

FORUM REVIEW ARTICLE

Imaging and targeting coronary artery inflammation

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Abstract

Significance

Coronary artery disease (CAD) continues to be a leading cause of morbidity and mortality across the world despite significant progress in prevention, diagnosis and treatment of atherosclerotic disease.

Recent Advances

The focus of the cardiovascular community has shifted towards seeking a better understanding of the inflammatory mechanisms driving residual CAD risk not modulated by current therapies. Significant progress has been achieved in revealing both pro-inflammatory and anti-inflammatory mechanisms, and how shift of the balance in favour of the former, can drive the development of disease.

Critical Issues

Advances in the non-invasive detection of coronary artery inflammation have been forthcoming. These advances include multiple imaging modalities, with novel applications of computed tomography both with and without positron emission tomography, and experimental ultrasound techniques. These advances will enable better selection of patients for anti-inflammatory treatments and assessment of treatment response. The rapid advancement in pharmaceutical design has enabled the production of specific antibodies against inflammatory pathways of atherosclerosis, with modest success to date. The pursuit of demonstrating efficacy and safety of novel anti-inflammatory and/or pro-inflammation resolution therapies for atherosclerotic CAD has become a major focus.

Future Directions

This review seeks to provide an update of the latest evidence of all three of these highly related but disparate areas of inquiry: Our current understanding of the key mechanisms by which inflammation contributes to coronary artery atherosclerosis, the evidence for non-invasive assessment of coronary artery inflammation, and finally, the evidence for targeted therapies to treat coronary inflammation for the reduction of CAD risk.

Introduction

Coronary artery disease (CAD) describes a constellation of closely related clinical entities predominantly driven by arteriosclerosis and resulting in a narrowing of the coronary vessel to restrict the flow of blood to the downstream heart muscle. An umbrella term itself, atherogenesis describes the process of thickening and hardening of an artery wall, while a hardening of an artery specifically due to an atheromatous plaque is typically called atherosclerosis (from Ancient Greek athéra, meaning 'gruel'). There has been considerable interest in the origins of atherosclerosis since the condition was first described by Fallopius in the 15th century, who noted macroscopically the 'degeneration of arteries into bone', referring no doubt to calcified plaque (25). This understanding was furthered by Crell in the 17th century who postulated that these hardening were not bone but rather thickenings derived from pus, presumably he was observing pre-calcified fibro-fatty deposits in vessels. Soon after von Haller first called these arterial lesions 'atheroma', and in the 18th century Frédéric Lobstein first used the term arteriosclerosis when describing the pathology of calcified arterial lesions in his *Traites d'Anatomie Pathologique* (97).

In more modern times, the term atherosclerosis has been favoured and has been widely considered a degenerative condition driven by the accumulation of cholesterol within the vessel intima. Despite the incredible advancements in managing cardiovascular risk during the last decade, this remains the world's biggest killer (189). The residual cardiovascular risk we consistently fail to target with the current therapeutic strategies, is now widely accepted to be driven by vascular inflammation (10).

The inflammatory hypothesis for atherosclerosis is not new (147), however it has gained significant traction in recent decade due to developments in both our understanding of the mechanisms of inflammation in the vascular system and the possibility of anti-inflammatory treatments that could be applied into the CAD field (143). Currently, most of the science to understand the core mechanisms of inflammatory processes in the vascular wall, and to treat them, has occurred in murine models, which has posed significant challenges to translation of findings into humans. Despite this, there is significant hope that through better understanding of inflammatory mechanisms in atherosclerosis, headway can be made on identifying and treating the significant residual cardiovascular risk.

This review outlines the current state of the art in three key areas of interest to scientists and clinicians working on CAD. First, our current understanding of the key mechanisms by which inflammation contributes to atherosclerosis is outlined. Secondly, the techniques that are currently available, and those that are in development, for the non-invasive visualisation of coronary artery inflammation are discussed. Finally, the work to develop therapies for targeting coronary inflammation for the reduction of CAD risk is reviewed.

Core mechanisms of coronary artery inflammation

There are several specific but highly inter-related molecular mechanisms driving the influence of inflammation on atherosclerotic disease, the majority of these are underpinned by the pro-inflammatory role of lipoproteins. Here we outline the major mechanisms that have been demonstrated to have the most significant influence on coronary artery atherosclerotic disease, while also discussing relevant evidence that has arisen from non-coronary artery atherosclerosis research. The focus of this review is on mechanisms of inflammation beyond the role of the lipoprotein. Others have provided comprehensive reviews of the pro-inflammatory nature of lipoproteins within the arterial wall (20). A large portion of the evidence discussed here comes from murine models. This has posed significant challenges in the translation of this science to humans, as discussed later within the review. Table 1 provides a summary of the mechanisms through which inflammation is driving residual CAD risk, while Figure 1 provides a visual representation of the major mechanisms driving inflammatory risk, and presents possible patient trajectories in regards to the mechanisms, the non-invasive detection of this risk and its possible clinical treatment.

Lipoprotein mediated inflammation

CAD is characterised by the sub-endothelial retention of plasma derived apolipoprotein B-containing lipoproteins within and adjacent to the coronary artery intima. A range of lipoproteins are sufficiently small to pass from circulation into the arterial intima.

It has been found that at all stages of atherosclerosis, from initial lipid entrance to the vessel intima, through to the rupturing of unstable coronary plaque, CAD can also be

characterised by inflammatory cell infiltration along with the lipid accumulation (55,95). The cellular components of the inflammatory response are not unique to atherosclerosis but are found in all inflammatory processes across all bodily tissues (176). Most notably, the migratory immune cells include monocyte derived macrophages (179), T lymphocytes and mast cells (69), as well as monocyte derived dendritic cells (126) and certain B lymphocytes (83). In advanced atherosclerotic lesions, neutrophils are strongly implicated in plaque progression (28), and along with all cells aforementioned, have been shown to contribute to the development of clinically significant vessel disease via inflammatory means. New technologies such as single cell sequencing and cytometry by time of flight are shedding light on the true diversity of the immune cells that are implicated in the disease (181). Recently, alteration in intracellular metabolic pathways of immune cells has been shown to drive survival, growth and pro- versus anti-inflammatory function in atherosclerosis (84). The relationship between lipid accumulation in the arterial wall and inflammation is also evident in the formation of a cell type almost unique to atherosclerotic inflammation – the foam cell (20). Foam cells can rapidly become harmful and contribute to the pro-inflammatory processes driving atherosclerosis (122,158). This occurs when the continual uptake of excess unesterified cholesterol, which necessitates ATP-demanding hydrolysis and de-esterification, leads to premature cell death (31). This process occurs predominantly in larger and advanced atherosclerotic lesions, and leads to the release of the foam cells cholesterol load into the extra-cellular space – ensuring a potentially pro-inflammatory end to what was initially a protective anti-inflammatory process.

The role of pro-inflammatory chemokines and cytokines in atherosclerosis is significant and is of utmost clinical importance given their possible role as

therapeutic targets, discussed at length below. The majority of these chemokines and cytokines are secreted by inflammatory cells within the intima of the vessel in response to the pro-inflammatory stimulus of modified lipoproteins (20).

Lipoprotein modifications occur in the extra-cellular space and appear to occur very early in the atherosclerotic process (20). Lipoproteins are vulnerable to modification from oxidizing agents, frequently proteases and lipases (73), all of which induce LDL aggregation, which has been shown to be associated with elevated risk of cardiovascular disease (CVD) death (149). Modified lipoproteins are found in aggregates within atherosclerotic lesions and often contain highly inflammatory cholesterol crystals (91). The lipoprotein modification process itself has been shown in-vitro to induce the formation of cholesterol crystals (64). Oxidative lipoprotein modification results in oxidized phospholipids (oxPLs) that can themselves act to induce inflammation through promoting leukocyte recruitment into the intima and the subsequent activation of these cells. The modified lipoproteins trigger both the local innate and adaptive immune responses (127) that drive the chemokines and cytokine release that in turn stimulate both the local endothelial cells (152) and the circulating immune cells. This drives further migration of circulating immune cells into the intima, particularly monocytes with inflammatory potential, such as the CD14⁺⁺CD16⁻ lineage (184). This process is severely damaging as the recruited monocyte-derived cells secrete the lipoprotein-modifying enzymes that drive the formation and eventual degradation of foam cells, while the modified lipoproteins themselves stimulate further secretion of the lipoprotein modifying enzymes (127). This creates to a pro-atherogenic positive feedback loop driving arterial plaque formation through both lipid and inflammatory means (127).

The inflammasome

Inflammasomes are multi-protein oligomers of the innate immune system. They are responsible for the activation of a range of inflammatory responses, including the secretion of cytokines interleukin 1 β (IL-1 β) and IL-18 (136,187). The NLRP3 inflammasome is the most studied of all and is strongly implicated in coronary atherosclerosis (20). Once activated, the NLRP3 inflammasome secretes pro-inflammatory cytokines such as IL-1 β and IL-18 and drive a form of cell death known as pyroptosis (125).

Importantly, oxLDL, lipolysed LDL, electronegative LDL and various other modified lipoproteins have been demonstrated to act as both a primer and activator of the NLRP3 inflammasome (59). This is an important finding as it demonstrates a clear link between intimal accumulation of lipids, both intra- and extra-cellular, and inflammasome mediated inflammation. The mechanism by which oxLDL can prime the inflammasome is through binding to CD36-toll-like receptor 4-TLR6 signalling complexes on macrophages and subsequent incorporation into the lysosome where it can induce the inflammasome via activation of cholesterol crystallisation (133).

The role of cytokines

The important role that cytokines play in both the pro- and anti-inflammatory milieu of CAD has been thrown into sharp relief by the magnitude of possible therapeutic targets that have already been explored or are currently under examination as possible druggable targets to reduce CAD risk (175). The major classes of cytokines are the interleukins (IL), chemokines, tumor necrosis factors (TNF), colony-stimulating factors (CSF), the interferons (IFN) and transforming growth factors (TGF). Classes are often sub-classified again, such as the chemokines, an important

class of hemo-attracting cytokines with great importance in initiating the inflammatory response in atherosclerosis.

The majority of cytokines can be secreted by macrophages, lymphocytes, natural killer cells and also vascular smooth muscles cells in the arterial wall (177). As discussed already IL-1 β and IL-18 are often secreted after activation of an inflammasome. Activation of the p38 mitogen activated protein kinase (p38MAPK)/nuclear factor kappa-light-chain-enhancer of the activated B-cell (NF- κ B) pathway is the predominant means through which tumour necrosis factor- α (TNF- α) and IL-1 signalling occurs. This pathway influences the majority of cells located in the vascular intima, and is a potent promoter of inflammatory cytokines at all stages of atherosclerosis (20). Other pro-atherogenic cytokines are mediated through different means. IL-6 is activated through its own receptor and gp130, a signal transducing protein that activates Janus kinase 1 (JAK1) and activators of transcription 1 and 3 pathways (195). IL-6 is a cross-over cytokine, as it also exhibits some anti-inflammatory roles given it induces the synthesis of IL1ra and the release of soluble TNF receptor, reducing the activity of other pro-inflammatory cytokines (192). Circulating levels of IL-6 have been demonstrated to be an independent risk factor for CAD (168), and it have frequently been included in trials assessing drugs aimed at combating atherosclerosis through anti-inflammatory means (143).

Anti-atherogenic cytokines confer a protective function against the development of atherosclerotic plaque. An example is transforming growth factor- β (TGF- β) which is secreted by immunomodulatory T-regulatory (T-reg) cells. T-reg cells secrete a range of anti-inflammatory cytokines beyond TGF- β , including IL-10 and IL-35, which have been implicated in the downregulation of pro-atherogenic cytokine such as TNF- α (124). This is an example of the profoundly complex and mostly poorly

understood relationships between individual cytokines in the inflammatory cascade and coronary atherosclerosis. This complexity translates to difficulty in the development of novel therapeutics that target relevant cytokines, as unexpected outcomes can occur for unknown reasons (175).

