Place du dosage des NETs dans la prise en charge des maladies critiques dont le sepsis

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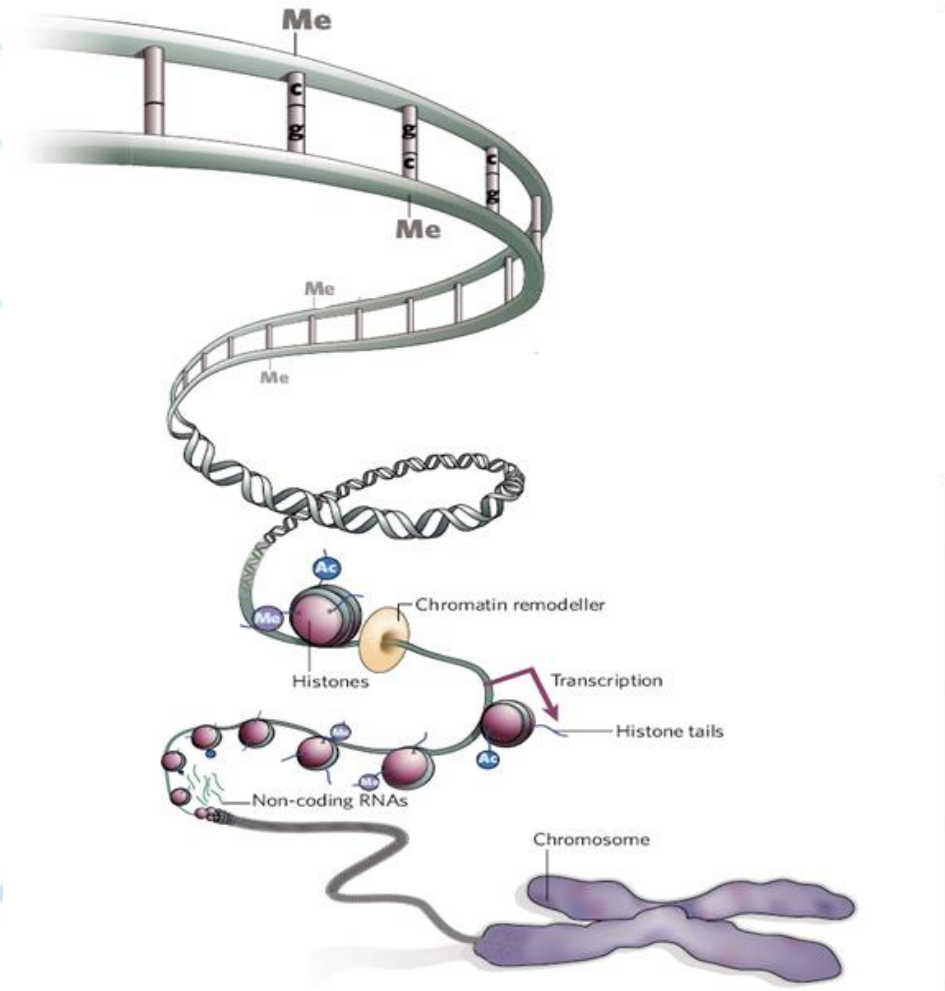
NETs - Définition

- **Signification ?**
  - Neutrophil Extracellular Traps.
- **Rôles?**
  - *Capture et destruction des agents pathogènes*
  - *Inflammation*
  - *Thrombose*
- **But?**

Nucléosome- Définition



- Les nucléosomes sont les unités fondamentales de la chromatine, composées d'un segment d'ADN enroulé autour d'un noyau d'histones (protéines).
- Les nucléosomes jouent un rôle crucial dans la compaction de l'ADN dans le noyau des cellules et dans la régulation de l'expression génique.

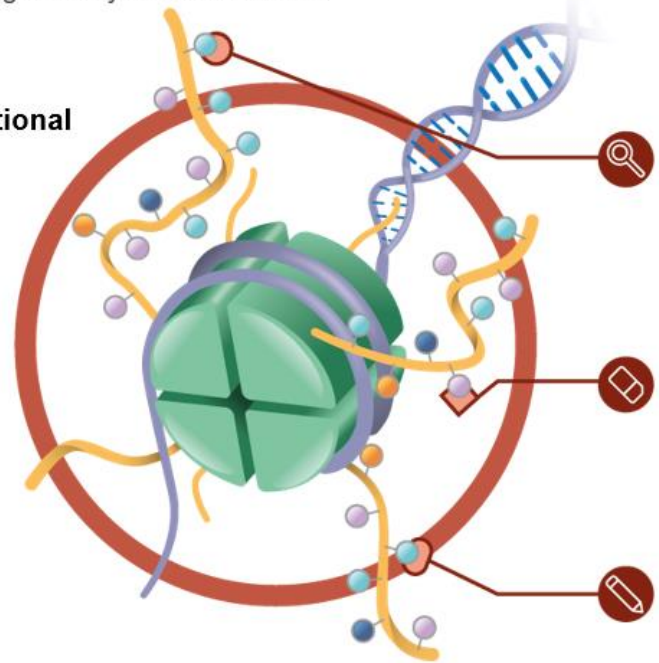
Nucléosome

Nucleosomes Contain A Variety of Modifications Reflecting Functional Activity & Drug Targets

Measuring and monitoring nucleosome levels and modifications in blood has the potential to aid diagnosis, prognosis and monitoring of many human diseases

Types of Post-Translational Modification (PTM)

- Me Methylation
- Ac Acetylation
- P Phosphorylation
- C Citrullination



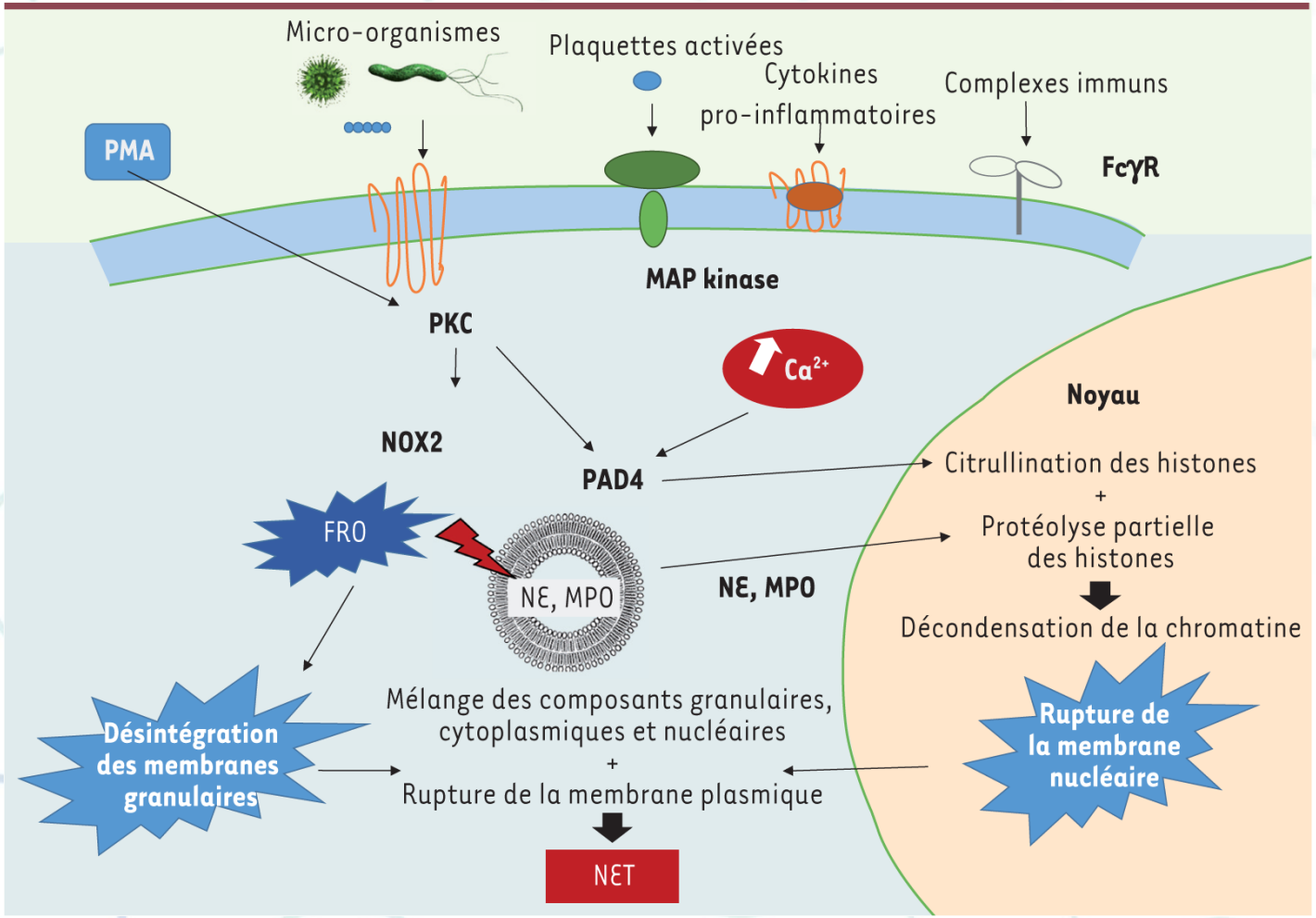
Writer
An enzyme that adds PTMs
Targets
EZH2, DNMT, DOT1L, PRMT

Eraser
An enzyme that removes PTMs
Targets
LSD1, HDAC, TET

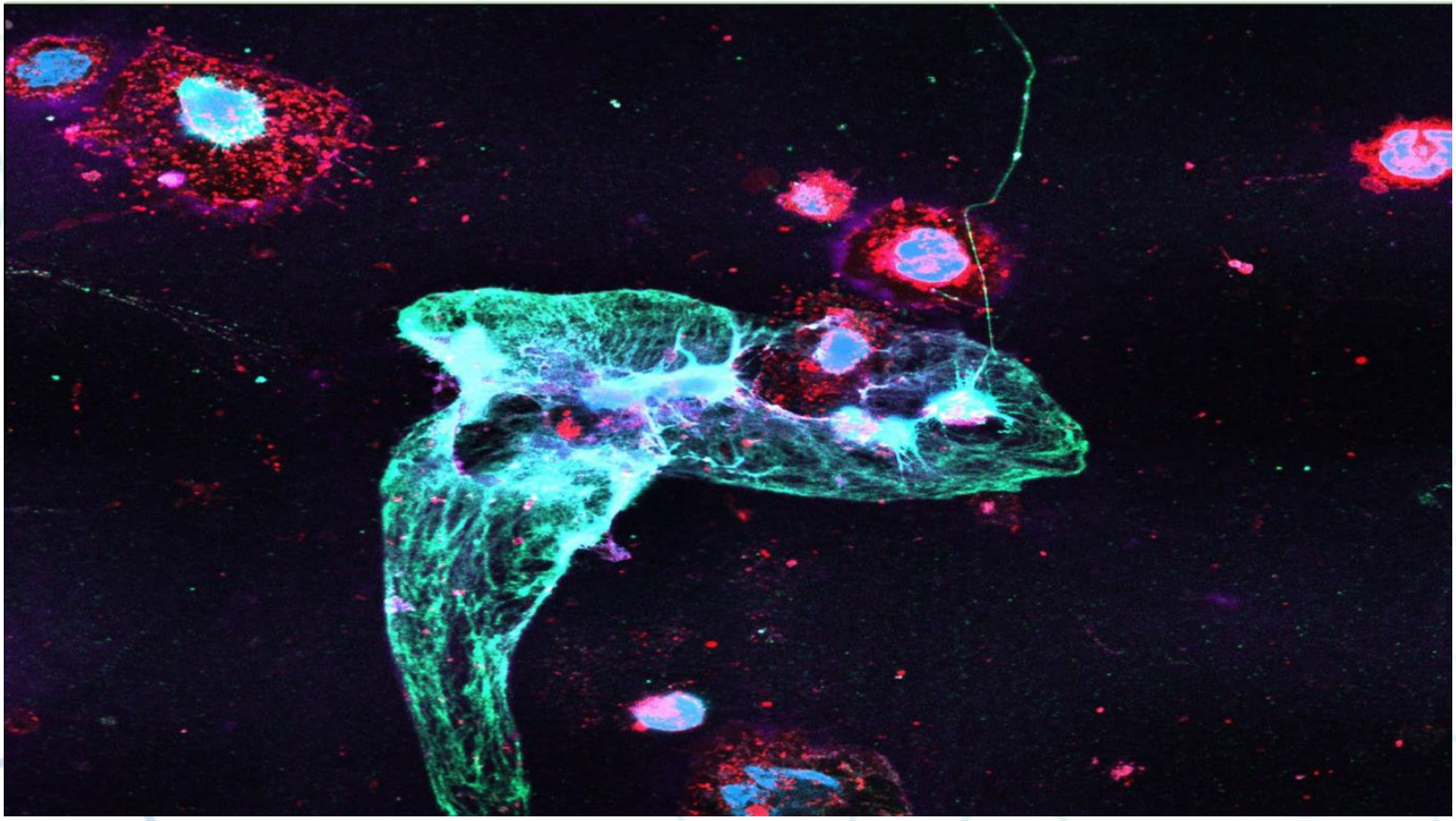
Reader
Proteins that recognize PTMs
Targets
BET

