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Editorial

Contemporary plaque imaging for risk stratification of coronary artery disease: Are we getting there?

Atherosclerosis is often termed a "silent killer". Atheromatous plaques build up over decades and rarely cause symptoms until vessel occlusion reaches a critical stage, or, most devastatingly, the fibrous cap of the lesion ruptures to precipitate a myocardial infarction or ischemic stroke. Understanding of why certain plaques are more vulnerable to rupture than others has increased vastly over the past decades. Ruptured plaques are characterised by specific morphological features such as large lipid cores overlaid with thinner fibrous caps, by a more inflammatory cell composition that favors cap fragility, and by discrete calcification patterns [1,2].

This latter feature is emerging as a critical determinant of plaque homeostasis [3,4]. Calcium deposition is commonly found in atherosclerotic lesions, and gross accumulation was considered for a long time to be a passive phenomenon of aging. This view has shifted, with lesion mineralisation now seen as an active process encompassing complex signaling, a bone-related gene program, and a bimodal impact on plaque stability. Of primary clinical importance in this context is the quality, rather than the quantity, of the calcium deposition. The current view is that dense macro-calcification confers stability to advanced atherosclerotic plaques, while micro-calcification, defined as discrete deposition hot-spots especially in the fibrous cap, renders plaques more fragile by reducing compliance and elasticity, and increasing local tissue stress beyond a certain cap-rupture threshold. An inflammatory microenvironment around the calcified region may further influence plaque vulnerability [5,6]. Calcium deposits in the form of hydroxyapatite tend to be associated with rupture-prone plaques, conversely, calcium oxalate deposition promotes a more stable lesion [7]; moreover, recent studies elegantly combining clinical and simulation data have demonstrated that even the shape and angle of the calcification can critically increase the destabilising stress on the cap [8,9].

Taken together, the constellation of calcification composition, location, size, shape and orientation may be a novel predictive feature of plaque vulnerability. Translation of this concept of plaque-based risk evaluation to the clinic requires sophisticated imaging modalities and pre-clinical models suited to assess the feasibility and effectiveness of candidate interventions and their time-points.

The classic experimental model of atherosclerotic plaque development is the ApoE-deficient mouse fed a pro-atherogenic high fat diet. While this model does not reproduce plaque rupture, it does provide a valuable tool to assess factors that determine plaque fate and the success of therapeutic approaches. In this Journal, MacAskill and colleagues [10] now provide an innovative study design to quantify and temporally characterise atherosclerotic plaque micro-calcification in ApoE-/- mice by non-invasive [^{18}F]NaF PET/CT imaging. [^{18}F]NaF is a gold-standard tracer to monitor vascular osteogenesis, with the advantage of intraperitoneal application, which allows for repeated measures and treatment interventions. This may not be possible with tracers applied intravenously. The authors could detect progressive micro-calcification over 12 weeks in all animals, mainly confined to the ascending aortic arch, with signal hot-spots doubling between the 6 and 12 week time-points. The findings were validated by traditional *ex vivo* PET/CT, which also documented macro-calcification in half of the animals studied at 12 weeks. The study design advances the state-of-the-art beyond traditional *ex vivo* and invasive assessment of intra-plaque calcification, and provides a quantitative tool for temporal characterisation of atherosclerosis-related micro-calcification, a predictive marker of plaque vulnerability. Conceivably, if translated to the clinic, the approach may provide the opportunity for timely detection of lesions at risk of rupture and effective intervention. This could be particularly useful in patients who underwent stent replacement, to monitor for rapid neo-atherosclerosis and vulnerable plaque development. In a just-published study, [^{18}F]NaF PET/CT imaging was applied to assess arterial micro-calcification and its relations to cardiovascular events in 80 healthy controls and 44 patients [11].

A recent integrated network analysis of the vascular "calcificasome" identified significant overlaps with endophenotype modules governing inflammation, thrombosis, and fibrosis [12]. Calcification in atherosclerotic aortae of ApoE-/- mice is closely linked to inflammatory macrophages, which drive osteogenic activity in early-stage atherosclerosis and precipitate pre-clinical micro-calcifications [13]. This phenomenon appears to be determined by the region-specific distribution of macrophage subpopulations in the atherosclerotic plaque. Both type-1 (MΦ1) and type-2 (MΦ2) macrophages are present in human plaques, but MΦ2 macrophages are localized predominantly to more stable locations within the lesion, while MΦ1 markers are highly expressed in vulnerable plaques [14].

The inflammatory state of an atherosclerotic plaque is thus also a key determinant of plaque vulnerability. Perhaps tandem imaging of the intra-plaque "inflammo-calcific axis" could further fine-tune risk plaque-dependent risk stratification? The established modality to monitor vascular inflammation *in vivo* is 18-fluorodeoxyglucose (FDG)-PET, a measure of glucose metabolism that

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is proportional to macrophage density in atherosclerotic lesions [15]. The combination of [^{18}F]NAF and [^{18}F]FDG PET/CT imaging has been applied in both atherosclerotic mice [16] and myeloma patients [17], and could provide a valuable non-invasive tool to identify vulnerable atherosclerotic plaques and their response to therapeutic intervention. Other novel complementary or alternative approaches for refined plaque risk assessment are in the pipeline, including bioengineered endogenous human ferritin nanocages [18], and fluorescently-labeled peptide amphiphile micelles [19], as well as innovative and optimised computing applications [20].

In summary, the present study by MacAskill and colleagues [10] provides a pre-clinical platform for temporal assessment of plaque micro-calcification. The approach has translational potential for plaque-based risk prediction, particularly if combined with imaging of inflammatory vascular burden.

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Declaration of Competing Interest

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

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Anke C. Fender *
Dobromir Dobrev
Institute of Pharmacology, West German Heart and Vascular Center, University Duisburg-Essen, Essen, Germany
* Corresponding author at: Institute of Pharmacology, University Medicine Essen, Hufelandstr. 55, 45122 Essen, Germany.
E-mail address: anke.fender@uk-essen.de (A.C. Fender)
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