Unravelling the role of macrophages in cardiovascular inflammation through imaging: a state-of-the-art review

Reece Parry 1,2*, Kamran Majeed 1,3, Fiona Pixley 4, Graham Scott Hillis 1,2, Roslyn Jane Francis 1,5, and Carl Johann Schultz 1,2

1School of Medicine, University of Western Australia, Perth 6009, Australia; 2Department of Cardiology, Royal Perth Hospital, 197 Wellington Street, Perth, WA 6000, Australia; 3Department of Cardiology, Waikato District Health Board, Hamilton 3204, New Zealand; 4School of Biomedical Sciences, Pharmacology and Toxicology, University of Western Australia, Perth 6009, Australia; and 5Department of Nuclear Medicine, Sir Charles Gairdner Hospital, Perth 6009, Australia

Received 6 February 2022; accepted 31 July 2022; online publish-ahead-of-print 22 August 2022

Cardiovascular disease remains the leading cause of death and disability for patients across the world. Our understanding of atherosclerosis as a primary cholesterol issue has diversified, with a significant dysregulated inflammatory component that largely remains untreated and continues to drive persistent cardiovascular risk. Macrophages are central to atherosclerotic inflammation, and they exist along a functional spectrum between pro-inflammatory and anti-inflammatory extremes. Recent clinical trials have demonstrated a reduction in major cardiovascular events with some, but not all, anti-inflammatory therapies. The recent addition of colchicine to societal guidelines for the prevention of recurrent cardiovascular events in high-risk patients with chronic coronary syndromes highlights the real-world utility of this class of therapies. A highly targeted approach to modification of interleukin-1-dependent pathways shows promise with several novel agents in development, although excessive immunosuppression and resulting serious infection have proven a barrier to implementation into clinical practice. Current risk stratification tools to identify high-risk patients for secondary prevention are either inadequately robust or prohibitively expensive and invasive. A non-invasive and relatively inexpensive method to identify patients who will benefit most from novel anti-inflammatory therapies is required, a role likely to be fulfilled by functional imaging methods. This review article outlines our current understanding of the inflammatory biology of atherosclerosis, upcoming therapies and recent landmark clinical trials, imaging modalities (both invasive and non-invasive) and the current landscape surrounding functional imaging including through targeted nuclear and nanobody tracer development and their application.

Keywords

- 68Gallium-DOTATATE positron emission tomography
- cardiovascular inflammation
- activated macrophages
- coronary artery disease
- optical coherence tomography
- coronary imaging

Introduction

Despite significant advances in the detection and treatment of cardiovascular disease, it remains the leading cause of death worldwide.1 The short- and long-term implications are wide-ranging—if a patient survives an acute coronary syndrome (ACS) there is a reduction in quality of life, increased risk of death, heart failure and arrhythmia, and a more than two-fold increased risk of mental health disorders including depression.2-7 Despite receiving optimal preventative therapies based on current evidence, up to 25% of patients have a repeated cardiovascular event within 5 years.8 Both primary and residual cardiovascular risks are driven by unmitigated thrombotic, inflammatory, and metabolic perturbations.

Atherosclerosis is a chronic inflammatory disease involving medium- and large-sized arteries that results from a diverse number of cellular and molecular processes. Tissue-resident macrophages occupy a central role in the maintenance of tissue integrity and healing where they switch between pro- and anti-inflammatory functions as required.9 When pathological stimuli modulate macrophage function, the subsequent loss of tissue homeostasis leads to a persistent inflammatory response and progressive injury. Advances in imaging continue to expand upon our understanding of the dysfunctional...
In this review, we discuss the role of macrophages in atherosclerosis, and imaging modalities used for studying macrophages with an emphasis on translation to in-human imaging and potential therapies.

**Macrophages and the developing plaque**

Recent macrophage studies in both animal and human models report a considerable functional spectrum between the classical extremes of M1 (pro-inflammatory) and M2 (anti-inflammatory) phenotypes. As atherosclerosis progresses and cholesterol crystals accumulate, incoming monocyte-derived macrophages and tissue macrophages adopt an M1-like phenotype on exposure to lipid-derived metabolites such as oxidized low-density lipoprotein (LDL), with activation of nuclear factor-κB, up-regulation of endothelial cell adhesion molecules and release of monocyte and macrophage chemokines such as chemokine (C–C motif) ligand 2 and colony-stimulating factor 1. These ‘activated’ macrophages upregulate inflammatory metabolic pathways leading to activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome, succinylation of intra-cellular proteins (including hypoxia-inducible factor-alpha), and release of interleukin-1β (IL-1β). Cytokines also attract other circulating immune cells such as neutrophils which scavenge, oxidize, and attempt to clear lipoproteins and other pro-inflammatory crystalloids (including calcium phosphate). The accumulation of oxidized phospholipids additionally results in the formation of M(ox) macrophages (which have weaker phagocytic capabilities) and the transdifferentiation of vascular smooth muscle cells into macrophage-like cells. Eventually, the loss of macrophage ability to create high-density lipoprotein (HDL) via reverse cholesterol pathways leads to their transformation into foam cells and eventually apoptotic cell death.

In the early atherosclerotic lesion, macrophage efferocytosis facilitates efficient clearance of apoptotic cells and lesioncellularity remains limited. As atherosclerosis progresses, efferocytosis becomes impaired due to macrophage cellular reprogramming, a failure of recruitment signal secretion and improper presentation of apoptotic body ligands. Eventually, the loss of effective efferocytosis leads to cell death by necrosis, the release of immunogenic cytoplasmic material and the formation of a necrotic core within atherosclerotic plaques. In a self-propagating cycle of inflammation, dead and dying macrophages release damage-associated molecular proteins, which promote local and systemic cytokine release, and impaired oxidative phosphorylation inhibits the ability of M1-like macrophages to switch back to the anti-inflammatory M2-like phenotype. The increased presence of M2-like macrophages with specialized iron handling functions at areas of intraplaque hemorrhage suggests ongoing attempts to arrest plaque progression, promote plaque regression and maintain tissue integrity. In addition, repeated exposure of macrophages to oxidized phospholipids induces innate immune memory via epigenetic reprogramming and leads to the increased production of pro-inflammatory cytokines, including tumour necrosis factor-alpha (TNFα) and IL-6. Finally, secretion of matrix metalloproteinases by M1-like macrophages degrades the extracellular matrix of the thin fibrous cap contributing to plaque instability, potential cap rupture, and ACS.

Therapies that have significantly improved outcomes over the last several decades have targeted multiple pathways within lipid and energy metabolism and thrombosis.

**Pharmacological therapy for cardiovascular inflammation**

A cornerstone of treatment for atherosclerosis involves the use of lipid-lowering therapies, including statins and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. These medications exert their effects in a pleiotropic fashion, largely through action on hepatic cholesterol biosynthesis and receptor recycling with subsequent reductions in LDL cholesterol levels. Inflammation and the role of the errant macrophage remain under-targeted, though recent studies demonstrate well-established lipid-lowering therapies exert unanticipated immunomodulating effects (such as inhibition of the NLRP3 inflammasome or toll-like receptor pathways), and reductions in plaque volume, macrophage burden, and modification of the thin fibrous (by intracoronary imaging).

In 2017, the randomized, placebo-controlled, double-blind Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) trial provided the first proof-of-principle data that targeted inhibition of inflammation by canakinumab, an IL-1β inhibitor, reduces cardiovascular event rates independent of lipid-lowering treatments. However, clinical application remains limited by the cost of the drug and an increased risk of severe respiratory infections although other immunomodulating medications are being evaluated. In the Cardiovascular Inflammation Reduction Trial in 2018, methotrexate failed to demonstrate an improvement in cardiovascular outcomes and the likely reasons have been extensively discussed.

The subsequent COLCOT study published in 2019 demonstrated a significant reduction in adverse cardiovascular events post-myocardial infarction with the use of low-dose colchicine (0.5 mg once daily). Patients with recent myocardial infarction were randomized to either placebo or colchicine in addition to guideline-directed medical therapy. After a median follow-up time of 22.6 months, there was a reduction in the composite primary outcome (death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, stroke, or urgent hospitalization for angina leading to revascularization) in the colchicine treated group of 23% compared with placebo, driven largely by reductions in stroke and urgent revascularization. Less than 5% of patients in the trial underwent biomarker assessment, limiting the overall interpretation of observed C-reactive protein (CRP) levels.

A more promising result was reported by the LoDoCo2 randomized clinical trial, which demonstrated a significant reduction in cardiovascular events with the use of colchicine at a low dose (0.5 mg once daily) in patients with stable coronary artery disease. Colchicine disrupts tubulin, thereby reducing the migration and replication of inflammatory cells, affecting endothelial function, and inhibiting the NLRP3 inflammasome, but is now also thought to have broader mechanisms of action that are incompletely understood. One effect is the indirect reduction in IL-1β activation and downstream reductions in IL-6 and CRP, inflammatory mediators which
are known to activate macrophages and propagate atherosclerosis. Following these results, colchicine now receives a Class IIb recommendation for the prevention of recurrent cardiovascular events in select high-risk patients. The observed trend towards increased non-cardiovascular and all-cause mortality in LoDoCo2 continues to be discussed, although long-term data from familial Mediterranean fever patients and a large metaanalysis published in 2021 are very reassuring.

