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Introduction to the Special Volume of Abstracts: 21st Congress of the ESS - 2025

Dear Readers,

We are proud to present this special volume, which includes the abstracts submitted to the 21st Biennial Congress of the European Shock Society (ESS), held in Dubrovnik, Croatia, from September 18 to 21, 2025.

The ESS has long been a hub for interdisciplinary research and clinical innovation in shock, sepsis, trauma, and critical care. As we move into our fifth decade, this collection reflects the continued evolution and expansion of our scientific mission. It brings together the work of researchers from across Europe and around the world—established experts, early-career investigators, and rising voices—united in their commitment to understanding and improving outcomes for critically ill patients.

This volume is more than a snapshot of current research. It is a symbol of the enduring commitment of the ESS to fostering excellence, dialogue, and inclusivity across disciplines and generations. We are especially proud to highlight new approaches rooted in multi-omics, precision medicine, systems biology, and innovative clinical strategies—approaches that are reshaping our understanding of pathophysiology and therapeutic intervention in acute care settings.

We extend heartfelt thanks to all authors who submitted their work, and to the reviewers and organizers who made this publication possible. We hope these abstracts serve not only as a preview of engaging presentations to come, but also as a source of inspiration and connection across our field.

Warm regards,
Borna Relja

[Please note that submissions received after the deadline could unfortunately not be considered for inclusion, and not all invited speakers were able to provide their abstracts.]

SEPSIS-INDUCED T LYMPHOCYTE ALTERATIONS: FROM PATHOPHYSIOLOGY TO THERAPEUTIC TRANSLATION

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Sepsis, a life-threatening organ dysfunction resulting from a dysregulated host response to infection, is characterized by profound alterations in the immune system, particularly affecting T lymphocytes. These changes play a critical role in the immunosuppression observed during and after the acute phase of sepsis, significantly contributing to increased morbidity and mortality. During sepsis, T lymphocytes undergo extensive phenotypic and functional changes, including increased apoptosis, exhaustion, and impaired cytokine production. A marked reduction in $CD4^+$ and $CD8^+$ T cell counts occurs early due to heightened apoptotic signaling, compromising the adaptive immune response. Surviving T cells often express high levels of inhibitory receptors such as PD-1 and CTLA-4, indicative of T cell exhaustion, which further impairs their effector functions. Additionally, an increased proportion of regulatory T cells contributes to a more immunosuppressive environment, further weakening antimicrobial defenses.

The mechanisms underlying these alterations are not yet fully understood and likely involve a complex interplay of pro- and anti-inflammatory cytokines, metabolic dysregulation, and dysfunction of antigen-presenting cells. Recent evidence also implicates mitochondrial dysfunction and epigenetic reprogramming in sustaining long-term T cell impairment. These immune derangements increase susceptibility to secondary infections and compromise long-term immune surveillance, including vaccine responses and tumor immunity.

A better understanding of the nature and mechanisms of T lymphocyte alterations in sepsis is essential for the development of targeted immunomodulatory therapies. Strategies aimed at reversing T cell exhaustion, inhibiting apoptosis, or restoring metabolic competence are under investigation, with promising findings emerging from preclinical and early clinical studies.

In this invited talk, we will present novel insights into both intrinsic and extrinsic mechanisms of sepsis-induced T lymphocyte dysfunction and discuss emerging clinical trials evaluating immune-adjuvant therapies in sepsis.

PRECISION MEDICINE IN SEPSIS

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Sepsis is a complex and heterogeneous syndrome characterized by a dysregulated host response to infection, leading to life-threatening organ dysfunction and high mortality rates. Traditional management strategies, while effective for some, often fail to address the variability in clinical presentation and therapeutic response among patients. Precision medicine offers a promising paradigm shift by aiming to tailor interventions based on individual patient characteristics, such as molecular, and clinical profiles. Advances in omics technologies and biomarker discovery have enabled the identification of distinct sepsis endotypes and phenotypes, facilitating more accurate patient stratification and the development of targeted therapies. This approach holds the potential to improve outcomes by delivering the right treatment to the right patient at the right time. Recent research has focused on integrating prognostic and predictive enrichment strategies, using biomarkers to identify high-risk individuals and those likely to benefit from specific interventions. Despite these advances, significant challenges remain, including the need for rapid bedside diagnostics, validation of novel biomarkers, and the translation of precision medicine approaches into routine clinical practice.

All abstracts are listed in alphabetical order based on the presenting author's last name.

1

IMPACT OF EARLY LIFE STRESS AND SEX ON IMMUNE CELL METABOLISM AFTER COMBINED ACUTE SUBDURAL HEMATOMA AND HEMORRHAGE IN SWINE

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Background: Upon activation immune cells undergo a metabolic switch from mitochondrial oxidative phosphorylation to a more glycolytic state. We recently demonstrated that ELS modified immune cell radical production in a sex-dependent manner in naïve, anesthetized swine without any further pathophysiological challenge. To investigate the effect of ELS and sex on immune cell metabolism in a long-term, resuscitated porcine model of combined acute subdural hematoma (ASDH)-induced brain injury and hemorrhage (HS).

Methods: In sexually mature male and female swine, ELS was induced by early (ELS) vs. standard (control) weaning on postnatal day 21 and 28-35, respectively (n = 11 in each group). Thereafter, anesthetized and (neuro)surgically instrumented animals underwent 2 hours of ASDH (subdural injection of autologous blood) and subsequent HS (passive blood removal), followed by up to 48 hours of "TBI-targeted" ICU-care comprising re-transfusion of shed blood, fluid resuscitation and continuous i.v. noradrenaline titrated to maintain cerebral perfusion pressure at baseline values. PBMC and granulocytes were isolated (ficoll density gradient centrifugation) before ASDH and HS as well as at 24 and 48 hours of ICU care. Mitochondrial respiration was quantified via High Resolution Respirometry, superoxide anion ($O2^{\bullet -}$) production was measured before and after stimulation with E. coli particles via Electron Spin Resonance.

Results: Both PBMC and granulocyte mitochondrial respiration and $O2^{\bullet -}$ production progressively increased over time. At 48 hours of ICU care, ELS females showed significantly lower granulocyte maximal mitochondrial electron transport capacity in the uncoupled state than controls, while there was no difference in males. Neither PBMC mitochondrial respiration nor $O2^{\bullet -}$ production showed any intergroup difference.

Conclusions: There was hardly any effect of ELS and sex on immune cell metabolism, possibly due to the ICU care-related continuous infusion of noradrenaline and/or *i.v.* anesthetics propofol, midazolam and remifentanil.

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2

CARDIAC MITOCHONDRIAL RESPIRATORY ACTIVITY DURING LONG-TERM, RESUSCITATED PORCINE ACUTE SUBDURAL HEMATOMA AND HEMORRHAGE: INTERPLAY OF NORADRENALINE INFUSION, ADRENERGIC RECEPTORS, MITOCHONDRIAL RESPIRATORY COMPLEXES AND ROS/RNS

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Background: Noradrenaline, the first-line drug for hemodynamic management of circulatory shock, was shown to be related to mitochondrial dysfunction and oxidative stress. We recently showed inverse relationships between myocardial mitochondrial respiration and both plasma catecholamine levels and β2-adrenergic receptor expression in naïve, anesthetized swine without any further pathophysiological challenge, i.e., at physiological catecholamine concentrations. To investigate the effect of supra-normal plasma noradrenaline levels on markers of oxidative and nitrosative stress, myocardial mitochondrial respiration, and β-adrenergic receptor and mitochondrial complex expression in a long-term, resuscitated porcine model of combined acute subdural hematoma (ASDH) and hemorrhage (HS).

Methods: 37 anesthetized and (neuro)surgically instrumented animals underwent 2 hours of ASDH and subsequent HS (passive blood removal), followed by up to 48 hours of "TBI-targeted" ICU-care comprising re-transfusion of shed blood, fluid resuscitation and continuous i.v. noradrenaline titrated to maintain cerebral perfusion pressure at baseline values. Blood samples (catecholamines: liquid-chromatography/tandem-mass-spectrometry; superoxide anion ($O_2^{\bullet -}$): electron spin resonance; isoprostanes: ELISA; NO metabolites $NO_2^- + NO_3^-$: chemoluminescence; DNA strand-breaks: "tail moment" of the alkaline "comet assay") were taken before, immediately after ASDH and HS and at the end of the experiment. In immediate *post mortem* heart apex specimen mitochondrial respiration (High Resolution Respirometry) and expression of β-adrenergic receptors, mitochondrial respiratory complexes and nitrotyrosine formation (immunohistochemistry) were measured.

Results: While neither myocardial oxidative phosphorylation nor maximum electron transfer capacity in the uncoupled state were related to noradrenaline levels ($r = 0.06$, $p = 0.744$), both were directly related to β1-adrenergic receptor expression ($r = 0.32$, $p = 0.068$ and $r = 0.38$, $p = 0.025$, respectively). In contrast, blood $O_2^{\bullet -}$ levels were directly related to noradrenaline concentrations and myocardial β1-adrenergic receptor expression ($r = 0.40$, $p = 0.018$, and $r = 0.44$, $p = 0.009$, respectively).

Conclusions: Catecholamine-induced mitochondrial alterations and oxidative stress could be mediated by β1-adrenergic receptor density independently of catecholamine level.

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3

USING EXTENDED INFLAMMATION PARAMETERS TO PREDICT SEPTIC SHOCK

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Background: Sepsis is a major cause of mortality and morbidity and early diagnosis and appropriate management of sepsis can help reduce mortality and morbidity. Various markers of sepsis like procalcitonin (PCT) and C-reactive protein (CRP), are used in clinical practice, but their specificity and sensitivity may vary depending on the type of infection and individual patient characteristics. A complete blood count (CBC) parameter, Monocyte distribution width (MDW) has shown similar performance to PCT and CRP as a diagnostic marker for identifying patients with sepsis and recently another set of CBC markers named as extended inflammation parameters (EIP) have been used to predict severe sepsis. The aim of our study was to assess the sensitivity and specificity of Extended inflammation parameters (EIP), done at the first presentation in Emergency Department, like neutrophil granularity intensity (NEUT-GI), neutrophil reactivity intensity (NEUT-RI); immature granulocyte count (IG#); and other EIP in early detection of severe sepsis/septic shock.

Methods: This was a retrospective electronic chart review of patient admitted to ICU in Tawam hospitals with sepsis between January 2023 to December 2023 and compared with medical clinic patient without any sepsis. The stored information from first CBC sample on first presentation was reviewed via XN-1000 haematology analyzer (Sysmex Corp., Kobe, Japan) and these parameters were calculated retrospectively and the predictive values of EIP to predict severe sepsis/septic shock was calculated using area under receiver operator curve (AUC).

Results: Various EIP parameters predicted severe sepsis, but IG# was the most sensitive EIP at cut-off of >0.03 (sensitivity 83.0 %, specificity 85.6%; AUC 0.909, $p < 0.0001$) and NEUT-RI was most specific at cut-off of >47.8 (sensitivity 66.0%, specificity 89.4%; AUC 0.793, $p < 0.0001$). Using a statistical prediction model a formula was developed i.e. IG# + -NEUT-RI/350 using a cut off >0.17 which outperformed all markers (sensitivity 84.9%, specificity 89.4%; AUC 0.922, $p < 0.0001$).

Conclusions: EIP novel markers especially IG# and NEUT-RI may predict severe sepsis with shock with reasonable accuracy.

4

TARGETING NECROPTOSIS AS A PROMISING PHARMACOLOGICAL STRATEGY TO COUNTERACT MULTIORGAN FAILURE IN SEPSIS

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Background: The necrosome complex, composed of Receptor Interacting Serine/Threonine-Protein Kinases 1 and 3 (RIPK1, RIPK3), along with Mixed Lineage Kinase Domain-Like (MLKL), orchestrates necroptosis, a programmed form of inflammatory cell death implicated in several pathological diseases. However, the role of the RIPK1-RIPK3-MLKL complex in the pathogenesis of sepsis remains poorly explored. This study investigates the therapeutic potential of GSK2593074A (GSK'074), a potent dual inhibitor of RIPK1 and RIPK3, to mitigate hyperinflammatory responses and associated multiorgan failure in experimental sepsis.

Methods: Experimental sepsis was induced by caecal ligation and puncture (CLP) in 30 male, four-month-old C57BL/6OlaHsd mice. One and six hours post-CLP or Sham surgery, mice were randomized to receive either GSK'074 (4.65 mg/kg) or vehicle via subcutaneous injection. At 24 hours, clinical severity score was assessed, and blood and tissue samples were collected for further analysis. Statistical significance ($p < 0.05$) was determined using one-way ANOVA with Bonferroni's post-hoc test.

Results: CLP-induced sepsis led to a marked multiorgan dysfunction, as evidenced by elevated plasma levels of the transaminases (ALT, AST), creatinine, urea, lactate dehydrogenase (LDH), and amylase, along with a robust systemic cytokine storm (a panel of 23 pro- and anti-inflammatory cytokines). GSK'074 treatment significantly attenuated all these pathological markers altered by sepsis. In liver tissue, sepsis triggered substantial neutrophil

infiltration and activation of the RIPK1-RIPK3-MLKL pathway, which was accompanied by enhanced nuclear translocation of NF-κB. GSK'074 effectively suppressed necrosome activation, thereby mitigating hepatic inflammation. Overall, GSK'074 improved the clinical severity score in septic mice.

Conclusions: Our findings underscore the central role of the RIPK1-RIPK3-MLKL necrosome complex in driving sepsis-associated inflammation and organ damage. Furthermore, GSK'074 emerges as a promising pharmacological candidate to counteract the hyperinflammatory response and multiorgan dysfunction in sepsis.

5

PHARMACOLOGICAL INHIBITION OF CDK9 AS A NOVEL THERAPEUTIC STRATEGY FOR SEPSIS-ASSOCIATED ENCEPHALOPATHY

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Background: Sepsis-associated encephalopathy (SAE) represents an acute manifestation of diffuse cerebral dysfunction driven by systemic inflammation in sepsis, contributing to increased mortality and prolonged ICU stays. Despite improved understanding of its pathophysiology, no specific pharmacological treatments are available. In this context, cyclin-dependent kinase 9 (CDK9), a ubiquitously expressed kinase, has emerged as a promising therapeutic target due to its central role in promoting inflammation across multiple diseases. However, its role in sepsis and SAE remains unexplored. This study investigates the effects of LDC000067, a selective CDK9 inhibitor, on neuroinflammation and blood-brain barrier (BBB) dysfunction associated with SAE.

Methods: Sepsis was induced by caecal ligation and puncture (CLP) in male C57BL/6OlaHsd mice. One hour post-CLP or sham surgery, animals received a single intravenous dose of LDC000067 (50 mg/kg) or vehicle. Samples were collected at 24 hours. In vitro, human brain microvascular endothelial cells were exposed to a septic-like stimulus (LPS +TNFα+IFNγ) with or without LDC000067. Data were analyzed by one-way ANOVA with Bonferroni post-hoc.

Results: In vivo, CDK9 inhibition significantly attenuated sepsis-induced multiorgan dysfunction and systemic cytokine storm. These anti-inflammatory effects translated into improved clinical severity scores in septic mice. Notably, sepsis induced a marked increase in CDK9 expression in the prefrontal cortex, with accompanying BBB disruption, evidenced by elevated plasma neurofilament light chain levels and pronounced microglial activation, as revealed by 3D microglial morphometric analysis. Treatment with LDC000067 effectively counteracted these brain abnormalities. In vitro investigation showed that LDC000067 attenuated claudin-5 degradation in brain microvascular endothelial cells exposed to septic-like stimuli. This effect correlated with a significant reduction in FITC-dextran permeability, indicating functional preservation of endothelial barrier integrity.

Conclusions: Our data show for the first time that CDK9 contributes to SAE by driving BBB dysfunction and neuroinflammation. Its pharmacological inhibition may represent a novel therapeutic strategy for SAE.

6

EFFECTS OF CELL-LINEAGE RESTRICTED PD-L1 GENE EXPRESSION ON EXPERIMENNTAL MURINE SHOCK/ SEPSIS INDUCED LUNG INJURY

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Background: Our laboratory and others have shown that Programmed cell death receptor-Ligand 1 (PD-L1), contributes to the development of shock/ sepsis induced morbidity/ mortality, but its role appears to vary across organ/cell type. Here we leverage the construction of Cre-lox mouse models to produce mice constitutively lacking either PD-L1 gene expression on endothelial cells (*ec*PD-L1$^{-/-}$) or neutrophils (*pmn*PD-L1$^{-/-}$), respectively, to test the hypothesis that endothelial cell as opposed to neutrophil deficiency PD-L1 differentially contributes to shock/ sepsis induced lung injury/ death.

Methods: Adult male C57BL/6 (WT), *ec*PD-L1$^{-/-}$, *pmn*PD-L1$^{-/-}$ and/ or mixed ^{flox}-no cre (Control) mice were subjected to either hemorrhage (Hem) followed 24 hrs by cecal ligation & puncture (CLP) (Hem/CLP) or sham Hem/ sham CLP (Sham). 15-day survival was followed and in a separate set of animals (assessed at 24 hrs post-procedure) blood, lung fluids (BALF) and lung tissues were harvested, processed/ stained for: flow cytometry, cytokine/ chemokine/ angiopoietin ELISAs and indices of organ injury.

Results: 14-day mortality in the *ec*PD-L1$^{-/-}$ mice was lower than in the Hem/CLP Control group, while the mortality rate was increased in the *pmn*PD-L1$^{-/-}$ vs. Controls. Lung vascular permeability was also markedly decreased in the *ec*PD-L1$^{-/-}$ Hem/CLP mice but no such decline was seen in the lungs of *pmn*PD-L1$^{-/-}$ mice. While Hem/CLP increased the lung tissue, BALF and blood levels of several cytokine/ chemokine/ angiopoietin levels, the concentrations of lung tissue, BALF MCP-1 and blood BUN markedly declined in the *ec*PD-L1$^{-/-}$ vs. Control mice. Alternatively, the lung levels of Angiopoietin-2 and BALF MIP-2 and IL-6 concentrations significantly increased in Hem/CLP *pmn*PD-L1$^{-/-}$ animals.

Conclusions: Taken together, these results support the hypothesis we have previously proffered that expression of PD-L1 on endothelial cells has a morbid impact. However, surprisingly, we have also uncovered a potential immune protective role of PD-L1 expression on neutrophils.

7

EARLY SEPSIS RISK ASSESSMENT IN MULTIPLE TRAUMA PATIENTS USING THE SEPSIS AS TRAUMA OUTCOME PREDICTION (STOP) SCORE – A PROGNOSTIC ACCURACY OF 59.4% WITHIN 24 HOURS POST-EMERGENCY DEPARTMENT ADMISSION

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Background: Sepsis is a critical complication observed in multiple-trauma patients. Early therapeutic decisions can influence the inflammatory response to traumatic injury, potentially promoting sepsis development. Thus, early recognition of patients at risk is crucial. This study focuses on identifying predictive parameters for sepsis risk within 24 hours following hospital admission in severely injured patients. To assess the risk of sepsis development in multiple trauma patients, we combined routinely collected clinical variables with a novel biomarker - the extracellular particle (EP) rate in plasma.

Methods: Patients aged 18 to 80 years with severe traumatic injury (injury severity score (ISS) $\geq$ 16) were included. Blood samples were collected immediately upon admission and subsequently processed by centrifugation. Plasma-EPs were stained with Calcein-AM and analyzed via flow-cytometry. Clinical parameters were assessed retrospectively. The prognostic value of significant variables was calculated using receiver operative characteristics analysis. Significant predictors from four categories were integrated into the calculation of the STOP score.

Results: Of the 124 patients included, 29 (23.4%) developed sepsis. Significant differences between sepsis and non-sepsis cohorts included

an increased ISS (34 vs. 24), decreased leukocyte counts on day one post-trauma (leukocytes d1; 7.42 vs. 10.11 x 10^9/L), higher packed red blood cell transfusion (PRBC) rates in the first 24 hours (5.36 vs. 2.48 units), and decreased rates of EPs (12418 vs. 16978 particles/μL). For STOP score calculation, two points were assigned for values below the cut-off in leukocytes (≤8.50) and EPs (≤12639), and one point each for 24 h-PRBC-transfusion (≥1.00) and ISS (≥25). A STOP score ≥ 4 had a positive predictive value of 59.4% for sepsis development, with a sensitivity of 65.5% and specificity of 86.3%.

Conclusions: Integrating ISS, leukocytes d1, 24 h-PRBC-transfusion rate and EP rate into the STOP score supports very early identification of multiple trauma patients at elevated risk for sepsis.

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DYNAMIC PROFILING OF TNF-PRODUCING IMMUNE CELLS PREDICTS SURVIVAL AND ORGAN DYSFUNCTION IN POLYTRAUMA

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Background: The cytokine Tumor Necrosis Factor (TNF) triggers pro-inflammatory activation of immune cells. The stimulated production of TNFa correlates with clinical outcome in septic and critically ill non-septic patients. Moreover, a reduced capacity to produce TNFα in LPS stimulated blood is predictive for patients' outcome in trauma, surgery and sepsis. We hypothesized whether the phenotypic characterization of TNF producing cells and the functional release of TNF within 24 hours serves to identify patients at risk for adverse outcome within 24 hours

Methods: Polytraumatized patients (n = 28; Injury Severity Score (ISS) ≥ 18) blood samples were collected in a level one academic trauma center on admission, after 8 and 24 hours. Systemic myeloid derived cells and their subtypes were phenotyped and quantified via counting and flow cytometry. Spontaneous and stimulated TNF production in whole blood (WB) was assessed via Enzyme Linked Immuno Spot (ELISpot). Alle values were used to predict in-hospital survival and multiple organ dysfunction syndrome within 24 hours using Receiver Operating Curves (ROCs) with an Area under the Curve (AUC) of > = 0.8.

Results: On admission the absolute systemic cell count of CD62L + ICAM1- neutrophils predicted survival with an AUC of 0.81 (p = 0,0229) whereas the best predictive value for MODS was the total expression of TFN after LPS stimulation (AUC 0.73, p = 0,0502). At 8 hours the PMA-stimulated TNF-production (spot size) predicted MODS with an AUC of 0.86 (p = 0,0017) whereas CD62L + ICAM1- neutrophils best predicted survival (AUC 0,79, p = 0,0458). At 24 hours ROC provided no AUC >0.8.

Conclusions: Our data suggests that the phenotypic and functional characterization of TNF producing cells serves as a predictive tool in polytraumatized patients within hours after injury. Interestingly in-hospital survival and MODS can be predicted at different timepoints underlining the importance of interpreting immundysfunction dynamically.

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DISTINCT HOST RESPONSE SIGNATURES ACROSS SEPSIS ETIOLOGIES IN INDIA

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Background: Sepsis is a heterogeneous condition caused by a range of pathogens, including bacteria and viruses. Most of our current understanding of sepsis comes from high-income settings, despite the highest burden being in low- and middle-income countries (LMICs). Here, we address this gap by directly comparing different infectious causes of sepsis in India to identify shared and pathogen-specific host response patterns.

Methods: Patients fulfilling the sepsis-3 diagnostic criteria were enrolled within 24 hours of ICU admission in Manipal, India. We measured 23 plasma biomarkers reflecting changes in key host response pathways implicated in sepsis pathophysiology: endothelial activation and coagulation, organ damage and inflammation, cytokine response, and chemokine release. We compared sepsis caused by pathogen groups and between 14 different pathogens. We applied variance partitioning analysis to quantify the pathogen share on host-response variance.

Results: We included 974 sepsis patients; in 54.1% a causative pathogen was identified (311 bacterial, 149 viral, 67 polymicrobial). Variance partitioning analysis revealed that, in addition to severity of disease, the causative pathogen independently explained a significant proportion of the variation in host response biomarker levels (4%-24%). Bacterial sepsis was associated with stronger activation of endothelial, coagulation, inflammation, and cytokine responses as compared to viral sepsis. Sepsis due to *Orientia tsutsugamushi* (scrub typhus) was particularly associated with profound endothelial cell activation (syndecan-1, VCAM-1), systemic inflammation (ferritin) and cytokine release (TNF, IFN-γ, GM-CSF). Sepsis caused by viral hemorrhagic fevers (dengue, Kyasanur Forest Disease) was accompanied by higher biomarker levels across all pathophysiological domains as compared to sepsis caused by SARS-CoV2 or influenza.

Conclusions: Distinct host response signatures across pathogens highlight the biological heterogeneity of sepsis in this LMIC cohort. These findings underscore the need for pathogen-aware diagnostics and tailored treatment strategies, particularly in tropical settings where a diverse range of infectious agents contribute to sepsis.

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WHEN WEIGHT FALLS, MITOCHONDRIA RISE: EARLY SEPSIS-INDUCED WEIGHT LOSS BOOSTS LONG-TERM MITOPHAGY AND MITOCHONDRIAL BIOGENESIS IN THE SKELETAL MUSCLE

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Background: Post-septic long-term muscle weakness is linked to mitochondrial dysfunction. However, regulation of mitochondrial biogenesis and mitophagy in skeletal muscle (SkM) after sepsis remains elusive. To assess long-term mitochondrial biogenesis (Pgc1a-Nrf1/2-Tfam) and mitophagy (Pink1-Parkin pathway) activity in SkM in a cecal ligation and puncture (CLP) mouse model of chronic sepsis.

Methods: 82 female C57BL/6 mice (3–4 months) underwent CLP and were monitored for body weight (BW), temperature, sickness, serial muscle μCT and RotaRod. At weeks (w) 1, 4, and 12, hindlimb SkMs were harvested for RT-qPCR, Western blot, histology, and high-resolution respirometry. For data analysis, CLP mice were retrospectively stratified into high- and low BW loss at w1 (high/low-BWL-w1) groups and compared to controls.

Results: CLP induced varying BWL, hypothermia, and elevated sickness scores at w1. μCT revealed reduced gastrocnemius volume (V_{GAS}) at w1, correlating with ΔBW. Both BW and V_{GAS} increased from w1 to w12 (independently of BWL-w1) accompanied by stable expression of Fbxo32/MURF1 atrophy markers. Mitochondria biogenesis-related mRNA of *Pgc1α* and *Tfam* remained unchanged. Conversely, Pgc1α protein level was elevated at w1 (~2.7-fold; higher in high-BWL-w1) and w4 (~2-fold); Tfam protein trended upwards to w12 (higher in high-BWL-w1). We observed upregulation of mitophagy-related mRNAs: *Pink1* (~2.25-fold), *Map1lc3a* (~3-fold) and *Map1lc3b* (~2.15-fold) at w1, especially in high-BWL-w1. Western Blot showed increased levels of: Pink1 (~2.4-fold) at w1, Sqstm1 (~2.15-fold) and Parkin (~1.8-fold) at w4 and w12. Their expressions were BWL-w1-dependent: at w4 (Sqstm1, Parkin; higher in high-BWL-w1) and w12 (Parkin; higher in low-BWL-w1). Respirometry demonstrated OXPHOS stability and maximal respiratory capacity gradually decreased from w1 to w12. RotaRod showed no functional differences.

Conclusions: CLP leads to a long-term upregulation of mitophagy and mitochondrial biogenesis pathways despite muscle mass/volume recovery. Importantly, long-term activities of both pathways appear to be more enhanced in high-BWL-w1 subgroup.

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ALCOHOL-DEPENDENT REGULATION OF ENERGY METABOLISM BY NF-KB SIGNALING IN HEPG2 CELLS

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Background: NF-κB signaling modulates both immune responses and metabolic pathways after trauma. Recent research indicates an alcohol-dependent regulation of NF-kB signaling, resulting in reduced immune response in the liver after trauma. Deubiquitinated enzymes such as A20 appear to play an important role in this regulation. While the interplay between NF-κB and metabolic pathways is recognized, and vice versa, the influence of alcohol on this interaction remains poorly understood. To investigate whether alcohol-dependent regulation of NF-κB signaling modulates the metabolic response in hepatocytes?

Methods: HepG2 wildtype and HepG2 A20 knockout cells (A20 KO) were stimulated for 24 h with a low (50 mM) or high (200 mM) concentration of alcohol. Metabolic activity was measured using the Seahorse XF Mito Stress test. The expression of glycolysis- and oxidative phosphorylation-related genes was evaluated.

Results: In wildtype cells, high dose alcohol significantly increased basal and ATP-linked respiration without affecting ATP production rates from glycolysis or oxidative phosphorylation. Gene expression of key metabolic markers such as *HK2*, *LDH*, *HSD17B10* or *FOXRED1* remained unchanged in wildtype cells. Alcohol stimulation in both concentrations had no influence on metabolism in HepG2 A20 KO cells. However, compared to HepG2 wildtype cells, HepG2 A20 KO cells show an increased basal respiration in unstimulated cells as well as in cells stimulated with low alcohol concentration. In addition, depletion of A20 leads to significantly increased energy production through glycolysis, in form of ATP, even without alcohol stimulation. Furthermore, *LDH* and *HSD17B10* gene expression levels were significantly higher in HepG2 A20 KO cells under both low and high alcohol stimulation compared to wildtype cells, while other genes measured remained unaffected.

Conclusions: Our data demonstrates that alcohol influences energy metabolism in hepatocytes in an A20-dependent manner. Given the central role of NF-κB signaling in the immune and metabolic response to trauma, these results suggest that alcohol-induced alterations in A20 activity may impair hepatic metabolic adaptation after trauma. This highlights A20 as a potential target for mitigating alcohol-related inflammatory and metabolic dysregulation in trauma patients.

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TRAUMA-INDUCED FUNCTIONAL CHANGES OF HUMAN DERMAL LYMPHATIC ENDOTHELIAL CELLS

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Background: The skin is an emerging target tissue after minor and major trauma which is the first layer that is exposed to the inflicting trauma vector. This may cause an enhanced susceptibility to infection and structure-function changes of the corresponding microcirculation including the lymphatic system. The lymphatic endothelium is not only involved in the immune response and barrier function but also in liquid homeostasis. However, little is known about the impact of danger- and pathogen-associated molecular patterns (DAMPS and PAMPS) on the lymphatic endothelium. Therefore, the aim of present study is to in vitro characterize the functional changes of the lymphatic endothelium in response to DAMP and PAMP exposure.

Methods: Human dermal lymphatic endothelial cells (HDLECs) from an adult donor were cultured and stimulated under four conditions, which included a DAMP trauma cocktail (including histones, mtDNA, IL-1ß, IL-6, and IL-8) and LPS as PAMP. In a time-dependent design, the gene expression was quantified by qPCR and the chemokine secretion was determined by ELISA and a LEGENDplex™. Statistical analyses were performed by ANOVA with post hoc testing.

Results: HDLECs responded to trauma cocktail exposure with significantly pro-inflammatory (e.g. ICAM-1, CXCL5, and IL-8) increase. Furthermore, angiogenetic markers (e.g. P*l*GF, sFlt-1, and KDR) were also significantly altered. Of note, the maximal effect within an incubation period of 24 h was seen in IL-8 secretion by an increase of more than nine times higher starting from the 6 h time point indicating a time-dependent pro-inflammatory monocyte attracting.

