**A novel assay of Neutrophil Extracellular Traps (NETs) formation independently predicts DIC and identifies the rationale for anti-IL-8 therapies**

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Background

Neutrophils are the first line of defence against bacterial infection and formation of neutrophil extracellular traps (NETs) is an important protective mechanism. However, NETs can also cause harm by promoting intravascular coagulation and multi-organ failure (MOF) in animal models. Although increasingly considered as important therapeutic targets, there is currently no robust and specific measure of NETs 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.

Methods

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

Results

We observed that NETs were directly induced by heterologous healthy neutrophils incubated with plasma taken from ICU patients, but not from healthy donors (unless incubated with 100 nM PMA). Using the novel assay, we could stratify ICU patients into 4 groups, those with absent (22.0%), mild (49.9%), moderate (14.4%) and strong (13.8%) NETs formation, respectively. Strong NETs formation was predominantly found in sepsis (P <0.0001) and was associated with higher SOFA scores on admission and throughout the study duration (72 hours post-admission). Adjusted by APACHE II, multivariate regression showed that measuring the degree of NETs formation in ICU admission could independently predict DIC and mortality whereas other NETs assays, e.g. cell-free DNA, myeloperoxidase and myeloperoxidase-DNA complexes, could not. Interleukin (IL)-8 levels were found to be strongly associated with NETs formation and inhibiting IL-8 significantly attenuated NETosis. MAPK activation by IL-8 has been identified as a major pathway of NETs formation in patients. Using mice models of sepsis, we specifically observed NETs positive staining (cit-H3) in the lung tissue. This was associated with increases in lung injury scores, along with circulating markers of liver (BUN [CLP: P=0.005, E.coli: P<0.001]), kidney (ALT [CLP: P=0.01, E.coli: P=0.002]) and cardiac injury (cTnI [(CLP: P<0.001, E.coli: P<0.001]). By targeting IL-8 we were able to significantly inhibit NETs formation, organ injury and also improved survival times in septic mice (P=0.004).

Conclusions

Our new NETs assay directly measures the NETs-forming capacity in patient plasma. This could guide clinical management and enable identification of NETs-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 NETs–induced organ damage and mortality.