In the earliest stages of atherogenesis, when lipids are initially entering the intimal layer of the artery, it has been demonstrated that cytokines play a significant role in the modulation of endothelial cell permeability (6). Both IFN- γ and TNF- α have been implicated in the necessary reorganisation of actin and tubulin cytoskeletons in the endothelial layer to facilitate the passage of lipids into the vessel intima (131). The role of chemokines in atherosclerosis has been of significant interest (161) and reviewed extensively due to their critical role in the migration of monocytes into the intima layer. There are at least 13 chemokines that play a role in this process (137), with the most important chemokines:chemokine receptors identified to date being CCL2:CCR2, CX3CL1:CX3CR1, and CCL5-CCR5. The inhibition of these chemokines has been demonstrated to lead to almost complete attenuation of atherosclerosis in hypercholesterolaemic murine models (40). Other important roles that have been identified in relation to chemokines in preliminary atherosclerosis include the prevention of reverse cholesterol transport through downregulating the expression of key proteins implicated in the efflux of lipoproteins from cells (74) and the discovery that some chemokines, such as CX₃CL1 can also serve in their own right as an adhesion molecule for monocytes (170).

The vascular redox state

Vascular oxidative stress has been identified as a significant pathophysiological mediator of inflammatory atherosclerotic disease progression (14). Oxidative stress

is a shift in the physiological balance between oxidants and antioxidants within a biological system, such as the arterial wall, toward a more pro-oxidant phenotype and oxidizing redox state. The reactive oxygen species (ROS) are critical in this process, as they are typically the primary molecules generated by aerobic cells that interact with endogenous molecules such as proteins or DNA to drive them towards an oxidative state. The vascular redox state is the balance between ROS generation through enzymatic sources and the correspondent scavenging by anti-oxidant mechanisms within the vascular wall (10). The ROS do play physiological signalling roles in many tissues but become pathological and contributory to the inflammation associated with atherosclerosis when in excess within the vascular wall (35). ROS mediate these harmful effects through reducing nitric oxide (NO) levels, dysregulation of sensitive translational pathways and often through direct cytotoxic effects on cells in the vascular wall (34,46). The ROS most often implicated in atherosclerosis is $O_2^{\bullet-}$ which is the product of the catalyzation of nicotinamide adenine dinucleotide phosphate (NADPH) to O_2 by NADPH oxidase (15). An example of the role ROS can play in driving atherosclerosis is through the loss of production of NO in the endothelium. Endothelial NOS (eNOS) is the major enzymatic source of NO in the endothelium and relies on a crucial cofactor, BH_4 to function (12,13). This cofactor is highly susceptible to oxidative degeneration in the presence of excess ROS (45). This uncoupling drives the eNOS to produce $O_2^{\bullet-}$ over NO and contributes to the overall pathological vascular redox state (34). There are other sources of ROS from a range of enzymatic sources in the vascular wall. These include xanthine oxidase, lipoxygenase, peroxidases, the mitochondrial electron transport system, the Fenton reaction of H_2O_2 , and cyclooxygenases, amongst others (62).

The balance of ROS with anti-oxidant defence mechanisms is pivotal in defining the role of vascular redox state in contributing to atherogenesis (159). The antioxidants of relevance to the vascular environment include enzymes such as glutathione peroxidase, glutathione reductase, catalase, heme oxygenase, non-enzymatic molecules such as albumin and bilirubin, and vitamins A, C and E (159). Importantly, diminished levels of these antioxidants in the vascular wall have been linked to atherosclerotic disease (65). Obesity is also implicated, having been shown to be associated with impaired antioxidant activity, suggesting a mechanistic connection between adipose tissue (AT) and dysregulated vascular redox state (27).

The role of AT in vascular redox state has recently become clearer (100). Alteration in AT due to obesity, insulin resistance and diabetes mellitus has been demonstrated to drive pathological changes in the AT adipokine secretion profile towards more pro-inflammatory adipokines. These include resistin, leptin, IL-6, TNF- α , and the chemokine CCL2 amongst others (123). Conversely, circulating levels of anti-inflammatory adipokines such as adiponectin and secreted frizzled-related protein 5 have been negatively correlated with AT accumulation (44). The release of adipokines from depots of AT has emerged as a player in atherosclerosis through the first vascular effects the molecules can exert promoting or protecting from oxidative stress. The role of peri-vascular AT (PVAT), that is the AT located adjacent to the tunica adventitia of arteries, in very close proximity to the site of possible atherosclerosis, has therefore become of interest (17,103). We have shown that PVAT can directly modify the biology of the adjacent vascular segment through adipokine-mediated paracrine mechanisms independently of any systemic, endocrine effects (7). There is evidence that there is bi-directional interplay between the vascular wall, in particular in regards to oxidative stress and inflammation, and

the PVAT (17,19). It has been found that local adiponectin levels in PVAT are positively correlated with $O_2^{\cdot-}$ production and eNOS uncoupling in the underlying vessels in patients undergoing coronary artery bypass grafting surgery. Ex-vivo study of these samples has revealed that adiponectin promoted eNOS coupling, a process protective from ROS and confirming the protective effects of adiponectin. Following this it has been demonstrated that the products of lipid peroxidation such as 4-hydroxynona diffuse from the vascular wall to adjacent PVAT and upregulate adiponectin expression, which can then work to limit $O_2^{\cdot-}$ production in the underlying vessel (103).

Building upon these findings, a subsequent study utilised internal mammary artery segments with associated PVAT to show that increased vascular oxidative stress as a result of increased NADPH oxidase activity results in upregulation of adiponectin expression in the neighbouring PVAT via the release and diffusion of oxidative products and a peroxisome proliferator-activated receptor gamma (PPAR- γ) mediated mechanism. Importantly, this study also demonstrated that adiponectin downregulated NADPH activity (17). These findings were only found in PVAT, and not distant AT depots, such as thoracic AT as was explored in this study, suggesting a paracrine signalling loop between PVAT and the vascular wall, where adipocytes function as a sensor of vascular oxidative stress and act to reduce this stress via the release of protective adiponectin. These findings are of importance to the management of CAD clinically due to further work that has translated these findings into valuable clinical imaging tools for coronary artery inflammation (19,119), discussed at length below.

The pro-resolving/pro-inflammatory (im)balance

As with most inflammatory processes, the inflammatory response within the atherosclerotic coronary intima layer is associated with specific and active resolution phase intended for repair of the damage (154). There are a host of pro-resolution mediators, including small lipid molecules, proteins and gases, all of which have been shown to be nearly ubiquitous throughout bodily tissues. This suggests that these molecules undertake a preparatory role, providing early response and resolution to inflammation. Resolution is achieved by these mediators by activating cell-surface receptors to block inflammatory cells influx, promote egress of inflammatory cells, slow the T cell response and promote efferocytosis (20) – all of which limits ongoing tissue damage and allows repair of damaged tissues such as the endothelium. The chronicity of coronary atherosclerosis is characterised by a failed resolution of inflammation, and it has become clear that the most clinically relevant plaques exhibit a complete failure of inflammatory resolution. This is usually through damage-associated molecular pattern mediated inflammation (163), a failure of efferocytosis, the development of a necrotic lipid core and subsequent thinning of the fibrous cap separating the atheroma from the coronary lumen.

There are several key pro-resolution mediators to be considered, with a brief summary of the evidence for the role of each in atherosclerosis proved here.

The fatty acids can be both pro- and anti-inflammatory in the setting of atherosclerosis. Free fatty acid receptor type 4 can be directly activated by omega-3 fatty acids, which is protective from inflammatory vascular injury (92), while the peroxidation products of fatty acids are often pro-inflammatory in the vascular wall (29). The specialised pro-resolving mediators (SPMs) are a class of fatty acid derived lipids which are synthesized in response to inflammation by a wide range of cells but primarily by macrophages and neutrophils (155). There are different SPMs

for omega-3 fatty acids (E-series and D-series resolvins, maresins and protectins), and omega-6 fatty acids (lipoxins) with the main enzymes involved in their synthesis being 5-, 12- and 15-lipoxygenase (LOX). The regulatory pathways of the LOX enzymes have essential roles in atherosclerosis and are heavily influenced by inflammatory states. The role of 5-LOX is a suitable example, with the location of the enzyme resulting in either pro- or anti-inflammatory actions. When located in the cytoplasm in neutrophils, mast cells and macrophages 5-LOX will synthesise the pro-resolving mediator lipoxin A4 from arachidonic acid (134). When located in the nuclear membrane the same enzyme will promote the conversion of arachidonic acid into leukotriene B4, which is pro-inflammatory (54). The nuclear membrane location of 5-LOX is itself a result of its phosphorylation by the enzyme MK2 (186). This inflammation promoting pathway has been found to be triggered by calcium-activated kinase CaMKII and inhibited by the presence of SPMs, as part of a pro-resolving amplification loop (54).

There are a number of non-lipid mediators of inflammation resolution which include select cytokines such as IL-10 (68), as discussed prior, and non-coding RNAs (56). Other pro-resolution mediators include hormones, such as α -melanocyte stimulating hormone, a peptide derived from adrenocorticotrophic hormone (ACTH), and the complete ACTH. These mediators can be delivered to developing atherosclerotic lesions through plasma extravasation or can be produced locally at an inflamed tissue site (129). Sex hormones have been shown to influence inflammation in a sex-specific way; where a low testosterone to oestradiol ratio is associated with systemic inflammation in men (180).

Finally, there are several gaseous transmitters that promote inflammation resolution. These include NO, hydrogen sulphide (H₂S) and carbon monoxide (CO) which are

capable of diffusing directly through cell membranes to influence cellular activity (182). The role of NO has already been discussed and a major endothelium-derived vasorelaxant factor with anti-inflammatory and anti-apoptotic properties (182). H₂S acts through different means, altering intracellular calcium and cAMP levels to promote post-translational protein sulfhydration. This can reduce lipopolysaccharide induced TNF levels and promote efferocytosis (49), CO is thought to contribute to anti-inflammatory processes by limiting leukocyte infiltration into the atherosclerotic lesion and suppressing cytokine production (182), and there is experimental evidence in murine models that inhaled CO increased pro-resolution macrophage activity and shortened time to resolution of acute inflammation (37).

Non-invasive assessment of coronary inflammation

There has been significant interest in the development of non-invasive techniques to visualise coronary artery inflammation. The non-invasive assessment of coronary artery inflammatory burden would provide significant opportunity for enhanced CAD risk stratification, personalised therapy decision making and enhanced monitoring of therapy efficacy (121).

Here, we provide a review of the imaging modalities available to researchers and clinicians to visualise coronary inflammation *in-vivo* with a focus on the molecular mechanisms that these imaging modalities rely on. Figure 2 provides an overview of these imaging modalities.

Molecular imaging using PET-CT

Positron emission tomography (PET) CT has provided the opportunity for molecular imaging using selective radiotracers for a direct assessment of vascular biology, including inflammation (183). The utility of PET-CT depends on the radiotracer employed. ^{18}F -FDG (fluorodeoxyglucose) is taken up by metabolically active inflammatory cells and has demonstrated affinity for inflamed and vulnerable coronary plaque in appropriately prepared patients (99). This preparation includes a strict low-carbohydrate and high fat diet preparation to suppress myocardial tracer uptake that would influence the results (191). Currently ^{18}F -FDG PET-CT is utilised clinically for assessment of myocardial viability and blood flow, and for the detection and monitoring of predominantly non-coronary conditions such as sarcoidosis and myocarditis. Another radiotracer that can provide similar inflammatory imaging is gallium-68-labeled DOTATATE (^{68}Ga -DOTATATE), which is utilised extensively in imaging of neuro-endocrine tumours and binds to the somatostatin receptor subtype-

2 that is expressed by M1 pro-inflammatory macrophages. PET-CT images with ^{68}Ga -DOTATATE have been shown to provide superior image quality to ^{18}F -FDG and can be utilised to identify inflamed coronary lesions (166) and residual inflammation within myocardial tissue following myocardial infarction (165).

Other radiotracers have also demonstrated good utility for the imaging of inflammation within the coronary wall. ^{18}F -NaF (sodium fluoride) has a high affinity for the vascular wall and has been demonstrated to incorporate into hydroxyapatite in areas of arterial wall micro-calcification. Imaging with ^{18}F -NaF has shown promising diagnostic accuracy of culprit coronary lesions as well as abdominal aortic aneurysms (135). There is promising evidence that ^{18}F -fluciclatide, an $\alpha_v\beta_3$ integrin-binding PET tracer, is useful for the identification of high risk coronary plaque (78). Endothelial $\alpha_v\beta_3$ is highly related to angiogenesis that can occur in atherosclerotic plaques due to the hypoxic plaque environment triggering new vessel demand (167). Further, $\alpha_v\beta_3$ is thought to play an inflammatory role in mediating mechanical shear stress induced pro-inflammatory signalling via the NF- κ B.