Conclusions: In conclusion, the lymphatic endothelium seems to play a crucial role in adhesion processes, permeability regulation and inflammatory response. However, the response to DAMPs and PAMPs appears different and requires a future in vivo translation of the findings.

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CHARACTERISING SEPSIS SHORT-TERM MORTALITY RISK USING TWO ORTHOGONAL TRANSCRIPTOMIC SCORES

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Background: Host responses during sepsis are highly heterogeneous, complicating the identification of patients at risk of short-term mortality or those potentially benefitting from targeted therapies. To identify blood gene expression scores that associate with 7-day mortality in sepsis.

Methods: Whole-blood transcriptomes were measured in patients with sepsis at ICU admission using microarray. Logistic regression was used to identify genes significantly associated with 7-day mortality. Genes with expression levels highest in non-survivors and lowest in health were defined as upregulated-severity (US) genes; genes with levels highest in health and lowest in non-survivors as downregulated-severity (DS) genes.

Results: We enrolled 1085 sepsis patients, in whom we identified 315 US genes and 754 DS genes. Gene set enrichment analysis showed that US genes most significantly associated with increased neutrophil degranulation and mitotic G1 phase and G1/S transition; DS genes with decreased interferon signalling. Principal component analyses were performed on the two gene sets separately; the first principal component score of each set was used as a patient's summary score for US genes (US-score) and DS genes (DS-score). US- and DS-scores showed a modest negative correlation ($r = -0.42$). We next determined orthogonal US- and DS-scores using a decorrelation transform, giving more weight to genes orthogonal across the two gene groups and less weight to more correlated genes. Patients within the highest orthogonal US- and DS-scores presented with the highest disease severity (SOFA), had the highest 7-day mortality and were more likely to have an abdominal source of sepsis. Only the orthogonal DS-score was associated with ICU-acquired infections ($P = 0.001$).

Conclusions: Distinct upregulated and downregulated gene sets in whole blood associate with 7-day mortality in sepsis. A newly developed score based on downregulated genes link with the subsequent occurrence of ICU-acquired infections. US- and DS-genes may indicate potentially modifiable targets for sepsis treatment.

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POST SEPSIS EXERCISE THERAPY ENHANCES CD4 T CELL RECOVERY IN THE LUNG

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Background: Sepsis survivors often develop persistent immunosuppression, increasing their risk of recurrent infections. Currently, no effective strategies exist to reverse this dysfunction. While exercise therapy aids post-sepsis recovery of muscular and cardiorespiratory functions, its effects on immune function remain unclear.

Methods: Using a murine model of sepsis induced by cecal ligation and puncture (CLP, 60% survival rate), we investigated the impact of post-sepsis exercise therapy (PSET) on immune recovery. After one week of recovery, survivors underwent four weeks of PSET or remained sedentary (Sed) before infection with Pseudomonas aeruginosa (PA, 5×10^7 CFU, i.n.). CD4 T cell percentages declined from 24 hours to five weeks post-CLP, recovering by 10 weeks, and IFN-γ^+ CD4 T cells remained suppressed, confirming the model's relevance to sepsis-induced immunosuppression.

Results: Notably, PSET improved survival after secondary PA infection by reducing bacterial load and IL-6 levels in the lung. PSET also increased CD4 T cell percentages in the lung compared to Sed. Single-cell RNA sequencing revealed that CD4 T cells from Sed mice exhibited upregulated ribosome biosynthesis pathways, whereas PSET shifted their transcriptomic profile toward naïve control levels and enhanced expression of genes linked to CD4 T cell activation and proliferation.

Conclusions: Our findings indicate that PSET protects against secondary PA infection by promoting CD4 T cell recovery in the lung. Understanding the mechanisms underlying this immunomodulation could inform exercise-mimicking therapies for exercise-intolerant patients to prevent and treat recurrent infections.

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THE EFFECT OF DIFFERENT ADRENORECEPTOR BLOCKERS ON FRACTURE HEALING OF NON-OSTEOPOROTIC AND OSTEOPOROTIC BONE

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Background: Osteoporosis is the most common metabolic bone disease characterized by low bone mass and increased fracture risk. Fracture healing is impaired in osteoporotic patients highlighting the urgent need for new therapeutic strategies. Recent findings suggest that sensory and sympathetic nerve fibers, and particularly adrenergic signaling, play a crucial role during fracture healing and may also contribute to the pathogenesis of osteoporosis. Supporting this, our RNASeq data revealed a marked enrichment of adrenergic signaling pathways in the fracture callus of osteoporotic mice. Moreover, osteoporotic mice displayed increased numbers of neutrophils in the early fracture hematoma. Notably, the treatment of non-osteoporotic mice with the non-selective β-blocker Propranolol immediately before fracture resulted in reduced neutrophil infiltration. We hypothesize that adrenergic signaling contributes to the disregulated early immune response, thereby impairing fracture healing in osteoporotic bone.

Methods: We investigated the effect of different adrenoreceptor blockers - Propranolol (unspecific β-blocker), Butoxamine (specific β2-blocker), and Phentolamine (unspecific α-blocker) - on fracture healing in non-osteoporotic and osteoporotic mice. Postmenopausal osteoporosis was induced by ovariectomy (OVX) at 12 weeks of age. A standardized femur osteotomy stabilized with an external fixator was performed four weeks later. The blockers were injected subcutaneously at the day of fracture and for the following three days. Fracture healing was analyzed at day 1, 3 and 21 using FACS, μCT and biomechanical testing.

Results: Short-term adrenoreceptor blockade did not influence the development of osteoporosis itself. However, β-blocker treatment impaired fracture healing in non-osteoporotic mice, while it mitigated the negative effects of postmenopausal osteoporosis on fracture healing observed by altered bending stiffness and bone formation, most likely due to reduced neutrophil infiltration. In contrast α-blocker treatment failed to improve fracture healing in osteoporotic mice.

Conclusions: Further in-depth analyses are required to determine the potential beneficial effects of β-blockers on osteoporotic fracture healing and to elucidate the underlying effects.

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TEMPORAL DYNAMICS OF INFLAMMATION AND WNT/β-CATENIN SIGNALING IN THE MURINE LUNGS AFTER POLYTRAUMA

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Background: Trauma remains the leading cause of death and disability among Europeans under 40 years of age and can result in direct and indirect lung injuries through complex pathophysiological mechanisms. Nevertheless, research focusing on long-term effects of polytrauma including the lungs is limited. The aim of this study was to investigate

the long-term effects of polytrauma on pulmonary inflammation and regeneration.

Methods: 96 male 12-weeks-old C57BL/6 N mice were randomly assigned to sham or polytrauma group. The polytrauma animals underwent hemorrhagic shock with resuscitation, blunt thoracic trauma, femoral osteotomy with external fixation, and laparotomy. Sham animals received only anesthesia and external fixation. Gene and protein expression in lung tissue and bronchoalveolar lavage fluid (BAL) were assessed on days 1, 3, 5, 7, 14, and 21 post-injury (dpi) by qRT-PCR and ELISA.

Results: CCL2 levels in BAL and lung tissues significantly increased one dpi and remained consistently low at all subsequent time points. A comparable but less pronounced early increase was also observed in sham animals. Polytrauma resulted in an early downregulation of IL-6 and IL-10 expression (1 and 3 dpi) compared to sham, but a biphasic increase at 5, 7 and 21 dpi. This late increase was not observed in BAL. Gene expression levels of *Wnt3a, Wnt10b* and *β-catenin* peaked in sham animals 3 dpi, and gradually decreased until 21 dpi. Same factors showed a peaking expression 5 dpi which remained elevated until 21 dpi in polytrauma. *Sost* expression remained constant in both groups and showed a significant decrease 21 dpi in sham and an increase in polytrauma.

Conclusions: Polytrauma induces a dynamic and prolonged regulation of pulmonary inflammation and repair mechanisms. The observed delayed cytokine responses and sustained activation of Wnt signaling pathway suggest a shift in the timing and nature of lung tissue regeneration following trauma.

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MAPPING THE HOST RESPONSE IN CRITICALLY ILL PATIENTS WITH ASPIRATION PNEUMONIA AT ICU ADMISSION

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Background: The immunopathology of aspiration pneumonia (AP) is poorly understood, even though AP accounts for up to 15% of all cases of community-acquired pneumonia. This hinders opportunities for precision medicine targeting the host response in AP. We aimed to characterize distinct features of the immune response in critically ill patients with community-acquired AP in comparison to non-aspiration community-acquired pneumonia (non-AP) at intensive care unit (ICU) admission.

Methods: We analyzed clinical characteristics, disease outcomes, 42 plasma biomarkers and whole blood transcriptomes in patients with community-acquired AP and non-AP upon admission to the ICU of two tertiary hospitals in The Netherlands. Plasma biomarkers were indicative of key pathophysiological mechanisms in pneumonia, including endothelial cell activation, coagulation activation, inflammation, and organ damage.

Results: A total of 197 patients were enrolled, out of whom 53 had AP and 144 non-AP. Immunosuppression and chronic respiratory insufficiency were more frequent premorbid conditions in the non-AP group. 30-day mortality rates did not differ significantly between groups (34% in AP versus 25.9% in non-AP). AP patients were characterized by reduced plasma biomarker levels indicative of systemic inflammation and endothelial activation. Gene set enrichment analysis of whole blood transcriptomes in patients with AP showed dampened gene expression of genes linked to hemostasis, as well as heightened expression of adaptive and innate immunity pathways.

Conclusions: To our knowledge, this is the first comprehensive multi-omics study on the host response to AP. Our analyses reveal that the immunopathology associated with AP differs considerably from that associated with non-AP upon ICU admission. Our findings may assist in identifying potential targets for future immunomodulatory trials in patients with AP.

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TISSUE FACTOR KNOCKOUT ABOLISHES THE LETHAL PRO-COAGULANT EFFECTS OF MSC-DERIVED EXTRACELLULAR VESICLES IN A RAT MODEL OF HAEMORRHAGIC-TRAUMATIC SHOCK

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Background: Haemorrhagic traumatic shock (HTS) frequently leads to systemic inflammation, coagulopathy, multiple organ failure and death. Mesenchymal stem cell (MSC)-derived extracellular vesicles (EVs) show therapeutic promise in chronic diseases but their potential in critical care remains unclear. We hypothesized beneficial effects of MSC-EVs on organ function and inflammation in a severe HTS rat model.

Methods: HTS was induced via controlled blood loss after laparotomy (targeted base excess ≤-8), followed by restrictive (RR) and full (FR) crystalloid resuscitation mimicking pre-/in-hospital care. Rats were randomized for placebo or 5×10^9 EVs/kg i.v. 20 minutes post-RR. We used two EV types: wildtype (EV^{wt}) and TF-depleted ($EV^{TF\text{-}ko}$). In a mild rat HS experiment, we repeatedly measured pro-coagulative effects of EV^{wt} administration. We separately assessed *ex vivo* impact of both EV types in rat and human blood.

Results: EV^{wt} exacerbated 7-day mortality by 60% (n = 9/group; 1 death in placebo) but without altering organ function and pro-inflammatory cytokine release (IL-1β, IL-6, TNF, MCP-1) and despite a 4-fold elevation of anti-inflammatory IL-10 ($p < 0.05$). EV^{wt} administration induced an immediate pro-coagulative effect in mild HS rats: clotting time (CT) and clot formation time (CFT) decreased by 10 and 40% ($p < 0.01$). Human/rat blood exposed *ex-vivo* to EV^{wt} showed similar effects ($p < 0.01$): decreased CT (47/67%) and CFT (44/41%), and increased MCF (15/7%). Next, we generated $EV^{TF\text{-}ko}$ by knocking out the F3 gene; a full TF loss at protein level was confirmed by Western Blot. In the repeated human/rat *ex vivo* experiments, administration of $EV^{TF\text{-}ko}$ (or TF-neutralizing antibodies) completely abrogated the pro-coagulative EV effects. Preliminary data show no detrimental short-term effects of $EV^{TF\text{-}ko}$ in the same rat model of severe HTS.

Conclusions: Early administration of wildtype MSC-EVs in severe HTS can be detrimental, likely due to TF-mediated pro-coagulative activity. This adverse effect was mitigated by genetic deletion of TF from the EVs.

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DEPLETION OF INFLAMMATORY AND PROCOAGULANT MEDIATORS BY CYTOSORB IN SEPSIS PATIENTS – AN INTERIM ANALYSIS

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Background: Sepsis is a life-threatening condition characterized by a dysregulated host response to infection, resulting in excessive inflammation and immunothrombosis. CytoSorb, an extracorporeal hemoadsorption system, has emerged as a potential therapeutic intervention to modulate the hyperinflammatory response. We aimed to analyze inflammatory mediators as well as coagulation-related parameters in sepsis patients undergoing CytoSorb treatment and to assess its immunomodulatory effects over time.

Methods: An interim analysis was performed after 24 sepsis patients had been enrolled in this observational study. Plasma samples were collected at baseline (0 h), after 6 and 24 hours of CytoSorb treatment. Cytokines, chemokines, and growth factors were analyzed using the Bio-Plex Pro™ human cytokine 17-plex bead array (Bio-Rad). Phosphatidylserine-exposing extracellular vesicles (Annexin5$^+$ EVs) and their cellular origin were analyzed by flow cytometry. Soluble tissue factor (TF) levels were quantified by ELISA, while the EV-associated TF activity was assessed via a factor Xa generation assay.

Results: In this cohort of sepsis patients, with a median SOFA score of 12 and an all-cause mortality of 41.7%, CytoSorb treatment was associated with a significant, progressive time-dependent reduction in inflammatory parameters, including interleukin (IL)-4, IL-6, IL-8, IL-10, tumor necrosis factor-α, and granulocyte colony-stimulating factor. EV-TF activity, though elevated in sepsis patients, showed a decreasing trend, which did, however, not reach significance. EV-TF activity correlated with soluble TF levels at 6 hours but not at baseline or 24 hours. EV levels significantly increased within the first 6 hours of CytoSorb treatment and returned to baseline after 24 hours, without affecting the relative abundance of EVs derived from platelets, red blood cells, leukocytes, or monocytes.

Conclusions: These interim findings suggest that CytoSorb may contribute to the attenuation of systemic inflammation and point towards a potential role in influencing immunothrombotic processes in sepsis. Further patients will be included and analyzed to confirm and expand upon these findings.

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REAL-TIME EXHALED METHANE MONITORING: A DYNAMIC, NON-INVASIVE TOOL TO DETECT AND DIFFERENTIATE BETWEEN MESENTERIC AND PULMONARY CIRCULATORY FAILURE

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Background: Ileal perfusion correlates with exhaled methane (Ex-CH_4) when pulmonary circulation is intact. However, intensive care patients often exhibit impaired perfusion in both pulmonary and gastrointestinal systems, and respiratory surface area and pulmonary blood flow are also known to influence Ex-CH_4 levels. We aimed to develop a method capable of detecting and distinguishing gastrointestinal and pulmonary perfusion disorders.

Methods: Anesthetized, ventilated rats received CH_4-enriched 0.9% saline (CH_4-NaCl; 6 ml/kg/h) either enterally via a gastric probe or intravenously. Mesenteric ischemia (MI; 60 min) was induced in Groups 1 (MI-en) and 2 (MI-iv). The left pulmonary artery was occluded (PuO) for 5 min in Groups 3 (PuO-en) and 4 (PuO-iv). In *Study I*, continuous CH_4-NaCl administration (enteral or intravenous, respectively) began 10 min before vascular occlusions, whereas in *Study II*, CH_4-NaCl administration was started during the occlusion phase. Both studies included four groups (n = 6 each). Online Ex-CH_4 measurements were performed using photoacoustic spectroscopy; microcirculatory changes were recorded with intravital videomicroscopy (Cytocam-IDF).

Results: *Study I:* Both enteral and intravenous CH_4-NaCl administration resulted in increased Ex-CH_4 levels; however Ex-CH_4 was significantly reduced by MI and PuO in the enterally treated groups but decreased only by PuO in the intravenous administration groups.

Study II: Enteral CH_4-NaCl administration elevated Ex-CH_4 only after MI resolution, whereas intravenous treatment resulted in an immediate increase in Ex-CH_4 during MI. Neither route increased Ex-CH_4 during PuO.

In both studies, Ex-CH_4 levels paralleled with the occlusion-induced microcirculatory dysfunction of the affected organ and recovered during reperfusion.

Conclusions: In this proof-of-concept study we have shown that monitoring Ex-CH_4 levels after combined application of enteral and intravenous methane supplementation reliably differentiates and tracks pulmonary and gastrointestinal perfusion disorders in real time and could guide interventions in hemodynamically unstable patients.

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SINGLE-NUCLEUS TRANSCRIPTOMICS REVEALS NEUROVASCULAR DYSFUNCTION IN SEPTIC PATIENTS

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Background: Sepsis is a systemic inflammatory response to infection and remains a leading cause of mortality in intensive care units. Sepsis-associated encephalopathy (SAE), a frequent complication, results from neurovascular injury and inflammation within the brain, often contributing to long-term cognitive impairments in survivors. Despite its prevalence, the specific dysfunctions of neurovascular cells during sepsis remain poorly understood. This study aims to characterize sepsis-induced alterations in neurovascular cell populations, with a focus on signaling dysregulation, cell reprogramming, and intercellular communication, particularly among astrocytes, microglia, and other vascular-associated cell types.

Methods: Postmortem hippocampal tissues from control individuals and sepsis patients were obtained from the NIH NeuroBioBank and MUSC Brain Bank. Single-nucleus RNA sequencing was performed by Novogene. Data analysis utilized the Seurat package in RStudio, and pathway enrichment was assessed using Gene Set Enrichment Analysis (GSEA) with Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.

Results: We identified profound transcriptional alterations across 21 distinct neurovascular cell populations. Astrocytes and microglia exhibited strong activation signatures, shifting toward neurotoxic A1 and pro-inflammatory M1 phenotypes. Communication analysis revealed intensified astrocyte-microglia crosstalk that amplified inflammatory cascades. Endothelial and mural cells displayed gene expression profiles consistent with blood-brain barrier (BBB) breakdown, oxidative stress responses, and compromised vascular stability. Trajectory analyses

indicated coordinated maladaptive reprogramming in both glial and vascular compartments under septic conditions. Pathways related to immune activation, reactive oxygen species, and vascular permeability were selectively enriched across cell types.

Conclusions: Our findings highlight a central role for aberrant glial-vascular interactions in driving neurovascular dysfunction during sepsis. This study provides novel insight into the mechanisms underlying SAE and points toward potential therapeutic strategies aimed at preserving or restoring neurovascular integrity in septic patients.

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EXTRACELLULAR VESICLES (EVS) CONTRIBUTE TO POST-TRAUMATIC CARDIAC DYSFUNCTION AND REMODELING BY PROMOTING OXIDATIVE STRESS AND REDUCING THE VIABILITY OF CARDIAC FIBROBLASTS

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Background: Cardiac dysfunction is a severe complication in polytrauma (PT) patients. Circulating extracellular vesicles (EVs) are emerging mediators of intercellular communication in systemic inflammation and organ damage. However, their role in post-traumatic cardiac remodeling and stress response, remains unclear. This study specifically explores how EVs influence cardiac fibroblasts, which are key players in the remodeling process. To investigate the effects of plasma-derived EVs from PT-patients on the viability and stress response of human cardiac fibroblasts (HCFs), aiming to elucidate the role of EVs in post-traumatic cardiac dysfunction and remodeling.

Methods: EVs were isolated from plasma of healthy donors (n=9) and PT patients with and without elevated Troponin (TnT) Levels as sign of cardia dysfunction (TnT_High >50 pg/mL, TnT_Low <12 pg/mL; n=21). PT plasma samples were collected at two time points: at emergency room (ER) and 24 hours post-injury and characterized via nanoparticle tracking analysis (NTA). HCFs were stimulated with EVs for 4 and 24 hours and cell viability was assessed via AlamarBlue-, apoptosis via Caspase-Glo® 3/7- and oxidative stress via CellROX® Green Reagent - assays.

Results: NTA revealed no significant differences in concentration or size distribution of plasma EVs from patients with TnT >50 pg/mL v.s. TnT <12 pg/mL. PT-derived EVs from the TnT_High_24h ($p=0.06$) and TnT_Low_ER ($p\leq0.05$) groups significantly reduced HCF viability compared to healthy donor EVs ($p\leq0.05$). Caspase 3/7 activity remained unchanged across all conditions. However, CellROX analysis showed a marked increase in oxidative stress following stimulation with TnT_High_24h EVs ($p\leq0.05$).

Conclusions: EVs circulating after polytrauma may contribute to early post-traumatic cardiac dysfunction by inducing oxidative stress in cardiac fibroblasts, potentially compromising their viability and thereby affecting subsequent cardiac remodeling. The active contribution of EVs to the development of cardiac dysfunction, however, needs further investigations. Additionally, the early stress response of other cardiac cell types, such as cardiomyocytes, should be explored following trauma.

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TWO-DECADE TRENDS IN INCIDENCE AND MORTALITY OF DISSEMINATED INTRAVASCULAR COAGULATION IN TRAUMA PATIENTS

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Background: Trauma is a major underlying cause of disseminated intravascular coagulation (DIC), a life-threatening condition associated with poor outcomes. We investigated temporal trends in the incidence and mortality of DIC among trauma patients over a 20-year period and evaluated its clinical impact.

Methods: This single-center retrospective study screened 2,028 trauma patients admitted to a tertiary emergency department (ED) between 2000 and 2020. Patients aged >16 years with at least one injury of Abbreviated Injury Scale >3 were included. DIC was diagnosed upon ED arrival. Trauma severity was assessed using the Injury Severity Score (ISS). Shock was defined as systolic blood pressure <90 mmHg and lactate >2 mmol/L on arrival. The study period was divided into four intervals: A (2000–2005), B (2006–2010), C (2011–2015), and D (2016–2020). Primary outcomes included in-hospital mortality and incidence of DIC. Data are presented as medians.

Results: A total of 737 patients met inclusion criteria (n per period: A=184, B=177, C=165, D=211). Patient age increased over time. In period D, both ISS (22) and shock incidence (12.3%) rose, and the use of tranexamic acid significantly increased from nearly 0% to ~60% after period C. Although the incidence of DIC (15.8%, 23.7%, 22.4%, 16.1%) and its mortality (17.2%, 38.1%, 10.8%, 14.7%) remained almost stable, mortality differences between DIC and non-DIC patients diminished across periods (A: 17.2% vs. 3.9%, $p=0.016$; B: 38.1% vs.8.1%, $p<0.001$; C: 10.8% vs. 7.0%, $p=0.491$; D: 14.7% vs. 5.1%, $p=0.055$).

Conclusions: Despite changes in trauma severity and treatment strategies, the incidence of DIC and its mortality among trauma patients remained relatively constant over two decades. Validation through large-scale multicenter studies is warranted to confirm the obtained results.

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SEX-SPECIFIC EFFECTS OF EARLY LIFE STRESS ON BONE FRACTURE HEALING

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Background: Early life stress affects approximately 40% of the world population and is an acknowledged risk factor for the development of several somatic and affective disorders, some of which are associated with elevated inflammation, osteoporosis and an increased bone fracture risk. Furthermore, the susceptibility to stress-related disorders was shown to be dependent on sex/gender. The purpose of this study was to test whether early life stress influences the inflammatory response and regeneration after bone fracture, and whether these effects might be sex-specific.

Methods: The maternal separation procedure (separation of pubs from their mother for three hours per day for the first 14 days of their life) was used to introduce early life stress in C57BL/6N mice. At the age of 12 weeks, a standardized right femur osteotomy was introduced and stabilized by an external fixator. Mice were euthanized three hours, 10 days and 21 days post-fracture. Depending on the time point, cytokine assays, histology, biomechanical testing and μCT measurements were used to characterize the inflammatory response, functional outcome and fracture callus tissue formation (n =4-11/group). Statistical analysis was conducted using unpaired Student's t tests.

Results: Male mice subjected to early life stress showed a reduced inflammatory response three hours after fracture, characterized by decreased expression of IL10, RANTES and TNFα in the fracture hematoma and plasma. However, the composition of the fracture callus and bending stiffness assessed at days 10 and 21 after fracture did not differ between stressed male mice and non-stressed controls. No significant

differences were seen in female mice for all three time points and assessed parameters.

Conclusions: Our results imply that early life stress affects the early inflammatory phase of fracture healing in a sex-specific manner, while these alterations did not result in long-term effects on callus tissue formation and functional outcome.

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PKCα-PHOSPHATASE FEEDBACK LOOPS IN SEPSIS-ASSOCIATED LIVER FAILURE: MOLECULAR INSIGHTS

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Background: Excretory liver failure, characterized by early canalicular membrane remodeling, involves the disruption of the brush border and impaired elimination of bile acids (BAs). Protein Kinase C-α (PKCα) knockout preserves canalicular structure and function, highlighting PKCα's central role in early hepatocellular remodeling during sepsis. This remodeling entails the dephosphorylation and mislocalization of Radixin, which anchors Multidrug Resistance-associated Protein 2 (MRP2) to the F-actin-rich canalicular membrane. The absence of Radixin phosphorylation, controlled by protein phosphatases (PPs), disrupts MRP2 localization and hinders BA clearance. Objective: Determine the molecular pathways by which PKCα induces dephosphorylation of Radixin, resulting in excretory liver failure.

Methods: In vitro, HepG2, HepG2-PKCα knockout, and HepaRG cells were treated with PMA or inflammatory cytokines. PKCα activation was monitored by FLIM-FRET-biosensors and validated via cell fractionation. Downstream signaling was analyzed using PP1/PP2-specific inhibitors. Fluorescence microscopy evaluated F-actin remodeling, MRP2 localization, Radixin dephosphorylation, and RAB11A-mediated endocytosis. Proximity ligation assays (PLA) were used to study protein-protein interactions. Image data were quantified using JIPIPE and analyzed with R. In vivo/Ex vivo, the peritoneal and contamination sepsis model was done on FVB/N and PKCα knockout mice. Liver tissues were collected 24-hours after infection to assess Radixin phosphorylation levels.

Results: In vitro, the FRET sensor detected PKCα activation within 15-minutes of PMA and 60-minutes after cytokine exposure, causing Radixin dephosphorylation, canalicular F-actin remodeling, and MRP2 endocytosis involving RAB11A. These effects were absent in PKCα-deficient cells. In vivo/Ex vivo, septic wild-type mice exhibited significant Radixin dephosphorylation, this change was absent in PKCα-deficient animals. Liver perfusion with PMA induced Radixin dephosphorylation, which was reversed by a PKCα inhibitor, Midostaurin.

Conclusions: PKCα promotes Radixin dephosphorylation, cytoskeletal remodeling, and MRP2 endocytosis that impair bile acid clearance in sepsis-induced liver failure. Inhibiting this pathway pharmacologically reverses effects, positioning PKCα as a therapeutic target for intervention with existing kinase inhibitor, Midostaurin.

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DESIGN OF ARMED TARGETED sEVs TO BLUNT SYSTEMIC PROPAGATION OF POST-TRAUMATIC INFLAMMATION

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Background: Traumatic injuries can cause high mortality, largely through uncontrolled systemic inflammation. We recently identified pro-inflammatory small extracellular vesicles (sEVs) released from endothelial cells as major amplifiers. Inhibiting their biogenesis with GW4869 normalized inflammation after thorax trauma in mice, but this inhibitor is not clinically applicable. To create a clinically relevant therapy option, we engineered endothelial-targeting sEVs armed with siRNAs against interleukin-6 (IL-6) and the sEV-biogenesis enzyme nSMase2 to blunt post-traumatic inflammation

Methods: We designed an expression vector fusing human or murine ICAM-1–binding peptides to a GPI-anchor consensus sequence, routing the peptides into the plasma membrane and sEVs. These sEVs were purified from HEK293T (human) or NIH-3 T3 (mouse) supernatants by size-exclusion chromatography and characterized according to MISEV guidelines. Uptake efficacy and specificity was quantified in HUVEC (human) or C166 (mouse) endothelial cells with ExoGlow-labelled sEVs, ± competing serum sEVs, and after in vitro trauma by a polytrauma cocktail (human PTC: IL-6, IL-8, IL-1β, C3a, C5a). sEVs were armed with siRNAs against IL-6 and nSMase2 by electroporation. Target depletion was assessed by ELISA.

Results: Presence of the ICAM-1-targeting construct in cells and sEVs was validated by Western blots, and sEVs met MISEV criteria. Uptake by endothelial cells was dose-dependent and remained efficient despite serum sEV competition in human/mouse in vitro systems, upon PTC-stimulation, significantly exceeding parental sEV. Preferential uptake in endothelial cells was also confirmed. Electroporated sEVs achieved marked reductions of IL-6 and nSMase2 within 4 h. Currently, targeted sEVs are produced for injections in mice 20 min after blunt chest trauma to confirm in vivo efficacy.

Conclusions: Targeted sEVs efficiently deliver siRNAs to endothelial cells and rapidly deplete IL-6 and nSMase2 despite competing serum vesicles, indicating the potential to dampen the early post-traumatic inflammatory surge. Pending validation in mice, this platform may offer translational relevance in abrogating post-traumatic inflammation.

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DEVELOPMENT AND VALIDATION OF A VASOPRESSOR STAGING SCORE (VSS) FOR SEPTIC SHOCK: A NON-INFERIORITY COMPARISON WITH THE NOREPINEPHRINE EQUIVALENT (NEE) SCORE

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Background: The norepinephrine equivalent (NEE) score quantifies the vasopressor and inotrope burden as a continuous measure of hemodynamic support to reflect illness severity. However, its clinical utility is limited by complex calculations, lack of standardization across studies, and poor adaptability to novel vasopressors. In the context of protocolized sepsis care—where vasopressor administration is increasingly standardized—we hypothesized that the number of concurrently administered vasopressors may serve as a pragmatic surrogate marker for illness severity. We therefore developed the Vasopressor Staging Score (VSS), a simplified ordinal score reflecting the number of vasopressors used. This trial aimed to evaluate whether the VSS demonstrates non-inferior predictive performance for 28-day mortality compared to the NEE score.

Methods: We analyzed a data of adult patients (≥18 years) with septic shock from a prospectively collected multicenter registry of the Korean Shock Society between October 2015 and December 2019. Patients were categorized into three groups based on the number of vasopressors administered during initial resuscitation: single, double, or triple agent use. The Vasopressor Staging Score (VSS) was constructed as

follows: single-agent use = 0–1 point, double-agent = 2 points, triple-agent = 4 points. The predictive performance of the VSS for 28-day mortality was compared to that of the NEE score using receiver operating characteristic (ROC) curve analysis and DeLong's test. A non-inferiority margin of $\Delta = 0.025$ for the AUC difference was predefined to determine statistical non-inferiority of the VSS score relative to the NEE score.