References: Li X and Li XD. Integrative Chemical Biology Approaches to Deciphering the Histone Code: A Problem-Driven Journey. Acc Chem Res 2021 54(19), 3734-3747; Regnier FE, Kim J. Proteins and Proteoforms: New Separation Challenges. Anal Chem 2018 Jan 2;90(1):361-373

Qu'est ce que la NETose?



Qu'est ce que la NETose?

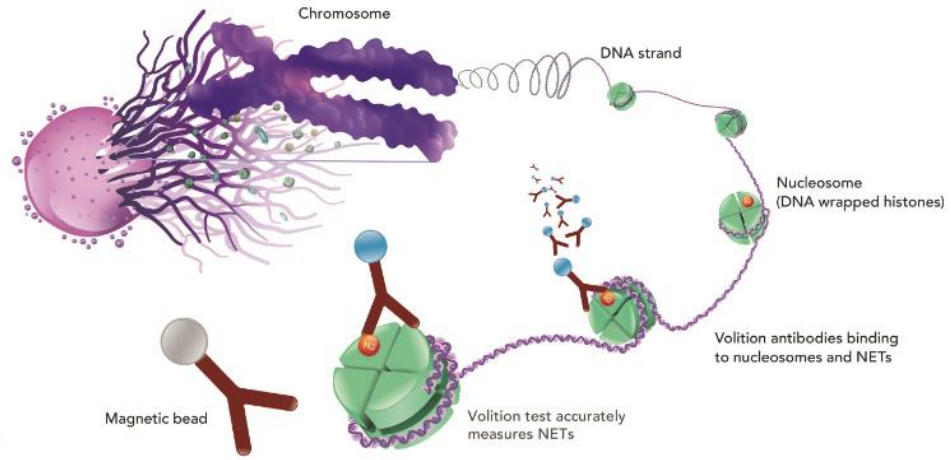


1. Neutrophil releasing NETs
Why Immune Cells Extrude Webs of DNA and Protein, The Scientist , Oct 1, 2019

Comment les doser?

Nu.Q® technology (across species)

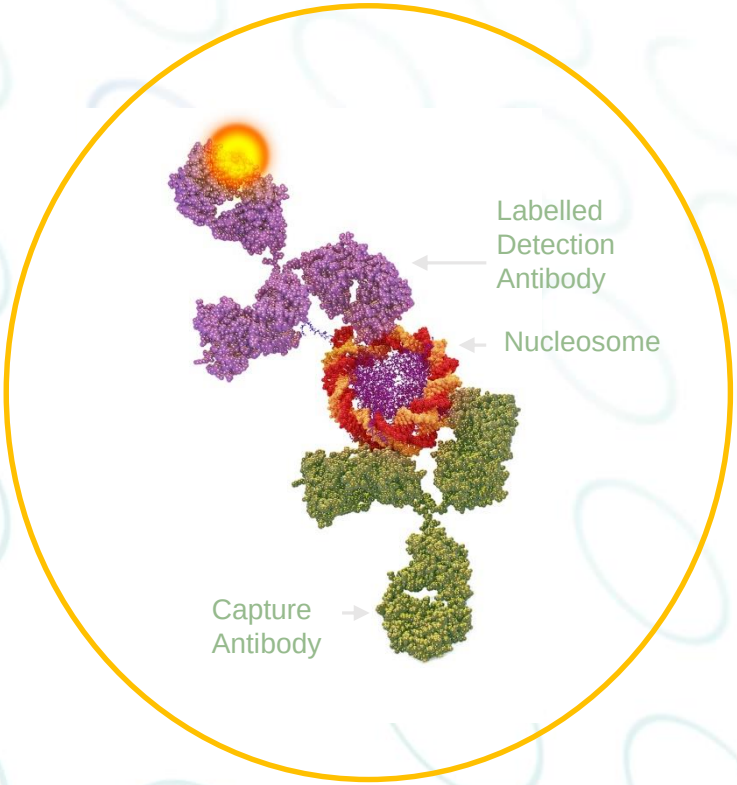
- Plate-forme d'immunodosage épigénétique
- Détermine les niveaux de nucléosomes circulants.
- Profils des épitopes des nucléosomes.
    - Modifications post-traductionnelles des histones.
    - Variants d'histones.
    - Modifications de l’ADN.
- La flexibilité de la plateforme et la diversité des modifications permettent de développer des panels spécifiques à certaines maladies.
- Facile à utiliser :
    - Plasma
    - Faible volume



Nu.Q® technology (across species)

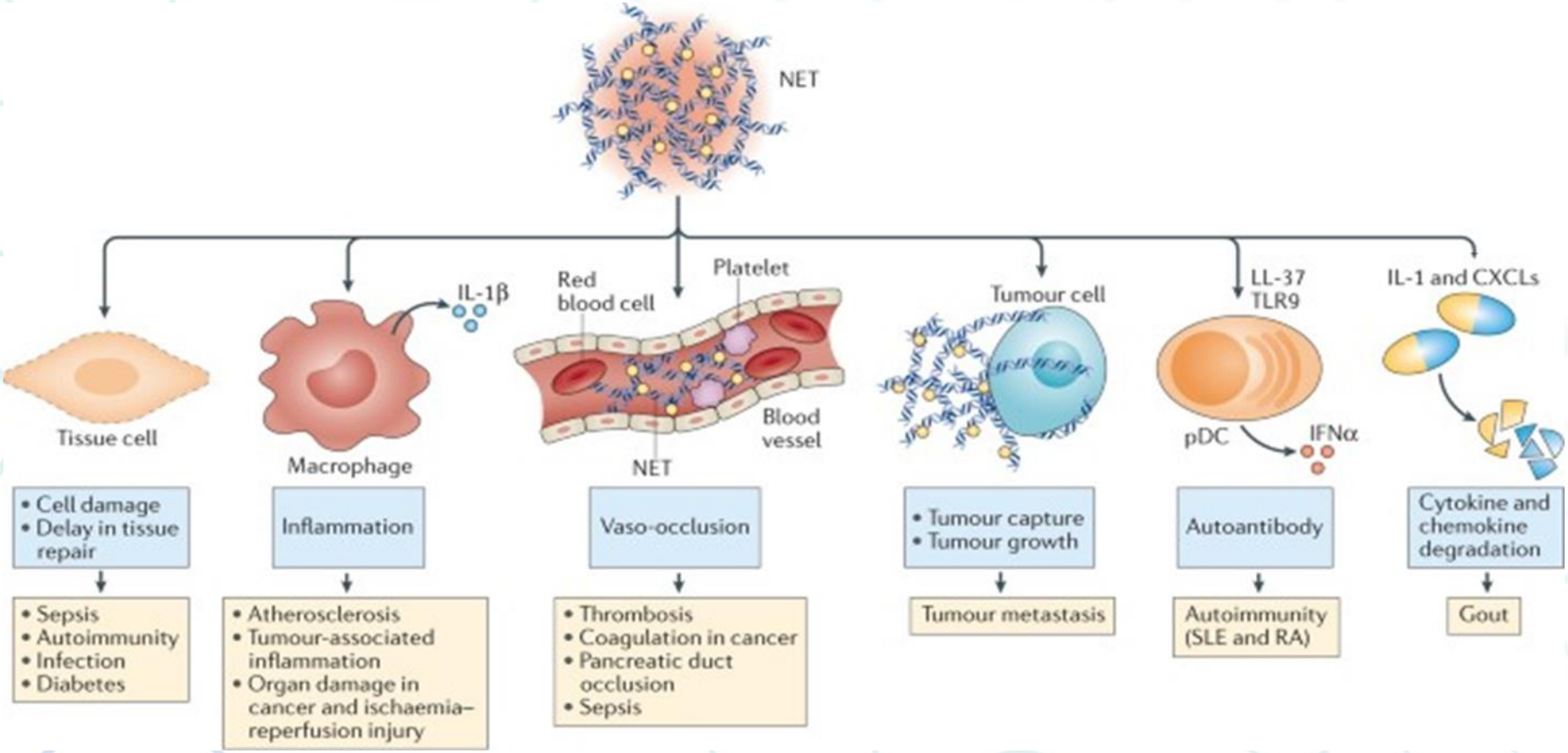
- ELISA plate format (colorimetric assay)

- Automated Magnetic beads format (chemiluminescence assay)

