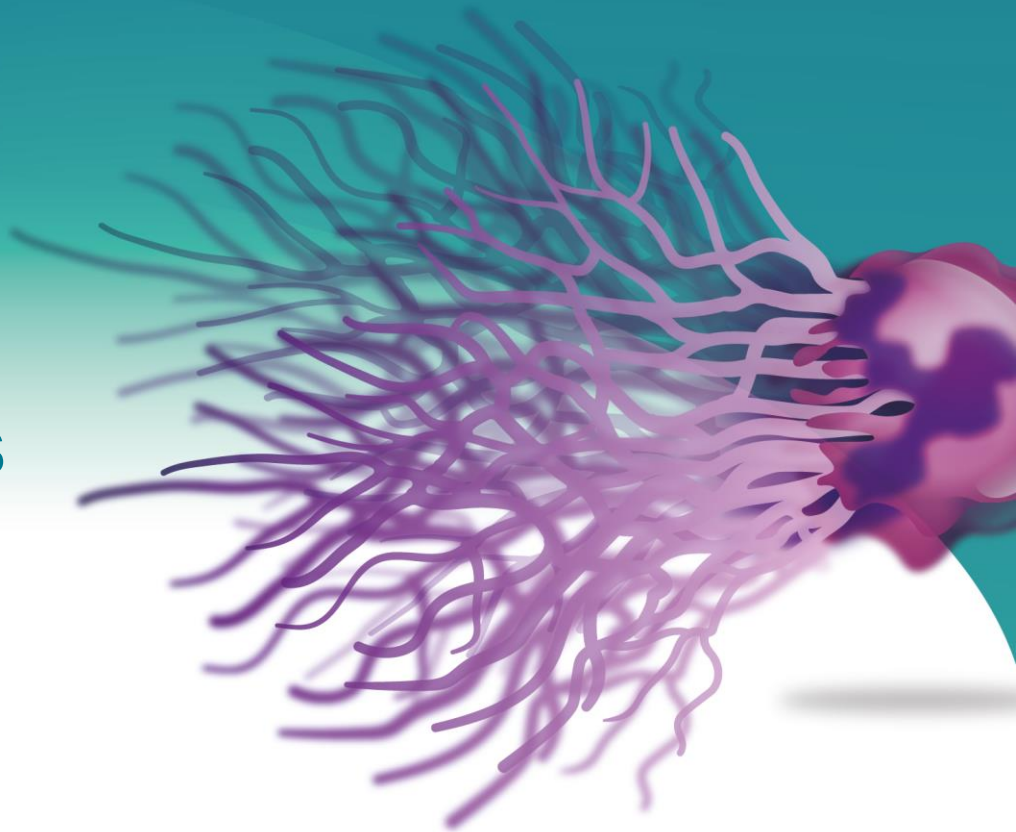
NETs: Casting a new light on sepsis management



Volition

Bienvenue!

Professor Djillali Annane

Volition

Neutrophil Extracellular Traps (NETs)

NETs:

- are produced by ejecting chromosomal material out of the cell
- catch and kill bacteria and viruses
- can sterilize blood in minutes
- first reported in 2004[1]
- now the subject of > 5000 publications

1. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. Science. 2004 Mar 5;303(5663):1532-5. DOI: 10.1126/science.1092385

1 in 5 deaths worldwide are associated with sepsis

Almost **50 million** cases resulting in **11 million** deaths

Over 40% of cases are children under 5 years of age

It's the **number 1...**

Cause of death in hospitals

Cause for hospital readmissions

Healthcare cost
($62bn in USA pa alone)

Over **40%** of survivors suffer from long-term physical or psychological effects

Rudd et al, The Lancet, 2020. https://doi.org/10.1016/S0140-6736(19)32989-7

Volition's Mission

Develop a low-cost, easy-to-use, rapid diagnostic test to save lives and improve outcomes for patients worldwide.

We are here to present Nu.Q® NETs H3.1 assay, a novel, clinically relevant biomarker which has the potential to change the management of patients with sepsis.

Executive Summary: consolidated conclusions

Results from three independent studies totalling over 3,000 patients

These findings are consistent across cohorts[1-3]

An elevated H3.1 level reflects a dysregulated immune response and is associated with:

- a risk of **increased mortality**
- an increased risk of **septic shock**
- an increased risk of **(multi-) organ failure**
- an increased risk of **ARDS**
- an increased risk of **renal failure**

…could be thought of as a Treatable Trait in sepsis management

1. German Data Set, data on file; 2. Amsterdam UMC Data Set, data on file; 3. RHU Records Data Set, data on file

Speakers

Dr. Andrew Retter

Clinical Lead in Critical Care Medicine, ECMO and Thrombosis

Chief Medical Officer at VolitionRx, UK

Terry Kelly, PhD

Chief Innovation Officer, VolitionRx,
USA

Dr. Caroline Neumann

Senior Consultant in Intensive Care Medicine,
Jena University Hospital, Germany

Why is H3.1 key: the biology & scientific rationale

Dr. Andrew Retter,

MBBS, MRCP, FRCPath (Haem), DICM, FFICM

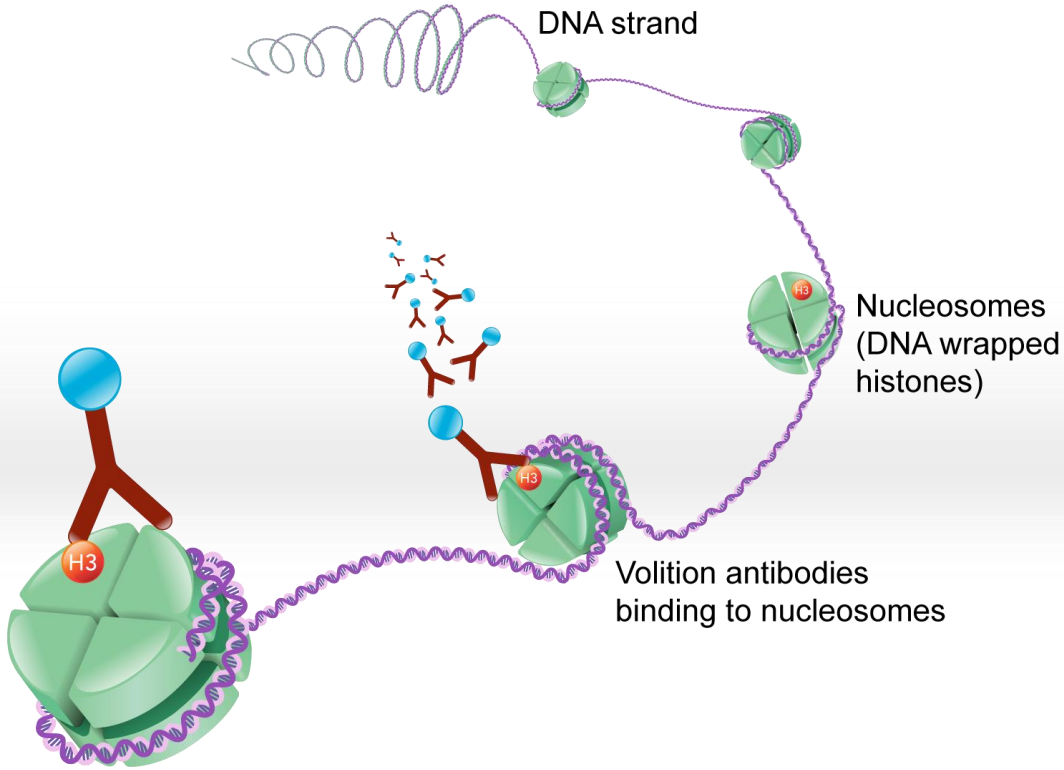
Clinical Lead in Intensive Care, ECMO and Thrombosis

Volition

Conflicts of interest to declare

- Employee and shareholder of VolitionRX Limited

Nucleosomes and histones:



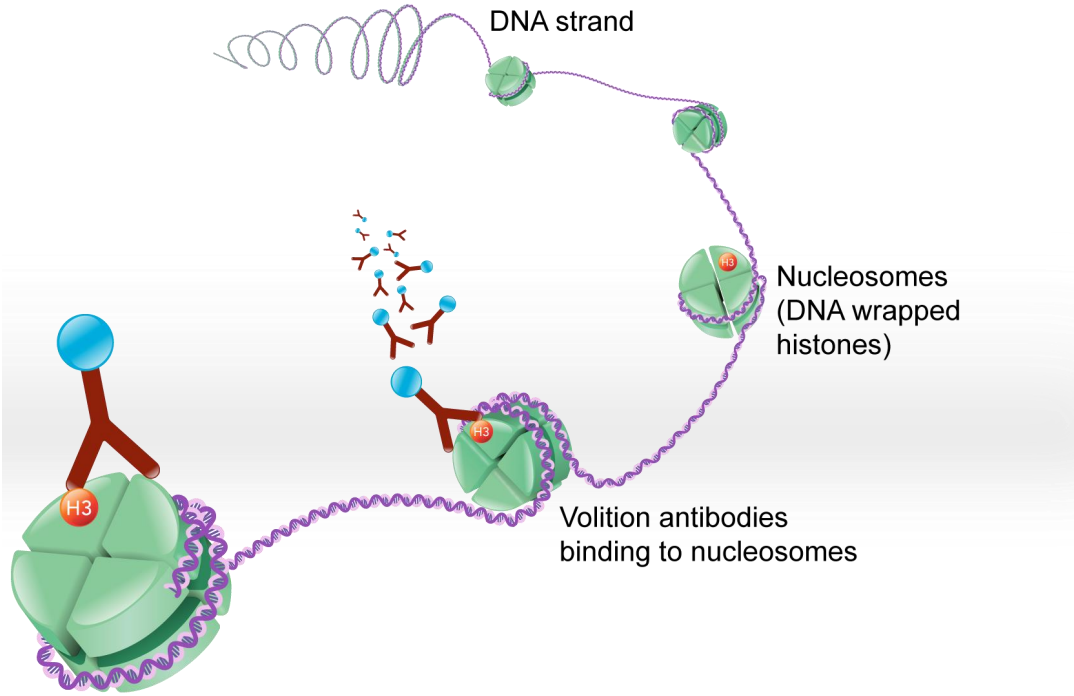
Nucleosomes and histones:

Key message:

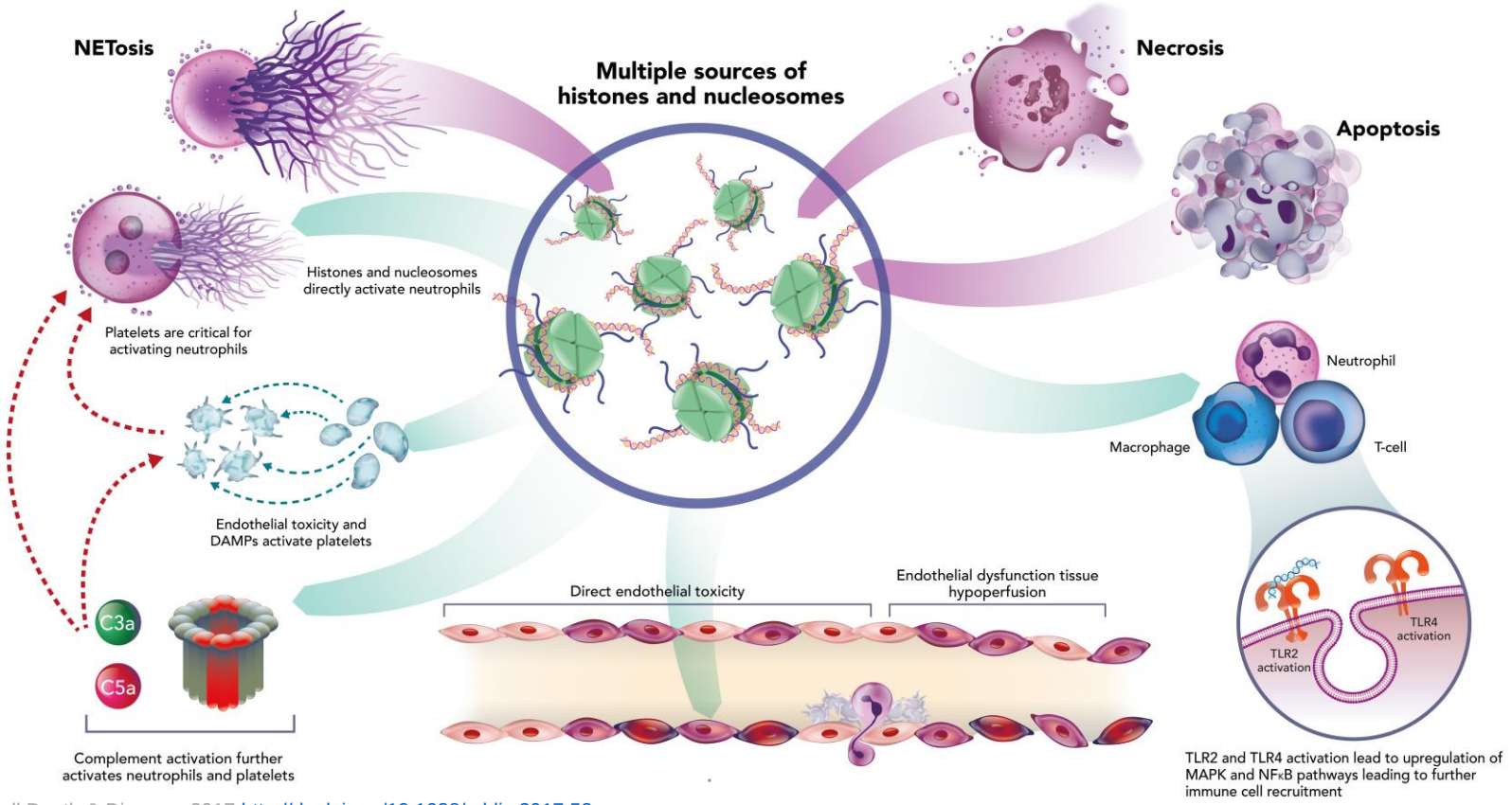
The H3.1 assay can detect nucleosomes using chemiluminescence technology and provide a result within 15 minutes

