


# Neutrophil Extracellular Traps in the Infarct-Related Coronary Artery—A Marker or Mediator of Adverse Outcome?

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In response to cell death or activation, neutrophils extrude modified chromatin, with a web-like structure, called neutrophil extracellular traps (NETs).<sup>1</sup> This involves citrullination of the histone tail of positively charged arginine residues by the calcium-dependent peptidylarginine deiminase 4 (PAD4), with subsequent nuclear delobulation and breakdown of the nuclear envelope, chromatin decondensation, and plasma membrane rupture.<sup>2,3</sup> This process is physiologically beneficial when released during an infection, since NETs can capture and kill bacteria. Yet, excessive NET formation or reduced NET breakdown by deoxyribonucleases (DNase) may be harmful and enhance atherothrombosis mechanistically and clinically, in a range of scenarios.<sup>4–7</sup>

Laboratory studies indicate that NETs exhibit potent prothrombotic and proinflammatory properties.<sup>2</sup> The DNA skeleton of NETs can attract and bind circulating proteins, acting as a scaffold for occlusive thrombus formation.<sup>8</sup> Such neutrophil-derived proteins include myeloperoxidase (MPO), serine proteinases such as cathepsin G and neutrophil elastase, and interleukin-1 $\alpha$ . NETs also recruit and express nonneutrophil-derived proteins, including functional tissue factor, as well as factor XII, histones H3 and H4, and fibrinogen, all of which favor thrombin generation and coagulation.<sup>9</sup> Extracellular NET histones bind von Willebrand factor and fibrin to recruit platelets and erythrocytes, also cause platelet activation via toll-like receptors. In turn, activated platelets induce NET formation. Furthermore, NETs impair endogenous fibrinolysis, with NET-bound neutrophil elastase cleaving tissue factor pathway inhibitor, a factor that inhibits coagulation,<sup>10</sup> and histones impair thrombomodulin-dependent protein C activation,<sup>11</sup> resulting in prolongation of clot lysis and enhanced clot stability.<sup>12</sup>

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In the current issue of *Thrombosis and Haemostasis*, Blasco and colleagues report on the analysis of coronary thrombi aspirated from 406 patients with ST-elevation myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention (pPCI).<sup>13</sup> Thrombus aspiration was performed for clinical indication, favoring the inclusion of those with large thrombus burden at angiography (62% of all pPCI). NETs were detected in 51% of aspirates, based on the colocalization of antibodies to citrullinated histone 3 (citH3) and MPO (i.e., double positivity). Over a median follow-up of 47 months, the presence of NETs was strongly associated with the occurrence of major adverse cardiovascular events (MACEs) in the first 30 days after infarction (hazard ratio [HR]: 2.82; 95% confidence interval [CI]: 1.26–6.35,  $p = 0.012$ ), mainly driven by cardiac death and stent thrombosis, especially in the first 7 days (HR: 3.23; 95% CI: 1.18–8.82,  $p = 0.022$ ) (–Fig. 1). The individual components of MACE associated with NETs were reinfarction (odds ratio [OR] 2.28; 95% CI: 1.08–4.81,  $p = 0.03$ ) and urgent revascularization (OR: 2.14; 95% CI: 1.11–4.13,  $p = 0.02$ ). Bacteria were detected in 45% of aspirated thrombi but were not associated with either NET presence or density.

This is the largest cohort study assessing NETs in coronary aspirates during STEMI.

However, earlier, smaller studies have also documented the presence of NETs in thrombi aspirated from the infarct-related artery in patients with STEMI and ischemic

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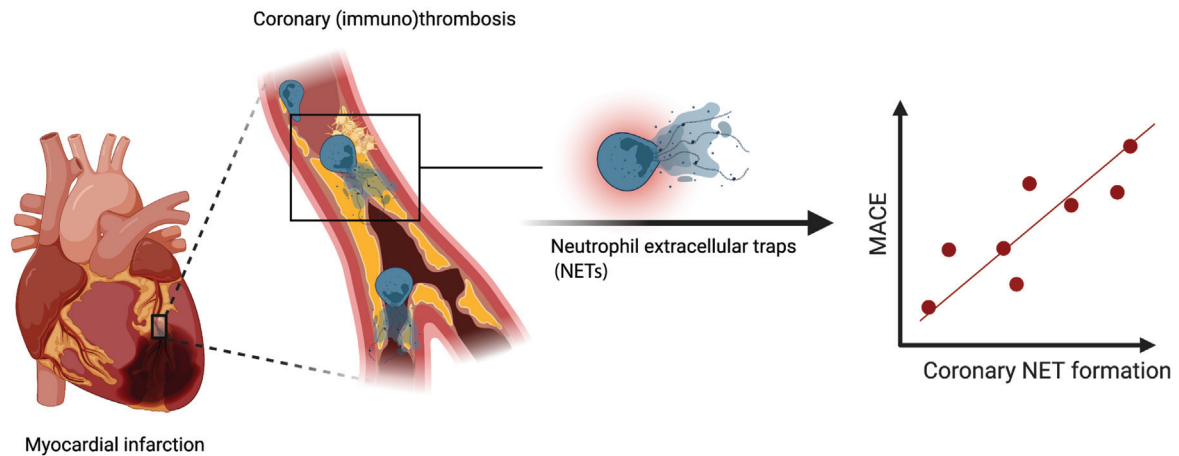
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**Fig. 1** During coronary atherothrombosis, neutrophils release prothrombotic extracellular traps and initiate a process termed immuno-thrombosis. In the study by Blasco and colleagues,<sup>13</sup> the presence of NETs in aspirated coronary thrombi was associated with the occurrence of MACEs in the first 30 days in patients with ST-elevation myocardial infarction. MACE, major adverse cardiovascular events; NETs, neutrophil extracellular traps. Figure adapted from BioRender.