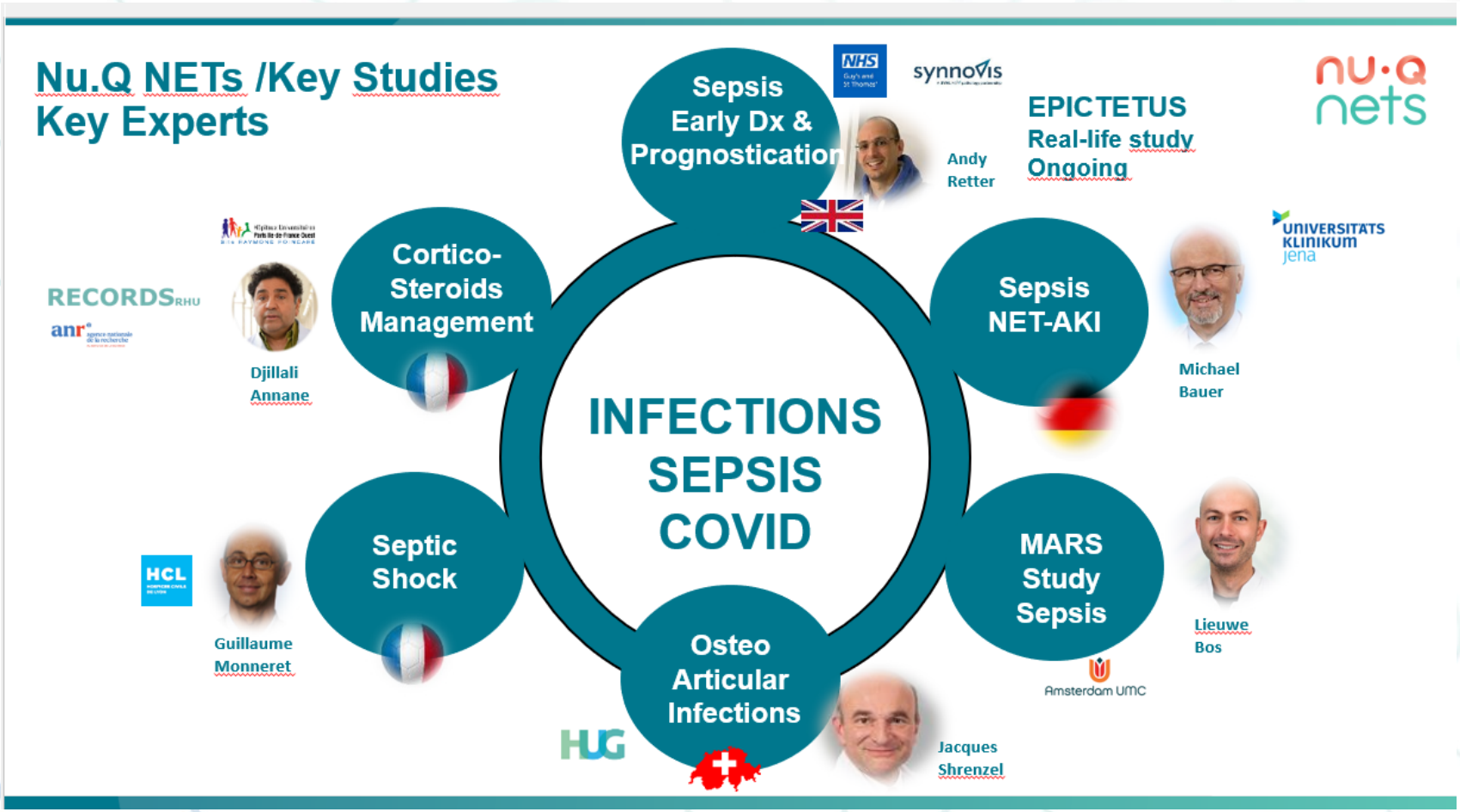


Quelles sont les applications?

NETs Clinical Use

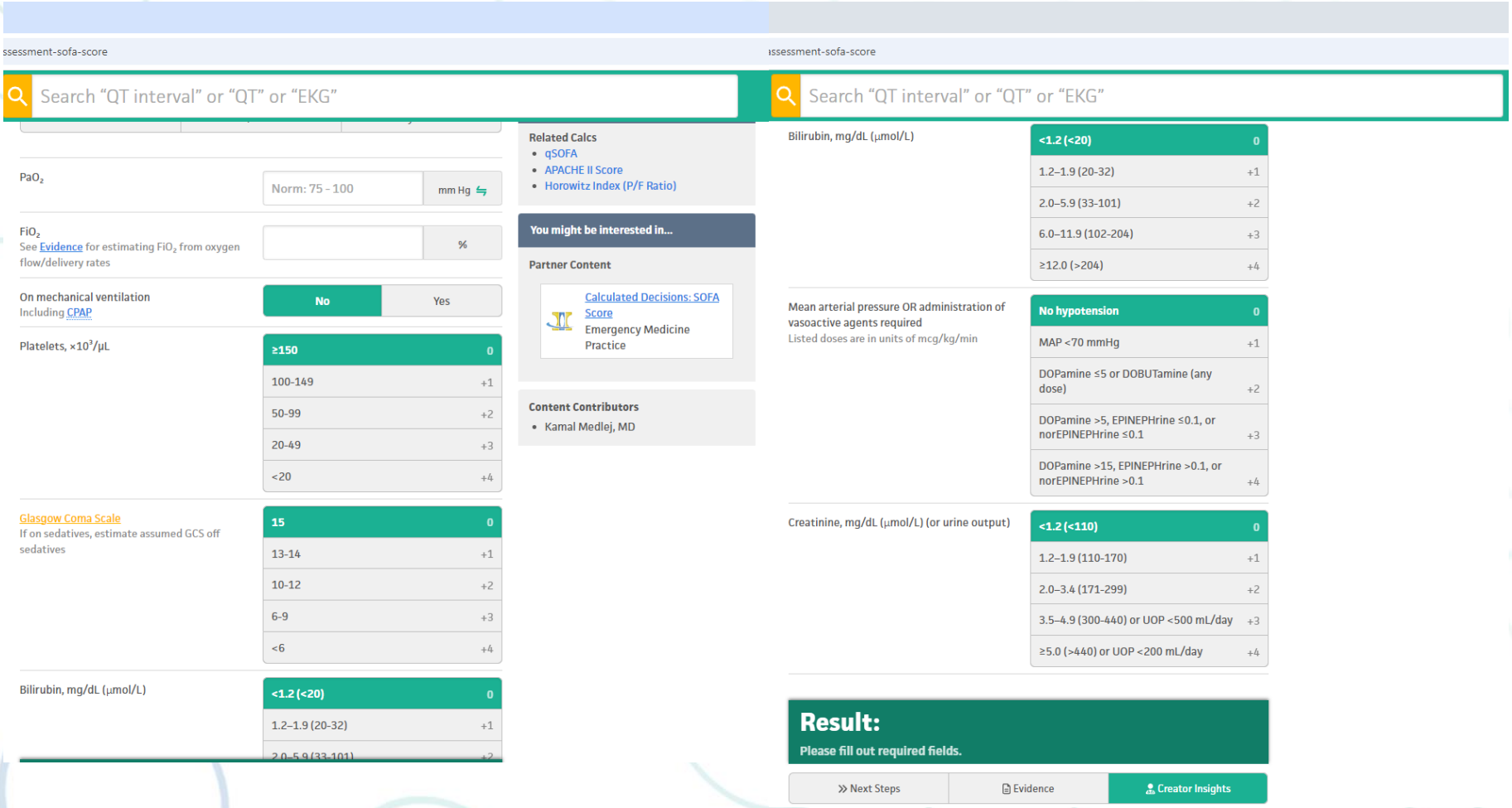


Applications cliniques



Applications cliniques
Littérature

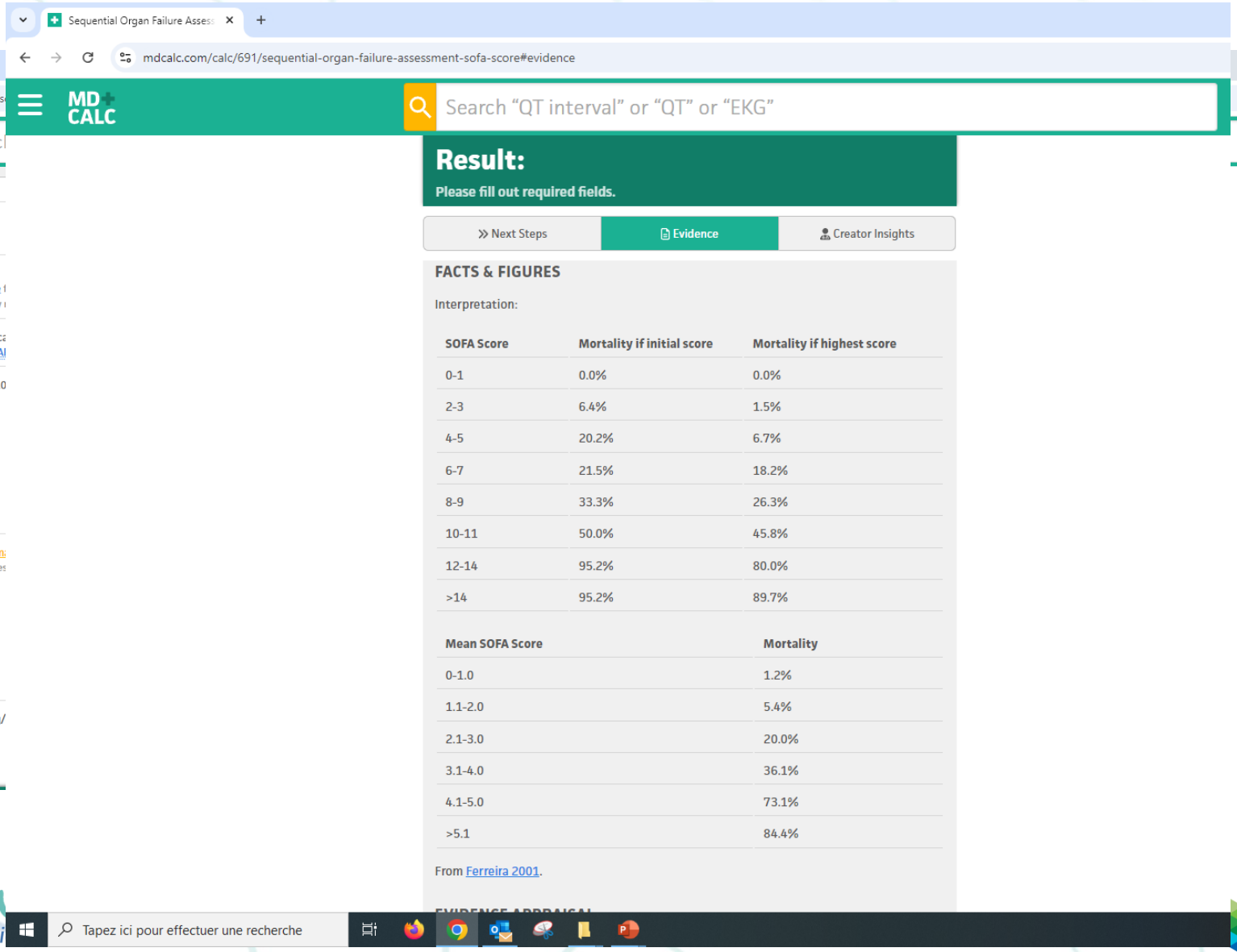
SOFA Score



https://www.mdcalc.com/calc/691/sequential-organ-failure-assessment-sofa-score

Sepsis –related Organ Failure Assessment

SOFA Score


Sequential Organ Failure Asses
mdcalc.com/calc/691/sequential-organ-failure-assessment-sofa-score#evidence
MD+ CALC
Search "QT interval" or "QT" or "EKG"
Result:
Please fill out required fields.
Next Steps
Evidence
Creator Insights
FACTS & FIGURES
Interpretation:
SOFA Score | Mortality if initial score | Mortality if highest score
0-1 | 0.0% | 0.0%
2-3 | 6.4% | 1.5%
4-5 | 20.2% | 6.7%
6-7 | 21.5% | 18.2%
8-9 | 33.3% | 26.3%
10-11 | 50.0% | 45.8%
12-14 | 95.2% | 80.0%
>14 | 95.2% | 89.7%
Mean SOFA Score | Mortality
0-1.0 | 1.2%
1.1-2.0 | 5.4%
2.1-3.0 | 20.0%
3.1-4.0 | 36.1%
4.1-5.0 | 73.1%
>5.1 | 84.4%
From Ferreira 2001.
Tapez ici pour effectuer une recherche

Circulating Nucleosomes as Potential Markers to Monitor COVID-19 Disease Progression

Etienne Cavalier[1†], Julien Guiot[2†], Katharina Lechner[3,4], Alexander Dutsch[3,4], Mark Eccleston[5], Marielle Herzog[5], Thomas Bygott[5], Adrian Schomburg[6,7,8], Theresa Kelly[9†] and Stefan Holdenrieder[10*†]