There has been preliminary work into the possible use of 18-kDa translocator protein as a target for demonstrating inflammation via a specific PET-CT tracer. This protein is present in macrophages that enter inflamed atherosclerotic lesions. ^{11}C -PK11195 has been utilised for this purpose, but predominantly in carotid disease as yet (42). Within a population with carotid plaque, PET-CT imaging with ^{11}C -PK11195 was demonstrated to distinguish between recently symptomatic and asymptomatic plaque (77).

Other experimental techniques have been trailed for the visualisation of coronary artery inflammation via PET-CT. Chemokine receptors that are upregulated in pro-

inflammatory macrophages can be visualised in experimental nanoplateforms (98) and endothelial activation and inflammation can be visualised by ^{18}F -labeled small vascular cell adhesion molecule type-1 (VCAM-1) affinity ligands (111).

Visualisation of inflammation has been achieved through utilising polysaccharide containing magnetofluorescent 20nm nanoparticles labelled with ^{64}Cu . These nanoparticles have been found to accumulate in macrophages in atherosclerotic lesions in ApoE-lipoprotein knock-out mice when utilising an approach that combines PET, magnetic resonance and optical detection technologies (139).

Another experimental approach to non-invasive visualisation of coronary artery inflammation is with single-photon emission computed tomography/CT imaging that utilises ^{111}In - and ^{123}I -radiolabelled compounds. These compounds target activated matrix metalloproteinases and allow their detection and tracking of the composition of plaques in a cross-sectional and longitudinal manner in response to treatment (67).

Beyond ^{18}F -FDG PET-CT for myocardial assessment, all these technologies are currently experimental only, and limited by their high costs, limited availability and lack of required expertise. From a clinicians' perspective the largest barrier to uptake is a paucity of clinical evidence to demonstrate the translational potential of these PET-CT techniques for reduction of either primary or secondary cardiovascular risk. There will likely be some growth in uptake of these technologies led by the pharmaceutical industry seeking techniques for assessing response to therapeutics targeting coronary artery inflammation.

Experimental ultrasound techniques

There has been research interest in the use of contrast enhanced ultrasound for the visualisation of inflammation in atherosclerosis, with the majority of work making use

of murine models utilising aortic tissue. The techniques explored are not currently translatable into clinical practice but provide relevant possible applications of a low-cost non-invasive imaging modality for the visualisation of vessel inflammation driving atherosclerosis. These techniques have predominantly focused on VCAM-1, which is expressed by activated endothelial cells and plays an important role in atherosclerotic plaque development through immune cell recruitment (81). The use of targeted contrast enhanced ultrasound has been successfully demonstrated to allow the assessment of inflammation in arterial lesions utilising a variety of predominantly VCAM-1 targeted microbubbles (82). These inflammatory changes can be imaged noninvasively before the development of advanced lesions in the murine aorta (81). These techniques have recently been further developed with microbubbles with a maleimide-thiol conjugation of an anti-VCAM-1 nanobody to detect VCAM-1 expression in both murine models of atherosclerosis and ex vivo in human carotid endarterectomy specimens using non-invasive ultrasound imaging. Importantly, this work showed that this enhanced contrast agent allowed detection of VCAM-1 on human arterial tissue with ultrasound imaging and has moved clinical translation of contrast enhance ultrasound to detect early inflammatory changes closer to reality (132). How these techniques could be replicated in-vivo within the coronary vasculature is yet to be explored and intra-vascular ultrasound has not yet been utilised with these techniques.

Cardiac computed tomography

Computed tomography (CT) has allowed the non-invasive examination of the coronary tree and revolutionised the assessment of CAD in clinical practice. Traditionally, coronary computed tomography angiography (CCTA) has been employed for its ability to provide an accurate assessment of the anatomical

structure of the coronary arteries including any atherosclerotic lesions that may be present within the walls of the vessels.

The field of cardiac CT has experienced a renewed interest in the visualisation of disease processes, such as inflammation, that are driving the development of clinically significant structural coronary disease, such as calcified plaque and vessel stenosis, that CCTA is traditionally employed for (121). CCTA is an existing and widely available imaging modality already cemented in the investigation arsenal of physicians. Importantly, CCTA is a part of clinical guidelines for the investigation of chest-pain across the world (63,86), and as such, any advances that allow these scans to be utilised for the visualisation of coronary inflammation would be a significant development in clinical practice. The rise of the field of radiomics and the harnessing of artificial intelligence techniques to interpret large imaging derived datasets has now seen the first such technology become available.

These advanced rely on the fundamental but often overlooked fact that CT scans, like all images, are datasets. Handling CT scans as datasets for analysis rather than images is the focus of the field of radiomics, which uses mathematic formulae to compute many hundreds of shape-, attenuation- and texture-related features for a given anatomical volume or segmentation (88). The field of radiomics was developed for the large-scale analysis of geo-spatial satellite imagery and first applied to healthcare in the field of cancer imaging. In this field, radiomic feature analysis has been found to reliably discriminate malignant from benign lesions (3). Radiomic approaches have recently been implemented in CCTA with the aim to detect biological mechanisms, such as coronary inflammation, that are driving the residual risk of CAD events (87).

To date, the major imaging technology that utilises CCTA for the detection of inflammation is the perivascular Fat Attenuation Index (FAI). This non-invasive CT-derived biomarker was described by the authors of this review and relies on radiomic phenotyping of peri-coronary adipose tissue composition to extract information about the inflammatory status of the adjacent coronary artery (19,102).

The premise of this work emerges from the understanding that adipose tissue is a key regulator of cardiometabolic health, as discussed already in regards to the role vascular redox state plays in coronary artery inflammation (21,118). The perivascular adipose tissue (PVAT) is the adipose tissue that forms a contiguous entity with the arterial adventitia and plays a key role in vascular homeostasis and atherogenesis by regulating the local microenvironment through the release of bioactive adipokines (17,103), gaseous and other lipid messengers (102,118). Studies which utilised ¹⁸F-FDG PET/CT to visualise inflammation in PVAT have demonstrated significant relationships between this inflammation and a range of clinical significant cardiovascular disease endpoints (117). Our group recently demonstrated that the paracrine interactions between the arterial wall and the PVAT are bi-directional (16,17,19,103). We have shown that in the presence of increased vascular oxidative stress, lipid peroxidation products such as 4-hydroxynonenal are increasingly produced and diffuse from the vascular wall to the PVAT. These substances activate PPAR- γ signal mechanisms in PVAT adipocytes and result in an upregulation in production of the antioxidant adiponectin. Adiponectin can then diffuse back to the vascular wall and proximal myocardial tissue and reduce superoxide production by NADPH oxidase activity (17,103). Importantly, we subsequently demonstrated that if vascular inflammation is present, the release of pro-inflammatory mediators such as TNF- α , IL-6 and interferon-gamma blocks the ability of perivascular pre-adipocytes to

differentiate into mature lipid-laden adipocytes (19). The perivascular FAI relies on both these findings and the observation that in paired PVAT biopsies from a site attached to the right coronary artery and 2cm away, perivascular adipocytes were significantly smaller and less well differentiated (as evidenced by a lower relative expression of the adipocyte differentiation markers PPAR- γ and fatty acid binding protein-4) compared to those from the non-perivascular site (19). This gradient in perivascular adipose tissue composition reflects the inflammatory burden of a given coronary segment and has highlighted PVAT as a sensor of coronary artery inflammation, and therefore a potential diagnostic and prognostic biomarker (19).

These laboratory findings have been translated through CCTA through the segmentation and analysis of PVAT along the coronary vessels utilising pre-defined validated Hounsfield unit (HU) cut-offs (-190 to -30HU) (19,101). The perivascular FAI utilises CCTA to track spatial changes in PVAT composition that are induced by inflamed coronary vessels as outlined above (19). The FAI relies on the concept that the inflammation-induced changes in adipocyte size are associated with a detectable shift in CT attenuation towards a less negative HU range (towards -30 HU). The perivascular FAI (calculated by the CaRi-HEART algorithm developed by the University of Oxford) captures these gradients in the attenuation of the perivascular space, with high perivascular FAI linked to a higher inflammatory burden (19). The proprietary CaRi-HEART algorithm has been trained to perform a number of adjustments related with technical CT scan characteristics, the local coronary anatomy, the background adipocyte size and others, interpreted using machine learning modelling. This adjusted measurement of FAI can be combined with the risk factor profile, the plaque characteristics and calcification on CCTA, to generate the

individualised risk of the patient for a fatal heart attack over a fixed period of time (using the CaRi-RISK calculator).

Critically, there is now excellent evidence that CAD is associated with higher perivascular FAI compared to healthy individuals (19). In addition, perivascular FAI is significantly increased around culprit/unstable lesions in patients presenting with acute myocardial infarction (19), and, perivascular FAI exhibits dynamic changes around culprit coronary lesions, decreasing significantly when measured five weeks after the index event (19). It is interesting to consider the concept of the vulnerable plaque in light of these findings, as this has dominated clinical thinking around acute coronary syndromes (ACS) for decades. How inflammatory imaging, such as FAI and others, can assist in identifying coronary lesions at increased risk of rupture or, perhaps more importantly, superficial erosion (94) mediated through inflammatory mechanisms, will be of tremendous importance. Whether CT based techniques may be useful in non-invasive differentiation of ACS because of plaque erosion vs rupture remains to be seen. Despite this, it is important to note that the perivascular FAI provides a measure of vascular inflammation and CAD risk regardless of the presence of any detectable coronary plaque, suggesting that patient vulnerability extends beyond immediately detectable plaque(s). Perivascular FAI has also been demonstrated to accurately predict plaque progression (19), which favours the utility of FAI for the detection of both global coronary artery inflammatory burden, and the detection of plaques that may be of significant clinical relevance. The potential clinical utility of perivascular FAI has become clear when FAI is incorporated into cardiac mortality risk calculators. The validation study for FAI demonstrated incremental prognostic value for FAI in both constituent cohorts (Erlangen and Cleveland Clinic). In Figure 3 we present a merged analysis of these cohorts as the

complete CRISP-CT cohort (n=3,912). There is incremental prognostic value of the perivascular FAI risk score (CaRi-RISK application) for cardiac mortality beyond best clinical risk prediction model.

Other groups have investigated the application of FAI to confirm the hypothesis behind the biomarker (57,70). In a validation of the link between coronary artery inflammation and PVAT phenotype, higher perivascular fat radiodensity has been shown to strongly correlate with increased plaque inflammation as assessed by ¹⁸F-NaF uptake on PET-CT imaging (90), as well as the progression of total and non-calcified atherosclerotic plaque burden in the adjacent vessel (58). It is noteworthy that in symptomatic patients undergoing cardiac CT the information captured by the perivascular FAI is independent of coronary calcification (19,102) or systemic markers of inflammation, such as high-sensitivity C-reactive protein (hsCRP) (102).

A proposed pathway as to how the assessment of inflamed coronary arteries via perivascular FAI can be incorporated in clinical practice is presented in Figure 4. This figure provides a means as to how FAI analysis, along with high-risk plaque features on CCTA, could be employed by clinicians as a companion diagnostic for secondary prevention of CAD. This approach could better select patients for the personalised deployment of high cost therapies such as canakinumab or PCSK9-inhibitors. Currently there is no direct test that clinicians can utilise to assess for potential response to these treatments.

Harnessing CT radiomics for the detection of coronary inflammation

Beyond the perivascular FAI, which utilises the power of a single radiomic feature for the assessment of changes in adipocytes that are sensing vascular inflammation, we recently reported the first study to apply complete radiomic feature quantification of

CCTA scans for the purpose of better detection of CAD processes (120). Radiomic signatures derived via a machine learning approach were able to detect coronary artery inflammation and significantly, features of radiomic texture were found to be related to adipose tissue fibrosis and vascularity, as measured through gene expression in tissue samples obtained during cardiac surgery. In this study we went on to apply an AI derived algorithm named the Fat Radiomic Profile (FRP) into the SCOT-HEART trial where it significantly improved major adverse cardiac event prediction beyond traditional risk stratification that included risk factors, coronary calcium score, coronary stenosis and high-risk plaque features on CCTA ($\Delta[C\text{-statistic}] = 0.126$, $P < 0.001$). This represented an improvement in CVD risk prediction beyond the current state of the art. Notably, it was found that FRP was unlike perivascular FAI in relation to its responsiveness to therapy, as the FRP was not altered up to six months following an index cardiac event while FAI was shown to improve during this time, suggesting that FRP is detecting PVAT changes beyond coronary inflammation alone, changes that are not captured by FAI and that are less susceptible to current treatment strategies for CAD. These findings are important in understanding the mechanistic pathways that are being detected in these radiomic analyses and the possible translational applications of the technology into specific clinical scenarios related to the detection and monitoring of coronary artery inflammation.

