

Therapeutic removal of NETs from blood in a pig model of sepsis

A. Aswani^{3,2}, D. Genkin², P. Skorup⁴, J. Micallef¹, M. Wagnies¹, R. Varsebroucq¹, M. Lipcsey⁵

¹ Belgian Volition SRL, Isnes, Belgium

² Santerus AG, Kusnacht, Switzerland

³ Guy's and St Thomas' NHS Foundation Trust, Department of Critical Care Medicine, London, United Kingdom

⁴ Uppsala University, Department of Medical Sciences, Uppsala, Sweden

⁵ Uppsala University, Department of Surgical Sciences, Uppsala, Sweden

ABSTRACT

Background

Neutrophil Extracellular Traps (NETs) are associated with sepsis. Histone H1 protein binds NETs and nucleosomes. We investigated the removal of NETs from the circulation of a pig model of sepsis using the Santerus NucleoCapture therapeutic plasmapheresis method based on recombinant histone H1 conjugated to a sepharose bead matrix. We monitored the therapy using Volition's 45-minute, automated Nu.Q™ immunoassay for cell free nucleosomes containing histone H3.1 in 50µl of plasma as a surrogate measure for circulating NETs levels.

Methods

We induced sepsis in 2 pigs by means of a 3-hour infusion of E. Coli. One pig was treated by H1-apheresis for 5 hours and the other by a sham treatment using unconjugated bead matrix. We measured nucleosome levels before and during infusion with E. Coli, and during apheresis in plasma samples taken directly from the pig and also taken from the apheresis instrument, both upstream and downstream of the H1 cartridge. The pigs were also monitored by a variety of clinical parameters.

Results

A single pass of NET-contaminated plasma through the H1 cartridge resulted in near quantitative removal (97.7-99.0%) of NETs.

The background levels of circulating nucleosomes measured in the H1-apheresis and sham treated pigs prior to the experiment were 7.0 and 7.5ng/ml respectively. Infusion of E. coli over 3 hours resulted in an increase in nucleosome levels to 74 and 58ng/ml respectively. The level of NETs in the sham treated pig rose continuously during the experiment reaching 356ng/ml. In contrast, removal of NETs prevented a continuous rise in the H1-apheresis treated pig with nucleosome levels plateauing at 120(±1)ng/ml.

The low NET levels in the treated pig were consistent with the attenuation of septic shock as evidenced by reduced lactate (4.3 vs 9.1mmol/l), reduced total noradrenaline required (120ug vs 12,800ug) and increased urine output (875ml vs 340ml).

Conclusions

In this small initial study, therapeutic plasmapheresis effectively removed NETs from the circulation in a pig model of sepsis, resulting in improved physiological and biochemical well-being indicators of the pig. We aim to progress the investigation to further animal models and are recruiting into our first human trial ([Link to Study](#)). Immunoassay of nucleosomes is a simple, low cost and effective measure of NETs that may be used to monitor treatment and to select subjects for treatment.

Overlooked Danger of Neutrophil Extracellular Traps

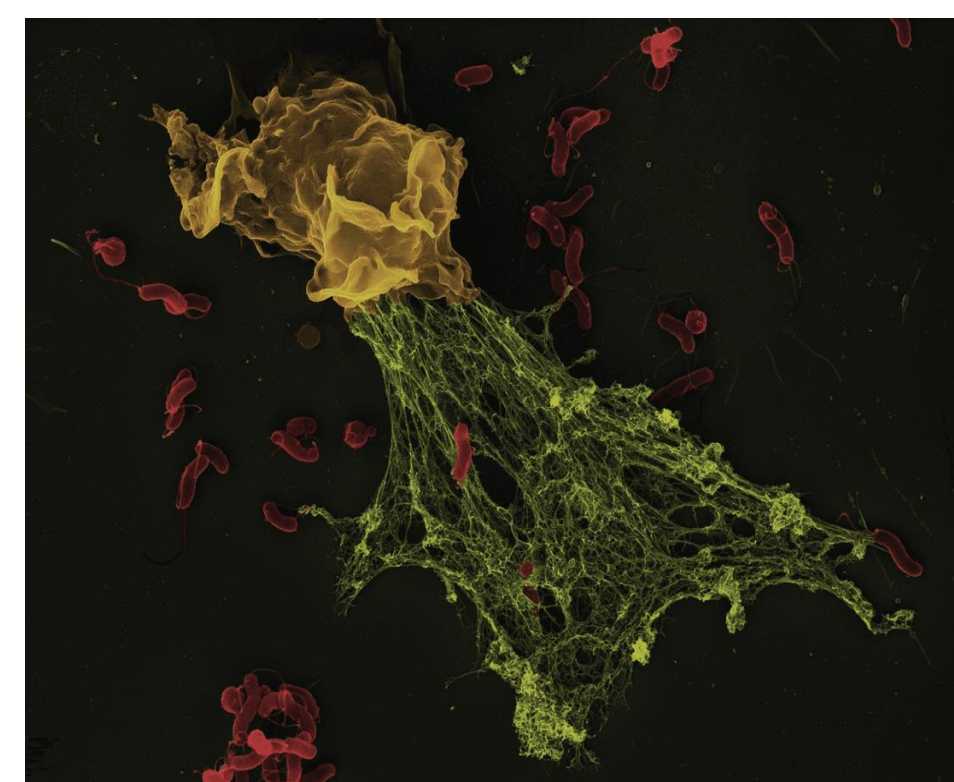
NucleoCapture has the potential to transform the treatment paradigm of unmet medical diseases