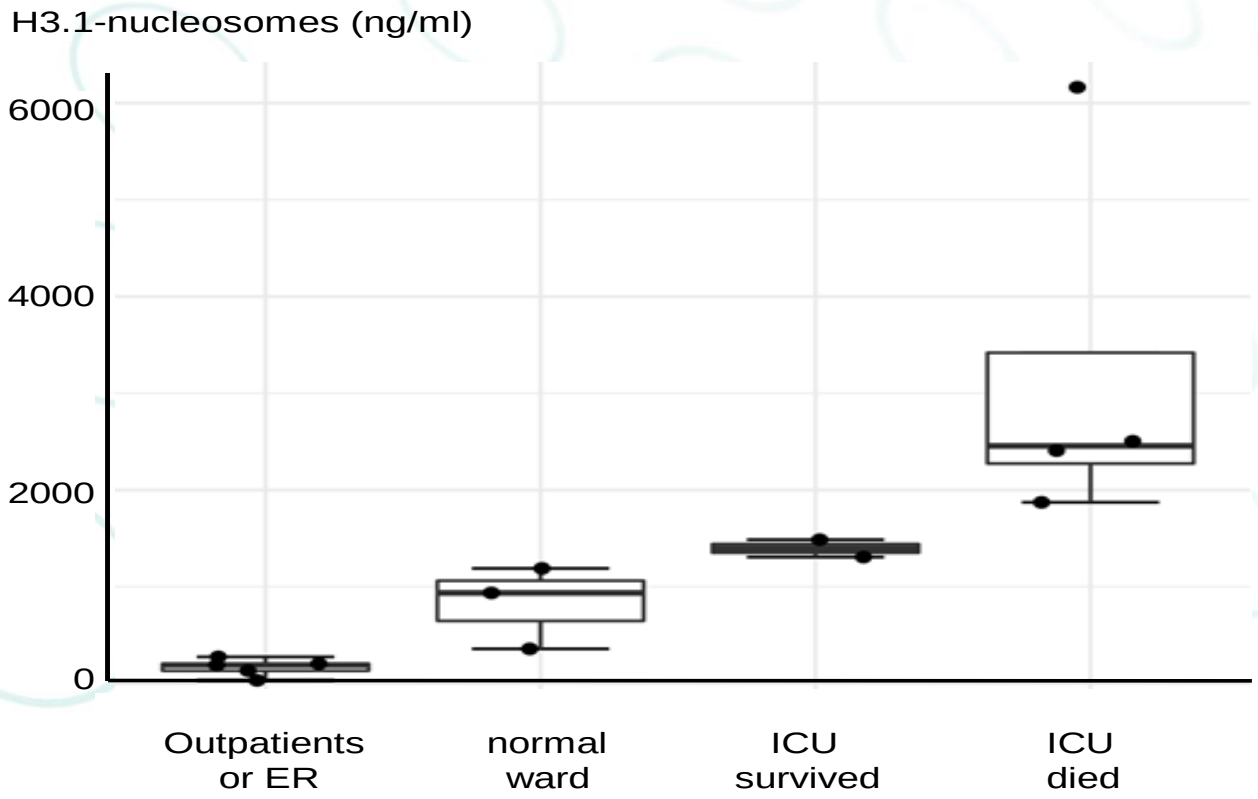
[1] Clinical Chemistry Department, Centre Hospitalier Universitaire de Liege, Domaine Universitaire Du Sart-Tilman, Liège, Belgium, [2] Pneumology Department, Centre Hospitalier Universitaire de Liege, Domaine Universitaire Du Sart-Tilman, Liège, Belgium, [3] Department of Cardiology, German Heart Centre Munich, Technical University Munich, Munich, Germany, [4] DZHK (German Centre for Cardiovascular Research), Partner Site Munich, Munich Heart Alliance,Munich, Germany, [5] Belgian Volition SRL, Isnes, Belgium, [6] Eisbach Bio GmbH, Munich, Germany, [7] Department of Physiological Chemistry, Biomedical Center (BMC), Faculty of Medicine, LMU Munich, Munich, Germany, [8] Volition Germany GmbH, Gräfelfing, Germany, [9] Volition America, Austin, TX, United States, [10] Institute for Laboratory Medicine, German Heart Centre, Technical University Munich, Munich, Germany

ABSTRACT:

The severity of coronavirus disease 2019 (COVID-19) varies significantly with cases spanning from asymptomatic to lethal with a subset of individuals developing Severe Acute Respiratory Syndrome (SARS) and death from respiratory failure. To determine whether global nucleosome and citrullinated nucleosome levels were elevated in COVID- 19 patients, we tested two independent cohorts of COVID-19 positive patients with quantitative nucleosome immunoassays and found that nucleosomes were highly elevated in plasma of COVID-19 patients with a severe course of the disease relative to healthy controls and that both histone 3.1 variant and citrullinated nucleosomes increase with disease severity. Elevated citrullination of circulating nucleosomes is indicative of neutrophil extracellular trap formation, neutrophil activation and NETosis in severely affected individuals. Importantly, using hospital setting (outpatient, inpatient or ICU) as a proxy for disease severity, nucleosome levels increased with disease severity and may serve as a guiding biomarker for treatment. Owing to the limited availability of mechanical ventilators and extracorporal membrane oxygenation (ECMO) equipment, there is an urgent need for effective tools to rapidly assess disease severity and guide treatment selection. Based on our studies of two independent cohorts of COVID-19 patients from Belgium and Germany, we suggest further investigation of circulating nucleosomes and citrullination as biomarkers for clinical triage, treatment allocation and clinical drug discovery.

Front. Mol. Biosci., 18 March 2021

Nu.Q® NETs levels are elevated in COVID-19 and are correlated with disease severity



Cavalier et al (2021)
Circulating Nucleosomes as Potential Markers to Monitor COVID-19 Disease Progression.
Front. Mol. Biosci. 8:600881. doi: 10.3389/fmolb.2021.600881

AIC Paper: Nu.Q® NETs associated with early mortality and 28-day morbidity

Haem Rahimi et al. Annals of Intensive Care (2023) 13:102
https://doi.org/10.1186/s13613-023-01204-y

Annals of Intensive Care

RESEARCH Open Access

Association of pronounced elevation of NET formation and nucleosome biomarkers with mortality in patients with septic shock

Muzhda Haem Rahimi[1,2], Frank Bidar[3], Anne-Claire Lukaszewicz[2,3], Lorna Garnier[4], Léa Payen-Gay[5], Fabienne Venet[1,6] and Guillaume Monneret[1,2*]

Abstract

Background Understanding the mechanisms underlying immune dysregulation in sepsis is a major challenge in developing more individualized therapy, as early and persistent inflammation, as well as immunosuppression, play a significant role in pathophysiology. As part of the antimicrobial response, neutrophils can release extracellular traps (NETs) which neutralize and kill microorganisms. However, excessive NETs formation may also contribute to pathogenesis, tissue damage and organ dysfunction. Recently, a novel automated assay has been proposed for the routine measurement of nucleosomes H3.1 (fundamental units of chromatin) that are released during NETs formation. The aim of the present study was to measure nucleosome levels in 151 septic shock patients (according to sepsis-3 definition) and to determine association with mortality.

Results The nucleosome H3.1 levels (as determined by a chemiluminescence immunoassay performed on an automated immunoanalyzer system) were markedly and significantly elevated at all-time points in septic shock patients compared to the control group. Immunological parameters indicated tremendous early inflammation (IL-6 = 1335 pg/mL at day 1–2) along with marked immunosuppression (e.g., mHLA-DR = 3853 AB/C and CD4 = 338 cell /µL at day 3–4). We found significantly positive correlation between nucleosome levels and organ failure and severity scores, IL-6 concentrations and neutrophil count. Significantly higher values (day 1–2 and 3–4) were measured in non-survivor patients (28-day mortality). This association was still significant after multivariate analysis and was more pronounced with highest concentration. Early (day 1–2) increased nucleosome levels were also independently associated with 5-day mortality. At day 6–8, persistent elevated nucleosome levels were negatively correlated to mHLA-DR values.

Conclusions This study reports a significant elevation of nucleosome in patients during a one-week follow-up. The nucleosome levels showed correlation with neutrophil count, IL-6 and were found to be independently associated with mortality assessed at day 5 or 28. Therefore, nucleosome concentration seems to be a promising biomarker for detecting hyper-inflammatory phenotype upon a patient's admission. Additional investigations are required to evaluate the potential association between sustained elevation of nucleosome and sepsis-induced immunosuppression.

Keywords Sepsis, Septic shock, Inflammation, NETs, NETosis, Nucleosome, Immunosuppression, mHLA-DR

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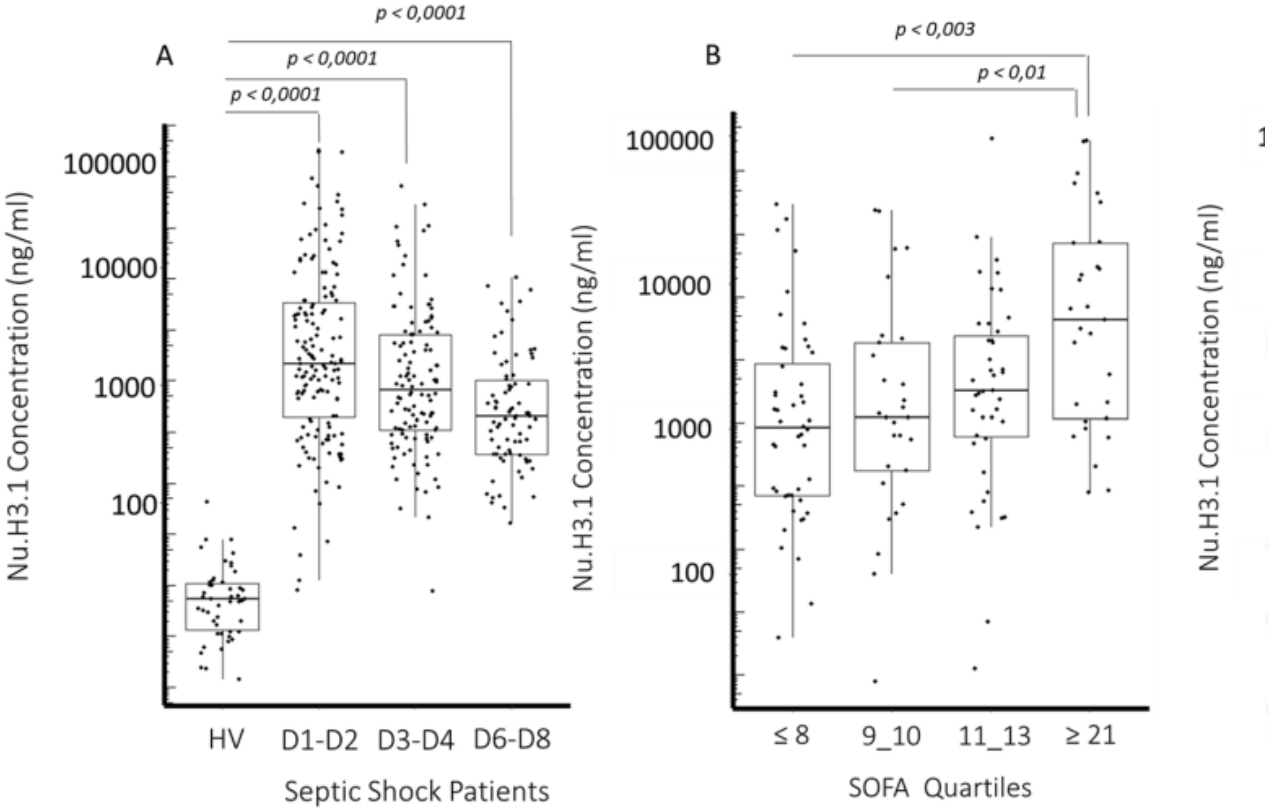
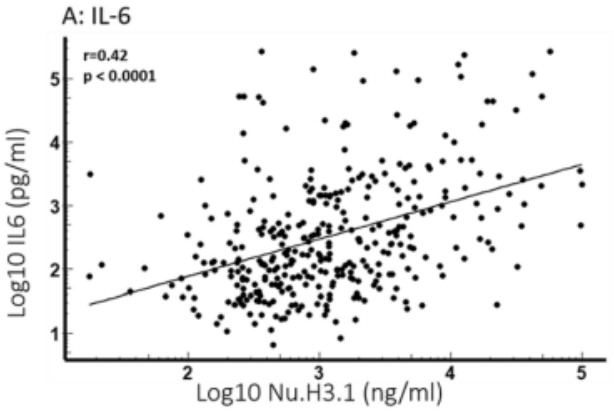
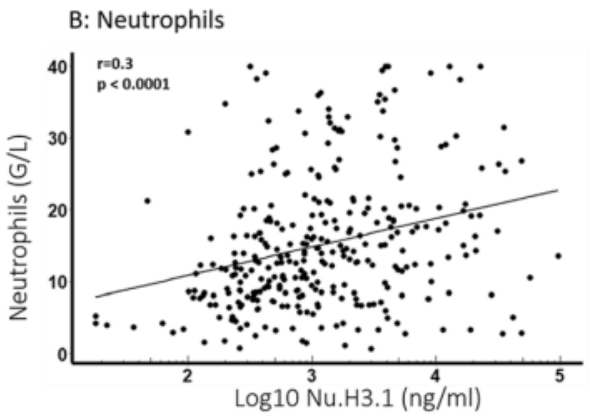