Therapy targeting coronary inflammation for clinical risk reduction

Given the compelling evidence implicating numerous inflammatory pathways in the development of atherosclerotic CAD, there has been a series of both observational studies and randomised controlled trials that have explored the efficacy of a number of different therapeutic agents for the reduction of coronary inflammation and hence CAD event risk. Many of the therapies discussed here are biological agents with limited clinical data available and this review does not explore the specific safety profiles of these agents, instead we focus on the inflammatory mechanisms targeted by each agent.

Figure 5 provides a summary of the major anti-inflammatory therapeutics that are discussed here as they have been specifically developed for, or are currently being trailed for, the reduction of CAD inflammatory risk. Experimental therapeutics not yet trialled in humans are also discussed here and included in Figure 5.

It is important to note that to date, only five large randomised controlled trials of anti-inflammatory medical therapy for the reduction of risk of atherosclerotic CAD have been completed, and the majority of data discussed here rely on animal models or surrogate markers of inflammation in humans.

Aspirin

The role of aspirin is typically discussed in cardiology as an anti-platelet therapy, however its mechanisms to achieved this anti-platelet effect – the irreversibly inhibition of cyclooxygenase and the suppression of the production of prostaglandins and thromboxanes - also exert an anti-inflammatory effect in the vascular wall (174), which has been well documented in murine models and increasingly in humans (41). The exact means through which aspirin may mediate these anti-inflammatory effects

in the vascular wall are not certain, however there is evidence for a number of different mechanisms that are likely operating in a dose-dependent manner (128). The influence of dosing on aspirin therapy is controversial. Balancing the anti-inflammatory effects of high dosing with the proven cardio-protective benefits of low-dosing is not well understood (110) and has been more extensively studied in regard to the anti-platelet effects of cyclooxygenase inhibition at differing doses. There are three mechanisms through which aspirin has been shown to exert vascular anti-inflammatory effects. Evidence from patients with stable angina provides the first mechanism. In this population aspirin has been demonstrated to reduce levels of inflammatory cytokines such as IL-6, hs-CRP and monocyte colony stimulating factor (75,141). Second, aspirin has been found to be protective of inflammation induced endothelial dysfunction in a small trial of 12 humans (85). Third, aspirin has been found to reduce chemerin (72), an important chemoattractant for macrophages and an adipokine that regulates adipocyte differentiation, and also reduce fractalkine, a chemokine implicated in inflammation and atherosclerotic plaque stability (96). Despite these findings, results from large scale observational studies that looked at high risk CVD patients have called into question the benefits of aspirin on both CVD events and mortality. The Coronary CT Angiography EvaluationN for Clinical Outcomes study (CONFIRM) (38) and the progression of arterial disease and diabetes (POPADAD) trial (22) failed to demonstrate benefit of aspirin in regards to mortality or rate of CVD events, respectively. This run of negative results continued with the publication of the very large, randomised, placebo controlled Aspirin in Reducing Events in the Elderly (ASPREE) trial which found regular low-dose aspirin did not provide any benefit for overall disability-free survival (106), and perhaps emphasises the relatively poor understanding that we have concerning the function

of aspirin beyond its anti-platelet roles. It has been suggested that investigating the effects of aspirin between groups with differing levels of circulating markers of inflammation, such as hs-CRP and IL-6, may be of interest (175), particularly in light of the observational data suggesting possible reductions of these cytokines with treatment (75).

Statins

Statins are HMG-CoA reductase inhibitors predominantly prescribed for their LDL-cholesterol lowering effects and have excellent evidence for their use to reduce the likelihood of CAD events in both primary and secondary prevention. Beyond cholesterol reduction, statins also have an anti-inflammatory effect (145) and have been found to influence levels of serum hs-CRP (160).

Statins have been shown to exert their pleiotropic effects through a very broad range of mechanisms (185). The majority of statins exert their anti-inflammatory effects in a dose-dependent manner, this is particularly true for atorvastatin and simvastatin (18). In brief, statins inhibit the Rho-GTPase isoprenylation through reducing geranylgeranylpyrophosphate production during cholesterol biosynthesis (33). This leads to increased expression of endothelial NOS and NO production (93). It has been demonstrated that statins directly improve vascular NO bioavailability and reduce vascular O_2^- through tetrahydrobiopterin-mediated endothelial NO synthase coupling (11). Beyond this, statins have also been shown to increase NO production via activation of the PI3K-Akt pathway through phosphorylation of Akt (89). In vivo, a single dose of simvastatin to either wild type or apoE^{-/-} mice increased endothelium-derived NO production (151). Moreover, statins can suppress the activity of pro-oxidant enzymes (such as NADPH oxidase) in the endothelium (104). Statins have

also been found to influence leucocyte migration at the site of vascular inflammation via reduction in the expression of intercellular adhesion molecule-1 (140).

hs-CRP had been independently shown to predict the risk of CAD events in observational studies (80,142). This relationship between CAD events, hs-CRP as a marker of inflammation and statin therapy was first explored in the Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) study in 2008, which examined the effects of rosuvastatin on apparently healthy adults with elevated hs-CRP but without hyperlipidaemia (144). They found that rosuvastatin significantly reduced the incidence of major cardiovascular events, despite the fact that nearly all study participants had lipid levels at baseline that were well below the threshold for treatment according to current prevention guidelines. Further analysis also demonstrated that a lower number of CVD events were observed in patients who achieved both very low LDL cholesterol (<1.81 mmol/l) and low hs-CRP (<1 mg/l) levels, suggesting that treating both blood lipids and vascular inflammation achieves better risk control than lipids alone (142).

More recently, the IMProved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) study re-examined this relationship between LDL cholesterol, inflammation as quantified by hs-CRP and CVD events. In IMPROVE-IT, 15,179 patients were randomised to either simvastatin monotherapy or combination therapy with simvastatin and ezetimibe, a cholesterol absorption inhibitor. It was found that the combination lipid-lowering therapy significantly increased the likelihood of reaching both the pre-specified LDL cholesterol target (<1.81 mmol/l) and the hs-CRP target (<2 mg/l). Those who reached both targets had significantly lower primary CVD

endpoints compared to those meeting neither target (38.9 % versus 28.0 %; adjusted HR 0.73; 95 % CI [0.66–0.81]; $p < 0.001$) (26).

It is important to note that not all available evidence suggests that statin therapy has significant effects on reducing the risk of CVD events through modulating inflammation. Trials similar to JUPITER, such as the Anglo-Scandinavian Cardiac Outcomes Trial, have been unable to reproduce the main findings (157). With the advent of proprotein convertase subtilisin/kexin type 9 (PCSK-9) inhibitors, a potent class of cholesterol reduction medications, important questions related the relationships between LDL reduction and inflammation, as assessed by hs-CRP have been addressed (153). PCSK-9 inhibition appears to have very little effect on hs-CRP despite very significant reduction in LDL cholesterol (24). These findings suggest that statins are indeed exerting direct anti-inflammatory effects that are mediated beyond reduced LDL cholesterol, and that inflammation, as assessed by hs-CRP, is not inevitably reduced with aggressive LDL cholesterol reduction.

Relevant to the role of both statins and aspirin in reducing inflammatory risk is findings from the validation study of perivascular FAI – the CRISP-CT study (Figure 6) (119) that among participants who received a clinical recommendation to initiate statins or aspirin following clinical CCTA, the perivascular FAI (measured before deployment of the new treatment) was no longer predictive of cardiac mortality (adjusted HR 2.97, 95% CI 0.46–19.35; $p = 0.25$). In comparison, among those who did not receive any recommendations for change of management after imaging, the predictive value of high perivascular FAI values for cardiac mortality was retained (adjusted HR 18.71, 95% CI 2.01–174.04; $p = 0.0101$), suggesting that the risk identified by the perivascular FAI could be modifiable with statin or aspirin therapy. This finding could suggest a role for perivascular FAI in guiding the initiation of

aspirin and/or statin therapy in patient who traditionally would not be considered candidates for such measures.

Biologics

Despite the results demonstrating modest success in reducing inflammation through statin therapy, the major issue of specifically targeting the residual risk of CAD through inflammatory pathways will not be addressed through studies examining statin therapy alone. The acceleration in discovery and use of biological active medications has offered a completely new therapeutic scope to researchers and clinicians. Specific anti-inflammatory interventions are now being studied and, in many cases prescribed, for a host of chronic inflammatory conditions such as rheumatoid arthritis, ankylosing spondylitis, psoriasis and inflammatory bowel disease. The availability of these anti-inflammatory agents has generated numerous hypotheses related to the suppression of inflammatory pathways for the reduction of CAD risk beyond the LDL cholesterol reduction paradigm.

TNF- α and IL-6

As discussed at length above, TNF- α and IL-6 are inflammatory cytokines strongly implicated atherosclerosis and therefore the role of blockade of these cytokines on CAD risk is of significant interest (113). No large randomised controlled trials of these agents have been initiated in the CVD field yet.

Most people currently receiving medications such as IL-6 receptor blocker tocilizumab or the TNF- α blocker etanercept are on treatment plans for rheumatoid arthritis or other non-CVD autoimmune conditions. The studies informing these treatment indications were never statistically powered for the assessment of CAD

endpoints and meta-analyses have also failed to elucidate the effects of these medications on hard CVD events (150). The focus has therefore been on surrogate markers of CVD risk in pharmaco-epidemiological studies that aim to elucidate the differences in CVD risk between different biological agents in patients already receiving therapy. For example, a recent observational study in individuals with severe psoriasis showed that biologic therapy was associated with favourable modulation of plaque indices (50). Such studies need to be considered carefully due to the fundamental risks of confounding due to participants disease risk factors and confounding by indication. A meta-analysis of observational studies in patients with rheumatoid arthritis, psoriatic arthritis and psoriasis found that those prescribed TNF- α blockers are at 30% lower risk (95% CI 0.54–0.90) of CVD than patients taking non-biological therapies (148), suggesting that there may be a useful role for TNF- α blockage in reducing CVD events in those with established non-cardiovascular inflammatory conditions. Similarly, analysis from the CORRONA database found that those taking TNF- α blockers (compared to those on non-biological and non-methotrexate based therapies) had a substantially decreased risk of CVD events (HR 0.39, 95% CI 0.19 to 0.82) (61), although many issues exists related to the sample populations in this study and these likely confounded the findings (185). The ENTRACTE trial directly compared IL-6 receptor blocker tocilizumab with TNF- α blocker etanercept in the presentation of CVD events in those with rheumatoid arthritis; however this study was not designed to test the inflammatory hypothesis, but to prove safety of tocilizumab in relation to risk of major adverse cardiovascular events (MACE) when compared to etanercept. The study found that rheumatoid arthritis patients treated with tocilizumab were 5% more likely to experience MACE compared with those treated with etanercept (HR of 1.05, CI 0.77–0.43). However,

the uncertainty around this estimate was wide enough that the true risk for MACE in the tocilizumab arm could have been anywhere from 43% higher to 23% lower than in the etanercept arm.

IL-1 β

In what was an eagerly awaited trial published in 2017, the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) (143) was the first study to directly assess a fully human monoclonal antibody, in this case anti-IL-1 β , in the prevention of cardiovascular events. This study was ground-breaking as it was the first true test of an anti-inflammatory biological therapy intended for the reduction of CAD events in a randomised, double-blind trial format. CANTOS included 10,061 high-risk CVD patients with known previous MI and an hs-CRP of $\geq 2\text{mg/l}$. Three doses of the agent were compared (50mg, 150mg and 300mg) with placebo therapy, all administered subcutaneously. The primary efficacy endpoint was nonfatal MI, nonfatal stroke, or cardiovascular death. Compared with patients receiving placebo (4.50 events per 100 person-years), both the 150 mg (3.86 events per 100 person-years; HR 0.85; 95% CI 0.74-0.98) and 300 mg (3.90 events per 100 person-years; HR 0.86; 95% CI 0.75-0.99) doses of canakinumab resulted in a significant reduction of the primary endpoint. Canakinumab was associated with a significantly higher incidence of fatal infection than was placebo (0.31 versus 0.18 events per 100 person-years). Importantly there was no change in LDL cholesterol and large concomitant reductions in hs-CRP and IL-6 were observed (143). Importantly, the secondary endpoint of urgent revascularisation returned a significant result, with a 17% relative risk reduction over the follow up period (median 3.7 years). In what is possibly a demonstration of the role in inflammation across numerous systems, there

was also a significant dose-dependent reduction in incident lung carcinoma and related mortality.

