Circulating eNAMPT as a Diagnostic Biomarker in the Critically Ill: Acute Pancreatitis, Sepsis, Trauma, and Acute Respiratory Distress Syndrome

Christian Bime
The University of Arizona Health Sciences
Nancy Gonzalez Casanova
University of Arizona Health Sciences Library: The University of Arizona Health Sciences
https://orcid.org/0000-0002-6363-2414
Sara M. Camp
The University of Arizona Health Sciences
Radu C. Oita
The University of Arizona Health Sciences
Juliet Ndukum
The University of Arizona Health Sciences
Vivian Reyes Hernon
The University of Arizona Health Sciences
Dong Kyu Oh
University of Ulsan College of Medicine
Yansong Li
USAISR: US Army Institute of Surgical Research
Phil J. Greer
University of Pittsburgh School of Medicine
David C Whitcomb
University of Pittsburgh School of Medicine
Georgios I. Papachristou
The Ohio State University College of Medicine
Joe G. N. Garcia (skipgarcia@email.arizona.edu)
The University of Arizona Health Sciences

Research

Keywords: NAMPT, eNAMPT, ARDS, pancreatitis, trauma, sepsis, biomarker.

Posted Date: August 31st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-847354/v1

Abstract

Background: Nicotinamide phosphoribosyltransferase (NAMPT) is a protein that exhibits dual functionality – as an intracellular enzyme which regulates nicotinamide adenine dinucleotide metabolism and as an extracellular protein (eNAMPT) secreted into the blood and functions as a cytokine regulator of innate immunity by activating NF-κB via binding to Toll-Like receptor 4. In limited preclinical and clinical studies, eNAMPT was implicated in the pathobiology of acute respiratory distress syndrome (ARDS) suggesting that eNAMPT could be a potential diagnostic and prognostic biomarker. Our aim was to investigate the feasibility of circulating eNAMPT levels to serve as a biomarker in an expanded cohort of patients with ARDS and ARDS-predisposing conditions such as acute pancreatitis, sepsis, and trauma when compared to controls.

Methods: 795 patients and 179 healthy controls were included in the discovery and validation cohorts. Plasma and serum eNAMPT levels were quantified using one of two complementary Enzyme-linked Immunosorbent Assays. After log base 2 variance stabilizing transformation of plasma/serum eNAMPT measurements, differences between healthy controls and each disease cohort were compared using linear regression or generalized estimating equation (GEE) model where applicable. Complementary analyses included sensitivity, specificity, positive predictive values, negative predictive values, and the area under the receiver operating curve.

Results: Compared to controls, circulating eNAMPT levels were significantly higher in patients with acute pancreatitis, with sepsis, with trauma, and with ARDS (all $p<0.01$). In the acute pancreatitis cohort, circulating eNAMPT levels positively correlated with disease severity ($p<0.01$).

Conclusions: Circulating eNAMPT levels are a potential novel diagnostic biomarker in the critically ill with acute pancreatitis, sepsis, trauma, and/or ARDS.

Introduction

Viral and bacterial sepsis, trauma, and acute pancreatitis are inflammatory disorders that commonly precede the development of acute respiratory distress syndrome (ARDS), a heterogeneous lung disorder characterized by an intense inflammatory response and affects nearly 300,000 patients annually in the United States[1]. Patients rapidly develop acute hypoxemic respiratory failure requiring mechanical ventilation and 30–40% die from the resulting multi-organ system failure[2]. Morbidity and mortality from of ARDS have dramatically increased world-wide due to the ongoing SARS-CoV-2/COVID-19 pandemic[2]. Diagnostic uncertainty is common in ARDS because of imprecise clinical and radiographic criteria, which further exacerbates heterogeneity[3]. The clinical and biological heterogeneity of ARDS makes it extremely challenging to investigate novel promising therapies in phase II/III clinical trials[4]. There is a compelling unmet medical need for biomarkers with pathophysiologic relevance that might guide subject stratification for enrollment in future clinical trials investigating personalized ARDS therapies.