Ann Intensive Care 2023, Haem-Rahimi et al

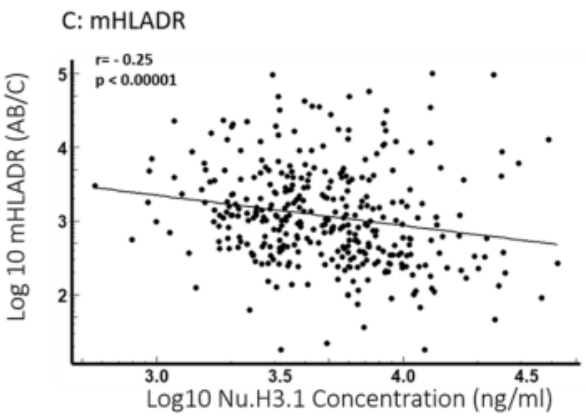
AIC Paper: Nu.Q® NETs associated with early mortality and 28-day morbidity



Time Point	r	p
D1-D2	0.35	< 0.0001
D3-D4	0.32	< 0.001
D6-D8	0.36	< 0.001



Time Point	r	p
D1-D2	0.1	ns
D3-D4	0.4	< 0.0001
D6-D8	0.5	< 0.0001



Time Point	r	p
D1-D2	- 0,005	0.94
D3-D4	- 0,4	< 0.0001
D6-D8	- 0,4	< 0.0001

Fig. 2 Correlation of nucleosome H3.1 levels with immunological parameters. **A** Correlation with IL-6 (345 samples). **B** Correlation with neutrophil count (314 samples). **C** Correlation with mHLA-DR (Spearman correlation test). Figures depict correlations including all samples plots. Below each figure (ABC), tables are presented, providing Spearman's correlation coefficient and p-value calculated at the different time points for the considered parameter

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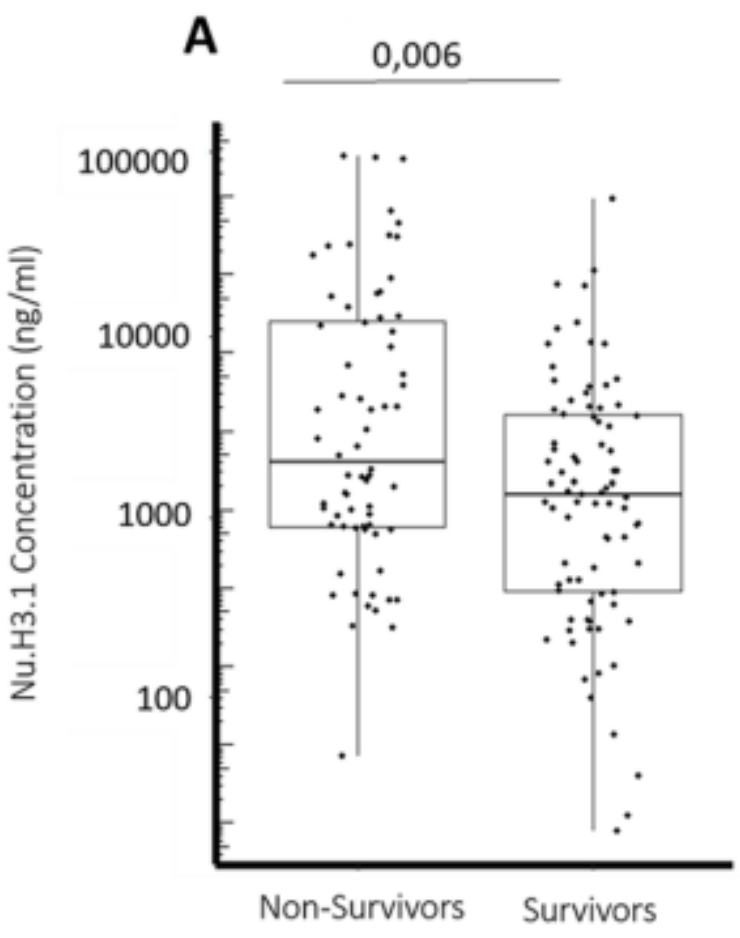
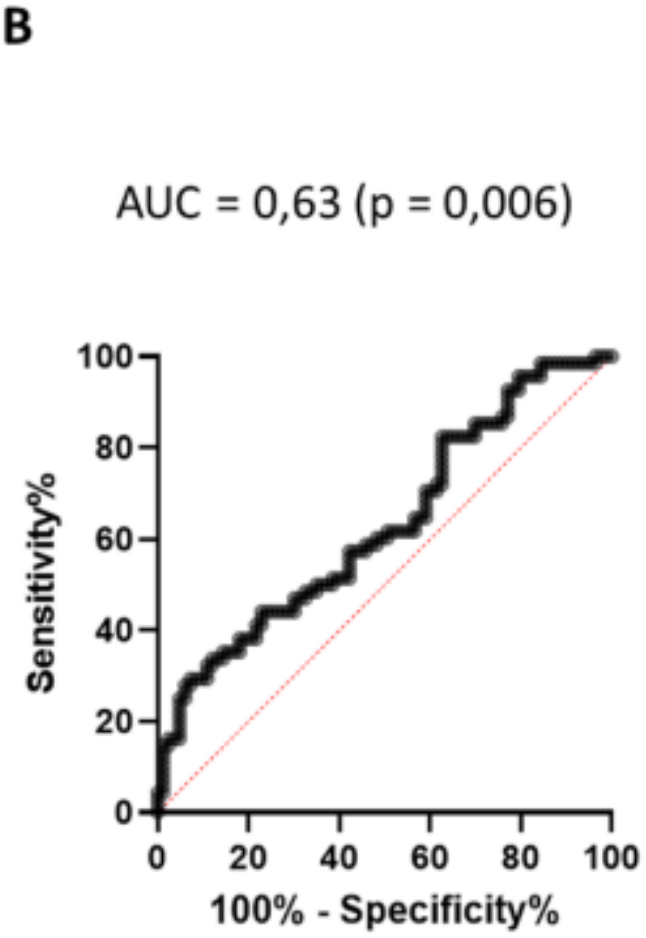
AIC Paper: Nu.Q® NETs associated with early mortality and 28-day morbidity





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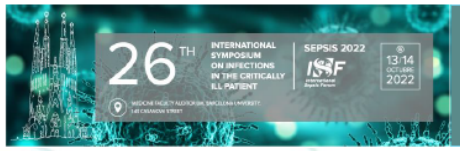
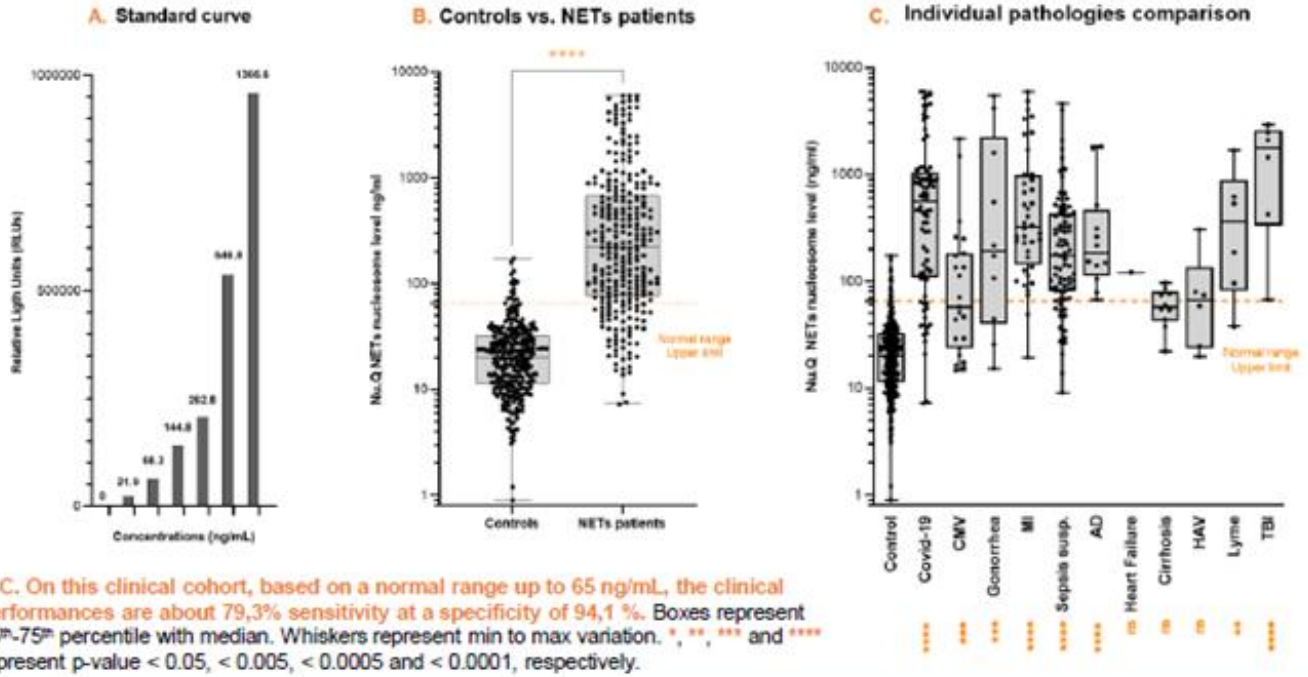


Detection and evaluation of diseases associated with NETosis in Human Plasma using Nu.Q® NETs ChemiLuminescence Immunofluorescent Assay.

Julie Candiracci[1], Guillaume Rommelaere[1], Robin Varsebroucq[1], Florian Jibassia[1], Olivia Thiry[1], Emilie Bauwens[1], Léa Payen[2], Marielle Herzog[1]
1. Belgian Volition SRL, Isnes, Belgium
2. Laboratoire de Biochimie et Biologie Moléculaire, Groupe Hospitalier Sud, Hospices Civils de Lyon, Pierre-Bénite, France

Assessment of circulating nucleosome levels in the control population, compared to the population of patients with NET-related pathologies. A. Standard curve of the Nu.Q® NETs kit. **B.** Significantly elevated levels of circulating nucleosomes were found in patients with NETs related diseases compared to control population (mean 732,2 ng/mL vs 26,1 ng/mL, p <0.0001). **C.** All pathologies were individually compared to controls.