The above-mentioned studies have established the importance of specific inflammatory pathways in atherosclerosis, such as IL-1β and downstream cytokines and the potential for therapeutic inhibition. However, not all patients benefit from immune modulation, which also carries inherent risks. Blood biomarkers have been used to try to identify populations with active inflammation who may be more likely to benefit, but they have been ineffective or lacked specificity thus far. For example, the greatest benefit in CANTOS occurred in those patients who had significant reductions in IL-6 and CRP. Surrogate measures of IL-1β activation such as high-sensitivity CRP, IL-18, and IL-6 have been shown to independently predict cardiovascular events, while others such as serum amyloid A, TNFα, and soluble intercellular adhesion molecule type 1 do not. Also moderated by IL-1β inhibition, a high neutrophil-lymphocyte ratio independently predicts cardiovascular risk and all-cause mortality though (similarly to soluble biomarkers) has not shown sufficient specificity to be widely applied in clinical practice.

Improved detection of specific inflammatory pro-atherosclerotic processes or components in vivo could better identify patients with a favourable benefit-to-risk ratio for immune-modulating treatments and offer potential intermediate endpoints for assessing promising therapies in human populations before proceeding to large outcome trials.

Current methods for imaging inflammation and cardiovascular macrophages

In vivo imaging of plaque in human atherosclerosis offers a novel approach to the assessment of inflammation and includes both non-invasive and invasive techniques that could have application in diagnosis, prognosis, and research settings. Indications for clinically available techniques vary widely depending on patient characteristics and clinical context (such as chest pain, ACS, or in guiding a revascularization strategy). In general, invasive coronary techniques are used in the setting of ACS, where the risk stratification with coronary angiography to guide decisions on revascularization improves prognosis. Non-invasive imaging approaches may image multiple vascular territories and may be more useful in imaging chronic cardiovascular syndromes. The following sections outline current methods for imaging inflammation and their potential for detecting macrophages, both directly and indirectly, to better understand their central role in the process of inflammation.

Optical coherence tomography

Coronary optical coherence tomography (OCT) is a catheter-based invasive imaging system used in adjunct to fluoroscopy during percutaneous coronary intervention (PCI). During automated pullback along the length of the artery, OCT uses near infrared light, to produce high-resolution, tomographic images and can be used to plan and optimize stent placement. Since the first in vivo characterization of coronary atherosclerotic plaque in 2002 using high-resolution OCT, analysis of various cellular structures such as macrophages, vasa-vasorum and cholesterol crystals in near-microscopic detail has been possible.

OCT assessment of macrophages is based on the characteristic visual appearance of punctate regions with high signal attenuation. Because of their size and the different refractory index of phagolysosomes and surrounding intra-cellular fluid, investigators have hypothesized that macrophages have a higher OCT signal than the neighbouring tissues. However, histologically correlated findings of macrophages on OCT can vary and include appearing as ‘bright spots’ or as ‘shadow/dark regions’. Some groups have tried to develop algorithms that automatically detect macrophage infiltrations on OCT and that may improve detection compared with the expert reader (see Figure 1).

The use of these specialized algorithms requires dedicated software such that automated real time quantification of plaque macrophages in the catheter laboratory is not yet possible. A summary of various studies using specialized off-line algorithms to study macrophages along with histological validation is presented in Table 1.

Visual detection and quantification of macrophages on OCT is reproducible and requires in-depth knowledge of the varied OCT appearance of numerous plaque features. Macrophage accumulation may also be confused on occasion with microcalcification, cholesterol crystals and the internal or external elastic membrane. Macrophages attenuate the OCT light significantly, and as a result, superficial macrophages can shadow underlying tissue, giving it the appearance of a necrotic core. Despite these limitations, the detection of macrophages on OCT is associated with clinical cardiovascular risk.

Prati et al. performed a prospective, observational study which included 1003 patients who were referred for coronary angiography and underwent OCT imaging in an untreated proximal left anterior descending coronary artery. This study elegantly explored whether multiple high-risk OCT plaque features predict cardiovascular events at the patient-level. The hard composite endpoint of cardiac death and target vessel myocardial infarction was 7.5 x higher in patients who had lesions with a thin cap fibroatheroma (TCFA), lipid arc >180°, minimum lumen area <3.5 mm² and macrophage accumulation, when compared with patients without high-risk plaque characteristics (18.9 vs. 3.0%). Notably, the prevalence of all four high-risk features was 3.6%, and 19% of these high-risk plaques caused a subsequent cardiovascular event within 1 year.

In a Retrospective2 centre study of patients, who were found to have plaque erosion and were studied with OCT, the presence of macrophages was a marker of cardiovascular events over the subsequent 2.5 years. However, the prevalence of macrophages is considerably lower in patients with erosion when compared with those with rupture as underlying mechanism for ACS, which would also attenuate the predictive value of macrophage-rich plaque morphology in the overall population.

The presence of plaque macrophages on OCT is strongly correlated with other high-risk features including lipids, calcification, thin fibrous caps, and vasa-vasorum so that it is not
known whether or not macrophages is an independent marker of risk. OCT is currently by far the most studied and validated method to depict macrophages and it is available for clinical use. Studies investigating the quantitative assessment of macrophages on OCT and associations with clinical coronary disease including predictive value are further summarized in Table 2. The subsequent section discusses other experimental techniques.

### Intracoronary polarimetry using modified optical frequency domain imaging

An emerging technology that utilizes the polarization of light is based on a modification of existing optical frequency domain OCT systems. The polarization of infrared light is influenced by microscopic structures of the arterial wall. Modification of the OCT apparatus and image reconstruction methods have allowed polarimetric measurements to be obtained simultaneously with conventional cross-sectional imaging through standard OCT catheters.

Villiger et al.\(^8\) performed the first histological validation of this technique utilizing coronaries arteries of 5 human cadaveric hearts, demonstrating different polarization signatures caused by various tissue types including macrophages. Subsequently, Otsuka et al.\(^8\) validated first-in-human intracoronary polarimetry in 12 patients with ACS and 18 patients with stable angina. This study demonstrated that culprit lesions in ACS and or ruptured plaques exhibited lower birefringence than in the caps of target lesions in patients with stable angina. Furthermore, birefringence of these fibrous caps was associated with increased normalized standard deviation (NSD) on OCT analysis, suggesting macrophage accumulation in these lesions. Current data are limited and further validation studies are needed.

### Combined OCT and near-infrared autofluorescence

Inflammation increases the risk of (and is exacerbated by) intraplaque haemorrhage, resulting in macrophage-dependent breakdown of extra-vascular red blood cells. Htun et al.\(^8\) in pre-clinical work demonstrated that near-infrared autofluorescence (NIRAF) can identify intraplaque haemorrhage and haeme-degradation products, a feature of plaque instability using a mouse model and 50 human carotid endarterectomy samples. Ughi et al.\(^8\) for the first time demonstrated the safety and feasibility of high-quality OCT and NIRAF images simultaneously, using a combined catheter in 12 patients undergoing PCI. High NIRAF signals (defined as maximum normalized NIRAF intensity >0.4) were associated with more vulnerable plaque phenotypes as characterized by OCT, namely TCFA and plaque rupture. Comparison of OCT and NIRAF lipid arcs revealed that high NIRAF intensity was restricted to regions of plaque that had dense macrophage accumulations, as determined by the OCT-based NSD parameters (see Figure 2). This report is of major interest because it indicates the ability to deliver complementary imaging techniques simultaneously and safely within the coronary arteries. These novel intracoronary optical imaging-based plaque characterization techniques have potential clinical applications in guiding treatment and prognostication but are awaiting longitudinal outcome studies to determine the potential additive clinical value.

Quantification of plaque characteristics on OCT including macrophages is time-consuming and remains at present a research tool.
| Author                  | Study aim                                                                                           | Sample                                                                 | Methods                                                                                                                                  | Results                                                                                                                                                                                                 |
|------------------------|-----------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Tearney et al (2003)   | To investigate the use of OCT for identifying macrophages in fibrous caps                           | 26 lipid-rich atherosclerotic arterial segments obtained at autopsy      | Macrophage density was quantified morphometrically by immunoperoxidase staining with CD68 and then compared with the NSD of the OCT signal intensity | (1) High degree of positive correlation between OCT and histological measurements of fibrous cap macrophage density ($R = 0.84, P < 0.0001$)  
(2) A range of OCT Signal SD thresholds (6.15–6.35%) yielded 100% sensitivity and specificity for identifying caps containing $>10\%$ CD68 staining |
| Phipps et al (2015)    | To quantitatively identify macrophages using a novel algorithm including tissue depth, distance from light source, and signal-to-noise ratio | Fresh 14 coronary arteries from 10 human hearts                         | Use of specific OCT algorithm (an intensity threshold was employed on the normalized algorithm OCT data, considering the distance between the catheter and the surface of the vessel) in 1599 OCT cross-sectional images and validated with histology | (1) Macrophages were present in 57% of bright spot-positive regions  
(2) Other aetiologies for bright spots included cellular fibrous tissue (8%), interface of calcium and fibrous tissue (10%), calcium and lipids (5%) and fibrous cap and lipid pool (3%)  
(3) Bright spots in the context of thin cap fibroatheroma were caused by macrophages in 94% of cases |
| Di Vito et al (2015)   | To investigate the capability of OCT to identify coronary plaque macrophage presence using tissue property indexes | 15 epicardial coronary arteries                                          | OCT analysis of histologically correlated regions of interest (ROI) was performed in a stepwise manner  
The ROI after histological evaluation were divided in to inflamed (ROI having a macrophage percentage $>10\%$) or non-inflamed (ROI having a macrophage percentage $<10\%$)  
The application of OCT-derived tissue property indexes including signal attenuation, NSD and granulometry index were again applied in a stepwise manner | (1) 43 paired samples (OCT frame and histology sections) were considered suitable as ROIs for analysis. 11 out of 43 ROIs were considered inflamed  
(2) ROC curve analysis showed that NSD, granulometry index and signal attenuation had a significant AUC (AUC = 0.906, 0.804 and 0.793 respectively)  
(3) a two-step algorithm requiring to first apply NSD with a cut off value of 0.0570 followed by granulometry index was able to identify an inflamed ROI with a sensitivity of 100% and a specificity of 96.8% |

