**Could targeting the macrophage urokinase-type plasminogen activator receptor be a bullseye for PET imaging of atherosclerotic plaque inflammation?**

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A myriad of imaging techniques and targets have been tested to identify high-risk plaques and ultimately improve patient cardiovascular risk-stratification.[1] In this issue of *Atherosclerosis*, Khare et al. examine the urokinase-type plasminogen activator receptor (uPAR) as a novel positron emission tomography (PET) imaging target in atherosclerosis.

[2] The findings of this initial proof-of-concept study are intriguing: could targeting uPAR be a bullseye for PET imaging of atherosclerotic plaque inflammation?

Precision imaging is an unmet need in cardiovascular disease.Landmark randomised, controlled trials such as ISCHEMIA and FAME-2 failed to show reductions in myocardial infarction or mortality following percutaneous revascularisation compared to medical therapy in patients with stable angina,

[3,4] even among individuals with extensive angiographic coronary disease and a high degree of myocardial ischemia.

[5] This insight underscores the urgent demand for better assessments of disease severity (and better treatments) than are currently available in mainstay clinical practice. Indeed, contemporary imaging studies have identified atherosclerotic plaque features such as lipid content and plaque burden measured e.g., invasively by NIRS-IVUS, or non-invasively by CT coronary angiography, as well as CT pericoronary fat attenuation, to be strongly associated with the risk of future major adverse cardiovascular events.

[6-9] The use of PET imaging to track inflammation, microcalcification, neoangiogenesis, and other active biological processes underlying these high-risk plaque features offers additional promise.

[10] Whether any of these imaging characteristics will lead to meaningful refinements in clinical decision making and improve prognosis for coronary disease remains unknown. However, beyond the current blanket of general secondary preventive measures applicable to all patients with atherosclerotic cardiovascular disease, there exists a fundamental gap in our understanding of which patients will require more advanced therapies. In particular, an accurate means of identifying and quantifying plaque inflammation is needed to identify patients who would most benefit from the array of emerging immunomodulatory strategies following the CANTOS, COLCOT, and LoDoCo-2 trials.[11-13] Although 18F-fluorodeoxyglucose and several more specifically targeted PET tracers have been examined for this use,[14] each approach has its own limitations and the need for new imaging targets persists, especially that can be used in the coronary vasculature.

The role of the urokinase system in the pathogenesis of atherosclerosis has been extensively reviewed elsewhere.

[15] Briefly, urokinase-type plasmin activator (uPA) is a serine protease enzyme produced by monocytes and macrophages, as well as by other cells including smooth muscle cells, vascular endothelial cells, and fibroblasts. uPA binds to the uPAR (CD87), which is a 55-60 kDa single chain polypeptide multifunctional receptor with three extracellular domains anchored to the cell membrane by glycosyl phosphatidyl inositol. The uPA/uPAR complex promotes fibrin proteolysis through the conversion of plasminogen to the active enzyme plasmin and activates matrix metalloproteases. It acts to i) facilitate monocyte adhesion by interacting with vitronectin to form complexes with integrins; ii) encourage cell migration and infiltration via pericellular proteolysis; iii) promote monocyte-to-macrophage differentiation; and iv) increase intracellular oxidative stress and lipid accumulation in macrophages - all contributing to atherogenesis. In addition, uPA simulates proliferation and migration of vascular smooth muscle cells causing neointimal formation and inward arterial remodelling. In response to oxidized LDL, uPAR associates with CD36 and TLR4 in human coronary vascular smooth muscle cells driving the transition into a pro-inflammatory secretory phenotype.[16]

Expression of uPA within the human arterial system has been previously characterised in excised ruptured carotid plaques,

[17] aortic atherosclerosis specimens,[18] and advanced coronary and peripheral atherosclerotic lesions.[19-21]

In murine models, overexpression of macrophage-specific uPA is associated with accelerated atherosclerosis and aortic dilatation,

[22] intraplaque haemorrhage, fibrous cap disruption, and increased matrix metalloprotease activity.

[23] In contrast, pharmacological inhibition of uPA decreases the extent of atherosclerosis in mice.

[24] A proteomic analysis comparing aortic samples from transgenic mice with macrophage-specific overexpression of uPA with human carotid plaques, identified basement membrane protein loss as a prominent feature connecting proteolysis with plaque rupture.