Previous genomic–intensive approaches have identified potentially novel therapeutic targets in sepsis and acute inflammatory lung disorders including ARDS[5–11]. Among them is the gene encoding nicotinamide phosphoribosyl transferase (*NAMPT*), a novel candidate gene in ARDS[5]. *NAMPT* encodes a protein that exhibits dual functionality – as an intracellular enzyme which regulates nicotinamide adenine dinucleotide metabolism and as an extracellular protein (eNAMPT) secreted into the blood and functions as a cytokine regulator of innate immunity by activating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) via binding to Toll-Like receptor 4 (TLR4)[12]. Extracellular NAMPT (eNAMPT) was among a panel of six biomarkers (Interleukin-6 [IL-6], Interleukin-8 [IL-8], Interleukin-1 receptor antagonist [IL-1RA], macrophage migration inhibitory factor [MIF], and Angiopoietin-2 [Ang-2]) shown to be highly predictive of 28-day mortality in ARDS[13]. Since the role of eNAMPT as a stand-alone diagnostic biomarker has not previously been studied, we investigated whether circulating eNAMPT levels were significantly elevated in patients with ARDS and ARDS-predisposing conditions such as acute pancreatitis, sepsis, septic shock, and trauma when compared to controls. Furthermore, we sought to determine the best cutoff point of plasma eNAMPT levels to distinguish patients with disease from control subjects.

Methods

Sources of Data. A total of 795 blood specimens from patients with ARDS, sepsis, acute pancreatitis, or trauma and 179 healthy controls were included in this study. The discovery cohort included a combination of serum and plasma samples, analyzed separately. The serum pool consisted of 59 subjects with acute pancreatitis and 11 from healthy controls from the University of Pittsburgh (IRB#STUDY20060223)[14, 15] and 100 subjects with sepsis and 20 controls from Asan Medical Center, Seoul, Korea (Approval #2001-0001). The plasma pool consisted of 67 trauma subjects from the US Army (IRB# L-12-004), 123 subjects with sepsis and septic shock, and 248 ARDS subjects from the Fluid and Catheter Treatment Trial (FACTT) study[16], the University of Arizona (IRB#1312168664R001) and University of Illinois (IRB #20120192) and 70 controls from the University of Arizona (IRB#1312168664R001). The validation cohort included 276 plasma specimens (100 ARDS, 98 sepsis, and 78 controls) from the University of Arizona (IRB#1312168664R001).

Participants. All patients with ARDS met diagnostic criteria per the Berlin Definition[3]. Patients with sepsis met the Consensus definition for sepsis guidelines[17] at enrollment. Acute pancreatitis was diagnosed based on the International Association of Pancreatology (IAP)/American Pancreatic Association (IAP/APA) guidlines[18]. Grading of the severity of acute pancreatitis was determined based on the Revised Atlanta Classification[19 20].

Blood collection and measurement of eNAMPT. We utilized two complementary eNAMPT ELISA assays in multiple well-phenotyped cohorts of ARDS, sepsis, trauma, and acute pancreatitis patients, including a discovery dataset and a validation cohort to confirm circulating eNAMPT as a diagnostic biomarker in ARDS.

Blood was collected in red-top and EDTA-treated tubes for serum and plasma respectively, centrifuged within 1 h from sample collection (2000Å~g for 20min, RCF) and stored at − 80°C. In the discovery cohorts, plasma and serum concentrations of eNAMPT were quantified using an in-house Enzyme-linked Immunosorbent Assay (ELISA)[21]. In the validation cohort, circulating levels of eNAMPT in plasma were quantified using MSD-Uplex assay electrochemiluminescent immunoassay predesigned panel from MesoScale (Meso Scale Discovery, MSD®)[22].

Statistical analysis.

Descriptive Statistics. Standard descriptive statistics were used to summarize the data for all cohorts. For the continuous variables, the mean, and standard deviation were calculated for the entire data. Counts and percentages were calculated for the categorical variables.

Comparison of circulating eNAMPT levels in disease and controls. For the plasma-based cohorts consisting of patients with sepsis, trauma, and ARDS (one-time measurements), a log base 2 variance stabilizing transformation was performed on the circulating eNAMPT measurements and linear regression models were used to compare the log-transformed eNAMPT measurement (response variable) between each disease category versus controls. Additionally, each log-transformed eNAMPT measurement was fit to the disease severity (for example: control vs. sepsis, septic shock; or control vs. trauma in lower, upper, and combined upper and lower parts of the torso).

For the serum pancreatitis cohort with repeated eNAMPT measurements, a similar log base 2 variance stabilizing transformation was performed on each eNAMPT measurements (i.e., first and second measurements). The transformed eNAMPT measurement was fit to the disease category (control, pancreatitis) as well as the time of measurement to ascertain if there was a difference in eNAMPT levels between the two groups or if the measurements varied because of the time of measurement. Generalized estimating equations (GEE) models were then used to fit the log-transformed eNAMPT level comparing controls to mild, moderate, and severe acute pancreatitis. Timing of measurement was included in the model to ascertain whether the severity of pancreatitis is affected by eNAMPT levels. We plotted receiver operating curves (ROC) and calculated area under the curves (AUC) to determine how well circulating eNAMPT levels distinguished acute pancreatitis from normal controls. The ROC analysis results were interpreted as follows: AUC < 0.70, low diagnostic accuracy; range of 0.70–0.90, moderate diagnostic accuracy; and AUC ≥ 90, high diagnostic accuracy.

