



Neutrophil extracellular traps: a new source of tissue factor in atherothrombosis

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This editorial refers to ‘Expression of functional tissue factor by neutrophil extracellular traps in the culprit artery of acute myocardial infarction’, by D.A. Stakos *et al.*, on page doi:10.1093/eurheartj/ehv007.

Atherosclerotic cardiovascular diseases are still major causes of morbidity and mortality worldwide. Sudden rupture of vulnerable atherosclerotic plaques expose to the flowing blood prothrombotic molecules that trigger platelet aggregates that grow in association with an increase in fibrin deposition and further entrapment of inflammatory and red blood cells.¹ The resulting thrombus may lead to blood flow cessation and subsequent acute ST-elevation myocardial infarction (STEMI).² Thrombus aspiration has offered a unique opportunity to characterize antemortem the culprit coronary thrombus. In this regard, within the last years several studies in coronary thrombectomy specimens extracted from occluded arteries have provided insights as to thrombus composition, reporting that platelets, erythrocytes, and activated neutrophils are major contributors of arterial thrombosis after plaque rupture.^{3–6}

Neutrophils are critical components of the innate immune system launching the first line of host defence against invading microorganisms. Seminal work by Brinkman and colleagues in 2004⁷ revealed that, in addition to the well-established mechanism of phagocytosis, activated neutrophils fight microbes through the release of web-like filamentous structures of decondensed chromatin (so-called neutrophil extracellular traps, NETs). NETs are composed of DNA and histones, and harbour granular components [such as myeloperoxidase (MPO), neutrophil elastase, and cathepsin G] that exert antimicrobial properties.⁸ During the last years, however, several *in vivo* models of thrombosis have described a critical role for neutrophils in linking sepsis and deep venous thrombosis through release of NETs.⁹ *In vitro* data have supported the ability of NETs to stimulate the activation of the coagulation cascade (both the extrinsic and the intrinsic pathway) and platelet adhesion. As such, isolated nucleic acids have been shown to activate the coagulation pathway by binding both factor XII and XI; purified histones have been shown to impair thrombomodulin-dependent protein C activation enhancing plasma thrombin generation; and neutrophil serine proteases

have been shown to inactivate tissue factor (TF) pathway inhibitor through cleavage, with an ensuing increase in factor Xa procoagulant activity.¹⁰ On the other hand, DNA and histones have been shown to interact and trap platelets most probably via electrostatic interactions or via Toll-like receptors (TLRs).

In 2012, the laboratory of K. Ritis reported seminal findings on the ability of isolated neutrophils from patients with sepsis to release TF through NETs, supporting a key role for neutrophils at the interface between septic inflammation and systemic coagulation (Figure 1).¹¹ In a study published in the current issue of the journal the group of K. Ritis now provides the first evidence of neutrophils and functional TF-bearing NETs at sites of atherosclerotic plaque rupture in STEMI patients, a feature not detected in patients that had undergone spontaneous thrombus resolution.¹² With these data, the authors describe a novel mechanism by which neutrophils may locally externalize functional TF in the culprit lesion. The authors analyse thrombotic material obtained from the infarct-related coronary artery (IRA) and non-IRA of STEMI patients and perform a series of well-conducted *ex vivo/in vitro* approaches with blood samples obtained in the vicinity of the culprit vessel. The researchers demonstrate that, despite STEMI-related up-regulation of TF in circulating neutrophils, NET release and subsequent active TF exposure is exclusively associated with neutrophils isolated from the IRA. In addition, Stakos and colleagues observe that NET structure integrity is critical for the functionality of NET-bound TF.¹²

The results also provide a hint on the potential triggers for NET release. Clark *et al.* reported that activation of platelet TLR4 favoured platelet/neutrophil interaction and subsequent NET production, thereby promoting bacterial trapping under severe septic infections.⁸ However, platelet binding to neutrophils in the presence of NETs was not completely prevented with TLR4-blocking antibodies, suggesting that alternative mechanisms regulate platelet activation and subsequent NET formation. Stakos and colleagues also demonstrate that thrombin-mediated platelet activation via protease-activated receptor (PAR-1) binding is critically involved in TF-bearing NET formation.¹² As such, the use of thrombin inhibitors and PAR-1 blockers abrogates NET generation and TF activity. These data underscore the importance of thrombin, generated at sites of plaque rupture,

