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Plaque erosion – new insights from the road less travelled by.

Bentzon JF[1,2*], Falk E[2]

[1]Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain
[2]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

Short title: New insights into plaque erosion

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*Correspondence to
Jacob F. Bentzon
Experimental Pathology of Atherosclerosis,
Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC)
Melchor Fernández Almagro 3, 28029 Madrid, Spain
Telephone: +34-914531200 (Ext. 2012)
Mobile: +34-608989770
Email: jfbentzon@cnic.es

The exploration of the steps that lead to coronary thrombi needs another chapter. Decades ago it was found that the majority (about ¾) of lesions underlying lethal thrombi featured a necrotic core into which blood had entered through a severed fibrous cap [1]. Sometimes bits of core material were embedded in the thrombus linking cap rupture and thrombosis closely in time. Like watching a crashed car or a broken plate, it took modest imagination to understand what must have happened. A thin fibrous cap had ruptured, ripping open the lesion and exposing the thrombogenic core to the hemostatic system. The lack of a credible alternative mechanism that could explain the observed has made it less of a problem that re-enactment of plaque rupture in model systems or formal proof for the sequence of events in humans have been difficult to achieve.

For the remainder of thrombi that are not formed on ruptured plaques, it is a completely different game. These cases give few signs away about what caused them. Endothelial cells (ECs) at the plaque-thrombus interface are generally absent, leading to the term *plaque erosion*, and macrophages are rarely conspicuous [2]. More notably, neutrophils are often present [3]. But autopsies cannot tell for sure whether the missing endothelium and neutrophil accumulation were the cause or result of thrombosis. This is not an academic question. EC sloughing and neutrophil invasion occur secondarily to stasis-induced venous thrombosis [4], and thrombi precipitated by plaque rupture can produce the appearance of plaque erosion in neighboring segments [2]. The idea that erosions are preceded by plaques deprived of large regions of its endothelial lining may thus be far too simple. Rather, processes of EC loss, neutrophil recruitment, and thrombosis may occur simultaneously facilitating growth of thrombus across an erosion-prone lesion surface.

This is part of the lesson that can be learnt from the work of Franck et al. in the present issue of *Circulation Research* [5]. In a murine model system that featured components associated with human plaque erosion, they describe a mechanism by which a hyaluronan (HA)-rich subendothelial matrix, disturbed blood flow and neutrophils cooperate to drive endothelial denudation and arterial thrombosis.

Whereas lesions producing thrombi by rupture are invariably fibroatheromas with thin caps, no distinct set of histological features have been identified for the precursors of erosions. They appear a mixed bag of pathological intimal thickenings and fibroatheromas, with variable degrees of superficial inflammation [1]. Yet one common theme may be the presence of tissues rich in SMCs and glycosaminoglycans, including HA, at the plaque-thrombus interface [6]. HA is an anionic, nonsulfated extracellular matrix glycosaminoglycan with important structural and signaling properties in multiple organisms and tissues. Its water retaining properties makes it an important constituent of articular cartilage and cosmetic dermal fillers, and fragments of HA, which is also present in the coat of certain bacteria, signal through Toll-like receptors, including TLR2, to facilitate inflammation [7].

Franck et al. show that eroded carotid lesions in humans, as well as non-thrombotic lesions with many apoptotic luminal ECs, have accumulation of superficial neutrophils. This extends their previous work that such lesions are characterized by superficial HA accumulation, and that HA in vitro signals through TLR2 to activate ECs leading to expression of neutrophil-recruiting chemokines and adhesion molecules [8].

To create vessels with HA-rich subendothelial matrix, Franck et al. allowed electrical injury of murine carotid arteries to heal with formation of smooth muscle cell-rich neointima and regenerated ECs. Subsequently, the healed segment was exposed to disturbed blood flow by the application of a constrictive perivascular cuff. The link between flow patterns and risk of erosion has not been well worked out in humans, but disturbed flow is common in severely atherosclerotic arteries. Because an eroding lesion provides a subtler thrombogenic stimulus than rupture, it makes sense that the other components of the triad of Virchow (flow disturbance and systemic thrombotic propensity) could be particularly important in this setting. Moreover, disturbed blood flow increases endothelial TLR2 expression and apoptosis thereby triggering the proposed HA-TLR2-neutrophil axis [9]. With these two arterial insults in place, neutrophil accumulation, EC apoptosis and mural thrombosis resulted.