Continued
Table 1  Continued

| Author            | Study aim                                                                 | Sample | Methods                                                                 | Results                                                                                                                                 |
|-------------------|---------------------------------------------------------------------------|--------|-------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| Gutierrez-Chico et al (2017) | To investigate whether imaging of macrophages in OCT could be enhanced by means of superparamagnetic nanoparticles | Cell pellets | Comparison of optical backscattering and attenuation of cell pellets containing RAW 264.7 macrophages with those of macrophagic cell pellets labelled with very small superparamagnetic oxidized nanoparticles (VSOP) by means of light intensity analysis in OCT. The backscattering was estimated by the peak normalization intensity, whilst the attenuation was estimated by the number of pixels between the peak and normalized intensity (PNI). | (1) VSOP-loaded macrophages have higher backscattering than the corresponding unlabelled macrophages (PNI 6.30 vs. 3.15). (2) There was slightly higher attenuation (PNI 61 vs. 66 pixels). |
| Shimokado et al (2018) | To assess agreement between OCT and healed coronary plaques (HCP) ex vivo, and to evaluate the prevalence and characteristics of HCP’s in vivo | In a subset clinical study 60 lesions (in 60 patients) were compared based on presence or absence of HCP. | 73 coronary arteries were examined by OCT from cadavers. The left main coronary and the proximal and middle portion of the 3 coronary arteries were examined. One or more heterogenous signal-rich layers of different optical density located close to luminal surface with clear demarcation from the underlying tissue, which was deemed OCT-derived HCP. | (1) In the autopsy study, the sensitivity, specificity, positive predictive value and negative predictive value of OCT-derived HCP to detect histologically defined HCPs were 81%, 98, 93 and 93% respectively. (2) In the clinical study 46 (77%) had OCT-derived HCPs. Microvessels and macrophages were more frequently identified in OCT-derived HCP’s compared with their counterparts (43 vs. 0%; P<0.01, 70 vs. 21%; P<0.01, respectively). |
| Author                    | Study aim                                                                 | Sample                                                                 | Methods                                                                                     | Results                                                                 |
|--------------------------|--------------------------------------------------------------------------|------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|------------------------------------------------------------------------|
| Rico-Jimenez et al (2019) | To introduce and validate a simple intravascular OCT image processing method for automated, accurate and fast detection of macrophage infiltration with atherosclerotic plaque | OCT images acquired from 28 cadaveric human coronary artery segments | The ratio of the NSD was estimated over two axially adjacent regions of interest in OCT cross-sectional images (B-Scan) When applied to entire OCT B-scans, the areas of plaque with high NSD ratio were highlighted, which was demonstrated to be correlated with the degree of coronary plaque macrophage infiltration | Using an optimized NSDRatio threshold value, coronary plaque macrophage infiltration could be detected with 88% sensitivity and specificity For comparison, using an optimized NSD threshold value (considered the standard OCT signature for macrophages), coronary plaque macrophage infiltration could be detected with only 55% sensitivity and specificity |
| Nicol et al (2021)       | To investigate whether tissue attenuation differs between regions with and without neointimal foam cell infiltration, and whether tissue attenuation index could reliably identify patients with neointimal foamy macrophage infiltration as an early sign of atherosclerosis | 13 autopsy samples of stented coronary arteries, 29 patients with in-stent restenosis undergoing OCT were included | 13 autopsy samples of stented coronary arteries were assessed to determine attenuation index of neointima with and without foam cells. Based on this, a threshold for homogenous and non-homogenous neointima was determined The established attenuation index derived from autopsy cases was applied to detect neointimal foam cells in clinical cases presenting with in-stent restenosis | ROC analysis of homogenous neointima using a threshold of −0.796 demonstrated an AUC of 0.87, a sensitivity of 0.93 and a specificity of 0.73 ROC analysis of nonhomogeneous neointima using a threshold of −1.93 demonstrated an AUC of 0.69, a sensitivity of 0.40 and a specificity of 0.95 In patients with in-stent restenosis, neointimal foamy macrophages were detected in 34.2% of homogenous and 43.6% of the non-homogenous neointimal ROIs that were evaluated |

AUC, area under the ROC curve; CD, cluster of differentiation; HCP, healed coronary plaque; NSD, normalized-intensity standard deviation; OCT, optical coherence tomography; PNI, peak and normalized intensity; ROC, receiver operating characteristic; ROI, region of interest; SD, standard deviation; VSOP, very small superparamagnetic oxidized nanoparticles.
**Table 2** Summary of studies investigating the clinical significance of macrophages in various coronary syndromes

| Author         | Aim                                                                 | Sample                                                                 | Methods                                                                                                                                                                                                 | Results                                                                                                                                                                                                 |
|----------------|----------------------------------------------------------------------|------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| MacMeill (2004) | Using OCT to investigate the relationship between macrophages distributions and clinical coronary syndromes | 49 subjects undergoing single vessel stenting for de novo coronary artery disease | OCT images were acquired at 4 frames/s during intermittent saline injections. After automated segmentation of fibroatheroma cap comparison between the surface (<50 μm from the lumen) and subsurface (>50 μm) macrophage densities of the was performed. Macrophage density was studied using the NSD of the OCT signal. | (1) Lipid-rich plaques demonstrated significantly greater macrophage density in both STEMI and ACS groups than stable angina patients. 
(2) In lipid-rich plaques, no significant difference was seen in macrophage density between the STEMI and the ACS groups. 
(3) Significantly higher macrophage density in fibrous plaques of the unstable group than in plaques of the stable group was found. |
| Raffel (2007)   | To evaluate the relationship between peripheral WBC count, plaque fibrous cap macrophage density and the presence of TCFA on OCT | 43 patients undergoing coronary angiography                             | Macrophage density was assessed as the average of the NSD values within the segmented/ROI of the cap. For individual plaques, the value of the highest macrophage density obtained from the 3 sampling sites was used for subsequent data analysis. WBC counts were obtained in all cases. | (1) Baseline WBC count correlated with macrophage density ($r = 0.48$, $P < 0.001$). 
(2) Both parameters correlated with lipid-rich plaque and correlated inversely with fibrous cap thickness. |
| Minami (2015)   | To investigate the clinical significance of bright spots in coronary plaque detected by OCT in patients with coronary artery disease | 112 patients (50 ACS and 62 SAP)                                        | Macrophage density was calculated as the number of bright spots divided by the total number of analysed pixels with the 5mm span of vessel centred around the culprit frame (a total of 25 cross-sectional frames, including the culprit frame and 12 frames on both proximal and distal sides). Macrophage location was considered superficial (≤250 μm) or deep (≥1000 μm). | (1) Bright spot density in the culprit lesion was significantly higher in ACS patients compared with those presenting with SAP, particularly in the subgroup with ruptured culprit plaque. 
(2) TCFA was associated with a trend towards a higher density of bright spots compared with non-TCFA plaques. |
| Galon (2015)    | To compare OCT volumetric quantification of fibrous cap and macrophage detection using visual assessment and automated image processing algorithms in non-culprit lesions | 67 consecutive patients with STEMI and SAP                              | FC was manually delineated by a computer-aided method and automatically classified into three thickness categories: fibrous cap <65 μm, TCFA 65–150 μm, and >150 μm. Automated detection and quantification of macrophage was also performed. | (1) STEMI patients had more absolute categorical surface area for TCFA, thinner minimum fibrous cap thickness, greater fractional luminal area for TCFA and greater macrophage index than compared with SAP. |