The lower limit of quantification is 20ng/ml

The upper limit of quantification is 20,000ng/ml



H3.1 as a Damage-Associated Molecular Protein

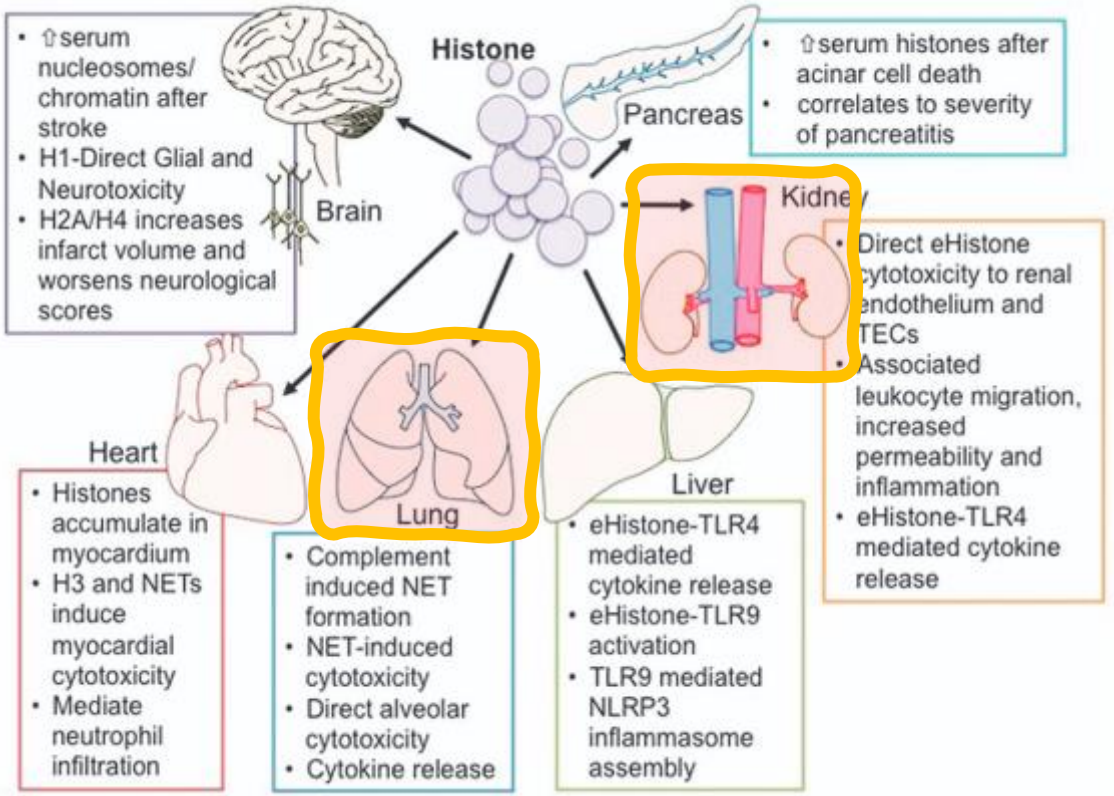


Silk et al, Cell Death & Disease, 2017 http://dx.doi.org/10.1038/cddis.2017.52

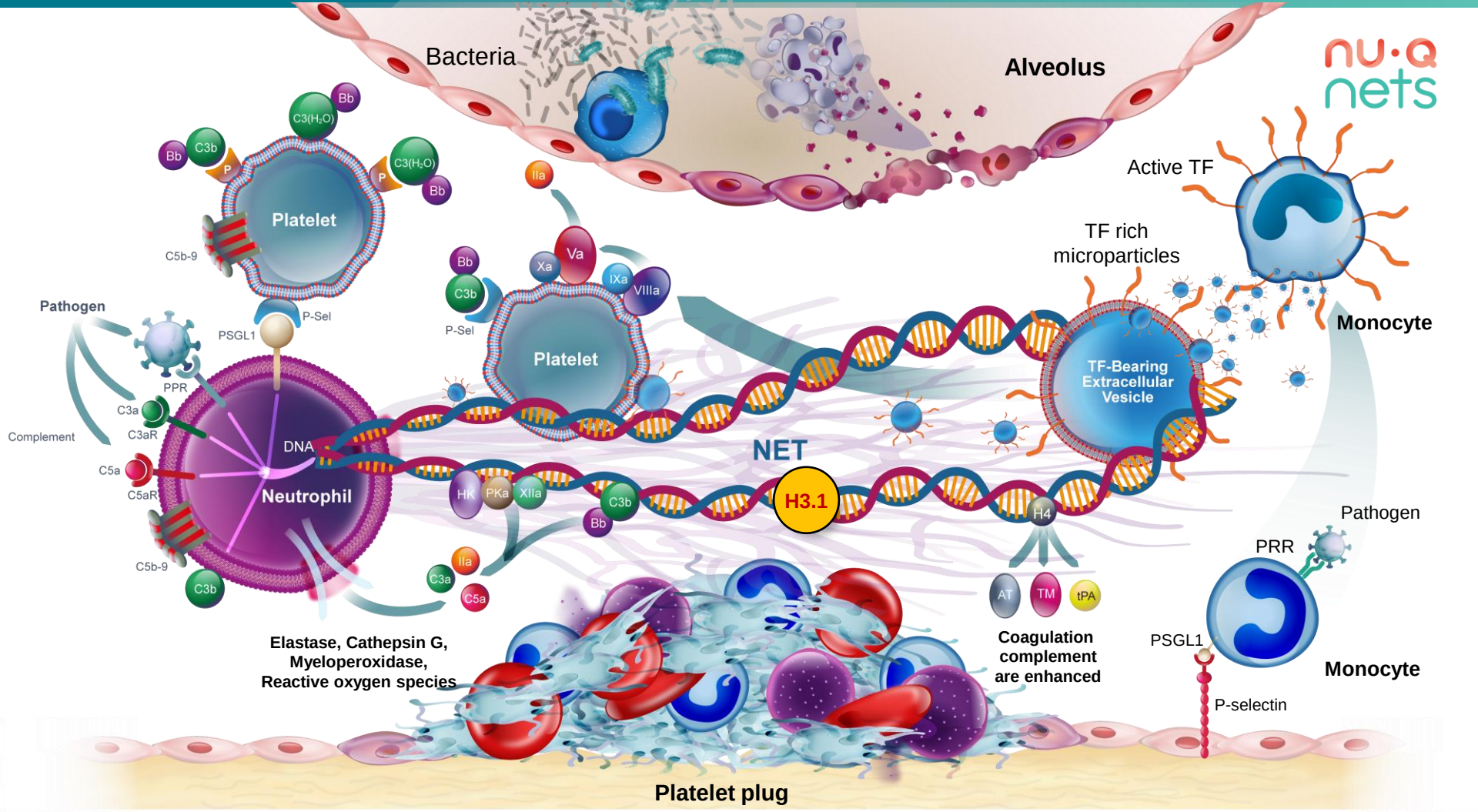
H3.1 sits as a triumvirate of innate immunity, inflammation and coagulation.

The majority of extracellular pathology is due to the indiscriminate binding of anionic components of the circulation and vasculature.

Extracellular Histones and Organ Injury



Silk et al, Cell Death & Disease, 2017 http://dx.doi.org/10.1038/cddis.2017.52


Bacteria
Alveolus
nu·Q nets
Active TF
TF rich microparticles
Monocyte
Pathogen
PSGL1
PPR
P-Sel
Platelet
C5b-9
C3(H2O)
Bb
C3b
P
Complement
C3a
C3aR
C5a
C5aR
DNA
Neutrophil
Va
Xa
IXa
VIIIa
IIa
HK
PKa
XIIa
NET
H3.1
H4
TF-Bearing Extracellular Vesicle
AT
TM
tPA
Coagulation complement are enhanced
Elastase, Cathepsin G, Myeloperoxidase, Reactive oxygen species
PRR
P-selectin
Platelet plug

The role of H3.1 in NETosis:

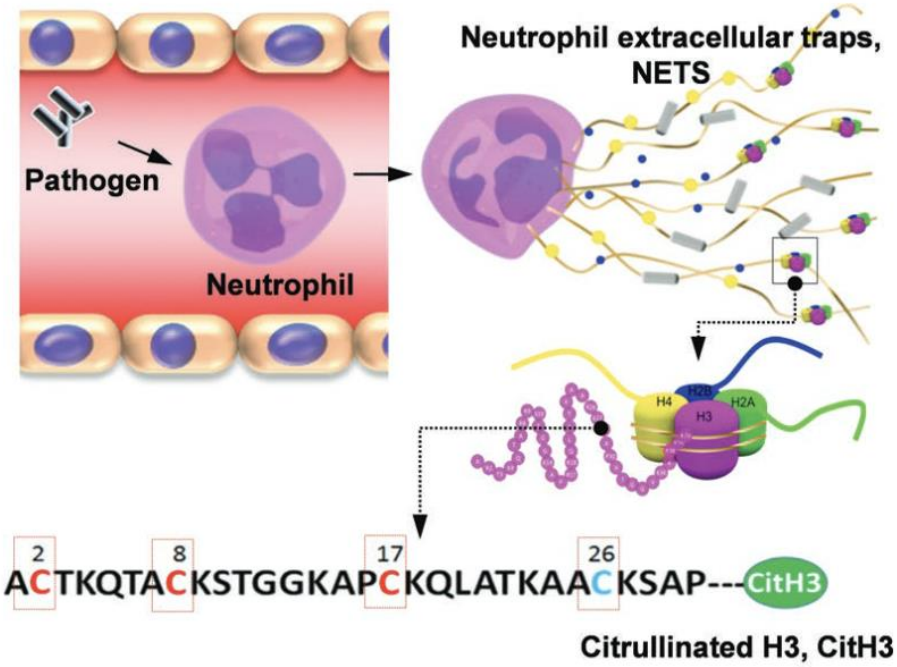
…proving we are measuring what we say we are measuring!

Terry Kelly, PhD.,

Chief Innovation Officer, Volition

Volition

NETs Contain Proteins and Trap Pathogens

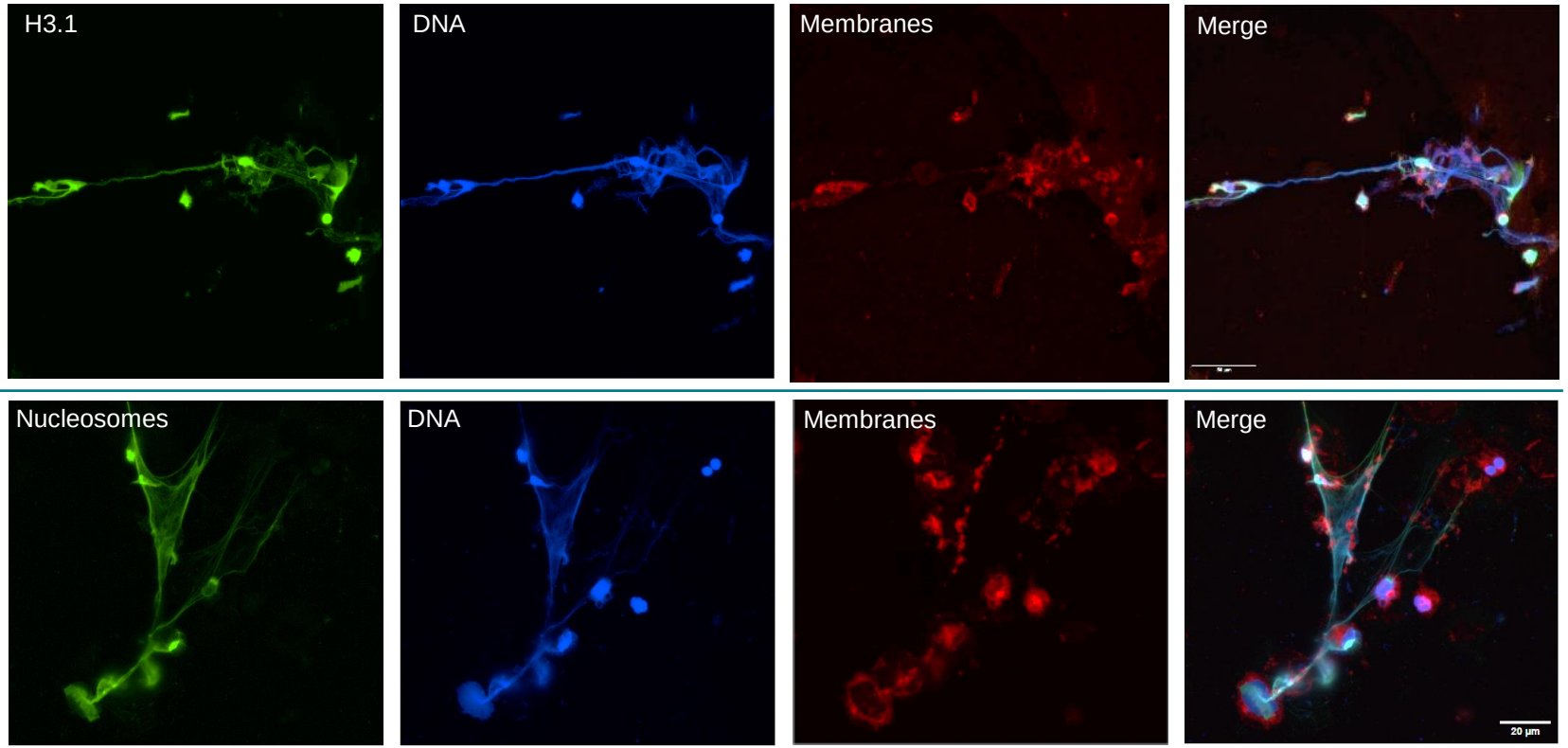


A neutrophil granulocyte (yellow) has ejected a NET (green) to capture bacteria (purple). A red blood cell (orange) is also trapped in the NET. Stained scanning electron microscope image by Volker Brinkmann.
© Volker Brinkmann / Max Planck Institute for Infection Biology

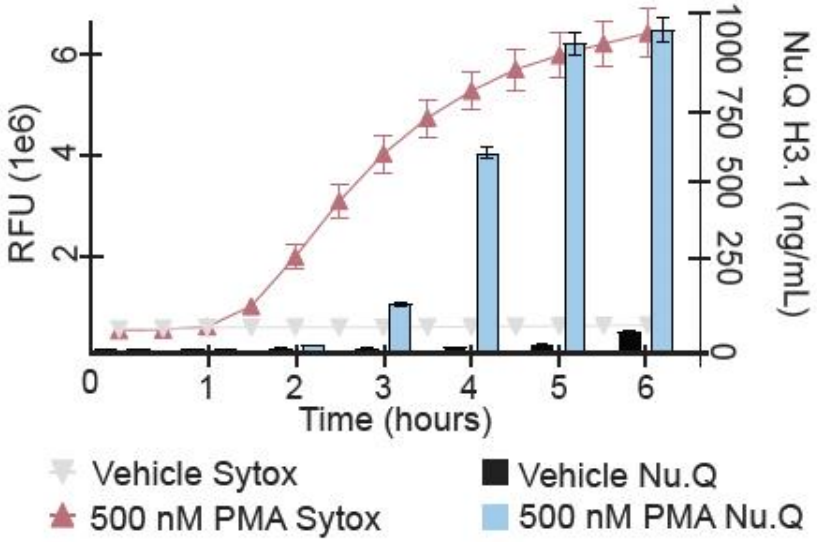
Y Park et al., Small, Jan 2020 https://doi.org/10.1002/smll.201905611

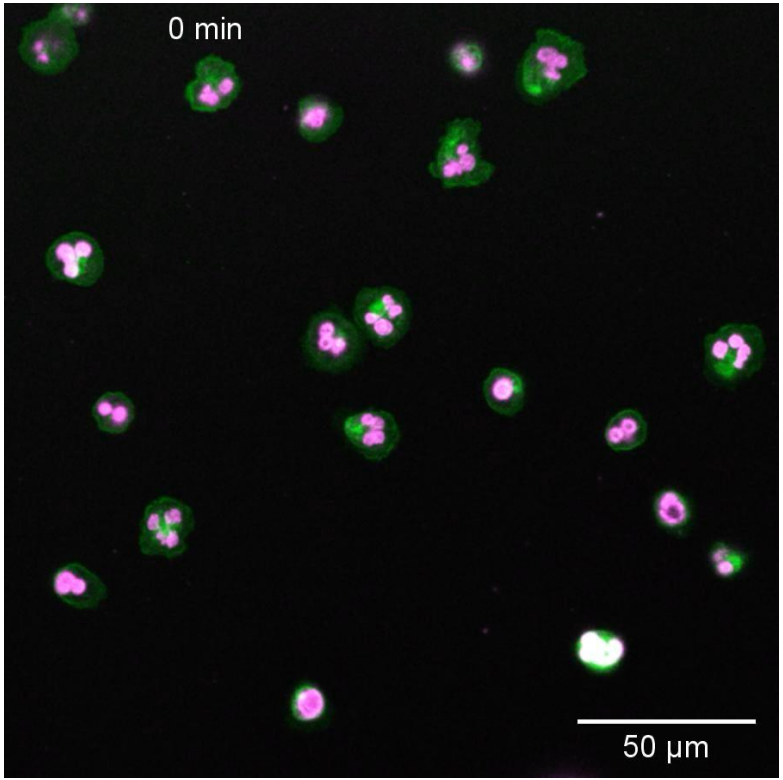
Apel et al, Science Signaling, March 2021. DOI: 10.1126/scisignal.aax7942

Nu.Q® Antibodies Label NETs in Isolated Neutrophils



H3.1 Nucleosome Levels Increase With NETosis





Zukas et al, Journal of Thrombosis & Hematology, 2024
https://doi.org/10.1016/j.jtha.2024.05.028

JTH Commentary, Sept 2024
https://doi.org/10.1016/j.jtha.2024.06.016

Kinetic Information

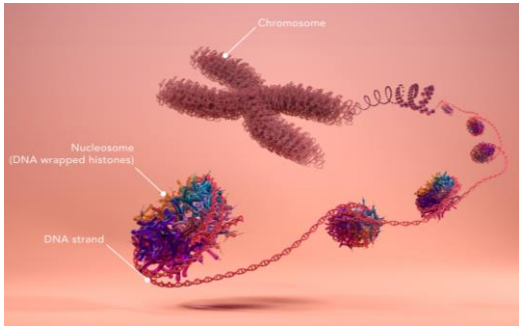
H3.1 is not impacted by height, weight, age, sex[1]

H3.1 is not impacted by the circadian rhythm[2]

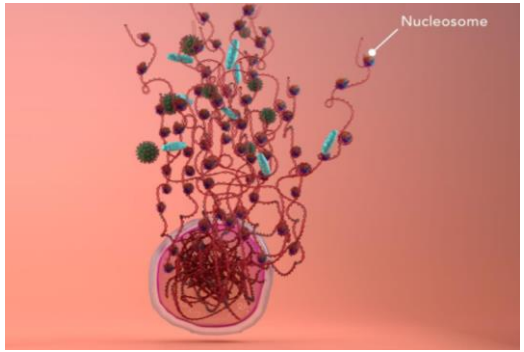
1. German Data Set, data on file; 2. RHU Records Data Set, data on file