Results: Of 2,724 patients screened, 594 with do-not-resuscitate orders were excluded, leaving 2,130 patients: 1,627 in the single group, 417 in the double group, and 86 in the triple group. The 28-day mortality rates were 5.0% in the single group, 9.8% in the double group, and 20.9% in the triple group. In the single group, norepinephrine alone was administered; vasopressin was used in 95.9% of the double group, and epinephrine was added in 88.4% of the triple group. The VSS demonstrated an AUC of 0.6352 for predicting 28-day mortality, which was not significantly different from the NEE score (AUC 0.6525; absolute difference = 0.0173, p = 0.16). The lower bound of the 95% confidence interval for the AUC difference remained within the predefined non-inferiority margin ($\Delta = 0.025$).

Conclusions: In hospitals with protocolized sepsis care, the VSS, a simplified ordinal measure based on the number of vasopressors used, reflected illness severity and was non-inferior to the NE score. It may serve as a practical tool for future research and risk stratification.

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ABSOLUTE LYMPHOCYTE COUNT DYNAMICS MAINLY DRIVEN BY CD4$^+$ T-CELL DEPLETION PREDICTS CLINICAL OUTCOME IN SEVERE TRAUMA

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Background: A reduced absolute lymphocyte count (ALC) has been associated with poor outcomes in critically ill patients, including multiple organ failure and increased mortality. However, standardized immunomonitoring methods for early high-risk stratification are still unavailable. Therefore, exploring the relationship between ALC dynamics and clinical outcomes, as well as potentially associated specific T-cell loss, remains crucial for further research. This study assessed the prognostic value of ALC dynamics after severe trauma and analyzed T-cell subset changes to explore their contribution to lymphopenia.

Methods: 38 polytraumatized patients (Injury Severity Score (ISS) $\geq$ 18) were enrolled. Blood samples were collected at 0, 8, 24, and 48 hours, and on days 5 and 10. Differential blood counts were performed. Nine healthy volunteers served as controls. Based on ALC kinetics at 48 h, patients were classified into four groups: persistent lymphopenia (PL), rapidly decreasing (RD), slowly rising (SR), and normal fluctuation (NF). Groups were compared regarding unfavorable physical clinical outcome (death or new long-term care), in-hospital mortality, intensive care unit length of stay (ICULOS) and incidence of multiple organ dysfunction syndrome (MODS). Flow cytometry was used to quantify total and naive CD4$^+$ and CD8$^+$ T-cells.

Results: ALC significantly declined in all patients over 10 days compared to controls. PL and RD correlated with unfavorable outcomes, increased in-hospital mortality, prolonged ICU-LOS and higher MODS incidence. CD4$^+$ T-cell trajectories closely mirrored ALC groupings. Comparing healthy subjects and patients revealed a significant reduction of CD4$^+$ T cell absolute and naive numbers. In contrast, CD8$^+$ T-cell dynamics were less pronounced; naive CD8$^+$ T-cells did not reveal a significant decrease.

Conclusions: ALC represents a simple and cost-effective early clinical predictor in trauma care. Its decline is mainly driven by CD4$^+$ T-cell loss, with a smaller contribution from CD8$^+$ T-cells. These findings support the clinical use of ALC as early high-risk predictor and underline the need to preserve T-cell subsets following severe trauma.

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ASSOCIATION BETWEEN EMERGENCY DEPARTMENT LENGTH OF STAY AND 28-DAY MORTALITY IN SEPTIC SHOCK: A MULTICENTER PROSPECTIVE REGISTRY STUDY IN KOREA

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Background: Reducing emergency department (ED) length of stay (LOS) is considered quality indicator, aiming to improve patient flow and outcomes. In Korea, the Ministry of Heath and Welfare recommends ED LOS for severe illness patients to be less than 5 hours. However, it is uncertain whether ED LOS impacts outcomes in septic shock. This study aimed to investigate whether ED LOS is independently associated with 28-day mortality in adult patients with septic shock, using data from a large multicenter Korean cohort.

Methods: This multicenter, prospective observational study analyzed 9,970 adult patients with septic shock enrolled between January 2016 and December 2023 from the Korean Shock Society (KoSS) septic shock registry. The registry includes data from 11 university-affiliated tertiary EDs in Korea. Baseline characteristics were compared between 28-day survivors and non-survivors. Univariate and multivariable logistic regression were used to assess the relationship between ED LOS and 28-day mortality. The multivariable model used Firth's penalized likelihood and adjusted for age, Sequential Organ Failure Assessment (SOFA) score, comorbidities, and infection source.

Results: The overall 28-day mortality rate was 24.1%. In univariate analysis, longer ED LOS was associated with a slight decrease in mortality risk (OR 0.996 per hour; 95% CI 0.993–0.998; $p < 0.05$). However, this association was not significant in the multivariable model (OR 1.000; 95% CI 0.999–1.001; p = 0.36). Independent predictors of mortality included older age, higher SOFA score, metastatic cancer, and respiratory infection. Hepatobiliary and urinary tract infections were associated with lower mortality.

Conclusions: In this large cohort study, ED LOS was not an independent predictor of 28-day mortality after adjusting for illness severity and comorbidities. These findings challenge the assumption that prolonged ED stay alone worsens clinical outcomes, suggesting that risk stratification and timely interventions may be more critical than absolute time thresholds in the ED.

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IRON NANOCLUSTER–MEDIATED PHOTODYNAMIC THERAPY ENABLES EFFICIENT AND SELECTIVE BACTERIAL KILLING

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Background: Growing antibiotic-resistance demands innovative and efficacious antibacterial strategies. Nanomaterial-based photodynamic therapy (PDT) represents a promising alternative to conventional treatments. This study investigates the antibacterial effects of hemoglobin-derived iron nanoclusters (FeNCs) as a novel photosensitizer for PDT against both Gram-negative and Gram-positive bacteria.

Methods: FeNCs were synthesized and characterized for their physicochemical and biological properties. Their antibacterial activity and bacterial reactive oxygen species (ROS) generation were assessed under varying concentrations, incubation times, and blue light exposure both against *Escherichia coli* (*E. coli*) and *Staphylococcus capitis* (*S. capitis*). Bacterial interactions with FeNCs were visualized using confocal and transmission electron microscopy (TEM).

Results: The ultrasmall FeNCs (<2 nm) exhibited a negative surface charge, water solubility, inherent autofluorescence, characteristic absorption/emission peaks at 460/565 nm, and non-toxicity in eukaryotic cells (human amniotic epithelial cells). FeNCs alone induced a 3.52-$\log_{10}$ reduction in *E. coli* colony-forming units (CFU), while combined with light achieved up to ≥5-$\log_{10}$ CFU reduction. In contrast, *S. capitis* had no response to either FeNCs or light alone; only the combined treatment showed reduction up to 1.42-$\log_{10}$. Importantly, FeNCs triggered ROS production in both strains under both photoradiaton and non-photoradiation conditions. The absence of ROS generation in control groups without FeNCs confirmed that ROS production was primarily mediated by FeNCs. Confocal imaging showed similar colocalization of FeNCs with both bacterial strains. TEM images showed effective bacterial killing and structural damage in E. coli with both FeNCs and FeNCs + light treatments, while a slight increase in the number of damaged or dead bacteria was observed in S. capitis with combined therapy.

Conclusions: To conclude, FeNCs combined with light offered a synergistic antibacterial effect against *E. coli* while *S. capitis* was less susceptible. These findings highlight the need to tailor nanomaterial-based therapies by bacterial strain and support further *in vivo* investigation.

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FIBRIN POLYMERS ARE ESSENTIAL IN THE SYNERGISTIC INDUCTION OF TNF BY ALIVE STREPTOCOCCUS PNEUMONIAE IN HUMAN WHOLE BLOOD

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Background: Streptococcus (S.) pneumoniae infection is a common cause of pneumonia and sepsis. TNF, thrombin and platelets contribute to the resistance to S. pneumonia infection in pneumosepsis models. In mice we showed that the procoagulant FV-Leiden mutation provides a major survival benefit in a preclinical model with antibiotic treatment of S. pneumoniae pneumosepsis. How blood coagulation affects the host response to S. pneumoniae is however still unclear. Objective: To determine the effect of coagulation on the host response to alive S. pneumoniae in a human whole blood model.

Methods: S. pneumoniae bacteria were grown, washed and mixed with freshly drawn citrated whole blood from healthy donors and calciumchloride for recalcification to induce clotting. Mixtures were incubated at 37C and clotted after 10 minutes (in the absence of anticoagulants) irrespective of the presence of bacteria. After 4 hours samples were put on ice and homogenized to measure cytokine release.

Results: S. pneumoniae induced robust TNF and IL-8 production in coagulated whole blood. Dual prevention of thrombin generation/activity by BAPA drastically reduced the S. pneumoniae induced TNF and IL-8 production by 95%. Prevention of fibrin polymerization with GPRP and fibrinolysis by tPA reduced TNF production by 85% but did not affect IL-8 levels. Block of fibrin crosslinking by FXIII inhibitor T101 and platelet GPIIbIIIa did not affect cytokine levels, while a CD11b blocking antibody reduced TNF production significantly. Inhibition of TNF expression by targeting thrombin and fibrin polymers was a result on TNF transcription as TNF mRNA levels were also 85% reduced. Inhibition with TLR inhibitors indicated that fibrin dependent TNF production by S. pneumoniae was dependent on intracellular TLR signalling.

Conclusions: Our study indicates that intact fibrin polymers form an important part of the host immune response to S. pneumoniae via TNF induced by synergistic signalling of intracellular TLR's and CD11b in leukocytes.

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ARDS INCIDENCE AND IN-HOSPITAL MORTALITY IN GERMANY: A NATIONWIDE ANALYSIS OF 87,488 ICU CASES

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Background: The acute respiratory distress syndrome (ARDS) gained significant attention during the COVID-19 pandemic. Although numerous studies, including observational multicenter studies, have provided valuable insights, significant uncertainties persist. This study retrospectively analyzed ARDS patients admitted to German intensive care units (ICUs) over a 15-year period.

Methods: Data from the German Federal Statistical Office covering all adult (≥ 18 yr) ARDS patients admitted to an ICU between 2008 and 2022 were used. Incidence, in-hospital mortality, comorbidities, complications, sex, and age distribution were analyzed.

Results: Of 87,488 ARDS patients admitted to ICUs from 2008 to 2022, 47.8% (41,816) survived. ARDS incidence increased slightly, peaking at 21.0/100,000 inhabitants during the COVID-19 pandemic in 2021. In-hospital mortality decreased from 54.2% in 2008 to 50.8% in 2018, but rose to 53.8% in 2021. Older age (60 vs. 68; $p < 0.0001$), a higher Elixhauser index (12 vs. 17; $p < 0.0001$), and severe ARDS (41.1% vs. 54.2%; $p < 0.0001$) were significantly associated with increased in-hospital mortality, while obesity appeared to have a protective effect (17.2% vs. 12.6%; $p < 0.0001$). Mortality was highest (82%) in ARDS patients aged 90-94. Men were more likely to develop ARDS, but sex did not significantly impact survival.

Conclusions: ARDS incidence and in-hospital mortality remain significant challenges. The underrepresentation of mild to moderate ARDS cases highlighted the need for better diagnostic tools and standardized criteria. In-hospital mortality rates in elderly are drastic and underscores the urgency for age-specific treatment approaches.

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SEX- AND AGE-RELATED DIFFERENCES IN LPS-INDUCED LUNG INJURY: ESTABLISHING A MOUSE INTENSIVE CARE UNIT

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Background: Mouse models are widely used to establish new therapy concepts for acute lung injury, but the transfer of therapeutic approaches into the intensive care unit often failed. Therefore, we establish a mouse intensive care unit to adequately reflect the patient's situation and to investigate sex- and age-related differences in response to lipopolysaccharide. The primary objective of this study was to investigate sex- and age-related physiological responses to i.t. LPS administration and MV by assessing disease manifestation, survival, and lung function parameters, including oxygenation, lung compliance, and respiratory mechanics.

Methods: For the establishment of a mouse intensive care unit, young (2-3 months) and old (15-18 months) mice of both sexes received continuous respiratory and cardiovascular monitoring for 6 h. Mimicking an acute lung injury by intratracheal lipopolysaccharide stimulation for 6 or 24 h, the impact of sex and age on survival and physiological parameters was evaluated.

Results: The establishment revealed sex- and age-related differences in physiological responses during mechanical ventilation, with old males requiring more noradrenaline to maintain stable hemodynamics. While young mice, irrespective of sex, developed acute lung injury 24 h after lipopolysaccharide administration, old mice exhibited a rapid systemic response, showing signs of lactic acidosis and endotoxemia. Among these, old females had the highest mortality risk, whereas in old males, mechanical ventilation provided effective support, contributing to improved survival outcomes.

Conclusions: We successfully established a mouse intensive care unit that integrated all critical aspects of a human intensive care unit simultaneously. By highlighting sex- and age-related differences following lipopolysaccharide stimulation and mechanical ventilation, our study underscored the need for diversity in preclinical models to improve translation of findings on critical illnesses like acute lung injury into clinical settings.

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THE SYSTEMIC ROLE OF OREXIN A, SUBSTANCE P, BRADYKININ AND DABK IN SEVERE COVID-19 AND 2.5-YEAR FOLLOW-UPS: AN OBSERVATIONAL STUDY

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Background: Orexin A regulates sleep-wake cycles, arousal, and energy homeostasis, linking it to the renin-angiotensin system (RAS) and substance P. Dysfunctions in these pathways occur in acute and long-term COVID-19, including post-COVID syndrome. The current study aimed to identify the clinical and physiological role of orexin A in severe COVID-19 patients and 2.5-year follow-ups, compared to healthy controls. Furthermore, we want to understand the interplay between orexin A, substance P, bradykinin and DABK and the immune-metabolic setting of those patients, as well as its effect on the unusual level of sedation in COVID-19 patients.

Methods: This observational study analysed plasma orexin A, substance P, bradykinin, and des-Arg9-bradykinin (DABK) in 78 ICU COVID-19 patients, 14 survivors of severe COVID-19 (2.5 years follow-ups), and 14 healthy controls.

Results: During acute COVID-19, bradykinin and substance P were significantly reduced, while DABK was elevated compared to healthy controls and 2.5-year follow-ups. Orexin A levels were noticeable and correlated with ICU survival (Cohen's $d = 0.4$), length of stay (LOS; $r = -0.26$, $p = 0.02$), and sedation levels (RASS). Intriguingly, substance P plasma levels were elevated in 2.5-year follow-ups. Plasma orexin A, substance P, and bradykinin increased with lower RASS, and this effect seemed to be independent of the number of anaesthetics. Thereby, combined orexin A, substance P, and bradykinin levels at RASS -3 to -5 distinguished survivors from non-survivors of COVID-19 when categorized by age.

Conclusions: Severe COVID-19 alters bradykinin axis, affecting substance P and orexin A signalling, influencing disease worsening, ICU LOS and survival. Elevated substance P levels in the 2.5-year follow-up cohort may be associated with physical, cognitive, and neuropsychological impairments commonly seen in Post-Intensive-Care Syndrome (PICS) and post-COVID syndrome. The predictive values of orexin A, substance P, bradykinin and DABK as well as the complex interplay between RAS and the orexinergic system in severe, critical illnesses or viral diseases will be investigated in future studies.

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FUNCTIONAL IL-10 PRODUCTION AS AN EARLY INDICATOR OF CLINICAL OUTCOME AFTER SEVERE TRAUMA

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Background: Severe trauma induces a dysregulated immune response with simultaneous hyperinflammation and immunosuppression. Interleukin-10 (IL-10) can limit cellular damage but may impair host defense if overproduced.

While high IL-10 levels correlate with increased mortality in critical illness and sepsis, the dynamics and cellular sources of IL-10 after trauma, remain poorly defined. This study evaluates systemic IL-10 levels and functional IL-10 production in trauma patients as an early predictor for clinical outcome and mortality.

Methods: In a monocentric prospective study blood samples were collected from healthy individuals ($n = 25$) and polytraumatized patients ($n = 34$; Injury Severity Score (ISS) ≥ 18) at admission, 8, 24, 48 hours, 5 and 10 days post-injury. Systemic IL-10 levels were measured. Enzyme Linked Immuno Spot (ELISpot) assays assessed LPS-stimulated IL-10 production in patients, healthy controls whole blood and in isolated immune cell types in healthy controls. Leukocyte subsets were quantified. Patients were stratified into three outcome groups: favorable discharge, adverse discharge (long-term functional impairment), and in-hospital death.

Results: Systemic IL-10 levels did not reveal significant differences within the first 8 hours. Functional IL-10 production differed significantly: patients with adverse discharge showed reduced LPS-stimulated IL-10 production compared to those with favorable discharge or death. Notably, IL-10 production was comparable between favorable and fatal

outcomes. Isolated monocytes and T cells were likely the predominant IL-10 source as when co-incubated produced the highest LPS-induced IL-10 positive cell numbers in healthy patients.

Conclusions: Early high functional IL-10 levels may distinguish favorable from adverse discharge but not from in-hospital death. These findings challenge the simplified association of high IL-10 levels with poor prognosis and suggest IL-10 as an early balancing immunosuppressive mediator post-trauma. Monocytes and T cells seem to be key IL-10 producers posttrauma. These findings highlight the need for deeper mechanistic insight to provide a rationale for targeting IL-10 therapeutically in trauma care.

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PYRROLIDINE DITHIOCARBAMATE ACCELERATES EARLY RECOVERY OF INTESTINAL MICROVASCULAR OXYGENATION IN A REVERSIBLE MODEL OF HEMORRHAGIC SHOCK IN RATS

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Background: Improving intestinal tissue oxygenation appears to be crucial for maintaining global homeostasis in hemorrhagic shock. Suitable pharmacological strategies could therefore be able to reduce organ dysfunction. Pyrrolidine dithiocarbamate (PDTC) reduces intestinal tissue injury in experimental hemorrhage via the induction of heme oxygenase-1 (HO-1). We investigated the effect of PDTC dependent HO-1 protein induction on intestinal microvascular oxygenation (μHbO_2) as a measure of regional tissue oxygenation in a rat model of hemorrhage-retransfusion.

Methods: 40 male, anesthetized Wistar rats were randomized into 4 experimental groups (n = 10). Hemorrhagic shock was induced by arterial blood withdrawal (MAP: 40 ± 5 mmHg; 1 h). Subsequently, the shed blood was retransfused and animals were observed for 2 h. Control animals were observed for 3 h without the induction of hemorrhagic shock. PDTC (100 $mg \cdot kg^{-1}$, i.p.) or saline were applied 24 h prior to hemorrhagic shock or control treatment. Colonic and ileal $\Delta\mu HbO_2$ were continuously evaluated by white-light spectrophotometry, calculated as difference from baseline, and analyzed using 2-way ANOVA with Bonferroni post-hoc test. HO-1 protein expression was determined in a subset of experiments and analyzed using an unpaired two-tailed t-test (n = 6). Data are expressed as mean ± SEM, $p < 0.05$.

Results: Intestinal μHbO_2 decreased after the induction of hemorrhagic shock. PDTC pretreatment significantly improved colonic μHbO_2 after 60 min of hemorrhagic shock (shock:-29 ± 1% vs. shock+PDTC:-21 ± 2%) and accelerated early recovery of colonic (shock:-10 ± 3% vs. shock+PDTC:-3 ± 3%) and ileal (shock:-14 ± 5% vs. shock+PDTC:-3 ± 4%) μHbO_2 after shed blood retransfusion. PDTC pretreatment led to a 5-fold increase of HO-1 protein levels in the ileum but not in colonic tissue.

Conclusions: The application of PDTC appears to represent a promising pharmacological strategy to improve intestinal oxygenation during hemorrhagic shock and subsequent blood retransfusion. The beneficial effects of PDTC on μHbO_2 can only partially be attributed to increased HO-1 protein expression. Therefore, HO-1-independent mechanisms underlying the effects of PDTC warrant further investigation.

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REMOTE ISCHEMIC PRECONDITIONING (RIPC) IMPROVES ILEAL MICROVASCULAR OXYGENATION DURING HEMORRHAGIC SHOCK IN RATS INDEPENDENT OF MICROVASCULAR PERFUSION

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Background: Remote ischemic preconditioning (RIPC) was reported to induce tissue protection under various conditions of restricted oxygen supply. These effects are in part attributed to mediators with vasoactive properties. However, the effect of RIPC on microvascular oxygenation and perfusion during hemorrhagic shock has been insufficiently described. In this context, maintenance of intestinal homeostasis is of special interest since intestinal dysfunction has been linked to adverse events and poor long-term outcome in critically ill patients.

Objective: We investigated the effect of RIPC on intestinal microvascular oxygenation (μHbO_2) and regional perfusion variables in a rat model of hemorrhage-retransfusion.

Methods: 48 male, anesthetized Wistar rats were randomized into 4 experimental groups (n = 12). RIPC was induced by bilateral hindlimb compressions (4x5 min ischemia+5 min reperfusion). Animals with control treatment did not receive RIPC. Hemorrhagic shock was induced by arterial blood withdrawal (MAP: 40 ± 5 mmHg; 1 h) followed by shed blood retransfusion. Animals were observed for further 2 h. Control animals were observed for 3 h without the induction of hemorrhage. Intestinal μHbO_2 was continuously evaluated by white-light spectrophotometry. Microvascular perfusion was assessed by laser Doppler flowmetry and incident dark-field imaging. 2-way ANOVA was performed in combination with Bonferroni post-hoc test. Data are expressed as mean ± SEM, $p < 0.05$.

Results: Intestinal μHbO_2 decreased after the induction of hemorrhagic shock ($control_{ileum}$:74 ± 3% vs. $shock_{ileum}$:34 ± 4%; $control_{colon}$:73 ± 2% vs. $shock_{colon}$:51 ± 2%). RIPC pretreatment significantly improved ileal ($shock_{ileum}$:34 ± 4% vs. shock+RIPC:48 ± 4%) but not colonic μHbO_2 during early hemorrhagic shock. Improvement of ileal μHbO_2 was independent of microvascular perfusion variables. After 60 min of hemorrhagic shock and after shed blood retransfusion RIPC did not reveal beneficial effects on intestinal μHbO_2 and microvascular perfusion.

Conclusions: The results demonstrate the potential of RIPC to improve intestinal μHbO_2 hemorrhage independent of regional microcirculation. However, RIPC seems to have limited effects in prolonged shock states.

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SINGLE-CELL PROTEOME PROFILING REVEALS DISTINCT IMMUNOLOGICAL PATTERNS IN THE LUNGS OF PATIENTS WITH ACUTE RESPIRATORY DISTRESS SYNDROME

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Background: Acute Respiratory Distress Syndrome (ARDS) is a life-threatening condition characterized by pulmonary inflammation and immune dysregulation. Current insights into ARDS immune responses

primarily derive from peripheral blood studies or transcriptomic analyses of lung tissues, which may not fully capture functional protein-level differences within the local lung environment. To characterize alveolar immune cell composition and function using high-dimensional proteomic cytometry by time of flight (CyTOF), assessing differences associated with ARDS etiology and patient outcomes.

Methods: In this observational cohort study, bronchoalveolar lavage (BAL) fluid was collected repeatedly from critically ill patients admitted to the intensive care unit (ICU). Leukocytes were isolated and analyzed using CyTOF. A 50-marker panel was used to assess the maturity and activation states of immune cells.

Results: A total of 128 bronchoalveolar lavage (BAL) fluid samples were collected from 91 intubated patients. Among these patients, 75 (82.4%) met criteria for ARDS, 64 (70.3%) had pneumonia, and 68 (74.7%) survived up to 28 days post-intubation. Patients with viral-induced ARDS exhibited a distinct immune response characterized by increased frequencies of effector-memory $CD8^+$ T cells expressing elevated levels of granzyme B and perforin, enrichment of CD169^high monocyte-derived macrophages, and heightened macrophage activation. Neutrophil activation was significantly increased in pneumonia compared to non-pneumonia controls but showed no significant differences among viral vs bacterial pneumonia. A joint model integrating longitudinal sampling data and survival outcomes revealed that higher frequencies of immature neutrophils were associated with increased mortality and declined over time, whereas patients exhibiting greater proportions of resident-like alveolar macrophages had improved survival.

Conclusions: Although alveolar immune cell frequencies in ARDS patients are similar across etiologies, differences in cell maturation and activation significantly correlate with patient outcomes. Assessing immune functionality rather than abundance alone is essential for understanding ARDS pathogenesis and guiding targeted therapies.

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PROTECTION OF RENAL MITOCHONDRIA BY METHANE-ENRICHED CUSTODIOL DURING COLD STORAGE

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Background: Cold ischemia encountered during organ preservation can severely impair mitochondrial integrity by depleting ATP reserves, disrupting the function of the respiratory chain, and increasing outer membrane permeability—all of which contribute to post-transplant graft dysfunction. Methane (CH_4) has emerged as a cytoprotective and mitoprotective agent capable of stabilizing mitochondrial function. Through these protective properties, methane may play a beneficial role in mitigating ischemia-induced damage, thereby improving organ preservation outcomes and enhancing graft viability following transplantation. The present study aimed to investigate whether enrichment of the histidine–tryptophan–ketoglutarate (HTK, Custodiol) preservation solution with CH_4 can maintain mitochondrial respiratory function and outer membrane integrity during static cold storage of porcine kidneys.

Methods: Bilateral nephrectomies were performed on anesthetized pigs (n = 5). The right kidneys were preserved in standard HTK solution, whereas the left kidneys were stored in HTK solution enriched with 2.2% CH_4 (administered at a flow rate of 1.5 L/min for 15 minutes). All kidneys were stored at 4 °C for 24 hours. Mitochondrial respiration was assessed in cortical and medullary homogenates using high-resolution respirometry (Oroboros O2k, Innsbruck, Austria). Parameters assessed included basal respiration, complex I- and II-linked oxidative phosphorylation (OXPHOS), and cytochrome c oxidase (complex IV, CIV) activity. Outer mitochondrial membrane integrity was calculated from the respiratory response to the addition of exogenous cytochrome c.

Results: CH_4 supplementation significantly enhanced complex I- and II-linked OXPHOS in both the cortex and medulla and increased CIV activity in the medulla. Furthermore, kidneys preserved with CH_4-enriched HTK exhibited a markedly reduced respiratory response to cytochrome c addition, indicating improved preservation of outer mitochondrial membrane integrity.

Conclusions: Methane enrichment of HTK preservation solution effectively attenuates mitochondrial dysfunction and membrane damage associated with cold ischemia. These protective effects may limit apoptosis and improve post-transplant graft quality in kidney transplantation.

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NOVEL GATEKEEPER IN POST-SEPTIC IMMUNE RESPONSES: MYELOID-DERIVED SUPPRESSOR CELLS (MDSCs)

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Background: Sepsis is a life-threatening condition characterized by an initial hyperinflammatory phase followed by immune paralysis, leading to increased susceptibility to secondary infections and high mortality. Despite advances in intensive care, effective rescue treatments remain limited. Myeloid-derived suppressor cells (MDSCs) have emerged as a promising immunomodulators exhibiting both immunosuppressive and immune-activating functions depending on the disease entities. This study investigates the therapeutic potential of MDSCs in modulating immune responses and improving survival following sepsis.

Methods: MDSCs were isolated from the bone marrow of C57BL/6 (CD45.1) mice and expanded by GM-CSF. Sepsis was induced in C57BL/6 (CD45.2) mice via cecal ligation and puncture (CLP). One day post-CLP, mice received adoptive MDSC transfer, along with fluid resuscitation and antibiotics to resemble the clinical condition. Mice were monitored for survival, immune responses, and bacterial clearance over seven days.

Results: MDSC therapy significantly improved survival and reduced systemic inflammation, as evidenced by lower IL-6 and TNFα levels. Additionally, MDSCs restored T cell immunity, reversing sepsis-induced T cell dysfunction and enhancing both polyclonal and antigen-specific responses. Transcriptomic analysis revealed that MDSCs promoted antimicrobial innate immunity and facilitated pathogen clearance. Notably, MDSCs enhanced the phagocytic capacity of macrophages, contributing to a marked reduction in bacterial load in serum and peritoneal fluid three days post-CLP.

Conclusions: MDSC therapy effectively reprograms the immune response in sepsis, restoring both innate and adaptive immunity. These findings highlight MDSCs as a promising immunotherapeutic strategy to prevent post-septic immune paralysis and improve clinical outcomes.

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OVERTRANSFUSION IN WOMEN? A REAL-WORLD DATA ANALYSIS ON THE NEED FOR GENDER-SPECIFIC TRANSFUSION THRESHOLDS

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Background: Blood transfusions in critically ill patients are primarily indicated for anemia and active bleeding. Restrictive transfusion regimens have been widely adopted in critical care, often using hemoglobin thresholds of <7–8 g/dL. While reference values for hemoglobin and hematocrit differ between men and women, current transfusion thresholds are not sex-specific. We investigated whether the use of uniform thresholds in burn patients may lead to overtransfusion in women by analyzing real-world data.

Methods: We performed a retrospective cohort study using TriNetX, a real-world database of deidentified patient records. Patients with >20% total body surface area (TBSA) burns were included (ICD-10 codes T31.2–T31.9). Identified patients were stratified into groups based on hemoglobin levels (g/dL): Hb <6.5, Hb 6.5–10, and Hb >10. Transfusion rates for the overall cohort and separately for men and women were calculated and compared using the chi-square test. Statistical significance was defined as $p < 0.05$.

Results: Cohort 1 (Hb <6.5 g/dL) included 1,079 patients with a mean age of 48 ± 21 years; 32.1% were female and 65.5% male. Overall, 54.1% of this cohort received a blood transfusion within one month of injury. Cohort 2 (Hb 6.5–10 g/dL) included 5,077 patients, of whom 36.6% received transfusions. The mean age was 50 ± 21 years; 29.8% were female and 67.6% male. Cohort 3 (Hb >10 g/dL) included 7,774 patients, with a transfusion rate of 23.2%. Transfusion rates in males were 53.61%, 42.48%, and 26.55%; in females 59.81%, 43.39%, and 29.72%, respectively. While differences in Cohorts 1 and 2 were not significant, Cohort 3 showed a significant difference ($p = 0.0264$).

Conclusions: Our findings highlight the need to reconsider gender-specific transfusion practices and evaluate their impact on outcomes in critically ill subpopulations.

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LONG-TERM IMPACT OF SEPSIS ON PEDIATRIC IMMUNE CELL FUNCTION: IMMUNOPHENOTYPIC AND METABOLIC SHIFTS IN MYELOID CELLS

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Background: Pediatric patients under five years of age represent up to 40% of total sepsis cases, many of whom suffer from lasting immunosuppression that complicates and prolongs their recovery process. Immune cells are activated by exposure to various stimuli as invading pathogens or cytokines, which is followed by their metabolic switch associated with phenotypic changes. Detailed characterization of immune cells' immunometabolic status and phenotypic alterations together with patient clinical status represent possible approach how to depict the sepsis dynamics, especially with emphasis to the long-term consequences of sepsis in pediatric patients.

Methods: Using state-of-the-art techniques – deep immunophenotypization and single-cell metabolic analysis 'SCENITH' we depicted specific sepsis-induced alterations in myeloid cells from blood of pediatric patients (n = 12), collected within three time points – TP1 – within 48 h after ICU admission, TP2 – 3-5 days after and TP3 ~ 6 months after sepsis onset.