*Continued*
| Author          | Aim                                                                 | Sample                  | Methods                                                                 | Results                                                                 |
|-----------------|----------------------------------------------------------------------|-------------------------|------------------------------------------------------------------------|------------------------------------------------------------------------|
| Park (2017)     | To investigate whether OCT markers of plaque vulnerability predict cardiac allograft vasculopathy after heart transplantation | 34 consecutive patients | Presence of vulnerability markers (lipid pools, TCFA, macrophages and microchannels) was assessed in 100 consecutive frames in 20 mm proximal segments of the LAD. The total number of appearances of vulnerable markers was defined as a vulnerability score (VS). Plaque volume (PV) was measured in the same coronary segment using IVUS at baseline and 1-year follow-up and the association between the baseline VS, and the change in percent PV (PV/ vessel volume x 100 [%PV]) was evaluated. | (1) There was a significant correlation between vulnerability score and %PV change ($r = 0.757$, $P < 0.001$)  
(2) On multivariable analysis, only VS correlated significantly with %PV. |
| Taglieri (2017)  | To evaluate the relationship between aortic inflammation (as assessed by 18F-FDG PET) and features of plaque vulnerability (as assessed by OCT) | 30 consecutive patients with ACS undergoing PCI | All patients underwent 3 vessel OCT before intervention and 18F-FDG-PET before discharge. 18F-FDG uptake in both ascending and descending aorta was measured as averaged mean and maximum TBR or as active slices (TBR$_{max}$ $\geq$ 1.6). | (1) TBR$_{mean}$ and TBR$_{max}$ in the descending aorta was associated with the number of lipid-rich plaques. TBR$_{max}$ in the descending aorta was also associated with lipid-rich plaque containing macrophages. |
| Lannaccone (2018) | To evaluate the clinical impact of OCT-detected culprit plaque features in ACS | 209 patients with ACS | Retrospective analysis of a multi-centre registry. All ACS patients undergoing OCT of culprit and non-culprit plaque with follow-up conducted through clinical visits or phone calls at 6 months. | (1) Culprit plaque rupture and necrotic core with macrophage infiltration were independent predictors for MACE.  
(2) Dual antiplatelet therapy with prasugrel/ticagrelor at discharge was protective. The protective impact of these drugs was reported only in patients with plaque rupture. |
| Joner (2018)    | To assess neo-atherosclerosis in patients presenting with stent thrombosis using OCT | 134 patients presenting with very late stent thrombosis | Plaque morphology was analysed in every other frame. The presence of fibroatheroma and signal attenuation indicative of macrophages (at least 1 quadrant) was recorded. | (1) In patients with neo-atherosclerosis, in-stent plaque rupture was the most frequent cause (40 cases, 69%) of very late stent thrombosis.  
(2) Macrophage infiltration was significantly more frequent in OCT frames with plaque rupture compared with those without (50 vs. 22%; $P < 0.0001$). |
| Okamoto (2019)  | To study the relationship between healed plaques and the development of significant coronary stenosis in SAP | 205 SAP patients in retrospective arm, 42 samples from 18 SAP in prospective arm | In the retrospective arm, the prevalence and clinical characteristics of OCT-based layered plaque at the culprit site in patients with SAP was assessed. In the prospective arm, the histopathological characteristics of layered plaques in SAP lesions were identified using DCA samples. | (1) The retrospective arm demonstrated that layered plaque was observed in 36.6% of patients. Furthermore, layered plaque was accompanied by higher plaque vulnerability and smaller luminal area.  
(2) In the histopathological study, the layered plaque had a significantly higher rate of intramural thrombus and macrophage infiltration. |
| Author          | Aim                                                                 | Sample                          | Methods                                                                 | Results                                                                 |
|-----------------|----------------------------------------------------------------------|---------------------------------|------------------------------------------------------------------------|------------------------------------------------------------------------|
| Wu et al (2019) | To investigate the correlation between insulin resistance and culprit plaque characteristics using OCT in patients with ACS | 145 patients with ACS           | 159 culprit plaques in 145 patients were identified. Patients were identified into 4 groups according to the homeostasis model assessment of insulin resistance (HOMA-IR) | (1) Minimal fibrous cap thickness was inversely correlated with HOMA-IR level  
(2) Spotty calcification prevalence was also significantly different among the 4 groups  
(3) Compared with the bottom quartile, patients with elevated HOMA-IR values had a higher prevalence of macrophage infiltration and microvessels |
| Raber et al (2019) | To study changes in OCT-defined morphological changes in patients with STEMI receiving high-intensity statin therapy | 103 patients with STEMI         | 103 patients with STEMI undergoing IVUS and OCT of two non-infarct related arteries and a repeat coronary angiography at 13 months were recruited. Co-primary endpoints for OCT analysis were change in minimum FCT and macrophage accumulation angle. Patients received 20 mg of rosuvastatin daily for the first 2 weeks and were then uptitrated to 40 mg daily | (1) There was a significant increase in FCT from 65 ± 20 µm to 88 ± 38 µm with a mean change of 24 µm (95% CI 7–42 µm)  
(2) Macrophage line arc was decreased significantly from 10°±13° to 6°±10° with a mean change of −3° (−5° to −2°) |
| Prati et al (2020) | To investigate the predictive value of high-risk OCT plaque features including macrophages | 1003 patients with coronary artery disease undergoing PCI | The predictive value of high-risk OCT plaque features including MLA, FCT, lipid arc circumferential extension and the presence of OCT-detected macrophages was studied in a proximal LAD segment. The primary endpoint of target-segment MI and/or cardiac death was studied at 1-year follow-up | (1) MLA <3.5 mm² [hazard ratio (HR) 2.1, 95% confidence interval (CI) 1.1–4.0], FCT <75 µm (HR 4.7, 95% CI 2.4–9.0), lipid arc circumferential extension >180° (HR 2.4, 95% CI 1.2–4.8), and OCT-defined macrophages (HR 2.7, 95% CI 1.2–6.1) were all associated with an increased risk of the primary endpoint |
| Gatto et al (2020) | To revise the clinical and demographic variables of patients who have coronary plaques with macrophages, and investigate the reproducibility of quantitative assessment | Post-hoc analysis of the CLIMA study | A total of 577 patients out of 1003 showed macrophage accumulation. Three groups were identified: Group 1 (426)—without macrophages  
Group 2 (296)—patients with low macrophage content (less than median value of 67° of circumferential arc)  
Group 3 (281)—patients with high macrophage content (more than median value of 67° of circumferential arc) | (1) Patients with macrophages (Group 2 and 3) showed a higher prevalence of family history for coronary artery disease and hypercholesterolaemia, and had a significantly larger body mass  
(2) Group 3 had higher rates of triple vessel disease and higher LDL cholesterol levels compared with the other two groups  
(3) The inter-observer agreement for macrophage interpretation was excellent: the R value was 0.97 for circumferential arc extension, 0.95 for minimum distance and 0.98 for mean distance |
| Author       | Aim                                                                 | Sample                                                                 | Methods                                                                 | Results                                                                 |
|-------------|----------------------------------------------------------------------|------------------------------------------------------------------------|-------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Taglieri et al (2020) | To investigate the prevalence and the features of OCT-detected macrophage accumulation in culprit plaques as compared with non-culprit plaques | 32 patients with an index NSTEMI who successfully underwent PCI of at least one coronary lesion | Patients underwent three vessel OCT at the time of PCI with a 20 mm/s pullback speed that was applied during automated injection of intracoronary iso-osmolar contrast media | (1) Macrophage accumulation was more frequent in culprit plaque (84 vs. 61%, *P* = 0.015) with higher circumferential extension  
(2) Macrophage accumulation extension had a higher association with culprit plaques (odds ratio = 4.42; 95% CI: 2.54–9.15; *P* < 0.001) than the mere presence of macrophages accumulation (odds ratio = 3.36; 95% CI: 1.30–8.66, *P* = 0.012)  
(3) Culprit plaques had a smaller minimal luminal area, a higher extension of lipid component and a thinner fibrous cap |
| Majeed et al (2020) | To study the association between NaF PET uptake and high-risk plaque features on OCT and CTCA, and the potential application to patient-level risk stratification | 62 patients with ACS were recruited prospectively | Patients underwent multivessel OCT at the time of angiography, with subsequent CTCA and NaF PET within a maximum time window of up to 4 weeks  
A low-risk cohort (n = 10) with no prior coronary artery disease and calcium score of 0 were used to determine the upper limit of the normal reference range of radiotracer activity for each coronary segment based on 18-segment model. After co-registration, a total of 286 coronary segments were analysed for association between NaF activity and high-risk OCT and CTCA plaque features  
Patient-level data were analysed based on number of coronary segments with NaF positivity | (1) Coronary segments with NaF activity demonstrated significantly higher lipid arc, calcium arc, cholesterol crystals and macrophages and lower plaque free wall, compared with those without NaF activity  
(2) Coronary segments with NaF activity were associated with more dense calcified plaque  
(3) The proportion of patients with calcium arc above group median and OCT-detected macrophages increased with higher NaF uptake. In addition, the proportion of plaque free wall above group median decreased with higher NaF uptake |

ACS, acute coronary syndrome; CI, confidence interval; CTCA, CT coronary angiography; FCT, fibrous cap thickness; HR, hazard ratio; IVUS, intravascular ultrasound; LAD, left anterior descending artery; MI, myocardial infarction; MLA, minimal luminal area; NaF, 18F-sodium fluoride; NSD, normalized-intensity standard deviation; OCT, optical coherence tomography; PCI, percutaneous coronary intervention; PET, positron emission tomography; ROI, region of interest; SAP, stable angina pectoris; STEMI, ST-segment elevation myocardial infarction; TBR, target-to-background ratio; TCFA, thin cap fibroatheroma; WBC, white blood cell.
Unravelling macrophages in cardiovascular inflammation

Figure 2 Combined intravascular coronary OCT with NIRAF demonstrates a fibrotic plaque with low NIRAF signal (A) and a fibroatheroma with high NIRAF signal corresponding to dense macrophage accumulation (B). Adapted with permission from Ughi et al.84 The asterisk (*) corresponds with catheter artefact. F, fibrotic; L, lipid.

Computed tomography imaging

Quantification of plaque burden using computed tomography (CT) calcium scoring predicts cardiovascular outcomes across multiple vascular beds including the aorta, carotid, femoral, and coronary arteries.86 Recent developments indicate that CT can also indirectly detect inflammation by examining perivascular fat. Perivascular fat exists in two phases, an ‘aqueous’ phase and a ‘lipid’ phase. Inflammation in epicardial fat causes the aqueous phase to dominate with an associated increase in X-ray attenuation. It has been proposed that this represents the effects of a nearby inflammatory coronary artery lesion (see Figure 3). Perivascular fat on CT independently predicts the progression of non-calcified and total plaque burden on follow-up CT, correlates with 18F-sodium fluoride (NaF) positron emission tomography (PET) uptake in coronary plaque and may improve risk prediction of cardiovascular mortality compared with standard risk assessment models.87–89 However, there are still inherent limitations including a lack of specificity (as it may simply represent the effects of visceral fat rather than arterial inflammation) and is not assessable in many vascular territories.87

Perivascular fat imaging with CT will be improved by machine learning methods to provide more accurate and less demanding workflows.90 While machine learning remains in its infancy, it is likely to surpass conventional image assessment.91 At present, conventional assessment of cardiovascular disease by CT is validated, yet imprecise at the patient-level, providing downstream assessment of already significantly advanced cardiovascular disease. Molecular imaging provides an alternative and uniquely targeted imaging approach to gain fine insight into coronary physiology and inflammation.

