with residual risk, is common among patients with CVD, and additional interventions to lower inflammation and decrease CV risk are an unmet need.

Our understanding of atherosclerotic cardiovascular disease (ASCVD) has evolved from being a disease of passive cholesterol accumulation, to a disease that is driven by chronic inflammation which initiates a plethora of biochemical and histologic phenomena that lead to atherosclerotic plaque formation and the triggering of plaque rupture events

Abbreviations

AFCAPS/TexCAPS	The Air Force/Texas Coronary Atherosclerosis Prevention Study
ASCVD	Atherosclerotic cardiovascular disease
CAC	Coronary artery calcium
CAD	Coronary artery disease
CANTOS	Canakinumab Anti-inflammatory Thrombosis Outcome Study
CD	Cluster of differentiation
cIMT	Carotid intima media thickness
CIRT	Cardiovascular Inflammation Reduction Trial
CRISP-CT	Cardiovascular RISk Prediction using Computed Tomography
CT	Computed tomography
CV	Cardiovascular
CXCR	Chemokine receptor
DOTATATE	[^{68}Ga] 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra-acetic acid
EPA	Eicosapentaenoic acid
EPIC-Norfolk	European Prospective Investigation Into Cancer in Norfolk Prospective Population Study
FDA	Food and Drug Administration
FDG	Fluorodeoxyglucose
FFP	Farnesylpyrophosphate
FMO3	Flavin containing monooxygenases-3
FOURIER	Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk
GGPP	Geranylgeranyl pyrophosphate
GTP	Guanine triphosphate
HIV	Human immunodeficiency virus
HMG-CoA	β-Hydroxy- β-methylglutaryl-CoA
hsCRP	High-sensitivity C-reactive protein
ICAM	Intercellular adhesion molecule
IL	Interleukin
IL-6R	IL-6 receptor
IMPROVE-IT	Improved Reduction of Outcomes - Vytorin Efficacy International
JUPITER	Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin
LDL-C	Low-density lipoprotein cholesterol
Lp-PLA$_2$	Lipoprotein-associated phospholipase-A2
MAPK/JAK	Mitogen-activated protein kinases/Janus kinases
MI	Myocardial infarction
MIP	Macrophage inflammatory proteins
MNS	Methylenedioxy-β-nitrostyrene
MPO	Myeloperoxidase
NF-kB	Nuclear factor kappa B
NHANES	National Health and Nutrition Examination Survey
NLRP3	Nod-like receptor protein 3
Ox-LDL	Oxidized Low-density lipoprotein
PCE	Pooled Cohort equation
PCI	Percutaneous coronary intervention
PESA	Progression of Early Subclinical Atherosclerosis
PET	Positron emission tomography
PRINCE	PRavastatin Inflammation CRP Evaluation
PROVE-IT	Pravastatin or Atorvastatin Evaluation and Infection Therapy
SMART	Strategies for Management of Antiretroviral Therapy
SPIRE	Studies of PCSK9 Inhibition and the Reduction of Vascular Events
SSTR2	Somatostatin receptor 2
STABILITY	Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy
TLR2	Toll-like receptor-2
TMAO	Trimethylamine-N-oxide
TNF-α	Tumor necrosis factor-α
TSPO	Targets macrophage translocator protein
VCAM	Vascular cell adhesion molecule
VIRGO	Variation in Recovery - Role of Gender on Outcomes of Young AMI
VLDL	Very low-density lipoproteins
WBC	White blood cell

[9]. Early investigations revealed significant inflammatory cell infiltrates in atherosclerotic plaque [10]. The Physicians' Health Study [11] and Women's Health Study [12] showed that inflammatory markers such as interleukin-6 (IL-6) and IL-1β are highly associated with ASCVD risk. The Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAP-S/TexCAPS) suggested that patients with evidence of low-grade inflammation might be at risk even when LDL-C is controlled [13]. In the Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial, patients with no prior CVD or diabetes with LDL-C <130 mg/dL but with hsCRP ≥2 mg/L benefited from 20 mg/day of rosuvastatin with a 44% relative risk reduction in a composite endpoint of myocardial infarction, stroke, arterial revascularization, hospitalization for unstable angina, or death from cardiovascular causes [14]. A 65% reduction in risk of vascular events occurred when both on-treatment LDL-C <70 mg/dL and hsCRP <2 mg/L were achieved [15]. Thus, JUPITER showed that measuring low-grade inflammation with hsCRP identified a subgroup of patients who would previously have not been considered for statins and were at increased CV risk and experienced benefit with statin therapy. However, it was difficult to ascertain whether the benefit with statin therapy was from the effects of statins on cholesterol or inflammation. The proof of concept trial that confirmed the role of inflammation in the causal pathway of ASCVD later came from the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) trial in which a monoclonal antibody, canakinumab, targeted against IL-1β, significantly reduced ASCVD events with no effect on LDL-C [16]. This was followed by the Low-Dose Colchicine after Myocardial Infarction (COLCOT) trial which showed that non-selective inhibition of inflammation using colchicine significantly reduced ASCVD-related events in patients with high ASCVD risk [17].

The purpose of this review is to summarize the role of inflammation in ASCVD and examine the current evidence for measuring and targeting inflammation in patients with and without ASCVD.

2. Inflammation and the pathobiology of atherosclerosis

Inflammation is a key driver of all the steps involved in athero-thrombosis (Fig. 1) [18]. At the inception of atherosclerotic lesions, endothelial dysfunction and subintimal cholesterol accumulation ignite a subintimal inflammatory response. Upregulation of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and a variety of selectins promote the binding, rolling, and transmigration of inflammatory cells such as monocytes and T helper cells to early plaque initiation sites. Infiltrating monocytes can become resident macrophages in the subendothelial space [19]. At a molecular level, the formation of the Nod-like receptor protein 3 (NLRP3) inflammasome in macrophages is a key step in propagating inflammation [20]. The inflammasome, which is a complex cytosolic multiprotein, is formed when macrophages primed through activation of the nuclear factor kappa B (NF-kB, a nuclear transcription factor
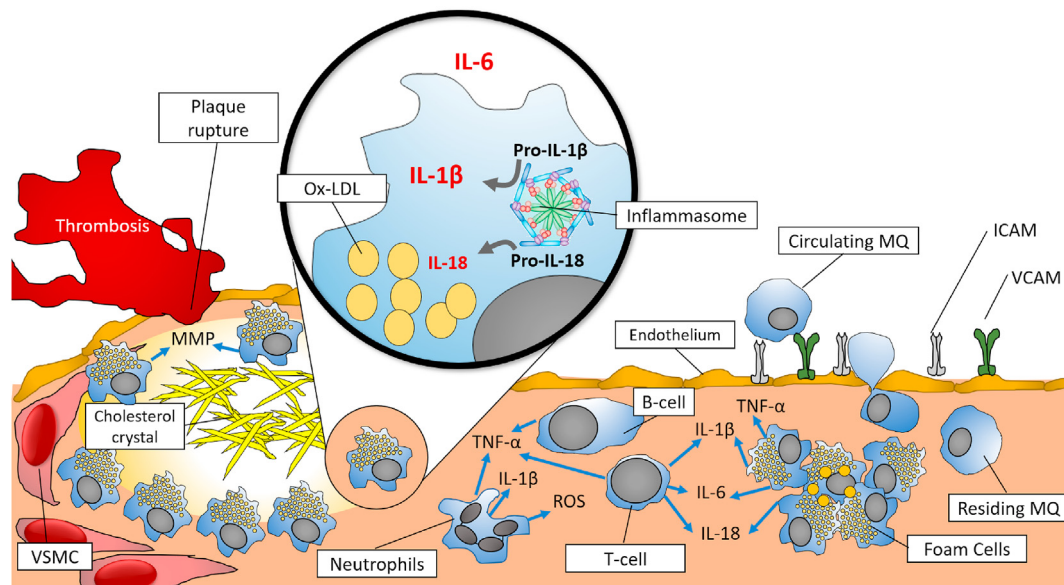
Fig. 1. Figure legend: inflammation plays a key role in all phases of atherosclerosis.
Abbreviations: ICAM, Intercellular adhesion molecule; VCAM, Vascular cell adhesion molecule; IL, interleukin; MQ, macrophage; MMP, metalloproteinases; VSMC, vascular smooth muscle cell; Ox-LDL, oxidized low-density lipoprotein.

regulating the expression of genes involved in inflammation) pathway receive a second hit such as from cellular hypoxia or engulfed cholesterol crystals. The result of the inflammasome formation is the production of IL-1β from pro-IL-1β [20]. Similarly, pro-IL-18 is cleaved to its active form IL-18. These cytokines are released to activate a variety of inflammatory cells and produce IL-6, which stimulates the production of CRP from the liver and amplifies the inflammatory cascade within the vessel wall [21].

Within the inflammatory cascade are layered and redundant mechanisms that add significant complexity to development and progression of atherosclerotic plaque. This involves upregulation of cytokines and interleukins, and production of such reactive oxygen species as peroxide, superoxide anion, and peroxynitrite [18,22]. Moreover, other inflammatory cells such as T-cells, mast cells, and dendritic cells contribute by amplifying cytokine production and signaling (e.g. by producing large amounts of interferon-γ and tumor necrosis factor-α [TNF-α]) that modulate plaque formation and growth [23,24]. Eventually, the accumulation of lipid-laden macrophages (also known as foam cells) leads to the formation of a necrotic lipid core secondary to impaired macrophage efferocytosis [25].

Inflammation also plays a key role in determining the architectural stability of complex atherosclerotic plaques by influencing the formation and destabilization of collagen in the fibrous cap. Cytokines released from foam cells, T-cells and other cells stimulate the migration of vascular smooth muscle cells into the intima and the production of interstitial collagens to form the extracellular matrix surrounding the necrotic lipid core [26]. IL-1β plays an important role in the production of matrix metalloproteases 1, 8, and 13, which degrade collagen in the fibrous cap. Together with lipid core growth, thinning of the fibrous cap leads to plaque instability with increased risk for rupture and formation of overlying thrombus formation, resulting in myocardial ischemia and acute coronary syndromes [26].

2.1. Inflammation and plaque calcification

Inflammation plays an important role in atheromatous plaque calcification. Proinflammatory cytokines released by activated macrophages in atherosclerotic plaques lead to vascular smooth muscle cell apoptosis and release of matrix vesicles high in calcium and phosphate that form the nucleation site for calcium deposition [27,28]. Furthermore,

proinflammatory cytokines such as TNF-α induce osteogenic differentiation of vascular smooth muscle cells into osteoblast-like cells that accelerate intimal calcification within the plaque [29]. These initial calcium foci are identified as areas of macrocalcification which can then propagate a persistent inflammatory response inducing further cell apoptosis, fibrous cap thinning, and an increase in mechanical stress within the plaque favoring plaque rupture [30]. Microcalcifications are often missed on contrasted computed tomography (CT) scanning but are more reliably detected using non-contrast cardiac-gated CT imaging with thin slice reconstruction ($\leq$0.5 mm slice thickness) or with molecular imaging techniques using positron emission tomography (PET)/CT with [18F] fluoride as a marker of newly forming microcalcification [31,32].