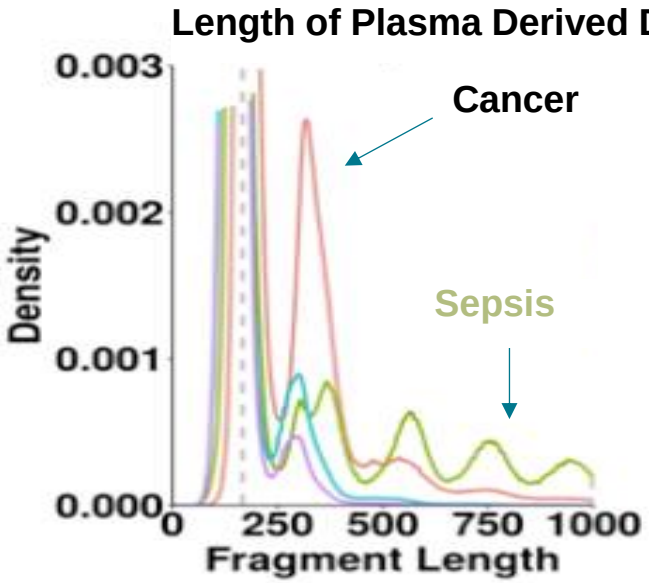
cfDNA Profiles Vary Across Disease and Cell Death Mechanisms

Apoptosis



NETs

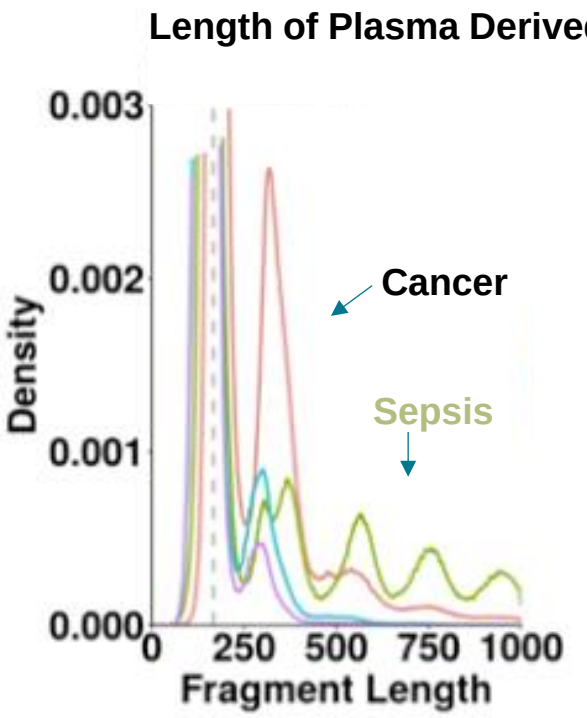




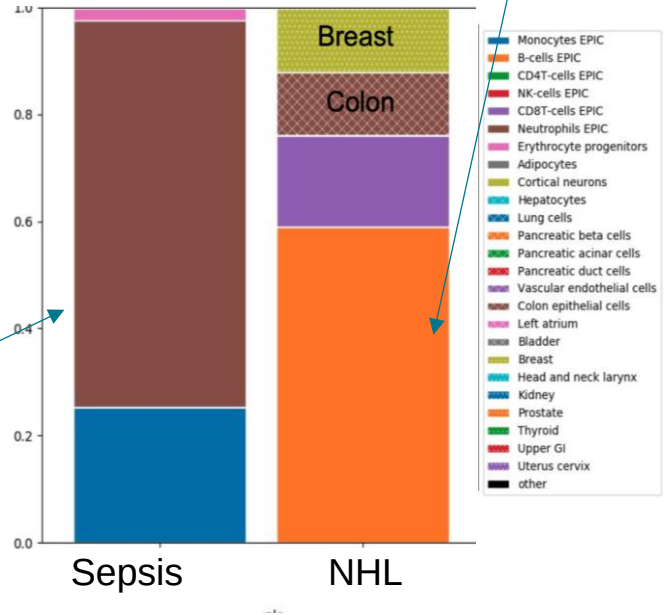
Circulating nucleosome levels increase as they are released faster than they can be removed

Data on file

Size Distribution Distinguishes Sepsis from Cancer & DNA Methylation Patterns Identify Cell of Origin



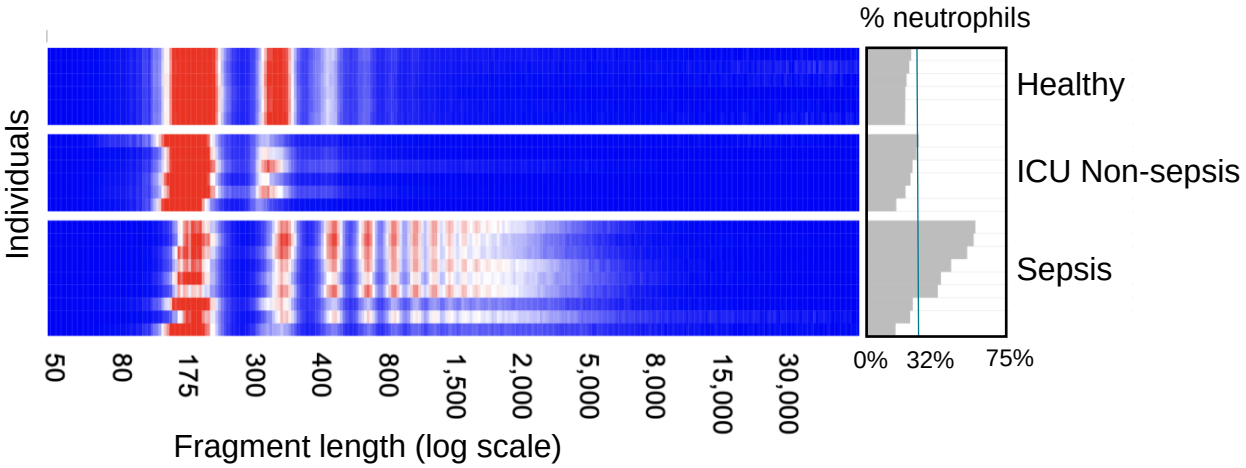
cfDNA from NHL sample primarily derived from B-cell (B-Cell Lymphoma)

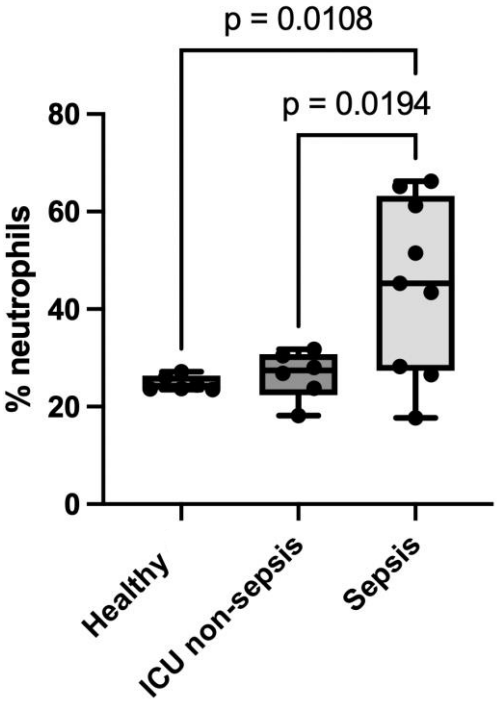


cfDNA from sepsis sample primarily derived from Neutrophils (i.e. NETs)

Data on file

cfDNA Derived From Neutrophils is Enriched in Sepsis Patients





Data on file

Clinical Data

Dr. Andrew Retter

KOL meeting: Sept 2024

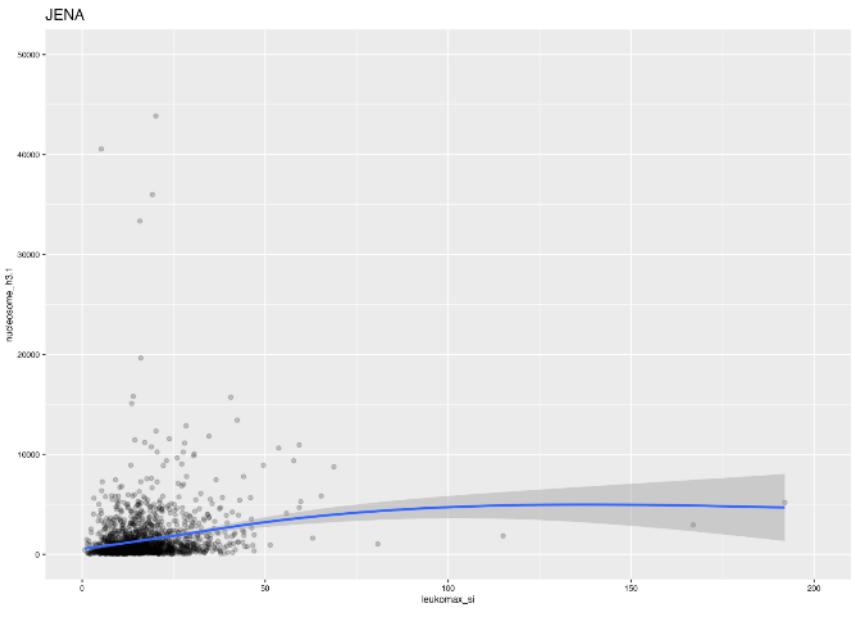
Chaired by **Professor Djillali Annane**, Professor of Medicine at University Paris Saclay-UVSQ.

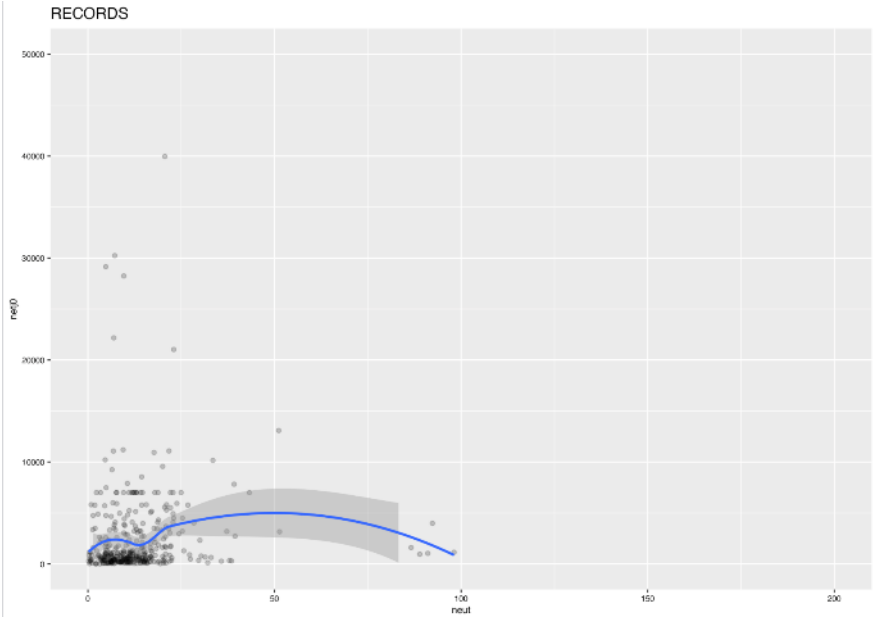
- **Professor Derek Angus**, Professor and Chair of the Critical Care Medicine Department at the University of Pittsburgh. (Partial attendance; virtual)
- **Professor Michael Bauer**, Professor and Chair of the Department of Anaesthesiology and Intensive Care Medicine at Jena University Hospital, Germany.
- **Dr. Lieuwe Bos,** Principal Investigator within the Intensive Care department at Amsterdam UMC, and associate professor at the University of Amsterdam. (Partial attendance)
- **Professor Luc de Chaisemartin,** Professor of Immunology at Paris-Cité University, and Head of the Biological Immunology Department at Bichat Hospital, Paris.
- **Dr. Charles Dehout**, attending physician at Erasmus Hospital in Brussels.
- **Professor Evangelos J. Giamarellos-Bourboulis**, National and Kapodistrian University of Athens, Greece and Chair of the European Sepsis Alliance.
- **Dr. Caroline Neumann,** Senior Consultant in Intensive Care Medicine at Jena University Hospital, Germany.
- **Dr. Andrew Retter**, Clinical Lead in Critical Care Medicine, ECMO and Thrombosis, and Chief Medical Officer at Volition.
- **Professor Mervyn Singer** OBE, University College London, UK. Co-chair of the Sepsis-3 Definitions International Task Force.

Studies at Centers of Excellence: >3000 patients

Study	Country	Description	Cohort Size
SISPCT	Germany	Retrospective analysis of prospectively collected cohort	971 intensive care patients Multiple timepoints
Amsterdam UMC	Netherlands	Retrospective analysis of prospectively collected cohort	1,713 intensive care patients Multiple timepoints
RHU RECORDS	France	Prospective, multi-center, placebo controlled, bio-marker-guided, adaptive Bayesian design basket trial	1,500 intensive care patients Interim analysis of 416 patients

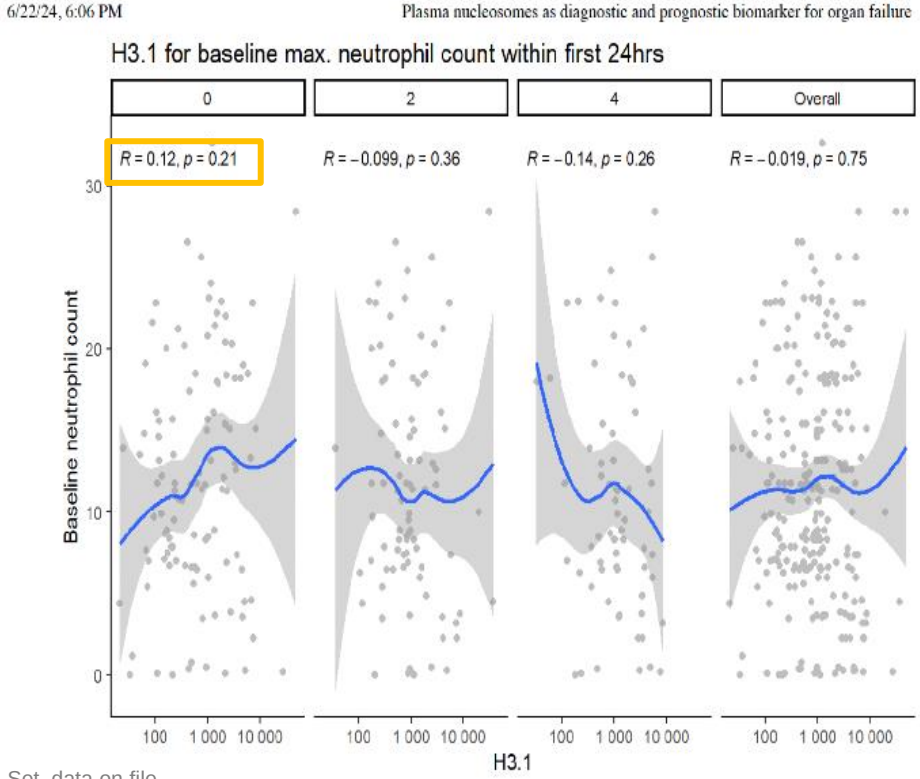
H3.1 only correlated weakly with the neutrophil count





1. German Data Set, data on file; 2. RHU Records Data Set, data on file

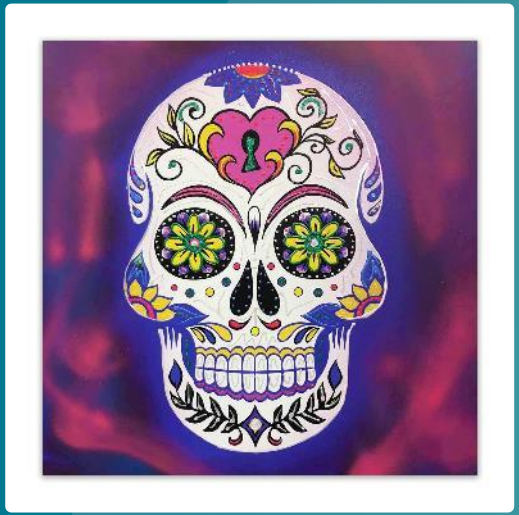
H3.1 only weakly correlated with the neutrophil count