Functional imaging of atherosclerotic inflammation by PET

Several PET tracers are being assessed for their role in understanding atherosclerosis, and to find translational opportunities for future clinical therapies. Many/most targets for molecular imaging have been developed through in vitro experiments and animal studies and have not been validated in humans in vivo. Tracers that have been studied in humans may be non-specific, e.g. targeting metabolism or a consequence of inflammation (such as minerals and hypoxia) whilst a small number detected macrophage-specific receptors or functions. These tracers and their targets will be discussed in more detail in the following section and are highlighted in Figure 4.

18F-fluorodeoxyglucose (FDG) is a tracer taken up by metabolically active cells due to its similar structure to glucose and is most commonly used for diagnosis and surveillance of cancer. It is widely available with well-established methods, is relatively undemanding to produce and has been used as an endpoint in trials of novel pharmaceutical agents.92,93 Uptake of FDG in large arteries was first reported in 2001, with a subsequent study demonstrating that patients with ≥1 cardiovascular risk factor showed higher FDG uptake in the iliac and femoral arteries (but not the abdominal aorta), when compared with patients without risk factors.94,95 A landmark prospective study in 2013 demonstrated that significant vascular uptake of FDG at baseline was associated with subsequent arterial calcification at the same location within a period of 1–5 years, a finding that reflects increasing cardiovascular risk.96,97 FDG uptake in large arteries is associated with increased macrophage accumulation and statin therapy results in a reduced arterial wall FDG uptake.98,99

Unfortunately, FDG PET has several significant limitations that prevent reliable assessment of coronary arteries. These include variable and high-intensity myocardial uptake of FDG with significant spillover preventing coronary assessment, poor tolerability and reliability of dietary techniques to switch the myocardium to free fatty acid metabolism, and lack of cell specificity due to FDG uptake in all metabolically active tissue.100–102 While FDG PET has served a valuable role in advancing our knowledge of vascular inflammation, a more specific and reliable tracer that can overcome these limitations is highly desirable.

18F-sodium fluoride (NaF) binds hydroxyapatite (the dominant form of calcium in the body) and localizes to microcalcifications in the arterial wall. In a prospective study, both FDG and NaF PET scans were performed in 80 patients with either ACS or stable angina. Culprit plaques showed significant NaF uptake in >90% of patients with ACS and in nearly half of patients with stable angina, with high-risk plaque characteristics by intravascular ultrasound (IVUS), such as positive remodelling, a large necrotic core and greater...
Figure 3  Perivascular fat attenuation analysis of the proximal epicardial coronary vessels as defined by fat within a radial distance of the diameter (d) of the vessel. Adapted with permission from Oikonomou et al.\textsuperscript{87} HU, Hounsfield units; RCA, right coronary artery; LAD, left anterior descending artery; LCx, left circumflex artery.

Figure 4  Macrophage PET tracer targets in atherosclerosis. Chemokine receptors, major regulator of macrophage chemotaxis; GLUT receptor, transport protein involved in glucose translocation across the cell membrane; Calcium crystals, accumulated intra-cellular calcium phosphate crystals; Methionine, essential amino acid associated with polarization of M1-like macrophages; Macrophage mannose receptor, acts as a pattern recognition receptor for various carbohydrates resulting in immune activation against a variety of pathogens; Folate receptor beta, high-affinity receptor for the uptake of folate into macrophages; Hypoxia, Hypoxia-inducible factor-1α is a key transcription factor in cellular responses to hypoxia and promotes M1-like polarization; SST2 receptor, influences cell proliferation, cytokine release and immunoglobulin production; TSPO transporter, mitochondrial membrane protein that handles cholesterol and impacts on immune cell function; cholesterol crystals, phagocyted intra-cellular cholesterol forming ‘foam cells’; VCAM-1 receptor, modulates cellular adhesion and facilitates migration of macrophages; αvβ3 integrin receptor, regulates macrophage differentiation and transendothelial migration; HDL particles, circulating lipoproteins that carry accumulated lipid molecules; Nanobodies; engineered high-affinity antigen-binding fragments.
Unravelling macrophages in cardiovascular inflammation

Microcalcification more likely to be demonstrated in these lesions. NaF PET was superior to FDG PET in identifying vulnerable coronary artery plaques, largely due to difficulties with interpreting FDG uptake despite techniques aimed to suppress myocardial activity. NaF uptake in coronary lesions is likewise associated with high-risk plaque features on OCT and IVUS, closely correlates to the Framingham risk score for the prediction of cardiovascular events and predicts 2-year cardiovascular event rates (see Figure 5). There are disadvantages—however—while NaF detects microcalcification activity, a process correlated with atherosclerosis, it does not directly measure inflammation but rather a downstream consequence of chronic inflammation. The assessment of structures adjacent to bone, such as the aorta, can also be difficult due to significant spillover from the bone. Nonetheless NaF imaging has conclusively shown that PET imaging can reliably detect high-risk plaque in coronary arteries and has led to the development and continued refinement of coronary-specific PET imaging, reconstruction, and image analysis techniques. Several centres around the world have independently implemented these approaches successfully, representing a breakthrough of our understanding of coronary PET imaging and which can also be applied to other PET tracers more specific to inflammatory markers.

Progress is also being made with volumetric quantification of tracer uptake rather than peak intensity measurements which, intuitively, is more relevant to vascular assessment.

Local lesional hypoxia has also led to hypoxia-specific PET tracers, currently used for oncology purposes, being examined in atherosclerosis. 18F-fluoromisonidazole (FMISO) is the first and most widely studied of these tracers and has shown promise in both rabbit and human models though does not seem to correlate with cardiovascular risk factors. Unfortunately, hypoxia-specific tracers such as FMISO may remain limited in their application to atherosclerosis by their poor sensitivity in identifying subtle vascular hypoxia, rather than hypoxia as typically identified in the necrotic tumour core. Additionally, the slow blood pool clearance of FMISO is unfavourable for patient imaging as a delay of several hours from injection to scanning is required, combined with a relatively high administered activity resulting in a moderate radiation dose to patients. More refined tracers that attempt to address these concerns continue to be developed in the hope of addressing these technical considerations.

Identifying macrophages using targeted tracers

Currently used in the diagnosis and monitoring of patients with neuroendocrine tumours, gallium-68-labeled DOTATATE is a PET ligand with high-specificity binding affinity to somatostatin receptor subtype-2 (SST2). Importantly, up-regulation of SST2 has been demonstrated on the surface of activated macrophages and the highly-specific binding of 68Ga-DOTATATE to M1 macrophages in humans was prospectively validated in 2017. Tarkin et al. examined 42 patients with atherosclerosis and compared the discriminatory ability of DOTATATE PET to FDG PET. In patients with symptomatic carotid artery disease, DOTATATE PET was able to discriminate culprit vs. non-culprit carotid specimens with histological localization to macrophages in the necrotic core and plaque shoulders. In patients with both stable angina and recent ACS, DOTATATE PET was able to correctly identify culprit plaques and was more accurate than FDG PET in discriminating high-risk vs. low-risk plaques. Promisingly, both inter- and intra-observer reliability was excellent and interpretation of DOTATATE uptake was possible in all coronary lesions without myocardial or bone spillover (as occurs with FDG and NaF PET, respectively). These very promising results are yet to be validated by other groups. Several other tracers also target SST2 receptors on macrophages and may have some advantages and disadvantages but are in earlier stages of development (see Table 3). Various other tracers are under development and have been further summarized in Table 3. Many remain in their infancy and are being studied in animal models of atherosclerosis. For example,
### Table 3  Functional radiological methods of assessing cardiovascular macrophages