When inflammation is reduced, vascular smooth muscle cell survival is enhanced and regulated mineralization prevails within the plaque, eventually causing calcium layering within the plaque leading to macrocalcification. As a result, macrocalcification often represents a more advanced stage of inflammation and is present in less inflamed plaques that are more stable and less likely to rupture [33]. This observation may, at least in part, explain why therapies with anti-inflammatory effects such as statins are associated with a reduction in CVD risk but progression of plaque macrocalcification as inflamed plaques become quiescent [34,35]. Interestingly, observational evidence suggests that the progression of macrocalcification seen with statin therapy may be attenuated by the concomitant use of PCSK9 inhibitors [36]. PCSK9 inhibitors attenuate local vascular inflammation and accelerate plaque delipidation when added to statins [37]. However, unlike statins, PCSK9 inhibitors have the benefit of lowering lipoprotein (a), a lipoprotein known to potentiate vascular calcification [38,39]. Macrocalcifications are best visualized on cardiac-gated CT imaging without contrast. The burden of coronary artery macrocalcification correlates highly with total atherosclerotic disease burden, including the burden of noncalcified plaque [40].

2.2. Inflammation post myocardial infarction

Data from animal models show that inflammation may play a significant role in accelerating atherosclerotic plaque growth in the immediate period after an MI. This effect persists for weeks post-infarction [41]. In an ApoE knock-out mouse model, the induction of MI by selective coronary artery ligation increased inflammatory gene expression and

resulted in enhanced inflammatory cell migration into atherosclerotic plaques [41]. Moreover, these mice post MI experience accelerated plaque growth with larger necrotic core volumes, higher protease activity, and reduced fibrous cap thickness in distant aortic plaques that persisted 3 weeks after MI. Compared with patients with stable angina, post-MI patients have increased inflammation within aortic atherosclerotic plaques as measured by 18-F PET imaging shortly after MI (median time between MI and PET scan = 11 days) despite the high use of aspirin and statin therapy [42]. Furthermore, [^{18}F] FDG PET uptake also increases in the bone marrow and spleen as mobilization of inflammatory cells occurs to aid in myocardial repair (termed the cardiosplenic axis) [43].

2.3. Inflammation and calcific aortic valve disease

Of interest, the role of inflammation in coronary artery calcification (CAC) is similar to that in aortic valve calcification. Contrary to the previous hypothesis that calcific aortic stenosis is a disease of passive wear-and-tear, more evidence suggests an active role of inflammation in disease progression [44]. Early evidence from pathology studies demonstrates the same atherosclerotic plaque components within the aortic valve leaflets, with an abundance of foam cells and an inflammatory infiltrate preceding calcification [45]. Subendothelial oxidized LDL (ox-LDL) induces increased proinflammatory cytokine expression, leading to immune cell recruitment including macrophages, T-cells, and B-cells with an in increase in IL-6 and TNF-α release [46]. Similar to coronary plaque, osteoprogenitor cells differentiate into osteoblast-like cells under the influence of the inflammatory mediators leading to calcium deposition [47]. Impaired clearance of calcium deposits promotes calcium layering and eventually reduced leaflet mobility and aortic valve stenosis with time [46].

3. The origin of inflammation

Several factors contribute to the development of inflammation (Fig. 2). These include modifiable and non-modifiable risk factors which are reviewed here.

3.1. The role of genes

Genetic traits modulate risk of CVD through multiple pathways related to cholesterol, salt balance, and inflammation [48]. Valuable insight has been obtained from population wide genetic studies on the role of genes encoding inflammatory mediators and other CVD risk markers. Several genetic polymorphisms determine the levels of circulating cytokines and inflammatory markers [49]. Studying differences in gene expression of inflammatory mediators may explain why some inflammatory responses vary between individuals with similar risk factors. In addition, Mendelian randomization studies offer insights into potential inflammatory targets contributing to CVD [50].

The genes encoding for IL-1α, IL-1β are present on chromosome 2. The genetic evidence linking polymorphisms of genes involved in IL-1 production have been conflicting [21]. In a Mendelian randomization study from the Interleukin 1 Genetic Consortium, genetic polymorphisms which increased the expression of IL-1Ra (an endogenous IL-1 inhibitor) were associated with an increased risk of developing coronary heart disease [51]. However, this finding has several limitations. First, increased IL-1Ra expression is also associated with higher levels of atherogenic lipids which may have independently increased the risk for coronary heart disease. Second, because IL-1Ra is an endogenous inhibitor of both IL-1α and IL-1β, genetically mediated elevation in IL-1Ra does not inform on the effects of selectively inhibiting IL-1α vs. IL-1β. Lastly, the lack of a standardized assay shared between studies used in the Mendelian randomization analysis may have limited the accuracy in estimating the associations between genetic variants of IL-1β and plasma IL-1β levels [21].

In a collaborative meta-analysis of 82 studies measuring IL-6 receptor (IL-6R) polymorphisms known to affect the IL-6R signaling pathway, polymorphisms at rs2228145 and rs7529229 were associated with lower
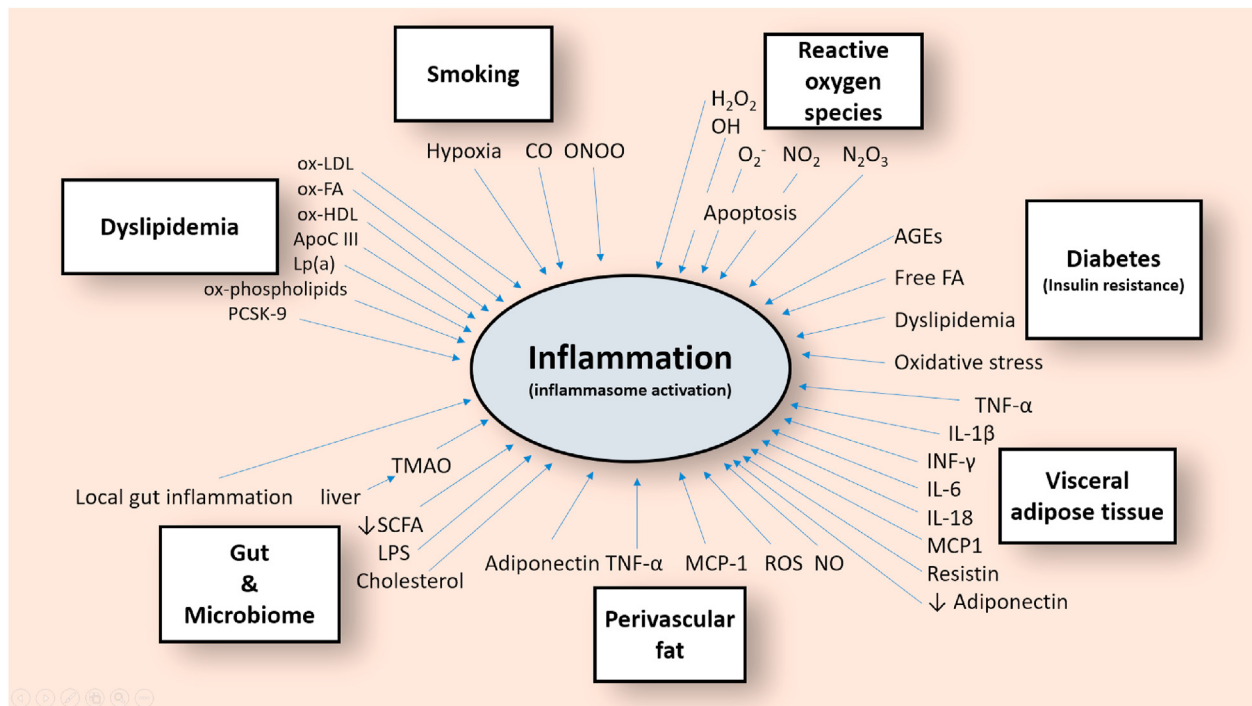

Fig. 2. Figure legend: Risk factors and mediators of inflammation.
Abbreviations: AGEs, Advanced glycation end products; FA, free fatty acids; TNF-α, tumor necrosis factor-α; IL, interleukin; INF-γ, interferon-γ; MCP-1, monocyte chemoattractant protein-1; ROS, reactive oxygen species; SCFA, short-chain fatty acids; LPS, lipopolysaccharides; TMAO, trimethylamine N-oxide; Ox-LDL, oxidized low-density lipoprotein; HDL, high-density lipoprotein; ApoC III, apolipoprotein C III; Lp(a), lipoprotein (a).

incident CAD with no alteration in lipid levels, blood pressure, or adiposity [52]. These selected polymorphisms were also associated with lower lifetime hsCRP levels as a downstream product of IL-6. This suggested a causal association between the IL-6R related pathway and incident CAD. Data on genetic polymorphisms that determine circulating TNF-α levels show conflicting results regarding its genetic association with MI [49]. TNF-β is closely linked to TNF-α and not associated with MI risk in genetic association studies. Similarly, polymorphisms of the TNF receptor 1 and 2 were not associated with the development of CAD [53].

Polymorphisms in genes involved in the production of cytokines may in part explain differences in ASCVD risk between different races. Van Dyke et al. studied 103 African American and 380 Caucasian women and found between-race differences in 52 single nucleotide polymorphisms associated with key inflammatory pathways. These findings suggest increased genetic expression of the proinflammatory IL-1 and lower expression of the anti-inflammatory IL-10 in African Americans compared with Caucasians [54]. In addition, a genome-wide association study involving 3109 African American and 6050 European Americans has found that ApoE ε2 rs7214 single-nucleotide polymorphisms in African Americans are associated with higher CRP levels compared with European Americans [55].

When taken together, findings from population based genetic association studies highlight the significant variation between individuals in levels of circulating biomarkers. Moreover, genetic polymorphisms may also determine the prognostic value of measuring circulating cytokines. Population based genetic association studies also demonstrate that single gene polymorphisms have limited effects on overall heritable traits and cytokine levels [56]. Therefore, single genetic polymorphisms have very limited contribution to overall CVD risk. Understanding genetic polymorphisms may shed light on potential mechanisms why some individuals may have variable responses to targeted anti-inflammatory therapies. For example, in the CANTOS trial, not all individuals randomized to canakinumab experienced hsCRP lowering [16]. Whether certain genetic traits contributed to this variation in treatment effect remains unclear.