1. Amsterdam UMC Data Set, data on file

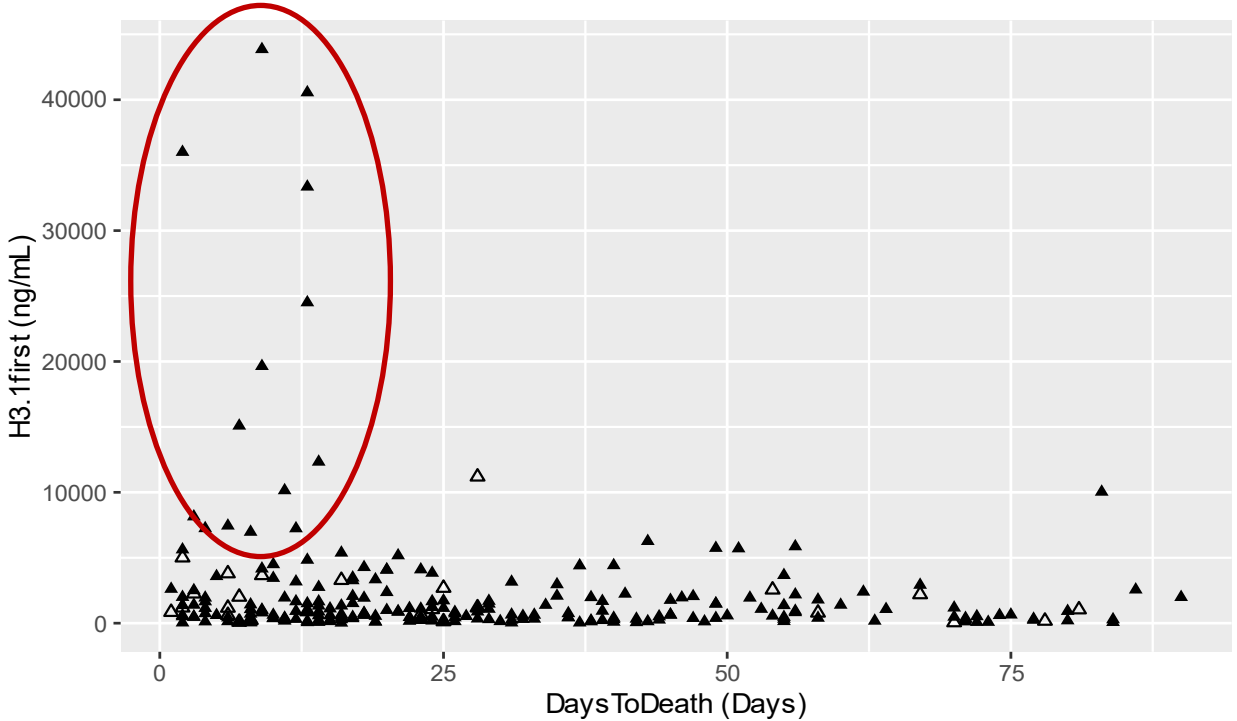
This is critical as H3.1 is giving us NEW information!

You don't just have to measure an FBC.



H3.1 predicts mortality

Initial H3.1 readings above 10,000 predicted mortality within 14 days



Overall survivors not plotted

German Data Set, data on file

All this work is based on admission H3.1

Mortality risk for different levels of H3.1 on admission

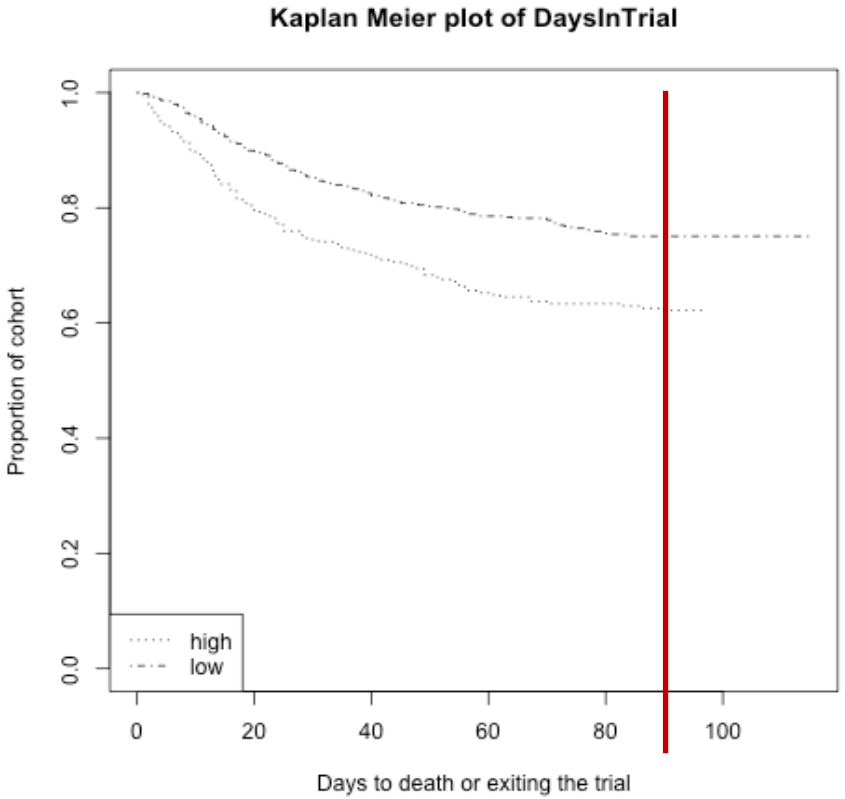
14 day mortality	Survivor			
Initial H3.1 (ng/mL)	Yes	No	Total	Risk
>20,000	0	5	5	100%
10,000-20,000	12	4	16	25%
1,000-10,000	264	36	300	12%
<1,000	508	40	548	7%
Total	**784**	**85**	**869**	**10%**

German Data Set, data on file

All this work is based on admission H3.1

Kaplan-Meier plot of survival based on initial H3.1 – high > 1,143.3 ng/mL

H3.1_first 7.080e-05 1.000e+00 1.195e-05 5.923 **3.16e-09 *****



German Data Set, data on file

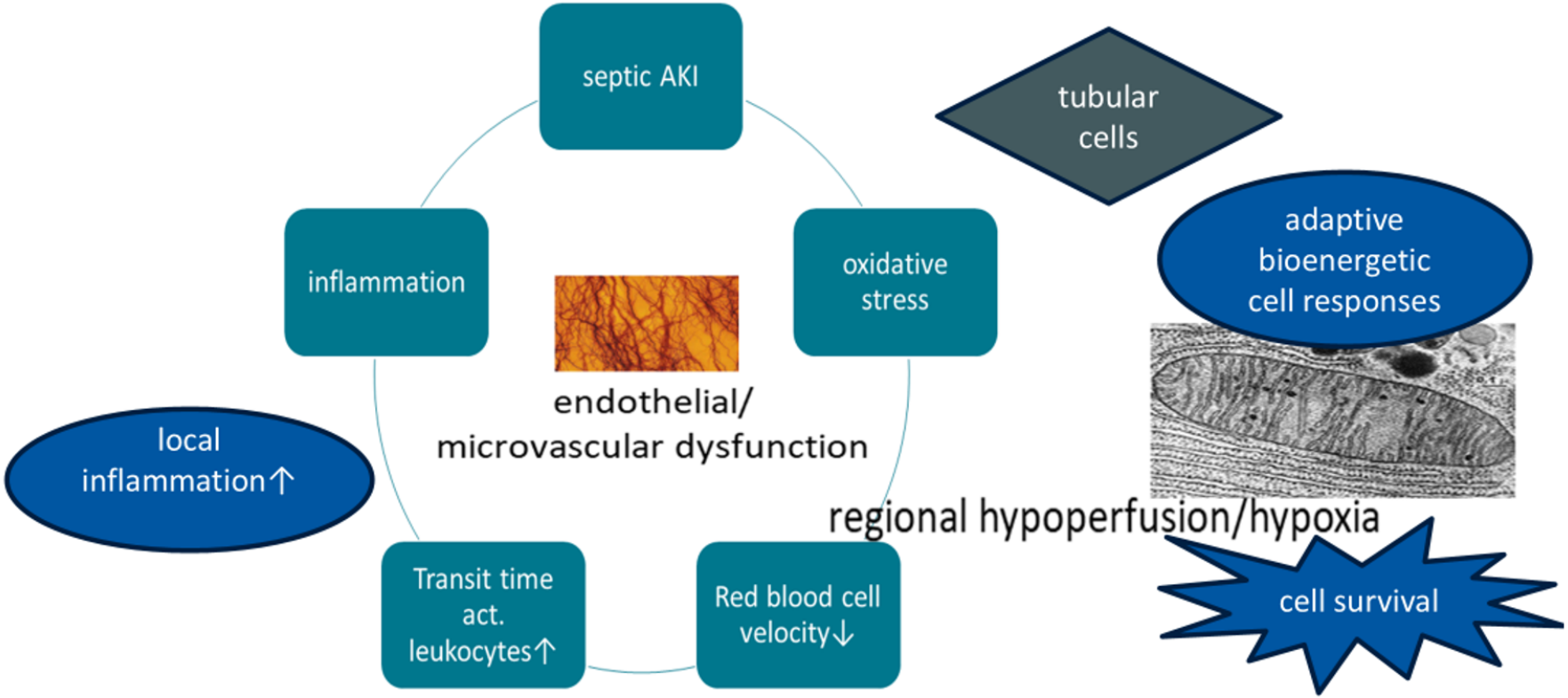
Clinical utility of Nu.Q® in septic Acute Kidney Injury (AKI): Data from SISPCT

Dr. Caroline Neumann,

DESA, EDIC, Infectious Diseases Specialist (DGI)
Senior Intensive Care Consultant

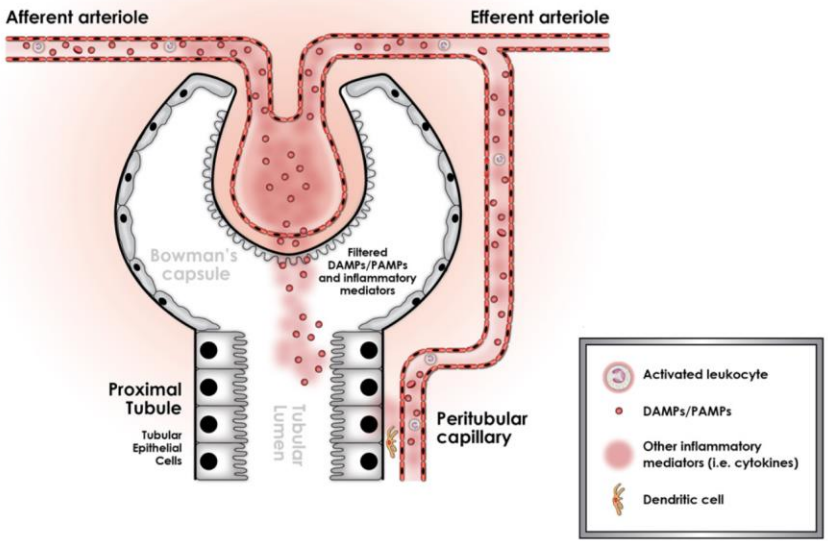
Volition

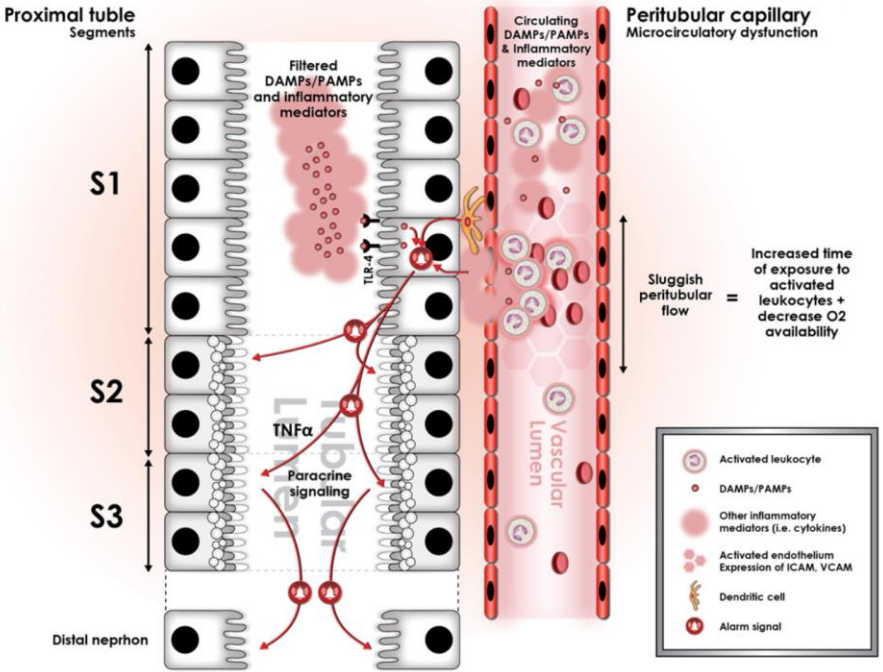
A Unified Theory of Sepsis-Induced AKI



Adapted from Gomez H, Ince C, De Backer D, Pickkers P, Payen D, Hotchkiss J, Kellum JA. A unified theory of sepsis-induced acute kidney injury: inflammation, microcirculatory dysfunction, bioenergetics, and the tubular cell adaptation to injury. Shock. 2014 Jan;41(1):3-11. doi: 10.1097/SHK.0000000000000052

A Unified Theory of Sepsis-Induced AKI

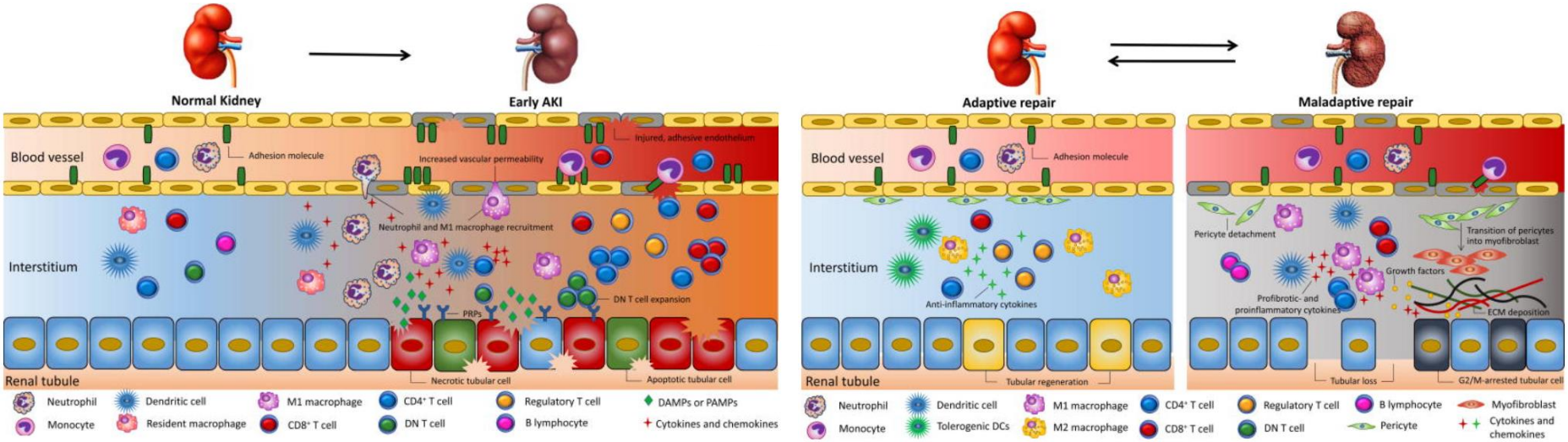




Gomez H, Ince C, De Backer D, Pickkers P, Payen D, Hotchkiss J, Kellum JA. A unified theory of sepsis-induced acute kidney injury: inflammation, microcirculatory dysfunction, bioenergetics, and the tubular cell adaptation to injury. Shock. 2014 Jan;41(1):3-11. doi: 10.1097/SHK.0000000000000052

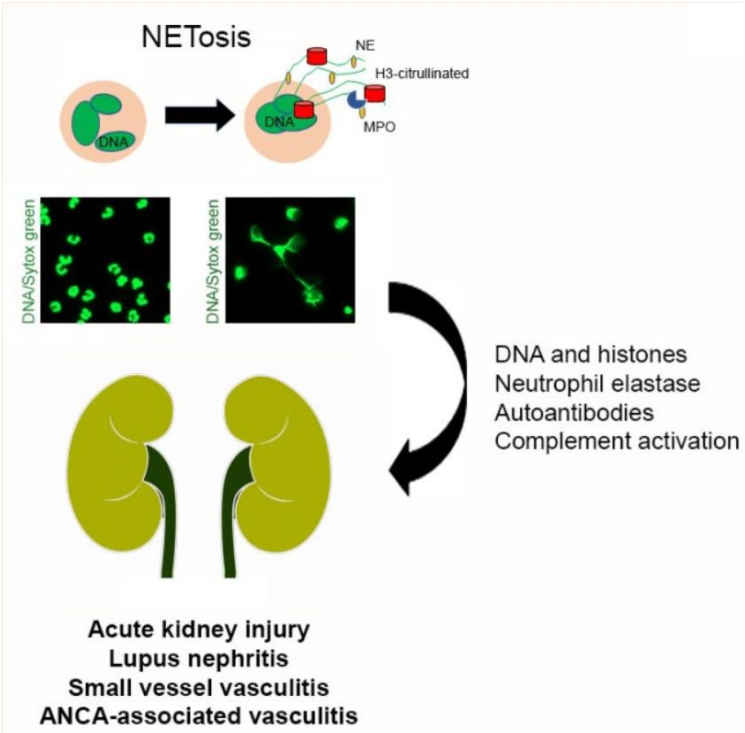
The role of immune cells in early injury and repair from AKI