stroke.<sup>14,15</sup> NETs were associated with more complex coronary plaques (intraplaque hemorrhages, erosions, and ruptures) than simple plaques, and more frequent in fresh rather than older, lytic thrombi.<sup>16</sup> An earlier, smaller study of 253 patients with stent thrombosis found NETs in only 23% of specimens.<sup>14</sup>

Several studies have examined the relationship of potential biomarkers of NETs (including MPO-bound to DNA, citrullinated histones, or less specific markers such as double-stranded DNA [dsDNA] or neutrophil elastase) in blood or aspirated thrombi and related these to surrogate markers of clinical outcomes. In patients with STEMI, higher NET burden and reduced DNase activity at the culprit site correlated with thrombus burden, microvascular obstruction and larger infarct size,<sup>17–19</sup> and reduced ST-segment resolution.<sup>17,18</sup> In smaller studies in STEMI patients, dsDNA and citH3 were increased at the culprit site and were positively correlated with infarct size and subsequent left ventricular dysfunction<sup>20</sup> and MACE.<sup>21</sup> Following STEMI, there appears to be an early increase in nucleosomes and MPO levels peaking at 3 hours after pPCI.<sup>22</sup> Furthermore, in a case-control study of patients with acute coronary syndrome, the composite of peripheral markers of NETs and platelet activation was a good predictor of recurrent MACE.<sup>22</sup>

The importance of the current study is that it adds significantly to the volume of data showing the presence of NETs in the infarct-related artery in patients with coronary thrombosis and shows a significant relationship with hard clinical outcomes beyond the acute presentation, but whether NETs are simply a marker of adverse prognosis or play a functional role is unclear. Laboratory studies indicate a direct functional role of NETs in thrombosis and impaired fibrinolysis. In mice, inhibition of PADs prevented NET formation and decreased atherosclerotic lesion size and carotid artery thrombosis.<sup>23</sup> Furthermore, addition of DNase to ex vivo thrombi accelerated thrombolysis.<sup>24</sup> In a recent murine

model, externalized histone H4 was shown to bind to and lyse atherosclerotic lesion smooth muscle cells (SMCs), leading to plaque destabilization; conversely, pharmacological (Cl-amidine treatment) or genetic (*PAD4 knockout*) blockade of NET release prevented SMC death and decreased overall plaque vulnerability.<sup>25</sup>

Two questions naturally arise in response to reading the paper by Blasco and colleagues. First, could NET markers help identify high-risk patients, and second, could NET inhibition offer a novel avenue to reduce cardiovascular risk? Since thrombus aspiration during pPCI is no longer routinely performed, could peripheral sampling be helpful? Several earlier studies indicate that NETs are increased in STEMI at the culprit site but not at remote sites, such as the femoral artery.<sup>20,21</sup> However, peripheral neutrophil count and neutrophil:lymphocyte ratios are well-recognized markers of adverse cardiovascular outcome in STEMI.<sup>26,27</sup> Similarly, NET-related markers such as MPO levels correlate with cardiovascular risk.<sup>28</sup> There are also data that suggest that markers of neutrophil activation, extracellular chromatin, and DNase activity correlate with C-reactive protein and interleukin-6.<sup>18</sup> Thus, to what extent circulating biomarkers of NETs reflect the NET burden within the thrombus and the relationship between NETs, peripheral markers, and outcome has not been fully elucidated in any of the above studies.

As shown by the study of Blasco and colleagues, and other STEMI studies, conventionally employed antithrombotic agents such as heparin and tirofiban appear to be ineffective against already established NETs. Novel therapeutic avenues include inhibition of the formation of and/or increasing the consumption of NETs. While some laboratory studies showed that specific or broad PAD inhibition attenuated NET formation, others have not replicated this.<sup>3</sup> In mice, inhibition of Janus kinase signaling with ruxolitinib decreased NET formation and venous thrombosis.<sup>29</sup> Inhibitions of MPO, colchicine, complement, or phosphodiesterase 4 are other

possible strategies.<sup>8,30</sup> Enhanced NET breakdown with DNase 1 has been shown to accelerate tissue plasminogen activator-mediated lysis of coronary thrombi *ex vivo*, improve coronary microvasculature patency, and attenuate infarct size and long-term left ventricular remodeling in rodents.<sup>31</sup> While recombinant DNase 1 has been used in cystic fibrosis patients to improve mucociliary clearance,<sup>32</sup> it has not been used to target NETs associated with cardiovascular thrombosis. Although there was no difference in pain-to-pPCI time between NET-positive and NET-negative samples, amongst those who were NET-positive, the timeline for peak NET formation is not known. This would be important to appropriately time the administration of any possible treatment to ameliorate the effects of NETs.

In conclusion, the study by Blasco and coworkers significantly strengthens the link between NETs, coronary thrombosis, and adverse outcome. Whether NETs are a marker or an active contributor to adverse outcome in humans remains unclear. Future studies are required to identify peripheral NET markers, the peak time window of NET formation, and to explore potential pharmacological strategies to inhibit NETs to improve cardiovascular outcome.

#### Conflict of Interest

None declared.

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