Determination of optimal disease-distinguishing plasma eNAMPT level. After log base 2 variance stabilizing transformation of all plasma eNAMPT measurements for each cohort and corresponding controls, the mean and standard deviation (SD) of the combined dataset was calculated. We used an empirical rule to calculate cutoffs at 0, 0.25, 0.5, 0.75, -0.25 SDs. Each cutoff was then converted back to the original units. For each cutoff value, a 2X2 contingency table was generated for each cohort and corresponding controls and the sensitivity, specificity, positive predictive value (PPV), negative predictive values (NPV) calculated.

Statistical analysis performed using Stata version 16.1 (StataCorp) and GraphPad Prism version 8.0 (Sn Diego Ca.) software.

Results

Characteristics of the cohorts. Demographic and clinical characteristics of each cohort are presented in Table 1. The discovery cohort included specimens from 690 subjects and the validation cohort from 276 subjects. The trauma group comprised of 67 samples from US Army trauma wounded soldiers, all males, with mean age of 25 years. Twenty-one trauma subjects experienced upper body injury, 31 with lower body injury, and 13 exhibited a combination of upper and lower body injury. The sepsis group from South Korea represented the oldest subset (mean age, 66.5 years). The pancreatitis cohort consisted of 59 subjects stratified by disease severity (29 mild, 14 moderate, and 16 severe). The highest mortality was observed in the ARDS cohort (21.7%), followed by the South Korean sepsis cohort (19%).

Table 1

Baseline characteristics of patient cohorts and control. Baseline characteristics of the discovery and validation cohorts included in the analysis. Age is reported in mean ± standard deviation (sd), mortality, gender and race are shown as percentages. Race abbreviations: White (W), African American (AA), Native American (NA), Asian- South Korean (A). Pancreatitis classification: Mild: no organ failure/ systemic complications. Moderate: transient organ failure local complications or exacerbation of comorbidities. Severe: persistent organ failure (> 48H). P = plasma, S = serum.

Disease	N	Age (mean±sd)	Mortality (%)	Sex (male, %)	Race (W/AA/NA/A)	Speci-men
Discovery Cohort						
Trauma	67	25 ± 5.8	10.4	100.0	NA	P
ARDS	248	50.3 ± 14.3	21.7	51.2	73.7, 18.5, 1.2, 0.4	P
Sepsis	123	56.7 ± 16.2	16.2	48.4	66.4, 33.4, 0.75, 0	P
Sepsis (SK)	100	66.5 ± 13.5	19	62.0	0, 0, 0, 100	S
Pancreatitis	59	51 ± 18.4	3.3	45.8	88.1, 6.7, 7.1, 0	S
Mild	29	50.8 ± 20.2	0	37.9	82.7, 10.3, 0, 0	S
Moderate	14	45.8 ± 16.6	0	42.9	85.7, 7.1, 7.1, 0	S
Severe	16	56.3 ± 15.2	12.5	62.5	100, 0, 0, 0	S
Healthy controls	70	62 ± 14	0	70	82.8, 10, 1.4, 0	P
Healthy controls	31	57.5 ± 20.9	0	32.3	83.9, 9.7, 3.2, 0	S
Validation Cohort						
ARDS	100	55.4 ± 15.3	37	52	88, 3, 7, 1	P
Sepsis	98	55.5 ± 17.6	14	48	84, 13, 1, 1	P
Healthy controls	78	55.2 ± 16.75	0	57.7	83, 10.2, 1, 0	P

Discovery Cohorts:

Circulating eNAMPT in acute pancreatitis. When compared to controls, circulating eNAMPT levels were significantly higher in patients with acute pancreatitis, (median 20.4 ng/ml, vs 26.84 ng/ml, respectively, $p < 0.01$) (FIGURE 1A**)**. Furthermore, the circulating eNAMPT levels were significantly different in the three severity categories of acute pancreatitis, with higher levels observed among subjects with severe pancreatitis compared to mild pancreatitis, (median 67.7 vs 17.6 ng/ml, $p < 0.01$) and higher in moderate pancreatitis compared to mild (median 40.4 ng/ml vs 17.6 ng/ml, $p < 0.01$), nonetheless, eNAMPT levels were not significantly different when moderate vs. severe pancreatitis subjects were compared (p-value = 0.24) (Table 2**).** Serum eNAMPT at baseline significantly distinguished patients with acute pancreatitis from healthy controls (AUC = 0.74, 95% confidence interval: 0.62–0.86, p = 0.009) and a high diagnosis

accuracy was observed between patients with severe pancreatitis from those with mild pancreatitis (AUC = 0.92, 95% confidence interval: 0.85–1.0, p-value < 0.01) (**FIGURE1B).**

Table 2
Circulating eNAMPT levels elevated in acute pancreatitis compared to healthy controls.