Results: We found specific cytokine patterns representing progression of sepsis and subsequent recovery. We also revealed the metabolic alterations across all innate immune cells represented by increased expression of CD36, CD38, and CD39. Moreover, neutrophils od septic patients showed increased mitochondrial dependence, whereas monocytes had increased glycolytic capacity. All markers were correlated to the severity of sepsis (pSOFA score) to obtain comprehensive information associated also with the clinical picture of patients. We also evaluated the decline of quality of life of these patients using self-reporting SF-36 questionnaire.

Conclusions: In summary, we showed a sepsis-induced changes in immunophenotype and immunometabolic profile of myeloid cells of pediatric patients within three timepoints with special emphasis to long-term immunosuppression ~6 months after sepsis onset indicating persisting changes in myeloid cell functionality. By further integration of all obtained data by machine learning we will evaluate the potential risk of severe complications' development and design timely treatment strategy to facilitate the patient full recovery.

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MORTALITY IN SEPTIC SHOCK ASSOCIATED WITH TYPE OF ANTICOAGULATION FOR THROMBOPROPHYLAXIS: A PROPENSITY-SCORE MATCHED ANALYSIS

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Background: Disseminated Intravascular disease (DIC) occurring in the context of sepsis consorts with more severe disease, serves as risk factor for mortality and discloses sepsis' concurrent coagulopathy (immunothrombosis). Heparin is widely used therapeutically, not only for its anticoagulant, but also for potentially exhibiting anti-inflammatory and immunomodulatory properties. We aimed to assess whether continuous intravenous injection of unfractionated heparin (UFH) leads to better outcomes compared to subcutaneous low molecular weight heparins (LMWHs) as thromboprophylaxis in septic patients.

Methods: 52 patients with septic shock were compared according to receipt of UFH or LMWH as thromboprophylaxis. Comparators (LMWH) were matched 4:1 with UFH cases based on Charlson comorbidity index (CCI) and Sequential Organ Failure Assessment (SOFA) score. Septic shock was classified by the Sepsis-3 definitions and only newly diagnosed cases (within the last 18 hours) were enrolled. The primary outcome was 28-day mortality.

Results: We analyzed 52 patients (13 under UFH, 39 under LMWH). Propensity scores did not differ in the matched comparisons. Overall, mortality of UFH-cases was 38.5% and it was similar to mortality of LMWH-cases (38.5% vs 41.03%; $p = 0.87$). Emerge of DIC in UFH was similar to that in LMWHs (p = N/S).

Conclusions: Among patients of similar severity and comorbidity burden, the use of intravenous UFH versus subcutaneous LMWH as thromboprophylaxis does not alter mortality or DIC rates in septic shock.

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INVESTIGATING THE ROLE OF HYDROGEN SULFIDE IN OSTEOMYELITIS USING Cse MOUSE MODELS

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Background: Osteomyelitis is a bone inflammation caused by pathogens like *Staphylococcus aureus* with anti-infective therapy being the primary treatment. However, increasing bacterial resistance highlights the need for novel therapies. Recently, cystathionine-γ lyase (CSE), an enzyme involved in hydrogen sulfide (H_2S) production, has emerged as a key immune modulator with anti-inflammatory effects. This study investigates the role of H_2S in osteomyelitis using $Cse^{-/-}$ mice to clarify the involvement of CSE in infection and inflammation to explore potential therapeutic targets.

Methods: A total of 72 transgenic mice (n = 18/group, 4 months old, both sexes) including $Cse^{+/+}$ (wild-type) and $Cse^{-/-}$ (knockout-KO) participated. Primary osteomyelitis was induced via intramedullary inoculation of 10^4 colony-forming unit (CFU) *Staphylococcus aureus* into the femur, while bacteremia was modeled by intravenous inoculation of 10^7 CFU/mouse into the tail vein. Animal survival and overall health status were monitored daily for 21 days, including clinical symptoms and weight measurements, prioritizing animal welfare. Bacterial loads were quantified in tissue samples (femur, kidney, liver and spleen) isolated from sacrificed animals. Additionally, blood and spleen were collected for immunological analysis through cytokine profiling (TNF-α, IL-6).

Results: Knockout mice exhibited higher mortality following intravenous inoculation and presented increased IL-6 in spleen and blood. Mortality in $Cse^{+/+}$ mice at 21 days was 5% in primary and 44.5% in secondary osteomyelitis (p-value = 0.02). In deficient mice, 5% fatality was noted in primary and 80% in secondary osteomyelitis (p-value <0.0001). Statistically significant differences in bacterial loads were observed in the liver and kidney. In $Cse^{+/+}$, the p-values were 0.008 for the liver and 0.003 for the kidney between primary and secondary osteomyelitis. Similarly, significant differences were observed in $Cse^{-/-}$ mice, with p-values <0.0001 for both tissues.

Conclusions: This gene plays a critical role in controlling systemic bacterial infection and modulating the host immune response.

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MUCOSAL IMMUNE RESPONSE IN RESPIRATORY VIRAL INFECTIONS

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Background: Ease to social restrictions lead to an increased prevalence of many other respiratory viruses besides SARS-Cov-2 and Influenza. Mucosal immune response to invading pathogens is of paramount importance as immune reactions prime diverse immune pathways. The aim of this study was to provide data regarding current epidemiological trends for respiratory viruses along with mucosal immune response to specific pathogens by measuring cytokines levels from nasopharyngeal swabs.

Methods: This is a multicenter, prospective study that was conducted in 7 study sites in Greece including patients with upper respiratory tract symptoms. The nasopharyngeal swab sample was analyzed for all the targets of the FilmArray upper respiratory panel (BioFire® Respiratory Panel 2.1 plus (RP2.1plus) and cytokines IL-1a, CXCL9, IP10, TNFα, TNFRSF1A, TNFRSF1B, IL-17 were determined with ELISA kits (Diaclone SAS) according to the instructions of the manufacturer. The Kolmogorov-Smirnov test was used to assess the normality assumption of the continuous variables; group comparisons were made using either a two-sample t-test or the Wilcoxon rank-sum test accordingly. The level of confidence was 5%.

Results: A total of 514 patients participated in the study from March to April 2023. SARS-CoV-2 was detected at 13.71% of patients while 17.75% had other respiratory viruses isolated. IL-6 levels were significantly higher in patients with Influenza virus compared to patients with SARS-CoV-2 (p = 0.0003) and patients with other respiratory viruses (p = 0.01). IL-8 levels were significantly higher in patients with Influenza virus compared to patients with SARS-CoV-2 (p = 0.033). For TNFRSF1A levels were higher in COVID-19 patients and patients with other respiratory viruses compared to patients with influenza (p = 0.01 and p = 0.02 respectively). Levels of TNF-α were higher among patients with SARS-CoV-2 and Influenza compared to other viruses.

Conclusions: SARS-CoV2, Influenza type A and B present a different and distinct pattern of mucosal response compared to other respiratory viruses, as depicted by relevant cytokine levels.

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EARLY SEPSIS DIAGNOSIS THROUGH VISCOELASTIC COAGULATION MONITORING

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Background: Early identification of sepsis in patients presenting with infection to the emergency department poses a significant clinical challenge. To this end, there is growing interest in the mechanisms of thromboinflammation, as there is close interplay between early inflammation and coagulation. Viscoelastic coagulation monitoring (VCM) provides point-of care insights into clot formation dynamics and may reflect the host's early response to infection. The objective of this study is to evaluate whether any of the eight coagulation variables captured by VCM can serve as early predictors of sepsis progression in patients presenting to the Emergency Department with suspicion of infection.

Methods: Vision Project (viscoelastic coagulation monitor as an early index of sepsis in patients admitted with infection at the emergency department) is an exploratory, prospective study aiming to provide proof-of concept for the use of VCM in the Emergency Department as a tool for early-stage sepsis diagnosis. VCM analysis will be conducted at the time of presentation and at three subsequent points. Progression to sepsis will be evaluated using SOFA score according to Sepsis-3 definitions.

Results: A total of 75 patients were included in the interim analysis. Preliminary findings indicate that the total number of out-of-range VCM variables was higher in patients who progressed to sepsis compared to non-septic patients. Specifically, septic patients exhibited a higher median number of out-of-range values (3 vs 1), with this difference reaching statistical significance (Mann-Whitney test, p = 0.002). Furthermore, ROC curve analysis demonstrated that the total number of out-of-range VCM variables has moderate discriminatory ability for identifying sepsis, with an area under the curve (AUC) of 0.734 (p = 0.001).

Conclusions: These findings suggest that early derangements in viscoelastic coagulation profiles may contribute to sepsis recognition shortly after the patient's presentation at the Emergency Department. While promising, the clinical implementation of VCM is still unfolding.

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DELETION OF LYSOSOMAL ASSOCIATED PROTEIN TRANSMEMBRANE 4 ALPHA (LAPTM4A) AMELIORATES ACUTE KIDNEY INJURY IN MICE WITH SHIGA-TOXIN-INDUCED HEMOLYTIC UREMIC SYNDROME

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Background: Infections caused by Shiga-toxin (Stx)-producing *Escherichia coli* can lead to typical hemolytic-uremic syndrome (HUS). The hallmarks of HUS are microangiopathic hemolytic anemia, thrombocytopenia and acute kidney injury. As there are no effective pharmacological treatments available, the management of HUS primarily relies on supportive care. A critical and fatal event in the pathogenesis of HUS is the binding of Shiga toxin to globotriasylceramide (Gb3), which leads to ribosomal inactivation and primarily endothelial cell death in the kidneys. Lysosomal-associated protein transmembrane 4 alpha (Lapm4a) is a key factor required the Gb3 synthase activity, and is exclusively responsible for Gb3 production. The effects of Laptm4a gene deletion (Laptm4a$^{-/-}$) on disease progression and kidney function in experimental HUS were evaluated, aiming to explore Gb3 synthase as a potential therapeutic strategy to mitigate Stx-induced renal damage.

Methods: Laptm4a$^{-/-}$ (provided by Dr. Proia from NIH, USA) and wild-type (WT) mice were examined on day 7 following repeated administration of Stx2 (registration number UKJ-22-018). Disease severity was evaluated based on clinical symptoms and hematological parameters. Kidney injury was determined in plasma by ELISA/colorimetric assay and in renal tissue by immuno- and histochemistry.

Results: Stx-challenged Laptm4a$^{-/-}$ mice exhibited improved survival compared to Stx-challenged WT mice (100% vs 93%). Consistently, levels of surrogate markers of kidney injury (NGAL, urea, renal KIM-1 and injury score (PAS)) were significantly lower in Laptm4a$^{-/-}$ mice than in WT mice with HUS. This was accompanied by less weight loss, decreased levels of plasma LDH and renal Ki-67 (proliferation marker) in Laptm4a$^{-/-}$ compared to WT mice with HUS. Consistent with these findings, hematological profiles (e.g. lymphocytes, monocytes, granulocytes, hemoglobin, red blood cells) also differed significantly between Stx-challenged mice groups.

Conclusions: This first study investigating the influence of Laptm4a deletion on the development of HUS in mice shows that Laptm4a plays an important role in HUS pathogenesis.

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LYTIC PHAGES TARGETING ABDOMINAL SEPSIS CAUSED BY ST218 KL57 CARBAPENEM-RESISTANT HYPERVIRULENT KLEBSIELLA PNEUMONIAE: EFFICACY AND RESISTANCE MECHANISMS

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Background: Abdominal sepsis caused by carbapenem-resistant hypervirulent *Klebsiella pneumoniae* (CR-hvKP) is gradually increasing around the world, and phage therapy is a viable application as an alternative to antibiotics. However, clinical application is still restricted by the phage resistance. In this study, we investigated the therapeutic potential of two novel phages, focusing on their *in vivo* efficacy through mouse model experiments, and elucidated the potential mechanisms of phage resistance.

Methods: This study established the mouse model of abdominal sepsis with a CR-hvKP clinical strain of ST218, KL57. Comprehensive biological profiling of two novel phages was performed by transmission electron microscopy, one-step growth curve assay, optimal multiplicity of infection, pH and temperature stability. We screened for phage-resistant strains *in vivo* and investigated their resistance mechanisms through whole-genome sequencing, metabolomic and transcriptomic analyses, and RT-qPCR.

Results: Two novel phages were successfully isolated from the hospital sewage, respectively named JLBP1001 and JLBP1002. Two phages exhibited specificity for the ST218 KL57 CR-hvKP, a clinical isolate obtained from a patient with intra-abdominal infection. Both phages completely suppressed bacterial growth within 12 hours *in vitro*. Assessment of survival rate, bacterial load, and hematoxylin and eosin staining of tissues from the abdominal sepsis mouse model demonstrated that treatment with JLBP1001 or JLBP1002 significantly enhanced survival outcomes. A total of 21 strains with complete or partial phage-resistant were identified and named RM01 to RM21. Whole-genome sequencing and bacterial variation analysis indicated that mutations in the capsular polysaccharides (CPS) gene cluster were present in 76.19% (16/21) of the strains, mainly including *wbaP*, *wzc*, and *wzy*. However, the strains RM01, RM02, and RM12 developed phage resistance by downregulating CPS and its transcriptional regulators, without any mutations in the CPS gene.

Conclusions: In summary, our findings provided further evidence in phage therapy, particularly in addressing the issue of abdominal sepsis caused by CR-hvKP.

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COMPETITIVE CXCR2 ANTAGONISM RESTORES NEUTROPHIL'S ABILITY TO CONTROL THE HOST RESPONSE TO INFECTION IN SURVIVAL AND SEVERITY STRATIFIED POLYMICROBIAL MOUSE SEPSIS

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Background: Sepsis remains a major cause of death due to dysregulated immunity. Since neutrophil dysfunction—mediated by CXCR2—is conserved across mice and humans, we tested CXCR2 blockade in stratified sepsis model as a precision therapy. To assess the pathogen-load-dependent effects of Danirixin (competitive CXCR2 antagonist) on survival, cytokine dynamics, bacterial clearance, and immune responses across organs in murine sepsis.

Methods: C57BL/6 J mice were subjected to peritoneal contamination and infection (PCI) with graded pathogen loads (60 μL for mild and 100 μL for severe sepsis), followed by treatment with Danirixin or vehicle. Survival was monitored for 7-days, along with clinical scoring, weight, and temperature. Cytokine levels (CXCL1, CXCL2, IL-6) were assessed longitudinally, and organ-specific bacterial counts, NETosis, and immune-cell infiltration and activation were analyzed at 24-h after infection.

Results: In severe sepsis, Danirixin significantly improved 7-day survival (87% vs. 52% in vehicle), with no changes in severity scores, weight loss, or hypothermia. Cytokine analysis revealed elevated CXCL1 at 24-h and sustained IL-6 at both 6- and 24-h in high-mortality sepsis. Danirixin treatment preserved or enhanced IL-6 levels at 24-h in high-pathogen sepsis while reducing IL-6 and CXCL2 at 48-h in low-pathogen sepsis. Across both peritoneum and spleen, Danirixin markedly reduced NETosis at 24-h in severe sepsis without impairing bacterial clearance, whereas in low-pathogen sepsis it suppressed NET formation but led to increased bacterial burdens in the peritoneum and circulation. Additionally, Danirixin enriched CXCR2^{+}CD34^{+} peritoneal macrophages in severe sepsis, without altering leukocyte infiltration across organs. A single 24-hour IL-6 measurement predicted survival with 75% accuracy, perfectly identifying survivors in the high-pathogen group.

Conclusions: Danirixin enhances survival in high-pathogen-load triggered sepsis by modulating neutrophil activity and immune homeostasis,

while effects in mild sepsis may risk immunosuppression. CXCR2 antagonism represents a promising precision strategy for hyperinflammation sepsis endotypes, warranting critical validation.

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TARGETING P2Y12 AS A SEX-SPECIFIC THERAPEUTIC STRATEGY TO DECREASE PLATELET-DRIVEN INFLAMMATION IN SEPSIS

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Background: Sepsis is characterized by a dysregulated immune response to infection, leading to organ failure and potentially death. Platelets play a role in sepsis. The ADP-receptor $P2Y_{12}$, essential for platelet biology, is activated during sepsis. Blocking $P2Y_{12}$ diminished cytokine storm and immune cell activation in a sex-related manner. However, how blocking $P2Y_{12}$ improves sepsis differently in males and females is still unknown. Objective: To investigate the effects of $P2Y_{12}$ blockade on survival and organ damage in male and female septic mice.

Methods: C57BL/6 J male and female mice underwent sham surgery or cecal ligation and puncture (CLP) to trigger sepsis. Post CLP, the mice received intraperitoneal injections of Ticagrelor (TIC - a $P2Y_{12}$ antagonist). We determined survival by monitoring mice for seven days post-surgery. Blood, kidneys, lungs, and bronchoalveolar lavage (BAL) fluid were collected 24 hours post-surgery. Platelet count was performed using Hemavet® Multispecies Hematology System. IL-6 levels were measured using an ELISA kit. Lung and kidney damage were evaluated using hematoxylin and eosin (H&E) staining. $P2Y_{12}$ content was determined using western blotting

Results: The CLP + TIC group showed an increased survival compared to the untreated CLP group in males but not females. IL-6 levels in the plasma and BAL were significantly lowered in TIC-treated septic males but not females. TIC lowered the sepsis-induced platelet infiltration into BAL fluid in males but not females. TIC prevented lung and organ damage in males, but not females. Increased levels of $P2Y_{12}$ protein in the lungs and kidneys were noted in septic males, but not females. Treatment with TIC decreased $P2Y_{12}$ expression in the lungs and kidneys of males, but not females.

Conclusions: Blocking $P2Y_{12}$ receptor improves survival and reduces organ damage exclusively in septic male mice. $P2Y_{12}$ expression was associated with organ damage, suggesting that $P2Y_{12}$ receptor could be a sex-specific biomarker in sepsis.

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DOBUTAMINE AND VASOPRESSIN: UNRAVELING THEIR EFFECTS ON SEPTIC MICROCIRCULATION

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Background: Sepsis is a life-threatening condition with systemic inflammation and microcirculatory dysfunction, leading to multi organ dysfunction syndrome. Gastrointestinal microcirculatory impairment plays a crucial role in sepsis progression by disturbing mucosal barrier integrity, leading to bacterial translocation, and exacerbating systemic inflammation. While dobutamine is commonly used to support cardiac output, its direct effects on intestinal microcirculation remain unclear. A similar uncertainty exists for addition of vasopressin, a second-line vasoconstrictor in septic shock. This study aimed to investigate the effects of dobutamine and vasopressin on gastrointestinal microcirculation in a rat model of abdominal sepsis.

Methods: 24 h after induction of sepsis (colon ascendens stent peritonitis, CASP), male Wistar rats (n = 44) were randomized into four groups: control (crystalloid vehicle), vasopressin, dobutamine, and a combination of both drugs. Microcirculatory variables, including oxygenation (μHbO_2) and perfusion (μFlow), were assessed using tissue-reflectance spectrophotometry and laser Doppler flowmetry, respectively for 90 min. Statistical analysis: two-way ANOVA for repeated measures followed by Tukey's and Dunnett's post-hoc tests (significance level $p < 0.05$) Data are expressed as mean $\pm$ SD.

Results: Dobutamine significantly improved intestinal microvascular oxygenation after 60 minutes ($\Delta\mu HbO_2$: 10.2 $\pm$ 13.4 % vs. control -2.3 $\pm$ 4.4 % and vs. baseline, $p < 0.05$) and blood flow ($\Delta\mu$Flow: 30 $\pm$ 21 AU vs. baseline, $p < 0.05$) in septic rats. The combination of dobutamine and vasopressin enhanced microcirculatory perfusion (28 $\pm$ 27 AU vs. baseline $p < 0.05$) but did not further increase tissue oxygenation. Vasopressin alone led to a transient increase in blood flow without significant effects on oxygenation.

Conclusions: Dobutamine improves gastrointestinal microcirculation in sepsis by improving oxygenation and perfusion. The combination with vasopressin also improves microcirculatory perfusion but does not further enhance tissue oxygenation. These findings support the use of dobutamine to counteract sepsis-induced microcirculatory failure.

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DEGREE OF IMMUNE DYSREGULATION AS A GUIDE FOR PROGNOSIS AND CORTICOSTEROID TREATMENT RESPONSE IN PNEUMONIA AND SEPSIS: A MULTICOHORT STUDY ACROSS DISTINCT CARE SETTINGS AND A RANDOMIZED CONTROLLED TRIAL

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Background: Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection. Yet, most immunomodulatory trials enroll patients based solely on clinical severity, overlooking the root cause of sepsis: the immune response. Existing phenotyping approaches focus only on sepsis patients and rely on transcriptomic or clinical data, thereby incorporating non-immune features and lacking specificity needed to guide personalized immunotherapy. Objective: To develop and validate a practical, immune-specific framework that quantifies the degree of host response dysregulation across the full spectrum of infection.

Methods: We measured 35 plasma biomarkers reflecting key pathophysiological pathways in 398 patients with community-acquired pneumonia across emergency, ward, and ICU settings. Using pseudotime trajectory inference, we modeled immune dysregulation as a gradient, identifying three distinct stages (minor, moderate, major) and a continuous score. After recursive feature elimination, we trained a 3-biomarker machine learning model (IL-6, procalcitonin, sTREM-1) to predict the full 35-biomarker based dysregulation score and translated this into an easy-to-use tool. Validation was performed in five independent cohorts (n = 1191). We assessed the value of the 3-biomarker tool for predictive enrichment in a RCT reanalysis.

Results: Clinical severity was an inadequate proxy for the underlying immune dysregulation. Patients with greater dysregulation had higher mortality and secondary infections rates, independent of disease severity. The 3-biomarker model accurately predicted immune dysregulation and was robust across diverse external cohorts with varying infections and care settings. In a reanalysis of a pneumonia-derived sepsis RCT, hydrocortisone improved survival only in patients with the most severe immune dysregulation, accompanied by faster resolution of the aberrant immune response. Stratifying by SOFA score or CRP did not predict response to hydrocortisone.

Conclusions: We introduce a 3-biomarker framework capturing immune dysregulation as a clinically meaningful continuum. This framework enables real-time immune profiling and offers a scalable tool to enrich trials and guide personalized immunotherapy.

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REVEALING RNA EDITING AS A MECHANISM OF MONOCYTE PLASTICITY IN AGEING AND COMMUNITY-ACQUIRED PNEUMONIA

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Background: RNA editing is an emerging post-transcriptional mechanism that may influence immune responses. In monocytes from patients with community-acquired pneumonia (CAP), an epigenetic signal was previously identified at a genomic locus harbouring RNA editing enzymes, associated with an immune-tolerant phenotype. Given the significant burden of CAP and immune dysfunction in older adults, we hypothesize that RNA editing contributes to immune heterogeneity in both ageing and infection. Objectives: To characterize messenger (m)RNA editing events in monocytes of elderly and young donors, as well as CAP patients. To assess mRNA expression of RNA editing enzymes in monocytes upon stimulation with lipopolysaccharide (LPS).

Methods: We enrolled 11 young (18–30 years) and 25 elderly individuals (>65 years) from the community or long-term care facility, following informed consent. Blood monocytes were purified for RNA-sequencing. Publicly available data from monocytes of 69 CAP patients and 41 controls were also included. RNA editing events were identified using GATK variant calling. To assess RNA editing enzyme inducibility, monocytes from young donors were stimulated with LPS followed by RT-qPCR analysis.

Results: We identified 184 mRNA editing events unique to monocytes from elderly donors, compared to 35 mRNA edits specific to young participants. In CAP samples, A-to-I and C-to-U editing frequencies were increased by 15% ($p < 0.05$) and 52% ($p < 0.0001$), respectively, with over one-third of these events located in unique exonic regions. Notably, 4,181 RNA editing sites were shared between the age-discordant cohort and CAP patients. Furthermore, LPS stimulation of monocytes from young donors induced differential expression of *ADAR* (A-to-I editor) and *APOBEC3G* (C-to-U editor) genes.

Conclusions: These findings uncover RNA editing as a potential post-transcriptional mechanism contributing to monocyte functional diversity in the context of ageing and infection. RNA editing pathways may represent novel targets for immune stratification and therapeutic intervention in acutely ill patients.

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PATHOGEN RNA IDENTIFIED IN THE BLOOD OF PATIENTS

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Background: Sepsis results in organ dysfunction due to an infection. Diagnosis of the causative pathogen can take days. RNA sequencing allows for the identification of all RNA in the blood of patients with sepsis. We hypothesize that RNA from pathogens can be identified in the blood of patients with sepsis.

Objective: Assess for the presence of RNA from pathogens in the blood of patients with sepsis.

Methods: Whole blood was collected in PAXgene tubes from patients with sepsis or suspected infection (bacteremia or pneumonia) after consent was obtained. This blood was used to obtain over 100 million RNA sequencing reads for each patient timepoint. The RNA was first aligned to the human genome and those reads are labeled as mapped. Then the unmapped reads (those not aligning to the human genome) were aligned to the genomes of Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus pyogenes. Read counts were correlated with culture data.

Results: RNA sequencing was done on 329 patient timepoints, either ER admission or day 0, 1, 3, 7 of ICU admission. Across all patients there were 574,182 reads to Escherichia coli, 678,850 reads to Klebsiella pneumoniae, 165,134 reads to Pseudomonas aeruginosa, 102,632 reads to Staphylococcus aureus, and 59,264 reads to Streptococcus pyogenes. There was no correlation between the number of reads to a pathogen in a patient and a positive culture for that pathogen.

Conclusions: RNA sequencing data from patients with sepsis has RNA that aligns to pathogens of clinical interest. However, simple correlation of the number of reads from the pathogen is not diagnostic. Further

work will be needed to assess the RNA from the pathogens for potential markers of infection.

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IMPACT OF POLYTRAUMA ON THE HEALTHY MUSCLE

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Background: Polytrauma can lead to complex systemic reactions and organ damage extending far beyond the directly injured areas. As the largest organ in the body, skeletal muscle can be affected by these systemic effects even if not directly damaged. The aim of this study was to investigate the effects of remote organ damage after experimental polytrauma on healthy skeletal muscle over a period of five days.

Methods: 12-week-old male C57BL/6 N mice were randomly assigned to polytrauma or sham group. The polytrauma model consisted of thoracic trauma, hemorrhagic shock, femur osteotomy with external fixator application, and laparotomy. 1, 3, and 5 days after trauma, the indirectly affected quadriceps femoris muscle was examined. Skeletal muscle analysis was performed using light microscopy, Western blot, and quantitative real-time PCR. The collected data were evaluated using two-way ANOVA.

Results: Histological examinations showed increased signs of muscle fiber damage in the polytrauma group, such as cell necrosis, interstitial edema formation and a decrease in fiber diameter. Gene expression analyses revealed significant upregulation of the atrogenes MuRF1 and Atrogin-1, especially on the first day after trauma. Increased expression of pro- and anti-inflammatory cytokines were observed with peak values of IL-6 on day 1 and IL-10 on day 3. Interestingly, the IGF-1/PI3K/Akt signaling pathway (measured by IR and IRS-1 expression), Akt phosphorylation and mTOR expression, showed no significant changes between groups. This suggests that the muscle atrophy and inflammation induced by polytrauma occur independently of the classical insulin-mediated signaling pathway.

Conclusions: Experimental polytrauma leads to time-dependent structural and molecular changes in indirectly affected skeletal muscle. The activation of atrogenes and increased expression of inflammatory mediators seem to play a central role, while the IGF-1/PI3K/Akt signaling pathway is not significantly affected. Further investigations are necessary to elucidate the underlying mechanisms and develop intervention strategies.

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TRAUMA-RELATED ACUTE KIDNEY INJURY (TRAKI) FOLLOWING FEMORAL FRACTURE: INVESTIGATING THE IMPACT OF SYMPATHETIC MODULATION

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Background: Femoral fractures are a rising public health concern, with high prevalence in the elderly. Trauma-related acute kidney injury (TRAKI) is a frequent complication, and even in cases of transient kidney dysfunction, increases the risk of poor outcomes. However, the mechanisms of fracture-related kidney injury, particularly the role of sympathetic activation in the renal response to trauma, remain poorly understood. This study aimed to assess the progression of TRAKI following femoral fracture and investigate the contribution of sympathetic modulation through targeted adrenergic receptor (AR) blockade.

Methods: Following femoral fracture and external fixation, mice received daily treatment with either Phentolamine, Propranolol, or Butoxamine, administered for the first three days after trauma. Kidneys were harvested 1 or 21 days post-fracture for histology or gene expression analysis. Blood urea nitrogen (BUN) levels were measured in blood samples to assess renal function. Statistical analysis was performed by one-way ANOVA testing plus post hoc analysis using Sidak's multiple comparison.

Results: One day after trauma, mice showed increased histological signs of kidney injury and elevated gene expression levels of the kidney injury marker neutrophil gelatinase-associated lipocalin (NGAL). However, BUN levels indicated no functional impairment. Activation of renal immune and oxidative stress responses was observed, along with increased proliferation and inflammation, but no development of fibrosis. By day 21, these alterations had resolved, indicating transient TRAKI without progression to chronic kidney disease. AR blockade did not provide sustained renal protection. Notably, Phentolamine treatment significantly delayed the upregulation of heme oxygenase 1 (HMOX1) and hypoxia-inducible factor 1-alpha (Hif1α), suggesting α-adrenergic involvement in the renal response to oxidative stress.

Conclusions: Femoral fracture induces a transient, mild form of TRAKI in mice without permanent functional impairment. While AR blockade did not prevent AKI, our findings point to a role for sympathetic signaling—especially α-adrenergic pathways—in shaping the renal response to trauma.

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EGFR ACTIVATION CORRELATES WITH INTRACRANIAL PRESSURE AND SURVIVAL IN A MIXED INTRACRANIAL BLEEDING PORCINE MODEL

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Background: The pig model is an advanced system for studying human brain trauma due to its anatomical similarities with the human brain, such as brain size, gyrencephalic structure, skull shape, and white-to-gray matter ratio. Ischemia, a common feature in fatal acute intracranial hemorrhage cases, occurs when brain tissue compression obstructs vasculature, reducing cerebral blood flow. This ischemia-driven injury is central to brain injury pathophysiology. This study investigates the role of receptor tyrosine kinases (RTKs) in the injury response and clinical outcomes, focusing on their potential as therapeutic targets for edema and reperfusion control after injury.

Methods: We developed a sustained, resuscitated pig model of acute mixed intracranial hemorrhage with ICP, providing a robust system for in-depth examination of brain injury. Multimodal brain monitoring and neurological assessments offered valuable insights into the progression of the injury. Macroscopic postmortem analysis, transcriptional evaluations combined with western blotting and protein arrays, allowed the the assessment of RTK pathway activation.