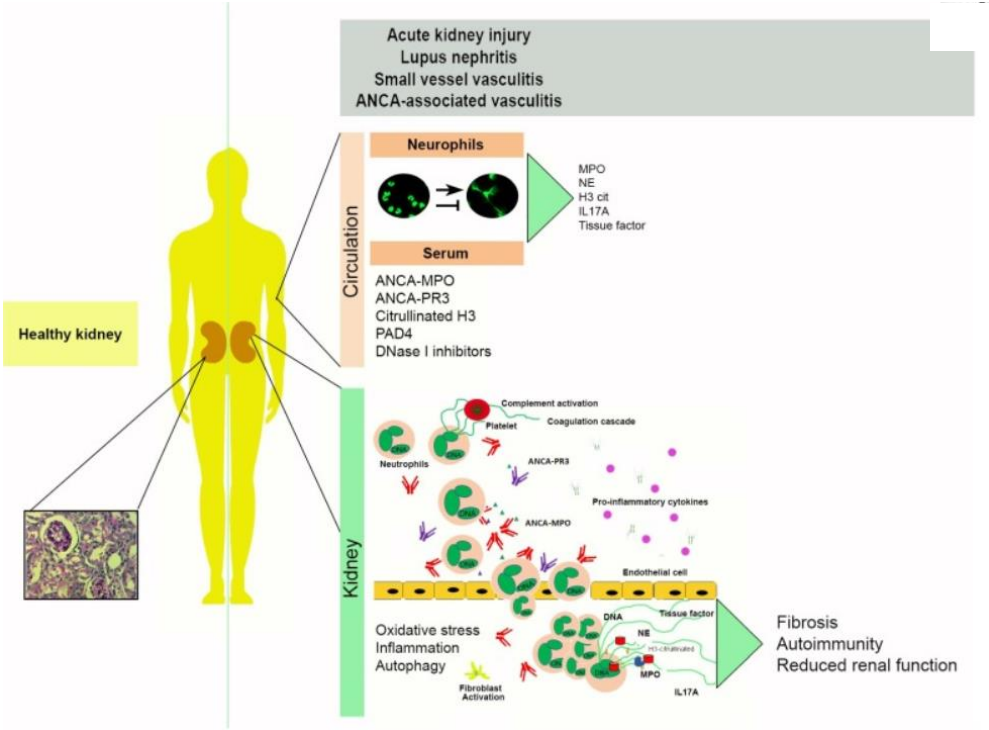
Role of Immune Cells in AKI and Repair



Lee SA, Noel S, Sadasivam M, Hamad ARA, Rabb H. Role of Immune Cells in Acute Kidney Injury and Repair. Nephron. 2017;137(4):282-286. doi: 10.1159/000477181

Neutrophil Extracellular Traps in the Establishment and Progression of Renal Disease





Salazar-Gonzalez H, Zepeda-Hernandez A, Melo Z, Saavedra-Mayorga DE, Echavarria R. Neutrophil Extracellular Traps in the Establishment and Progression of Renal Diseases. Medicina (Kaunas). 2019 Aug 2;55(8):431. doi: 10.3390/medicina55080431

How to assess damage and failing repair?

Observational study of the clinical utility of the nucleosome levels in septic acute kidney injury:
improved classification and targeted intervention in AKI?

Objectives and methods:

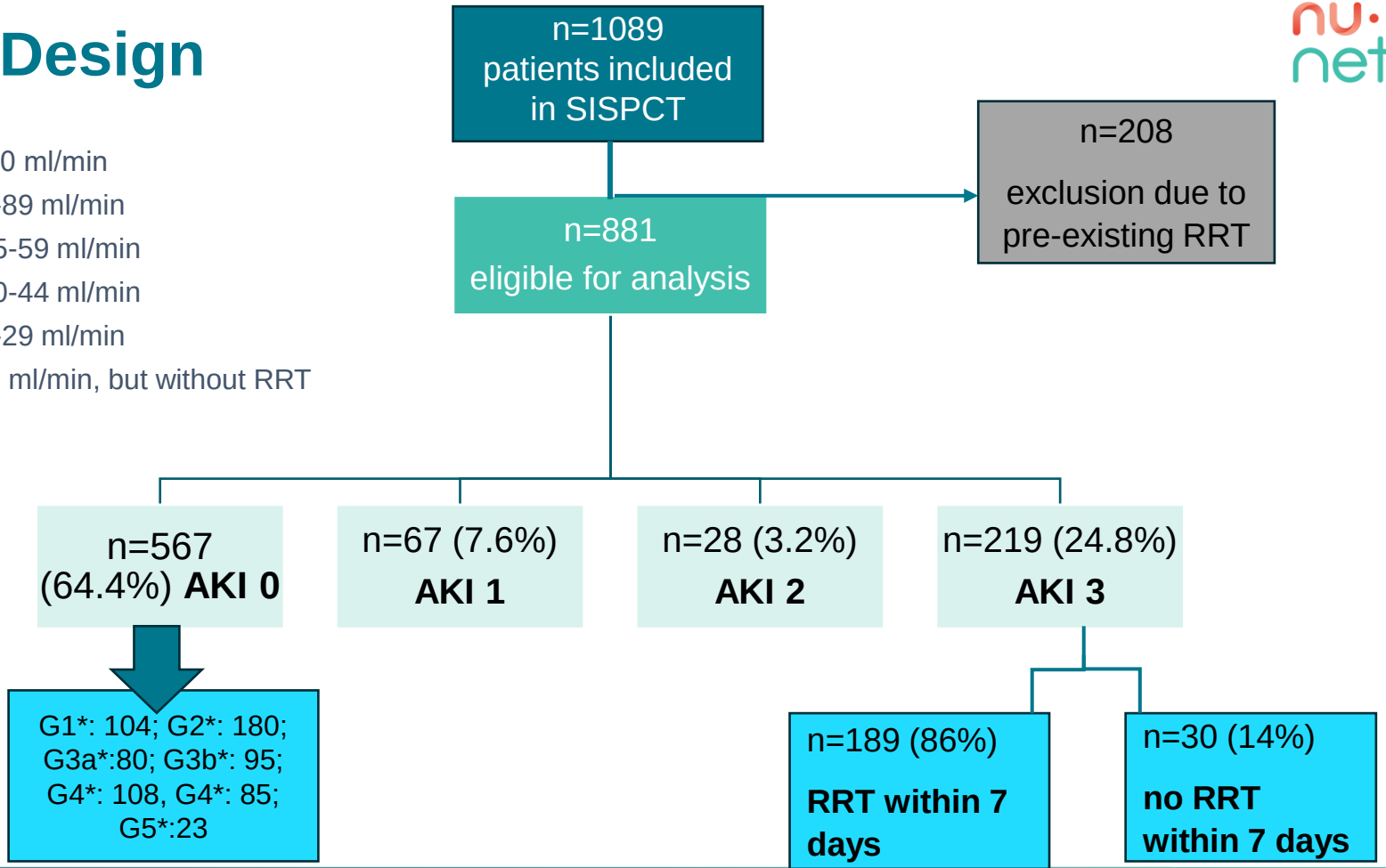
- secondary analysis of patients recruited for the SISPCT trial [1]

- evaluation of levels of circulating H3.1 nucleosomes (surrogate of NETosis):
  - association between the development of acute kidney injury (AKI) and its severity in patients with confirmed sepsis and septic shock

Bloos F, Trips E, Nierhaus A, Briegel J, Heyland DK, Jaschinski U, Moerer O, Weyland A, Marx G, Gründling M, Kluge S, Kaufmann I, Ott K, Quintel M, Jelschen F, Meybohm P, Rademacher S, Meier-Hellmann A, Utzolino S, Kaisers UX, Putensen C, Elke G, Ragaller M, Gerlach H, Ludewig K, Kiehntopf M, Bogatsch H, Engel C, Brunkhorst FM, Loeffler M, Reinhart K; for SepNet Critical Care Trials Group. Effect of Sodium Selenite Administration and Procalcitonin-Guided Therapy on Mortality in Patients With Severe Sepsis or Septic Shock: A Randomized Clinical Trial. JAMA Intern Med. 2016 Sep 1;176(9):1266-76. DOI: 10.1001/jamainternmed.2016.2514

Study Design

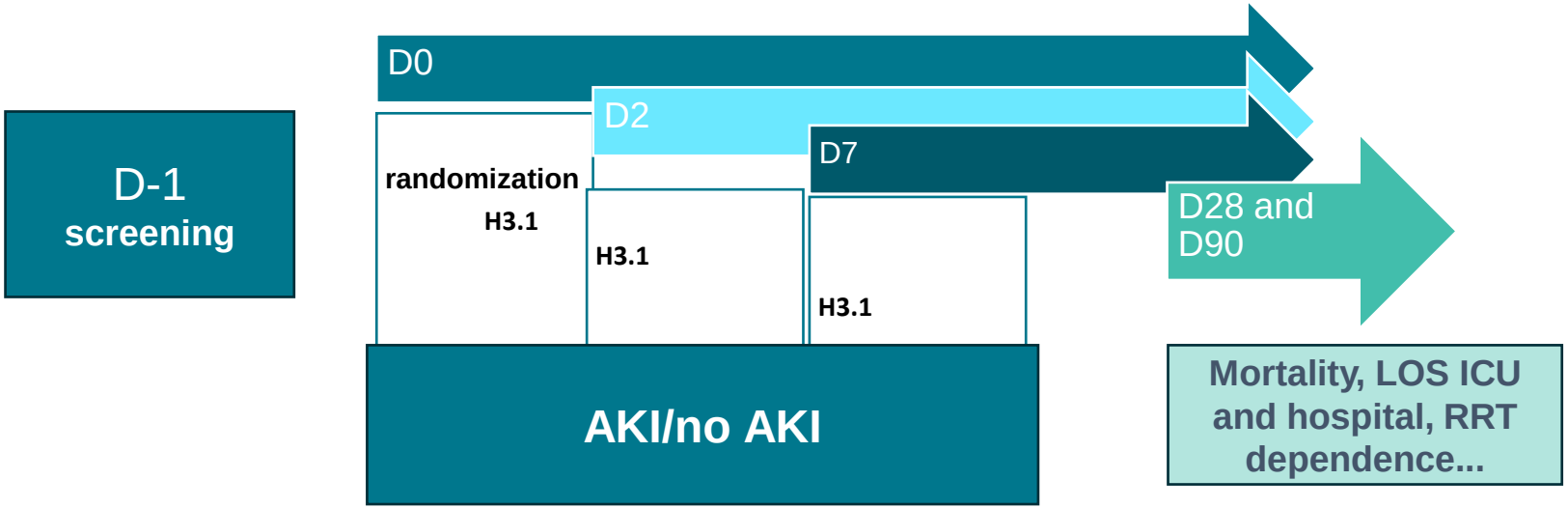
*G1: GFR ≥ 90 ml/min
*G2: GFR 60-89 ml/min
*G3a: GFR 45-59 ml/min
*G3b: GFR 30-44 ml/min
*G4: GFR 15-29 ml/min
*G5: GFR<15 ml/min, but without RRT



German Data Set, data on file

Measurement of H3.1 using Nu.Q® H3.1 Assay

H3.1 nucleosome levels were analyzed at admission and serially in frozen citrate plasma samples



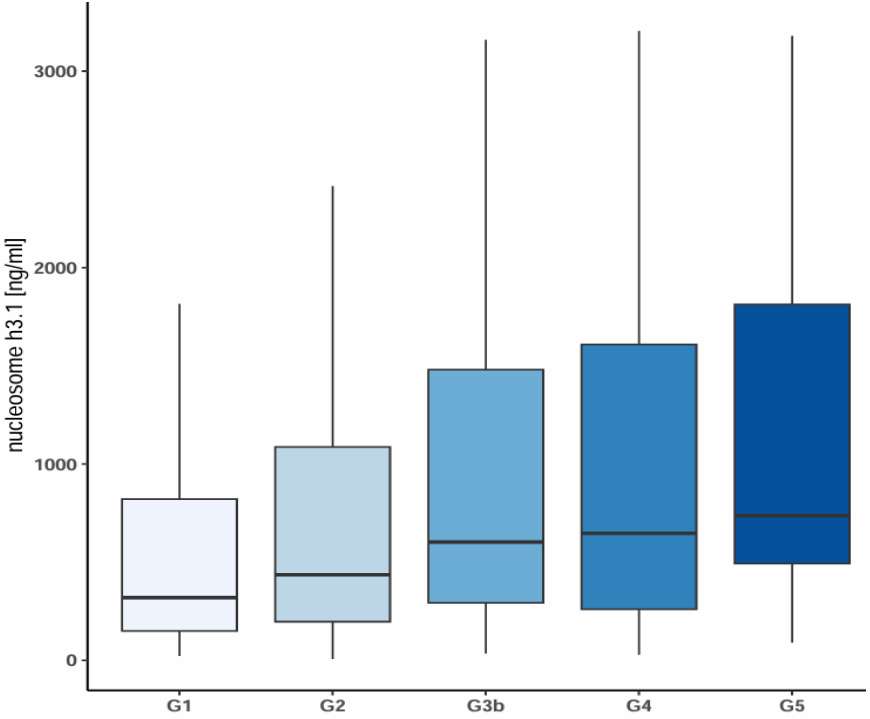
German Data Set, data on file

SISPCT sub-cohort baseline characteristics

Variable	AKI 0 (n=567)	AKI 1 (n=67)	AKI 2 (n=28)	AKI 3 (n=219)
Male sex, No. (%)	365 (64%)	46 (69%)	18 (64%)	143 (65%)
Age, med. (SD), y	67 (56, 75)	71 (60, 77)	66 (56, 74)	70 (60, 76)
SOFA	9 (7, 11)	9 (8, 11)	8 (7, 10)	11 (9, 15)
Scr 24 h before study inclusion (µmol/l)	97 (71, 150)	80 (62, 115)	75 (49, 98)	194 (116, 278)
Max. Scr (µmol/l)	104 (71, 150)	141 (97, 186)	185 (104, 220)	248 (186, 351)
Lactate, med. (mmol/l)	2.2 (1.4, 3.6)	3 (1.7, 4.3)	1.8 (1.4, 4.1)	3.4 (2, 6.1)
proADM, med. (nmol/l)	3.4 (2, 5.5)	4.7 (2.3, 7.3)	3.8 (2.4, 5.9)	9.1 (6.1, 14)
Septic shock	270 (48%)	38 (57%)	11 (41%)	151 (70%)
Invasive mechanical ventilation	387 (68%)	51 (76%)	20 (71%)	176 (80%)

German Data Set, data on file

H3.1 levels in patients with preexisting impaired kidney function without evolving AKI (p<0.001)



H3.1 levels in patients with preexisting impaired kidney function without evolving AKI (p<0.001)

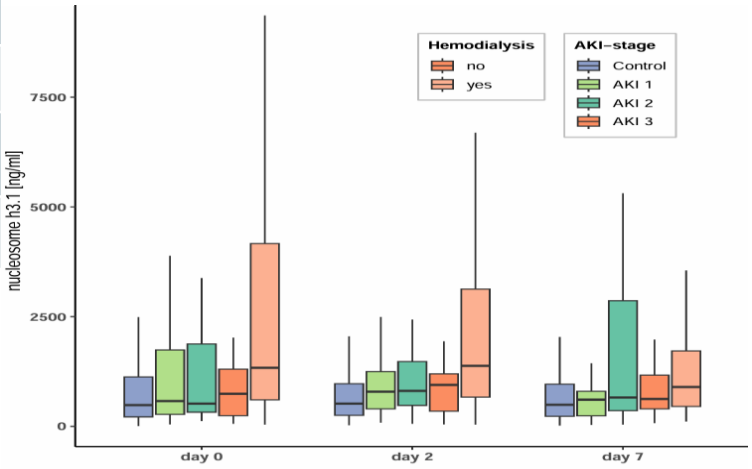
G1: GFR≥ 90ml/min
G2: GFR 60-89 ml/min
G3a: GFR 45-59ml/min
G3b: GFR 30-44ml/min
G4: GFR 15-29ml/min
G5: GFR <15ml/min