Neutrophils make up the largest fraction of blood cells produced by the bone marrow with a 100 billion cells produced daily. This amount might triple in certain conditions including infection, inflammation, autoimmunity and cancer.

NETosis is a unique form of neutrophil cell death that is characterized by the release of Neutrophil Extracellular Traps (NETs) composed of DNA web-like structures decorated with highly cytotoxic protein components.

Recent studies have pointed to the massive release of NETs into the bloodstream as playing a pivotal role in the progression of certain acute, chronic debilitating and fatal conditions, costing healthcare systems around the world billions a year:

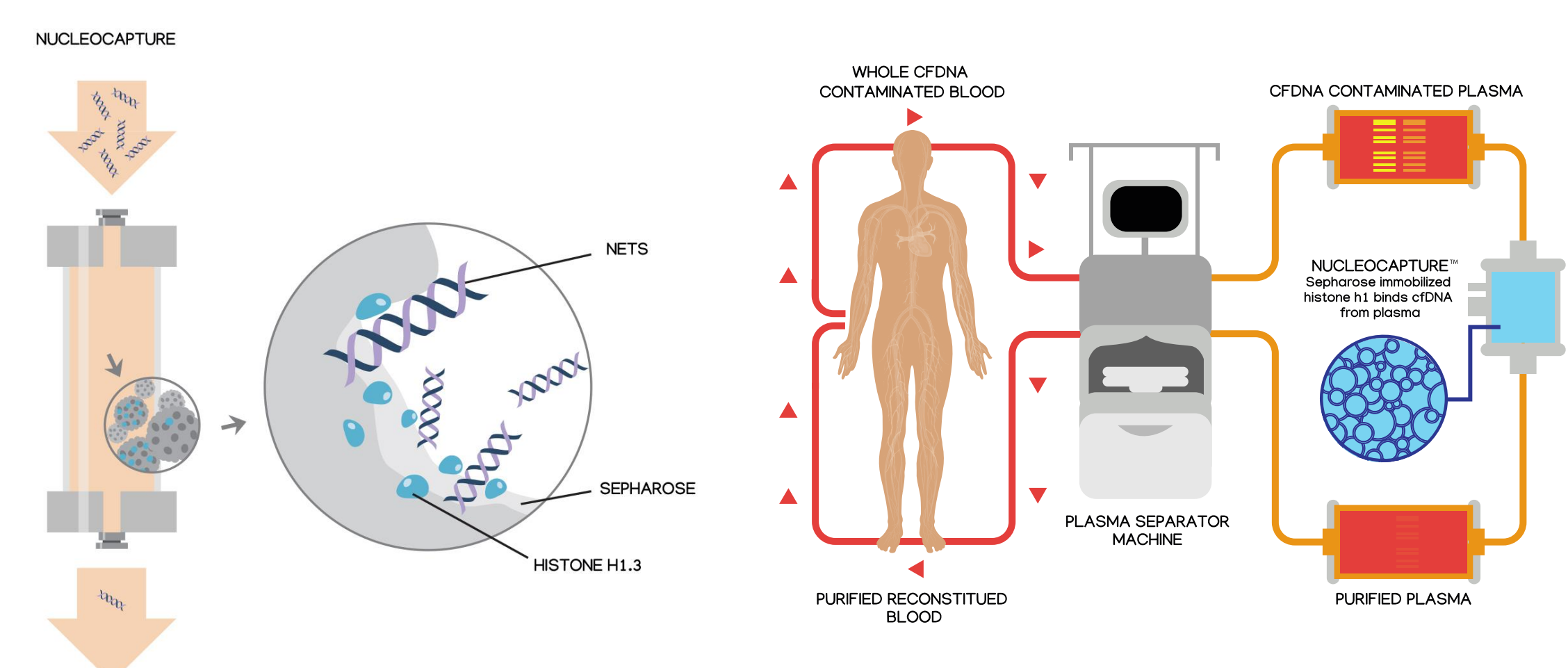
- Sepsis [42M cases, 11M deaths]
- Severe trauma [40M cases, 8M deaths]
- Metastatic Cancer [20M cases, 10M deaths]
- Alzheimer's Disease [44M cases, 2.4M deaths]