Results: Our findings showed that 44-54 hours post-injury, animals exhibited signs of hypoxia, neuroinflammation, and extensive tissue damage. Elevated HIF1-α expression in the ipsilateral hemisphere confirmed local hypoperfusion. Inflammatory markers such as TNF-α, CD68, and MMP-9 were upregulated in both hemispheres, reflecting a generalized neuroinflammatory response. Gene expression analysis

revealed increased markers of vascular, astrocytic, and neuroimmune activation, particularly related to endothelial integrity and astrocyte activation. RTK expression analysis showed increased levels of VEGFR1, VEGFR2, Tie-2, EGFR, and Axl in the injured cortex, with activation of EGFR/ErbB4 and HGFR/Met pathways. Hierarchical clustering of intrand astrocytic markers revealed distinct patterns of activation, highlighting the relationship between ICP severity and astrocyte response. Elevated EGFR phosphorylation correlated with astrocyte activation and ICP severity, survival, and Glasgow Coma Scale outcomes.

Conclusions: These findings suggest that modulating EGFR signaling may offer a therapeutic approach for managing ICP and improving outcomes in traumatic brain injury.

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SELECTED MARKERS OF ENDOTHELIAL DYSFUNCTION MAY BE UTILIZED AS MARKERS FOR SEPSIS TRIGGER AND DISEASE PROGRESSION

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Background: According to the sepsis-3 criteria, sepsis is an organ dysfunction caused by dysregulation of the host immune response. One important factor is endothelial dysfunction which consequences, such as hyperpermeability, pose severe problems. Previous clinical trials have tested different treatments for endothelial dysfunction during sepsis progression, but without satisfactory outcomes and further molecular elucidation of endothelial dysfunction is needed. This study aims to unravel the role of markers of endothelial dysfunction in depicting the outcome of sepsis disease progression and to help elucidate phenotypic differences between viral and non-viral sepsis.

Methods: In the course of two mainly monocentric clinical studies (ICROS and ICROVID; ethics board Jena: 5276-09/17 & 2020-2052-BO) 271 sepsis patients were monitored and blood samples taken during the acute phase of the disease progression (3 and 7 days post onset) and survivors followed up on up to one year post sepsis onset (6 and 12 months post onset). As a control, samples were taken from 81 comparable, healthy volunteers. Different markers of endothelial dysfunction were analyzed in plasma from all participants through ELISA measurements and their levels investigated for associations with mortality.

Results: Relative to the healthy control cohort some factors (e.g. angiopoietin-2, ICAM-1) were increased during the acute phase of sepsis, while others (e.g. angiopoietin-1, PAI-1) were decreased during the acute phase, before approaching control levels over a one-year period. In some cases, the triggers differentially influenced analyte levels. Especially for PAI-1 and angiopietin-2 non-viral sepsis patients showed higher levels compared to viral sepsis patients. While both angiopoietin-2 and PAI-1 are possible predictors of patient mortality, only angiopoietin-2 may be a feasible predictor for the duration of the hospital stay.

Conclusions: In conclusion, angiopoietin-2 and PAI-1 may become part of routine checks during sepsis disease progression to obtain a first indication on the outcome and to possibly adapt the medication plan of the patient.

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LEFT VENTRICULAR DAMAGE IN A PORCINE POLYTRAUMA MODEL – IS THE INHIBITION OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) A TREATMENT OPTION?

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Background: Cardiac damage after polytrauma (PT) is associated with increased mortality. Especially, a reduction of the left ventricular function is associated with an unfavorable outcome. The aim of the study was to validate the therapeutic effect of ISO-1, a macrophage inhibitory factor (MIF) inhibitor, on left ventricular damage following PT in pigs.

Methods: A porcine PT model was conducted in an Intensive Care Unit Setting with a follow-up period of 72 h. The following five groups, each with n = 6-8, were performed: minor trauma (MIMT: unilateral femur fracture and chest trauma); major trauma (MAMT: MIMT with a pressure-controlled haemorrhagic shock); a MAMT group with a single shot intraperitoneal application of ISO-1 (MIF-inhibitor, 5 mg/kg/kg bodyweight, MAMT+ISO-1); a MAMT group with the dilution media (MAMT+DMSO); and a sham group. Cardiac damage was measured via Troponin I (TnI), Growth Differentiation Factor (GDF)-15 and N-terminal pro b-type natriuretic peptide (NT-proBNP) ELISA. Histomorphological damage was evaluated with the Heart Injury Score, C5aR1, Nitrotyrosine, Caspase 3 and Connexin 43 immunohistochemistry. Finally, mRNA and miRNA expression (Next-Generation Sequencing) were compared among the groups.

Results: 1.5 h after trauma, a significant increase in TnI concentration was measured in both trauma groups, but not in the ISO-1 therapy groups ($p < 0.01$). NT-proBNP was slightly elevated in all animals. Histology of the left ventricle showed an increase in nitrotyrosine in the MIMT ($p < 0.001$) and MAMT animals ($p < 0.05$), as well as an increase in C5aR1 in the MAMT group ($p < 0.01$). The mRNA and miRNA profiles from the left ventricle, were dependent on trauma severity.

Conclusions: Cardiac damage was detected in PT groups by an increase in TnI, the activation of the complement system and an elevated level of nitrotyrosine. These changes were not observed in the therapeutic group. These findings suggest a potential protective effect of MIF inhibition on left ventricular function.

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DEVELOPMENT OF A NON-INTERVENTIONAL DIAGNOSTIC NGS-BASED ASSAY FOR RAPID PATHOGEN DETECTION IN BLOOD SAMPLES FROM SEPSIS PATIENTS

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Background: Conventional Standard of Care (SoC) culture methods for sepsis diagnosis suffer from long turnaround times, delaying appropriate antimicrobial therapy. Next Generation Sequencing (NGS) enables direct microorganisms characterization from clinical samples, offering rapid and comprehensive insights into microbial infections and antimicrobial resistance (AMR). Here we present PISTE™, an NGS-based approach for fast pathogen detection and AMR profiling in whole blood.

Primary objective of the study was to evaluate the diagnostic performance of PISTE^{TM} compared to SoC cultures in sepsis.

Methods: 100 patients with suspected sepsis were enrolled. Whole blood (20 ml) was drawn before antibiotic treatment and incubated at 37 °C (blood culture). After 6 hours, DNA was extracted for NGS library preparation, while the remaining volume was kept in incubation monitoring for microbial growth. Full-length 16S rRNA sequencing enabled species-level identification, followed by Shotgun Metagenomic Sequencing using the rapid PCR barcoding kit SQK-RPB114.24 (Oxford Nanopore Technologies) for deeper pathogen profiling and AMR gene detection. Data were analyzed using an in-house pipeline, and statistical analysis was performed with IBM SPSS.

Results: SoC identified 14 sepsis-positive patients. PISTE^{TM} successfully identified 13/14, achieving 91.7% Sensitivity, 96.5% Specificity, and 95.7% Accuracy. Turnaround time was ~6.5 h compared to ~30.5 h for SoC cultures. Shotgun sequencing was successful in 7/13 samples; failures were linked to low microbial biomass (below detection limit). Taxonomy profiles from both sequencing strategies showed full concordance. Predicted antimicrobial resistance profiles showed 100% Accuracy for Cephalosporins and Penicillins, 85.7% for Carbapenems, and 71.4% for Aminoglycosides, while lower Accuracy was measured for Tetracyclines and Quinolones (both 57.1%).

Conclusions: PISTE^{TM} technology provided rapid, accurate pathogen detection and AMR profiles in suspected sepsis cases, significantly reducing diagnostic turnaround. This approach supports, timely, targeted antibiotic therapy in critically ill patients and may substantially impact clinical management pathways for severe infections.

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HOST AQP4 GENETIC POLYMORPHISM AND GRAM-NEGATIVE BACTERIA PREDICT SEPTIC SHOCK AND OUTCOME IN PLEURAL EMPYEMA PATIENTS

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Background: AQP4 molecules compose water channels that control cell movement, migration and survival. AQP4 single-nucleotide genetic variants within in the regulatory 3' region associate with unfavorable course and outcome in post-stroke and sepsis patients. Whether the genetic variation within this region may serve as a source of prognostic biomarkers for severe complication of pneumonia, the pleural empyema and which infection, gram(-) or gram(+), affect the prognosis, have remained unexplored. The aim of the study was to test a hypothesis that AQP4 rs1058424 genetic variant in concert with gram (-) bacteria may serve as prognostic biomarkers in pleural empyema patients.

Methods: The study included 216 patients with pleural empyema aged M (IQR) 54 (41-66) years (women, 30%). On admission, M (IQR): SOFA score, 2 (2-2); APACHE-2, 5 (3-8); Charlson Comorbidity Index value, 2 (1-4). Septic shock was developed in 38 patients. Genomic DNA was isolated from 200 µl of whole blood, then a PCR product of 823 nucleotides in length was obtained using primers: 5`-CCGTGTGTCAAGATTTGGT -3` and 5`-GATTATCAACAAATGTCACGAG -3`. Genotypes of AQP4 rs1058424 were determined using Sanger sequencing.

Results: Detecting the gram-negative flora (mainly due to Klebsiella bacteria) associated with unfavorable both the course (development of septic shock) and the outcome of pleural empyema when compared with patients with only gram(+) bacteria ($P < 0.0001$, $n = 216$) or patients with no Klebsiella detected (HR = 7.1, 95% CI:2.7-19.1, $P = 0.021$; $n = 69$. The distribution of AQP4 rs1058424 genotypes corresponded to the Hardy-Weinberg law and did not differ from the distribution in Moscow healthy population. Minor allele T *AQP*4 rs1058424 exhibited significant protection against septic shock development ($P = 0.002$; OR = 22,5, 2,4-207,7, Fisher test, $n = 38$) and lethal outcome (HR = 8.9, 95% CI:3.5-22.5; $P = 0.009$).

Conclusions: Data demonstrate the contribution of AQP4 rs1058424 genetic variant to decreasing the risk of unfavorable course and outcome in pleural empyema patients infected with Klebsiella.

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ROLE OF PENTRAXIN-3 ON IMMUNE PHYSIOLOGY IN (EXPERIMENTAL) MELIOIDOSIS

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Background: Pentraxin-3 (PTX3) is an acute-phase protein that modulates protection against infectious bacterial diseases. In bacterial sepsis, PTX3 plasma levels are associated with severity, patient survival, and response to therapy. We aimed to assess the role of PTX3 in melioidosis, an important cause of pneumosepsis.

Methods: PTX3 levels were quantified in plasma of 34 culture-confirmed melioidosis patients and 31 matched healthy controls from Sunpasitthiprasong Hospital, Ubon Ratchathani, Thailand. In additional experiments, PTX3 expression were assessed in tissue from melioidosis patients after H&E- and immuno-staining. Further, wild-type C57Bl/6j mice were intranasally inoculated with *Burkholderia pseudomallei* strain 1026b and PTX3 levels and tissue pathology were assessed in organs harvested after 24-, 48-, and 72-hours post infection.

Results: Melioidosis patients exhibited elevated PTX3 levels in plasma, significantly decreasing after 10-14 days of admission. No correlation with mortality was found. PTX3 expression in stained-tissue samples from patients was prominent around lung infection sites with influx of inflammatory cells including neutrophils and macrophages. Experimental melioidosis revealed increased PTX3 levels in murine plasma, bronchoalveolar lavage fluid, lung and liver samples over time. Histopathology of *B. pseudomallei*-infected mice tissues mirrored patterns observed in patient tissues.

Conclusions: Our findings demonstrate PTX3 as a potential novel biomarker for melioidosis. Although PTX3 was not associated to mortality in our cohort, classical expression patterns and infection severity during disease was reflected. Similar trends were observed during experimental melioidosis. Future studies will utilize data from mice models treated with recombinant human PTX3, administered either prophylactically and/or therapeutically, to elucidate the functional role of this protein during experimental melioidosis and explore its therapeutic potential.

Data form this work were previously presented at the World Melioidosis Congress 2024.

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ACCELERATING PATHOGEN DETECTION IN SEPSIS: A NOVEL BIO-ELECTRODYNAMIC METHOD FOR SAMPLE PREPARATION

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Background: A rapid diagnosis of sepsis is difficult, given that pathogen detection is frequently inaccurate and requires 24-72 h. Cellectric Biosciences develops a technology by reduces blood matrix effects while maintaining the viability of the pathogens. This project evaluates a preparation method using a BaseStation device and compares it to the gold standard.

Methods: Blood samples from healthy human volunteers (HB; n = 15), mild trauma patients (MTB; n = 20), and mice (n = 7) subjected to cecal ligation and puncture (CLP) sepsis were used. Human blood was spiked with *E. coli* and *B. cereus,* whereas CLP samples contained live polymicrobial pathogens. Samples were processed in three groups: i) non-processed (control = NP) and exposed to ii) high voltage (cell lysed = HV), and iii) zero voltage (non-lysed = 0 V) in the BaseStation. Samples were cultured to evaluate the impact of the BaseStation workflow on bacterial quantity and viability. Used assays included FACS, colony forming unit (CFU) counts, matrix-assisted laser desorption/ionization (MALDI), and Fourier-transform infrared spectroscopy (FTIR).

Results: We demonstrated an efficient BaseStation-dependent lysis of erythrocytes (98%) in blood samples subjected to HV, with similar differences in conductivity between HB and MTB samples (range 4,69 – 8,65 mS/cm; n = 17). Comparison of CFUs in HB and MTB spiked samples demonstrated similar bacterial concentrations in all three groups (NP, 0 V, and HV), regardless of the pathogen used. In the blood of CLP mice, 13 different pathogens (most commonly *E. gallinarum, E. coli, K. oxytoca*) were detected. In CLP mice with positive NP cultures, more than 50% of pathogens were the same between NP and HV in 71% of mice, and between NP and OV in 57%. The FTIR spectral profiles were acquired independently for MTB samples spiked with *E. coli*, *B. cereus,* and CLP mice, with each showing similar clustering.

Conclusions: Our findings suggest that the BaseStation neither impedes the viability of the pathogens nor alters their biochemical composition.

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ELEVATED PLASMA SOLUBLE PROGRAMMED DEATH LIGAND-1 (PD-L1) CONCENTRATIONS ASSOCIATE WITH HYPERINFLAMMATORY AND ENDOTHELIAL CELL ACTIVATION SIGNATURES IN COMMUNITY-ACQUIRED PNEUMONIA

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Background: Soluble programmed death ligand-1 (sPD-L1) is widely considered a marker of immunosuppression in infection and sepsis, primarily based on the role of cell-surface PD-1/PD-L1 in downregulating immune responses. However, whether circulating sPD-L1 reflects true activation of this pathway or broader immune dysregulation remains unclear. The coexistence of hyperinflammation and immunosuppression in sepsis complicates its interpretation, and its relevance in milder infections is unclear. Objective: To characterize immune response patterns associated with sPD-L1 in moderately severe community-acquired pneumonia (CAP).

Methods: Plasma sPD-L1 and 60 other host response biomarkers were measured in hospitalized ward CAP patients, sampled within 16 hours of admission, and in non-infectious controls. Associations with biomarkers reflecting inflammation, vascular response, coagulation, and cytokine/chemokine release were assessed through dimensionality reduction (PCA) across sPD-L1 tertiles. Individual biomarker associations were assessed using effect sizes, regression and correlation analyses. Associations between sPD-L1 and PD-1/PD-L1 expression on peripheral blood mononuclear cells (flow cytometry) and blood transcriptomic profiles (RNA sequencing) were examined in subsets.

Results: sPD-L1 levels were elevated in CAP patients (n = 226) versus controls (n = 28; p = 0.0021). PCA revealed that higher sPD-L1 was significantly associated with increased inflammation and organ damage (e.g., TREM-1, β2-microglobulin), elevated cytokine release (e.g., IL-1α, IFNγ), and enhanced endothelial activation (e.g., angiopoietin-2, syndecan-1). IL-10, reflecting immunosuppression, was not associated with sPD-L1. Monocyte surface PD-1/PD-L1 expression was significantly increased in CAP versus controls, but did not correlate with sPD-L1. Neither PD-1 nor PD-L1 gene expression was associated with sPD-L1. Transcriptomic pathway analyses revealed significant enrichment of hemostasis (platelet activation and clotting), endothelial activation (vascular wall interactions), and immune response (antigen presentation and interferon signaling) in blood.

Conclusions: In moderately severe CAP, plasma sPD-L1 concentrations do not correlate with monocyte PD-1/PD-L1 expression and do not signal immune suppression; rather, sPD-L1 is reflective of inflammation and endothelial activation in this condition.

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POLYTRAUMA-INDUCED ALTERATIONS IN THE GUT MICROBIOME AND THEIR ASSOCIATION WITH SYSTEMIC INFLAMMATION IN MICE

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Background: The gut harbors a diverse microbial community essential for metabolism and immune regulation. Disruption of this ecosystem, or dysbiosis, has been linked to various inflammatory diseases. Trauma triggers excessive inflammation, which affects organs beyond the primarily site of injury, including the gut. This study aimed to characterize changes in gut microbiota after polytrauma in mice.

Methods: C57BL/6 N mice were assigned to four groups: control (no intervention), sham (anesthesia, catheterization, external fixation), fracture (sham + femoral osteotomy), and polytrauma (fracture + hemorrhagic shock, laparotomy, thoracic trauma). Feces were sampled for 21 days post injury (dpi) (n = 6). Serum levels of 45 cytokines and chemokines were analyzed at days 1, 3, 5, 7, 14, and 21. Feces bacterial composition was identified by Illumina paired-end raw 16S-rRNA-sequencing and analyzed using QIIME2 (v.2023.2) within the Galaxy platform. After filtering and quality control, 285 samples and 6,056,880 reads were retained. Taxonomic assignment of 3,860 representative sequence sets with 99% identity, phylogenetic analysis, and diversity metrics (alpha and beta diversity) were evaluated using lme4-R-package (v.1.1-31), R-package MicrobiotaProcess (v.1.10.3), Bray Curtis, Jaccard, Unweighterd-Unifrac, Weighte- Unifrac. Maaslin2 (v.1.12.0) R-packages.

Results: Alpha-diversity analysis by longitudinal and general linear-mixed-effects modeling showed no significant within- or between-groups differences. However, beta-diversity metrics revealed significant bacterial compositional shifts between control and the other three experimental groups. In polytraumatized mice, several bacterial genera were significantly upregulated (e.g. *Eubacterim coprostanolignes, Clostridia UCG.014, Candidatus Arthrombomitus, Muribaculaceae*) or downregulated

(*Gastranaerophilales, Lachnospiraceae A2, NK4A136*). Multiple bacterial genera correlated positively (*Rikenellaceae*) or negatively (*Oscillospirales, Butyricicoccaeceae, Lachnospiracea*) with serum CCL2 levels, specifically in polytraumatized animals.

Conclusions: Polytrauma caused significant alterations in the intestinal microbiota, with increasing changes correlating to injury severity. Several bacterial genera were closely linked to the systemic inflammatory marker CCL2, suggesting a potential role in trauma-induced immune modulation.

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THE COMBINATION OF MCP-1, IL-9 AND IL-10 ON ADMISSION IDENTIFIES DIVERGING OUTCOMES ACROSS DIVERSE COHORTS OF POLYTRAUMA PATIENTS

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Background: Polytrauma remains a leading cause of mortality worldwide. Critical immune mechanisms following injury are yet to be fully understood. Cytokine biomarkers hold the promise of providing biological insights and predicting outcomes. This study aimed to identify immune mediators associated with unfavorable outcomes in polytrauma patients and their optimal combination for patient clustering.

Methods: Patient data from two multicenter studies of polytrauma patients were collected. "PAMPer" randomized trial included patients at risk of hemorrhagic shock (Sperry et al., 2018), and "PRECISE" was a prospective observational study containing a diverse set of injured patients (McKinley et al., 2022). Two subsets of patients, propensity score matched by age, gender, ISS, AIS head, and shock index, were selected. Measurements for 21 immune mediators at admission were performed using the Luminex assay. The most promising mediator was identified by measuring the correlation of mediators with outcome variables. After the patients were grouped by the first mediator, an iterative process was used to reach the most informative second mediator for combination.

Results: After matching, patients from both cohorts demonstrated similar patterns in mediator correlations. MCP-1 showed the strongest association with adverse outcomes, including ICU length of stay in the PAMPer cohort (Spearman's $\rho = 0.36$, $p < 0.01$) and development of multiple organ dysfunction syndrome (MODS) in the PRECISE cohort ($\rho = 0.48$, $p < 0.01$). Following stratification based on median MCP-1 levels, IL-10 proved to be a key secondary mediator: elevated IL-10 in both strata was associated with longer ICU stays in PAMPer and higher organ failure scores in PRECISE. In contrast, IL-9 consistently correlated with favorable outcomes, suggesting a potentially protective role.

Conclusions: We show that a limited set of immune mediators can classify polytrauma patients into clusters with diverging trajectories. Further validation can illuminate how similar sets of markers could guide diagnosis and personalized therapy.

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PHARMACOLOGICAL TARGETING OF RSK AS INNOVATIVE STRATEGY TO AMELIORATE CARDIAC INJURY EVOKED BY EXPERIMENTAL SEPSIS

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Background: p90 ribosomal S6 kinase (RSK) is a key signaling node in numerous pathologies. Well studied in cancer and known to play a role in cardiovascular diseases, RSK is emerging as central player in inflammatory disorders. This study uncovers RSK involvement in a severe inflammatory condition: sepsis. The purpose of this study is to investigate the effects of RSK pharmacological inhibition on sepsis-induced hyperinflammation and multi-organ failure, with a focus on cardiac injury.

Methods: C57BL/6 J mice underwent cecal ligation and puncture (CLP) or sham surgery. 1 h and 5 h after surgery, we administered intravenously 10 mg/kg and 5 mg/kg of the RSK inhibitor BI-D1870 or vehicle. 24 h after surgery, we assessed sepsis severity, body temperature and free fluids. We quantified biomarkers of organ damage and inflammatory mediators in plasma. In the heart, we assessed tissue morphology, RSK phosphorylation and expression of inflammatory genes.

Results: BI-D1870 markedly improved the clinical score of septic mice, while reducing sepsis-induced increase in body temperature and edema. RSK inhibition lowered the systemic levels of a panel of 23 cytokines and chemokines and biomarkers of multi-organ damage, among which cTnI, NT-proBNP and LDH. The heart of CLP + BI-D1870 mice showed improved fiber structure compared with CLP mice. In the heart, the treatment reduced also the phosphorylation, and consequent activation, of RSK2, and reduced the expression of pro-inflammatory cytokines and chemokines.

Conclusions: These findings suggest RSK as a promising therapeutic target for sepsis-induced injury, especially cardiac. RSK is associated with the immune response and the contribution of non-myocyte cells in cardiomyopathies is becoming of growing interest. For this reason, we are evaluating the effect of RSK inhibition on leukocyte infiltration into the inflamed heart tissue. In addition, by proving RSK involvement in sepsis, RSK inhibitors that are being developed for other pathologies could be repurposed for this disease.

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LARGE ANIMAL MODEL OF KIDNEY TRANSPLANTATION TO OPTIMIZE STATIC COLD STORAGE

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Background: Kidney transplantation remains the gold standard treatment for end-stage renal dysfunction. However, the prolonged shortage of suitable donors further compounded by the increasing utilization of extended-criteria donors emphasizes the urgent need for advancements in organ preservation techniques. While static cold storage (SCS) is widely adopted due to its cost-effectiveness and simplicity, it still requires further optimization. Preclinical evidence suggests that methane (CH_4) has potent anti-inflammatory, antioxidative, and mitochondrial-stabilizing properties, indicating potential utility as a bioactive additive in preservation solutions. This study aimed to develop a clinically relevant porcine kidney transplantation model to investigate the efficacy of methane-enriched Custodiol (HTK) solution in improving SCS outcomes.

Methods: Under general anesthesia and mechanical ventilation, pigs underwent left nephrectomy followed by recovery (approval no. V/3262/2022). Retrieved kidneys were perfused and stored for 16 hours at 4 °C in either standard HTK or methane-enriched HTK solution

(HTK + CH_4; enriched with 25 L of 2.1% CH_4-air mixture; n = 5/group). Kidneys were then reimplanted, and contralateral nephrectomy performed following reinduction of anesthesia. Animals were monitored for 24 hours postoperatively. Primary endpoints included renal artery flow (RAF), urine output (UO), and serum creatinine levels. Secondary parameters included systemic hemodynamics and requirement for diuretic interventions.

Results: Kidneys preserved in HTK + CH_4 demonstrated superior perfusion, evidenced by consistently higher RAF values across the postoperative period. Moreover, serum creatinine levels at endpoint were significantly reduced in the methane group (4.01 ± 0.2 vs. 4.7 ± 0.3 mg/dL), indicating improved renal function. Although UO remained similar between groups, the standard HTK group required more frequent furosemide administration to maintain diuresis.

Conclusions: This translational porcine model effectively facilitates the evaluation of organ preservation strategies. Our findings suggest that methane-enriched HTK solution confers significant renoprotective effects, enhancing graft function and perfusion in the context of kidney transplantation.

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EXTRACELLULAR VESICLES AND miRNA SIGNATURES: A TRANSLATIONAL APPROACH FOR NEW BIOMARKER CANDIDATES IN TRAUMATIC BRAIN INJURY AMONG POLYTRAUMA PATIENTS

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Background: Severe traumatic brain injury (TBI) in polytrauma (PT) represents a major medical and socioeconomic challenge due to high morbidity and mortality. For the first time, comprehensive data on epidemiology, treatment and long-term outcomes are available thanks to the new established TBI database (DB) in the German TraumaRegistry (TR-)DGU®. The complex pathophysiology of TBI in PT makes diagnosis and treatment difficult, which often leads to insufficient accuracy of biomarkers. Extracellular vesicles (EVs) and miRNA, involved in intercellular communication, have potential as biomarkers. We hypothesized that the characteristics of the injured tissue are reflected in the cell-specific epitopes of EVs and that the miRNA composition in plasma and EVs are signatures that change specifically after injury. The identification of TBI-specific EVs and miRNA profiles serve as potential biomarkers under the translational validation using clinical TBI-DB TR-DGU® data.

Methods: The study includes patients with isolated TBI ($AIS_{Head} \geq 4$; n = 10) and PT without TBI (Injury Severity Score ≥ 16, $AIS_{Head} = 0$; n = 10). EVs were isolated from patient`s plasma at admission and 48 hours later. Healthy volunteers (n = 10) were used as controls. EV surface marker quantification was performed using the *MACSPlex EV Neuro Kit*, with differentially expressed epitopes validated via Western blot. Additionally, miRNA content in plasma, EVs, and neuron-derived EVs (nEVs) was analyzed using real-time PCR after. nEVs were enrichment with L1CAM antibody magnetic beads. All findings were correlated with clinical data from TBI-DB.

Results: Analysis revealed 10 previously undescribed markers (e.g., MOG+, C49+) differing between groups, with five showing TBI specificity. These markers correlate with clinical outcome (e.g., Glasgow Coma Scale), indicating their potential as indicators of varying phases of brain injury. miRNA expression pattern (e.g. miR21-5p) in plasma, (n)EVs change depending on injury and time point. Regression analyses identified markers associated with clinical outcomes (e.g., Glasgow Coma Scale).

Conclusions: The TBI-DB will facilitate continuous evaluation of TBI epidemiology and treatment in Germany. Our findings suggest EVs and their miRNA content may be valuable biomarkers for severe TBI and PT, ultimately enhancing patient care.

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EXPLORING THE ROLE OF LUNG ASYMMETRY IN AMPLIFYING THE PENDELLUFT EFFECT AND PULMONARY STRESS

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Background: Anatomical asymmetry in humans is a normal variation that has been well documented in the literature, yet the consequences of this asymmetry have not been explored. We will explore the effects that normal asymmetry has on the Pendelluft effect. The Pendelluft effect is characterized by the intrapulmonary movement of air during the respiratory cycle that is not involved with respiration. This effect can lead to increased tissue stress and lung damage. In hematogenous diseases, there is an increase in the Pendelluft effect, leading to even greater discrepancies in gas exchange. This study investigates how natural anatomical asymmetry in human lungs affects the Pendelluft effect and contributes to the progression of hematogenous lung diseases. It hypothesizes that normal asymmetry may influence the lung's mechanical stress and decrease respiratory efficiency in hematogenous diseases.

Methods: The hypotheses outlined above are based on a review of existing literature regarding airflow mechanics, bronchial pressure, and changes in compliance in the human lung. Previous studies included both observational and theoretical data that form the basis for the proposed relationship. We investigate how normal anatomical variation may affect airflow and pressure, influencing the Pendelluft effect. Furthermore, alterations in compliance and their impact on gas exchange are examined in relation to hematogenous diseases.

Results: In the preliminary research it is suggested that the anatomical asymmetry leads to airflow and pressure variation, amplifying the Pendelluft effect, especially in hematogenous inflammation. This increase in the Pendelluft effect elevates lung tissue stress, leading to progressive alveolar damage.

Conclusions: By further understanding the relationship between normal anatomical asymmetry and the Pendelluft effect, we may gain greater insight into disease progression in hematogenous lung conditions. This relationship may improve diagnostic and therapeutic strategies for uneven ventilation in vulnerable lung regions.

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MACRO- AND MICROSTRUCTURAL BRAIN ALTERATIONS IN FEMALE SEMI-PROFESSIONAL SOCCER PLAYERS

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Background: Repetitive head impacts (RHI) from soccer headers could be associated with neurotrauma and neurodegeneration, but distinct effects among young athletes are widely unexplored. It has been proposed that particularly female athletes may have worsened outcome following neurotrauma, thus represent a vulnerable but underrepresented cohort in research and clinical management. Objective: To explore the impact of RHI on the brain's macro- and microstructure, and to investigate associations between soccer headers, brain volume alterations, and white matter (WM) changes.

Methods: In this prospective study, 20 semi-professional female soccer players (mean age: 22.4 ± 4.1 years) and 15 female non-contact-sport controls underwent 3-Tesla magnetic resonance imaging (MRI) of the brain (3D T1-weighted and diffusion-weighted sequences). Players were assessed for measures of exposure (including field position and subjective header frequency). Subcortical grey matter and corpus callosum WM volumes were calculated from T1-weighted images, and whole brain-based spatial statistics (WBSS) were performed for diffusion MRI. Group differences in brain volumes (macrostructure) as well as fractional anisotropy (FA) from WBSS (microstructure) were assessed. Moreover, associations with exposure measures were analyzed, and results were controlled for multiple comparisons.

Results: No significant group differences in overall brain regional volumes were found, while players showed statistically significant FA changes within the thalamus ($p < 0.0001$). Among players, higher number of headers correlated with lower posterior corpus callosum volumes ($r = -0.57/p = 0.013$). Field position was linked to volumes of the right nucleus accumbens ($r = 0.58/p = 0.011$), hippocampus ($r = 0.72/p = 0.001$), and putamen ($r = 0.59/p = 0.010$), suggesting that higher header frequency was associated with lower respective volumes.

Conclusions: Heading the ball during soccer may result in RHI that could lead to impaired microstructure of the thalamus, the brain's critical relay station involved in many functions such as consciousness, learning, and memory. Furthermore, higher header frequency may be linked to reduced subcortical and WM volumes in a vulnerable cohort of young female athletes.