German Data Set, data on file

H3.1 Nucleosome levels over time

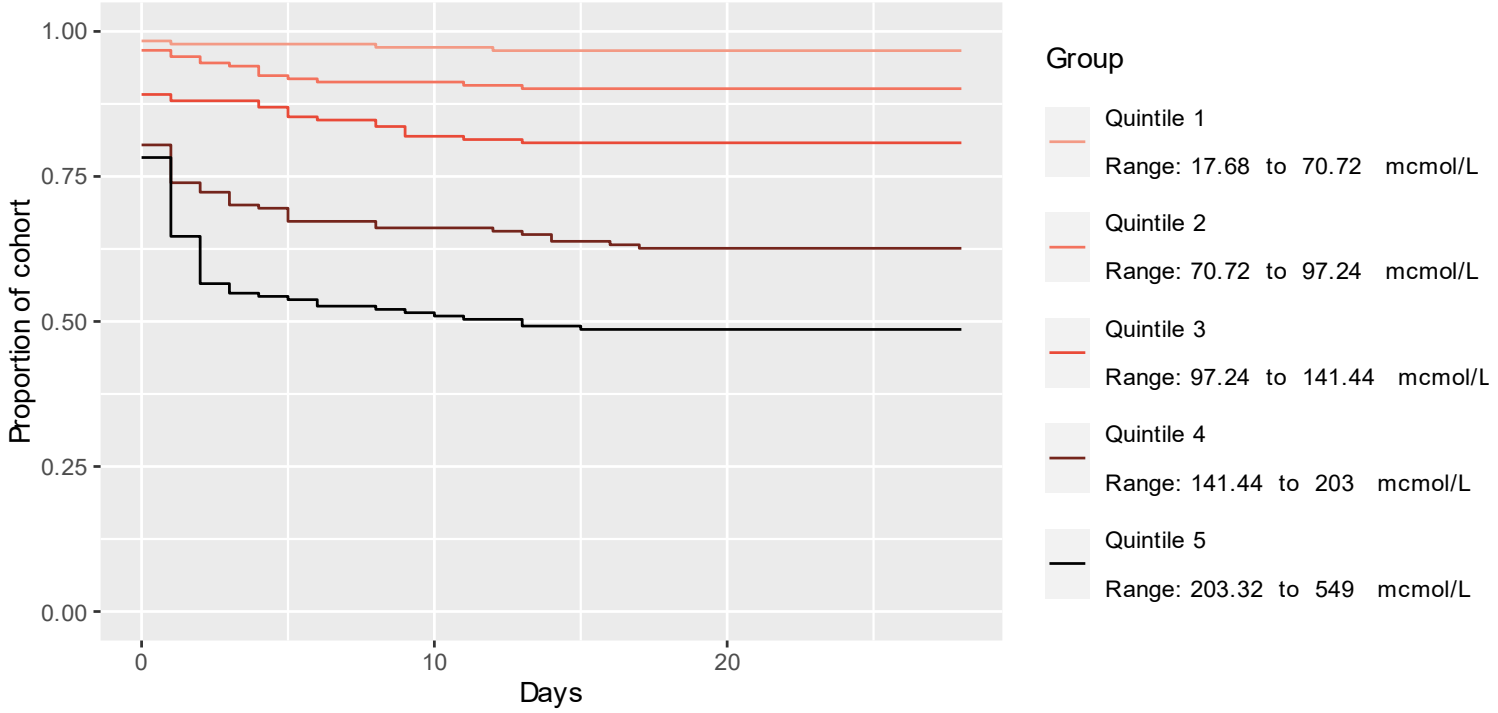
AKI stage	D0 (Nu.Q® Level)	D2 (Nu.Q® Level)	D7 (Nu.Q® Level)	p-value
0	484 (216-1127)	518 (249-974)	492 (229-969)	0.785
1	577 (266-1881)	790 (393-1319)	608 (234-820)	0.084
2	518 (319-1917)	809 (477-1477)	658 (356-2864)	0.574
3	1151 (509-3797)	1169 (611-2881)	885 (438-1641)	0.001
3 without RRT	741 (242-1362)	944 (345-1198)	625 (399-1166)	0.924
3 with RRT	1335 (604-4165)	1378 (654-3133)	898 (447-1778)	<0.001

initiation of RRT ?



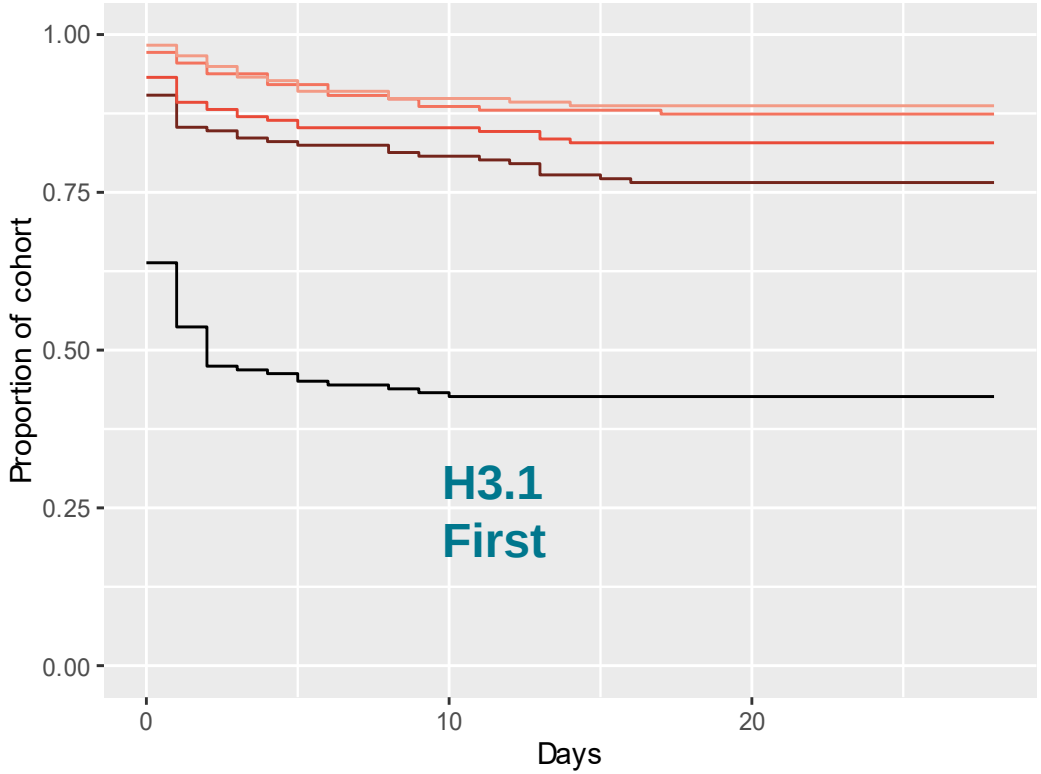
German Data Set, data on file

Kaplan-Meier: Quintiles of Creatinine first for AKI Stage 4 at 28 days



German Data Set, data on file

Kaplan-Meier: Quintiles of H3.1 First for AKI Stage 4 at 28 days



Group

Quintile 1
Range: 0 to 709.57 mL/24h

Quintile 2
Range: 720 to 1330 mL/24h

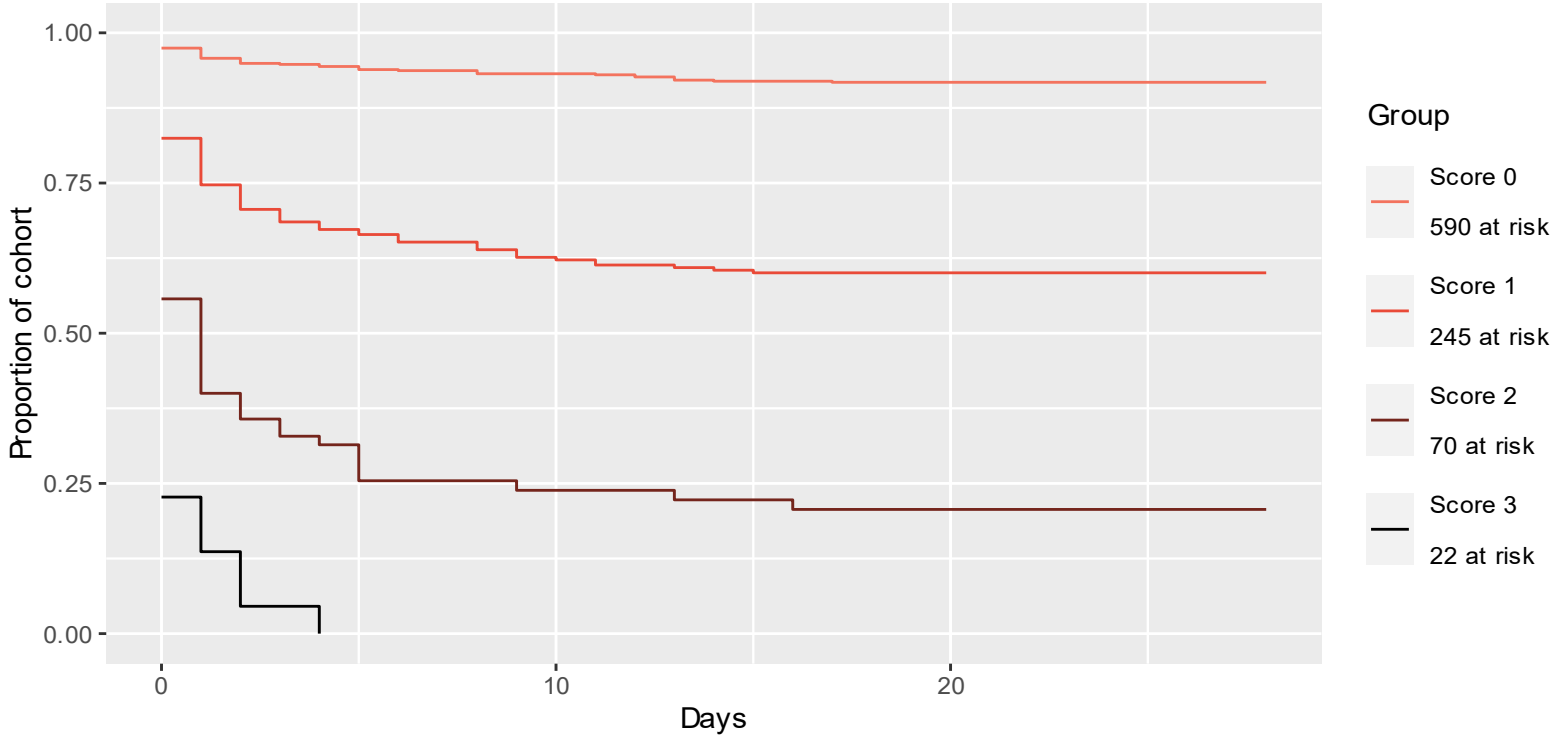
Quintile 3
Range: 1334.11 to 2000 mL/24h

Quintile 4
Range: 2000 to 3000 mL/24h

Quintile 5
Range: 3000 to 13680 mL/24h

German Data Set, data on file

Clinical Model of H3.1 Dose + Platelets + Urine24hr for AnyRRT28



German Data Set, data on file

Kidney Failure: summary

- Sepsis-induced AKI involves complex pathophysiology, with NETs playing a crucial role.
- Nu.Q® H3.1, as a marker of NETosis, shows promise as a biomarker in this context.
- H3.1 levels correlate with AKI severity and show distinct temporal patterns, particularly in severe AKI requiring RRT.
- Compared to creatinine, H3.1 offers improved early risk stratification for RRT requirements.
- A clinical model incorporating H3.1, platelet count, and urine output demonstrates strong predictive performance for RRT needs.

➢ **These findings open up exciting possibilities for improving the management of sepsis-induced AKI.**

Respiratory Failure

Dr. Andrew Retter

ELSEVIER

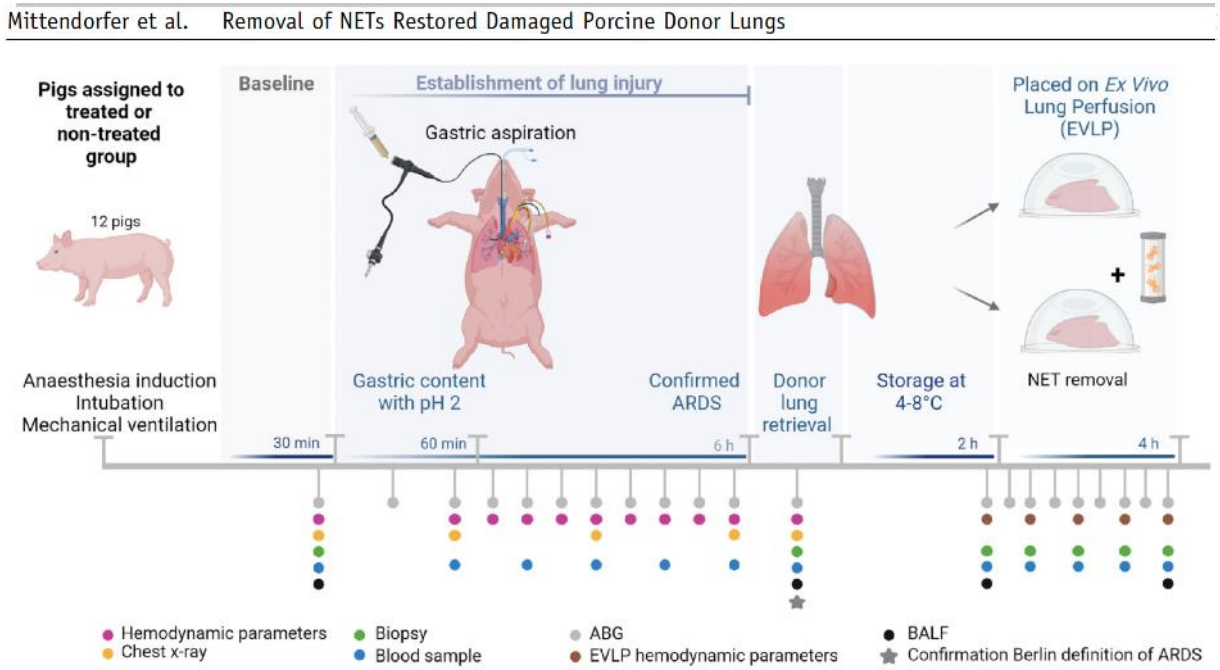
The Journal of Heart and Lung Transplantation
http://www.jhltonline.org

ISHT MEETING

Restoring discarded porcine lungs by ex vivo removal of neutrophil extracellular traps

Margareta Mittendorfer, MSc,[a,b,c,d] Leif Pierre, PhD,[b,c,d] Tibor Huzevka, MD,[a,b,c,d] Jeremy Schofield, MD,[e] Simon T. Abrams, PhD,[e] Guozheng Wang, PhD,[e] Cheng-Hock Toh, MD, PhD,[e,f] Nicholas B. Bèchet, PhD,[a,b,c,d] Ilma Caprnja, MD,[a,b,c,d] Gunilla Kjellberg, PhD,[g] Andrew Aswani, MD, PhD,[h,i] Franziska Olm, PhD,[a,b,c,d] and Sandra Lindstedt, MD, PhD[a,b,c,d]

Restoring discarded porcine lungs by ex vivo removal of neutrophil extracellular traps. Mittendorfer, Margareta et al. The Journal of Heart and Lung Transplantation, Volume 0, Issue 0.
https://doi.org/10.1016/j.healun.2024.07.007