	Log (eNAMPT ng/ml)		
Predictor	**Estimates**	**95% CI**	**P-Value**
Intercept	2.68	2.14–3.17	< 0.0001
Mild Pancreatitis	0.196	-0.22–0.85	0.24
Moderate Pancreatitis	1.23	0.59–1.81	< 0.0001
Severe Pancreatitis	1.66	1.21–2.54	< 0.0001

Circulating eNAMPT levels in sepsis and septic shock. There were two independent cohorts of sepsis/septic shock and controls. In the first group, when compared to the 70 healthy controls, median plasma eNAMPT levels were significantly higher in 123 subjects with sepsis or septic shock (51.5 ng/ml vs 20.4 ng/ml, p < 0.01). (FIGURE 2A**)**. There was no significant difference in plasma eNAMPT levels between patients with sepsis and those with septic shock (median 47.2 ng/ml vs 51.5 ng/ml; p = 0.4). Similarly, there was no significant difference in circulating eNAMPT levels between sepsis survivors and non-survivors (p value = 0.35). Plasma eNAMPT significantly distinguished patients with sepsis and septic shock from healthy controls (AUC = 0.89, 95% confidence interval: 0.85–0.93; p < 0.01) **(**FIGURE 2B**).** In the second group which analyzed serum from South Korean subjects, compared to the 31 healthy controls, the median serum eNAMPT levels were significantly higher in the 100 subjects with sepsis or septic shock (34.7 ng/ml vs. 1.3 ng/ml, p < 0.01) (FIGURE 2C**).** Plasma eNAMPT levels accurately distinguished patients with sepsis/septic shock from healthy controls, median of 34.68 ng/ml in sepsis vs 1.27 ng/ml in controls (AUC 0.93 95%CI 0.86–0.99, p < 0.01) (FIGURE 2D**).** In South Korean cohort, there was no significant difference in plasma eNAMPT levels between subjects with sepsis and septic shock (p value = 0.15) or between survivors and non-survivors (p value = 0.5).

Circulating eNAMPT in trauma. When compared to controls, median plasma eNAMPT levels were significantly higher in trauma subjects (20.4 ng/ml vs 54 ng/ml; p < 0.01) (FIGURE 2A**).** There was no significant correlation between body part affected by trauma and circulating eNAMPT levels. Plasma eNAMPT at baseline significantly distinguished patients with acute trauma from healthy controls with high diagnosis accuracy (AUC = 0.94, 95% CI: 0.90–0.97; p < 0.01) (FIGURE 2B**).**

Circulating eNAMPT in ARDS. When compared to controls, median plasma eNAMPT levels were significantly higher in patients with ARDS (20.4 ng/ml vs. 60.7 ng/ml; $p < 0.01$ respectively). Plasma eNAMPT at baseline significantly distinguished patients with ARDS from healthy controls (AUC = 0.86, 95% confidence interval: 0.82–0.90, $p < 0.01$) FIGURE 2B

Validation Cohort:

Circulating eNAMPT in ARDS and sepsis patients: In this validation cohort, median plasma eNAMPT levels, measured with MSD ELISA assay, were significantly higher in ARDS patients when compared to controls (3.78 ng/ml vs. 1.2 ng/ml; $p < 0.01$) and in sepsis patients when compared to controls (4.7 ng/ml vs. 1.2 ng/ml; $p < 0.01$) (FIGURE 3A**).** Plasma eNAMPT at baseline significantly distinguished patients with ARDS and healthy controls (AUC = 0.85, 95% CI: 0.8–0.9, p value < 0.01), and patients with sepsis and healthy controls (AUC 0.87 95% CI: 0.82–0.92, p value < 0.01) (FIGURE 3B**).** No significant differences were present comparing ARDS and Sepsis subjects.