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EX-VIVO AND IN-VIVO OPPORTUNISTIC ASSESSMENT OF VERTEBRAL BONE MINERAL DENSITY USING COMPUTED TOMOGRAPHY – ACCURACY AND CLINICAL IMPLICATIONS IN PATIENTS WITH VERTEBRAL FRACTURES

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Background: Assessment of osteoporosis currently requires dedicated examinations by dual-energy X-ray absorptiometry (DXA) or quantitative computed tomography (QCT). However, with methods of artificial intelligence (AI), opportunistic assessment (i.e., measurements of volumetric bone mineral density [vBMD] in non-dedicated routine CT data acquired for other purposes than osteoporosis screening such as oncological staging) becomes feasible. Objective: 1) Ex-vivo: To investigate lumbar vBMD from opportunistic CT scans using different protocols, and compare them to dedicated QCT. 2) In-vivo: To opportunistically assess vBMD in CT scans from patients with/without vertebral fractures (VFs).

Methods: For opportunistic vBMD measurements, a newly developed AI framework based on convolutional neural networks (CNNs) was used for fully automated spine identification, vertebral body labeling/segmentation, VF detection, and contrast agent correction. Cadavers from two donors were scanned (L1–L5) using six different CT protocols (80–140kVp, 72–200mAs, 0.75–1 mm slice thickness) and one standard QCT scan. Furthermore, clinical routine CT data (maximum coverage T1–L6) from 794 patients (mean age: 67.8 ± 0.9 years) were screened and divided into VF and non-VF groups.

Results: Strong correlations were observed between vBMD from opportunistic CT and reference QCT ex-vivo ($\rho = 0.869/p < 0.01$). Bland-Altman analysis showed a mean bias of 3.1 mg/cm^3, with all data points falling within the limits of agreement. In-vivo, the framework worked in all patients for vBMD extraction of each imaged vertebral body, showing significantly lower mean vertebral vBMD in patients with at least one VF (104.5 ± 36.6 vs. 79.0 ± 33.1 mg/cm^3, $p < 0.01$; measured in non-fractured vertebral bodies).

Conclusions: Opportunistic vBMD measurements of vertebrae using our AI framework may provide reliable consistency and accuracy across various scan parameters when compared to dedicated QCT. In-vivo, it reliably worked for opportunistic vBMD extractions, indicating significantly lower vBMD in patients with VFs. Thus, it may serve as a screening tool for osteoporosis and fracture risk in the future.

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A PORCINE MODEL OF SEPTIC SHOCK WITH FLUID OVERLOAD AND PROLONGED RESUSCITATION

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Background: Fluid overload in sepsis, driven by capillary leakage, poses a major challenge in managing septic shock. Capillary leakage is a central mechanism, but experimental data to guide targeted therapeutic approaches remain limited. It is therefore essential to develop robust preclinical models in order to explore strategies aimed at limiting its deleterious effects. We therefore developed a translational porcine model of Pseudomonas aeruginosa-induced septic shock with fluid overload, resuscitated over 24 hours.

Methods: Sixteen female pigs (55 kg) were divided into three groups. control (CT, n = 4, no bacteria), the two other groups received an IV infusion of *Pseudomonas aeruginosa* (5×10^8 CFU/mL) at short dose (SD, n = 6, 0.3 mL/20 kg/min for 90 min), and continuous dose (CD, n = 6, 0.1 mL/kg/h with hourly doubling over 6 h). Upon hypotension (MAP <65 mmHg), resuscitation followed a standardized protocol (fluids ± norepinephrine). Data were collected over 24 hours from the start of bacterial infusion.

Results: No sepsis was observed in the CT group, while all pigs in the SD and CD groups met sepsis criteria based on the porcine-adapted five-domain SOFA score. Septic shock developed in 33.3% of SD and 100% of CD animals. Twenty-four-hour survival was 100% (CT), 83.3% (SD),

and 66.7% (CD). Significant differences were observed in fluid balance (1230, 5650, 7808 mL; $p < 0.001$), lung wet/dry ratio (2.35, 6.57, 5.75; $p = 0.0008$), kidney wet/dry ratio (2.78, 5.78, 5.09; $p = 0.02$), and combined pleural/peritoneal fluid accumulation (11, 255, 974 mL; $p = 0.003$).

Conclusions: Continuous *P. aeruginosa* infusion effectively induced septic shock with significant fluid overload, providing a robust porcine model of sepsis-induced capillary leakage. However, the resuscitation protocol requires refinement to improve survival.

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INFLUENCE OF ACUTE ALCOHOL-INTOXICATION ON NEUTROPHIL-DERIVED EXTRACELLULAR VESICLES IN POSTTRAUMATIC INFLAMMATION IN-VITRO

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Background: Severe traumatic injuries lead to a tremendous release of damage-associated molecular patterns, initiating a prompt and serious innate immune reaction. This activation facilitates the development of sepsis and multi organ failure. Neutrophil Granulocytes are key modulators of the early inflammatory phase, potentially using small Extracellular Vesicles (EVs) for remote intercellular communication. Interactions of these EVs with the liver could contribute to an excessive systemic inflammation. Therefore, in this study we focussed on the interplay between Neutrophil-derived small EVs and hepatocytes, and the effects of the known immune regulator ethanol, as ethanol intoxication is frequently observed in trauma patients. Our study investigated the effects of small EVs released by stimulated Neutrophils with or without ethanol on HepG2-hepatocytes in-vitro.

Methods: Neutrophils from healthy volunteers were isolated and seeded for 1 h with N-formylmethionine-leucyl-phenylalanine/phorbol 12-myristate 13-acetate (f/P) or without stimulus in combination with and without ethanol (f/P-nEVs, spontaneous (s)-nEVs, f/P-C2-nEVs or C2-nEVs). EVs <200 nm were collected from supernatant by size exclusion chromatography. The protein amount of nEVs was determined. A concentration of 1 μg/ml nEVs from differentially stimulated Neutrophils was used to stimulate HepG2 for 24 h. Gene expression of interleukin (IL)-6, IL-1β and tumor necrosis factor (TNF)-α in HepG2 was analysed by real-time quantitative polymerase chain reaction (qPCR) and the expression of intercellular adhesion molecule 1 (ICAM-1) by flow-cytometry.

Results: Incubation of HepG2 with f/P-nEVs lead to a significant increase of IL-6 gene expression with similar trends seen for IL-1β compared to unstimulated cells and cells stimulated with s-nEVs. Stimulation with f/P-C2-nEVs and C2-nEVs showed comparable results as s-nEVs. Gene expression of TNF-α trended to decrease following incubation with f/P-nEVs with further reduction in s-nEVs.

Conclusions: Neutrophil-derived EVs regulate hepatic inflammation in a neutrophil-stimulus dependent pattern. Co-stimulation of Neutrophils with ethanol attenuated the proinflammatory effects of stimulated nEVs.

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BIOELECTRICAL IMPEDANCE ANALYSIS DERIVED PHASE ANGLE IS A PREDICTOR OF LONG-TERM OUTCOME IN PATIENTS WITH SEPSIS

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Background: The long-term prognosis after sepsis is influenced by various factors, including pre-illness functional status. Bioelectrical impedance analysis (BIA) employs whole-body resistance and reactance to obtain phase angle (PA). PA has been demonstrated to predict mortality in different cohorts; however its prognostic value for long-term outcome in sepsis has not been investigated. The objective was to evaluate the prognostic value of BIA-derived PA for 365-day mortality and for physical outcome of patients with sepsis treated on intensive care unit (ICU).

Methods: In this post-hoc analysis of a monocentric cohort study, BIA was conducted in ICU patients with sepsis or septic shock (n = 137) in the acute phase (T1:3 ± 1 and T2:7 ± 1 days) and long-term course (T4:6 ± 2 and T5:12 ± 2 months), and in healthy controls (n = 81). Six-minute walk test was performed at T4 and T5. The primary endpoint was to ascertain the prognostic value of PA for 365-day mortality (Cox-regression). The longitudinal changes in PA and walking distance were compared with controls (t-test). The association between PA and walking distance was evaluated (multiple linear regression).

Results: 365-day mortality of sepsis was 37.2% (n = 48/116). Adjusted for age, sex, comorbidities and disease severity, PA_{T1} was associated with 365-day mortality (hazard ratio [95% confidence interval], 0.65 [0.45–0.92], $P = 0.016$). PA_{T1} (mean ± standard deviation, 3.6 ± 1.1°) was lower in septic patients compared to controls (6.5 ± 0.9°, $P < 0.001$) and remained decreased up to one year (5.8 ± 1.5°, $P = 0.035$). Walking distance was significantly reduced one year after sepsis (467.5 ± 122.8 m *vs* 601.7 ± 111.0 m, $P < 0.001$). Adjusted for age, sex, and comorbidities, PA_{T1} was associated with walking distance after six months (β_z [95% confidence interval], 0.46 [0.14–0.78], $P = 0.007$).

Conclusions: Lower PA was prognostic for 365-day sepsis mortality and impaired physical outcome. The long-term reduction in PA may indicate the persistence of an underlying impairment. BIA may prove to be a valuable tool to guide treatment and rehabilitation in sepsis.

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EFFECTS OF METHANE INHALATION ON CEREBELLAR AND RENAL MITOCHONDRIAL FUNCTION IN A RAT SEPSIS MODEL

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Background: Mitochondrial dysfunction and activation of neutrophil granulocytes (NGs) constitutes a central pathological mechanism underlying organ injury in sepsis. Methane (CH_4) has emerged as a biologically active gas with anti-inflammatory and mitochondria-protective properties. This study aimed to examine the impact of CH_4 inhalation, administered at distinct post-insult intervals, on organ function, oxygen utilization, and mitochondrial respiration and NG activation in a rodent model of intra-abdominal sepsis.

Methods: Adult male Sprague Dawley rats (n = 44, 380 ± 30 g) underwent sepsis induction (n = 36) or sham operation (n = 8). Septic animals were randomized to untreated or CH_4-treated groups (2.2% CH_4 in normoxic air at 3–6, 16–19, or 19–22 h post-induction; n = 9/group). Rat-specific sickness (RSS) and organ failure-related (ROFA) scores, plasma MPO activity, CitH3 levels, renal and cerebellar mitochondrial respiration (Complex I–II), oxygen extraction, and pCO_2-gap were assessed. Neutrophil populations (intact, ING; degraded, DNG; neutrophil extracellular traps, NETs) were isolated by Ficoll gradient, identified with DNA-binding fluorescent dyes.

Results: Sepsis markedly increased RSS and ROFA scores, impaired hemodynamics, mitochondrial respiration, and oxygen extraction. CH_4 inhalation at 3–6 h improved clinical status and survival, but had modest effects on organ function. At 16–19 h improved cerebellar mitochondrial respiration and, at 19–22 h with the most robust effects, decreased ROFA, MPO and CitH3 levels, and preserved cerebellar and renal mitochondrial function. All CH_4-treated subgroups demonstrated enhanced renal function and improved pCO_2-gap. ING counts improved, while the ratios of DNG and NETs were significantly reduced in treated animals compared to untreated septic animals.

Conclusions: Methane inhalation therapy, particularly when administered during the latest stages of sepsis, has potential as an adjunctive therapy, preserving oxygen dynamics, reducing inflammation and NG activation and protecting mitochondrial function in both the kidney and cerebellum.

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PROGNOSTIC INDICATORS OF MORTALITY IN CRITICALLY ILL PATIENTS WITH SECONDARY HLH: A UNIVARIATE CORRELATION ANALYSIS

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Background: Secondary hemophagocytic lymphohistiocytosis (sHLH) is a rare but severe hyperinflammatory syndrome frequently requiring intensive care unit (ICU) support. Despite prompt recognition and treatment, mortality remains high. Early identification of risk factors for poor outcomes, especially death, is crucial to optimize management in critically ill patients. Objective: To identify clinical and biological variables associated with mortality in ICU patients diagnosed with sHLH, aiming to improve prognostic assessment and guide targeted interventions.

Methods: We performed a retrospective study of 35 ICU patients with a confirmed diagnosis of sHLH. Clinical parameters, laboratory findings, and pathway-specific data were collected upon ICU admission. Univariate Spearman correlation analyses were conducted to evaluate associations between individual variables and in-hospital mortality.

Results: At ICU admission, patients frequently presented with elevated inflammatory markers, cytopenias, and signs of multiorgan failure. Correlation analyses identified several clinical and molecular parameters associated with mortality. Notably, markers indicative of immune dysregulation, impaired cell growth control, and cellular stress responses showed moderate to strong correlations with the risk of death. While not all associations reached statistical significance, the patterns suggest biologically plausible pathways linked to poor outcomes.

Conclusions: In this cohort of critically ill patients with sHLH, several clinical and biological indicators were associated with increased mortality. These findings highlight the relevance of immune and metabolic dysregulation in disease progression and underscore the need for integrative prognostic tools. Larger, prospective studies using multivariate analyses are needed to validate these associations and to inform risk stratification and personalized therapeutic strategies in the ICU setting.

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RELEVANCE OF HISTOPATHOLOGICAL FINDINGS FOR PREDICTIVE SCORING OF SHORT-TERM TREATMENT RESPONSE TO PLASMA EXCHANGE IN CRITICALLY-ILL ANCA-ASSOCIATED RENAL VASCULITIS PATIENTS

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Background: Rapidly progressive glomerulonephritis (RPGN) is a severe clinical manifestation of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), leading to rapid kidney function decline and high morbidity. Therapeutic plasma exchange (PLEX) is frequently used in severe cases, yet predicting treatment response remains challenging. A novel scoring system has been proposed to estimate short-term outcomes under PLEX therapy, but external validation is limited. Objective: To validate a recently developed PLEX response score for predicting short-term treatment outcomes in patients with severe renal AAV presenting with RPGN, and to compare its performance with established renal AAV classification tools, including histopathological findings.

Methods: We conducted a retrospective analysis of 53 patients with biopsy-proven RPGN due to severe AAV, all treated with PLEX according to contemporary guidelines. Clinical, laboratory, and histopathological data were collected. The predictive accuracy of the PLEX score was assessed and compared to conventional renal AAV classification systems. Stepwise multivariate regression was employed to identify the most robust predictors of poor short-term outcomes.

Results: The PLEX score demonstrated strong predictive power for identifying patients at risk of adverse short-term outcomes ($p < 0.0001$). When combined with histopathological findings from kidney biopsies, predictive accuracy further improved, as confirmed by multivariate regression analysis ($p < 0.0001$).

Conclusions: Our study supports the utility of the PLEX score as a practical tool for early risk stratification in patients with severe renal AAV and RPGN. The additive value of kidney biopsy findings emphasizes their continued importance, even in critically ill patients, despite procedural challenges. Prospective validation in larger, independent cohorts is warranted to confirm these findings and inform individualized treatment strategies.

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CHARACTERIZING THE HOST RESPONSE TO SEPSIS AMONG ICU PATIENTS WITH CONCURRENT MALIGNANCIES

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Background: One in every five patients with sepsis requiring treatment on an Intensive Care Unit (ICU) has a concurrent malignancy. Malignancy is associated with a one-third higher risk of poor sepsis outcomes. Knowledge of the impact of concurrent malignancy on the host response during sepsis is highly limited. This study aims to characterize the host response to sepsis in patients with concurrent malignancy and compare it to sepsis patients without malignancy.

Methods: This investigation used data of patients with sepsis admitted to the ICU of two Dutch academic hospitals between 2011 and 2013. The host response was characterized by measurements of plasma biomarkers and the blood transcriptome (microarray) on ICU admission.

Results: We included 482 patients with sepsis: 94 patients with an active hematological malignancy, 147 with an active solid malignancy and 241 controls with sepsis without malignancy matched for age, sex and comorbidities. Patients with concurrent hematological malignancy had increased plasma concentrations of interleukin (IL)-6, IL-8 and IL-10). Targeted gene set enrichment analysis showed that pathways related to hemostasis, cytokine signaling, innate immunity and programmed cell death were strongly downregulated in blood of these patients. Compared

to patients without malignancy, patients with concurrent hematological or solid malignancy had higher 30-day mortality (50.0% vs. 32.7 vs. 27.5% respectively; p = 0.001).

Conclusions: Hematological but not solid malignancy strongly impacts sepsis-induced host response dysregulation.

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PERSISTENT SARS-COV-2 INFECTION: A RETROSPECTIVE COHORT STUDY FROM A DUTCH TERTIARY CARE CENTER

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Background: In most individuals, SARS-CoV-2 RNA is cleared from the upper respiratory tract within 30 days after symptom onset. However, some patients, particularly the immunocompromised, exhibit prolonged detection of SARS-CoV-2 RNA, indicative of persistent infection. These prolonged infections pose therapeutic and public health challenges, yet comprehensive cohort-level data remain limited. Objective: To identify and characterize clinical features and outcomes of patients with persistent SARS-CoV-2 infection at a Dutch tertiary care center. Patients were compared to those who tested positive for SARS-CoV-2 by RT-PCR but did not meet criteria for persistent infection.

Methods: In this retrospective cohort study, we analyzed clinical and virologic data from all adults with a positive SARS-CoV-2 RT-PCR on nasopharyngeal swab between March 2020 and January 2025 at a Dutch tertiary care center. Persistent infection was defined as at least two positive SARS-CoV-2 RT-PCR results 30 or more days apart, with less than 90 days between two consecutive tests.

Results: A total of 12.986 patients tested positive for SARS-CoV-2 by RT-PCR of whom 277 (2.1%) patients met the criteria for persistent infection. The median length of infection was 46 days (IQR 37.0-67.0). The median age was 60 years (IQR 42.0-72.0) and 124 (44.8%) were female. Immunocompromising comorbidities were prevalent; 65 (23.5%) patients had a hematologic malignancy, 22 (7.9%) had received a solid organ transplant, and 103 (37.2%) used immunosuppressive medication. Among the 277 patients, 127 (45.8%) patients were hospitalized and 35 (12.6%) required intensive care unit admission. Comparative data from the 12.709 patients without persistent infection will follow.

Conclusions: Immunocompromising comorbidities are prevalent among this cohort with persistent SARS-CoV-2 infection. Ongoing analyses aim to identify clinical and virological risk factors associated with persistent SARS-CoV-2 infection in both hospitalized and ambulatory settings. These findings may support the development of targeted surveillance and treatment strategies for high-risk populations.

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TOGETHER WE ARE STRONGER: GLUCOCORTICOIDS AND CATECHOLAMINES ORCHESTRATE THE RESOLUTION OF INFLAMMATION

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Background: Glucocorticoids (GCs) are widely prescribed drugs to reduce inflammatory disease, despite their severe adverse effects (diabetes, liver steatosis, osteoporosis, muscle atrophy). During inflammation macrophages are one of the first innate immune cells that respond to the inflammatory stimulus and by their actions regulate the inflammatory response. Objective: The modulation of macrophages to an anti-inflammatory and pro-resolving phenotype supports the resolution of inflammation and sickness.

Methods: Murine-bone marrow derived macrophages as well as human macrophages were used for *in vitro* studies and analyzed with quantitative realtime-PCR and flow cytometry. In functional assays (efferocytosis, chemotaxis) and in an in *vivo* model of lung inflammation the objective was investigated.

Results: Co-treatment of primary murine macrophages with glucocorticoid (GC) and catecholamine (Cat) enhanced the ligand bound Glucocorticoid receptor (GR) nuclear translocation, as well as induction of anti-inflammatory genes and repression of inflammatory genes compared to the single GCs or Cat treatments. In addition, the macrophage migration and efferocytosis was significantly upregulated after GCs + Cat co-treatment. All the GC + Cat mediated *in vitro* findings in the murine system are translatable to primary human monocytes differentiated to macrophages, indicating that this might be a conserved mechanism. Furthermore, in a model of murine lung inflammation GCs in combination with Cat reduced $Ly6C^+$ and $MHCII^+$ macrophages in the lung, reduced inflammatory TNFα and induced anti-inflammatory CD163 expression in the lung significantly compared to the single treatments. However, in mutant mice lacking the Glucocorticoid receptor or the adrenergic receptor in macrophages the shift to a resolving phenotype could not be detected during lung inflammation.

Conclusions: This treatment regime shows the combinatorial treatment of glucocorticoids with catecholamines resolves inflammation faster compared to the single treatment. This might be a new therapeutic strategy to reduce GC doses to restrict the GC mediated side effects and combine the treatment with catecholamines.

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A20 MODULATES ETHANOL-INDUCED SUPPRESSION OF NF-κB-MEDIATED HEPATIC INFLAMMATION IN IN VITRO POLYTRAUMA CONDITIONS

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Background: Approximately 50% of polytraumatized patients present with acute alcohol intoxication (AAI) upon admission, which significantly alters hepatic immune responses. The transcription factor NF-κB plays a central role in regulating inflammation after trauma. Its activity is tightly controlled by regulatory mechanisms, including deubiquitinating enzymes (DUBs) such as A20, which are essential for modulating NF-kB activation and maintaining immune homeostasis. This study investigates the regulatory role of the DUB A20 in modulating NF-κB-mediated hepatic inflammatory responses to polytrauma in the context of AAI.

Methods: HepG2 wild-type and A20-knockout cells were treated with 200 mM ethanol for 4 hours, followed by exposure to a polytrauma-simulating inflammatory cocktail (PTC, containing C3a, C5a, IL-6, TNF, IL-1ß, IL-8) for additional 20 h. Ethanol concentration and treatment duration were optimized based on cell viability assessed by MTT assay. Inflammatory responses and NF-κB signaling were evaluated by gene expression analyses, cytokine secretion profiling and western blotting.

Results: 200 mM EtOH was identified as non-toxic over 24 hours. In wild-type cells, PTC increased phosphorylation of NF-kB proteins p150, p65, and IkBα, and induced IL-1β expression. Ethanol co-exposure

reduced phosphorylation of p65, p50 and IkBα, but not p105 compared to PTC alone. A20-knockout cells showed no significant effects of PTC and/or ethanol on pp65 or pp105, but PTC co-exposure with ethanol significantly increased p50 and IκB phosphorylation ratios. PTC upregulated *TNF*, *CXCL1*, and *CXCL5* gene expression, and IFN-α/γ and CCL2 secretion in wild-type cells—effects dampened by ethanol. A20-deficient cells exhibited reduced expression and secretion of inflammatory factors in response to PTC compared to WT, with ethanol increasing IL-10 and IL-8 levels.

Conclusions: Ethanol attenuates the inflammatory response in WT cells by reducing NF-κB activation and cytokine secretion. The absence of A20 alters cytokine signaling dynamics, highlighting its role in balancing inflammation under combined trauma and ethanol exposure.

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GLYCOCALYX INJURY IN BACTERIAL BLOODSTREAM INFECTION: PLASMA SYNDECAN-1 ASSOCIATES WITH MORTALITY AND HOST RESPONSE ABERRATIONS INDEPENDENT OF THE CAUSATIVE PATHOGEN

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Background: Damage to the endothelial glycocalyx plays a key role in the pathogenesis of sepsis. Syndecan-1 is a widely used biomarker of glycocalyx degradation. It is unclear to which extent the causative pathogen drives glycocalyx disruption. Objective: To determine the contribution of the causative pathogen to glycocalyx disruption in critically ill patients with bacterial bloodstream infection (BSI) and to assess the association of plasma syndecan-1 levels with outcome and host response changes implicated in the pathogenesis of sepsis.

Methods: We included intensive care unit (ICU) patients with BSI (positive blood culture from one day before to one day after ICU admission). Blood samples for host response analyses were collected ± one day relative to blood culture draw. We determined plasma levels of 24 biomarkers reflecting inflammation, coagulation, and endothelial function, and in a subset of patients blood transcriptomes. Syndecan-1 was analysed both as a continuous variable and by tertile grouping.

Results: We included 188 patients with bacterial BSI. The most prevalent pathogens were *Escherichia coli* (n = 38), *Streptococcus* (n = 34), *Enterococcus* (n = 25) and *Staphylococcus aureus* (n = 23). Higher syndecan-1 was associated with increased 30-day mortality, independent of disease severity. Syndecan-1 levels did not differ between pathogen groups. Variance partitioning analysis indicated that disease severity rather than the causative pathogen explained a significant proportion of the variance in syndecan-1 levels. High syndecan-1 associated with host response changes, particularly those related to endothelial dysfunction, followed by inflammation and coagulation. Transcriptome analysis revealed enhanced expression of hemostasis-related pathways, notably platelet degranulation, in patients with elevated syndecan-1 levels.

Conclusions: In ICU patients with BSI, glycocalyx disruption as measured by plasma syndecan-1 is associated with poor clinical outcomes and host response aberrations, especially endothelial activation. Glycocalyx disruption in BSI is mainly driven by severity of disease, not by the causative pathogen.

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SURVIVAL RATE AND FUNCTIONAL OUTCOMES BETWEEN IN-HOSPITAL AND OUT-OF-HOSPITAL SUDDEN CARDIAC ARREST: A COMPARATIVE STUDY

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Background: Cardiac arrest has been categorized as in-hospital and out-of-hospital based on the location of the occurrence. Studies on the comparison of these 2 types of cardiac arrest in Chinese population is limited. This study aimed to compare the survival rate and neurological functional outcomes between in-hospital and out-of-hospital cardiac arrest in Chinese population.

Methods: Data from adults with cardiac arrest during May 2020 an July 2024 were retrospectively collected and analyzed. Patients were categorized as in-hospital or out-of-hospital, and comparisons were made with regard to 30-day survival rate, return of spontaneous circulation, and neurological functional outcomes as defined by percentages of patients modified Rankin scale >2.

Results: A total of 173 cases of cardiac arrest were documented, including 109 (63.01%) out-of-hospital and 64 (36.99%) in-hospital. The two groups were similar with regard to patient age (63 ± 11 vs 61 ± 13, P = 0.28), sex (men 66/109 vs 41/69, P = 0.88), and prevalence of comorbid cardiovascular diseases (33/109 vs 19/64, P = 0.94). Patients with in-hospital cardiac arrest had significantly better 30-day survival rate (51/64 vs 70/109, P = 0.03) and percentages of return of spontaneous circulation than out-of-hospital cardiac arrest (49/64 vs 63/109, P = 0.01). The percentages of modified Rankin scale >2 were similar between the in-hospital and out-of-hospital group (18/51 vs 26/70, P = 0.83).

Conclusions: Compared to the out-of-hospital cardiac arrest, Chinese patients with in-hospital showed between survival rate but comparable neurological functional outcome.

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THE CARDIOVASCULAR RISK PROFILE IS ASSOCIATED WITH THE APPEARANCE AND SEVERITY OF CARDIAC DAMAGE AFTER POLYTRAUMA

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Background: Cardiac injury is a known predictor of poor outcomes following polytrauma (PT). In an aging population, the presence of cardiovascular risk factors (smoking, hypercholesterolemia, and pre-existing heart disease) may predispose individuals to post-traumatic cardiac dysfunction, which can be detected through systematic transthoracic echocardiography (TTE) after PT. The aim of this study was to assess whether the cardiovascular risk profile is associated with cardiac injury and clinical outcome in PT patients.

Methods: In this prospective, non-randomized study at a German Level 1 Trauma Centre, 59 PT patients (ISS ≥ 16) were enrolled upon Emergency department admission. Blood samples were collected at six timepoints (Emergency Room, 24 h, 48 h, days 3, 5, and 10). Cardiac injury was assessed using serum levels of Troponin T (TnT), NT-proBNP, and CK-MB. TTE was performed shortly after admission and 48 hours thereafter within the ICU setting. Cardiovascular risk was assessed using "SCORE 2" algorithm (10-year risk of cardiovascular disease) and

lipoprotein(a) [Lp(a)] levels. Spearman correlation analysis was used to examine associations between these parameters and echocardiographic findings, arrhythmias, and ischemic events.

Results: Overall, arrhythmias were observed in 38.9%, and acute ischemia (e.g., ST-segment elevation) in 18.6% of patients. TTE revealed wall motion abnormalities in 11.9%, diastolic dysfunction in 10.2%, and right ventricular dysfunction in up to 5.1% of cases. SCORE 2 and Lp(a) levels significantly correlated with TnT, NT-proBNP, and CK-MB. Higher Score 2 values were significantly associated with non-survival ($p \leq 0.01$), diastolic dysfunction ($p \leq 0.05$), and demonstrated a trend toward association with ischemia ($p = 0.06$). Lp(a) showed no significant correlation with echocardiographic findings.

Conclusions: Cardiovascular risk, as measured by SCORE 2, was significantly associated with cardiac injury markers, echocardiographic abnormalities, and mortality in PT patients.

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FROM BEDSIDE TO BOTTLENECK – WHY SEPSIS THERAPY REMAINS REACTIVE: INSIGHTS FROM CLINICAL PRACTICE AT UNIVERSITY MEDICAL CENTER GÖTTINGEN

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Background: Although standardized protocols have improved the management of sepsis, outcomes remain suboptimal. In daily ICU practice, decisions are still often reactive and symptom-driven. A mechanistic or personalized therapeutic approach is rarely applied, particularly in immune-dysregulated or diagnostically complex cases.

Methods: This narrative, case-based presentation illustrates typical challenges in sepsis management and outlines how we aim to implement structured, mechanistic decision-making in the future—based on experiences from clinical practice at University Medical Center Göttingen.

Results: Key challenges emerged across cases: (1) High clinical complexity often obscures therapeutic priorities. (2) Lack of immune literacy among treating teams impairs the use of biomarkers like mHLA-DR or endothelial markers. (3) New biomarkers face implementation hurdles, while existing ones (e.g., PCT) are variably interpreted. (4) Immune dynamics are not systematically discussed, even during ABS or interdisciplinary rounds. (5) Pilot initiatives such as immune conferences or structured screening exist—but lack integration into everyday clinical workflows.

Conclusions: This presentation uses real cases to highlight why sepsis therapy often remains reactive—and how we envision more structured, personalized strategies in the future. These include embedding immune diagnostics into clinical pathways, improving interdisciplinary communication, and defining biomarker-triggered decision points. Translating immunological insight into daily ICU decisions will be key to overcoming today's therapeutic bottlenecks.

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BEYOND THE ONCOTIC PRESSURE – IS ALBUMIN A REGULATOR OF ENDOTHELIAL CELL INTEGRITY AND LYMPHOCYTE TRAFFICKING?

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Background: Hypoalbuminemia is frequently observed in critically ill patients, but the clinical value of albumin replacement remains debated. Beyond its role in oncotic pressure, human serum albumin (HA) acts as a carrier for sphingosine-1-phosphate (S1P), a bioactive lipid involved in immune regulation and endothelial integrity. While S1P circulates bound primarily to HDL and albumin, the impact of HA supplementation on this distribution and its physiological consequences is poorly understood.

Methods: From March 2022 to February 2023, a prospective observational cohort study (AlbuS1P Study) was conducted in an intensive care unit. Forty-seven ICU patients were stratified into three groups: Group A (hypoalbuminemia treated with 180 g HA over three days), Group B (hypoalbuminemia untreated), and Group C (normoalbuminemia untreated). Blood samples were collected at multiple time points. Plasma S1P levels were quantified, immune cells analyzed by flow cytometry, and Flow-Induced Dispersion Analysis (FIDA) used to assess S1P binding to HDL or albumin. In vitro assays examined endothelial barrier function and B-cell chemotaxis in response to patient plasma.