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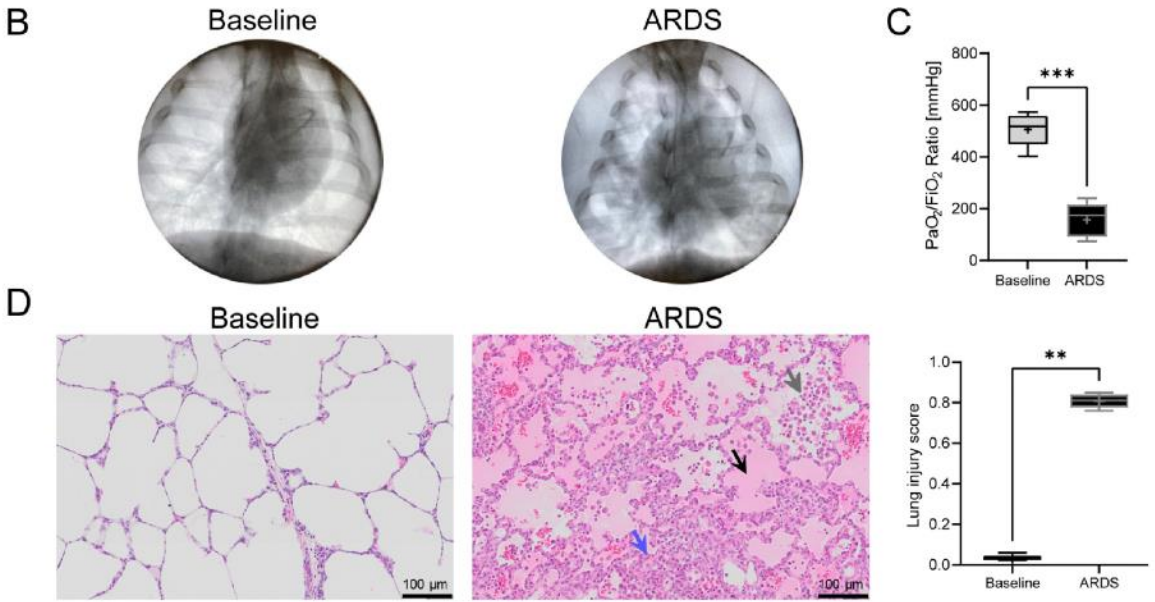

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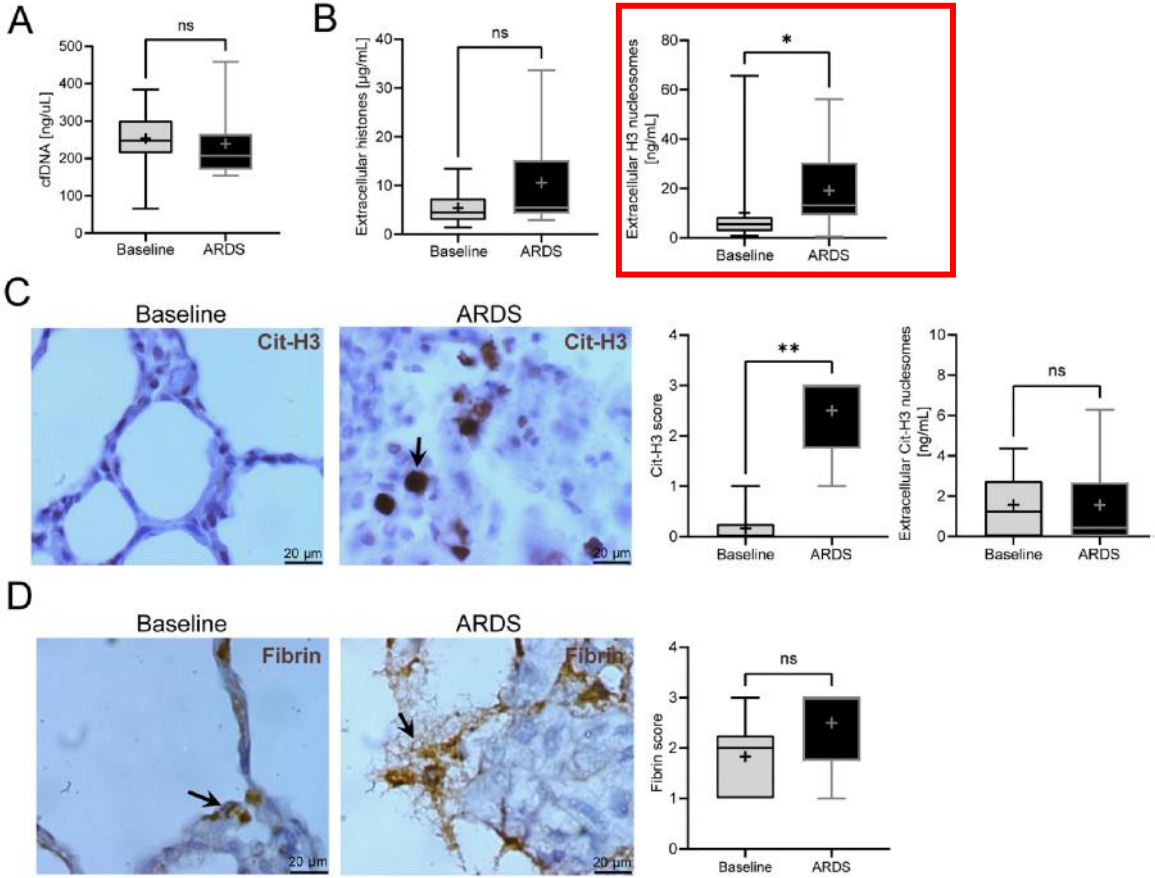

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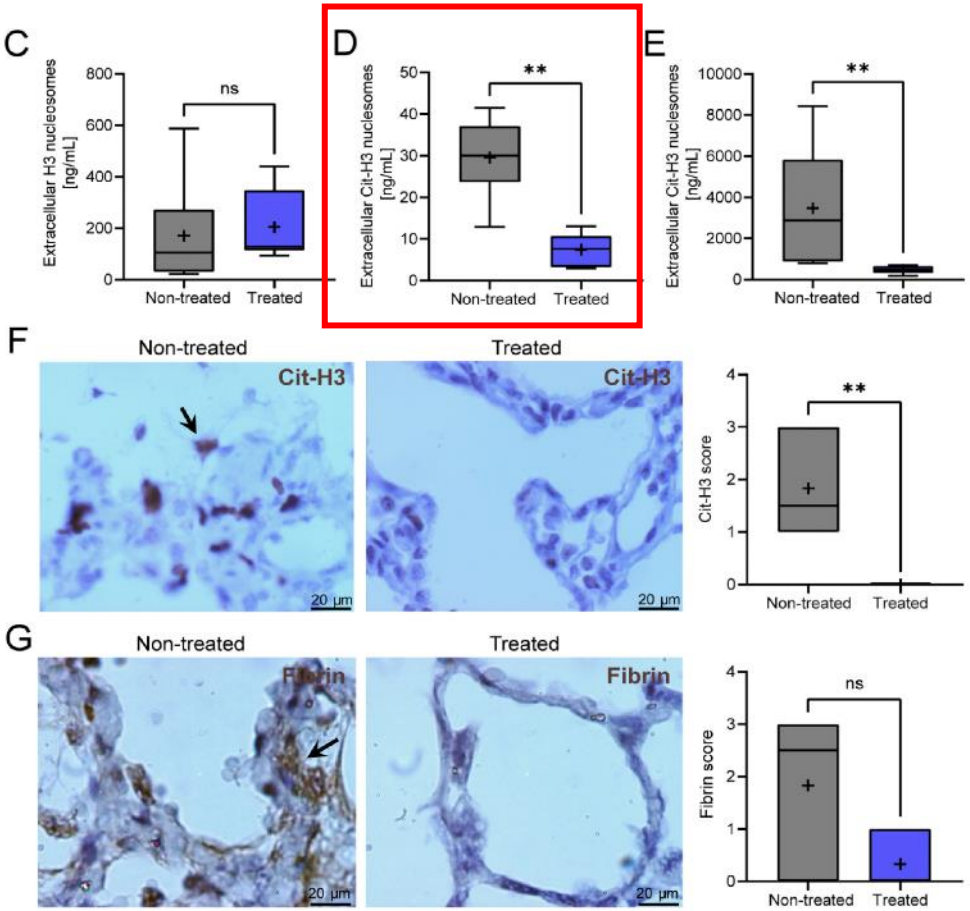

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Our human data is consistent with this animal model of ARDS

Respiratory parameters at time of admission and ARDs

				Nu.Q® H3.1 levels Sepsis	Nu.Q® H3.1 levels Septic Shock	Statistical
No ARDs	138 (14.2%)	70 (15.8%)	63 (12.1%)	285.7	647.8	0.0017
Mild ARDs	201 (20.7%)	102 (23%)	96 (18.5%)	396.3	646.7	0.0044
Moderate ARDs	436 (44.9%)	193 (43.6%)	243 (46.7%)	465.5	921.6	***
Severe ARDs	196 (20.2%)	78 (17.6%)	118 (22.7%	540.1	1,306	***

Data on file

Results from other Clinical Studies

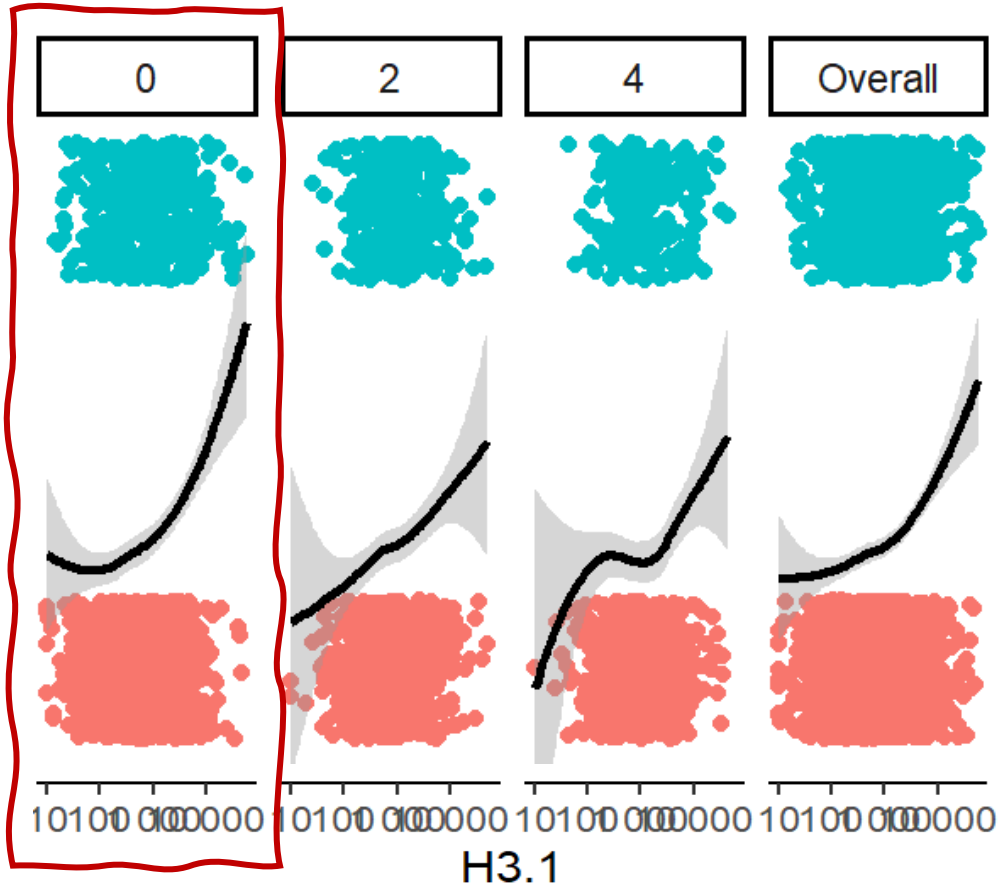
Dr. Andrew Retter

Volition

Studies at Centers of Excellence: >3000 patients

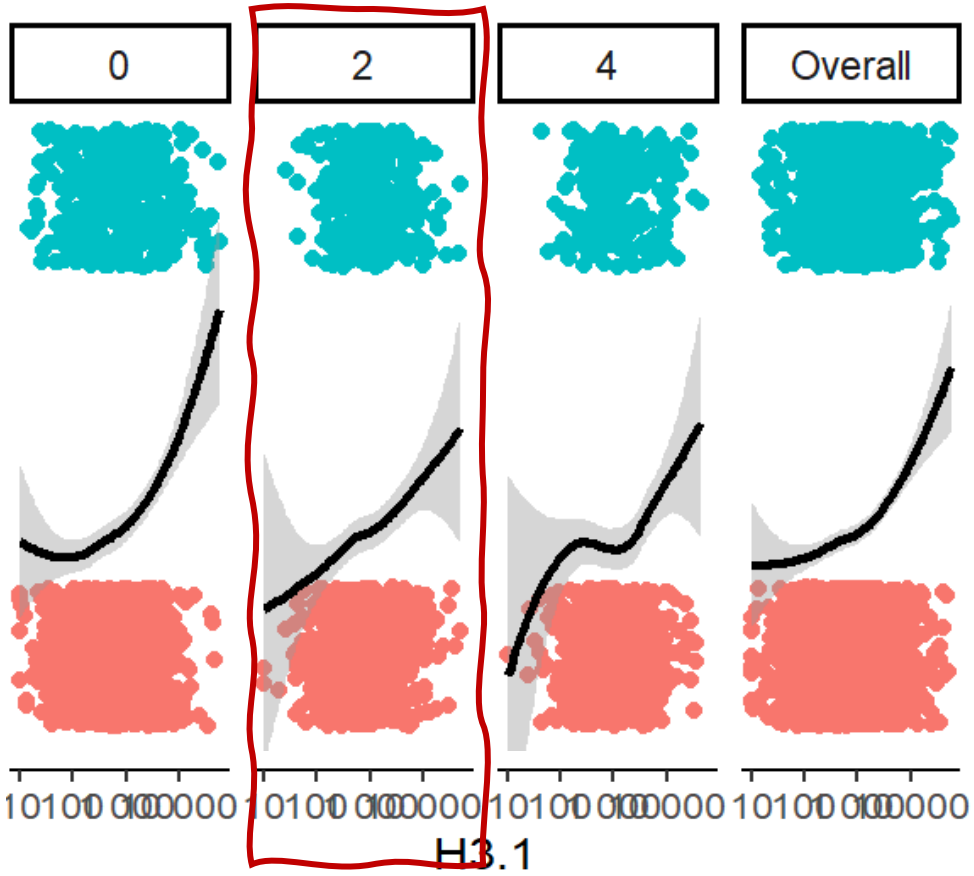
Study	Country	Description	Cohort Size
SISPCT	Germany	Retrospective analysis of prospectively collected cohort	971 intensive care patients Multiple timepoints
Amsterdam UMC	Netherlands	Retrospective analysis of prospectively collected cohort	1,713 intensive care patients Multiple timepoints
RHU RECORDS	France	Prospective, multi-center, placebo controlled, bio-marker-guided, adaptive Bayesian design basket trial	1,500 intensive care patients Interim analysis of 416 patients