| Imaging modality       | Mechanism of action                          | Advantages                                                                 | Disadvantages                                                                                                                                 |
|------------------------|----------------------------------------------|-----------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| PET tracers            |                                              |                                                                             |                                                                                                                                                |
| Cell metabolism        | Uptake into cells with increased metabolic rates | Inexpensive and clinically available Prospectively studied in multiple human trials | Myocardial spillover frequently precludes coronary assessment Poor specificity—uptake by all metabolically active cells                    |
| 18F-FDG                |                                              |                                                                             |                                                                                                                                                |
| 11C-methionine         | Amino acid metabolism of macrophages          | Strongly correlated with cardiovascular risk factors and CRP                | Limited observational human data Uptake also seen in other cells (such as myofibroblasts)                                                   |
| 18F-NaF                | Microcalcification as a result of necrosis or inflammation | Widely available with well-established safety                               | Downstream assessment of effects of chronic inflammation Validity of risk prediction and longitudinal monitoring capabilities not yet established |
| Hypoxia                | Trapped within cells in hypoxic state         | Correlates well with areas of vessel hypoxia and increased macrophage density | Indirect assessment of atherosclerosis Requires further prospective and comparative research                                               |
| 18F-HX4                |                                              |                                                                             |                                                                                                                                                |
| 18F-FMISO              |                                              |                                                                             |                                                                                                                                                |
| 62Cu-ATSM              |                                              |                                                                             |                                                                                                                                                |
| Hypoxia                |                                              |                                                                             |                                                                                                                                                |
| 68Ga-DOTATATE          | Activated macrophages expressing somatostatin receptor 2 (SST2) | Widely available with well-established safety                               | Validity of risk prediction and longitudinal monitoring capabilities not yet established                                               |
| 64Cu-DOTATATE          |                                              |                                                                             |                                                                                                                                                |
| 68Ga-DOTANOC           |                                              |                                                                             |                                                                                                                                                |
| 18F-FDR-NOC            |                                              |                                                                             |                                                                                                                                                |
| 68Ga-DOTATOC           |                                              |                                                                             |                                                                                                                                                |
| 68Ga-pentixafor        |                                              |                                                                             |                                                                                                                                                |
| Hypoxia                |                                              |                                                                             |                                                                                                                                                |
| 64Cu-DOTA-DAPTA-comb nanoparticles |                                              |                                                                             |                                                                                                                                                |
| Imaging modality                          | Mechanism of action                  | Advantages                                                                 | Disadvantages                                                                 |
|------------------------------------------|--------------------------------------|-----------------------------------------------------------------------------|------------------------------------------------------------------------------|
| $^{64}$Cu-DOTA-ECL1 $^{133,134}$         | C-C chemokine receptor 2 (CCR2)      | Validated in mice experiments of aortic transplantation and plaque regression | No in-human data available, further studies required to assess fate and role of cells highlighted |
| $^{64}$Cu-DOTA-vMIP-II $^{135}$          | Multiple simultaneous chemokine receptors | Broad chemokine receptor targeting improves specificity and sensitivity     | No in-human data available                                                   |
| $^{64}$Cu-vMIP-II-comb nanoparticles $^{136}$ | Folate receptor β selectively expressed on macrophages | Rapid blood pool clearance, low liver, lung and myocardial uptake          | Limited immunohistochemical correlation data available, further assessment of receptor expression required in human atherosclerosis |
| $^{18}$F-FOL $^{137}$                   | Vascular cell adhesion molecule-1 (VCAM-1) | Highly correlated with increasing disease severity in animal models of atherosclerosis | No in-human data                                                             |
| $^{68}$Ga-MMR $^{139}$                  | Macrophage mannose receptor (MMR)    | Correlates well when compared with $^{18}$F-FDG in rabbit aortic atherosclerosis | Poorly established role in the assessment of atherosclerosis given predominant uptake in M2-like macrophages |
| $^{18}$F-4V $^{138}$                    | Upregulated αβ integrin receptor at areas of intraplaque angiogenesis and neovascularization | Highly correlated and co-localizes with macrophage-rich atherosclerotic plaque | Validity of risk prediction and longitudinal monitoring capabilities not yet established |
| Mitochondrial membrane protein $^{144}$ | Translocator protein (TSPO)          | Highly expressed in human macrophages (though not uniquely) Rapid blood clearance | Low target-to-background ratio (similar to FDG) Genetic polymorphisms influence binding in some tracers High uptake in lung and myocardium |
several tracers targeting either single or multiple chemokine receptors require further assessment in humans, although the precise and complex role of chemokine receptors and their variable expression across the macrophage polarization spectrum may make interpretation difficult. Other approaches make use of nanoparticles to utilize the phagocytic properties of macrophages.

**PET/magnetic resonance imaging and nanomaterials**

Most clinical nanomaterials are between 10 and 300 nm in size. Large nanoparticles preferentially accumulate in lung and liver tissue while nanoparticles <8 nm can be filtered through the kidneys. Rapid filtration of tiny nanoparticles makes this platform an attractive modality for imaging in atherosclerosis due to their rapid clearance from the blood pool and lack of uptake in structures adjacent to the vasculature (such as the lungs). \(^{18}F\)-Macroflor utilizes lysine cross-linked low molecular weight carboxymethyl polyglucose polymers, each containing 22 glucose units as a basis for a 5 nm nanoparticle. Due to the rapid blood pool clearance, the nanoparticle can be radiolabelled with \(^{18}F\) (which has a short half-life of 110 min) to facilitate the short tracer injection to scanning time required. A comprehensive series of proof-of-principle animal studies performed by Keliher et al.\(^{149}\) reported rapid excretion of \(^{18}F\)-Macroflor by the kidneys in mice, rabbits, and primates, with tracer uptake visualized in cardiac macrophages and confirmed by autoradiography and fluorescence. Translatability to humans as well as toxicology studies are required but, overall, the safety of nanoparticles studied thus far is reassuring. Longer-term safety data will also be important as the effect of high concentrations of nanoparticles in tissue (particularly the renal tubules) remains unknown. With the development of hybrid PET and magnetic resonance imaging (MRI) platforms, ultrasmall superparamagnetic iron oxide particles can be combined with radionuclide tracers to increase sensitivity and reduce the required dose for optimal resolution given the soft-tissue resolution capabilities of MRI.\(^{151}\)

These paramagnetic nanoparticles accumulate at sites of high macrophage concentration, including high-risk or ruptured atherosclerotic plaques.\(^{149}\) Continued assessment and refinement of multimodality tracers in humans remains in its infancy though is among the most promising vectors for unravelling the complexity of cardiovascular inflammation and personalizing diagnosis and therapy.

While beyond the scope of this review, technological advances in PET with total body PET (which encompasses the whole body in the field of view to improve image acquisition time, reduce radiation exposure and improve image quality), new image reconstruction techniques and hybrid tracer imaging will continue to improve sensitivity.\(^{154,155}\) This is particularly relevant in fields such as atherosclerosis where small lesions are prone to the partial volume effect and low target-to-background ratios and the close proximity of the blood pool can make assessment challenging.

**Conclusion**

While cost and access to PET limits use in routine, large-scale clinical practice, identification of high-risk individuals who would benefit from aggressive treatment to reduce cardiovascular events may
lead to focused adoption, individualized cardiovascular care and an overall reduction in cost—especially considering the worldwide economic impact of cardiovascular disease over the next decade is expected to exceed US$1 trillion annually. New pharmacotherapies that are likely to come with considerable prices require precise strategies to quantify individual and residual inflammatory risk in a variety of clinical settings. As outlined in Figure 6, a suite of imaging modalities is available to consider with further advances in invasive imaging, total body PET, molecular targeted tracers, simultaneous contrast techniques, integrated high-resolution PET/CT and PET/MRI imaging platforms, machine learning and concurrent targeted therapy with nanoparticles in our near future. The identification of those patients who are most vulnerable to the devastating effects of uncontrolled inflammation is closer than ever.

Acknowledgements
We wish to thank James Down for his artistic contribution to this manuscript. All authors contributed to the final manuscript and approved the use of their respective names for publication.

Conflict of interest: None declared.

Funding
This research is supported by a Western Australian Department of Health Research Registrar Fellowship, an Australian Government Research Training Program Scholarship and grants from the University of Western Australia and the Royal Perth Hospital Medical Research Foundation.

Data availability
No new data were generated or analysed in support of this research.

References
1. World Health Organization. World Health Statistics 2019: Monitoring Health for the SDGs, Sustainable Development Goals. Geneva: World Health Organization; 2019.
2. Beck CA, Joseph L, Bélisle P, Pilote L. Predictors of quality of life 6 months and 1 year after acute myocardial infarction. Am Heart J 2001;142:271–9.
3. Bata IR, Gregor RD, Wolf HK, Brownell B. Trends in five-year survival of patients discharged after acute myocardial infarction. Can J Cardiol 2006;22:399–404.
4. Stone GW, Witzenbichler B, Guagliumi G, Peruga JZ, Brodie BR, Dudek D, et al. Heparin plus a glycoprotein IIb/IIIa inhibitor versus bivalirudin monotherapy and paclitaxel-eluting stents versus bare-metal stents in acute myocardial infarction (HORIZONS-AMI): final 3-year results from a multicentre, randomised controlled trial. Lancet 2011;377:2193–204.
5. Ziegelstein RC. Depression in patients recovering from a myocardial infarction. JAMA 2001;286:1621–7.
6. Rudsch B, Nemeroff C. Epidemiology of comorbid coronary artery disease and depression. Biol Psychiatry 2003;54:227–40.
7. Ormel J, Von Korff M, Burger H, Scott K, Demytenaere K, Huang Y-Q, et al. Mental disorders among persons with heart disease — results from World Mental Health surveys. Gen Hosp Psychiatry 2007;29:325–34.
8. Ferrari R, Ford I, Fox K, Challeton JP, Corrêas A, Tendler M, et al. Efficacy and safety of trimetazidine after percutaneous coronary intervention (ATPCI): a randomised, double-blind, placebo-controlled trial. Lancet 2020;396:630–8.
9. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. Immunity 2016;44:450–62.
10. Xue J, Schmidt SV, Sander J, Draflleh A, Krebs WA, Quester I, et al. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. Immunity 2014;40:274-88.

11. Chistakou DA, Bobryshev YV, Nikiforov NG, Elizova NV, Sobenin IA, Orekhov AN. RETRACTED: macrophage phenotypic plasticity in atherosclerosis: the associated features and the peculiarities of the expression of inflammatory genes. Int J Cardiol 2015;184:e36-45.

12. Park SH. Regulation of macrophage activation and differentiation in atherosclerosis. J Lipid Atheroscler 2021;1:251-67.

13. Khallou-Laschet J, Varshaman A, Fornasa G, Compain G, Gaston A-T, Clement M, et al. Macrophage plasticity in experimental atherosclerosis. Plastir ONE 2010;5.e5852.

14. Gubbene AM, García-Cardenía AG. Endothelial cell dysfunction and the pathobiology of atherosclerosis. Circ Res 2016;118:620-36.

15. Chen Y, Yang M, Huang W, Chen W, Zhao Y, Schulte ML, et al. Mitochondrial metabolic reprogramming by CD36 signaling drives macrophage inflammatory responses. Circ Res 2019;125:1087-102.