Results: In 42 evaluable patients, HA therapy significantly increased serum albumin (+0.7 g/dL, +40%) but did not alter total plasma S1P levels. However, it shifted S1P binding from HDL to albumin. This redistribution was associated with reduced CD4+ T and CD19+ B cell counts, suggesting altered immune cell migration. In vitro, HA-treated plasma did not impair endothelial barrier function or S1P-dependent chemotaxis.

Conclusions: HA modulates S1P carrier dynamics without reducing overall S1P levels. This redistribution may influence immune cell behavior without compromising vascular stability. Targeted studies are needed to identify subgroups that might benefit from HA's immunomodulatory effects.

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EPIDEMIOLOGICAL CHARACTERISTICS AND CLINICAL OUTCOMES OF ABDOMINAL SEPSIS: A SECONDARY ANALYSIS OF A NATIONAL MULTICENTER PROSPECTIVE COHORT STUDY

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Background: The abdomen is the second most common source of sepsis. Abdominal sepsis is associated with high mortality risk, and surviving patients often experience severe complications, imposing a substantial burden on global healthcare systems. However, the clinical characteristics of abdominal sepsis remain unclear in China. The primary objectives of this study were to analyze the clinical features, pathogen distribution, and antimicrobial resistance in patients with abdominal sepsis and to identify mortality risk factors associated with abdominal sepsis for improved clinical management.

Methods: Based on a national multicenter prospective cohort study in China (Epidemiology, antimicrobial resistance and outcomes of intra-abdominal infections, ChiCTR2300072253), we report the clinical characteristics of abdominal sepsis. The primary outcome was 28-day mortality. We employed multivariate logistic regression analyses to identify independent risk factors for mortality in patients with abdominal sepsis.

Results: A total of 1620 intra-abdominal infection patients were included in the analysis, among whom 357 were diagnosed with abdominal sepsis. The abdominal sepsis group showed significant differences ($P < 0.001$), including ICU admission, mechanical ventilation, vasopressor use, and drug-resistant bacterial infections, along with distinct comorbidities (chronic pulmonary, cardiac, and renal diseases; malignancy) and

lifestyle risk factors compared to non-septic patients. Source control was implemented in 84.88% of cases, with 51.26% undergoing surgical intervention. Among patients who received source control, 82.12% achieved successful control by day 14, as evidenced by the absence of persistent inflammation or organ dysfunction. The mortality rate among patients with abdominal sepsis was 22.4% (80/357), with independent risk factors for death identified as invasive mechanical ventilation, drug-resistant bacterial infections, and renal replacement therapy.

Conclusions: Findings from this study delineate the clinical characteristics and systematically elucidate the etiological profile of abdominal sepsis in China. Identification of key mortality risk factors provides critical insights for optimizing prognostic evaluation and clinical management strategies.

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TOPICAL TREATMENT OF THE SPLEEN WITH $NaHCO_3$ IMPROVES THE SURVIVAL OF RATS WITH SEVERE INTRA-ABDOMINAL SEPSIS

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Background: Sodium bicarbonate (SB) is used to treat metabolic acidosis in patients with septic shock, but its effectiveness on survival is not well established. Recently, oral bicarbonate sodium (Bicarb, NaHCO3) was found to activate splenic anti-inflammatory response. We hypothesized that topical NaHCO3 treatment of the spleen during source control laparotomy would inhibit the spleen-mediated inflammatory response and improve survival of rats with feculent peritonitis.

Methods: Feculent peritonitis was induced by cecal ligation and incision (CLI) in male Sprague Dawley rats. Two hours after CLI, peritoneal washout at a dose of 0.5 ml/g was performed with normal saline (NS, control group) or 0.1 meq/ml NaHCO3 (SB washout group), or NS washout plus topical NaHCO3 (1 meq/ml) rinse of the spleen at a dose of 0.5 ml (Topical SB group).

Results: Median survival was 9.0 hours in control rats versus 13.5 hours in SB washout rats ($p = 0.01$ vs. control). All control and bicarb washout rats died within 13 hours and 36 hours respectively after CLI. However, topical splenic bicarb rats had a 60% survival over 10 days following CLI. Another group of rats received topical splenic rinse with 5.8% saline (same osmolality as 1 meq/ml NaHCO3) and showed a median survival of 10.2 hours. All CLI rats developed combined acidosis. Topical SB did not improve pH, but significantly improved BE, $HCO3^-$, sO2, and lactic acid. Analysis of the plasma and splenic tissues showed that topical splenic SB inhibited spleen-mediated inflammatory responses.

Conclusions: SB peritoneal washout significantly prolonged the survival duration of rats but did not have survival benefit. Topical SB rinse of the spleen significantly improved the survival. Topical SB rinse modulates the spleen to an anti-inflammatory mode by inhibiting the Caspase1-NLRP3-IL-1β pathway and attenuates systemic inflammatory responses.

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MYOCARDIAL PYRUVATE DEHYDROGENASE KINASE 4 DRIVES SEX-SPECIFIC CARDIAC RESPONSES TO ENDOTOXEMIA

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Background: Sepsis-induced cardiac dysfunction is more severe in males than in females, but the underlying mechanisms remain poorly understood. Identifying the molecular drivers of these sex-specific differences is essential for developing precise and effective therapeutic strategies tailored to sepsis patients. Pyruvate dehydrogenase kinase 4 (PDK4), a key regulator of glucose metabolism in mitochondria, may contribute to these disparities by modulating pyruvate dehydrogenase (PDH) activity and mitochondrial function. This study aimed to determine whether PDK4 plays a critical role in the sex-based differences in cardiac responses to septic challenges.

Methods: We used a mouse model of endotoxemia in which acute inflammation was induced by a sublethal dose of lipopolysaccharide (LPS). We evaluated cardiac function, metabolic alterations, and mitochondrial integrity in wild-type, cardiac-specific PDK4-overexpressing, and PDK4-knockout mice of both sexes.

Results: LPS caused significant cardiac dysfunction and increased myocardial PDK4 expression in males but not females. Cardiac-specific PDK4 overexpression exacerbated LPS-induced dysfunction in both sexes, whereas PDK4 knockout was protective. In wild-type males, LPS disrupted cardiac metabolism by reducing PDH activity and fatty acid oxidation (FAO) while increasing lactate levels, indicating a metabolic shift from oxidative phosphorylation to glycolysis. These effects were aggravated by PDK4 overexpression but alleviated by its knockout. In females, LPS had minimal metabolic impact, except for a reduction in FAO with PDK4 overexpression. Furthermore, PDK4 overexpression exacerbated LPS-induced mitochondrial damage in both sexes, leading to disrupted cristae, impaired membrane potential, increased fragmentation, and reduced mitophagy, along with heightened oxidative stress and inflammation. PDK4 knockout mitigated these detrimental effects.

Conclusions: These findings establish PDK4 as a key driver of sex-specific metabolic and mitochondrial responses in sepsis-induced cardiac dysfunction and suggest it as a potential therapeutic target.

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ELEVATED TISSUE FACTOR–POSITIVE EXTRACELLULAR VESICLES AT ADMISSION IDENTIFY TRAUMATIC BRAIN INJURY AND CO-SHAPE ACUTE HEMOSTATIC–IMMUNE DYNAMICS IN SEVERE TRAUMA

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Background: Traumatic brain injury (TBI) exposes tissue factor (TF, CD142)-rich neuronal tissue, triggering robust coagulation and immune responses. We examined whether TF-bearing Extracellular Vesicles (EVs) in plasma could serve as a clinically relevant indicator for

confirming TBI and whether temporal fluctuations in platelet-, innate-, and adaptive-immune markers on EVs mirror the evolving host response to a major traumatic incident.

Methods: We analyzed 42 severely injured adults (Injury Severity Score > 15), enrolled in the bicentric PRIME study (NCT06314841), classifying them as TBI (n = 21) or non-TBI (n = 21) based on clinical and imaging assessments. Blood samples were obtained at admission (0 h), 24 h, 48 h, and 96 h. EV characterization in plasma used the MACSPlex EV Kit, measuring markers of hemostasis (CD41b, CD42a, CD62P, CD142), innate immunity (CD14, CD56), adaptive immunity (CD3, CD4, CD8, etc.), and cellular regulation (CD9, CD63, CD81). Semi-quantitative epitope-specific signals were recorded as median fluorescence intensity (MFI). Signals were compared with relevant surface antigens on circulating leukocytes. Kruskal–Wallis tests with Dunn's post hoc correction determined significance ($p < 0.05$).

Results: At 0 h, TBI patients showed higher CD142+ EV levels than non-TBI (MFI, median [IQR]: 3450 [2047–5278] vs 1814 [1531–3354], $p = 0.024$), and receiver operating characteristic (ROC) analysis confirmed their moderate diagnostic value (AUC = 0.703) for TBI. No differences were observed in other antigens or at 24 h, 48 h, and 96 h. In all patients, platelet-associated EV markers were elevated at admission, indicating an acute procoagulant state. Over 24–96 h, markers of innate immunity (e.g., myeloid, NK cell–associated signals) and later adaptive and regulatory pathways (T-cell and antigen presentation) increased, reflecting a shift from immediate hemostatic activation toward inflammatory and reparative processes. EV-associated antigen levels partially paralleled the expression of the same surface antigens on circulating leukocytes.

Conclusions: A transient surge in TF-bearing EVs at admission prompts an early procoagulant activation secondary to TBI. Subsequent changes in platelet, innate, and adaptive EV markers in the entire patient cohort indicate a complex immuno-regenerative interplay following major trauma. EV-based phenotyping may complement existing neuronal TBI markers, accelerate its detection and better characterize evolving host responses.

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THE ESS SUMMER SCHOOL 2025: POLYTRAUMA – BRIDGING EMERGENCY MEDICINE AND CUTTING-EDGE RESEARCH OF THE FOR 5417

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The management of polytrauma patients involves several factors that can affect the risk of developing critical complications. In this endangered patient population, the emergency management and initial operative treatment can substantially influence the clinical course. Consequently, in emergency settings, prompt treatment decisions are necessary, and an in-depth understanding of underlying pathophysiological responses is needed. Thus, researchers within the DFG FOR 5417 are focusing on evaluating the complex reactions associated with polytrauma to improve understanding and influence the treatment of polytrauma patients. The Summer School program is designed to emphasize pathophysiological and immunological considerations in trauma care, in addition to the novel research foci within the DFG FOR 5417.

This year's Summer School at the ESS 2025 invites young basic scientists to engage with the clinical context of their work and at the same time addresses young healthcare professionals and motivated students to deal with hot topics in basic science in the polytrauma research field.

The course is organized by clinician scientists with expertise in trauma surgery and basic research. In four different sessions, specific aspects of central clinical phases in the treatment of polytrauma patients will be highlighted. Hands-on sessions will cover acute treatment concepts (e.g. preclinical bleeding management or osteosynthesis via external fixator) for polytrauma patients with the focus of linking clinical practice with trauma and sepsis research.

The sessions provide insights into prehospital and acute in-hospital medical care, emergency surgery concepts as well as intensive care treatment dealing with post-traumatic complications and immune dysregulation. It will be emphasized that our current treatment concepts are based on basic scientific knowledge.

Our diverse program focuses on key clinical aspects and hands-on learning, with the purpose of facilitating translation of basic science into routine clinical practice.

The Summer School is supported by the DFG FOR 5417.

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PROTEOMICS ASSOCIATED TO AI FOR STRATIFICATION OF SEVERITY IN INFECTION

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Background: Early identification of patients at risk of severe infection is essential to improve clinical outcomes. Human and microbial proteomic profiling offers a promising approach to identify new biomarkers that reflect the host response to infection. When integrated with artificial intelligence (AI) techniques, this approach allows the identification of complex host response signatures capable of stratifying infection severity. Such a strategy holds great promise for transforming patient management by allowing early data-driven assessment of disease progression and prognosis. This study aims to identify proteomic signatures associated with infection severity, using high-throughput proteomics platforms combined with AI-based models, to enable early detection and stratification of infection.

Methods: Two proteomic strategies were employed: (1) targeted quantification of specific host immune response-related proteins using Ella-SimplePlex™ system (Bio-Techne) in plasma of critically ill COVID-19 patients; and (2) large-scale proteomic profiling of over 3,000 plasma proteins using the Olink® Explore 3072 panels in patients with infection from the emergency department (ER). Clinical data and biomarker measurements were analyzed using advanced machine learning techniques, including clustering analysis, to identify biomarker signatures predictive of infection severity and clinical outcomes.

Results: Using an integrative IA approach combing pathogen and host-response biomarkers of different biological functions, we identified subgroups of patients with different immune responses and clinical trajectories. In the COVID-19 study, we identified a biological signature, defined by low pathogen burden, robust adaptative immunity and limited inflammatory response, associated with better outcomes. This approach was also reproduced in the ER study, demonstrating their applicability across different clinical settings.

Conclusions: The combination of host-response and microbial data by machine learning techniques enables better characterization of subgroups of patients with different prognosis following infection. The integration of high-throughput proteomics and AI allows for robust stratification of infection severity, supporting early detection and the development of personalized therapeutic strategies.

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THE USE OF MULTI-OMICS TO BRING PRECISION MEDICINE TO TRAUMA

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Multi-omics has led to an in-depth understanding of the biology of trauma and has identified some biomarkers that define outcome-based patient sub-groups. Here we present three examples of how multi-omics data can lead to precision medicine for trauma. First, multi-omics can be used to identify endotypes that are associated with outcomes. We have defined endotypes based on single-cell multi-omics as well as targeted proteomics from trauma patients. The identification of these endotypes will be useful for the design of trials targeting specific mediators or pathways. Second, omics from observational and interventional studies have identified treatment response sub-groups. Specifically, analysis of omics data has shown patient subsets that are responsive to early plasma administration. Thirdly, the omics of human injury can be used to develop animal models that replicate the key processes in the human injury response. This, in turn, can be used to identify therapeutic targets based on mechanistic studies and representative animal models. An example will be presented demonstrating the identification of program cell death pathways that are involved in early mortality after trauma/hemorrhage.

In the future, large scale multi-omics studies will continue to advance our understanding of both the biology and therapeutic targets in severely injured humans.

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POLYTRAUMA MODELING

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Background: Polytrauma is the leading cause of death under the age of 50 and 60% of survivors suffer from relevant disabilities years after polytrauma. Due to the Berlin definition from 2014, a polytrauma is defined as relevant injuries with an Abbreviated Injury Score (AIS) ≥ 3 and an additional parameter (>70 years, blood pressure < 90 mmHg, unconsciousness, acidosis, coagulopathy). However, as the details of the interplay are not yet known, an experimental model is needed to study the fundamental basics, which reflects clinical reality. The aim was to investigate an experimental polytrauma mouse model that is comparable to the clinical reality in the long-term - over a post-traumatic course of 21 days.

Methods: 12-week-old male C57BL/6 N mice were used and kept in individual housing. The polytrauma model consisted of thoracic trauma, hemorrhagic shock, abdominal injury (laparotomy) and femur osteotomy stabilized via external fixator. All operation procedures were conducted under deep inhalation anesthesia with isoflurane. A multimodal analgesia was reached by subcutaneous injection of Carprofen (5 mg/kg) and Butorphanol (1 mg/kg) directly after anesthesia induction and by local anesthesia of the affected skin areas and periosteum. After surgery, the animals were subjected to indication-independent controls including scoring (health, lameness) according to a defined regimen. In addition, they received further analgesia for 3 days post-operatively and additionally, if necessary.

Results: This polytrauma model causes a mortality of 15-22%. Of the survivors, most animals show reduced activity one hour after surgery due to severe blood loss. After 4 hours, almost all polytrauma animals are fully active again. Results after 6 hours showed a good comparability to humans systemically, as well as local in liver and lung.

Conclusions: A model has been developed that accurately reflects the clinical reality of polytrauma, at least in the short term. Results regarding the long-term outcome are still pending.

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GETTING TO THE CORE OF ENDOTOXIN: IMPLICATIONS FOR SEPSIS PREVENTION AND THERAPY

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Background: Sepsis is a potentially lethal complication of Gram-negative bacterial infection. Despite the availability of potent antimicrobial agents and supportive care, the mortality from sepsis is unacceptably high. After decades of research, there is no approved immunotherapy adjunct for sepsis. In a large, multicenter study published 40 years ago, administration of antibodies to a highly conserved region in the core region of Gram-negative bacterial lipopolysaccharide (LPS) to patients with GNB bacteremia improved survival (NEJM 1982). While other investigators tried to confirm this observation, no study reproduced the clinical design. Consequently, the concept of a broadly-acting anti-LPS antibody as a therapeutic adjunct to sepsis has not taken hold. Given the dearth of effective antibiotics, especially against multi-resistant GNB, it may be time to reconsider this approach. The goal of this review is to re-assess the use of anti-core LPS antibodies as an immunotherapeutic adjunct to the treatment (or prevention) of GNB sepsis.

Methods: The data are based on review of the literature and the authors' published work

Results: The scientific rationale for the original core LPS antibody (J5) study will be discussed as well as the J5 study itself. The subsequent attempts to assess the efficacy of anti-core endotoxin antibodies will be reviewed. A LPS subunit vaccine derived from the original heat-killed bacterial vaccine was developed and shown to broadly protect against a broad range of GNB in a wide range of preclinical rodent models. This vaccine has undergone Phase 1 testing and is safe and well-tolerated.

Conclusions: The use of anti-core LPS antibodies merits further investigation.

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GLIAL CELL REACTIVITY IN A MOUSE MODEL OF TRAUMATIC BRAIN INJURY: INSIGHTS FROM THE CLOSED-HEAD INJURY PARADIGM

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Traumatic brain injury (TBI) is a leading cause of morbidity and mortality worldwide, and is categorized according to the severity in mild, moderate and severe. Interestingly, even patients with mild TBI are under risk of cognitive impairment that can greatly diminish the quality of life in the short and long-term post-injury. The lack of effective treatment to improve the cognitive and emotional function after TBI highlights the need for pre-clinical research that aims to investigate the mechanisms that determine injury outcomes.

Despite the ongoing debates on the translational value of the several available TBI animal models, these have offered valuable insights into the mechanisms that influence the TBI outcome and progression. Among the most commonly used and reproducible TBI models are the fluid percussion injury (FPI), the controlled cortical impact (CCI), and the stab wound injury (SWI) models. In the meantime, the closed-head injury (CHI) model, which is considered the most translational one, has been refined to stimulate varying degrees of injury severity, enhancing our understanding of outcome determinants.

TBI induces complex secondary injury mechanisms, mostly involving glial cells; astrocytes, microglia, and NG2-glia (oligodendrocyte progenitor cells). As key regulators of brain homeostasis, glial cells become

reactive following injury and contribute to neuro-inflammatory cascades that can either support tissue repair or exacerbate neuronal damage.

In this work, glial reactivity was investigated in adult male mice, after inducing TBI with the CHI model. Immunohistochemical analysis for markers associated with gliosis was performed to assess glial activation, while cell proliferation was evaluated using Ki67 and the thymidine analogues BrdU and EdU. A thorough investigation of the parameters influencing the TBI outcome may pave the way for the development of novel therapeutic strategies.

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DECISION MAKING IN POLYTRAUMA – QUO VADIS?

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Recent advances in trauma surgery have significantly refined decision-making strategies, particularly through a deeper understanding of the immunological and physiological processes underlying the "two-hit" response. This concept describes a biphasic pattern where an initial traumatic insult ("first hit") primes the immune system, followed by a secondary challenge ("second hit") – such as surgery, infection, or further injury – that can precipitate an exaggerated systemic inflammatory response, leading to complications like multiple organ dysfunction syndrome (MODS). Recognizing and mitigating the second hit has become pivotal in modern trauma care. Innovations in biomarker profiling, real-time physiological monitoring, and personalized resuscitation protocols now inform surgical timing and intervention strategies. These advances enable trauma surgeons to tailor operative decisions—such as choosing between definitive repair (Early Total Care – ETC) and staged damage control surgery (DCO – Damage Control Orthopedics) – based on a patient's immunological state and risk of second-hit exacerbation. The evolving knowledge of the two-hit model is reshaping trauma surgical decision-making, improving outcomes through precision-based approaches and better-informed clinical judgment.

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FOSTERING RESEARCH AND QUALITY – AUC CENTER FOR CLINICAL STUDIES AS A CATALYST IN GERMAN TRAUMA SURGERY

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Background: The considerable demands on clinically active trauma surgeons in German trauma centers—irregular working hours, staff shortages, and high patient volumes—combined with significant organizational and regulatory hurdles, limit the engagement of research-oriented clinicians and complicate activities beyond routine care. However, research, knowledge transfer, and quality assurance are essential prerequisites for delivering high-quality, progressive care to the injured. In 2018, the German Society for Trauma Surgery established the position of Research Coordination to promote, in particular, clinical multicenter studies. The primary aim was to develop a lasting clinical study center equipped with the necessary resources and expertise to support research within the professional society's context.

Methods: Experience was gained in securing third-party funding, preparing ethics applications, and negotiating cooperation agreements with clinical sites, considering the heterogeneous institutional requirements. A formal link to the Scientific Committee ensured strategic alignment. An advisory board was also established, comprising experts from diverse fields who contribute their knowledge on an honorary basis.

Results: As of June 2025, the center leads or contributes to eight clinical studies across up to 46 study sites, with up to €3,2 million in external funding and up to 1400 enrolled patients. It also supports the development of two S3 and one S2k clinical guidelines, all externally funded. A trust center and infrastructure for health data management have been established. Collaborations with statutory health insurers, the German Pension Insurance, university hospitals, and BG clinics are in place. The center has expanded its role in developing national and international scientific registries for various medical societies. Close integration with German trauma surgery clinics enables effective translation of research into practice.

Conclusions: Future goals include projects involving industry and outpatient care, acquisition of multinational trials, centralized follow-up for patient-reported outcomes, long-term institutionalization of the center, and closer collaboration with medical technology R&D.

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EUROPEAN TRAUMA CORE DATASET 2025: STANDARDISED REPORTING OF MAJOR TRAUMA IN REGISTRIES

Christine Hoefer[1], Kjetil Ringdal[2], Luke Leenen[3], Tim Coats[4], Expert Panel*, and Rolf Lefering[5]. [1]AUC – Academy for Trauma Surgery, Munich, Germany; [2]Department of Prehospital Care, Division of Emergency and Critical Care Medicine, Vestfold Hospital Trust, Tønsberg, Norway; [3]University Medical Centre Utrecht, Utrecht, The Netherlands; [4]Department of Cardiovascular Sciences, University of Leicester, Leicester, UK; [5]Institute for Research in Operative Medicine, University of Witten-Herdecke, Cologne, Germany; *Expert Panel: Germany, Sebastian Imach; Germany, Rolf Lefering; Germany, Christine Hoefer; Finland, Mikko Heinanen; France, Arthur James; France, Tobias Gauss; Austria, Andreas Hartmann; Austria, Wolfgang Voelckel; Switzerland, Kai Jensen; Switzerland, Yannik Kalbas; Spain, Alfredo Echarri; Spain, Fernando Rosell Ortiz; Spain, Mario Chico Fernández; Netherlands, Luke Leenen; Netherlands, Mariska de Jongh; Netherlands, Erna Krusemann; UK, Tim Coats; UK, Antoinette Edwards; Schweden, Folke Hammarqvist; Ireland, Conor Deasy; Ireland, Pamela Hickey; Norway, Olav Roise; Norway, Veslemoy Nordset; Norway, Kjetil Ringdal; Portugal, Antonio Marques; Belgium, Philip Verdonck; Denmark, Frederik Borup Danielsson; Denmark, Soren Rudolph; Israel, Arielle Kaim; Alpenländer, Simon Rauch; Cyprus, Chrysanthos Georgiou; Hungary, Petra Hartmann; Hungary, Tibor Donka; Turkey, Melih Ünal; Turkey, Alper Kati; Scottland, Thijmen Breeschoten; Scottland, Dean Kerslake; Scottland, Malcolm Gordon; Scottland, Angela Khan; Italy, Stefania Cimbanassi; Italy, Osvaldo Chiara; Slovenia, Matevz Tomazevic; Slovenia, Drago Brilej

Background: Trauma registries are established sources for generating new scientific insights and hypotheses in the field of major trauma. In 1999, the "Utstein Style Template for Major Trauma" was introduced as a minimum core dataset to standardise the documentation of patient data. Revised in 2008, the "Utstein Trauma Template" (UTT) reflects expert consensus on uniform data collection, enabling accurate description of patient populations, treatment processes, and outcomes. Since its publication, healthcare systems and treatment protocols have evolved considerably, while hospital staff now operate under altered conditions, influenced by demographic shifts and novel trauma mechanisms. This project aims to develop and implement an internationally agreed, updated European Trauma Core Dataset (EuTCoDaS). The dataset is intended to reflect current clinical realities and research priorities, support cross-border scientific collaboration, and offer a standardised framework for assessing treatment processes and quality in major trauma care. It is designed to be adoptable by multiple national trauma registries.

Methods: A structured international consensus process will be conducted via a three-round Delphi method (Classical and Argumentative), scheduled between March and October 2025. Forty-four experts (clinicians and scientists) from 22 predominantly European countries and 19 trauma registries have confirmed participation. All panel members possess experience in the use, management, or development of trauma registries. A five-member steering committee oversees the project. In the initial round, the panel assessed the 40 existing UTT parameters based on relevance, feasibility, timeliness, and definitional clarity.

Results: Thirty-six experts (82%) from 21 countries (95%) shared their opinion and completed the first questionnaire. Health-related quality of life and consideration of elderly trauma patients emerged as key priorities for inclusion in EuTCoDaS.

Conclusions: There is strong international support for updating the Utstein Trauma Template for major trauma documentation. The forthcoming Delphi process will refine it into the European Trauma Core Dataset 2025.

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AGING AND BURNS

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Older adult burn patients, in particular, face alarmingly high mortality rates due to their inability to initiate the self-preserving stress responses commonly seen in younger adults. However, the relationship between aging and burns is not one-sided. Burn survivors often experience increased hospital admissions for age-related conditions such as cardiovascular disease, diabetes, and musculoskeletal disorders, along with higher long-term mortality. The reasons for this remain unclear, but burns and aging share common pathogenic mechanisms, including chronic systemic inflammation, oxidative stress, mitochondrial dysfunction, and cellular senescence. These overlapping processes suggest that burn injuries may not only be influenced by aging but could also accelerate the aging process itself.

Over the years, our research has aimed to unravel the complex relationship between burns and aging. Initially, we focused on understanding how older adults fail to mount typical post-burn stress responses. We found that older burn patients exhibit immunosenescence, characterized by a weakened immune response, and a failure to activate critical processes like WAT browning, which is essential for metabolic adaptation after injury. Additionally, we observed hypometabolism and a hypoinflammatory response, both of which contribute to the poor healing and long-term outcomes in these patients. Building on these findings, we have demonstrated that burn injuries increase DNA damage and reactive oxygen species (ROS) production, leading to changes in DNA methylation patterns and the emergence of epigenetic aging footprints. This new insight suggests that burns don't just occur within the context of aging—they may also be pushing the body down a path of accelerated aging, intensifying the underlying mechanisms that drive age-related diseases. Through this work, we continue to explore how burns and aging are intertwined, shedding light on potential therapeutic targets to improve outcomes for older adult burn patients and, more broadly, advance our understanding of the aging process itself.

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FUNCTIONAL IMMUNE PHENOTYPING OF SEPSIS PATIENTS: INTEGRATING MICROPHYSIOLOGICAL ASSAYS, OMICS AND IN SILICO MODELING

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Background: Sepsis is characterized by life-threatening organ dysfunction caused by a dysregulated host response to infection. In sepsis, dysregulated neutrophil-endothelial cell interactions play a critical role in organ damage. Therapeutic development is hindered by the heterogeneous nature of sepsis and the diverse host response to infection. We utilized a synergistic combination of our organ-on-chip, clinical data, proteomics, and *in silico* modeling to identify neutrophil functional phenotypes in sepsis. Bioinformatic analysis was used to identify FDA-approved therapeutics that target differentially expressed proteins in neutrophil phenotypes.

Methods: Following informed consent, neutrophils were isolated from sepsis patients and healthy controls. Employing organ-on-chip analysis, functional neutrophil phenotypes were identified based on *ex vivo* neutrophil adherence/migration patterns. Differentially expressed proteins were identified by proteomic analysis. We leveraged proteomic analysis in a protein-protein interaction network to prioritize target proteins in each neutrophil phenotype. The Harvard/MIT Broad Institute Drug Repurposing Hub and Drugbank databases were used to identify FDA-approved therapeutics.

Results: Three different neutrophil functional phenotypes (Hyperimmune, Hypoimmune and Hybrid) were identified that were associated with distinct proteomic signatures and differentiated sepsis patients by important clinical parameters related to disease severity. Functional enrichment analysis highlighted several biological processes and pathways that impacted adhesion/migration patterns. Neutrophil pathway analysis highlighted nine differentially expressed proteins that were directly implicated in known neutrophil processes related to sepsis. These findings were leveraged to identify FDA-approved therapeutics that potentially could be repurposed to target proteins within each phenotype. We identified three distinct drug targets across phenotypes that could modulate the immune response in sepsis: VTN in the Hybrid phenotype, TRPV2 in the Hypoimmune phenotype and H2AC21 in the Hyperimmune phenotype.

Conclusions: Thus, we identified critical cellular processes impacting neutrophil phenotypes in sepsis and identified FDA-approved therapeutics that could potentially be prioritized for future validation in the treatment of sepsis.

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PERSONALIZED IMMUNOTHERAPY: WHERE DO WE STAND NOW

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Background: Over the past decades, immunomodulatory studies in sepsis targeting dysregulated host response, failed to show benefit, mainly due to patients' heterogeneity. Sepsis classification into endotypes is of paramount importance in terms of precision immunotherapy. The extremes of the immune dysregulation phenotypes in sepsis are a state of hyperinflammation, characterized as MALS (macrophage-activation syndrome) and a state of sepsis-induced immune paralysis, both deleterious to short- and long-term outcomes. Recently, a new study has described a unique endotype existing in sepsis called IFNγ-driven sepsis (IDS) endotype, defined as IFNγ >3 pg/ml and chemokine CXCL9 > 2,200 pg/ml. The rationale of personalized immunotherapy is a therapeutic strategy based on biomarkers that classify patients to specific endotypes. These biomarkers are informative about a specific pathway that its implication is detrimental for the patient's outcome. Personalized immunotherapy intervenes to modulate this specific pathway with available drugs.

Methods: Presentation of relevant clinical studies; A personalized randomized trial of validation and restoration of immune dysfunction in

severe infections and sepsis (The PROVIDE trial/ NCT03332225), Personalized immunotherapy in sepsis: a multicenter and multinational, double-blind, double-dummy randomized clinical trial (The IMMUNOSEP trial/NCT04990232), Emapalumab treatment for anticipated clinical benefit in sepsis driven by the interferon-gamma endotype (The EMBRACE trial/ NCT06694701).

