

Nature or The Natural Evolution of Plaque: What Matters?

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ABSTRACT

Progression to major acute cardiovascular events often is triggered by an atherosclerotic plaque complicated by rupture or erosion, namely the vulnerable plaque. Early and secure identification of these plaques would allow the development of individualized therapeutic and pharmacological strategies, applied in a timely manner. Imaging methods have a huge potential in detecting and monitoring the evolution of vulnerable plaque. Even though there are multiple invasive and noninvasive techniques, clinical application is for now a matter of choosing the relevant imaging feature for the prognosis, the methodology of study and the target population.

Keywords: vulnerable plaque, imaging techniques, imaging targets, major acute cardiovascular events

Modern cardiology benefits from undeniable advancements in atherosclerosis (ATS) research, translated into saving million of lives in clinical practice. Despite the diagnosis and therapeutic progress, a substantial number of patients continue to suffer major acute cardiovascular events (MACE). It has been demonstrated that such events are most commonly caused by an atherosclerotic plaque, hemodynamically insignificant but susceptible to rupture or erosion, namely on the vulnerable plaque.

Putting practical reality into conceptualization meant an important shift of paradigm, where the assessment of plaque instability in-

stead of detection the critical stenosis has become the cornerstone. This is an important aspect from an epidemiological perspective as MACE are responsible for almost half of all deaths and is the major cause of cardiovascular morbidity, while the uncomplicated evolution of atheromas represents only 3% of the annual rate of mortality caused by cerebrovascular and coronary ischemic injury (1).

It is a major issue for a clinician in which patients, when and how do we identify the vulnerable plaque? It is obvious that the population at risk is more numerous than that with MACE, most probably because ATS has a pronounced inter-individual variability of the rate and type of progression. Despite the efforts to

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identify and treat high-risk patients on current cardiovascular risk scores, the estimation of clinical evolution remains imperfect, especially in young patients. New integrated scores, including biomarkers (reactive protein C, fibrinogen and fibrin degradation products, shock protein HSP70, etc), non-invasive imaging methods, especially coronary computed tomography, are also considered to have a very low predictive value to be used in current practice (2,3).

Imaging of vulnerable plaque: which targets?

Technological progress offers a large variety of invasive and non-invasive methods which allow direct visualization of the atherosclerotic plaque evolution in fascinating details. In terms of practice, we talk about unrefined area for screening, timely detection and individualized preventive therapeutic attitude. The challenge remains in identifying a critical feature of vulnerable plaque, with a secure predictive value for MACE. The diagnostic optimization is necessary because only a reduced fraction of vulnerable plaques will likely progress to rupture, and of those that rupture, just a minority will cause a clinical event (4).

Imaging targets for defining vulnerability are based on morphological criteria and molecular features, focused on the immunobiology of vascular endothelium.

The morphological traits typically associated with vulnerable plaque are found in lesions usually referred to as thin-cap fibroatheroma. The main features are a thin fibrous cap ($<65\ \mu\text{m}$) heavily infiltrated with macrophages and inflammatory cells, usually associated with a lipid-rich large necrotic core, which represents $>30\%$ of the plaque area. Some other features like neovascularization and intraplaque hemorrhage, adventitial inflammation and “spotty” calcifications are added (5). Recent studies suggest the plaque instability is to be viewed not as a consequence of fibrous cap thickness alone but rather as a combination of structural features and biomechanical synergic interactions. Plaques with relative necrotic core thickness $>50\%$ and low arterial remodeling index are more prone to rupture, having a higher critical thin-cap thickness values (6). There are also less defined mechanisms of vulnerability, out of which the plaque erosion is the most common, present in about 25% of patients, generally women (7). Erosion-prone vulnerable plaque remains a delicate issue for structural imaging,

as stable plaque differentiation is difficult. These types of vulnerable plaque are heterogeneous and defined only by the presence of mural thrombus (8).

Molecular imaging is obviously a technological progress, which is addressed both to early phases of ATS and to critical events associated with vulnerability such as inflammation, remodeling of fibrous cap and of necrotic lipid core. Thus, the production of markers such as adhesion molecules (especially VCAM-1), selectins, chemokines (fractalkine CX3CR1 which mediates the adhesion of monocytes and T cells), macrophages, monocytes and foam cells are valuable targets for detecting inflammation. Macrophages from vulnerable plaques typically release cytokines and proteases (metalloproteinases, serine and cysteine proteases), attractive biomarkers by engaging in the process of fibrous cap remodeling and destabilization. Apoptosis of smooth muscle cells and of mononuclear phagocytes, as a feature of remodeling, can be intercepted by detecting a specific protein – the annexin V, typically externalized in the vulnerable plaque (9,10). Integrins as markers of neoangiogenesis, myeloperoxidase, lipid components are additional promising molecular targets for imaging vulnerable coronary plaques. Consequently, developing molecular imaging strategies based on the increased knowledge in vascular biology could have the potential to assess the “regional risk” that is specific to an atherosclerotic lesion, which can be then used in concert with traditional risk factors to individualize the preventive therapeutic decision.