Neutrophil releasing NETs
Why Immune Cells Extrude Webs of DNA and Protein, *The Scientist*, Oct 1, 2019

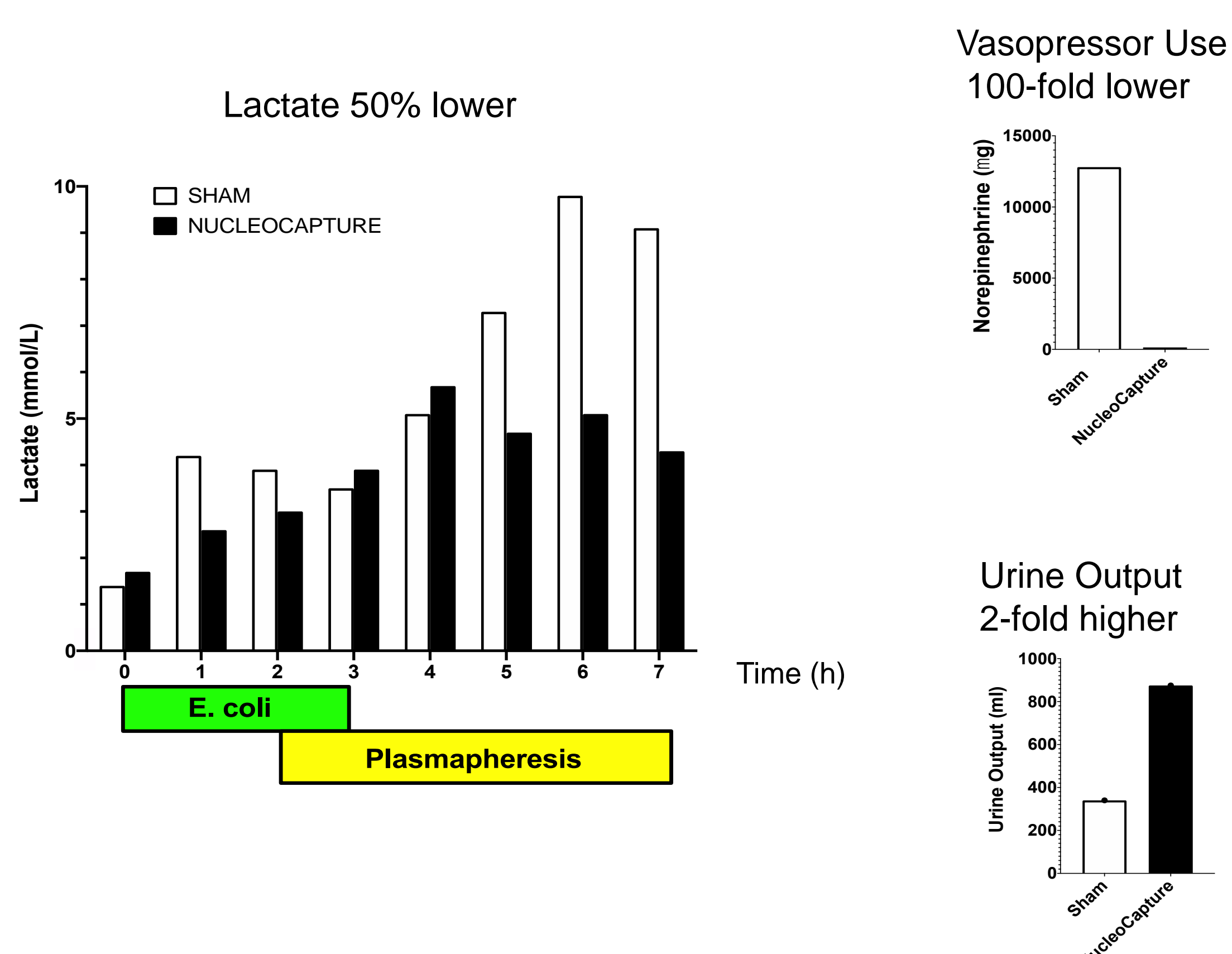
NucleoCapture: Novel first-in-class NET depletion Utilising Linker Histone H1.3 binding