In some quarters, this study has been declared the final piece of evidence required to prove the inflammatory basis of residual atherosclerotic disease risk (79), and indeed, this study does provide irrefutable evidence that modulating inflammatory pathways relevant to atherosclerosis can reduce CVD risk. Despite this, canakinumab will not yet be included among the suite of medications commonly utilised for secondary prevention of CAD and further studies are required in low risk and other diverse populations before wide-spread changes in practice will occur. The observed association of canakinumab with a higher risk of fatal infections also necessitates further studies to prove the safety of this therapy. Moreover, further studies to potentially identify other anti-inflammatory treatments with a more favourable risk/benefit ratio will be crucial.

Relevant to this discussion is the decision of the USA Federal Drug Administration to reject a 2018 application by the manufacturer of canakinumab for an approval as a cardiovascular medication (108), and the withdrawal by the manufacturer of their European Medicines Agency application (105). This highlights the difficulty in bringing novel CVD anti-inflammatory agents to the market, particularly when the pathways being targeted are still contested as to their exact roles in CAD. The specific applications brought before the regulators in regards to canakinumab were not broad, and targeted a specific population from within the CANTOS study who they termed 'responders' to the treatment. They proposed trialling patients with a single dose of the agent to assess their suitability for a complete course based upon hs-CRP level reduction, as a surrogate for vascular inflammation response. This approach emerged from subgroup analysis that showed patients who achieved a

substantial reduction in hs-CRP (to <2.0mg/l) had greater benefit from the treatment, including the most significant reduction in cardiovascular mortality (143).

PLA₂ superfamily

There are many different groupings within the PLA₂ enzyme superfamily. The most studied in regards to atherosclerosis include group II secretory PLA₂ (sPLA₂), and PAF acetylhydrolase, also known as lipoprotein-associated (Lp)-PLA₂ (146). Some PLA₂ enzymes have been found to be protective against atherosclerosis, so effects need to be well understood before the initiation of human trials to avoid unexpected results (4). There has been significant interest in the development of novel selective inhibitors for PLA₂ isoforms to act as anti-inflammatory agents relevant for atherosclerotic CAD. There is animal model evidence for the anti-atherosclerotic effects of the sPLA₂ inhibitor varespladib. When apoE^{-/-} mice were treated with varespladib there was a significant reduction in atherosclerotic plaque development (53). Another similar agent is darapladib, which is a selective inhibitor of Lp-PLA₂. In diabetic and hypercholesterolemic pig models this agent was found to reduce the development of coronary atherosclerosis, including reduction of plaque necrotic core area (190).

Observational data in humans suggests that both sPLA₂ and LP-PLA₂ inhibitors may be clinically useful for the reduction of CAD risk (171). Despite this, for darapladib, results in early clinical trials have been mixed. In a phase 2 trial, darapladib did reduce circulating IL-6 and hs-CRP significantly after 12 weeks of therapy (109), and halted progression of the necrotic core of atherosclerotic plaque (156). The phase 3 STABILITY Trial (188) included 15,828 individuals with 3.7 year follow-up and the SOLID-TIMI 52 Trial (116) which enrolled 13,026 patients with ACS with 2.7 year

follow-up, failed to show any reduction in risk for a composite CVD endpoint. Both trials have effectively ended current investigation into the role these agents could play in atherosclerotic disease management.

IL-12 and IL-23

IL-12 is a cytokine strongly implicated in the inflammatory response to atherosclerosis development, predominantly through INF- γ production and polarisation of CD4⁺ T-cells into pro-inflammatory phenotypes (169). Selective inhibition of IL-12 in immune cells has been shown in familial hypercholesterolemia murine models to dramatically decrease aortic plaque size (194). The link between IL-23 and CAD is not clear, however there is active research into its possible role in atherosclerosis given it has been implicated in carotid disease (2).

Ustekinumab and briakinumab are antibodies developed for the treatment of psoriasis that bind the p40 subunit, which both IL-12 and IL-23 possess, and theoretically would act as inhibitors of these pro-inflammatory cytokines. No clinical data is available as yet to suggest these agents are effective at reducing inflammatory pathways relevant for CAD risk reduction, and concerns have been raised regarding their safety after a meta-analysis found increased major adverse cardiac events following their administration in trials for other chronic inflammatory conditions (178).

Non- biological agents

Methotrexate

Methotrexate is a folate antimetabolite that inhibits DNA synthesis, repair, and cellular replication. Given these properties, methotrexate was originally an anti-cancer therapy that was found to also possess anti-inflammatory mechanisms

independent of its anti-folate activity and gained widespread acceptance as an important anti-rheumatic therapy. The mechanism by which methotrexate exerts anti-inflammatory effects in the vascular system remains to be fully elucidated. There is ample evidence to suggest that methotrexate does act on mechanisms fundamental to atherogenesis, with key work in both murine and leporine models indicating that obese mice treated with methotrexate produce less pro-inflammatory TNF- α , IL-6, MCP-1, IL-1 β and leptin, and more anti-inflammatory adiponectin and IL-10 (130). In leporine models, a high dose regime of intra-venous methotrexate demonstrated a 65% reduction in aortic atherosclerotic lesion size (32), although how relevant such findings are for humans is questionable. Similar to biological agents already discussed, observational data in those receiving methotrexate for rheumatological indications has indicated that there may be a reduction in CVD events in those on therapy (107).

Given these laboratory and observational findings, the Cardiovascular Inflammation Reduction Trial (CIRT) tested the benefit of low-dose methotrexate, in combination with folate, for the reduction of CV events in a randomised double-blind, placebo-controlled setting. Similar to the CANTOS Trial, the population CIRT selected were all stable but high-risk CVD patients suitable for secondary prevention therapy. In contrast to CANTOS, CIRT found that low-dose methotrexate treatment did not reduce levels of circulating IL-1 β , IL-6, or hs-CRP protein and, importantly, was not associated with fewer cardiovascular events than placebo. There are numerous possible reasons why CIRT failed to demonstrate benefit when CANTOS did. The obvious reason being that the pathways targeted by each drug are fundamentally different and that not all downregulation in pro-inflammatory pathways will be relevant for atherosclerotic disease. The selection of patients between the studies is

also of significance, as although similar, the population was different in that the CANTOS trial only enrolled those with elevated hs-CRP (median baseline hs-CRP 4.2mg/l) while CIRT did not focus on identifying those with elevated hs-CRP but instead enrolled only those with either diabetes or metabolic syndrome. This resulted in a median baseline hs-CRP of 1.6mg/l. How this patient group with lower systemic inflammatory burden would have responded to canakinumab had they been included in the CANTOS study is not known, but raises important questions about patient selection for anti-inflammatory treatments and the applicability of both studies in the population at large.

Colchicine

Colchicine is a longstanding anti-inflammatory drug predominately used for gout and pericarditis. Colchicine disrupts cytoskeletal functions by inhibiting β -tubulin polymerization into microtubules, preventing activation, degranulation, and migration of neutrophils in a range of tissues. Colchicine has also been found to interfere with intracellular assembly of the inflammasome complex present in neutrophils and monocytes that mediate activation of IL-1 β (23). These mechanisms are of significant interest for atherosclerotic CAD given the known roles of neutrophils in the pro-inflammatory milieu once they infiltrate CAD lesions (112).

The Low-Dose Colchicine (LoDoCo) trial enrolled 532 patients with stable CAD receiving aspirin and/or clopidogrel and statins who were then randomly assigned colchicine 0.5 mg/day or no colchicine and followed for a median of 3 years (115). This small non-placebo controlled study demonstrated that the addition of colchicine to standard therapy in patients with stable coronary disease significantly reduced the risk of a composite end-point including ACS and out-of-hospital cardiac arrest

(hazard ratio: 0.33; 95% CI: 0.18 to 0.59; $p < 0.001$; NNTT: 11). The benefits of colchicine were achieved on a background of widespread use of effective secondary prevention strategies, including high-dose statins and the patient sample was selected regardless of baseline levels of circulating inflammatory markers.

The Low Dose Colchicine after Myocardial Infarction (LoDoCo-MI) trial recently explored the use of colchicine further in the secondary prevention population (71). This trial was not designed to detect differences in CV events but instead investigated the effect of low-dose colchicine on hs-CRP in comparison to placebo. With a short follow-up of 30 days, they found no significant difference in hs-CRP in this high-risk, secondary prevention population.

In a further recent trial, the Colchicine Cardiovascular Outcomes Trial (COLCOT) undertook a randomized, double-blind trial involving patients recruited within 30 days following a MI. Patients received either low-dose colchicine (0.5 mg once daily) or placebo. The primary efficacy endpoint was a composite of death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, stroke, or urgent hospitalization for angina leading to coronary revascularization. This was the largest trial of colchicine for CVD prevention to date, with 4,757 patients enrolled, with no regard for baseline hs-CRP or other circulating inflammatory markers. After follow-up for a median of 22.6 months, the primary composite endpoint occurred in 5.5% of the patients in the colchicine group, as compared with 7.1% of those in the placebo group (hazard ratio, 0.77; 95% CI, 0.61 to 0.96; $P=0.02$). This result was predominantly mediated through a reduction in strokes and urgent hospitalisations for angina leading to coronary revascularisation.

From a patient perspective, the acceptability of colchicine for secondary prevention of CAD following MI is yet to be determined, with rates of significant gastrointestinal upset across all the colchicine trials discussed here ranging from 9% to 20%.

Epigenetic targets

Recent advances in our understanding of the epigenome has opened the possibility that cardiac specific microRNAs have potential as diagnostic biomarkers and treatment targets for CAD (48). Epigenetic alterations include DNA methylation, histone modification, microRNA and lncRNA, which are fundamental to the expression of genes for normal cellular function. One such alteration of note for CAD is acetylation of lysine residues on histone proteins. Bromodomain and extra-terminal (BET) proteins are a family of epigenetic readers that detect and bind to acetylated lysine residues, thereby forming molecular scaffolds between chromatin and transcriptional machinery. The recruitment of BET proteins has been found to enhance the expression of genes that drive maladaptive responses in atherosclerosis, including inflammation and oxidation (30,66). The possible clinical role of BET proteins has now been explored in a phase 3 clinical trial which recently reported its findings. This trial was established after pooled analysis of 3 placebo controlled phase 2 trials demonstrated benefit for the BET inhibitor apabetalone in regards to MACE (114). The BETonMACE trial was a randomised, double blind, placebo-controlled trial which saw 2,425 patients with recent ACS, type 2 diabetes and low HDL-cholesterol levels randomised on a 1:1 basis to apabetalone or placebo. Following a median period of 26.4 months, no difference was found between the groups for MACE (138). This study targeted a very specific high-risk population in an attempt to best make use of the known mechanism of the agent

being tested (114) and to select a population with a likely high inflammatory burden, but despite this did not demonstrate benefit.

Experimental therapeutics

There is considerable interest in the development of therapeutic approaches to the reduction of CAD risk through anti-inflammatory means beyond what has been included in this review. Despite the majority of inquiry to date focussing on therapeutics that inhibit the function of certain implicated cytokines, there are other possible approaches (137). Murine models have been utilised to explore the harnessing of the protective function of T-reg cells and the anti-inflammatory cytokines they secrete such as IL-10 and TGF- β (5). Techniques trailed to date include the administration of anti-CD3 antibodies that stimulate T-reg function (162) and the local delivery of IL-2 (43), both of which have suppressed atherogenesis in murine models.

Alternative approaches have investigated the stimulation of immune tolerance to common antigens found in atherosclerotic lesions and the direct delivery of anti-inflammatory cytokines. In early work, utilising adenovirus', the delivery of anti-inflammatory cytokine IL-19 has been successfully demonstrated to reduce intimal thickening and vascular smooth muscle cell proliferation in a dose dependent manner (173). Another approach has been via stealth liposomes, that is liposomes with inclusion of the synthetic polymer polyethylene glycol in their composition that remain in circulation longer and encapsulate the active nano-cargo, in this case the anti-inflammatory IL-10 (76). When delivered by stealth liposomes, IL-10 was reliably delivered to sites of atherosclerosis in mice (8), however no findings regarding improvement in disease states have been reported.