[25] Interestingly, statins and ezetimibe seem to modulate uPAR on endothelial cells and platelets.[26, 27] Finally, several population-based cohort studies have shown that the soluble bioactive form of uPAR is associated with the presence of cardiovascular risk factors including coronary artery calcification,[28] and is also an independent predictor of future cardiovascular events.[29-31]

Hence, the uPAR targeting PET tracer 64Cu-DOTA-AE105 that has been successfully developed for cancer imaging could also have an important role in cardiovascular disease. The authors of the article should be congratulated on taking the first step towards the clinical translation of uPAR PET imaging in atherosclerosis. In an elegant study, taking a bench-to-beside approach, increased macrophage uPAR expression was shown using flow cytometry in cells stimulated *in vitro* and confirmed in excised carotid endarterectomy tissue. uPAR expression within carotid plaques was also linked to several important macrophage activation markers using microarray data with gene ontology analysis. Lastly, clinical imaging data from ten patients grouped by coronary calcification score who underwent PET/CT imaging with 64Cu-DOTA-AE105 as part of a separate oncology trial showed differences in aortic tracer uptake.

These findings are exciting and should encourage further evaluation of uPAR PET imaging in cardiovascular disease. The *in vivo* findings provide a unique first glimpse into the potential clinical utility of this novel approach for vascular imaging, from the first-in-human oncology trial to test this tracer. The imaging characteristics of 64Cu-DOTA-AE105 appear to be well-suited for vascular imaging and are likely to be further optimised by using dedicated cardiovascular imaging protocols. Low tracer binding in the healthy myocardium also offers the possibility of unimpeded coronary artery imaging, though this has yet to be further explored and was sadly not reported in the study. The experimental components of the study suggest macrophage uPAR to be a potentially attractive candidate molecular imaging target for assessing inflammation, and the combination of cell- and tissue-based assays establishes a robust methodological pipeline for testing new targets.

However, as with any early study there are several limitations to consider. These drawbacks include a very small number of carotid artery samples included in the analysis as compared with other studies in the literature,

[17] and a limited number of cell markers and genes analysed by flow cytometry and micro-array analyses. This point is important because uPAR is known to be expressed by other plaque cells besides macrophages, and the contributions of these cells to arterial uPAR PET signal should also be examined. For instance, uPAR has been previously detected in vascular smooth muscle cells using multiple techniques.[21] The expression profiles of macrophages cultured from immortalized THP-1 cells in this study might also differ from primary blood-derived monocytes/macrophages, even if this is a common experimental technique. A very polarized macrophage phenotype was used, which we now know, thanks to single-cell RNA sequencing studies, only represents a small population among the panoply of cells in atherosclerotic lesions. Moreover, although the presence of uPAR within the plaque core was confirmed in a single carotid specimen, co-staining with additional cell markers in different plaque types would have been very informative. Lastly, due to the observational nature of the clinical imaging component of the study, which was confined to a very small retrospective cohort imaged following oncological acquisition and reconstruction protocols, without electrocardiographic gating or contrast-enhanced CT angiography, these findings should be appreciated primarily as hypothesis-generating data that lay the groundwork for future dedicated cardiac imaging trials.

The re-purposing of PET tracers for imaging atherosclerotic inflammation has also led to several other lines of current investigation, including chemokine receptor CXCR4 and somatostatin receptor binding tracers, which have been reviewed elsewhere.[14] Whether uPAR offers a more cell- or inflammation-specific PET imaging target than these other experimental tracers remains to be determined. For example, uPAR expression in vascular smooth muscle cells undergoing chronic vascular remodelling could, in theory lead to increased arterial PET signal in the absence of macrophage-driven plaque inflammation. Ongoing research using whole transcriptome RNA sequencing of atherosclerotic plaques with single-cell or spatial transcriptomic techniques will undoubtedly shed further light on the cellular expression profiles of these and other potential PET imaging targets (Figure 1).

In the future, an accurate means to identify patients with so-called “residual inflammatory risk” despite conventional treatments will be critical, given the changing landscape of atherosclerosis, and the rise of non-traditional risk factors such as the metabolic syndrome, triglyceride-rich lipoproteins, and abnormalities of the gut microbiome that are associated with inflammation.[32] At present, there are no circulating biomarkers, clinical or polygenic risk scores, nor imaging tests that can mirror the complex and dynamic cross-talk between cellular, biomechanical, environmental, and psycho-social factors driving the risk of an atherosclerotic plaque event in an individual. Moreover, due to the limitations of the current imaging techniques there remains a paucity of data from large clinical trials to describe the effects of disease modifying strategies that lower inflammation on plaque architecture and the micro-environment. For these reasons, there is an ever-increasing requirement for more precise imaging tools to inform and improve the management of cardiovascular disease.

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**Graphical user interface

Description automatically generated with low confidence**

**Figure 1. uPAR expression in the human vasculature.** Publicly available single-cell RNA sequencing data from the Tabula Sapiens consortium showing uPAR (PLAUR) expression localised predominately to macrophages in human aortic and coronary specimens, with lower expression in fibroblasts, endothelial cells, and vascular smooth muscle cells.