16. Folco EJ, Suhroka GV, Quillard KT, Libby KP. Moderate hypoxia potentiates interleukin-1β production in activated human macrophages. Circ Res 2014;115:875-83.

17. Nidorf SM, Fiolet A, Abela GS. Viewing atherosclerosis through a crystal lens: how the evolving structure of cholesterol crystals in atherosclerotic plaque alters its stability. J Clin Lipidol 2020;14:619-30.

18. Kadi A, Meher AK, Sharma PR, Lee MY, Doran AC, Johnstone SR, et al. Identification of a novel macrophage phenotype that develops in response to atherogenic phospholipids via H protestors. Circ Res 2010;107:737-46.

19. Wang Y, Dublind JA, Allahverdian S, Asoyne E, Sahin B, Jaw E, et al. Smooth muscle cells contribute the majority of foam cells in Apoe (apolipoprotein E)-deficient mouse atherosclerosis. Arterioscler Thromb Vasc Biol 2019;39:876-87.

20. Janoudi A, Shamoun FE, Kalavakunta JK, Abela GS. Cholesterol crystal induced arterial inflammation and destabilization of atherosclerotic plaque. Eur Heart J 2016;37:1959-67.

21. Kruth HS, Skarlatos SI, Gaynor PM, Gamble W. Production of cholesterol-enriched nascent high density lipoproteins by human monocyte-derived macrophages is a mechanism that contributes to macrophage cholesterol efflux. J Biol Chem 1994;269:24511-8.

22. Jerome WG. Advanced atherosclerotic foam cell formation has features of an acquired lysosomal storage disorder. Rejuvenation Res 2006;9:245-55.

23. Tabas I. Consequences and therapeutic implications of macrophage apoptosis in atherosclerosis: the importance of lesion stage and phagocytic efficiency. Arterioscler Thromb Vasc Biol 2005;25:2355-64.

24. Vandivier RW, Henson PM, Douglas IS. Burying the dead: the impact of failed apoptotic cell removal (efferocytosis) on chronic inflammatory lung disease. J Clin Invest 2011;121:361-70.

25. De Souza AV, Westra J, Limberg PC, Bijl M, Kallenberg CGM. HMGB1 in vascular diseases: its role in vascular inflammation and atherosclerosis. Autoimmun Rev 2012;11:909-17.

26. Van den Bossche J, Baardman J, Otto NA, van der Velden S, Neele AE, van den Berg SM, et al. Mitochondrial dysfunction prevents repolarization of inflammatory macrophages. Cell Rep 2016;17:684-96.

27. Bories G, Colin VS, Vannoue J, Derudas B, Copin C, Fanchon M, et al. Reduction of the E1-E2 subunit of Complex I reduces mitochondrial dysfunction and reduces inflammation in atherosclerotic mouse atherosclerosis. Circ Res 2015;115:358-66.

28. Kouoski K, Shahbaz SK, Mashayekhi K, Sadeghi M, Zayeri ZD, Tabas M, et al. Anti-inflammatory action of statins in cardiovascular disease: the role of inflammation and toll-like receptor pathways. Clin Res Allergy Immunol 2021;60:175-99.

35. Kwon O, Kang SJ, Kang SH, Lee PH, Yun S-C, Ahn J-M, et al. Relationship between serum inflammatory marker levels and the dynamic changes in coronary plaque characteristics after statin therapy. Circ Cardiovasc Imaging 2017;10:e005934.

36. Räber L, Ueki Y, Otsuka T, Losdat S, Hänner JD, Lombarjo A, et al. Effect of alirocumab added to high-intensity statin therapy on coronary atherosclerosis in patients with acute myocardial infarction: the PACMAN-AMI randomized clinical trial. JAMA 2022;327:5771-81.

37. Ridker PM, Everett BM, Thuren T, Macfadyen JG, Chang VH, Ballantyne C, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med 2017;377:1119-31.

38. Ridker PM, Everett BM, Pradhan A, Macfadyen JG, Solomon DH, Zalenski H, et al. Low-dose methotrexate for the prevention of atherosclerotic events. N Engl J Med 2019;380:572-62.

39. Tardif JC, Koup S, Waters DD, Bertrand OF, Diaz R, Magnoni AP, et al. Efficacy and safety of low-dose colchicine after myocardial infarction. N Engl J Med 2019;381:2497-505.

40. Nidorf SM, Fiolet ATL, Mostard A, Elkeboom JW, Schur A, Opstal TJJ, et al. Colchicine in patients with chronic coronary disease. N Engl J Med 2020;383:1388-43.

41. Leung YT, Yao Hui LL, Kraus VB. Colchicine—update on mechanisms of action and comparison with other anti-inflammatory agents. Semin Arthritis Rheum 2019;48:1075-86.

42. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory capases and processing of pro-IL-1β. Mol Cell 2002;10:417-26.

43. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, et al. 2021 ESC guidelines on cardiovascular disease prevention in clinical practice: developed by the Task Force for cardiovascular disease prevention in clinical practice with representatives of the European Society of Cardiology and 12 medical societies With the special contribution of the European Association of Preventive Cardiology. Eur Heart J 2021;42:3227-337.

44. Andreas A, Imazio M, Avondo S, Casula M, Panea E, Piroli F, et al. Adverse events of colchicine for cardiovascular diseases: a comprehensive meta-analysis of 14188 patients from 21 randomized controlled trials. J Cardiovasc Med (Hagerstown) 2021;22:1637-44.

45. Ben-Chetrit E, Levy M. Colchicine prophylaxis in familial Mediterranean fever: reappraisal after 15 years. Semin Arthritis Rheum 1991;20:241-6.

46. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:863-6.

47. Ridker PM, Macfadyen JG, Thuren T, Libby P. Residual inflammatory risk associated with interleukin-18 and interleukin-6 after successful interleukin-1β inhibition with canakinumab: further rationale for the development of targeted anti-cytokine therapies for the treatment of atherosclerosis. Eur Heart J 2020;41:2153-63.

48. Adamstein NH, Macfadyen JG, Rose LM, Glynn RJ, Dey AK, Libby P, et al. The neutrophil-lipoyxymytox ratio and incident atherosclerotic events: analyses from five contemporary randomized trials. Eur Heart J 2021;42:896-903.

49. Bezerra HG, Costa MA, Guagliumi G, Rollins AM, Simon DI. Intracoronary optical coherence tomography: a comprehensive review clinical and research applications. JACC Cardiovasc Interv 2009;2:1035-46.

50. Teaney GJ, Regar E, Alaszka T, Adriaenssens T, Barlis P, Bezerra HG, et al. Consensus standards for acquisition, measurement, and reporting of intravascular optical coherence tomography studies: a report from the International Working Group for Intravascular Optical Coherence Tomography Standardization and Validation. J Am Coll Cardiol 2012;59:1058-72.

51. Jang IK, Bouma BE, Kang DH, Park SJ, Park SW, Seung KB, et al. Visualization of coronary atherosclerotic plaques in patients using optical coherence tomography. J Am Coll Cardiol 2002;39:604-9.
Unravelling macrophages in cardiovascular inflammation

e523

57. Shimokado A, Matsuo Y, Kubo T, Nishiguchi T, Tawakol A, Ren J, Lippok N, Tawakol A, Fayad ZA, Mani V, Woodward M, Kallend D, Abt M, Burgess T, Alon A, Klimas MT, Dansky H, et al. Relationship between coronary plaque morphology and features of plaque vulnerability in patients with high-risk atherosclerotic plaques: A new endogenous contrast mechanism for optical frequency domain imaging. JACC Cardiovascular Imaging 2018; 11:1666–76.

58. Otsuka K, Villiger M, Karamanos A, Doradla P, Ren J, Lippok N, Tawakol A, Fayad ZA, Mani V, Woodward M, Kallend D, Abt M, Burgess T, Alon A, Klimas MT, Dansky H, et al. Relationship between coronary plaque morphology and features of plaque vulnerability in patients with high-risk atherosclerotic plaques. Circulation 2019; 139:290–81.

59. Hnt HN, Ben YC, Lim B, Schiller T, Maghal GJ, Huang AL, et al. Near-infrared autofluorescence induced by intraplaque hemorrhage and heme degradation as marker for high-risk atherosclerotic plaques. Nat Commun 2017; 8:785.

60. Uchi Y, Wang H, Gerbarg E, Gardexi JA, Fard AM, Hamidi E, et al. Clinical characterization of coronary atherosclerosis with dual-modality OCT and near-infrared autofluorescence imaging. JACC Cardiovascular Imaging 2019; 12:1304–14.

61. Balaji A, Kelsey LJ, Majeed K, Schultz CJ, Doyle BJ. Coronary artery segmentation from intravascular optical coherence tomography using deep capsules. Artif Intell Med 2021; 116:100270.

62. Spence JD, Eliaissi M, DiCicco M, Hackam DG, Galli R, Lohmann T. Carotid plaque area: a tool for targeting and evaluating vascular preventive therapy. Stroke 2003; 34:2916–22.

63. Okiomomou EU, Marwan M, Desai MY, Manico J, Alisha A, Hutt Centeno E, et al. Non-invasive detection of coronary inflammation using computed tomography and prediction of residual cardiovascular risk (the CRISP CT study): a post-hoc analysis of prospective outcome data. Lancet 2018; 392:929–39.

64. Goessler M, Tamarappoo BK, Wkan AC, Cadet S, Commandeur F, Raziapour A, et al. Relationship between changes in pericoronary adipose tissue attenuation and coronary plaque burden quantified from coronary computed tomography angio. Eur Heart J Cardiovascular Imaging 2019; 20:636–43.