The direct manipulation of intra-cellular signalling has been preliminary explored as a possible novel approach to limit atherosclerosis, and other work has occurring on relevant molecules, such as JAK3, in other inflammatory conditions (36). In atherosclerosis relevant work the in vivo transfection of decoy oligodeoxynucleotides (ODN) with a high affinity for NF- κ B, a critical transcription factor in the activation of cytokines and adhesion molecules in atherosclerosis, into balloon-injured rat carotid artery resulted in the inhibition of neointimal formation at 14 days after injury as compared with vessels transfected with scrambled ODN ($P < 0.01$) (193). Beyond this, non-coding ribonucleic acid (RNA), and in particular microRNA, has also emerged as a potential therapeutic target for curtailing cytokine-mediated inflammation in atherosclerosis (52). An example is exploiting the reduction in inflammatory response when there is a deficiency (47). The delivery of miRNA therapies directly to the plaque may be assisted with the development of novel nanomedicine (39).

The companion utility of non-invasive assessment of coronary inflammation

To date, there has not been a dedicated anti-inflammatory drug trial for CAD that has utilised non-invasive means to directly interrogate changes in coronary inflammation.

Given that the degree of coronary inflammation prior to and after acute coronary syndromes is highly heterogeneous, it is perhaps not surprising that clinical trials involving largely unselected patients with MI have had variable results to date. Novel imaging techniques may therefore have an early role in the identification of patient subgroups with the highest degree of coronary inflammation, who might be expected to derive the most benefit from immunomodulatory therapies.

As already discussed, there are two realistic techniques available to trialists for this to be applied into the right clinical setting and balance of ethical considerations. Currently, the two techniques that would be achievable are PET-CT, most likely with ^{18}F -FDG as the radiotracer, and, perhaps more achievable, the perivascular FAI, which relies on simple cardiac CT.

There is one sub-study underway which is employing ^{18}F -FDG PET-CT to detect changes in coronary inflammation with low dose methotrexate treatment (1). This is a secondary study of the CIRT imaging study and the investigators plan to utilise serial PET-CT imaging in a subset of patients enrolled in the main CIRT trial to directly visualize vascular inflammation. 123 subjects, who are all high risk for CVD and enrolled in the main CIRT trial, have been consented for baseline imaging and follow up imaging that will be performed approximately 8 months after enrolment. This study is yet to report results, however with the negative findings of the main CIRT analysis, it is thought unlikely that a significant reduction in identifiably coronary inflammation will be found.

It has been shown in a small study of 41 patients that measurement of PVAT attenuation around high-risk plaques also correlates with ^{18}F -sodium fluoride uptake on PET-CT (90). This proof-of-principle study confirmed that severe vascular inflammation drives changes in PVAT attenuation, as detected by FAI. The investigators of this work focused their analysis on high-risk plaques, as identified by conventional coronary CTA image analysis, and demonstrated that lesions with increased activity of ^{18}F -NaF had higher surrounding PVAT density (a crude measure of perivascular FAI) than those without (-73HU; IQR: -79 to -68 vs. -86; IQR -94 to -80; $p < 0.001$). This finding suggests that it would be unnecessary to examine both imaging biomarkers of coronary inflammation, and that the more

readily available perivascular FAI marker would likely be the first choice of trialists wishing to visualise the effects of interventions on coronary inflammation, in particular around high-risk plaques.

The perivascular FAI is yet to be tested in a clinical trial setting to assess the reversibility of coronary inflammation in response to pharmacological therapy of any sort (10). Despite this, data does exist to demonstrate the modifiable nature of FAI. In patients who received advice to commence treatment with statins or aspirin after CCTA, the perivascular FAI (measured before deployment of the new treatment) was no longer predictive of cardiac mortality (adjusted HR 2.85, $p=0.25$) while those who did not receive any recommendations for change of management after CCTA, the predictive value of high perivascular FAI values for cardiac mortality was retained (adjusted HR 18.71, $p=0.01$) (102). In further evidence of the modifiable nature of FAI in response to CAD therapy perivascular FAI measured around culprit lesions during ACS changes dynamically post-event, with significant changes being detectable as early as 5 weeks post-acute coronary syndrome and following the initiation of optimal secondary preventative therapies (19).

Beyond these findings, the best evidence to date that novel imaging techniques can be utilized to track response to interventions aimed at reducing the risk that inflammation conveys in CAD has come from an observational cohort of 134 patients with moderate to severe psoriasis but low CVD risk. In this cohort treatment with anti-inflammatory biological agents (anti-TNF- α , anti-IL17 and anti-IL12/23) was found to be associated with a significant reduction in perivascular FAI at one year compared to individuals treated with topical or ultraviolet B phototherapy (treatment group median FAI -71.22 HU [interquartile range (IQR), -75.85 to -68.11 HU] at baseline vs non-treatment group -76.09 HU [IQR, -80.08 to -70.37 HU] at 1

year; $P < .001$) (51). Figure 7-A provides a density plot demonstrating the changes in FAI between those who did and did not receive anti-inflammatory biologics, while Figure 7-B provides CCTA images of the coronary arteries with FAI demonstrated for patients who had a great, moderate or modest response to the biological therapy.

Innovation

Within cardiovascular science, better understanding the inflammatory pathology leading to atherosclerotic disease is somewhat of a holy-grail. This is due to the profound clinical impact that is possible with the identification and targeting of previously unknown pathways or mechanisms that influence patient risk. There is deep innovation in the development of novel biological agents to modulate coronary artery inflammation, however no single agent has yet proven viable for widespread clinical adoption. The non-invasive detection of coronary artery inflammation is a field of rich innovation, the perivascular Fat Attenuation Index being an excellent example within this field.

Conclusions

There is no doubt that inflammatory mechanisms play a fundamental role in the initiation and development of coronary artery atherosclerotic disease. The inflammatory mechanisms discussed here include the modification of lipoproteins within the arterial intima, the formation of foam cells, defective efferocytosis, the influence of vascular redox state and an imbalance in favour of pro-inflammation mediators over endogenous anti-inflammatory mechanisms. Importantly, these mechanisms are all triggered by the highly pro-inflammatory stimulus of lipid accumulation in the vessel wall, and efforts to reduce cholesterol levels should not be curtailed. This review outlined the efforts to develop novel techniques for both the visualisation of coronary inflammation and its treatment with agents that act as pure anti-inflammatories, over lipid reduction – such as through the inhibition of IL-1 β with canakinumab. The results of such trials to date have been informative but also highly demonstrative of the issues in modulating inflammatory mechanisms. The increase in fatal infections in the CANTOS trial is explainable by the host-defense compromising potential of this agent. Moving forward, continued efforts are required to describe the contributory mechanisms driving pro-inflammatory mechanisms in CAD. Given the extreme on-going risk to life that CAD carries, this work must focus on identifying possible targets for pharmaceutical intervention. Moreover, we require a focus on how trials for novel CAD therapies, be they anti-inflammatory or pro-resolution therapies, can better utilise non-invasive inflammation detection techniques, as discussed here, for both the initial selection of patients for therapy and the monitoring of the efficacy of such treatments. We hope that such an

approach will lead to greater success in the development of the next generation of agents to continue the struggle against the residual inflammatory risk of CAD.

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Disclosures: The methods for analysis of the perivascular Fat Attenuation Index described in this report are subject to patents EP3179917 - PCT/GB2015/052359 and PCT/GB2017/053262, while the methods for AI/radiomic analysis of perivascular space or adipose tissue are subject to patent applications GB2018/1818049.9, GR2018/0100490 and GR2018/0100510. CA is a founder and shareholder of Caristo Diagnostics Ltd., a CT image analysis company.

Figures and Legends

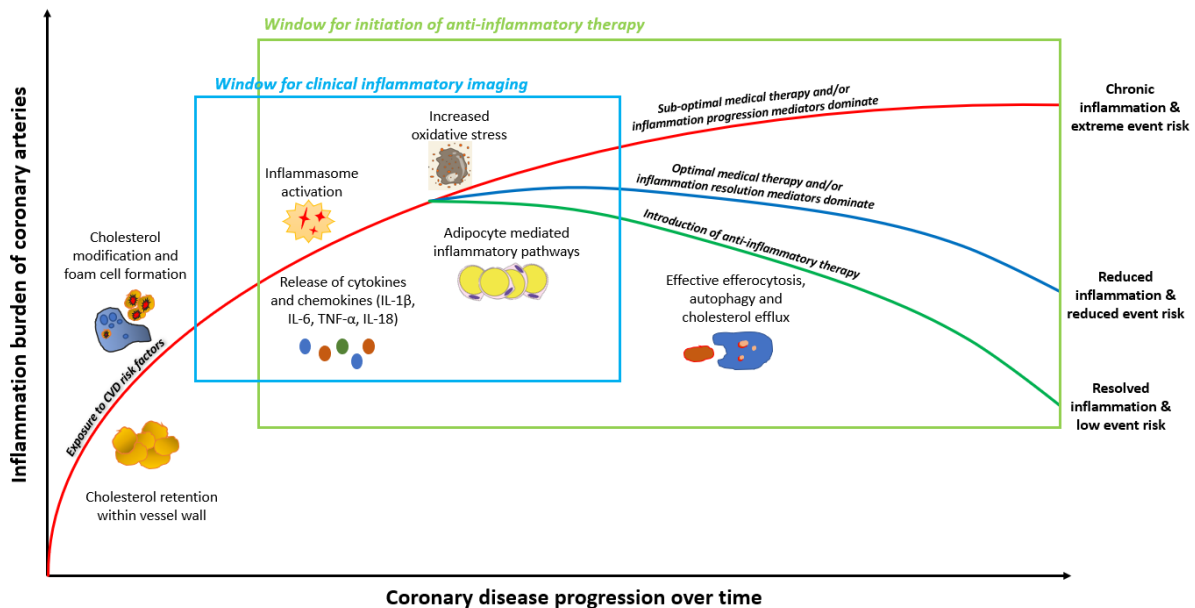


Figure 1. The theoretical progression curves of coronary artery inflammatory burden over time

Coronary inflammation increases over time with exposure to the traditional modifiable and non-modifiable risk factors for coronary artery disease (CAD). Pro-inflammatory mechanisms contributing to risk of CAD begin with the retention of cholesterol within the arterial wall, the pathological modification of these cholesterols and the formation of foam cells. Subsequent pro-inflammatory mechanisms include the release of cytokines and chemokines by immune cells within the vessel wall, and the activation of inflammasomes. Oxidative stress and the effects of peri-vascular adipose tissue, mediated through paracrine signalling, also significantly contribute to the overall pro-inflammatory state of the vessel wall. The window for the clinical detection of coronary inflammation is demonstrated in the light blue box, with the window for possible clinical treatment of this inflammation demonstrated in the light green box. Possible patient trajectories include maintaining a high degree of

inflammation (red) with likely adverse CAD outcomes. Patients on optimal lifestyle and medical therapy and/or those who through a pro-inflammation resolution genetic disposition have a reduced risk of CAD events (dark blue). Patients who through careful selection are initiated on anti-inflammatory treatment for CAD risk may have an even lower risk of CAD events (dark green).