SELECTIVE CAPTURE OF NETS WITH NUCLEOCAPTURE

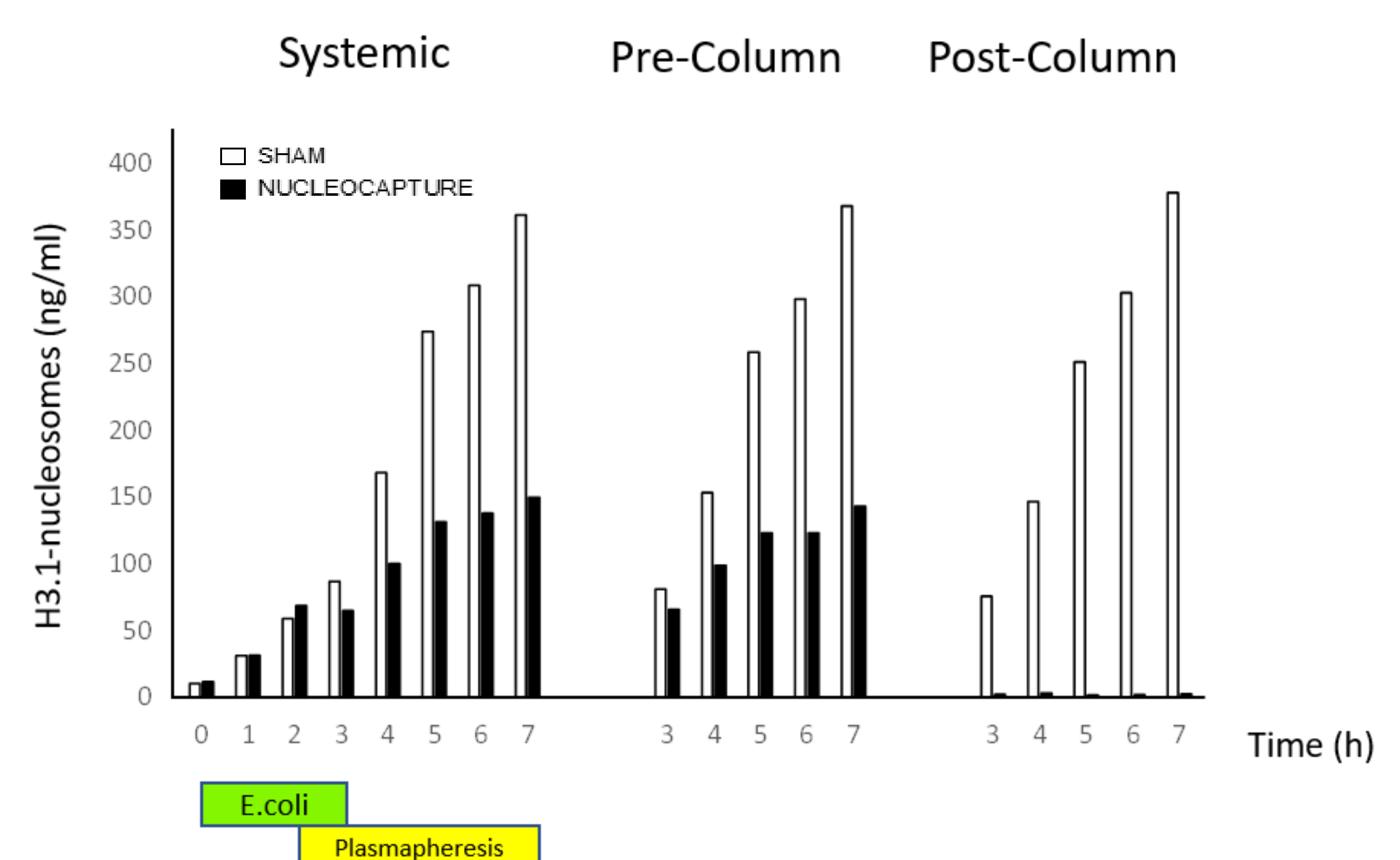


- In contrast to any potential NET-focused pharmacological interventions NucleoCapture allows the safe removal of excess NETs from blood without compromising the defensive functions of neutrophils
- NucleoCapture was evaluated in a clinically relevant porcine critical care model of sepsis

Shock Resolution With NucleoCapture



Marked NET Clearance with NucleoCapture: Up to 99% removed from Plasma



Immunoassay of cell free nucleosomes using the Volition Nu.Q®-H3.1 assay was found to be the best proxy measurement for monitoring levels of circulating NETs