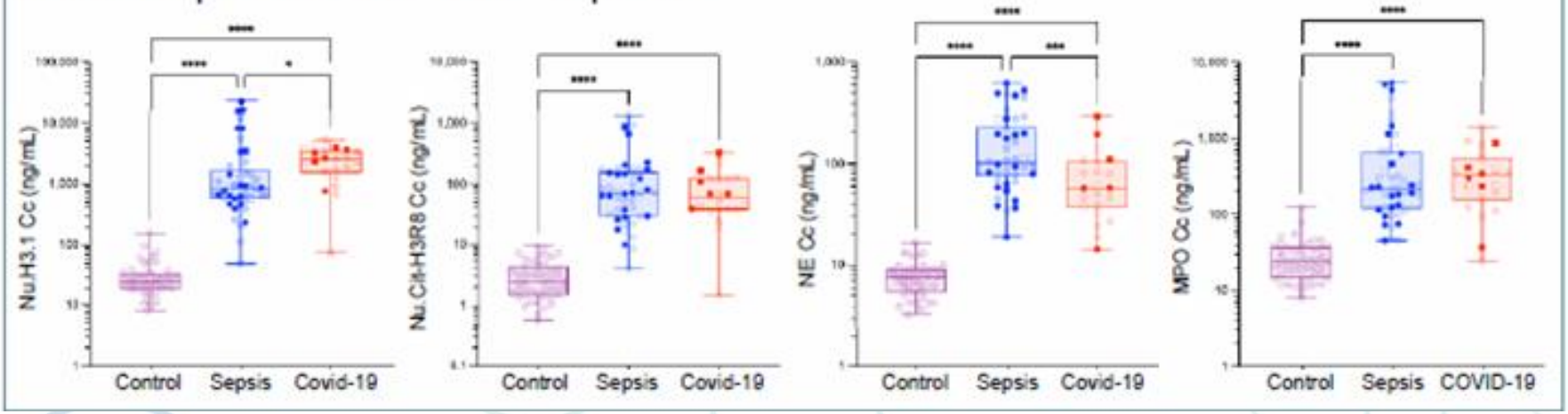
B-C. On this clinical cohort, based on a normal range up to 65 ng/mL, the clinical performances are about 79,3% sensitivity at a specificity of 94,1 %. Boxes represent 25th-75th percentile with median. Whiskers represent min to max variation. *, **, *** and **** represent p-value < 0.05, < 0.005, < 0.0005 and < 0.0001, respectively.

Circulating nucleosomes are markers of NETosis and correlate with SOFA scores in sepsis

Marielle Herzog[5], Laure Morimont[1,2], Mélanie Dechamps[3,4], Clara David[1], Céline Bouvy[1], Constant Gillot[2], Hélène Haguet[2], Julien Favresse[2], Lorian Ronvaux [5], Julie Candiracci[5], Pierre-François Laterre[3,4], Julien de Poortere[4], Sandrine Horman[4], Christophe Beauloye[4,6], and Jonathan Douxfils[1,2]

1.Qualiblood s.a., Research and Development Department, Namur, Belgium, 2.Department of Pharmacy, Namur Thrombosis and Hemostasis Center, Namur Research Institute for Life Sciences, University of Namur, Belgium, 3.Cardiovascular Intensive Care, Cliniques Universitaires St Luc, Brussels, Belgium, 4.Pôle de Recherche Cardiovasculaire, Institut de recherche expérimentale et clinique (IREC), Université Catholique de Louvain, Brussels, Belgium, 5.Belgian Volition SRL, Parc Scientifique Crealys, Isnes, Belgium, 6.Division of Cardiology, Cliniques Universitaires St Luc, Brussels, Belgium

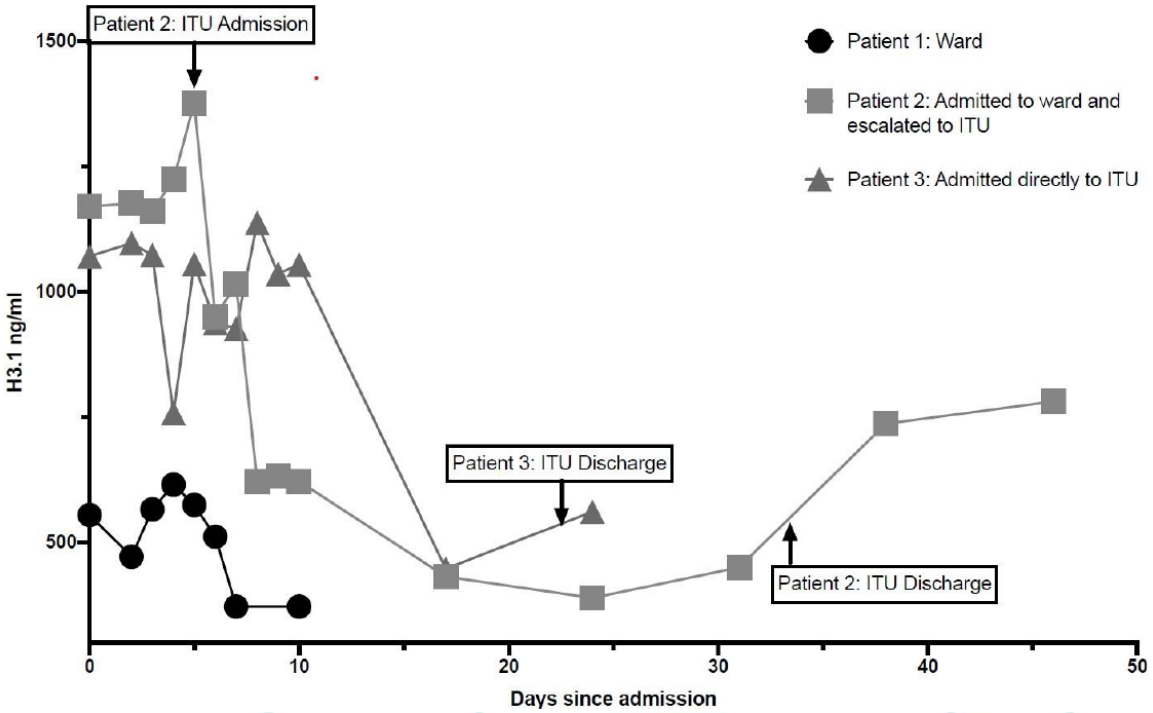
Nu.Q®H3.1, Nu.Q® Cit-H3R8, NE and MPO were compared. All markers were statistically different in septic shock and critical COVID-19 compared to controls. Only Nu.Q®H3.1 and NE were different between septic shock and COVID-19 patients.



Identifying tools to track hypercoagulability in COVID-19 patients: Exploring global haemostasis (ROTEM) and neutrophil extracellular traps (NETs) immunoassays

S.N. STANFORD[1], C. REA[4], A. ROY[1], B. HARRIS[2], T. ASHTON[2], S. MANGLES[3], T. EVERINGTON[3], K.CHANDRAKUMARAN[1], E. ARBUTHNOT[1], T.CECIL[1]

[1]Peritoneal Malignancy Institute, Hampshire Hospitals NHS Foundation Trust, Basingstoke, Hampshire, UK; [2]Anaesthetics and Critical Care, Hampshire Hospitals NHS Foundation Trust, Basingstoke, Hampshire, UK
[3]Haemophilia, Haemostasis and Thrombosis Centre, Hampshire Hospitals NHS Foundation Trust, Basingstoke, Hampshire, UK; [4]Kings College Hospital NHS Foundation Trust, London, UK



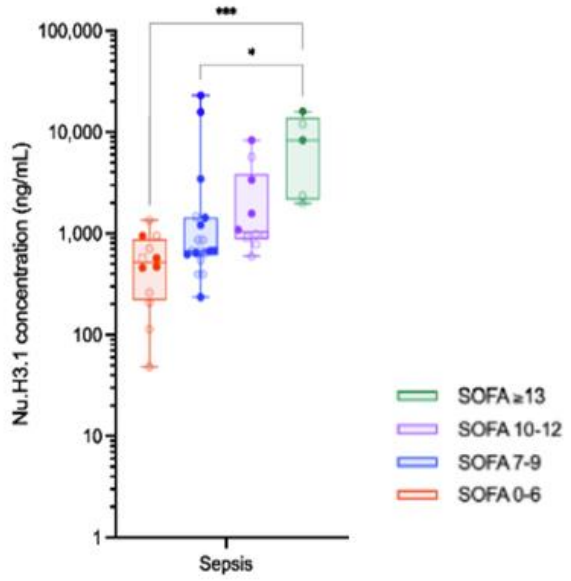
CONCLUSIONS

- COVID-19 patients demonstrated a hypercoagulable state compared to healthy controls as measured by ROTEM with increased MCF and hypofibrinolysis.
- Although this is a small, exploratory study, the H3.1 nucleosome findings suggest that it may be able to risk stratify on admission and track clinical course.

Corrélation avec le SOFA score

Nu.Q® NETs s levels correlate with SOFA Score in ICU patients with Sepsis

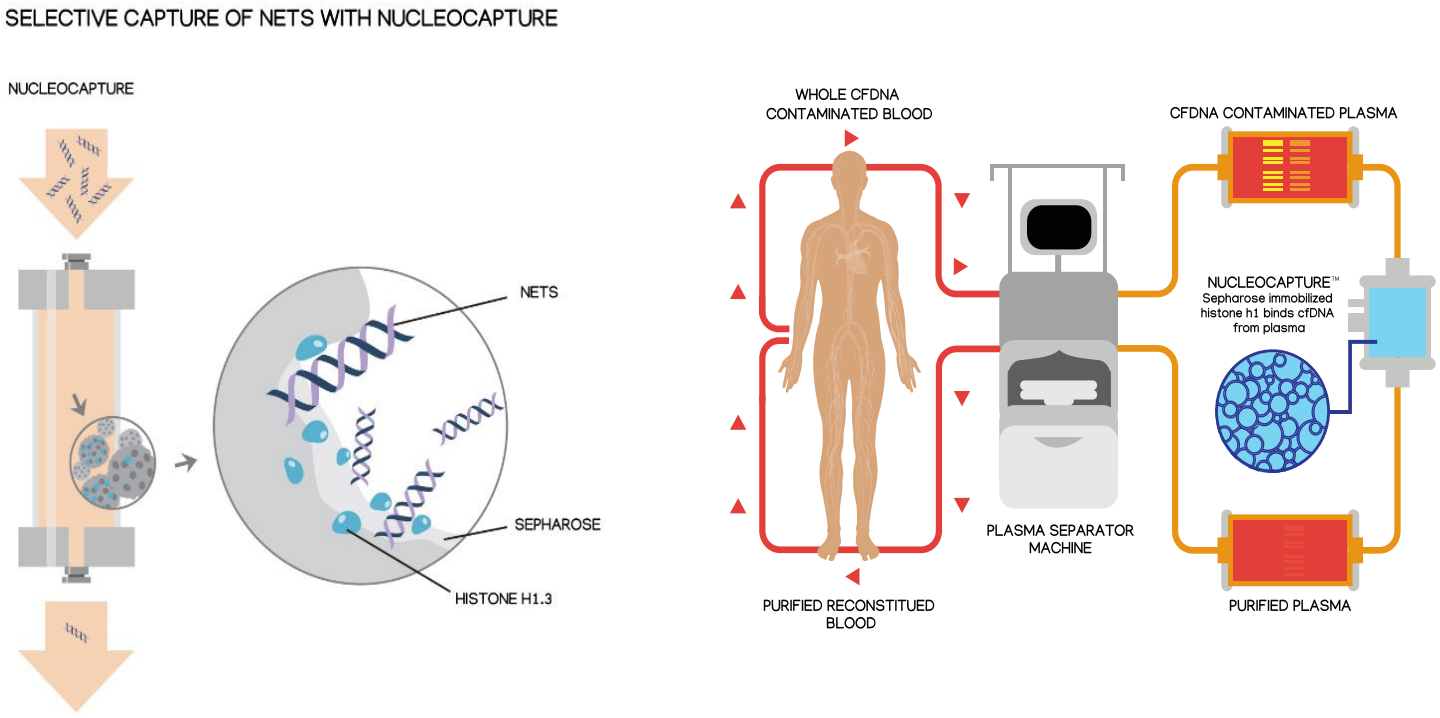
	APACHE-II 0–15	APACHE-II 16–25	APACHE-II 25–35	SOFA 0–6	SOFA 7–9	SOFA 10–12	SOFA ≥ 13
Nu.H3.1 (ng/mL)							
Septic shock	666.4 (133.7–1257.8)	670.0 (215.9–4898.9)	1575.3 (641.4–19,955.7)	517.9 (62.6–1213.9)	673.0 (396.9–15,775.5)	1032.8 (612.7–7993.8)	8285.6 (1980.4–16,068.7)