While we cannot be sure that the thrombotic mechanism in this cuffed neointima model replicates the one in human plaque erosion, it copies several components, and importantly, it offers opportunities to establish cause-effect relationships and directionality among them. From the experiments by Franck et al. we learn that TLR2 signaling is upstream of neutrophil accumulation, which in turn is important for EC loss and thrombosis [5]. The relatively restricted thrombus formation seen in the model may be expected. In our own experience, mechanical rupture of advanced lesions in the mouse carotid bifurcation also rarely lead to more than a small mural thrombus [10].

The cuffed neointima model is related to and may shed light over the thrombosis mechanism in two previously published animal models. Femoral artery stenosis in rabbits induces downstream neointima and thrombus, possibly by similar mechanisms [11]. Carotid tandem stenosis in hyperlipidemic mice has been introduced as a model of plaque rupture, but it is plausible that the thrombotic mechanism may include additional mechanisms [12].

The interesting work of Franck et al. is from a road less travelled by in atherosclerosis research. As the authors rightly note, there is a scarcity of investigation into plaque erosion, which is completely out of proportion with the importance of the problem. Perhaps it is partly the lack of a relevant model system that has discouraged more researchers from delving into the subject. If that is the case, the cuffed neointima model may inspire others to take on the problem.

Going forward the established model may offer possibilities to test the causal role of other determinants of erosion-proneness in the arterial wall or circulating cells, such as additional matrix components, secreted pro- or antithrombotic factors, and the role of EC phenotype. Previous studies have indicated that injured arteries heal with dysfunctional ECs [13]. Could this contribute to the thrombosis-proneness of the cuffed neointima? Is EC senescence resulting from decades of increased EC turnover over atherosclerotic lesions important? Can it be ameliorated?

In a broader perspective, it would be interesting to explore whether the thrombotic mechanism described here has a facilitating role in cases where it is not itself sufficient to precipitate thrombosis. Not all plaque ruptures cause clinical symptoms and even fewer lasting damage or death. Others heal silently with only mural or transient thrombus. Probably rheological forces and systemic thrombotic propensity are important factors, but one may also speculate that the vulnerability of the neighboring plaque surface for neutrophil-driven endothelial denudation could be a determining factor between the different outcomes. The

often protracted development of thrombus and the presence of neutrophils in the plaque-thrombus interface of ruptured plaques lend some support to this idea [3,14]. Clinical studies using intravascular imaging with optical coherence tomography (OCT) to detect plaque rupture have found that rupture is the most frequent substrate of STEMI, whereas NSTEMI often develops without OCT-detectable rupture [15]. Decline in age-adjusted incidence of ACS over recent decades has been particularly clear for STEMI [15]. These observations indicate that what has been achieved so far in primary and secondary prevention of ACS has been especially effective in counteracting plaque ruptures. It also suggests that the erosion-prone lesion is the next frontier in combating clinical complications from coronary atherosclerosis. Such an endeavor is best helped by a clear understanding of the mechanisms underlying thrombosis with plaque erosion.

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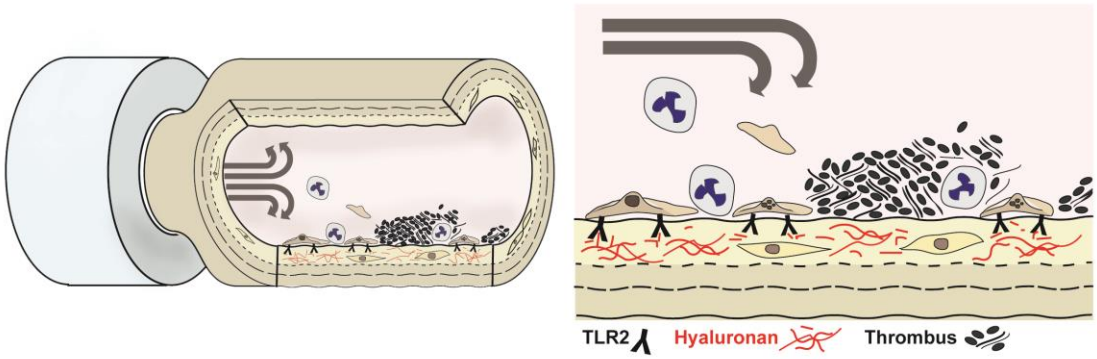

Figure. Schematic of the cuffed neointima model and the proposed thrombotic mechanism. TLR2 signaling, possibly evoked by hyaluronan fragments in the sub-endothelial matrix, together with disturbed blood flow leads to neutrophil recruitment, EC apoptosis/sloughing, and thrombus formation.