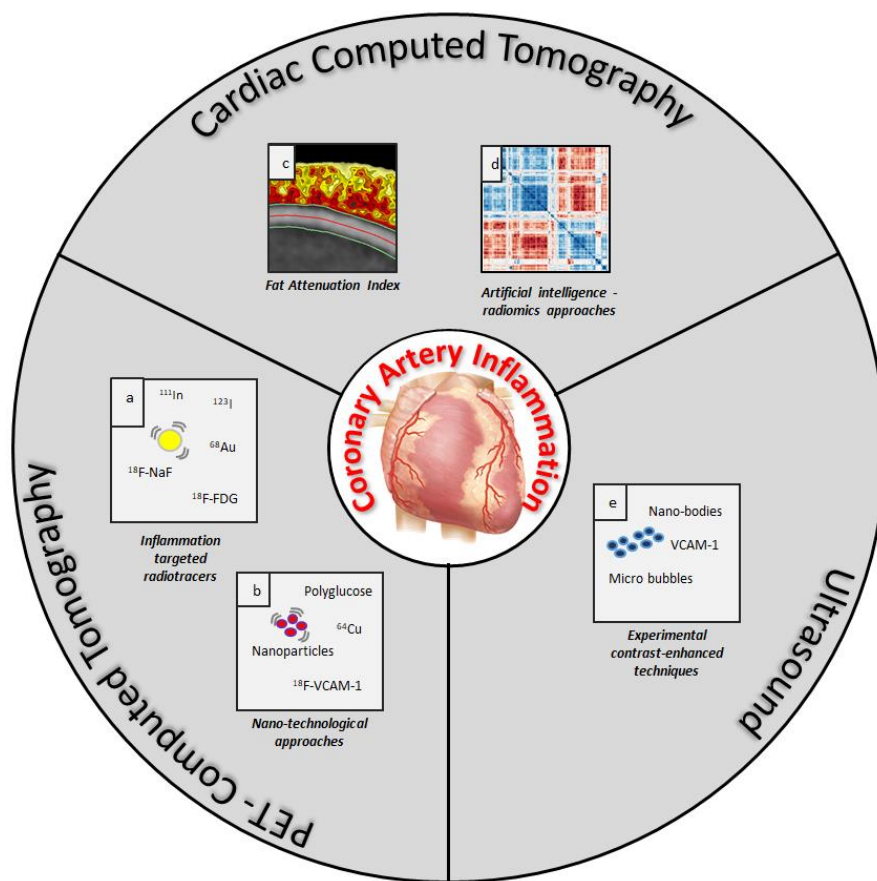


Figure 2. Non-invasive imaging modalities for the assessment of coronary artery inflammation

There are currently three modalities of imaging available to the scientist and clinician for the non-invasive assessment of inflammatory mechanisms occurring in the coronary arteries. Traditionally, cardiac computed tomography has been used to identify anatomically significant coronary lesions and quantify the degree of luminal stenosis caused by their presence. However, with the use of CT contrast agents, molecular CT and fusion positron emission tomography/CT with clinical or experimental radiotracers the in-vivo visualization of the earliest pathophysiological processes leading to atherosclerosis are becoming detectable. Such processes include inflammatory driven apoptosis and cell migration **(a)**. These techniques have been further developed into experimental models that utilise nanoparticles and

radiotracers that target inflammation specific receptors such as VCAM-1 **(b)**. The perivascular Fat Attenuation Index (FAI) can be measured on traditional cardiac CT with and without contrast administration **(c)**. FAI has demonstrated that coronary inflammation can be detected at an early stage by identifying spatial changes in perivascular fat attenuation. FAI is dynamic and can reflect response to changes in the inflammatory status of coronary lesions. The field of cardiac CT also enables radiomics based approaches to inflammation detection **(d)**, the recently developed Fat Radiomic Profile (FRP) captures inflammation and fibrotic changes around the coronary vasculature through applying artificial intelligence analysis to radiomic data. The experimental use of contrast-enhance ultrasound in murine models has proven the ability to visualise inflammation **(e)**, however how these techniques could be applied in humans is yet to be proven.

PET: positron emission tomography; VCAM-1: vascular cell adhesion molecule 1.

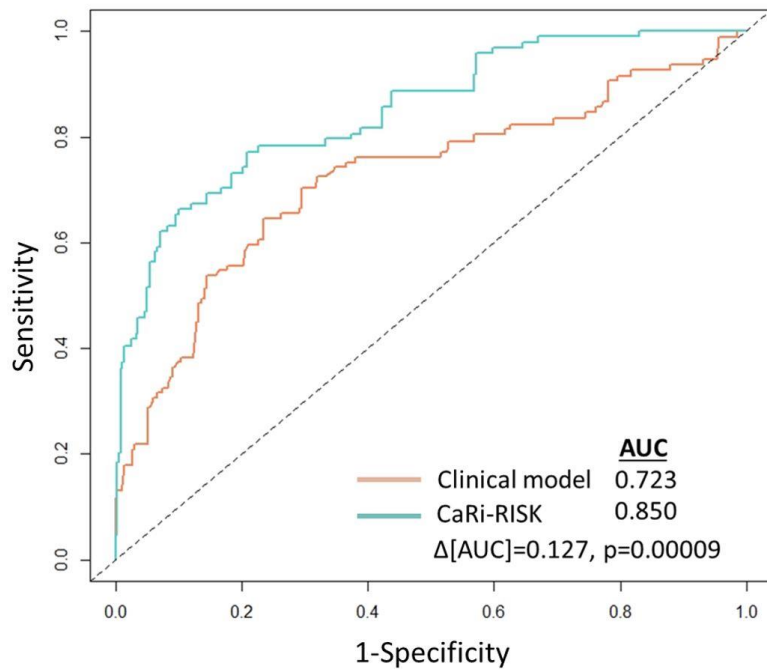


Figure 3. The incremental prognostic value of perivascular FAI for cardiac mortality beyond current clinical risk stratification.

Clinical model: includes the typical clinical factors included in the classic risk scores used in clinical practice: age, sex, smoking, hypercholesterolemia, diabetes and hypertension. The CaRi-RISK model is the clinical model plus the input of perivascular FAI assessment as extracted from CCTA images via the CaRi-HEART algorithm. CaRi-RISK is a proprietary CAD risk assessment product owned by Caristo Diagnostics Limited. Presented here is a time dependent ROC curve (at $t_0=6$ years) and respective AUC for the two nested models (before and after the addition of perivascular FAI information) for discrimination of cardiac mortality in the complete CRISP-CT cohort. AUC increased from 0.723 to 0.850 ($p_{\Delta AUC}=0.00009$) with the addition of perivascular FAI data.

AUC: area under the curve; CAD: coronary artery disease; CCTA: coronary computed tomography angiography; CI: confidence interval; FAI: fat attenuation index; HU: Hounsfield Units; ROC: receiver operating characteristic.

Original data published in the validation study of perivascular FAI (119).

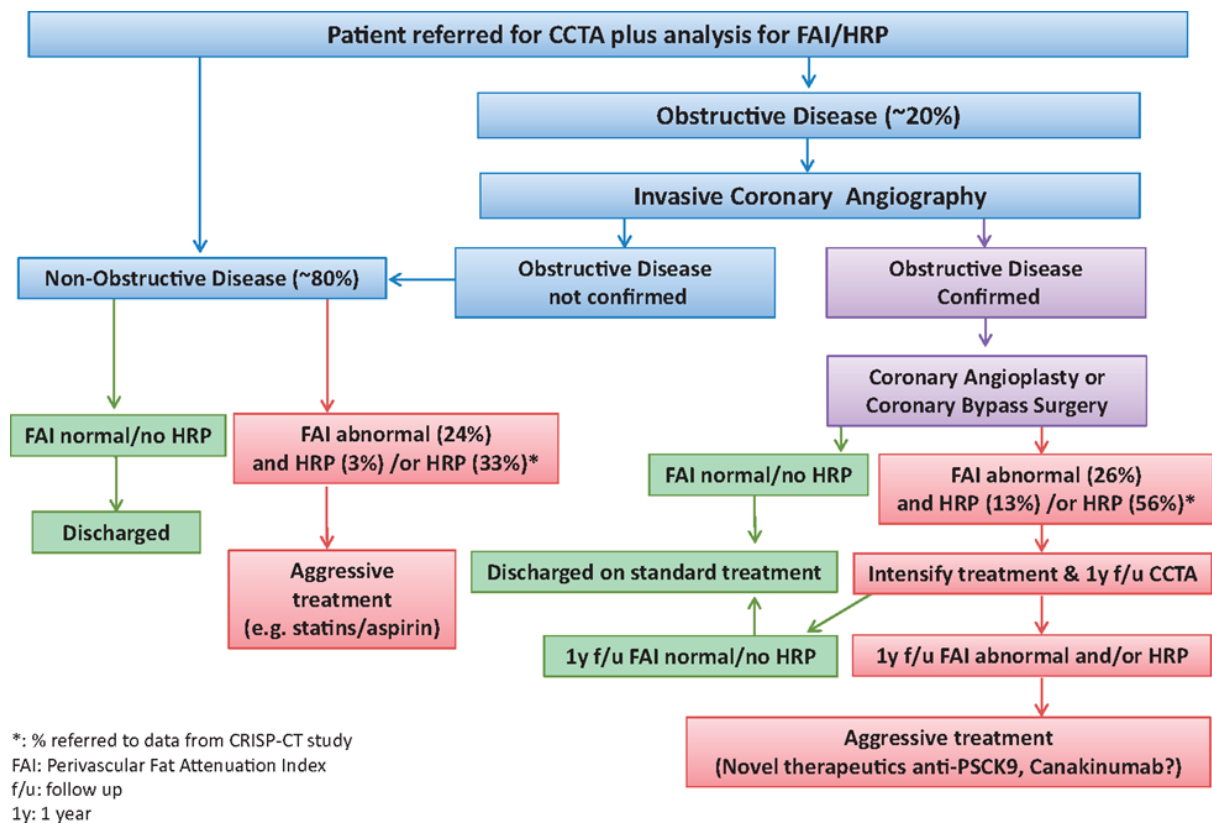


Figure 4. Vision for implementing personalised assessment of the perivascular fat attenuation index (FAI) and high-risk plaque features (HRP) within clinical practice.

CAD, coronary artery disease; CV, cardiovascular; OMT, optimal medical treatment; anti-PCSK9, pro-protein convertase subtilisin/kexin type 9 inhibitors

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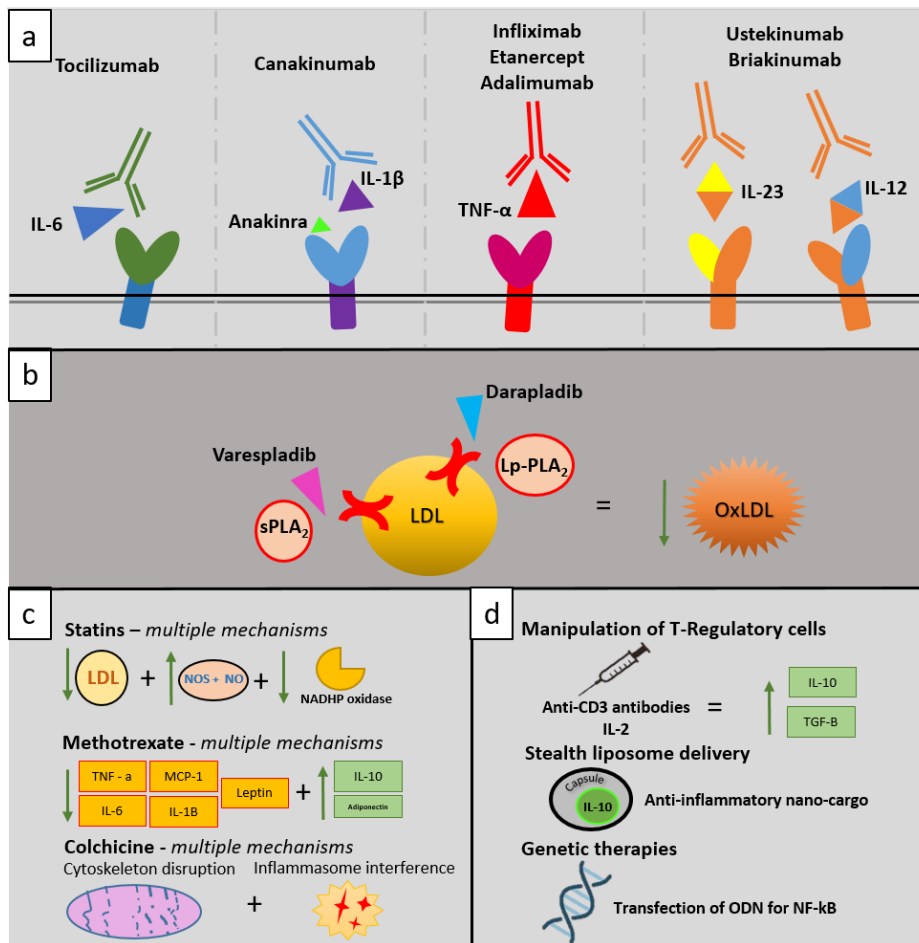


Figure 5. Summary of anti-inflammatory therapies for atherosclerosis

The current pharmaceutical approach to targeting inflammation in coronary artery disease (CAD) can largely be broken into biological agents and non-biological agents. Parts **(a)** and **(b)** outline the biological agents that have been trailed for CAD anti-inflammatory purposes to date. The majority are antibodies (a) which act by inhibiting the binding of a pro-inflammatory interleukin upon its respective receptor, while varespladib and darapladib (b) are not antibodies. To date, canakinumab has been the most successful agent in randomised trials, although no biologicals currently possess regulatory approval for CAD risk reduction. Varespladib and darapladib act by inhibiting the binding of phospholipases to reduce the oxidative modification of LDL to OxLDL, a highly inflammatory molecule. Non-biological agents

currently prescribed for CAD risk reduction and other indications include the medications listed in **(c)**. Statins are the mainstay medication for cholesterol reduction and have well described anti-inflammatory effects through a variety of mechanisms including through mediating an increase in nitric oxide synthase and nitric oxide. Methotrexate and colchicine have both recently been trailed for their anti-inflammatory effects in the CAD setting, with mixed results. Their exact mechanisms are complex however methotrexate exerts significant effects on cytokine regulation while colchicine has been shown to disrupt the cytoskeleton of migratory immune cells and interfere with the pro-inflammatory effects of inflammasomes. Other experimental therapeutics not trialled in humans are outlined in **(d)**, and include the manipulation of the anti-inflammatory actions of T-regulatory cells to influence anti-inflammatory cytokine release, the delivery of anti-inflammatory cytokines such as IL-10 in encapsulated stealth liposomes and the prospect of genetic therapies to influence the transcription of specific genes associated with atherogenesis.

