

A feedback loop between platelets and NETs amplifies inflammation in severe sepsis

Sepsis-derived S100A8/A9 induces GSDMD-dependent platelet pyroptosis via the TLR4–ROS–NLRP3–caspase 1 pathway, leading to the release of oxidized mitochondrial DNA that contributes to the formation of neutrophil extracellular traps (NETs). NETs in turn release S100A8/A9 and accelerate platelet pyroptosis, forming a positive feedback loop and thereby amplifying the production of proinflammatory cytokines.

This is a summary of:

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The question

Severe sepsis is a leading cause of morbidity, mortality and healthcare utilization for children worldwide. Platelets have emerged as key inflammatory cells that are implicated in the pathology of sepsis¹, but their contribution to rapid clinical deterioration and dysregulated inflammation remains obscure. Pyroptosis, a form of inflammation or infection-induced cell death, is crucial for immunity². Some essential signaling components of pyroptosis are expressed in platelets; however, no direct evidence existed that platelet pyroptosis occurs in sepsis and, if present, whether pyroptosis participates in the inflammatory response in sepsis.

The discovery

Combining clinical samples with basic research, we investigated the role of platelets in severe sepsis. We initially performed unbiased proteomic screening of patients with sepsis, compared with matched controls, from our hospital cohort (Fig. 1a). We confirmed that the expression of essential signaling components of pyroptosis was increased in severe septic platelets. To understand the role of these 'pyroptotic components' in severe septic platelets, we used three transgenic mouse models with platelet deficiency of key pyroptotic components (*Gsdmd*^{-/-}, *Tlr4*^{-/-} and *S100a9*^{-/-}). Injection of lipopolysaccharide (LPS) or cecal ligation and puncture (CLP) was used to reproduce the sepsis process in mice.

In our cohort of patients with severe sepsis, thrombocytopenia, thrombocytopeny and excessive release of inflammatory cytokines were significantly increased ($P < 0.05$). Platelet proteomic analysis revealed significant upregulation ($P = 0.007$) of the key pyroptotic component gasdermin D (GSDMD) in severe septic platelets (Fig. 1a). Using the three transgenic mouse models, we demonstrated that sepsis-derived S100A8/A9 induces GSDMD-dependent platelet pyroptosis via the TLR4–ROS–NLRP3–caspase 1 pathway. This pyroptosis causes release of oxidized mitochondrial DNA, and consequently leads to the formation of neutrophil extracellular traps (NETs). Through a series of further experiments, we showed

that NETs go on to affect platelet pyroptosis by releasing S100A8/A9, forming a positive feedback loop that contributes to excessive inflammatory cytokine release in severe sepsis (Fig. 1b).

The implications

The tightly interconnected nature of hemostasis, thrombosis and inflammation, as well as the central role of platelet activation in these processes, are increasingly recognized³. Platelets have a key involvement in thromboinflammation, linking coagulation to immune responses in various infections. The positive feedback loop between platelets and NETs amplifies inflammation in severe sepsis, further supporting the crucial role of thromboinflammation in severe infections. Similar to sepsis, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection also results in excessive inflammation and thrombocytopeny, and can lead to cytokine storms and organ failure in patients with COVID-19 (ref. 4). Thus, platelet pyroptosis might also be an essential component of COVID-19 pathology by mechanisms similar to those demonstrated in sepsis. Therefore, assessment of platelet pyroptosis not only serves as a prognostic marker for patients with severe sepsis, but also might be central to other infections such as COVID-19.

Our study showed that the immunomodulatory compound paquinimod (probably by several mechanisms) effectively inhibited platelet pyroptosis by targeting platelet TLR4, thereby reducing NET formation and inflammation. As a result, improved survival was recorded in CLP-induced sepsis mice.

As paquinimod might be a potential adjunct therapeutic agent for severe sepsis, further investigations are needed to fully understand its mechanism and pharmacodynamics in sepsis.

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EXPERT OPINION



This is a novel and impressive study providing new insights into the

proinflammatory role of platelet pyroptosis in severe sepsis." **An anonymous reviewer.**

FIGURE

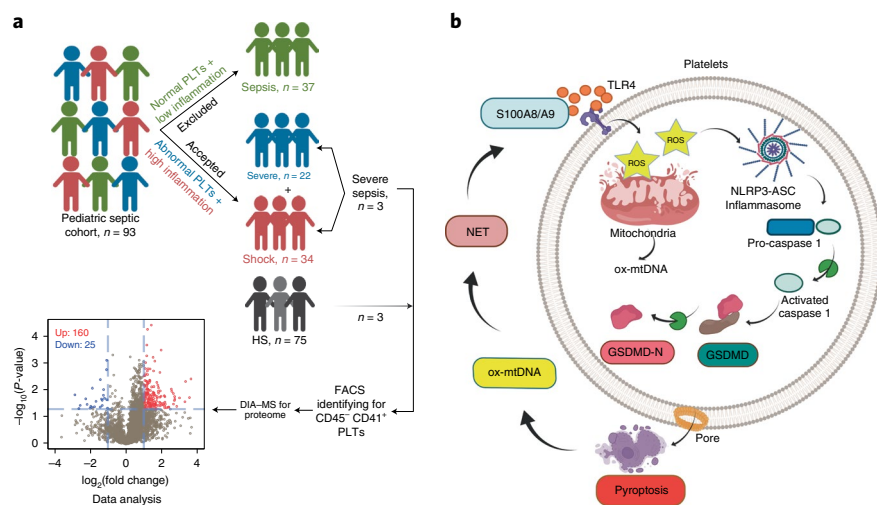


Fig. 1 | Platelet pyroptosis exacerbates NET-mediated inflammation in severe sepsis. **a**, Top, schematic diagram of the experimental design for data-independent acquisition mass spectrometry. Bottom, volcano plot with significantly increased (red) and decreased (blue) expression of proteins from healthy individuals (HS) and patients with sepsis ($n = 3$, fold change cut-off > 2 , $P < 0.05$). **b**, A positive feedback loop exists between platelet pyroptosis and NETs. S100A8/A9 induces GSDMD-dependent platelet pyroptosis via the TLR4–NLRP3 pathway in severe sepsis, which exacerbates NET formation via the release of oxidized mitochondrial DNA (ox-mtDNA). NETs in turn release S100A8/A9, thereby inducing platelet pyroptosis in severe sepsis. © 2022, Su, M. et al.

BEHIND THE PAPER

The identification of the patient with severe sepsis or septic shock at triage is challenging, particularly for children, owing to the low specificity of abnormal vital signs, so the severity of sepsis was not recognized in the course of sample collection. Therefore, we had to screen all the samples collected in our cohort for markers of platelet pyroptosis

to initiate the study. As platelets are easily activated and aggregated, platelet samples must be freshly used for the experiments to avoid unnecessary clotting of samples. Also, in the course of our study, venous blood samples from patients with severe sepsis are valuable and difficult to obtain, particularly for pediatric patients. **W.H.T.**

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FROM THE EDITOR



This study clarifies the key role of platelets in severe sepsis. The death of inflammation-induced platelets exacerbates inflammation through a positive feedback loop that could be potentially targeted to attenuate the rapid health decline in patients. The genetic or pharmacological inhibition of this pathway has revealed positive results in a mouse model of sepsis." **Elvira Forte, Associate Editor, Nature Cardiovascular Research**