Morimont et al. NETosis and Nucleosome Biomarkers in Septic Shock and Critical COVID-19 Patients: An Observational Study. *Biomolecules* **2022**, *12*, 1038. https://doi.org/10.3390/biom12081038

NucleoCapture: nouvelle thérapie

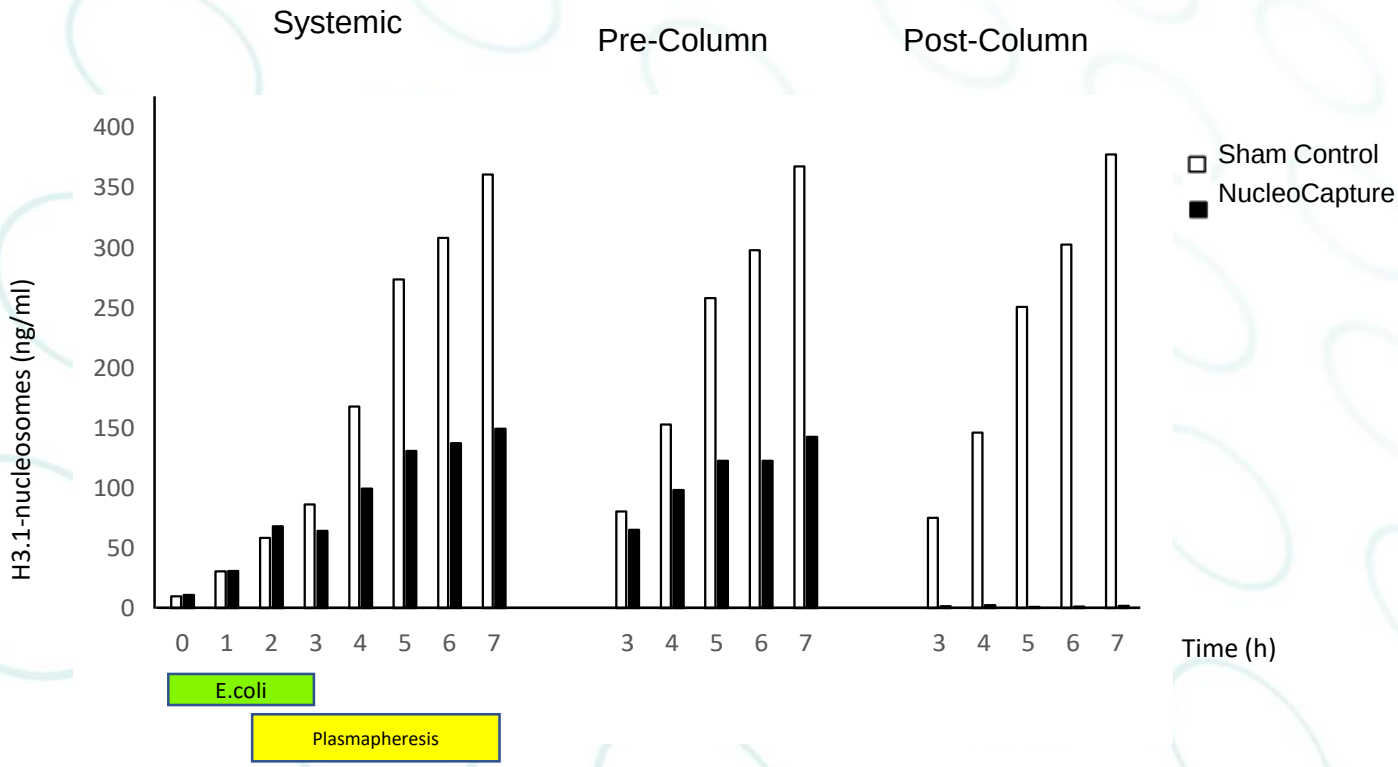
NucleoCapture: Novel first-in-class NET depletion
Utilising Linker Histone H1.3 binding



- 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

NucleoCapture:
Up to 99% removed from Plasma



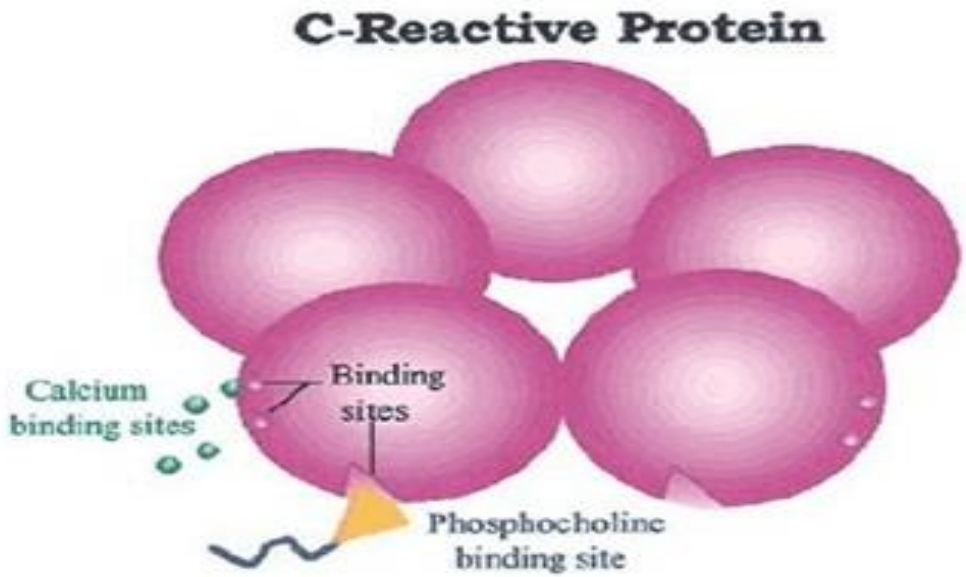
Immunoassay of cell-free nucleosomes using the **Volition Nu.Q®-H3.1 assay** was found to be the best proxy measurement for monitoring levels of circulating NETs

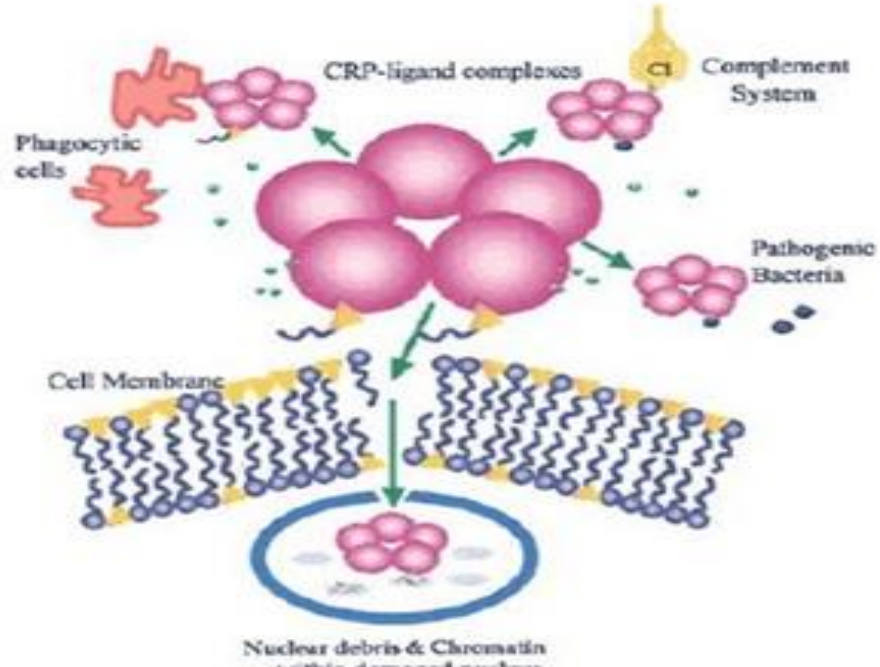
Et par rapport aux marqueurs connus?

CRP

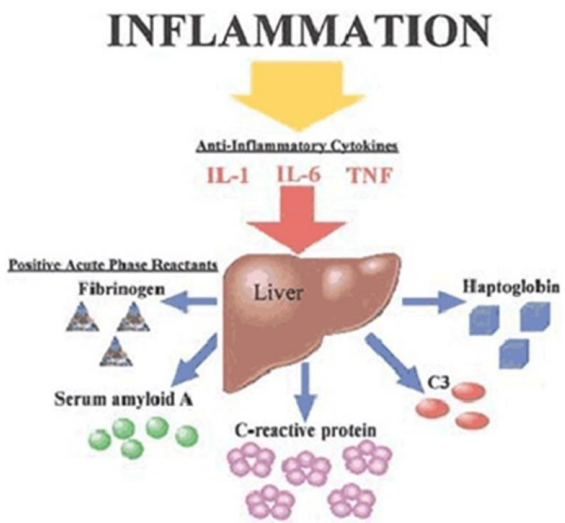
La CRP:

- Appartient à la famille des pentraxines, protéines calcium-dépendantes spécialisées dans la fixation de ligands
- Est composée de 5 monomères identiques, (207 AA, masse moléculaire : +/- 23kDa) qui s'organisent en anneau autour d'un pore central





CRP



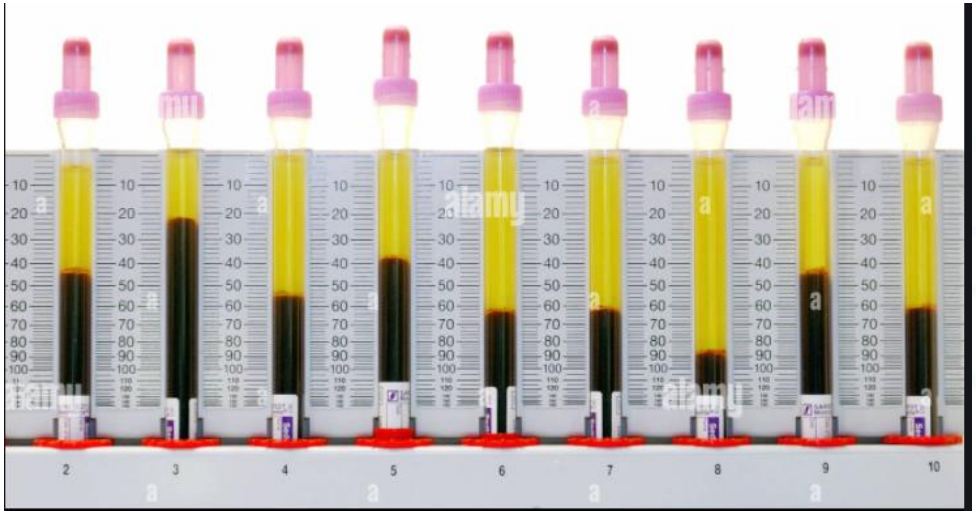
- **Mécanisme :** La CRP est une protéine produite par le foie en réponse à l'inflammation.
- **Spécificité :** La CRP est un marqueur non spécifique de l'inflammation.
- **Rôle :** La CRP participe à l'opsonisation des agents pathogènes, facilitant leur élimination par les phagocytes.
- **Comparaison avec les NETs :** Contrairement aux NETs, la CRP n'implique pas directement l'activation des neutrophiles ni la formation de structures complexes. Les NETs sont plus spécifiques aux réponses neutrophiles et aux infections, tandis que la CRP est un indicateur général d'inflammation.