Relationship with mortality



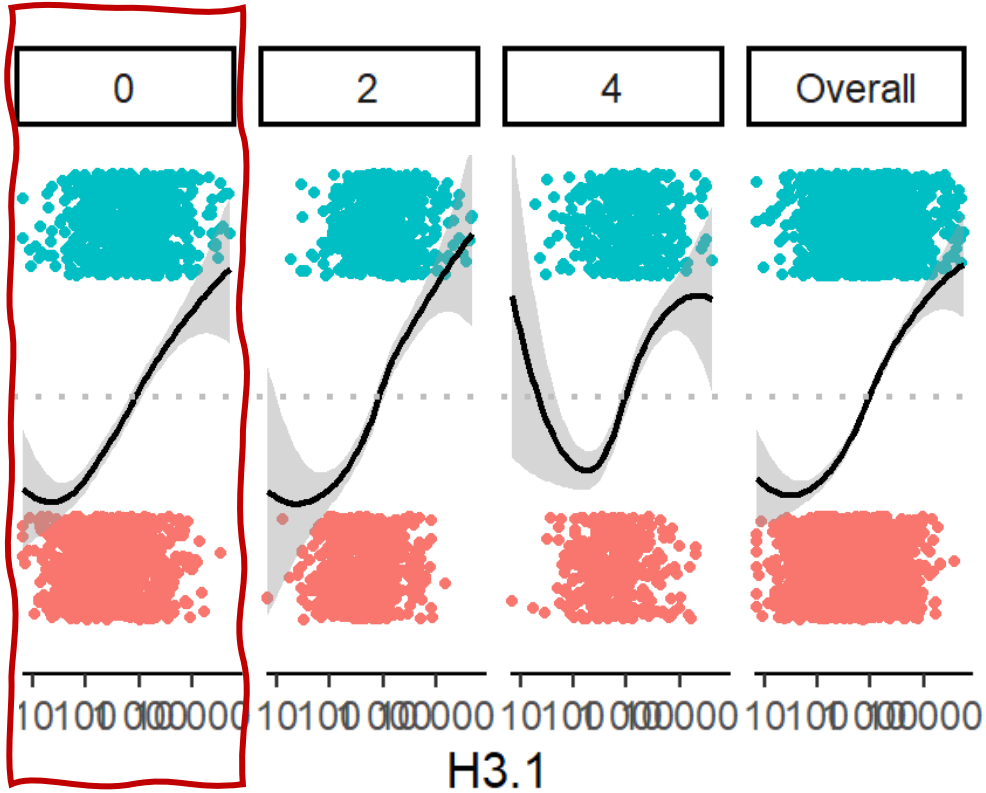
Amsterdam UMC Data Set, data on file

Relationship with mortality



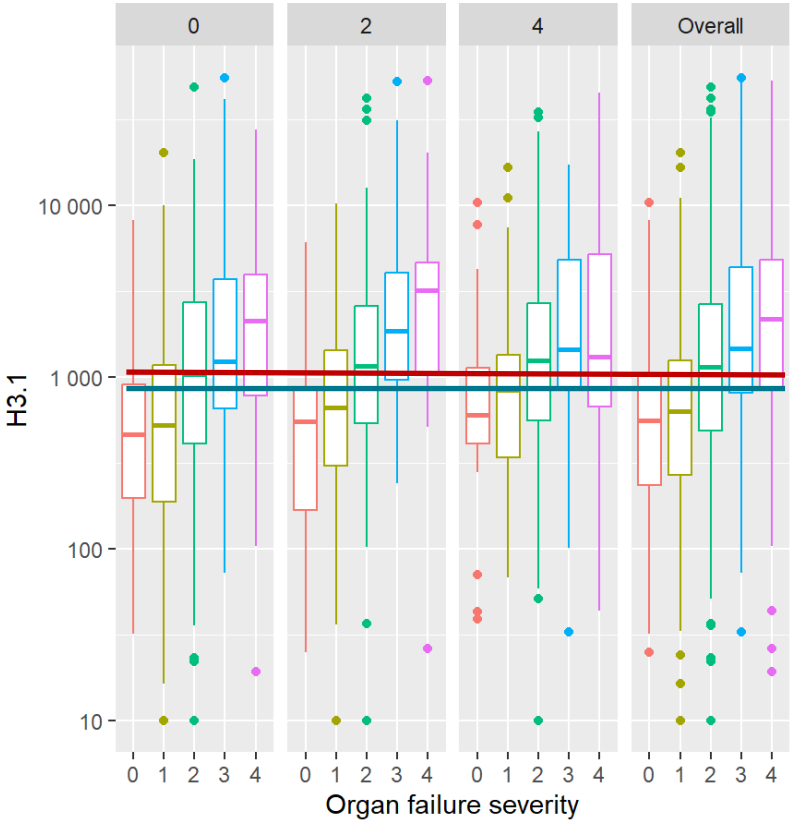
Amsterdam UMC Data Set, data on file

Relationship with AKI



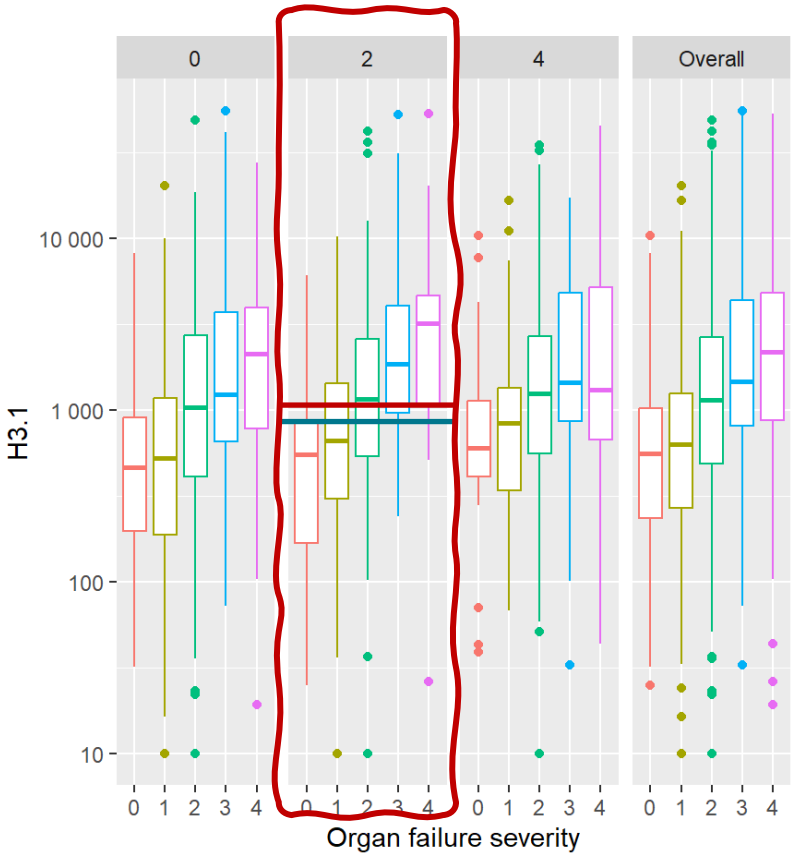
Amsterdam UMC Data Set, data on file

Total number of organ failures



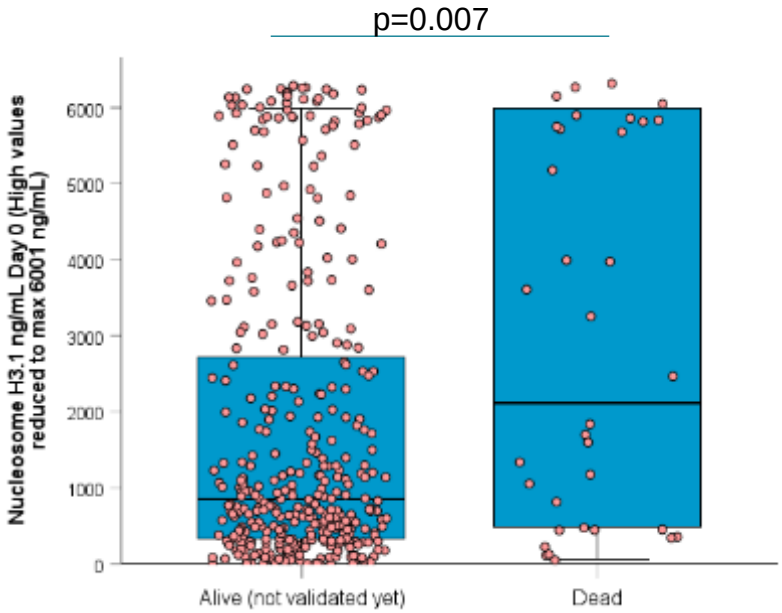
Amsterdam UMC Data Set, data on file

Total number of organ failures



Amsterdam UMC Data Set, data on file

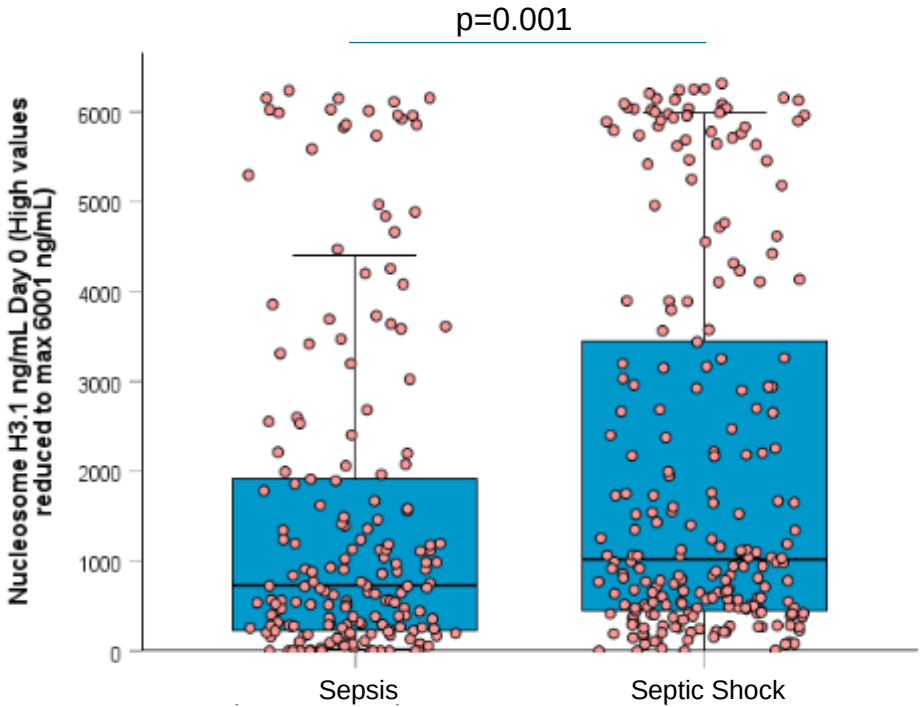
H3.1 levels and outcome: Higher initial level of H3.1-nucleosomes in patient who will die (7 days follow-up)



	Alive	Dead
n=	374	34
Median (ng/ml)	846	2106,8

Records Data Set, data on file

Higher level of H3.1 in septic shock group

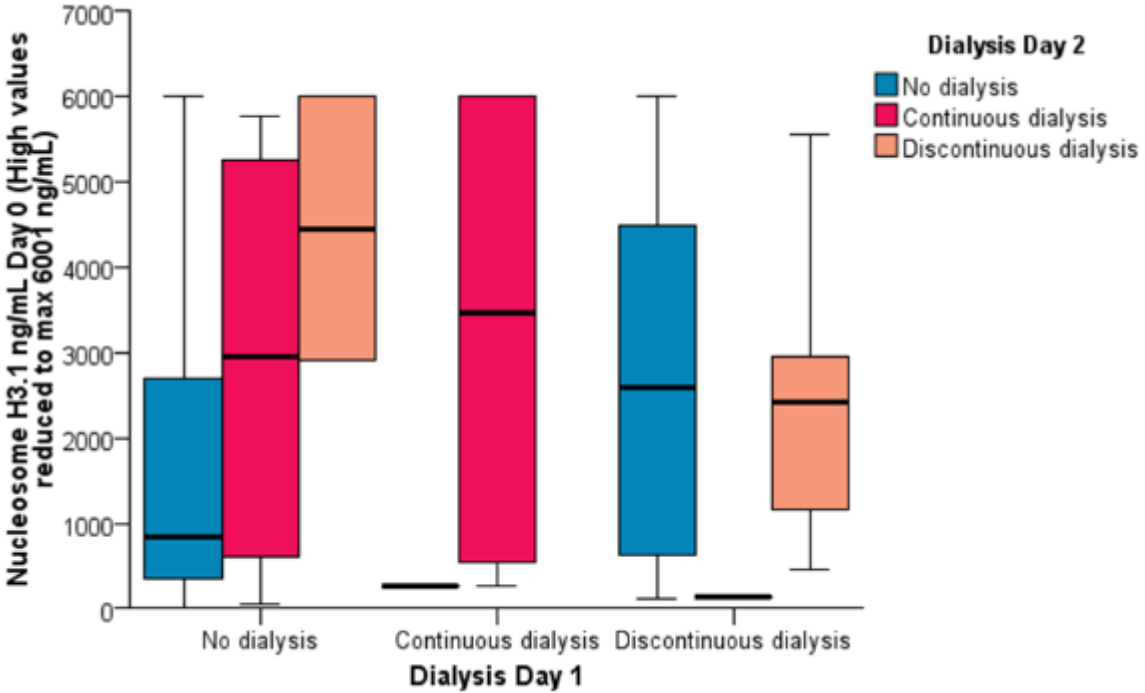


RECORDS

	Sepsis	Septic Shock
n=	183	233
Median (ng/ml)	727	1029

Records Data Set, data on file

H3.1 levels at admission is higher in patient who will need RRT at Day 1 and/or Day 2



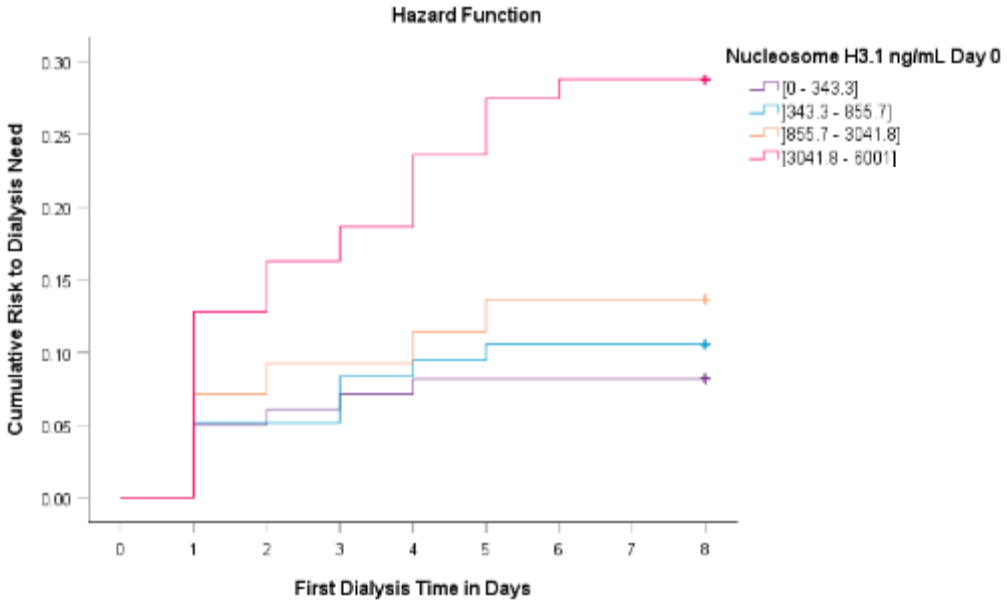
DAY 1	No dialysis	Continuous	Discontinuous
n=	375	18	11
Median (ng/ml)	847,1	3460	2424,4

Records Data Set, data on file

The risk to need any RRT increases with the level of nucleosomes at Day 0

56 patients had at least one (any) RRT within 7 Days

	Frequency	Percent
No dialysis	348	83.7
Dialysis	56	13.5
Unknown	12	2.9
Total	416	100.0



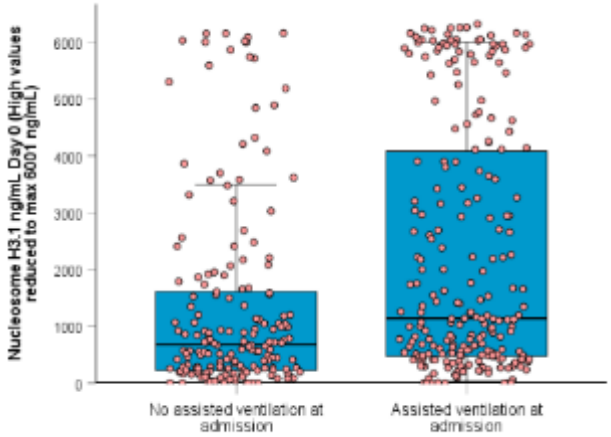
➔ The distributions are **significantly ≠** between categories (p = 0,002)

Records Data Set, data on file

Higher level of H3.1-nucleosomes in patient who required respiratory support (at admission)

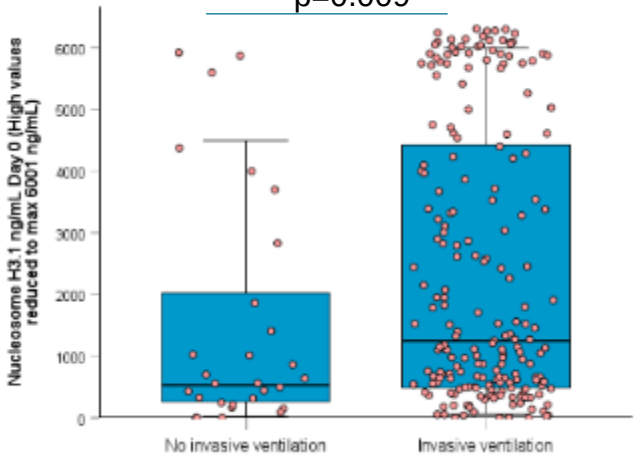
RHU RECORDS

p<0.001



	No assisted ventilation	Assisted ventilation
n=	181	235
Median (ng/ml)	673,4	1132,4

p=0.009



	No invasive ventilation	Invasive ventilation
n=	29	206
Median (ng/ml)	528,6	1261,9

Records Data Set, data on file

Concluding Remarks

Professor Djillali Annane

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Executive Summary: consolidated conclusions

Results from three independent studies totalling over 3,000 patients

These findings are consistent across cohorts[1-3]

An elevated H3.1 level reflects a dysregulated immune response and is associated with:

- a risk of **increased mortality**
- an increased risk of **septic shock**
- an increased risk of **(multi-) organ failure**
- an increased risk of **ARDS**
- an increased risk of **renal failure**

…could be thought of as a Treatable Trait in sepsis management

1. German Data Set, data on file; 2. Amsterdam UMC Data Set, data on file; 3. RHU Records Data Set, data on file

Question & Answer Session

Volition