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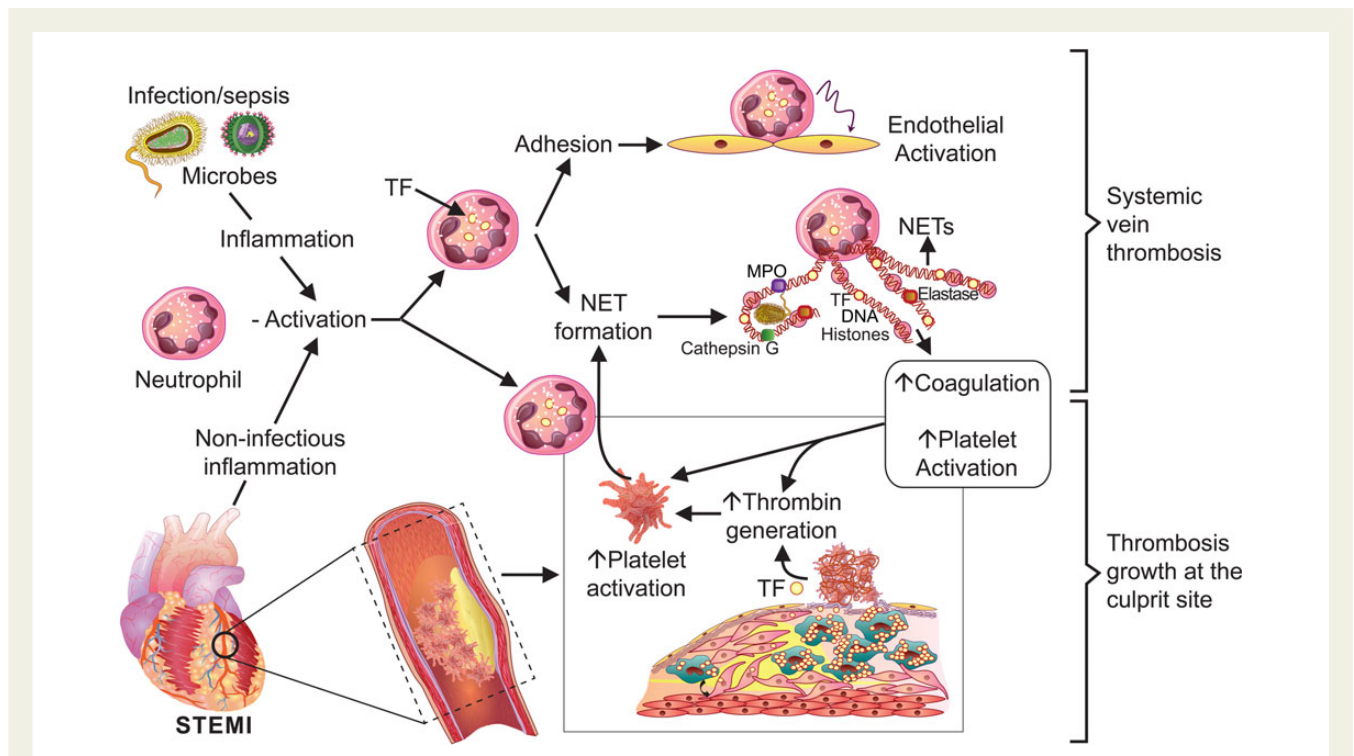


Figure 1 Neutrophils contribution to thrombosis via tissue factor-bearing neutrophil extracellular traps release. Neutrophil activation in response to an inflammatory stimuli (infectious or not) induces tissue factor formation and neutrophil extracellular traps extrusion. Neutrophil extracellular traps are composed of chromatin decorated with granular proteins and functional tissue factor capable of promoting thrombin generation (favoring systemic vein thrombosis) and platelet activation. At the culprit coronary site, generated thrombin boosts platelet activation which, in turn, enhances neutrophil extracellular trap-bound tissue factor release favoring thrombus growth on the ruptured atherosclerotic plaque.

as an initial trigger for platelet-derived neutrophil extrusion of NETs. In this regard, atherosclerotic-related TF may play a critical role.¹³ Upon vulnerable plaque disruption, extracellular TF, mainly expressed in foam cells and lipid-laden vascular smooth muscle cells, interacts with plasma factor VII/VIIa. The resulting TF–FVIIa complex activates FX that forms the prothrombinase complex with cofactor FVa transforming prothrombin into thrombin. Thrombin generated at the sites of plaque rupture triggers both the formation of a fibrin monolayer covering the surface of the exposed vascular damage and platelet activation.¹ Activated platelets may then stimulate neutrophil release of functional TF-bearing NETs, promoting a positive feedback loop to boost thrombin generation and subsequent thrombus growth (Figure 1). Moreover, NETs may also provide a scaffold to enable platelets, coagulation factors, and red blood cells to adhere, further contributing to the obstruction of coronary blood flow and even thrombus stabilization.⁹ Hence, the presented data are of particular importance since they propose that besides TF exposed by atheromatous plaque components, neutrophils may contribute to thrombosis at culprit sites via NET-bound TF release. Moreover, this study certainly engenders interesting questions about the mechanism by which activated platelets signal neutrophils subsequently to release NETs. Are platelet mediators involved or is it a process which relies on platelet–neutrophil interaction? In fact, interesting *in vitro* data have shown that interleukin-8 (IL-8) and reactive oxygen species, both released upon myocardial ischaemia, are

also able to induce NET extrusion, widening the spectrum of potential triggers.¹⁴ Likewise, more needs to be learned about the signalling mechanisms involved in NET release. So far, the use of chemical genetics screening has allowed the identification of the MAPK/ERK pathway as a potential signal transduction pathway involved in NET formation.¹⁵ While this topic certainly deserves further investigation, overall the study by Stakos and colleagues advances our understanding of the contribution of the innate immune system to coronary thrombosis and may pave the way to innovative treatment strategies and improved therapeutic options in human atherothrombotic diseases.

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