3.2. How lifestyle contributes to inflammation

3.2.1. Smoking

Smoking is the number one modifiable ASCVD risk factor. Tobacco smoke contains more than 6000 compounds which include direct mutagenic, carcinogenic, and cytotoxic agents as well as antigenic compounds and free radicals [57]. These compounds ignite a substantial inflammatory response and modulate the immune system, leading to simultaneous dysfunction and immunosuppression of cells regulating immunity [58]. By increasing the burden of free radicals, smoking induces genetic mutations and unregulated activation of pro-inflammatory genes and expression of pro-inflammatory cytokines [59]. Smoking is also associated with an increase in release of intracellular antigens and dysregulation of B-cell activity [60]. Smoking results in an acute rise in neutrophil and circulating T-cell counts and activity [61,62]. Nicotine may independently increase the production of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 through inhibition of the α7 nicotinic acetylcholine receptor in macrophages [63]. In addition to the systemic pro-inflammatory effects of compounds associated with smoking, smoking induces significant local inflammation in the lung parenchyma [64]. This results in local pro-inflammatory cytokine production which then spills-over to the systemic milieu contributing to systemic inflammation [64]. Smoking also has deleterious effects on innate and acquired immunity leading to immunosuppression. As a result, smoking is associated with higher rates of clinical and subclinical cancers and infections [58].

Multiple large prospective studies confirm the strong association between smoking and elevated inflammatory markers and ASCVD risk [65,66]. The strongest evidence is for the association between smoking and IL-6 levels and white blood cell (WBC) count. Data from

observational studies show persistently elevated hsCRP levels in smokers or former smokers with multiple cardiac risk factors [67,68]. In 843 self-reported current smokers enrolled in the Multi-Ethnic Study of Atherosclerosis (MESA), hsCRP was the most sensitive biomarker of low-grade inflammation in smokers [69]. In a study of 2920 men with no history of ischemic events, current cigarette smokers had significantly higher levels of hsCRP, and fibrinogen and a higher WBC count compared with participants who never smoked [68]. Ex-smokers had intermediate levels of these inflammatory markers. Observational data also support a strong dose-dependent association between smoking intensity measured by the number of cigarettes smoked per day and elevations of blood IL-6 and hsCRP levels [70].

3.2.2. Diet

Several observational studies have shown an association between unhealthy dietary habits and low-grade inflammation. In a randomized cross-over design study, a diet high in saturated fat (39% energy from fat) was associated with an increase in CRP and E-selectin compared to a control diet (30% energy from fat) in healthy male adults [71]. In individuals with type 2 diabetes, a high saturated fat diet was associated with higher IL-6, TNF-α, ICAM-1, and VCAM-1 levels [72]. Dietary hydrogenated *trans* fats are associated with increased vascular inflammation by inducing NF-kB and the production of IL-6 [73]. Furthermore, high intake of *trans* fats was found to be associated with higher CRP and fibrinogen levels [74] and increased risk of ischemic heart disease [75].

By contrast, a diet higher in polyunsaturated fatty acids such as alpha-linoleic acid was associated with a reduction in CRP, VCAM-1, and E-selectin compared to a diet higher in linoleic acid [76,77]. A high-fiber diet is associated with lower CVD in epidemiological studies. In a large US sample, dietary fiber was associated with lower levels of CRP, suggesting that lower levels of inflammation may mediate the beneficial effect of high-dietary fiber [78]. Higher intake of sugar is directly correlated with plasma CRP, IL-6, ICAM-1 and VCAM-1 levels [79]. Similarly, higher consumption of high-glycemic index foods is associated with increased levels of oxidative stress markers [80] as well as increases in NF-kB activation in mononuclear calls [81]. In a meta-analysis of intervention studies of dietary sugar intake, substituting glucose sugars with dietary fructose (either alone or as found in high-fructose corn syrup) was associated with a nonsignificant decrease in hsCRP levels (mean difference in hsCRP = −0.3 mg/L; 95% CI, −0.52 to 0.46) [82].

3.3. Sedentary lifestyle and lack of exercise

Observational data suggests a strong association between lack of exercise and markers of inflammation [83]. In a study of 285 adults in the ACTivity in Diabetes trial, greater sedentary time was directly associated with higher levels of IL-6, and a reduction in sedentary time was associated with a reduction in hsCRP levels after controlling for potential confounders [84]. Similarly, in a diabetes prevention program including 558 participants, activity recorded using a wearable accelerometer was inversely associated with IL-6, CRP and leptin levels [85]. Interestingly, moderate-to-vigorous physical activity ameliorated the effects of sedentary time on CRP and leptin levels but not IL-6 levels, which continued to be independently associated with sedentary time after adjusting for the amount of recorded physical activity, HbA1c levels, and adiposity measured by body mass index [84].

3.4. Obesity and inflammation

Insulin resistant adipose tissue is metabolically active and releases a variety of inflammatory mediators, such as IL-1, IL-6 and TNF-α, contributing to low-grade inflammation. Through the production of MCP-1, adipocytes potentiate local inflammation and recruit monocytes/macrophages and other inflammatory cells into the interstitial space of adipose tissue [86]. Evidence from FDG-PET imaging shows high levels of local inflammation in visceral fat, including omental and hepatic fat.

Of note, subcutaneous fat does not exhibit the same degree of local inflammation compared to omental fat [87]. In an interesting study of obese patients undergoing surgical weight reduction, large-volume abdominal liposuction was not associated with significant reduction in inflammatory markers. By contrast, obese patients who experience weight loss with bariatric surgery have significant improvement in levels of inflammatory biomarkers [88]. Increasing visceral adiposity is associated with a significant increase in blood biomarkers of inflammation secondary to the development of insulin resistance and potentially leptin resistance [89].

Several inflammatory pathways also link obesity to metabolic syndrome and increased ASCVD risk. As an example, TNF-α released by macrophages infiltrating adipose tissue activates intracellular kinases leading to serine phosphorylation of insulin receptor substrate-1 (IRS-1); this in turn impairs insulin signaling in the liver, skeletal muscle, and other tissue causing insulin resistance. Moreover, advanced glycation end products from insulin resistance and free fatty acids released from adipose tissue promote both inflammation and oxidative stress [86].

3.5. Dyslipidemia contributes to inflammatory risk

Dyslipidemia contributes to inflammation by several mechanisms. In the setting of insulin resistance, insulin cannot appropriately inhibit hormone sensitive lipase, an intracellular enzyme that hydrolyzes stored triglycerides within adipocytes and releases free fatty acids [86]. Circulating free fatty acids may trigger inflammation by activating toll-like receptor-2 and nod-like receptors on cells surfaces leading to NF-kB pathway activation [86,90].

Circulating free fatty acids are also taken up by hepatocytes where they are converted into triglycerides and released back into the circulation within apolipoprotein-C III (ApoC III) laden very low-density lipoproteins (VLDL) particles [91]. The expression of ApoC III on VLDL is enhanced in the setting of insulin resistance [92]. ApoC III contributes to inflammation by activating the toll-like receptor-2 (TLR2) signaling pathway [86]. This leads to upregulation of NF-kB and cytokine production. ApoC III also enhances the adhesion of monocytes to endothelial cells by upregulating the expression of VCAM-1 [93].

In addition to driving reverse cholesterol transport, HDL particles and ApoAI exert anti-inflammatory effects [94]. HDL inhibits the expression of adhesion molecules by inhibiting endothelial cell sphingosine kinase, a key enzyme in the TNF-α pathway [95]. HDL also inhibits MCP-1 expression in response to oxidized LDL [96]. Moreover, HDL protects LDL particles from oxidative damage and prevents the formation of oxidized and peroxidized LDL via the action of paraoxonase and glutathione selenoperoxidase-3, among other enzymes [97]. ApoAI is anti-oxidative secondary to its redox-active methionine residues [97].

4. Dysregulated immunity and accelerated atherosclerosis

4.1. Rheumatologic diseases

Patients with chronic inflammatory rheumatic diseases have higher systemic levels of inflammatory markers such as hsCRP, TNF-α and IL-6 [98]. This is associated with a higher prevalence of vascular inflammation and arterial macrophage accumulation. Furthermore, low-grade inflammation associated with rheumatological illnesses is associated with the development of insulin resistance and oxidative stress. As a result, patients with chronic inflammatory rheumatic diseases suffer accelerated atherosclerosis. Early observational evidence suggested a strong independent association between chronic inflammatory rheumatic diseases and CVD morbidity and mortality [98]. On average, patients with rheumatoid arthritis have 1.5 times the risk of CVD death compared with the general population [99]. Similarly, patients with systemic lupus erythematosus have 2–3 times the risk of CVD death compared with the general population [100]. For this reason, the 2019 American College of Cardiology (ACC)/American Heart Association

(AHA) Guideline for the Primary Prevention of CVD considers auto-immune and inflammatory diseases as a "risk enhancer" that would favor statin initiation or intensification in primary prevention patients [101].

4.2. HIV and atherosclerosis

Patients with human immunodeficiency virus (HIV) infection have higher risk for developing ASCVD. Several factors such as persistent viral replication contribute to this increased risk by inducing a state of chronic inflammation in HIV patients [102]. In the Strategies for Management of Antiretroviral Therapy (SMART) trial, continuous HIV viral suppression was associated with a significant decrease in a composite endpoint of cardiovascular, renal or hepatic disease. The benefit of viral load suppression was overall consistent when each of the components of the endpoint were analyzed individually [103]. Chronic HIV infection leads to simultaneous CD4$^+$ T-cell activation and T-cell suppression. In HIV infected patients, CD4$^+$ T-cell count strongly predicts CVD risk independent of traditional risk factors [104]. Chronic inflammation in HIV infected individuals leads to endothelial dysfunction and inappropriate endothelial activation as well as expression of VCAM-1 molecules [105, 106]. This activation of endothelial cells is reversed with proper HIV antiviral therapy [107]. When HIV infection is left untreated, higher interferon-α levels are associated with higher triglyceride levels which may induce inflammation directly through ApoC III [108]. Similar to autoimmune disorders, the 2019 ACC/AHA Primary Prevention Guideline also considers HIV infection as a "risk enhancer" that would favor more intensive preventive interventions [101].

5. Infections and atherosclerosis

Observational evidence supports a link between chronic infections and atherosclerosis [109]. However, the strength of association varies by organism [109]. Infections caused by viruses such as the cytomegalovirus, influenza A, and hepatitis C and bacteria such as Chlamydia pneumoniae and *Helicobacter pylori* have been associated with the development of atherosclerosis through local and systemic pathways. These pathways include upregulation of adhesion molecules on endothelial cells, increased local and systemic production of pro-inflammatory cytokines such as IL-1β and INF-γ, and increased expression of heat shock protein [109]. It remains unclear whether the increase CVD risk associated with infection is a direct and specific consequence of the involved pathogen itself or secondary to the heightened systemic inflammation associated with infection [109]. Interestingly, there is evidence that high level of inflammation driven by infection in the presence of few CVD risk factors may not be adequate to increase CVD risk. This is exemplified in the Tsimane population, a forager-horticulturalist population in Bolivia, in which the burden of chronic inflammation is high (prevalence of hsCRP > 3 mg/L was 51%) due to a high burden of mostly chronic parasitic infections, but the prevalence of CAD measured by CAC is among the lowest recorded in human populations [110].

