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A novel assay of neutrophil extracellular trap (NET) formation identifies anti-IL-8 therapies to reduce disseminated intravascular coagulation and mortality in the intensive care unit

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Introduction

Neutrophils are the first line of defence against bacterial infection, and formation of neutrophil extracellular traps (NETs) is an important protective mechanism. NETs can also be harmful by inducing intravascular coagulation and multi-organ failure (MOF) in animal models.[1–6] Although increasingly considered as important therapeutic targets,[7–9] there is currently no robust and specific measure of NET formation to inform clinical care and enable precision medicine in patients on the intensive care unit (ICU). The aim of this study is to establish a novel assay for measuring NETs and assess its clinical significance.

Materials and methods

A prospective cohort of 341 consecutive adult ICU patients was recruited at the Royal Liverpool University Hospital, following written informed consent. The NET-forming capacity of ICU admission blood samples was semi-quantified by directly incubating patient plasma with isolated healthy neutrophils *ex vivo*. Associations of NET-forming capacity with sequential organ failure assessment (SOFA) scores, disseminated intravascular coagulation (DIC) and 28-day mortality were analysed and compared with available NET assays. Cytokine analysis and inhibitor studies were performed to determine the driving factors of NET formation in patients. To determine the pathological relevance of NETs, complementary *in vivo* studies were performed in mouse models of sepsis (caecal ligation and puncture (CLP) or intraperitoneal injection of *Escherichia coli*), without or with anti-NET therapy.

Results and discussion

We observed that NETs were directly induced by heterologous healthy neutrophils incubated with plasma taken from ICU patients on ICU admission, but not from healthy donors (unless incubated with 100 nM PMA). Using this novel assay we could stratify patients into four groups: those with absent (22.0%), mild (49.9%), moderate (14.4%) and strong (13.8%) NET formation. Strong NET formation was predominantly found in sepsis ($p<0.0001$) and was associated with higher SOFA scores. Adjusted by APACHE II, multivariate regression showed that measuring the degree of NET formation on ICU admission could independently predict DIC and mortality, whereas other NET assays, eg cell-free DNA, myeloperoxidase and myeloperoxidase–DNA complexes, could not. Interleukin (IL)-8 levels were found to be strongly associated with NET formation, and inhibiting IL-8 significantly attenuated NETosis.

Using mouse models of sepsis, we could monitor NET formation using plasma, which was associated with NET-positive staining (cit-H3) in the lung tissue. This was associated with increased fibrin deposition within the lung tissue, along with lung injury scores and circulating markers of liver (blood urea nitrogen; CLP: $p=0.005$, *E coli*: $p<0.001$), kidney (alanine aminotransferase; CLP: $p=0.01$, *E coli*: $p=0.002$) and cardiac injury (cardiac troponin I; CLP: $p<0.001$, *E coli*: $p<0.001$). By targeting IL-8 (using a clinically relevant compound, reparixin) in septic mice, we were able to significantly inhibit NET formation, fibrin deposition and organ injury, and improve survival times ($p=0.004$).

Conclusion

Our new NET assay directly measures the NET-forming capacity in patient plasma. This could guide clinical management and enable identification of NET-inducing factors in individual patients for targeted treatment and personalised ICU medicine. We identify IL-8 as a major driving factor in sepsis, with anti-IL-8 therapy in septic mice significantly reducing NET-induced organ damage and mortality. ■

Conflicts of interest

None declared.

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