Results: In the PROVIDE study, using ferritin >4,420 ng/mL and < 5,000 HLA-DR receptors/monocytes as biomarkers, patients were classified into MALS, immunoparalysis, and intermediate. Patients with macrophage activation-like syndrome (MALS) or immunoparalysis were randomized to treatment with anakinra or recombinant interferon gamma or placebo. Survival after 7 days with SOFA score decrease was achieved in 42.9% of patients of the immunotherapy arm and 10.0% of the placebo arm ($p = 0.042$). IMMUNOSEP study has completed enrollment, and results are highly anticipated, where EMBRACE study started recruiting March 2025.

Conclusions: Personalized immunotherapy is crucial for an improved chance of success in the realm of sepsis immune dysregulation. The use of biomarkers for patients' classification to endotypes and subsequent targeted immunotherapy represent a promising strategy that may change clinical practice.

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DIGGING DEEP INTO THE COAL MINE (OR GOLD MINE) OF LACTATE PRODUCTION IN SEPSIS IN MICE

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Background: Sepsis kills 11 million people each year. The key mechanism leading to lethality may be related to lactate, but there remain many open questions. After we described, 4 years ago, that blood lactate appears to be increased based on production, but also based on a lack of gluconeogenic clearance, due to problems with the glucocorticoid receptor, we now focused on the production of lactate and wanted to discover its primary molecular source.

Methods: We applied a lethal mouse peritoneal sepsis model, CLP, and focused on pyruvate metabolism in the liver using a plethora of techniques, in mice, ex vivo and using purified mitochondria and Seahorse techniques (measuring oxygen consumption rates in vitro). We also used C13 labeled metabolite tracing and inhibitors of specific enzymes.

Results: Our data suggest that lactate production is the result of a problematic pyruvate oxidation in mitochondria. Using C13 labeled pyruvate, we found that entry into mitochondria is fine, and that pyruvate carboxylation (to oxaloacetate) is not hampered. However, the pyruvate dehydrogenase complex (PDC) is no longer functioning well, and we found that the mechanism is different than PDC phosphorylation by PDKs. I will display the details during the presentation. Cells appear to try to remove pyruvate and keep the TCA cycle turning, by importing glutamine, whereby pyruvate is consumed and turned into alanine and oxoglutarate is formed. As expected, acetyl-CoA levels are low in septic mitochondria, due to the PDC issues, but probably also by reduced beta oxidation.

Conclusions: Our data shed new light on the mechanism of pyruvate oxidation problems and they have allowed to develop two new therapeutic techniques which will be discussed.

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INNATE EXHAUSTION MEMORY IN SEPSIS AND REJUVENATION STRATEGIES

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Background: Innate immune exhaustion, reflected in reduced monocyte differentiation; pathogenic inflammation and immune suppression, underlies the pathogenesis of sepsis. Despite their pathological significance, mechanisms responsible for monocyte exhaustion is not well understood. Defining such mechanisms will aid future therapeutic rejuvenation interventions of trauma, shock and sepsis. Our planned studies aim at characterizing molecular and cellular mechanisms responsible for the generation and propagation of monocyte exhaustion in vitro and in vivo, in the context of sepsis pathogenesis and treatment. We further aimed at genetic and chemical strategies of rejuvenating innate monocytes and neutrophils.

Methods: We established a robust in vitro culture model for the generation of exhausted monocytes and neutrophils. Through genetic approaches, we compared the exhaustion profiles of monocytes and neutrophils harvested from wild type and TRAM knockout mice by integrated flow cytometry and functional studies. To test the propagation of exhaustion among neighboring cells, we perform co-culture studies with naïve and exhausted monocytes in vitro. Further, we tested the in vivo propagation of monocyte exhaustion through transfusing in vitro exhausted monocytes into naïve recipient mice. Genetic and chemical approaches were tested for the rejuvenation of innate monocytes and neutrophils.

Results: We observed that TRAM deletion largely erased the exhaustion phenotype, and prevented the exhaustion propagation. We also demonstrated that CD38 expression mediated by TRAM-STAT1 axis is responsible for the inter-cellular propagation of monocyte exhaustion. Genetic deletion of TRAM or chemical reprogramming with 4-PBA leads to the generation of rejuvenated leukocytes with active potential of reducing inflammation and alleviating sepsis severity.

Conclusions: Our studies revealed novel mechanisms involved in the generation of innate exhaustion and their propagation in vitro as well as in vivo. Our data pave the way for future effective intervention of innate exhaustion and treatment for trauma, shock and sepsis.

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INDUCTION OF ARDS STANDARDIZED BY A MINIMAL INVASIVE MURINE MODEL

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Background: The excessive inflammatory reaction in sepsis often leads to end organ damage in the lung, causing acute respiratory distress syndrome (ARDS). The underlying pathophysiology of ARDS is not completely understood and the mortality rate remains high. Therefore, it is essential to identify effective therapeutics that are currently lacking. For that, animal models offer the best opportunity to depict the complexity of the immune system and the clinical picture of ARDS. Animal experiments must be conducted in accordance with the 3 R´s principles and should be highly standardized to allow the best possible comparison of results among researchers. We perform a minimal invasive method to induce ARDS in a murine model under consideration of highest animal welfare and reproducibility.

Methods: To induce ARDS, lipopolysaccharide (LPS) is injected into the trachea via an endotracheal tube. Therefore, the mouse is subjected to general anesthesia and positioned on an inclined intubation stand. An intravenous catheter is used as an endotracheal tube. It is mounted on a fiber-optic light source that enables it to simply advance the tube into the trachea through the oral cavity without any further invasive measures. Following successful intratracheal intubation, the fiber-optic mandrin is retracted. A syringe can be attached to the tube and 3 mg/kg LPS are applied intratracheally. Then the mouse is carefully extubated and allowed

to recover from anesthesia. We recommend a single dose of a painkiller to counteract the irritation of the trachea. The immune reaction following ARDS is already significant after 24 hours.

Conclusions: Although other methods exist, this model has clear advantages for standardization of ARDS induction and research. It is comparatively easy to establish and perform. The provoked reaction reliably reproduces the clinical picture of ARDS, while the burden for the animals is reduced to an unavoidable minimum.

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LIFE AFTER MAJOR TRAUMA – PATIENT AND EXPERT INTERVIEWS

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Background: Increasingly patients survive a major trauma, but only recently research tries to explore the health and work outcomes of those survivors. The aim was to explore lived experiences of major trauma survivors and healthcare professionals / expert experiences with the care of major trauma survivors in Germany.

Methods: We conducted pair or single exploratory interviews with nine major trauma survivors (18-55 years; Injury Severity Score ≥ 16) and group interviews with 39 experts from 11 German medical societies including 14 occupational groups. The interviews were semi-structured in three blocks: (1) experiences from trauma to current daily life, (2) ideas for optimization of healthcare processes & structures, and (3) work ability & quality of life. For the analysis the International Classification of Functioning, Disability and Health framework (ICF) was used for patient interviews and a system mapping approach for the expert interviews.

Results: Communication and collaboration were major topics in patient and expert interviews concerning direct patient-doctors communication and interdisciplinary work. The importance of a contact person and social support throughout the recovery process was highlighted. The latter not only provides emotional and physical support but also for bridges gaps in the healthcare system. The support by employers and colleagues seemed to be beneficial for patients in relation to return to work.

Further, psychological consequences of trauma and the role of psychological/psychosomatic treatment were discussed as well as bureaucratic and rehabilitative obstacles.

Further, psychological consequences of trauma and the role of psychological/psychosomatic treatment were discussed as well as bureaucratic and rehabilitative obstacles.

Conclusion: During their recovery, major trauma survivors experience a wide range of services and sectors. Several factors influenced this recovery. Some factors may be modifiable by changes to processes and structures of the German healthcare system (e.g. contact person, access to psychological care), others through direct interventions with patients and health care personnel (e.g. communication, better integration of the social network of patients).

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FROM MOUSE-TO-HUMAN DIAGNOSTIC TRANSLATION STORY: THE NOVEL BIOMARKER T6A ACCURATELY IDENTIFIES SEPSIS AT ADMISSION

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Background: Diagnosis of sepsis at the ICU admission is burdened by uncertainty. There is a need for an accurate identification of patients with sepsis from those with non-infectious trauma. We tested diagnostic utility of a novel biomarker, nucleoside t^6A (N 6 - threonylcarbamoyladenosine) in animal sepsis and in adult patients with sepsis compared to two different patient cohorts with elective coronary artery bypass graft surgery (CABG) and polytrauma.

Methods: We performed a multicenter retrospective comparative observational study in adult intensive care units (ICUs) at three university hospitals: 81 patients presenting to the ICU with sepsis; 81 patients after CABG, 64 patients with polytrauma (Injury Severity Score > 15) and 49 patients with COVID-19. Animal modeling was performed by a research institution.

Results: Circulating t^6A measured accurately identified patients with sepsis at the ICU admission when compared to CABG (AUC 95%) and polytrauma (AUC 97%) patients and it was superior to procalcitonin (PCT) with an AUC 88% ($p < 0.05$) by ROC test. In identification of 49 COVID-19 patients at the ICU admission, t^6A reached AUC of 88%. t^6A was released to circulation approximately 6–8 h post-infection onset as demonstrated in the baboon *E. coli* septic shock and non-lethal mouse sepsis. While PCT declined, t^6A median concentration was constantly above the optimal ROC diagnostic threshold until day 10 post-admission. Both t^6A (AUC 62%) and PCT (AUC 72%) poorly predicted sepsis outcome at-admission. There was no increase of t^6A concentration in pig trauma. Compared to a normal human value, circulating t^6A was of similar magnitude in the healthy baboon, pig, dog, rabbit, rat and mouse.

Conclusions: t^6A accurately detected sepsis at the ICU admission compared to surgical CABG and severe polytrauma patients. t^6A rapid rise at the sepsis onset and its prolonged elevation makes t^6A an apt marker for diagnosis of sepsis.

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THE VAGUS NERVE AND CHOLINERGIC SIGNALING IN THE REGULATION OF INFLAMMATION

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Inflammation is a complex, protective response that is triggered by infection and injury, and a variety of other stimuli and conditions. Precisely controlled, localized and timely-resolved inflammation results in restoration of physiological homeostasis. However, dysregulated inflammatory responses and excessive or non-resolved inflammation are implicated in the pathogenesis of a broad spectrum of diseases. Discoveries during the last 20 years have revealed that brain and autonomic nervous system neuronal circuits, including the vagus nerve play key roles in the regulation of cytokine levels and inflammation. This knowledge led to utilizing electrical vagus nerve stimulation and pharmacological cholinergic modalities in treating various inflammatory conditions in preclinical settings and in recent successful clinical trials. We have identified the dorsal motor nucleus of the vagus (DMN) as a major brainstem source of efferent cholinergic fibers with an anti-inflammatory output. We have also recently revealed a specific role of the

celiac-superior mesenteric ganglion complex (CSMGC) that receives vagal efferent innervations in the regulation of cytokine responses and inflammation. I will summarize recent findings that advance our understanding of the brain and peripheral neuronal mechanisms controlling inflammation.

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PKCα-PHOSPHATASE FEEDBACK LOOPS IN HEPATOCYTES: A THERAPEUTIC LINK TO SEPSIS-ASSOCIATED LIVER FAILURE

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Background: The liver is a regulatory hub for infection. It clears endogenous and exogenous molecules, balances inflammatory and anti-inflammatory signals, and orchestrates the host's immune response. In life-threatening infections progressing to sepsis, liver failure emerged as an independent risk factor for mortality. Objective: Translating the molecular processes underlying sepsis liver dysfunction into targeted therapeutics.

Methods: Molecular investigations were conducted using HepG2 cells and mice with a Protein Kinase C (PKC) α knockout, alongside treatment with the clinically approved PKC inhibitor Midostaurin. In vitro, HepG2 cells were treated with cytokines and lipopolysaccharide (LPS) to induce inflammation. In vivo, sepsis was induced in mice via an analgesic-, antibiotic-, and fluid-resuscitation-based peritoneal contamination and infection model. Molecular and functional changes were assessed through RNA sequencing, spatial transcriptomics, histology, electron microscopy, immune cell profiling (flow cytometry), cytokine response analysis, and experimental liver function tests. In vitro studies included protein-interaction assays and molecular localization analyses.

Results: Mice with PKCα knockout or Midostaurin treatment post-infection onset exhibited no morphological or functional impairments in liver tissue. While the immune response remained functional in Midostaurin-treated animals, phagocytosis was reduced in PKCα knockout mice. Both interventions improved seven-day survival. In vitro studies confirmed that selective PKCα inhibition targets key pathways, leading to remodeling of the excretory canalicular pole, which impaired its function and contributed to liver dysfunction. Pathway analysis revealed that PKCα activates regulatory feedback loops via interaction with Protein Phosphatases. This interaction induces dephosphorylation of membrane anchors (e.g., Radixin) in hepatocytes, resulting in loss of canalicular morphology and excretory function through MRP2 retrieval.

Conclusions: PKCα signaling impacts the cytoskeleton, membrane anchor complexes, and trafficking machinery—key components of membrane remodeling in hepatocytes. Its activation during sepsis, driven by cytokine signaling, accelerates hepatic remodeling and liver failure. However, small-molecule pharmaceuticals such as Midostaurin can effectively inhibit these processes.

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CELLULAR SENESCENCE LANDSCAPE IN SEPSIS LIVER

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Cellular senescence, a hallmark of aging, is a state of irreversible growth arrest induced by telomere shortening or other stressors. The senescence phenotype is heterogeneous. We recently provided evidence to show an inverse relationship in the expression pattern of the two murine variants of p21 (v1 and v2) in aging and hemorrhagic shock, an acute injury. Further, we recently demonstrated a rapid onset of cellular senescence in the liver of mice subjected to CLP-induced sepsis, with upregulation of senescence markers and an increase in p21 expression predominantly contributed by the p21 v1. Using RNAscope, confocal microscopy, and single-cell sequencing, senescence onset was confirmed in several cell types in the liver. The landscape of senescent phenotype in the liver investigated by single-cell sequencing showed that this cell fate is not confined to any particular cell type but displays a heterogeneous distribution. We also observed a large influx of macrophages into the sepsis liver with a phenotype switching to an inflammatory state and expressing senescent markers. When sepsis was induced in mice treated with senolytics (Dasatinib and quercetin) we found a significant reduction in mortality. In summary, our experiments demonstrated a rapid development of cellular senescence with sepsis and, for the first time, described the senescence landscape in the sepsis liver and the possible role of senescent cells in the outcomes after sepsis.

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PERSONALIZED THERAPIES EFFECTIVE IN PRE-CLINICAL MODELS OF SEPSIS

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Sepsis continues to exact a substantial burden on patients with a 40% increase between 2016 to 2021. While the surviving sepsis guidelines have recommendations about antibiotics, fluid resuscitation and vasopressors, other personalized therapies for sepsis do not exist although ongoing clinical trials are underway. Animal models offer the advantage of stratifying sepsis subjects to tailor therapies based on easily measured biomarkers. Some measure physiologic parameters while others require a blood sample. Prior publications show that using an identical therapy reduces mortality in a high lethality model, while increasing lethality in a low lethality model. Biomarkers were measured in mice where sepsis was induced using the cecal ligation and puncture (CLP) model treated with fluid resuscitation and antibiotics resulting in 50% mortality over 28 days. Multiple biomarkers collected within the first 6 hours stratify mice who will die from those who will live. The blood level of IL-6 collected 6 hours (6@6) after the onset of sepsis accurately predicted mortality over the next three days with an Area Under the Curve (AUC) of 0.974. Renal injury commonly occurs in sepsis and BUN measured at 6 hours accurately predicted mortality: AUC = 0.96. Heart rate also accurately predicted survival: AUC = 0.83. A further refinement used a dual cutoff level of IL-6 where mice were stratified into 3 groups Live-predicted High IL-6 (<12 ng/ml) 100% sensitivity, Die-predicted (Die-P) Low IL-6 (>1.6 ng/ml), 100% sensitivity. The value of stratification was shown in studies where therapies given after mortality prediction improved survival, but only in those Die-P. These therapies include dexamethasone, hydrocortisone, ascorbic acid and thiamine, IL-18 binding protein, and a substance P antagonist. When sepsis subjects were not stratified, these therapies did not improve survival and may have increased mortality in Live-P mice.

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PROTEOMICS PROFILES OF SEPSIS

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Background: Sepsis is a life-threatening condition characterized by a dysregulated host response to infection, leading to organ dysfunction and high mortality rates. Despite significant advancements in clinical management, early and accurate diagnosis of sepsis remains a major challenge. In this context, proteomics has emerged as a promising tool to uncover novel molecular signatures associated with sepsis pathophysiology, which may support the discovery of more precise diagnostic and prognostic biomarkers. Objective: Identify protein biomarkers with differential expression between patients with sepsis and those with NISIRS. A secondary goal is evaluating the diagnostic performance of these biomarkers using machine learning-based feature selection and exploring the functional interactions among the identified proteins to better understand their role in sepsis.

Methods: Prospective observational study including adult patients diagnosed with sepsis through activation of the hospital Sepsis Code and patients with NISIRS admitted to the ICU at Vall d'Hebron University Hospital between 2016 and 2017. Plasma samples were analyzed using mass spectrometry-based proteomics. Recursive feature elimination (RFE) combined with cross-validated logistic regression was employed to identify proteins with the highest discriminative power. Protein-protein interaction networks were explored using STRING database analysis.

Results: We identified 25 proteins with statistically significant differential expression between groups, achieving a classification accuracy of 0.960 (95% CI: 0.936–0.983), specificity of 0.920 (95% CI: 0.859–0.980), sensitivity of 0.973 (95% CI: 0.945–1.00), and AUC of 0.985 (95% CI: 0.972–0.997). Among them, 14 proteins (e.g., vWF, PPBP, C5, FCN3, SAA2) were more strongly associated with sepsis, while 11 proteins (e.g., FN1, IGFALS, SERPINA4, APOE, SAA1) were enriched in NISIRS. Functional analysis revealed involvement in immune response, complement activation, coagulation, and lipid metabolism.

Conclusions: Distinct proteomic profiles differentiate sepsis from NISIRS with high accuracy. These findings support the potential utility of selected plasma proteins as novel biomarkers to improve early diagnosis and risk stratification in sepsis.

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IN VITRO MODELLING OF TRAUMA AND SHOCK – ADVANCED TOOLS FOR MECHANISTIC INSIGHT AND TRANSLATIONAL RESEARCH

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Trauma and shock are critical clinical challenges due to their complexity and involvement of multi-organ injuries. While animal models have been instrumental in advancing our understanding of trauma-related pathophysiology and informing treatment strategies, they are limited by interspecies differences, ethical concerns, and the difficulty of isolating defined biological mechanisms. In this context, advanced in vitro models have increasingly gained importance and offer a promising alternative, enabling controlled, adaptable human-relevant conditions and high-throughput screening. Advances in biomaterials, microfluidics, and the use of human-derived cells have led to the development of more physiologically relevant in vitro systems. These include co-culture systems that incorporate multiple cell types such as parenchymal, immune, and endothelial cells—as seen in liver models—and organ-on-a-chip technologies that mimic inter-organ communication. The use of primary human cells, including induced pluripotent stem cells further enhance the relevance and customizability of these models, allowing for the creation of complex tissue structures and organoids. To mimic localized trauma, at the cellular level, researchers apply methods such as mechanical stimulation, hypoxia, ischemia-reperfusion, or creation of defects such as wounds. Alternatively, systemic responses to injury are modeled by exposing cells to damage-associated molecular patterns, inflammatory cytokines, or complex inflammatory mixtures combining cytokines and complement factors that mimic the post-traumatic systemic inflammatory milieu. For sepsis modeling, pathogen-associated molecular patterns or trauma-relevant inflammatory cocktails are employed. This review explores and discusses key strategies for replicating both local tissue damage and systemic immune response in vitro. Emphasis is placed on how these models can bridge the gap between limited cell culture and complex in vivo conditions, supporting the development of targeted therapies and advanced translational trauma research.

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BILE ACIDS MEDIATE LIVER-FAILURE ASSOCIATED IMMUNOSUPPRESSION VIA TGR5-MEDIATED cAMP/PKA-SIGNALLING

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Background: Liver failure in critical illness is associated with immune dysfunction in patients with poor prognosis and correlates with the systemic accumulation of bile acids. In addition to their surfactant properties, bile acids signal via nuclear and G protein-coupled receptors (GPCR), potentially modulating the patients' immunity. We have documented a role for the TGR5 GPCR in the immunosuppressive action of bile acids on monocytes. However, it is not known to what extent and through which TGR5-dependent intracellular signals bile acids exert their immunosuppressive effect. Objective: To understand the relevance of TGR5 in bile acid-induced monocyte suppression, and the intracellular signalling network that mediates bile acid-driven TGR5-dependent immunosuppression.

Methods: Serum from critically ill patients with/without liver failure (e.g., ACLF, sepsis) was analysed for bile acid concentration and composition. Individual immunosuppressive scores of bile acid compositions were determined using a previously developed algorithm. Monocyte function was monitored by cytokine release and other assays. Using genetically engineered THP-1 monocytes with or without TGR5 expression, we characterised TGR5 signalling in an unbiased format (phosphoproteomic screen) and by direct assessment of GPCR-targeted pathways.

Results: In critically ill patients with liver failure, high serum levels of immunosuppressive bile acid signatures correlate with features of monocyte immunosuppression and increased mortality. Bile acid-induced, TGR5-mediated monocyte suppression was recapitulated in a THP-1 model, to dissect the immunosuppressive signal transduction pathways downstream of TGR5. Unbiased phosphoproteomics and signal transduction analysis illustrated a major role of the cAMP/PKA pathway

downstream of TGR5. PKA activation propagates the signal to immunity-relevant nodes like the CREB transcription factor or the cell adhesion regulator Rap1.

Conclusions: Pathogenic levels of serum bile acids correlate with immunosuppression and poor prognosis. In a cell biological model of monocyte function, bile acids induce strong cAMP/PKA signalling via the TGR5 GPCR. The precise consequences of cAMP/PKA signalling for monocyte immunity remain to be elucidated.

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OPEN INNOVATION TO IMPROVE POST-SEPSIS PATHWAYS, LESSONS LEARNED FROM CHRONIC WOUNDS

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Background: While critical care excels at saving lives during emergencies, long-term recovery is often poorly integrated into healthcare systems. Survivors of sepsis and septic shock are frequently discharged from hospital without a structured follow-up and insufficient information. Complications such as Persistent Inflammation, Immunosuppression, and Catabolism Syndrome (PICS) remain underdiagnosed, undocumented, and unsupported in many national frameworks. Objective: Translating formats from an Open Innovation program in chronic wound care to improve post-sepsis care.

Methods: The analysis draws from over 20 expert interviews with international sepsis experts in clinical settings, combined with a thorough review of Austrian and Czech public policy documents, legal texts, clinical guidelines, and scientific literature. Key findings are complemented by recent field insights into critical care and post-sepsis recovery models in Austria and Czechia.

Results: Like chronic wounds, post-sepsis syndrome requires a long-term, multidisciplinary treatment approach, and suffers from a lack of visibility in many healthcare systems. In Austria, new National Guidelines for "Sepsis prevention, diagnosis, treatment and follow-up" have been published, but less than 3% are dedicated to the follow-up. Sepsis survivors consistently express the need for clearer information on their treatment and health status at discharge. Long-term care pathways lack concise coordination. Initial comparisons between countries with and without national sepsis frameworks suggest that structured policies support better continuity of care. Local solutions such as brochures for survivors show promise if distributed and supported by healthcare professionals and facilities.

Conclusions: Open Innovation offers a practical framework for cross-sector collaboration. Improving outcomes for survivors requires more than clinical excellence - it demands systemic coordination, defined responsibilities, and attention to the entire patient journey.

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STANDARDIZED BURN TRAUMA – A RELIABLE MODEL FOR INDUCTION OF POST TRAUMATIC IMMUNE RESPONSE

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Background: Since trauma and its immunological complications are one of the main causes of death in young patients and a causal therapy is still missing, scientific research in this field remains crucial. Animal models are still indispensable in this context, as the full complexity of the immune system can only be represented in this setting. A highly used model in trauma immunology is the Burn Trauma Model. The method described in the following is one example for a standardized burn injury model for induction of full scald burn of 20 % of the body surface area (TBA).

Methods: Previous to the induction of the burn injury the mouse should be anesthetized using a fully antagonizable anesthesia (Medetomidine/Midazolam/Fentanyl) combined with isoflurane as hypnotic agent against the highly awakening properties of the burn procedure. The dorsum of the anesthetized mouse is shaved, and the animal is placed into a standardized template (based on a 50 ml syringe) revealing 20 % TBA. The template is immersed into a 90 °C water bath for 9 seconds. To reduce possible complications such as intestinal perforation, mice should have a body mass of at least 23 g, and the mouse spine should be exactly aligned with the median line of the burn template during positioning. To terminate narcosis, only partial antagonization should be administered to maintain the painkilling effect of fentanyl until it is directly displaced by the higher affinity of buprenorphine as further analgesia. After burn mice are rehydrated and closely observed for the following hours.

Conclusions: Full scald burn trauma of at least 20 % TBA is capable of inducing post-traumatic immune response such as SIRS and CARS. Burn trauma is easy to standardize and can be used stand alone or as a part of 2-hit trauma or polytrauma models.

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MANAGEMENT OF A C-PROBLEM IN SEVERELY INJURED PATIENTS WITH PELVIC FRACTURES: DOES THE VOLUME OF PELVIC HEMATOMA CORRELATE WITH HEMODYNAMIC INSTABILITY?

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Background: Severe trauma remains a leading cause of death in individuals under 40 in Western countries. 25% present with severe pelvic fractures (AIS $\geq$ 3), of which 3–9% are hemodynamically unstable due to concomitant vascular injuries. Mortality in such cases reaches 18–20%, rising to 33–40% with hemodynamically significant pelvic vascular injury. Rapid, individualized damage control is crucial, especially given the high-volume blood loss into pelvic and soft tissue compartments. Most bleeding in complex pelvic trauma causing hemorrhagic shock originates from venous injury or the fracture site (80–90%), complicating detection and treatment. Following the introduction of the Pelvic Vascular Injury Score (P-VIS) to determine the risk of peripelvic vascular injury preclinically, this study aimed to correlate pelvic hematoma volumes with hemodynamic instability, thereby identifying patients requiring urgent interdisciplinary management.

Methods: Data from 467 severely injured patients (ISS $\geq$ 16; $AIS_{Pelvis} \geq 3$) at a Level I Trauma Centre were analysed, including imaging, clinical records, and source of bleeding. 79 patients showed pelvic haematoma, its volume was measured via multiplanar reconstruction of initial CT-scans. Multivariate analysis examined correlations between hematoma volume, P-VIS, and hemodynamic parameters.

Results: Among the 79 patients with hematoma, only 40% exhibited significant hemodynamic compromise (C-problem). Of these, 60% had hematoma volumes >200 cm^3, compared to 10% without C-problems. Mean P-VIS scores were 7 in patients with C-problems and 4 in those without. Patients with volumes >200 cm^3 demonstrated significantly greater hemodynamic deterioration within 48 hours.

Conclusions: While pelvic hematoma volume alone does not reliably predict hemodynamic instability, volumes >200 cm^3 are associated with worsening circulatory status. Early identification of the presence of vascular injury in patients with unstable pelvic injury via P-VIS >3 and/or ultrasound-estimated hematoma volume should prompt immediate measures, including pelvic binding, tranexamic acid, structured resuscitation,

and transfer to a Level I Trauma center with comprehensive surgical, endovascular, and transfusion resources.

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ALTERATIONS IN THE PLASMA METABOLOMIC PROFILE IN SEPTIC PERITONITIS DUE TO PERFORATION OF THE LOWER DIGESTIVE TRACT: A CLINICAL AND LABORATORY-BASED STUDY

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Background: Perforation of the lower digestive tract causes severe hyperinflammation and deteriorates to worse outcomes. It is, therefore, crucial to detect useful biomarkers to prognose the outcome of septic conditions. Objective: To clarify the characteristics of metabolites in both patients and mice with sepsis due to perforation of the lower digestive tract by metabolomic analysis using plasma.

Methods: Ten septic patients who received open abdominal surgery for perforation of the lower digestive tract were consecutively included in the present analysis. Seven patients who underwent laparotomy for intestinal obstruction, or non-perforating ileus, were set as non-septic control. We collected plasma samples from these patients admitted to the intensive care units following emergency surgery. As for the murine sepsis models, we performed cecal ligation and punctures and sham operations as controls with a single laparotomy. We sacrificed them at 0, 8, and 24 hours to collect plasma samples (n = 4, in each group). Metabolome analysis was performed using capillary electrophoresis with electrospray ionization time-of-flight mass spectrometry. We investigated the metabolites and lipid mediators involved in peritonitis and performed clustering analysis using a multivariate integrative method to reveal sepsis-related metabolites and lipid mediators in both species.

Results and Conclusions: Patient metabolomic analysis annotated 251 metabolites and 331 lipid mediators. Five metabolites and 20 lipid mediators contributed to the separation of sepsis and non-sepsis in discriminant analysis using MINT. These data suggest that hyperinflammation-induced oxidative and mitochondrial damage reduces fatty acid oxidation in the mitochondria of sepsis patients, and treatment with LPE could be beneficial for sepsis by preventing further deterioration of mitochondrial function. Metabolome analyses revealed that several factors, including important metabolites and lipid mediators, were involved in the aforementioned sepsis pathophysiology. Further analyses are needed to combine the animal data and clarify whether these biomarkers are viable, potentially leading to novel treatments.

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ANTIBIOTICS FOR SEPSIS: SAVE YOUR ANAEROBIC MICROBIOTA!

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Background: Antibiotics are central to sepsis management. However, while anaerobic bacteria are rarely the primary cause of sepsis, anti-anaerobic agents are often included in empirical regimens. This routine practice may inadvertently harm patients by disrupting the gut's commensal anaerobic microbiota, which plays a key role in immune homeostasis. Objective: To challenge the routine empirical use of anti-anaerobic antibiotics in sepsis and promote awareness of the risks associated with anaerobic microbiota depletion.

Methods and Results: A synthesis of preclinical and clinical studies reveals that anti-anaerobic antibiotics significantly reduce beneficial gut anaerobes, leading to impaired immune responses and increased susceptibility to secondary infections. Observational data further associate this disruption with higher mortality in patients with sepsis. Despite these findings, empirical use of broad-spectrum antibiotics—including anaerobic coverage—remains widespread, often without confirmed need. The traditional "better safe than sorry" rationale may, in this context, result in greater harm than benefit (PMID: 39401510).

Conclusions: Empirical antibiotic choices in sepsis should be critically evaluated. Avoiding unnecessary anaerobic coverage can help preserve microbiota integrity, support host immunity, and improve clinical outcomes. Increased awareness of these issues is essential for more targeted, evidence-based sepsis therapy.