6. Measuring inflammation

A few clinical scenarios in the current practice of cardiovascular medicine warrant measuring inflammation. One example is the use of CRP in risk stratifying patients to guide statin therapy in primary prevention. Several biomarkers have been tested and are reviewed here:

6.1. Blood biomarkers of inflammation

6.1.1. CRP
CRP is one of the few biomarkers of inflammation that moved from the lab to clinical workflow. The majority of CRP is produced by the liver as an acute phase protein in response to circulating cytokines, especially IL-6. Although many observational studies have shown strong

associations between CRP levels coronary atherosclerosis [111], and vascular risk [112], CRP itself is not causally linked to atherothrombosis [111]. This is supported by evidence from Mendelian randomization studies showing that genetic polymorphisms associated with high CRP levels in humans were not associated with a significant increase in coronary heart disease [113].

Compared to other inflammatory biomarkers such as interleukins, the hsCRP assay has favorable test characteristics. hsCRP levels are generally stable over time with repeated measures and add incremental value to other lipid biomarkers in predicting CVD [114,115]. The Reynolds risk score is the only risk score that incorporates hsCRP measurement into ASCVD risk prediction. Both the Reynolds and Pooled Cohort equation (PCE) have high agreement on risk prediction in low risk patients [116]. However, this agreement diminishes with high risk levels. The utility of measuring hsCRP for risk stratification was highlighted by the JUPITER trial (described above) as it identified a subset of patients at risk for CVD who would not have been considered for statin treatment at the time of the trial but benefited when randomized to statin therapy [14]. In the context of ASCVD prevention, the 2019 ACC/AHA guideline on primary prevention of CVD considers elevated hsCRP, if measured, a risk-enhancing factor that would favor statin therapy when the 10-year ASCVD risk estimates are uncertain and patients fall into a borderline (5% to <7.5%) or intermediate ($\geq$7.5% to <20%) estimated risk category [101].

Although hsCRP predicts vascular risk, the predicted increased risk associated with elevated hsCRP levels in those with known risk factors is only modest. In a study by the Emerging Risk Factors Collaboration, adding hsCRP to a CVD prediction model based on traditional risk factors was associated with a net reclassification improvement of 1.52% in those with low (<10%) and intermediate (10–20%) estimated 10-year CVD risk [114]. Moreover, this improvement in risk prediction with adding hsCRP measurement was more prominent in males than females, patients who smoked at the time of measurement than non-smokers, and in patients with higher estimated 10-year ASCVD risk (>10%) than those with low estimated risk (<10%). Observational evidence from the Multi-Ethnic Study of Atherosclerosis suggested that hsCRP predicted all CVD events only in Caucasians but not in African American, Hispanic or Chinese participants [117].

With respect to secondary prevention, the utility of measuring hsCRP is currently unclear in the absence of approved anti-inflammatory therapies based on hsCRP measurement. However, as therapies targeting inflammation in secondary prevention become available for clinical use the role of measuring hsCRP may become more important in the future.

Inflammatory biomarkers upstream of CRP have also been linked to vascular risk. In both the Women's Health Study and Physicians' Health Study, healthy individuals with the highest quartile of IL-6 levels at baseline had around 2 times the risk of cardiac events compared to those in the lowest quartile [11,112]. In patients with acute coronary syndromes, levels of IL-6 were an independent predictor of increased mortality [118]. Unlike CRP, IL-6 is partially linked to the causal pathway in atherosclerosis as IL-6 has been shown in animal models to contribute to atherosclerotic plaque initiation and progression [21]. By contrast, there is a strong evidence of the causal link between IL-1 and atherosclerosis [21]. However, IL-1 measurement in epidemiological studies has been limited. Measuring biomarkers upstream of CRP (e.g. IL-1, IL-6, TNF-α) did not have similar success transitioning into clinical workflow due to several factors. These include biomarker instability in collected blood, limited assay availability, lack of assay standardization to allow comparisons, low assay precision and cost of testing.

6.1.2. Myeloperoxidase (MPO)

Myeloperoxidase (MPO) is a homodimer protein which catalyzes the oxidation and peroxynitrosylation of molecular targets [119]. MPO is locally released by inflammatory cells in response to bacterial infections but is also known to induce LDL oxidation and alter ApoA1 leading to the inactivation of HDL [120,121]. Higher free plasma levels of MPO have

been associated with vascular risk in observational studies. In apparently healthy individuals with low CVD risk in The European Prospective Investigation Into Cancer in Norfolk Prospective Population Study (EPI-C-Norfolk), elevated MPO predicted the risk of CAD [122]. In patients with chest pain or prior MI, plasma levels of MPO predicted the future risk of major adverse cardiac events [123]. To date, there are no clinical cardiovascular trials targeting MPO levels and with the current evidence available, the clinical utility of measuring MPO is questionable.

6.1.3. Lp-PLA2

Lipoprotein-associated phospholipase-A2 (Lp-PLA$_2$) is another plasma biomarker of inflammation with predictive value. Lp-LPA$_2$ is produced by macrophages or by macrophage-derived foam cells within atherosclerotic plaques [124]. This enzyme catalyzes the catabolism of oxidized phospholipids and release of pro-atherogenic proinflammatory oxidized fatty acids and lysophosphatidylcholine [125]. As a result, Lp-PLA$_2$ induces oxidative stress and contributes to the progression of atherosclerotic plaque [126]. When released into the circulation, Lp-PLA$_2$ is mainly carried on LDL and is bound to its apoprotein B100 component [125]. In patients $\geq$55 years old enrolled in the Rotterdam Study, higher plasma levels of Lp-PLA$_2$ were associated with a greater likelihood of developing CAD events independent of non-HDL-C levels [127]. The Integrated Biomarker and Imaging Study 2 suggested that Lp-PLA$_2$ inhibition using darapladib, a selective oral inhibitor of Lp-PLA$_2$, reduced plaque volume over 12 months [128]. However, darapladib failed to demonstrate benefit in large cardiovascular outcome trials. In the Phase III Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy (STABILITY) trial, 15,828 patients with stable CAD were randomized to darapladib vs. placebo [129]. After a median of 3.7 years, those randomized to darapladib did not experience benefit with regards to the primary composite outcome of cardiovascular death, myocardial infarction, or stroke. However, darapladib was associated with a nominal reduction in the prespecified secondary composite endpoint of major coronary events (HR, 0.90; 95% CI, 0.82 to 1.00; P = 0.045). Therefore, in the absence of an effective targeted therapeutic with clear ASCVD benefit, the utility of measuring Lp-LPA$_2$ remains uncertain.

6.1.4. Trimethylamine-N-oxide (TMAO)

Recent attention has focused on the role of TMAO in accelerating atherosclerosis. TMAO levels are elevated in individuals with higher dietary consumption of L-carnitine and choline. Both L-carnitine (mostly present in red meat) and choline (found in eggs) are present in high levels in animal-based food but are also present in small quantities in plant-based foods. After ingestion, bacteria residing in the gut convert these two compounds into trimethylamine, which is absorbed and converted by flavin containing monooxygenases-3 (FMO3) in the liver to TMAO.

TMAO has been implicated in inflammation and accelerating atherosclerotic plaque formation and enhancing platelet activity [130, 131]. Although TMAO levels do not increase LDL-C levels, in animal models, TMAO has been shown to up-regulate macrophage scavenger receptors CD36 and SR-A1, thereby increasing LDL-C uptake by macrophages and accelerating the formation of foam calls [132]. This is attenuated by administering poorly absorbed broad-spectrum antibiotics which inhibit bacteria forming TMAO precursors in the gut [132,133]. TMAO also promotes atherosclerosis by activating the cluster of differentiation (CD)36-dependet mitogen-activated protein kinases/Janus kinases (MAPK/JAK) pathway [134] and inducing the expression of pro-inflammatory cytokines and adhesion molecules through a NF-kB mediated pathway [135]. TMAO enhances platelet activation and is linked to thrombosis in vivo [136].

In humans, the levels of TMAO were associated with atherosclerotic CAD burden [137] and mortality in CAD patients in a dose-dependent fashion [138]. In a systematic review of 19 prospective studies, elevated TMAO and its precursors were associated with all-cause mortality and major adverse CV events [139]. The utility of measuring TMAO

levels in clinical practice is unclear, especially in the absence of treatments to lower TMAO levels beyond a heart healthy diet, which should be recommended broadly for patients with and without ASCVD. Moreover, some of the challenges in measuring TMAO includes the lack of standardized tests, and lack of association studies between TMAO and CVD in non-white populations.

6.2. Imaging biomarkers of coronary inflammation

Non-invasive imaging of inflammation has the advantage of localizing inflammation and providing functional and structural assessment of the coronary arteries. The advent of novel tracers which facilitate the visualization of arterial inflammation has promoted a better understanding of the role of inflammation in CVD and how it changes with therapy.

6.2.1. PET imaging

Positron emission tomography (PET) using [18F] FDG radiotracer has been widely applied in clinical studies measuring vascular inflammation in atherosclerotic plaque. [18F]FDG is a glucose analog that identifies metabolically active cells such as macrophages. As a result, inflamed atherosclerotic plaques with high macrophage density have higher uptake of [18F]FDG on PET imaging. [18F]FDG on PET may identify different stages of atherosclerosis, including fatty streaks, before they are detectable by other forms of structural imaging [140]. In a subgroup analysis of the Progression of Early Subclinical Atherosclerosis (PESA) study, individuals with higher numbers of CVD risk factors had higher [18F]FDG uptake in the femoral, iliac, aortic or carotid arteries [141]. Compared to healthy controls, the measured uptake of 18F-FDG for the carotid arteries and ascending aorta is >52% higher for patients with a Framingham risk of >10% at increased risk of CVD, and >67% in patients with known CVD [142]. PET FDG has also been used to demonstrate response to therapies with anti-inflammatory effects. In a meta-analysis of 7 statin studies, PET FDG showed a reduction in arterial wall inflammation that was independent from hsCRP or cholesterol levels [143]. Coronary plaques with high [18F]FDG were also more likely to have high-risk features such as positive remodeling, microcalcification, and necrotic core on intravascular ultrasound imaging compared with lesions with no [18F]FDG uptake [144]. Some of the challenges in PET FDG imaging reside in the lack of standardized imaging protocols to allow wide comparison of results and the lack of specificity in FDG uptake. A recent report showed that FDG uptake is not different in lesions with various densities of macrophages [145]. These challenges have led to the development of other more specific tracers such as [68Ga] 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTATATE), which targets somatostatin receptor 2 (SSTR2) found on macrophages in atherosclerotic lesions. Other PET tracers such as [11C] PK11195, which targets macrophage translocator protein (TSPO), and [68Ga]pentixafor,81–83 targeted to the macrophage chemokine receptor (CXCR)-4, recently gained attention but are still under investigation (Table 1) [146].