Imaging of vulnerable plaque: what method, when, and for whom?

Even if there are sophisticated, effective methods, now we cannot discuss about an ideal technique applied in daily clinical routine. Prospective randomized, multicenter, controlled studies are needed to establish the accuracy of predictive value for MACE, whether by choosing a relevant imaging target for the long-term prognosis, or by implementing a multimodal imaging protocol. Developing a reproducible, available, affordable method, without notable adverse effects is essential to enable its use in serial screening.

Intravascular imaging, even if it cleared the way in assessing plaque vulnerability, it is adequate only in secondary prevention, taking into account that some aspects are not com-

pletely elucidated. First, selection method of the investigated population with high risk remains difficult to implement. In the COURAGE trial, stable patients with coronary artery disease treated aggressively had a subsequent MACE risk of 18.5% over a median 4.6-years follow-up (11), while acute coronary syndromes revascularized successfully in the PROSPECT study had a 3.4-year event rate of 11.6% caused by destabilization of angiographically mild atherosclerotic plaques (12). Second, it is obvious that such methods have a small but present risk for vascular damage, are labour intensive and time consuming in spite of developing new approaches, algorithms and advanced software. Thirdly, the accuracy of diagnostic criteria for the identification of prone-rupture plaques is insufficient in accordance with the PROSPECT study. Thus, only 10% of plaques labeled as vulnerable using new techniques of intravascular ultrasound (IVUS) lead to clinical events, and rate of MACE is relatively low (10-18% depending on plaque features). The dynamic character of atherosclerosis, which evolves in repeated sequences rupture-healing-rupture, can be a plausible explanation (13). But, the major limitation is most probably our inability to accurately detect vulnerable plaques in humans *in vivo*, partly because the precise mechanisms of progression from an asymptomatic stable to high-risk plaque are incompletely understood. The importance of detecting the thin fibrous cap and the necrotic core to characterize the vulnerable plaque calls for dedicated methods in clinical research, such as new techniques of IVUS, optical coherence tomography and near-infrared spectroscopy

IVUS remains the method to which all imaging intravascular advancements relate. Beyond its clinical use, IVUS constitutes a valuable tool in research, as it is the gold standard for studying plaque vulnerability and has been used to investigate the role of local hemodynamics in plaque destabilization. The method has also the advantage of assessing the effectiveness of pharmacological treatments on the volume of plaque and vascular remodeling, in at least 20 trials, which demonstrate the benefit of statins, pioglitazone, amlodipine on coronary ATS and quantifies the role of new medication as reconstituted HDL (CSL-111), darapladib (a lipoprotein-associated phospholipase A2 inhibitor) and pactimibe (a non-selective inhibitor of acyl-coenzyme A: cholesterolacyl-

transferase) (14). IVUS gray-scale boundaries, the method originally used, is largely overcome by new techniques. Applications of IVUS-radiofrequency (RF) analysis (virtual histology, integrated backscatter analysis, wavelet analysis) provide detailed information on plaque composition. Virtual histology technique provides most evidence of diagnostic accuracy being validated against histological studies, but remains limited in describing the major criteria of vulnerability – thickness of fibrous cap, as well as in assessing the minor criteria of vulnerability (15). IVUS elastography (palpografia), also validated, completes the information by measuring the local rate of plaque deformation (strain), which is proportional to the degree of infiltration with macrophages and lipid content (2). New technical options as contrast-enhanced IVUS detect neovascularization (16), while IVUS-guided photoacoustic imaging proposes new imaging targets, such as, deep disposal of intraplaque lipid deposits. Biomechanical interpretation of vulnerability finds its application in quantification of stiffness distribution across the plaque, using strain map (elastogram) and elasticity map (modulogram) (5).

Optical coherence tomography (OCT) has the advantage of high spatial resolution in *ex vivo* experiments and has been shown to identify plaque morphology with a sensitivity between 71-96% and specificity between 90-98% (2). The method has a higher accuracy than IVUS as it measures exactly the thickness of fibrous cap and plaque volume, the presence of calcium deposits, identifies rupture and thrombus. Additionally, it is the only method that can detect eroded plaque. A major limitation of OCT remains the assessment of true vessel size and plaque burden. Combining OCT and IVUS-RF or the use of new techniques, such as, spectral radar OCT, parallel ultrasound beam, image processing etc offer further possibilities to assess vulnerable plaque (14).

Near-infrared spectroscopy (NIRS), validated against histology in SPECTACL study, has the advantage of superior assessment of lipid content (18). The method is available in the first FDA-approved hybrid imaging system combining NIRS with IVUS (InfraReDx) (18). Its prognostic value will probably be elucidated by the prospective initiative named European Prospective Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclero-

sis-Intravascular Ultrasound Study (19). To date, experimental animal studies have demonstrated feasibility of a derived method - intravascular near-infrared fluorescence molecular imaging. Clinical translation appears promising and targets various molecules such as cysteine-protease, adhesion molecules, macrophages, activity of thrombin (20).