Circulating eNAMPT cut-off determinations in critically ill subjects and controls. For the pancreatitis cohort, circulating eNAMPT cutoff values 26 ng/ml and 32.5 ng/ml exhibited the best estimates of sensitivity, specificity, NPV, and PPV [**SUPPLEMENTAL TABLE 1]**. For the sepsis cohort, eNAMPT cutoffs of 33.8 ng/ml, and 41.4 ng/ml exhibited the best estimates of sensitivity, specificity, NPV, and PPV [**SUPPLEMENTAL TABLE 2]**. For the trauma cohort, eNAMPT cutoffs 36.6 ng/ml and 44.4ng/ml demonstrated the best estimates of sensitivity, specificity, NPV, and PPV [**SUPPLEMENTAL TABLE 3]**. For the ARDS cohort, the eNAMPT cutoffs 38.1 ng/ml and 46.9 ng/ml, exhibited the best estimates of sensitivity, specificity, NPV, and PPV **(SUPPLEMENTAL TABLE 4)**. Taken together, a plasma eNAMPT level of 35–45 ng/ml (In House ELISA) would be a reasonable cutoff point to distinguish controls from patients with acute inflammatory conditions such as sepsis, trauma, acute pancreatitis, and ARDS.

Discussion

The activation of evolutionary-conserved inflammatory cascades, such as the pathogen-receptor recognition TLR4 pathway, triggered by multiple acute inflammatory conditions such as sepsis (viral, bacterial, fungal), acute pancreatitis, and trauma directly contribute to the multi-organ failure and high mortality associated with ARDS[23,24]. In addition to the intense inflammatory cascade that characterizes ARDS pathogenesis, endothelial and epithelial cell injury, dysregulated coagulation, apoptosis, and fibrosis are prominent features[25]. Accordingly, numerous potential biomarkers are generated by the acute dysregulation of multiple biochemical and cellular pathways characteristic of ARDS pathobiology[25,26]. Extracellular NAMPT was identified as one such potential biomarker and druggable target based on extensive research including preclinical mechanistic, genomic, and multi-species ARDS models[5,12,27,28]. The transcriptional regulation and blood//lung protein expression of *NAMPT* are highly induced by damage-associated molecular pattern proteins (DAMP) and multiple ARDS-relevant stimuli including

bacterial infection, shock, trauma, hypoxia, and excessive mechanical stress[29–32]. Upon binding to TLR4, eNAMPT elicits a profound cytokine release that is mediated by proinflammatory transcription factors such as NFκB and leads to increased vascular permeability and ultimately multi-organ dysfunction[12,29]. The role of eNAMPT as a potential standalone diagnostic or prognostic biomarker in ARDS has not previously been demonstrated although eNAMPT was among a panel of six biomarkers that predicted mortality in ARDS[13]. An ideal diagnostic biomarker for ARDS would identify early stages of the syndrome, minimize disease heterogeneity, reflect the natural history of the syndrome, and be a potential target for a clinical trial[4]. A prognostic biomarker would provide information that addresses the overall outcome of ARDS and be potentially useful in stratifying patients for enrollment in clinical trials, thus enhancing the ability to detect beneficial effects from novel therapies[4].

We have confirmed and validated, for the first time, that circulating eNAMPT is a potential diagnostic biomarker in ARDS and several ARDS-predisposing systemic acute inflammatory conditions including acute pancreatitis, sepsis, septic shock, and trauma. In addition to demonstrating that median circulating eNAMPT levels were significantly higher in ARDS and these ARDS-inducing acute inflammatory conditions when compared to healthy controls, we identified the range of eNAMPT values between 26 ng/ml and 33 ng/ml based on our in-house colorimetric ELISA assay to represent the best possible cut-off for distinguishing patients from healthy controls.

The translational utility of circulating eNAMPT as a diagnostic biomarker in ARDS include early diagnosis, minimizing disease heterogeneity, and facilitating the selection of subjects for enrollment in clinical trials, especially for therapies targeting this pathway[28,33]. An interesting finding in our analysis was the positive correlation between circulating eNAMPT levels and severity of acute pancreatitis. This also highlights the potential prognostic utility of eNAMPT as a biomarker. We did not replicate this positive correlation with disease severity in the sepsis, trauma, and ARDS cohorts, likely because of suboptimal classification of disease severity, non-standardized timing of specimen collection, and heterogeneity of cohorts.

The strengths of our report include, the inclusion of a large and diverse population of patients and controls in the discovery cohort, a robust validation with an independent cohort (patients with ARDS and sepsis), and use of two novel complementary ELISA assays, the MSD-Uplex assay electrochemiluminescent immunoassay, MesoScale (Meso Scale Discovery, MSD®)[22] and our validated in-house ELISA assay[30]. Our findings are also strengthened by the consistency across all the cohorts where circulating eNAMPT levels were significantly higher in patients with acute systemic inflammatory states (sepsis, trauma, acute pancreatitis, and ARDS) when compared to controls.