6.2.2. Perivascular adipose tissue

Perivascular adipose tissue imaging has gathered interest as a novel biomarker of coronary inflammation. Local coronary inflammation inhibits perivascular adipogenesis [147]. This is detected as a lower attenuation fat when measured by coronary computed tomography. In the Cardiovascular RISk Prediction using Computed Tomography (CRISP-CT) study, high perivascular fat attenuation around the left anterior descending and right coronary arteries was associated with higher risk of all-cause and cardiac mortality [148]. Furthermore, measuring perivascular fat attenuation improved risk prediction of cardiac events above and beyond traditional risk factors combined with CT measured cardiac risk marker such as CAC and plaque burden. Interestingly, perivascular fat attenuation weakly correlated with hsCRP levels and lost its predictive ability in those who were started on statins or aspirin after CT imaging. However, the application of perivascular fat attenuation in risk stratification and clinical workflow may be limited due to the complexity in measuring perivascular fat attenuation and the need for dedicated image processing software and workstations. Nonetheless, perivascular fat attenuation may be a useful biomarker in clinical trials for the detection of response to novel therapies.

6.3. The utility of measuring inflammation beyond markers of plaque burden

Both blood and imaging biomarkers are useful in predicting ASCVD risk. However, the utility of combining both in risk prediction is less clear. A question that remains is whether markers of inflammation may provide added benefit in risk prediction over markers of plaque burden.

A recent analysis from the Bioimage study compared multiple negative risk markers for cardiovascular risk [149]. Among adults >55 years of age with a majority (86%) having a 10-year ASCVD predicted risk of ≥7.5%, an hsCRP lower than the 25th percentile (<0.09 mg/L) was a better negative predictor of risk than other blood markers such as having NT-proBNP or ApoB below the <25th percentile of the population, an imaging biomarker such as carotid intima media thickness (cIMT) < 25th percentile of the population, or a normal ankle brachial index (ABI). However, compared with low hsCRP, having CAC ≤10 or no carotid plaque was a better predictor of low CAD and CVD event rates. When comparing the negative diagnostic likelihood ratios for CAD of hsCRP to other imaging tests, low hsCRP (mean diagnostic likelihood ratio [DLR], 0.60) was a better predictor of low risk than having cIMT <25th percentile and a normal ABI, but was not better than a CAC of zero (mean DLR, 0.20) or ≤10 (mean DLR, 0.20) or no carotid plaque (mean DLR, 0.39) after adjustment for potential confounders.

6.3.1. Targeting inflammation in clinical practice

To date, there are no guidelines addressing low-grade inflammation for the prevention of ASCVD. However, several therapeutic options have been shown to have favorable effects and are reviewed here (Fig. 3).

Table 1
PET Tracers for vascular inflammation imaging.

Tracer	Molecular target	Comments
FDG	GLUT transporters	Activated cells and macrophages
18F-fluoromethylcholine	Choline analogues	Correlates with phospholipid metabolism in many cell types
68Ga-DOTATATE	Somatostatin receptor 2	Surrogate to M1 macrophages activity
64Cu-DOTATATE	Somatostatin receptor 2	Surrogate to M1 macrophages activity
68Ga-DOTATATE	Somatostatin receptor 2	Surrogate to M1 macrophages activity
68Ga-pentixafor	CXCR4	CXCR4 present on lymphocytes and monocytes/macrophages
11C-PK11195	Translocator protein	Surrogate to macrophages activity
11C-PK11195	Translocator protein	Surrogate to macrophages activity
18F-fluorothymidine	Thymidine analog	Different inflammatory cell type proliferation
18F–4V	VCAM-1	Endothelial cells expressing VCAM-1

Abbreviations: FDG, 18F-fluoro-2-deoxyglucose; GLUT, glucose transporter; DOTATATE, [1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid]-d-Phe1,Tyr3-octreotate; CXCR, CX chemokine receptor; VCAM, Vascular cell adhesion molecule.

6.3.2. Non-pharmacological options

There is ample evidence that adopting a heart healthy lifestyle is associated with significant reduction in ASCVD and inflammatory risk. Physicians' and other healthcare professionals should advocate for a heart healthy lifestyle as the foundation for improving ASCVD risk and reducing the burden of low-grade inflammation. Often this requires multi-level strategies to increase the likelihood of a positive sustainable change [150].

6.4. Diet

A healthy diet may have favorable effects on inflammation through weight loss-dependent and independent pathways. A Mediterranean diet is associated with a significant reduction in circulating levels of hsCRP, as well as interleukins 6, 7, and 18 [151]. In a meta-analysis of 17 clinical trials, a Mediterranean diet was associated with significant reductions in CRP, IL-6 and ICAM-1 levels and improvement in adiponectin levels [152]. In a recent open-label single blinded randomized controlled trial of patients with coronary artery disease (CAD), randomization to 8 weeks of a vegan diet was associated with a significant 32% reduction in hsCRP compared with a heart healthy AHA recommended diet [153]. However, a vegan diet did not provide better glycemic control, weight loss or improvement in the lipid profile compared with an AHA recommended diet. For patients at risk of CVD, the current 2019 ACC/AHA Primary Prevention Guideline recommends emphasizing dietary vegetables, fruits, legumes, nuts, whole grains, and fish to reduce CVD risk [101]. Furthermore, minimizing meat, refined carbohydrates, and replacing saturated fat with dietary monounsaturated or polyunsaturated fat may be beneficial in further reducing CVD risk.

6.5. Physical activity and exercise

The 2019 ACC/AHA guideline on primary prevention recommends at least 150 min of moderate-intensity or 75 min of vigorous intensity exercise per week [101]. Regular exercise lowers systemic inflammation. However, this anti-inflammatory effect occurs via complex interactions between the type and level of physical activity, muscles, and inflammatory cytokines [154]. In observational studies, regular exercise is associated with an inverse, dose-dependent association with inflammatory biomarkers [155]. In a systematic review of 83 randomized and non-randomized controlled trials, CRP levels decreased following exercise training [156]. Data from the Greek ATTICA study showed that adults free of CVD who reported high-physical activity (>7 kcal/min expended) had lower levels of inflammatory mediators including 20% lower TNF-α, 32% lower IL-6, 29% lower CRP, and 20% lower SAA levels compared with those with sedentary lifestyle [157]. These findings were independent of age, sex, smoking status, lipid parameters, or BMI. Similar findings were seen in those with and without metabolic syndrome [158], those with CAD [159], or chronic heart failure [160,161].

The inflammatory response to exercise is multiphasic. During acute exercise, IL-6 levels significantly increase proportional to exercise intensity and duration and the mass of muscle recruited, but independently of muscle injury [162]. Some evidence suggests that IL-6 is produced directly from muscle groups involved in the exercise. This is supported by a small study which showed increased expression of IL-6 mRNA within activated muscle and a localized increase in circulating IL-6 concentrations obtained from selective catheterization of veins draining activated muscle [163]. Both IL-1β and TNF-α levels increase but to a much lesser degree compared with IL-6 [154]. During exercise, there is also an increase in the production of anti-inflammatory mediators that balance the initial inflammatory response. This includes the production of IL-1Ra (which inhibits circulating IL-1), IL-10 (which inhibits the production of cytokines by monocytes and type 1 T-cells), and the production of macrophage inflammatory proteins 1-alpha and 1-beta (MIP 1-α, MIP 1-β) [164]. Other cytokines such as IL-8 are released in response to exhaustive exercise and play a role in promoting angiogenesis [165]. The acute release of IL-6 during exercise has a systemic effect which induces lipolysis, stimulates fatty acid oxidation, and increases insulin sensitivity [154].

Repeated exercise modulates the pro-inflammatory response seen with exercise through IL-6 dependent and independent pathways. Regular exercise is associated with a reduction in weight and an increase in adiponectin production which has both anti-inflammatory and insulin-sensitizing effects [166]. Moreover, exercise was associated with a greater decrease in hsCRP levels when a reduction in body mass index occurred. A notable finding is that 11% and 6.6% of the variation of the effect of exercise on hsCRP levels is explained by changes in body mass index and % body fat, respectively. Exercise also improves the lipid profile and blood pressure. All these effects are associated with reduced levels of systemic inflammation. However, in observational and experimental studies, the association between exercise and lower inflammation was independent from adiposity, insulin sensitivity, and other lifestyle
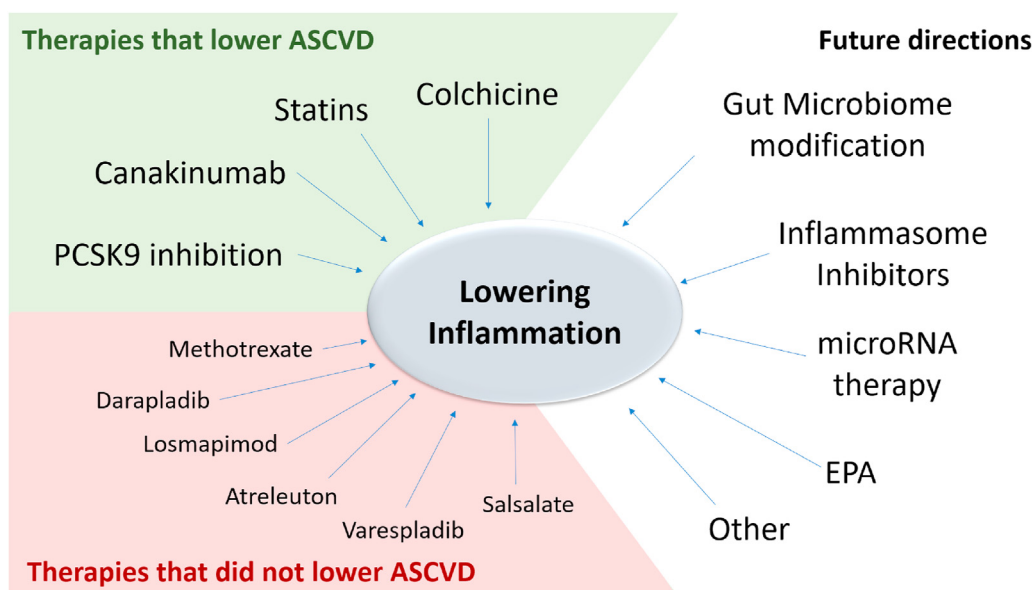