IL: interleukin; LDL: low-density lipoprotein; Lp-PLA2: Lipoprotein-associated phospholipase A2; MCP-1: monocyte chemoattractant protein-1; NO: nitric oxide; NOS: nitric oxide synthase; ODN: oligodeoxynucleotides; OxLDL: oxidised low-density lipoprotein; sPLA2: secreted phospholipase A2; TGF-B: transforming growth factor beta; TNFa: tumour necrosis factor alpha.

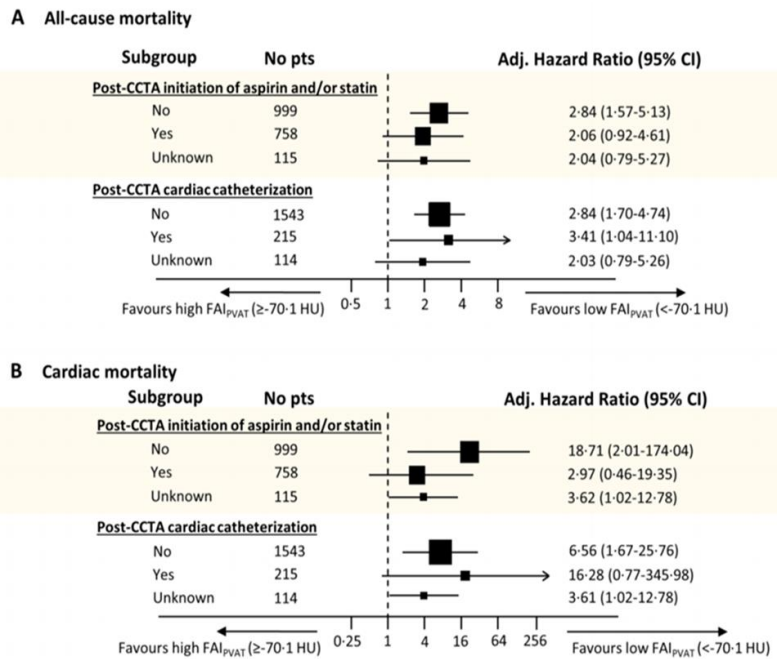


Figure 6. The predictive value of perivascular FAI based on post-CCTA changes in medical management

In the validation study of the perivascular FAI it was explored whether different clinical recommendations post-CCTA may have affected the predictive value of perivascular FAI for all-cause (A) and cardiac mortality (B), subgroup analysis was performed in the derivation cohort using adjusted Cox regression models (adjusted for age, sex and epicardial adipose tissue volume) stratified by the following post-CCTA recommendations: i. treatment with statin and/or aspirin; and ii. referral for cardiac catheterization. FAI retained its positive association with the prospective risk of both all-cause (A) and cardiac mortality (B) in all subgroups. Nevertheless, it is worth highlighting that FAI was strongly associated with cardiac mortality events in patients that did not receive recommendations for treatment with statins or aspirin, while the association in the group that did receive such recommendations was non-significant. This may suggest a role for FAI in guiding the deployment of secondary prevention measures in patients that do not qualify for medical treatment based on

conventional CCTA analysis and highlights a significant room for improvement in current clinical practice.

CCTA: coronary computed tomography angiography; CI: confidence interval; FAI: fat attenuation index.

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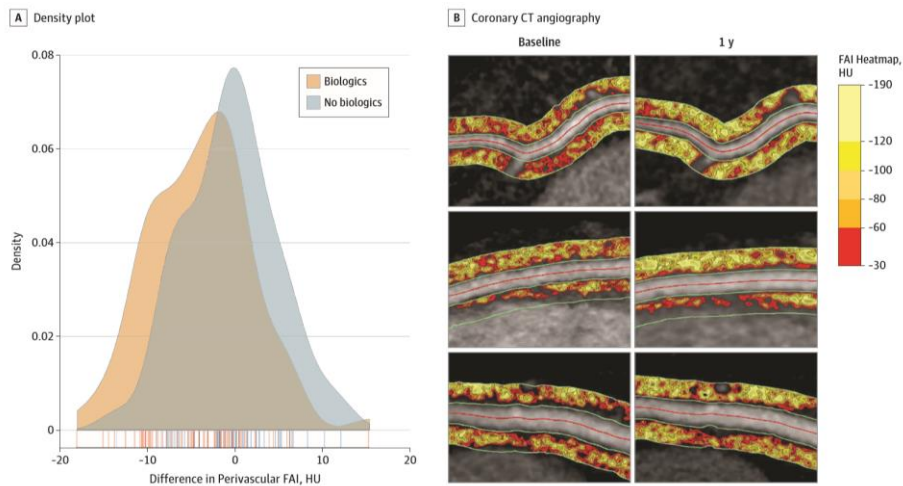


Figure 7. Capturing a reduction of coronary artery inflammation via the perivascular Fat Attenuation Index in patients with psoriasis

A) Density plot depicting the changes in perivascular fat attenuation index (FAI) in patients with treated and untreated psoriasis during 1 year (FAI at follow-up minus FAI at baseline; $p=0.001$).

B) CCTA images of the coronary arteries depicting the perivascular FAI before and after biologic therapy at 1 year of follow-up for patients with excellent response (top), moderate response (middle), and modest response (bottom) to biologic therapy. HU indicates Hounsfield units.

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Tables

Table 1. Summary of main pro-inflammatory mechanisms in atherosclerosis and key publications for each mechanism		
<i>Inflammatory mechanism</i>	<i>Main role in atherosclerosis</i>	<i>Key publications</i>
Accumulation of lipoprotein in arterial intima driving migration of immune cells	<p>The uptake and retention of LDL particles by arterial wall proteoglycans and the subsequent modification of the retained LDL products is a potent trigger of inflammatory mechanisms. Circulating monocytes are highly responsive to inflammatory signals and invade the arterial intima in which the cholesterol of locally modified LDL particles accumulates.</p> <p>In the atherosclerotic intima, monocytes differentiate into macrophages, which are then exposed to a milieu of growth factors, cytokines and specialized pro-resolving mediators. Different combinations of these factors determine the macrophage polarization into anti-inflammatory M2-like or pro-inflammatory M1-like phenotypes.</p>	Galkina et al (55); Libby et al (95)
Formation of foam cells and necrotic core	Migratory macrophages ingest the modified LDL particles via scavenger receptor-mediated endocytosis and can become foam cells. Pro-inflammatory forms of cell death further promote inflammation and generation of a large necrotic lipid core.	Shashkin et al (158); Oorni et al (122)

Defective efferocytosis	If cellular functions are intact then pro-inflammation resolving mediators, apoptosis and autophagy-associated cell death are favoured and efferocytosis of the dead cells can lead to resolution of inflammation. Efferocytosis can fail if the tyrosine-protein kinase receptor MERTK on the macrophage is cleaved, which can occur through inflammatory means, resulting in continual accumulation of cellular debris and the necrotic lipid core.	Green et al (60); Tajbakhsh et al (164); Thorp et al (172)
Inflammasome formation	Modified lipoproteins and cholesterol crystals induce the activation of inflammasomes, typically the NLRP3 inflammasome. Both the priming and activation steps required for inflammasome activity can be achieved by the products of inflammation in the atherosclerotic lesion, most commonly oxLDL and lipopolysaccharides. Inflammasomes then facilitate the secretion of pro-inflammatory cytokines IL-1 β and IL-18.	Westerterp et al (187); Rajamäki, et al (136)
Cytokine secretion and signalling	Cytokines are critical in the promotion of both pro-inflammatory mechanisms and anti-inflammatory mechanisms. Cytokine are implicated at all stages of atherosclerotic disease including the initiation of atherosclerosis and the progression of plaque into a clinically significant lesion. Cytokine secretion, both the molecules secreted and the quantity, is highly influenced by the prevailing inflammatory conditions of the vascular wall.	Ait-Oufella et al (6); Tousoulis et al (175); Turner et al (177)

Dysregulation of vascular redox state	There is a cycle of vascular inflammation and vascular oxidative stress driven by the production of inflammatory cytokines and the production of reactive oxygen species. The predominant mechanism is through pro-inflammatory cytokines, such as TNF- α and IL-6, effecting ROS generation from resident vascular wall cells. This oxidative stress activates redox-sensitive transcription factors leading to the upregulation of pro-inflammatory and pro-oxidant gene expression and the downregulation of anti-inflammatory and antioxidant genes, therefore enhancing local inflammation.	Antoniades at al (9); Antonopoulos at al (17); Margaritis et al (103)
Failure of pro-inflammation resolving lipid mediators, proteins and signalling gases	The balance of pro-inflammatory and anti-inflammatory processes controls the resolution of the lipid-driven inflammation in atherosclerotic lesions. The key pro-inflammation resolution mechanisms act to block inflammatory cell migration, modulate T-cell response to inflammation and promote effective efferocytosis. These mechanisms can be hijacked by both non-inflammatory and inflammatory mechanisms and contribute to the progression of atherosclerotic plaque towards clinical significance.	Serhan (154); Bäck et al (20)
eNOS: endothelial nitric oxide synthase; IL: interleukin; LDL: low-density lipoprotein; NADPH: nicotinamide adenine dinucleotide phosphate; oxLDL: oxidized low-density lipoprotein; ROS: reactive oxygen species; TNF- α : tumour necrosis factor alpha;		

Abbreviations

ACS	acute coronary syndrome
ACTH	adrenocorticotrophic hormone
AT	adipose tissue
BET	bromodomain and extra-terminal proteins
CAD	coronary artery disease
CCTA	coronary computed tomography angiography
CD	cluster of differentiation
CI	confidence interval
CSF	colony stimulating factor
CT	computed tomography
CVD	cardiovascular disease
DNA	deoxyribonucleic acid
eNOS	endothelial nitric oxide synthase
FAI	F at attenuation index
FDG	fluorodeoxyglucose
FRP	fat radiomic profile
hs-CRP	high sensitivity- c-reactive protein
HU	Hounsfield units
IFN	interferons
IL	interleukin
JAK1	janus kinase 1
kDa	kilodaltons
LDL	low-density lipoprotein

LOX	lipoxygenase
MI	myocardial infarction
NADPH	nicotinamide adenine dinucleotide phosphate
NF- κ B	nuclear factor kappa-light-chain-enhancer of the activated b-cell
NNTT	number needed to treat
NO	nitric oxide
ODN	oligodeoxynucleotides
oxLDL	oxidized low-density lipoprotein
oxPL	oxidized phospholipids
p38MAPK	p38 mitogen activated protein kinase
PCSK-9	proprotein convertase subtilisin/kexin type 9
PET	positron emission tomography
PLA	phospholipase a
PPAR	peroxisome proliferator-activated receptors
PVAT	perivascular adipose tissue
RNA	ribonucleic acid
ROS	reactive oxygen species
sPLA2	group ii secretory pla ₂
SPM	specialised pro-resolving mediator
STAT	signal transducers and activators of transcription
TGF	transforming growth factor
TLR	toll like receptor
TNF	tumour necrosis factor
T-reg	t-regulatory cells
VCAM-1	vascular cell adhesion molecule type-1

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