A limitation of our analysis, which is inherent with the use of previously bio-banked specimens, is the heterogeneity of our cohorts as shown by variability in mortality rates. We did not compare the diagnostic performance of circulating eNAMPT to other diagnostic biomarkers in ARDS, such as soluble receptor for advanced glycation end products (sRAGE)[34] and Angiopoeitin-2[35,36]. We had limited information on

generic severity of illness scales such as APACHE IV scores, sequential organ assessment (SOFA) scores or multiple organs dysfunction (MOD) scores, which limited the ability to better classify the patients within each cohort and further refine the diagnostic value of eNAMPT.

Conclusion

In summary, we have shown that circulating eNAMPT is significantly elevated in ARDS and ARDS-predisposing conditions and is a promising diagnostic biomarker in ARDS that could have utility as a stratification tool for enrollment of subjects in clinical trials targeting eNAMPT neutralization.

List Of Abbreviations

Acute Respiratory Distress Syndrome (ARDS), nicotinamide phosphoribosyl transferase (*NAMPT*), White (W), African American (AA), Native American (NA), Asian- South Korean (A), positive predictive value (PPV), negative predictive values (NPV), receiver operating curves (ROC) and calculated area under the curves (AUC).

Declarations

Ethical Approval and Consent to participate.

Institutional review boards at each institutions approved the study protocols. Research was carried our according to the principles of the Declaration of Helsinki, informed consent was obtained.

Consent for publication

Manuscript was reviewed and approved by all named authors

Availability of data and materials

The datasets generated during a the current study are available from the corresponding author on reasonable request.

Competing interests

The authors have declared no potential conflicts of interests

Funding

This work was supported by K08 HL141623 (CB); P01 HL126609 (JGNG); R41 HL147769 (JGNG); R42 HL145930 (JGNG).

Authors' contributions

CB, NC, JGNC, SMC- study design, analysis, data interpretation, drafting and revision of the manuscript, approval of final version.

JN, NC, PJG, DCW, GIP- data analysis and collection, revision of the manuscript.

RCO, VRH - assay performing, data collection and manuscript revision.

DKO, YL - GIP, data and specimen collection, assisted with processing and manuscript revision.

Acknowledgements Not applicable

Authors' information (Optional) Not applicable

References

1. Bellani G, Laffey JG, Pham T, et al. Epidemiology, Patterns of Care, and Mortality for Patients With Acute Respiratory Distress Syndrome in Intensive Care Units in 50 CountriesTrends in Acute Respiratory Distress Syndrome in 50 CountriesTrends in Acute Respiratory Distress Syndrome in 50 Countries. JAMA. 2016;315(8):788–800.
2. Tzotzos SJ, Fischer B, Fischer H, Zeitlinger M. Incidence of ARDS and outcomes in hospitalized patients with COVID-19: a global literature survey. Critical Care. 2020;24(1):516.
3. Ranieri VM, Rubenfeld GD, Thompson BT, et al. Acute respiratory distress syndrome: the Berlin Definition. JAMA. 2012;307(23):2526–33.
4. Bime C, Camp SM, Casanova N, et al. The acute respiratory distress syndrome biomarker pipeline: crippling gaps between discovery and clinical utility. Translational Research. 2020;226:105–15.
5. Ye SQ, Simon BA, Maloney JP, et al. Pre-B-cell colony-enhancing factor as a potential novel biomarker in acute lung injury. Am J Respir Crit Care Med. 2005;171(4):361–70.
6. Sun X, Ma SF, Wade MS, et al. Functional promoter variants in sphingosine 1-phosphate receptor 3 associate with susceptibility to sepsis-associated acute respiratory distress syndrome. American journal of physiology Lung cellular molecular physiology. 2013;305(7):L467–77.
7. Sun XBC, Shimizu Y, Wade MS, Ma SF, Liu B, Zhang W, Wang T, Garcia JG.. Function analysis of myosin light chain kinase gene promoter and inflammatory lung disease associated polymorphisms. *AJRCMB (submitted) 2015.* 2015.
8. Sammani S, Moreno-Vinasco L, Mirzapoiazova T, et al. Differential effects of sphingosine 1-phosphate receptors on airway and vascular barrier function in the murine lung. American journal of respiratory cell molecular biology. 2010;43(4):394–402.
9. Sun X, Singleton PA, Letsiou E, et al. Sphingosine-1-phosphate receptor-3 is a novel biomarker in acute lung injury. Am J Respir Cell Mol Biol. 2012;47(5):628–36.
10. Bime C, Pouladi N, Sammani S, et al. Genome-Wide Association Study in African Americans with Acute Respiratory Distress Syndrome Identifies the Selectin P Ligand Gene as a Risk Factor. Am J

Respir Crit Care Med. 2018;197(11):1421–32.