Fig. 3. Figure legend: Therapies targeting inflammation for atherosclerotic cardiovascular disease prevention. Abbreviations: ASCVD, atherosclerotic cardiovascular disease; EPA, eicosapentaenoic acid.

factors suggesting a direct anti-inflammatory effect of exercise. In an human experiment by Starkie et al., 3 groups of healthy men received E. coli endotoxin after either resting for 3 h (group 1), exercising for 3 h (group 2), or receiving recombinant IL-6 infusion for 3 h (group 3). Compared to the group which rested, the increase in TNF-α levels in response to E. coli endotoxins was two-fold lower in the exercise and recombinant IL-6 groups [167]. Similarly in a rat model, response to administration of bacterial lipopolysaccharides was blunted up to 6 h after exercise compared to rats that did not exercise [168]. Exercise training lowers the levels of TNF-α, IL-1β and IL-6 produced from muscle [160]. Furthermore, exercise training modulates monocyte cytokine production so that the production of anti-inflammatory cytokines (IL-10, IL-4, transforming growth factor-beta-1) is favored and proinflammatory cytokines (IL-1α, TNF-α and interferon-γ) are reduced. Exercise also improves endothelial function, and attenuates endothelial cell activation as well as the expression of adhesion molecules and chemokines [161]. To further elucidate the mechanisms by which exercise impacts the body, current ongoing projects including The Molecular Transducers of Physical Activity Consortium are expected to add valuable insight on the molecular and cellular changes associated with exercise with the aim of allowing physician to make tailored exercise recommendations for their patients [169].

6.6. Weight loss

Lifestyle changes aimed at reducing weight have been shown to reduce inflammatory markers. The 2019 ACC/AHA guideline on primary prevention recommends that overweight and obese individuals lose weight through exercise and dietary changes to improve ASCVD risk [101]. In a randomized controlled trial of 60 obese women with no diabetes, hypertension or hyperlipidemia, randomization to 10% weight reduction through a low-energy Mediterranean style diet and increased physical activity was associated with a significant 1.1 pg/ml reduction in serum levels of IL-6 and a 1.6 mg/L reduction in CRP while adiponectin levels increased by 2.2 mcg/mL [170]. In patients with morbid obesity, the effects of bariatric surgery on lowering ASCVD risk and inflammation are significant. In a systematic review of 116 studies, bariatric surgery was associated with significant reductions in hsCRP (pooled effect size, −5.3 mg/L; 95% CI -5.46 to −5.15), IL-6 (pooled effect size, - 0.58 pg/ml; 95% CI, - 0.64 to - 0.53)and TNF-α (pooled effect size, - 0.20 pg/ml; 95% CI - 0.39 to - 0.02) [171].

6.7. Smoking cessation

Smoking cessation significantly reduces ASCVD risk through multiple pathways including substantially reducing inflammation. In an analysis from the Third National Health and Nutrition Examination Survey (NHANES III) database, there was an inverse dose-dependent relationship between years of smoking cessation and levels of hsCRP, WBC count, fibrinogen and traditional risk factors such as total cholesterol, triglycerides, systolic blood pressure and an increase in HDL-C levels [66]. Furthermore, the improvement in inflammatory markers after smoking cessation preceded the improvement in other traditional risk factors. However, the measured inflammatory markers only returned to normal after an average of 5 years. This reduction in inflammatory markers and improvement in traditional risk factors paralleled a decrease in the observed ASCVD event rates. These findings have been replicated in multiple longitudinal studies [70,172,173].

In a 2 by 2 factorial randomized control trial in sedentary women, smokers randomized to a smoking cessation program with exercise had a significant reduction in inflammation measured by WBC counts [174]. In a study of 784 smokers, smoking cessation achieved with bupropion therapy was associated with a significant reduction in hsCRP levels 7 weeks after a baseline measurement. Continuing bupropion therapy was associated with sustained low levels of WBC and neutrophil cell counts [175] due to the substantial ASCVD benefits of smoking cessation. All smokers should be firmly advised to quit smoking to reduce ASCVD risk. This could be facilitated by the use of FDA approved cessation medications including nicotine replacement therapy, bupropion or varenicline [101].

6.8. Impact of lifestyle modification on CRP in UCC-SMART

The Utrecht Cardiovascular Cohort Second Manifestations of ARTerial disease (UCC-SMART) cohort evaluated the impact of a variety of lifestyle modifications on hsCRP levels. Smoking cessation was associated with a 0.40 mg/L reduction in hsCRP levels (β-coefficient −0.40; 95%CI -0.73,-0.07). Physical activity also associated with reductions in hsCRP: for every standard deviation (SD) increase in MET hours per week, hsCRP decreased by 0.09 mg/L (β-coefficient −0.09; 95%CI -0.17,-0.01) Weight loss in this study also correlated with reductions in hsCRP. For each SD in weight loss the hsCRP concentration decreased by 0.25 mg/L (β-coefficient −0.25; 95%CI -0.33,-0.16).

6.8.1. Pharmacological therapeutics

Therapeutics could either be targeted or non-targeted. However, to date, there are no Food and Drug Administration (FDA) approved agents for specifically reducing inflammation.

6.9. Lipid lowering therapies with anti-inflammatory effects

In addition to the cholesterol lowering effects of statin therapy, statins have pleiotropic effects and lower inflammation through lipid dependent and independent pathways [176]. Statins inhibit β-Hydroxy-γ-β-methylglutaryl-CoA (HMG-CoA) reductase, which catalyzes the rate limiting step in hepatic cholesterol biosynthesis. Statin therapy concomitantly reduces intracellular isoprenoid intermediates such as farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) and post-translational modifications of proteins involved in the pro-inflammatory response [176]. This includes the inhibition of several intracellular signaling pathways mediated through small guanine triphosphate (GTP)-binding proteins, including Rho, Ras, and Rac proteins [176]. Eventually this leads to down-regulation of pro-inflammatory cytokine expression such as IL-1β, TNF-α, and IL-6 [176]. Furthermore, statins reduce vascular wall inflammation by downregulating the expression of the adhesion molecules ICAM-1 and VCAM-1 [177]. Statins also improve nitric oxide bioavailability by upregulating endothelial nitric oxide synthase [178,179] and improve intracellular redox status which down-regulates the transcription of proinflammatory genes [180,181]. This in turn has favorable effects on vascular smooth muscle cells and the structure of the extracellular matrix by inhibiting the activation of NF-kB [181] and down regulating matrix metalloproteinase-9 expression [182]. Additionally, statins may attenuate inflammation by inhibiting the oxidation of LDL particles and reducing the expression of soluble CD40L and the activation of its proinflammatory receptor CD40 [183].

Observational studies and prospective randomized clinical trials show an independent association between statin therapy and lower levels of inflammatory markers in persons with CAD [176]. The PRavastatin Inflammation CRP Evaluation (PRINCE) trial was among the first to show a reduction in hsCRP independent of LDL-C achieved in patients with and without CVD [184]. Statins differ in their ability to reduce inflammation when controlling for factors such as lipid levels achieved. For example, in the PROVE-IT trial, among individuals who achieved an LDL-C of <70 mg/dL, a higher percentage of individuals randomized to atorvastatin (80 mg daily) also achieved on-treatment hsCRP levels <2 mg/L compared with individuals randomized to pravastatin (40 mg daily) [7]. In the JUPITER trial, patients randomized to low-dose rosuvastatin 20 mg daily had a 37% reduction in hsCRP and a 50% reduction in LDL-C concentration throughout the trial and a significant 44% relative risk reduction in ischemic events compared with placebo [14]. The greatest benefit with rosuvastatin was when both LDL-C <70 mg/dL and hsCRP

<1 mg/L were achieved [15]. Similar results were obtained in secondary prevention trials. In the PROVE-IT trial, achieving and LDL <70 mg/dL and hsCRP <1 mg/L was more likely among those randomized to atorvastatin 80 mg (80.6%) than in pravastatin 40 mg group (19.4%) [7]. Achieving both LDL-C <70 mg/dL and hsCRP <1 mg/L was associated with 35% risk reduction in recurrent MI or cardiovascular death compared with neither goal having been achieved. Similar results have been shown in the Aggrastat-to-Zocor trial where achieving both low LDL-C and hsCRP was associated with the lowest cumulative probability of death or MI compared with having only low LDL-C or neither goals achieved [185]. An exploratory analysis of the IMPROVE-IT trial of simvastatin plus ezetimibe vs. simvastatin alone yielded similar results with 18% lower risk of ischemic events starting 1 month after randomization in patients who had on-treatment LDL-C <50 mg/dL and CRP<1 mg/L regardless of treatment assignment [6].

The anti-inflammatory benefit with statin therapy is systemic. Using data from the Beyond Endorsed Lipid Lowering With Electron Beam Tomography Scanning (BELLES) trial, Raggi et al. showed that both atorvastatin and pravastatin reduce peripheral inflammation measured by epicardial adipose tissue attenuation independent of achieved LDL-C levels [186]. Statins inhibit Rac 1 which promotes the expression of neuroprotective genes that prolong neural cell survival [187]. In patients with rheumatoid arthritis, simvastatin inhibits the activation of NF-kB in synovial tissue and reduces the local production of TNF-α [188].

As noted earlier, patients treated with statins have significant residual ASCVD risk. The IMPROVE-IT trial randomized patients with an acute coronary syndrome to either ezetimibe plus simvastatin or simvastatin alone. After a median follow-up 6 years, the combination of ezetimibe and simvastatin was associated with a significant 6% reduction in the hazard of the primary composite endpoint of cardiovascular mortality, major cardiovascular events, or nonfatal stroke compared with statin monotherapy (HR, 0.94; 95% CI, 0.89–0.99) [189]. While CRP was lowered in both treatment groups, the addition of ezetimibe to simvastatin further lowered hsCRP by 0.3 mg/L at 1-month (16% reduction from baseline) and 0.3 mg/L for the duration of the study (14% reduction from baseline) compared with simvastatin alone [6]. As a result, a greater number of patients on ezetimibe plus simvastatin were able to achieve both LDL-C <70 mg/dL and hsCRP <2 mg/L targets (50% vs 29% with simvastatin alone, P < 0.001) [6]. In a pooled analysis of randomized controlled trials, the addition of ezetimibe to baseline statin therapy was associated with a significant 10% incremental reduction in CRP levels [190]. However, when used as monotherapy, ezetimibe was associated with a nonsignificant 6% reduction in CRP levels compared with placebo (P = 0.09). The synergistic effect of ezetimibe and statins on inflammation may be in part due to the inhibitory effects of ezetimibe on the expression of adhesion molecules and the NF-kB pathway resulting in lower plasma TNF-α levels [189,191].

The Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trial showed significant LDL-C lowering and improvement in CVD events with evolocumab in statin treated or statin intolerant patients with ASCVD who had LDL-C ≥70 mg/dL [192]. In addition to the known cholesterol lowering effects of PCSK9 inhibition, recent studies also report direct anti-inflammatory properties of PCSK9 inhibition. PCSK9 stimulates macrophage production of pro-inflammatory cytokines such as IL-1β and TNF-α and inhibits the synthesis of anti-inflammatory cytokines [193]. By contrast, PCSK9 inhibition is attenuates arterial wall inflammation [194] and reverses endothelial dysfunction [106]. Although PCSK9 inhibition with monoclonal antibodies does not lower systemic marker of inflammation, the cardiovascular benefit associated with PCSK9 inhibition is more apparent in individuals with higher markers of inflammation. In an analysis from FOURIER stratifying patients by baseline hsCRP levels, evolocumab was associated with the largest absolute risk reduction in those with hsCRP >3 mg/L [195]. Moreover, doubling of hsCRP levels at baseline was associated with a 9% higher risk of events at 3-year follow-up. In Studies of PCSK9 Inhibition and the Reduction of Vascular

Events (SPIRE)-1 and SPIRE-2 trials of bococizumab, there was no change in baseline hsCRP after 14-week follow-up. However, those randomized to statins plus bococizumab had a significant linear increase in event rates with higher on-treatment hsCRP levels [196]. Patients with on-treatment hsCRP >3 mg/L had 3.59 events per 100 person-years compared with those with hsCRP <1 mg/L who had 1.96 events per 100 person-years. The difference in event rates were significant after adjustment for on-treatment LDL-C and other potential confounders (adjusted HR, 1.62; 95% CI, 1.14–2.30). Therefore, low-grade inflammation measured by hsCRP is predictive of events even when aggressive LDL-C lowering is achieved with lipid-lowering therapy.

6.10. IL-1β inhibition

The most compelling evidence for targeting inflammation to reduce ASCVD risk comes from the CANTOS trial using canakinumab, a high-affinity human monoclonal antibody against IL-1β [16]. The role of IL-1β in the pathobiology of atherosclerosis is well-documented in both animal and human studies [21]. As discussed earlier, activation of the inflammasome converts pro-IL-1β to its active form IL-1β which then promotes amplification of the inflammatory signal leading to the progression of atherosclerosis and structural changes in plaque leading to architectural instability and rupture. IL-1β also stimulates the production of IL-6, which acts through the liver to induce CRP production and secretion [21].

Canakinumab is a human monoclonal antibody against IL-1β. Its structure allows it to bind specifically to human IL-1β and thus blocks the interaction of this cytokine with its receptors, with no effect on IL-1α. Prior to CANTOS, canakinumab had been used for the treatment of cryopyrin associated periodic syndrome and acute gout flares [197].

The CANTOS trial was designed to test the hypothesis of whether reducing residual inflammation in ASCVD patients through the inhibition of IL-1β would lead to reductions in major adverse cardiovascular events (MACE) events [197]. A total of 10,061 stable CAD patients with elevated hsCRP ≥2 mg/L despite aggressive secondary prevention strategies were randomized to placebo or one of three dosages of canakinumab (50 mg, 150 mg, or 300 mg). Patients had a median LDL-C level of 82.4 mg/dL and hsCRP of 4.2 mg/L. After a median follow up of 3.7 years, canakinumab at a dose of 150 mg every 3 months significantly reduced MACE by 15% compared to placebo [16]. The effect was primarily driven by a lower incidence of MI, with no effect on cardiovascular mortality. This beneficial effect on MI occurred with significant reductions in hsCRP levels and no change in LDL-C. Therefore, this trial offered a proof of concept that targeting inflammation lowered CVD risk and affirmed the independent role of inflammation in ASCVD. In a subsequent secondary prespecified analysis according to on-treatment hsCRP, a significant 25% risk reduction in MACE occurred only when canakinumab was associated with a drop in on-treatment hsCRP levels to <2 mg/L; this suggested that targeting inflammation through inhibiting the IL-1β to CRP pathway was important. In the CANTOS trial, canakinumab was associated with a significant increase in fatal infection or sepsis which may have contributed to the FDA's decision to not approve canakinumab as targeted therapy for patients with ASCVD.

6.11. Colchicine

Colchicine is the only non-targeted anti-inflammatory therapy to date shown to reduce ASCVD events in patients with established CVD. Colchicine is a broadly available drug with a well-established safety profile [198]. Relevant to inflammation in atherosclerotic plaque, colchicine exerts its effects through two mechanisms [199]. First, it inhibits microtubule polymerization which, in immune cells, impairs cellular mobility, adhesion, and activation. Colchicine also has an anti-inflammatory effect by inhibiting the inflammasome pathway. Studies have shown that similar to uric acid crystals in gout, cholesterol crystals in atherosclerosis triggers the release of IL-1β from macrophages

by activating the NLRP-3 inflammasome [200]. By modulating gene expression of the inflammasome components and inhibition of caspase-1 activity, colchicine reduces the production of IL-1β and IL-18. Colchicine was shown to have favorable effects on atherosclerosis in animal models. In rabbits fed with a high-lipid diet, 0.2 mg/kg colchicine supplementation twice a week reduced atherosclerotic plaque volume in the aorta [201].

In an open label pilot study of 64 patients with stable CAD with hsCRP ≥2 mg/L despite aspirin and high-dose atorvastatin therapy, randomization to colchicine 0.5 mg twice daily resulted in a 60% reduction in hsCRP levels compared with 11% reduction in placebo group [202]. These data were encouraging and led to the Low-dose-colchicine for CVD prevention (LoDoCo) trial. In the LoDoCo trial, 532 patients with stable CAD on statin and antiplatelet therapy were randomized to 0.5 mg of colchicine daily vs no colchicine in an open-label design [203]. After a median follow up of 3 years, those randomized to colchicine had a significant 67% relative hazard reduction in the composite primary endpoint of incident acute coronary syndrome, cardiac arrest, and non-cardioembolic ischemic stroke (HR, 0.33; 95% CI, 0.18 to 0.59; P < 0.001; NNT = 11). The observed reduction in events was almost entirely driven by a reduction in nonfatal cardiovascular events. Only a trend toward lower mortality was seen in LoDoCo (10 deaths in controls vs. 5 in colchicine arm).

Further evidence supporting the cardiovascular benefit with colchicine comes from the COLCOT trial and the more recent low-dose colchicine 2 (LoDoCo2) trials [17,204]. In the COLCOT trial, a total of 4745 patients who sustained an MI within 30 days prior to randomization and on intensive statin therapy were randomized to either 0.5 mg of colchicine daily or placebo and followed for a median of 1.9 years. Compared with the placebo group, those randomized to colchicine had a significant 23% (HR = 0.77; 95% CI, 0.61–0.96) reduction in the hazard of the composite primary endpoint of cardiovascular death, cardiac arrest, MI, stroke, or urgent hospitalization for revascularization. This was mostly driven by a reduction in stroke (HR, 0.26; 95% CI, 0.10–0.70) and urgent revascularization (HR, 0.50; 95% CI, 0.31–0.81). A subsequent analysis of COLCOT showed that the benefit with colchicine was restricted to those who were initiated on colchicine within the first 3 days post-MI (HR, 0.52; 95% CI 0.32 to 0.84), while those starting therapy 4–7 days or ≥8 days post-MI did not experience benefit [205]. These findings suggest that the timing of anti-inflammatory initiation post-MI was clinically important. Although colchicine was well tolerated and the rates of any adverse events were not different in the two treatment arms, it is worth highlighting that there was a significant increase in the incidence of pneumonia with colchicine compared with placebo (event rate 0.9% vs. 0.4%; p = 0.03).

In the LoDoCo2 trial, 5522 patients with stable CAD were randomized to either 0.5 mg of colchicine daily vs matching placebo [203]. After a median follow up period of 28.6 months, low-dose colchicine was associated with a 31% hazard reduction (HR, 0.69; 95% CI, 0.57 to 0.83; P < 0.001) in the composite primary outcome of cardiovascular death, spontaneous (nonprocedural) MI, ischemic stroke, or ischemia-driven coronary revascularization. When analyzed separately both the spontaneous MI (30% hazard reduction) and revascularization (25% hazard reduction) endpoints were significantly lower in those randomized to colchicine compared with placebo. However, there was a non-significant trend towards higher death from any cause in the colchicine groups compared with placebo (HR, 1.21; 95% CI, 0.86 to 1.71) driven by higher incidence of noncardiovascular deaths in the colchicine arm.

Several trials are ongoing to replicate the benefit that was observed with prior colchicine trials. This includes the Colchicine and Spironolactone in Patients With STEMI/SYNERGY Stent Registry (CLEAR-SYNERGY; ClinicalTrials.gov identifier: NCT03048825t), and Colchicine for Prevention of Vascular Inflammation in Non-cardio Embolic Stroke (CONVINCE; ClinicalTrials.gov identifier: NCT02898610t) trials.

7. ANTI-INFLAMMATORY agents WHICH failed to IMPROVE CVD

7.1. Low-dose methotrexate

Methotrexate is widely used in rheumatologic conditions. Initial reports of CVD benefit with methotrexate came from observational studies in psoriatic and rheumatoid arthritis patients. In an analysis from a nationwide database of patients with severe psoriasis from Denmark, being on methotrexate was associated with up to 50% reduction in composite endpoint of cardiovascular death, MI, and stroke [206]. Similarly, in rheumatoid arthritis patients longitudinally followed in the Questionnaires in Standard Monitoring of Patients with Rheumatoid Arthritis (QUESTA-RA) study, 15% risk reduction of CV events was noted in those on methotrexate [207]. In animal models, methotrexate reduced atheroma formation by 75% in New Zealand rabbits fed a high cholesterol diet [208]. In a study of human umbilical vein endothelial cell line treated with TNF-α, methotrexate was associated with downregulation of pro-inflammatory genes including TNF-α and, IL-1β [208]. Such findings provided the rationale for testing whether or not low-dose methotrexate reduces risk for ASCVD-related events in humans.

In the Cardiovascular Inflammation Reduction Trial (CIRT), 4786 patients with a history of MI or multi-vessel CAD plus type 2 diabetes mellitus or metabolic syndrome were randomized to either low-dose methotrexate (target dose of 15–20 mg weekly) or placebo. At baseline, 86% of patients were on statin therapy with a mean LDL-C of 68 mg/dL. The median hsCRP level was 1.5 mg/L. At 8 months, low-dose methotrexate did not reduce levels of IL-1β, IL-6, or hsCRP compared with placebo. However, there was a significant reduction in baseline WBC count with low-dose methotrexate compared to placebo. After a median follow up period of 2.3 years, low-dose methotrexate did not reduce the composite primary endpoint of nonfatal MI, nonfatal stroke, or cardiovascular death compared to placebo (HR, 0.96; 95% CI 0.79 to 1.16). CIRT was terminated after the study met a prespecified threshold for futility. At the end of the trial, patients randomized to low-dose methotrexate had a 72% higher relative rate of developing cancer (mostly non–basal-cell skin cancers) compared with the placebo group.