65. Kweecki J, Dey D, Cadet S, Lee S-E, Okabi Y, Huynh PT, et al. Peri-coronary adipose tissue density is associated with 18F-sodium fluoride coronary uptake in stable patients with high-risk plaques. JACC Cardiovascular Imaging 2019; 12:2000–10.

66. Okiomomou EU, Williams MC, Kotanidis CP, Desai MY, Marwan M, Antonopoulos AS, et al. A novel machine learning-derived radiotranscriptomic signature of peri-vascular fat improves cardiac risk prediction using coronary CT angiography. Eur Heart J Cardiovascular Imaging 2019; 40:3529–43.

67. Cheng K, Lin A, Vuyar J, Nicholas SJ, Wong DTL. Cardiac computed tomography radiomics for the non-invasive assessment of coronary inflammation. Cells 2021; 10:879.

68. Fazay ZA, Mani V, Woolquod M, Kallend D, Abt M, Burgess T, et al. Safety and efficacy of dalcetrapib on atherosclerotic disease using novel non-invasive multi-modality imaging (dal-PLAQUE): a randomised clinical trial. Lancet 2013; 381:929–39.

69. Rudd JHF, Myers KS, Bansilal S, Machac J, Rafique A, Farkouh M, et al. Intensification of high-intensity statin therapy (IBIS-4): a serial optical coherence tomography study. J Am Coll Cardiol 2017; 69:628–40.

70. van Soest G, Regel E, Goderie TP, Gonzalez N, Koljenovic S, van Leenders GJ, et al. Pitfalls in plaque characterization by OCT: images art fact in native coronary arteries. JACC Cardiovascular Imaging 2011; 4:810–3.

71. Prati F, Romagnoli E, Gatto L, Da Mann A, Burzotta F, Ozaki Y, et al. Relationship between coronary plaque morphology of the left anterior descending artery and 12 months clinical outcome: the CLIMA study. Eur Heart J 2020; 41:383–91.

72. Montone RA, Vetrugno V, Camilli M, Russo M, Fracassi F, Khan SQ, et al. Macrophage infiltration in coronary plaque erosion and cardiovascular outcome in patients with acute coronary syndrome. Atherosclerosis 2020; 311:158–66.

73. Zhao X, Wang Y, Chen R, Li J, Zhou J, Cui P, et al. Prognostic value of characteristics of plaque combined with residual syxial score among patients with STEMI undergoing primary PCI: an intravascular optical coherence tomography study. Thromb J 2021; 19:85.

74. MacNeill BD, Jiang K, Bouna BE, Ilitna N, Takano M, Yabushita H, et al. Focal and multifocal plaque macrophage distributions in patients with acute and stable presentations of coronary artery disease. J Am Coll Cardiol 2004; 44:972–9.

75. Raffel OC, Teamey G, Gauthier DD, Halpern EF, Bouma BE, Jang I-K. Relationship between transmural coronary marker, plaque inflammation, and plaque characteristics determined by intravascular optical coherence tomography. Arterioscler Thromb Vasc Biol 2007; 27:1820–7.

76. Minami Y, Phipps J, Hoyt T, Milner TE, Ong DS, Soeda T, et al. Clinical utility of quantitative bright spots analysis in patients with acute coronary syndrome: an optical coherence tomography study. Int J Cardiovascular Imaging 2015; 31:1479–87.

77. Galon MZ, Wang Z, Bezerra HG, Lemnos PA, Schnell A, Wilson DL, et al. Differences determined by optical coherence tomography volumetric analysis in non-culprit lesion morphology and inflammation in ST-segment elevation myocardial infarction and stable angina pectoris patients. Catheter Cardiovasc Interv 2015; 85:108–15.

78. Park KH, Sun T, Liu Z, Yang SW, Lennon RJ, Lerman LO, et al. Relationship between markers of plaque vulnerability in optical coherence tomography and atherosclerotic progression in adult patients with heart transplantation. J Heart Lung Transplant 2013; 32:178–93.

79. Taglieri N, Nairst C, Ghetti G, Bonfiglioli R, Sia F, Bacci Reggiani ML, et al. Relation between thoracic aortic inflammation and features of plaque vulnerability in the coronary tree in patients with non-ST-segment elevation acute coronary syndrome undergoing percutaneous coronary intervention. An FDG-position emission tomography and optical coherence tomography study. Eur J Nucl Med Mol Imaging 2017; 44:1878–87.

80. Iannaccone M, Soutyrand G, Niccoli M, Marcone M, Sardella G, Tamburino C, et al. Clinical impact of optical coherence tomography findings on culprit plaque in acute coronary syndrome: the OCT-FORMIDABLE study registry. Catheter Cardiovasc Interv 2018; 92:E486–92.

81. Joner M, Koppara L, Byrne RA, Castellanos MJ, Lewerich J, Novotny J, et al. Neatherosclerosis in patients with coronary stent thrombosis: findings from optical coherence tomography imaging (a report of the PRESTIGE consortium). JACC Cardiovascular Imaging 2018; 11:1340–50.

82. Okiomomou EU, Marwan M, Desai MY, Manico J, Alashia A, Hutt Centeno E, et al. Near-infrared autofluorescence induced by intraplaque hemorrhage and heme degradation as marker for high-risk atherosclerotic plaques. Circulation 2019; 139:290–81.

83. Wu Y, Liu W, Ma Q, Yu W, Guo Y, Zhao Y, et al. Association between insulin resistance and coronary plaque vulnerability in patients with acute coronary syndromes: insights from optical coherence tomography. Angiology 2019; 70:539–46.

84. Fazay ZA, Mani V, Woolquod M, Kallend D, Abt M, Burgess T, et al. Safety and efficacy of dalcetrapib on atherosclerotic disease using novel non-invasive multi-modality imaging (dal-PLAQUE): a randomised clinical trial. Lancet 2011; 378:1647–59.

85. Jacobs PC, Prokop M, van Der Graaf Y, Gondrie MJ, Janssen KJ, de Koning HJ, et al. Comparing coronary artery calcium and thoracic aorta calcium for prediction of non-invasive detection of coronary inflammation using computed tomography and prediction of residual cardiovascular risk (the CRISP CT study): a post-hoc analysis of prospective outcome data. Lancet 2018; 392:929–39.

86. Goessler M, Tamarappoo BK, Wkan AC, Cadet S, Commandeur F, Raziapour A, et al. Peri-coronary adipose tissue density is associated with 18F-sodium fluoride coronary uptake in stable patients with high-risk plaques. JACC Cardiovascular Imaging 2019; 12:2000–10.
Unravelling macrophages in cardiovascular inflammation

146. Hellberg S, Silvola JMU, Liljenbäck H, Savisto N, Li X-G, et al. 18-kDa translocator protein ligand 18F-FEMPA: biodistribution and uptake into atherosclerotic plaques in mice. *J Nucl Cardiol* 2017;24:862–71.

147. Hellberg S, Liljenbäck H, Eskola O, Morisson-Iveson V, Morrison M, Trigg W, et al. Positron emission tomography imaging of macrophages in atherosclerosis with 18F-GE-180, a radiotracer for translocator protein (TSPO). *Contrast Media Mol Imaging* 2018;2018:9186902.

148. Majmudar MD, Yoo J, Keliher EJ, Truelove JJ, Iwamoto Y, Sena B, et al. Polymeric nanoparticle PET/MR imaging allows macrophage detection in atherosclerotic plaques. *Circ Res* 2013;112:755–61.

149. Keliher EJ, Ye Y-X, Wojtkiewicz GR, Aguirre AD, Tricot B, Senders ML, et al. Polyglucose nanoparticles with renal elimination and macrophage avidity facilitate PET imaging in ischaemic heart disease. *Nat Commun* 2017;8:1–12.

150. Pérez-Medina C, Binderup T, Lobatto ME, Tang J, Calcagno C, Giesen L, et al. In vivo PET imaging of HDL in multiple atherosclerosis models. *JACC Cardiovasc Imaging* 2016;9:950–61.

151. Nahrendorf MMD, Zhang HP, Hembrador SBS, Panizzi PP, Sosnovik DEMD, Aikawa EMDP, et al. Nanoparticle PET-CT imaging of macrophages in inflammatory atherosclerosis. *Circulation* 2008;117:379–87.

152. Kooi ME, Cappendijk V, Cleutjens K, Kessels A, Kitaar P, Borgers M, et al. Accumulation of ultrasmall superparamagnetic particles of iron oxide in human atherosclerotic plaques can be detected by in vivo magnetic resonance imaging. *Circulation* 2003;107:2453–8.

153. Satomi T, Ogawa M, Mori I, Ishino S, Kubo K, Magata Y, et al. Comparison of contrast agents for atherosclerosis imaging using cultured macrophages: FDG versus ultrasmall superparamagnetic iron oxide. *J Nucl Med* 2013;54:999–1004.

154. Cherry SR, Jones T, Karp JS, Qi J, Moses WW, Badawi RD. Total-body PET: maximizing sensitivity to create new opportunities for clinical research and patient care. *J Nucl Med* 2018;59:3–12.

155. van der Vos CS, Koopman D, Rijnsdorp S, Arends AJ, Boellaard R, van Dalen JA, et al. Quantification, improvement, and harmonization of small lesion detection with state-of-the-art PET. *Eur J Nucl Med Mol Imaging* 2017;44:4–16.

156. Bloom DE, Cafiero E, Jané-Llopis E, Abrahams-Gessel S, Bloom LR, Fathima S, et al. The global economic burden of noncommunicable diseases. Program on the Global Demography of Aging; 2012.