11. Lynn H, Sun X, Casanova N, Gonzales-Garay M, Bime C, Garcia JGN. Genomic and Genetic Approaches to Deciphering Acute Respiratory Distress Syndrome Risk and Mortality. Antioxid Redox Signal. 2019;31(14):1027–52.
12. Camp SM, Ceco E, Evenoski CL, et al. Unique Toll-Like Receptor 4 Activation by NAMPT/PBEF Induces NFkappaB Signaling and Inflammatory Lung Injury. Scientific reports. 2015;5:13135.
13. Bime C, Casanova N, Oita RC, et al. Development of a biomarker mortality risk model in acute respiratory distress syndrome. Crit Care. 2019;23(1):410.
14. Langmead C, Lee PJ, Paragomi P, et al. A Novel 5-Cytokine Panel Outperforms Conventional Predictive Markers of Persistent Organ Failure in Acute Pancreatitis. Clin Transl Gastroenterol. 2021;12(5):e00351.
15. Whitcomb DC, Muddana V, Langmead CJ, et al. Angiopoietin-2, a regulator of vascular permeability in inflammation, is associated with persistent organ failure in patients with acute pancreatitis from the United States and Germany. Am J Gastroenterol. 2010;105(10):2287–92.
16. Wiedemann HP. A perspective on the fluids and catheters treatment trial (FACTT). Fluid restriction is superior in acute lung injury and ARDS. Cleve Clin J Med. 2008;75(1):42–8.
17. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801–10.
18. Working Group IAPAPAAPG. IAP/APA evidence-based guidelines for the management of acute pancreatitis. Pancreatology. 2013;13(4 Suppl 2):e1–15.
19. Foster BR, Jensen KK, Bakis G, Shaaban AM, Coakley FV. Revised Atlanta Classification for Acute Pancreatitis: A Pictorial Essay. Radiographics. 2016;36(3):675–87.
20. Banks PA, Bollen TL, Dervenis C, et al. Classification of acute pancreatitis–2012: revision of the Atlanta classification and definitions by international consensus. Gut. 2013;62(1):102–11.
21. Oita RC, Camp SM, Ma W, et al. Novel Mechanism for Nicotinamide Phosphoribosyltransferase Inhibition of TNF-alpha-mediated Apoptosis in Human Lung Endothelial Cells. Am J Respir Cell Mol Biol. 2018;59(1):36–44.
22. Bastarache JA, Koyama T, Wickersham NE, Ware LB. Validation of a multiplex electrochemiluminescent immunoassay platform in human and mouse samples. J Immunol Methods. 2014;408:13–23.
23. Gong T, Liu L, Jiang W, Zhou R. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. Nature Reviews Immunology. 2020;20(2):95–112.
24. Bime C, Casanova NG, Nikolich-Zugich J, Knox KS, Camp SM, Garcia JGN. Strategies to DAMPen COVID-19-mediated lung and systemic inflammation and vascular injury. *Translational research: the journal of laboratory and clinical medicine.* 2020.
25. Blondonnet R, Constantin JM, Sapin V, Jabaudon M. A Pathophysiologic Approach to Biomarkers in Acute Respiratory Distress Syndrome. Dis Markers. 2016;2016:3501373.