7.2. Comparing CANTOS and CIRT

When comparing CANTOS and CIRT, key differences may account for the contrasting results (Table 2). First, both trials predominantly enrolled patients already receiving statin therapy. However, CIRT had better controlled LDL-C levels with a mean that falls below the current LDL-C guideline target (<70 mg/dL). Second, patients in CANTOS had to

Table 2
Comparison between CANTOS and CIRT trials.

	CANTOS (n = 10,061)	CIRT (n = 4786)
% on statins	91%	86%
Baseline LDL-C	82 mg/dL	68 mg/dL
Baseline hsCRP	4.2 mg/L (high residual inflammatory risk)	1.5 mg/L (Low residual inflammatory risk)
% smokers	22%	11%
Inflammatory biomarkers		
Change in IL-1β	Inhibited	No change
Change in IL-6	Significant reduction	No change
Change in hsCRP	Significant reduction	No change

Abbreviations: CANTOS, Canakinumab Anti-inflammatory Thrombosis Outcome Study; CIRT, Cardiovascular Inflammation Reduction Trial; LDL-C, low-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; IL, interleukin.

have hsCRP values of ≥2 mg/L to be included in the trial, whereas CIRT did not require elevated hsCRP levels for inclusion. As a result, compared with CIRT, the baseline values of hsCRP in CANTOS were higher, reflecting a population with higher levels of residual inflammatory risk. Third, because smoking is associated with higher levels of inflammation and CANTOS had a higher percentage of smokers than CIRT, patients in the CANTOS trial had higher residual risk. Perhaps the most important difference was the way these two trials treated inflammation. While CANTOS directly modified the IL-1β pathway and reduced its downstream mediators IL-6 and hsCRP, CIRT lowered inflammation (lower WBC) but did not change IL-1β, IL-6, or hsCRP. Therefore, not all anti-inflammatory therapies are alike and the mechanism by which inflammation is reduced likely determines whether or not a given anti-inflammatory drug will reduce CV events.

Unlike canakinumab, several agents with promising preclinical experimental data failed to show benefit when tested in human clinical trials. Examples of such agents include infliximab, tocilizumab, and salsalate. Several reasons may contribute to the challenge in translating laboratory discoveries to humans, some of which are summarized in Table 3.

8. Potential future directions

Several new blood and imaging biomarkers have been the focus of recent attention.

8.1. GlycA

GlycA levels represent a composite of serum concentrations of 5 abundant acute-phase reactants including α1-acid glycoprotein, α1-antitrypsin, α1-antichymotrypsin haptoglobin, and transferrin which are produced in the liver in response to inflammation [209]. Levels of GlycA are associated with increased risk of incident CVD and all-cause mortality [210]. The clinical utility of measured GlycA is unclear and further research regarding its predictive ability is needed. However, one major limitation to its use will be that current measurement assays rely on nuclear magnetic resonance spectroscopy, which is not widely available.

8.2. MicroRNAs

MicroRNAs are short non-coding RNAs which have been shown to modulate gene expression [211]. More than 2000 microRNAs have been described in humans [212]. Recent research suggests a significant role of microRNAs in regulating inflammation. Both immune and bystander cells may express microRNA which then participates in an inflammatory feedback loop that would either potentiate or suppress inflammation depending on the type of microRNA produced [213]. Through targeting messenger RNA or by modulating signal transduction proteins involved in the inflammatory response, microRNA may exert anti-inflammatory effects. Numerous pro-inflammatory and anti-inflammatory microRNA's have been described [214,215]. This opens the potential for suppressing inflammation by sites of active inflammation [214]. The cost of microRNA drug development and modes of administration of microRNA remains a significant barrier at this time.

8.3. Targeting the NLRP3 inflammasome pathway

Data from CANTOR and CIRT suggest that inhibiting the inflammasome to CRP pathway improves vascular risk [216]. As a result, several studies are investigating the role of NLRP3 inhibition directly. Inhibiting the inflammasome directly may have the advantage of avoiding unintended immunosuppression as seen with targeting cytokines systemically. Several direct and indirect inhibitors of the inflammasome are under investigation. Direct inhibitors such as MCC950, 3,4-Methylenedioxy-β-nitrostyrene (MNS), and CY-09 have less off-target immunosuppression compared with indirect inhibitors. Levels of IL-18 which is activated

Table 3

Key differences between human and animal models of atherosclerosis that may contribute to the difficulty in translating discoveries to clinical practice.

	Humans	Animal model
Mechanism of atherosclerosis	Multifactorial and complex	Single defined mechanism (e.g. monogenic mutation)
Time to disease	Decades	Weeks to months
LDL-C levels	Low-moderate (rarely very high e.g. familial hypercholesterolemia)	Extremely high
Immune system	FOXP3 expression has limited role Th1 and Th2 populations of cells lie on a spectrum	FOXP3 expression important for Treg development and function Th1 and Th2 are different
Coronary atherosclerosis	Main site	Coronary plaque (minor) Aortic plaque is mainly studied
Mechanism of plaque rupture	Spontaneous plaque rupture	Surgical ligation (most common)
Plaque rupture with occlusive thrombosis	Common	Rare

Abbreviations: LDL-C, low-density lipoprotein cholesterol; FOXP3, forkhead box P3; Th, T helper cell; Treg, regulatory T cell.

by the inflammasome, but not inhibited by IL-1β inhibitors, have been shown to predict CVD but to a lesser extent than IL-1β or IL-6 [217].

8.4. Omega-3 fatty acids

In the REDUCE-IT trial, patients with established CVD or individuals with diabetes and additional ASCVD risk factors who had moderately elevated triglyceride levels (135–500 mg/dL) despite statin therapy and were randomized to highly purified eicosapentaenoic acid (EPA) in the form of icosapent ethyl at 4 g/day experienced a significant 25% reduction in ischemic events and mortality compared with placebo [218]. Interestingly, this beneficial effect occurred irrespective of baseline or achieved serum triglyceride levels. The potential mechanisms accounting for this benefit are not fully understood. However, among those randomized to icosapent ethyl there was a significant decrease in hsCRP levels compared with placebo (median percent change from baseline, −12.6% vs. 29.9%; $P < 0.001$) suggesting a potential anti-inflammatory effect with EPA supplementation. Some of the potential anti-inflammatory effects of EPA are mediated through a reduction in triglyceride levels. Moreover, EPA is a precursor of a family of specialized pro-resolving lipid mediators known as resolvins that inhibit inflammation and help resolve chronic inflammation [219]. Omega-3 fatty acids are known to reduce cytokine release and reduce endothelial and platelet activation which may account for some of the anti-inflammatory effects seen [220]. Further studies are ongoing in an effort to help delineate the precise mechanisms by which EPA modulates inflammation.

8.5. Microbiome modification

Accumulating evidence supports the complex interaction between the gut, its microbial ecology, and the immune system. Changes in the composition of the gut microbiota may predispose the gut into an inflammatory state leading to heightened systemic inflammation. The interaction happens on multiple levels. Data from animal models and observational studies in humans suggest a role of the microbiome in determining mucosal integrity. The microbiome ferments dietary fibers, leading to the production of butyrate and short chain fatty acids (SCFA) which are the main source of energy for the mucosal epithelium of the colon [221]. As such, low dietary fiber may impair the production of

butyrate and SCFA and increase epithelial permeability by impairing epithelial metabolism. This in turn causes the translocation of bacteria and associated lipopolysaccharides and both a local and systemic inflammatory response. A study comparing patients with symptomatic CAD to healthy controls found a negative correlation between bacterial genetic markers associated with the production of butyrate in the gut and blood hsCRP levels in humans [222]. Butyrate also modulates the local gut inflammatory response and immunologic homeostasis by affecting colonic regulatory T-cells [223]. Interestingly, alteration in the gut inflammatory response propagates changes in the microbial composition in a feedback loop that culminates in a chronic low-grade inflammatory state [224]. The microbiome also mediates some of the effect of an unhealthy western diet such as in the case of producing TMAO precursors from choline, betaine, and carnitine found in red meat, eggs, fish, and poultry.

The current analysis of the role of the microbiome in low-grade inflammation and CVD has been mostly based on cross-sectional studies. There is a need for more prospective studies to confirm these findings. Current research is exploring ways that would help regulate the microbial environment in the gut and break the potential feedback loop between gut inflammation and systemic inflammation. Potential mechanisms under investigation includes dietary intervention and the use of pre-biotics, which aim to favorably alter the composition of microbial communities and improve epithelial integrity to reduce inflammation systemically.

9. Conclusion

Chronic inflammation is a key mechanism driving ASCVD. The intersection between research on inflammation and vascular biology may prove transformative in our understanding of cardiovascular health and disease. ASCVD risk attributed to inflammation remains elevated in patients with or at risk of ASCVD despite guideline-proven therapies.

Although several inflammatory biomarkers are available, hsCRP is the most widely used. Based on recent studies and the 2019 ACC/AHA guideline on primary prevention of CVD [101], identifying low-grade inflammation using hsCRP may be reasonable to reclassify ASCVD risk and guide primary prevention efforts in patients when traditional risk estimates are unclear. However, the current evidence does not support the use of any of the inflammatory biomarkers to guide secondary ASCVD prevention. Finding the ideal inflammatory biomarker that reflects risk and response to treatment is an ongoing challenge. Given the complexity of the underlying mechanisms of inflammation, it is unclear whether a single biomarker will be adequate. The successful biomarker candidates would need to be associated with a known mechanism in the causal pathway of inflammation and ASCVD, be sensitive and specific allowing accurate reflection of inflammatory risk and change in risk in response to treatment. Other considerations include having reasonable analytical stability over time, and a widely available, accurate, reproducible, and cost-effective analytic technique.

A heart healthy lifestyle including dieting, exercise, weight loss and abstaining from smoking have been shown to reduce serum levels of inflammatory biomarkers. Beyond non-pharmacological interventions, a limited number of anti-inflammatory agents have shown promising results for lowering ASCVD risk. However, safely lowering inflammation without immunosuppression is a barrier to their wider utilization. To date, there are no Food and Drug Administration (FDA) approved lipid-lowering agents for specifically reducing inflammation. The quest for safe and effective therapies targeting inflammation to reduce ASCVD is still ongoing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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