Non-invasive imaging has been developing as an attractive alternative in the study of vulnerable plaque, with a huge potential to extensively use in the near future, as it targets asymptomatic individuals at high risk to develop an acute vascular event and multiple molecular targets. The methods are already successfully used for carotid imaging, but are not well-suited for the coronary arteries, owing mainly to the small size and continuous movement of imaging targets (2,21).

Coronary computed tomography angiography (CTA) is the only robust method applied currently to coronary artery territory, considered reproducible and adequate for clinical trials. It has an accuracy of 94% in characterizing plaque vulnerability in comparative studies with IVUS-virtual histology (5,9) and uses criteria with significant prognostic value documented by prospective studies (low-attenuation plaque <30 Hounsfield units, spotty calcification, positive arterial remodeling) (22). Its potential in molecular imaging, based on detection of inflammation with iodine nanoemulsions accumulated selectively in activated macrophages, completes the undeniable value of the method (23). The issue of detection and longitudinal assessment of vulnerable plaque, given the risks of radiation exposure, remains a problem in clinical practice.

Positron emission tomography is a method specifically and primarily addressed to identifying inflammation commonly associated with plaque vulnerability based on radiotracer uptake by metabolically active cells, such as, macrophages (5,24). Detection of molecular picomolar targets allows the use of significantly lower doses of contrast, although the hybrid imaging that combines the method with CAT or magnetic resonance imaging is needed to overcome the limitations of spatial and temporal resolution (5). The use of method for coronary plaque assessment based on the most validated radiotracer FDG (fluoro-deoxy-glucose labeled with ^{18}F) is not considered appropriate due to the concomitant uptake by adjacent myocar-

dium, although a recent prospective study demonstrated its feasibility (25). New radiotracers addressed to other specific molecular targets such as monocytes, oxidized LDL, metalloproteinase activity, VCAM, integrin $\alpha(v)\beta_3$ (3), somatostatin or benzodiazepine receptors are under investigation in experimental studies (9).

Magnetic resonance imaging (MRI) is considered the most promising method for complex and complete characterization of the plaque content, with well-proved usefulness in the study of large static arteries. Even if there are clear indications of the benefit in assessment of edema and local vascular inflammation in acute coronary syndromes (26), its use in this field needs the increase of technology efficiency, improvement of reproducibility and, obviously, determination of clinical predictive value for MACE (21). Current literature views as premature the routine application of molecular imaging, but the method certainly has potential, taking into account new techniques which intercept inflammation with superparamagnetic nanoparticles of iron oxide accumulated in macrophages or vectors specific for VCAM-1 and neoangiogenesis with paramagnetic particles (5). It has been demonstrated that MRI has as well as IVUS the ability to evaluate the role of local hemodynamic in plaque destabilization through a direct relationship with the endothelial shear stress and positive segmental remodeling (27).

Imaging of coronary vulnerable plaque: Quo Vadis?

Although the development of imaging techniques remains the cornerstone for the recognition of vulnerable plaques, the strategy that will allow a correct identification of the target population remains an issue in clinical practice. Carried out with this aim, The BIOIMAGE study which is part of the public health program "The High Risk Plaque Initiative" is unique and innovative in identifying and recruiting asymptomatic population with MACE risk (28). The primary objective of the study is to identify imaging biomarkers that predict near-term (3 years) acute vascular events within an algorithm applied to healthy adults with, at least, one cardiovascular risk factor. The Working Group of the National Heart, Lung and Blood Institute considers that the introduction into routine practice of imaging will be justified only after its superior predictive value compared to existent

methods will be proved to be more efficient in identifying the high-risk population, which means, at least, 2-fold better predictive power for invasive methods and several-fold for non-invasive ones (5). Most likely, a multimarker strategy that includes risk factors, molecular and genetic biomarkers, non-invasive imaging markers, will be a truly viable option in terms of practice. Till then, experimental studies with adequate animal models are needed, which would allow a further understanding of the role of the plaque microenvironment (cellular immune response, neovascularisation and intra-plaque hemorrhage, extracellular matrix composition, biomechanical stress, etc). PROSPECT-type studies are also needed to identify vulnerable plaque features that are the focal cause of future MACE, and also, to treat

and monitor some therapeutic interventions adapted to regional risk and to high-risk patients.

All in all, we should consider that the detection and the characterization of vulnerable plaques *in vivo* is becoming a reality thanks to the various imaging methods. Before these techniques can be used into daily clinical practice, their diagnostic and predictive value need to be investigated in prospective, multicenter, randomized, controlled human clinical imaging trials. Ultimately, these advances could strengthen preventive care and enable early initiation of personalized therapy before irreversible damage occurs.

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