Vitesse de sédimentation

- Dérive de la méthode de Westergreen (1920) : lecture à 1 heure de la hauteur de la colonne de plasma au-dessus des hématies qui ont sédimenté dans un tube

3 phases :

1. formation de rouleaux
2. sédimentation rapide
3. tassement final de la masse des érythrocytes.



- Bonne sensibilité:98%; mauvaise spécificité: 50%
- VPP:
  - **Mauvaise (46%) chez pts asymptomatiques**
  - **Bonne (89%) chez des pts symptomatiques**

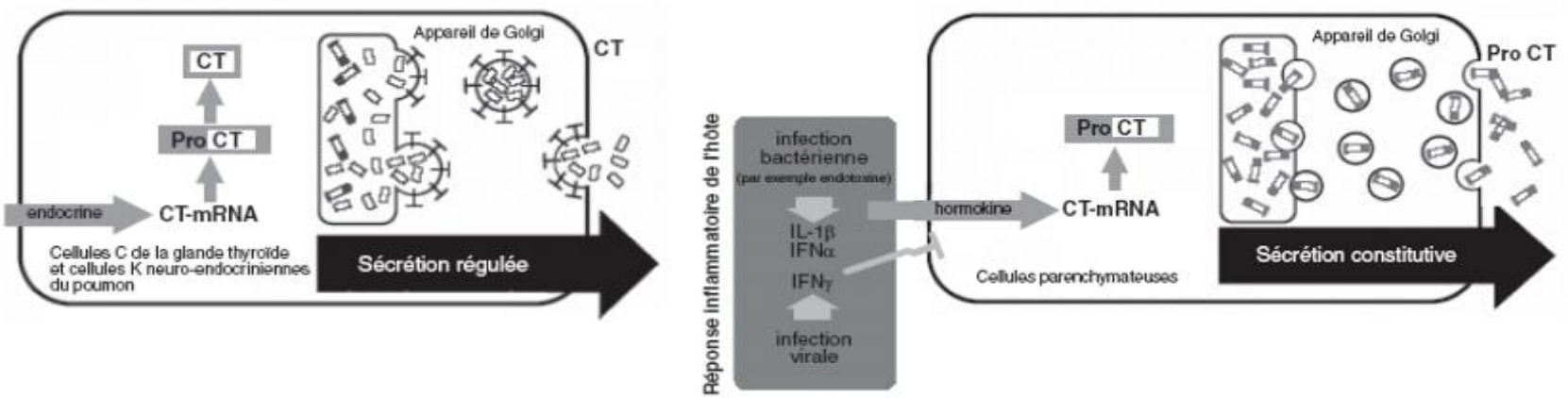
Vitesse de sédimentation

- **Mécanisme :** Mesure de la vitesse à laquelle les GR se déposent au fond d'un tube en une heure.
- **Spécificité :** Marqueur non spécifique. Il est élevé dans diverses conditions inflammatoires, notamment les infections chroniques, les maladies auto-immunes, et les cancers.
- **Rôle :** Pas de rôle direct dans la réponse immunitaire mais sert de mesure indirecte de l'inflammation.
- **Comparaison avec les NETs :** Ne fournit pas d'informations spécifiques sur le type de cellules impliquées dans l'inflammation, contrairement aux NETs qui sont directement liés à l'activité des neutrophiles.

Procalcitonine

LA PROCALCITONINE: UNE PRO-HORMONE DE LA CALCITONINE

Morgenthaler N. et al., Clin Lab 2002



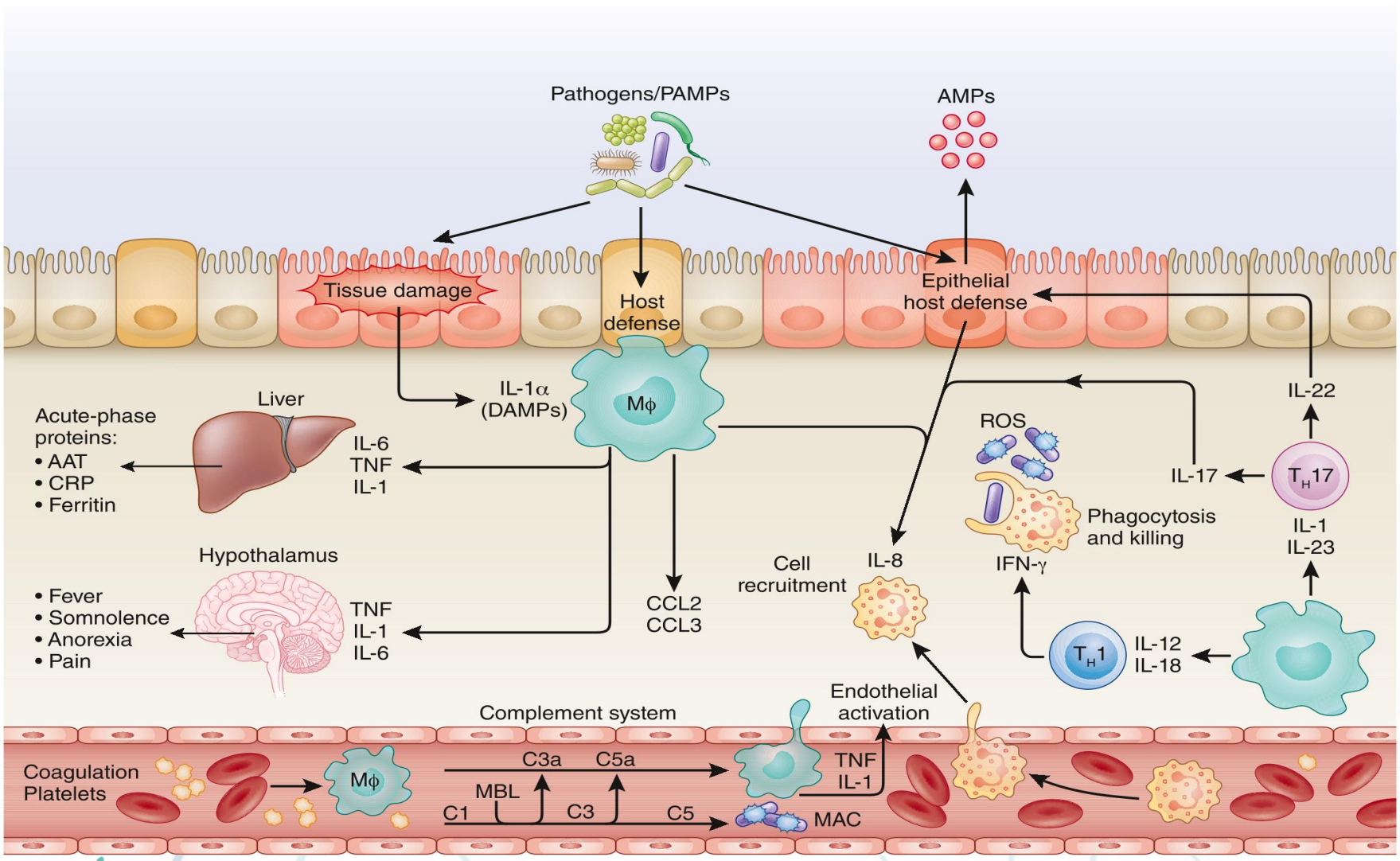
CT secrétée par cellules C de la thyroïde, en réponse à un stimulus hormonal

PCT secrétée par d'autres types cellulaires, en réponse à une stimulation pro-inflammatoire, notamment d'origine bactérienne

Procalcitonine

- **Spécificité :** Elle est plus spécifique aux infections bactériennes par rapport à la CRP ou à la VS.
- **Rôle :** Elle aide à évaluer la gravité de l'infection et est utilisée pour guider la thérapie antibiotique.
- **Comparaison avec les NETs :** Alors que la PCT est un biomarqueur indiquant la présence d'une infection bactérienne, les NETs sont une réponse active des neutrophiles contre les infections, impliquant directement la capture et la destruction des agents pathogènes.

Cytokines (IL-6, TNF-α, IL-1β)



Debbie Maizels/Springer Nature

Cytokines (IL-6, TNF-α, IL-1β)

- **Mécanisme :** Les cytokines comme l'IL-6, le TNF-α et l'IL-1β sont des médiateurs de l'inflammation. Elles sont produites par diverses cellules immunitaires (y compris les neutrophiles, les macrophages, et les lymphocytes) en réponse à des stimuli inflammatoires.
- **Spécificité :** Plus spécifiques que la CRP ou la VS, car elles sont directement impliquées dans l'initiation et la propagation de l'inflammation.
- **Rôle :** Elles orchestrent l'inflammation en activant les cellules immunitaires, en induisant la fièvre, en augmentant la production de protéines de phase aiguë (comme la CRP), et en modulant la réponse immunitaire.
- **Comparaison avec les NETs :** *Les cytokines sont des signaux solubles, agissant souvent à distance, tandis que les NETs sont des structures physiques qui piègent directement les pathogènes. Les NETs sont donc plus directement impliqués dans la neutralisation des agents infectieux, tandis que les cytokines modulent la réponse immunitaire de manière plus large.*

Les applications futures

1. Évaluation des Infections

Utilité : Les NETs jouent un rôle clé dans la réponse immunitaire aux infections bactériennes.

Leur dosage pourrait servir à évaluer la présence et la gravité des infections, en particulier dans des situations où d'autres marqueurs, comme la CRP ou la procalcitonine, ne fournissent pas suffisamment d'informations.

2. Identification du Risque Thrombotique

Utilité : Les NETs sont associés à la formation de thromboses, y compris dans des conditions telles que la thrombose veineuse profonde et l'embolie pulmonaire.

Le dosage des NETs pourrait aider à identifier les patients à haut risque de développer des événements thrombotiques.

Les applications futures

3. Diagnostic et Suivi des Maladies Auto-immunes

Utilité : Les NETs sont impliqués dans la pathogénie de diverses maladies auto-immunes, telles que le lupus érythémateux systémique (LES) et la polyarthrite rhumatoïde.

Le dosage des NETs pourrait aider au diagnostic et à surveiller leur progression ou la réponse au traitement.

4. *Suivi des Maladies Cardiovasculaires*

Utilité/ Avantage : Les NETs ont été impliqués dans la pathogenèse de maladies cardiovasculaires, y compris l'athérosclérose. La mesure des NETs pourrait fournir des informations sur l'activité ou la progression de la maladie.

Conclusion

Prédiction

Suivi de la progression

IDENTIFICATION DES PATIENTS À HAUT RISQUE

Évaluation de l'inflammation

Surveillance de l'efficacité des traitements

Prévention des complications post-opératoires

Approche personnalisée des traitements

Merci beaucoup pour votre attention!

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