26. Spadaro S, Park M, Turrini C, et al. Biomarkers for Acute Respiratory Distress syndrome and prospects for personalised medicine. Journal of Inflammation. 2019;16(1):1.
27. Sun X, Elangovan VR, Mapes B, et al. The NAMPT promoter is regulated by mechanical stress, signal transducer and activator of transcription 5, and acute respiratory distress syndrome-associated genetic variants. Am J Respir Cell Mol Biol. 2014;51(5):660–7.
28. Quijada H, Bermudez T, Kempf CL, et al. Endothelial eNAMPT Amplifies Preclinical Acute Lung Injury: Efficacy of an eNAMPT-Neutralising mAb. *Eur Respir J*. 2020.
29. Adyshev DM, Elangovan VR, Moldobaeva N, Mapes B, Sun X, Garcia JGN. Mechanical Stress Induces Pre–B-cell Colony-Enhancing Factor/NAMPT Expression via Epigenetic Regulation by miR-374a and miR-568 in Human Lung Endothelium. American journal of respiratory cell molecular biology. 2014;50(2):409–18.
30. Oita RC, Camp SM, Ma W, et al. Novel Mechanism for Nicotinamide Phosphoribosyltransferase Inhibition of TNF-α–mediated Apoptosis in Human Lung Endothelial Cells. American Journal of Respiratory Cell Molecular Biology. 2018;59(1):36–44.
31. Moreno-Vinasco L, Quijada H, Sammani S, et al. Nicotinamide phosphoribosyltransferase inhibitor is a novel therapeutic candidate in murine models of inflammatory lung injury. American journal of respiratory cell molecular biology. 2014;51(2):223–8.
32. Wu G-C, Liao W-I, Wu S-Y, et al. Targeting of nicotinamide phosphoribosyltransferase enzymatic activity ameliorates lung damage induced by ischemia/reperfusion in rats. Respiratory Research. 2017;18(1):71.
33. Sun BL, Sun X, Casanova N, et al. Role of secreted extracellular nicotinamide phosphoribosyltransferase (eNAMPT) in prostate cancer progression: Novel biomarker and therapeutic target. EBioMedicine. 2020;61:103059.
34. Jabaudon M, Berthelin P, Pranal T, et al. Receptor for advanced glycation end-products and ARDS prediction: a multicentre observational study. Scientific Reports. 2018;8(1):2603.
35. Wada T, Jesmin S, Gando S, et al. The role of angiogenic factors and their soluble receptors in acute lung injury (ALI)/ acute respiratory distress syndrome (ARDS) associated with critical illness. Journal of Inflammation. 2013;10(1):6.
36. Agrawal A, Matthay MA, Kangelaris KN, et al. Plasma Angiopoietin-2 Predicts the Onset of Acute Lung Injury in Critically Ill Patients. American Journal of Respiratory Critical Care Medicine. 2013;187(7):736–42.

Figures

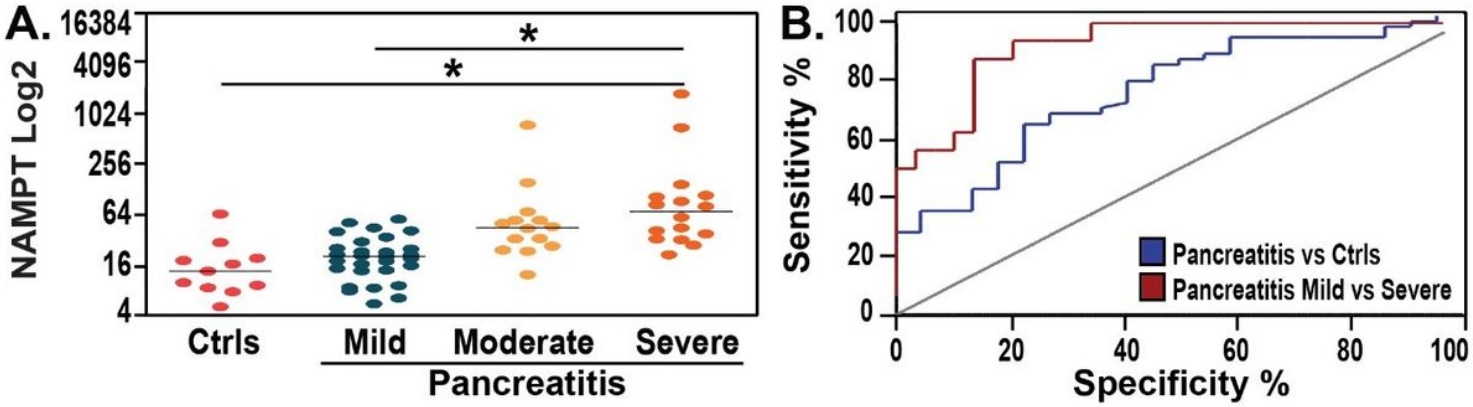

Figure 1

Discovery Cohort: Circulating eNAMPT levels elevated in acute pancreatitis compared to healthy controls. Circulating eNAMPT levels are significantly elevated in acute pancreatitis compared to controls. Positive correlation with disease severity. A. Y-axis represents of log base 2 transformation of plasma eNAMPT values; X-axis group comparison: healthy controls and pancreatitis by severity groups – mild, moderate, severe. Comparisons of medians between pancreatitis and healthy controls and pancreatitis by severity subgroups significantly differ (p-value<0.01) Kruskal-Wallis test. B. Pancreatitis ROC plots and the corresponding AUCs. eNAMPT distinguishes acute pancreatitis from healthy subjects (blue) (AUC=0.74, 95% confidence interval: 0.62-0.86, p 0.009) and severe pancreatitis vs mild pancreatitis (red) (AUC=0.92, 95% confidence interval: 0.85-1.0, p-value < 0.01).

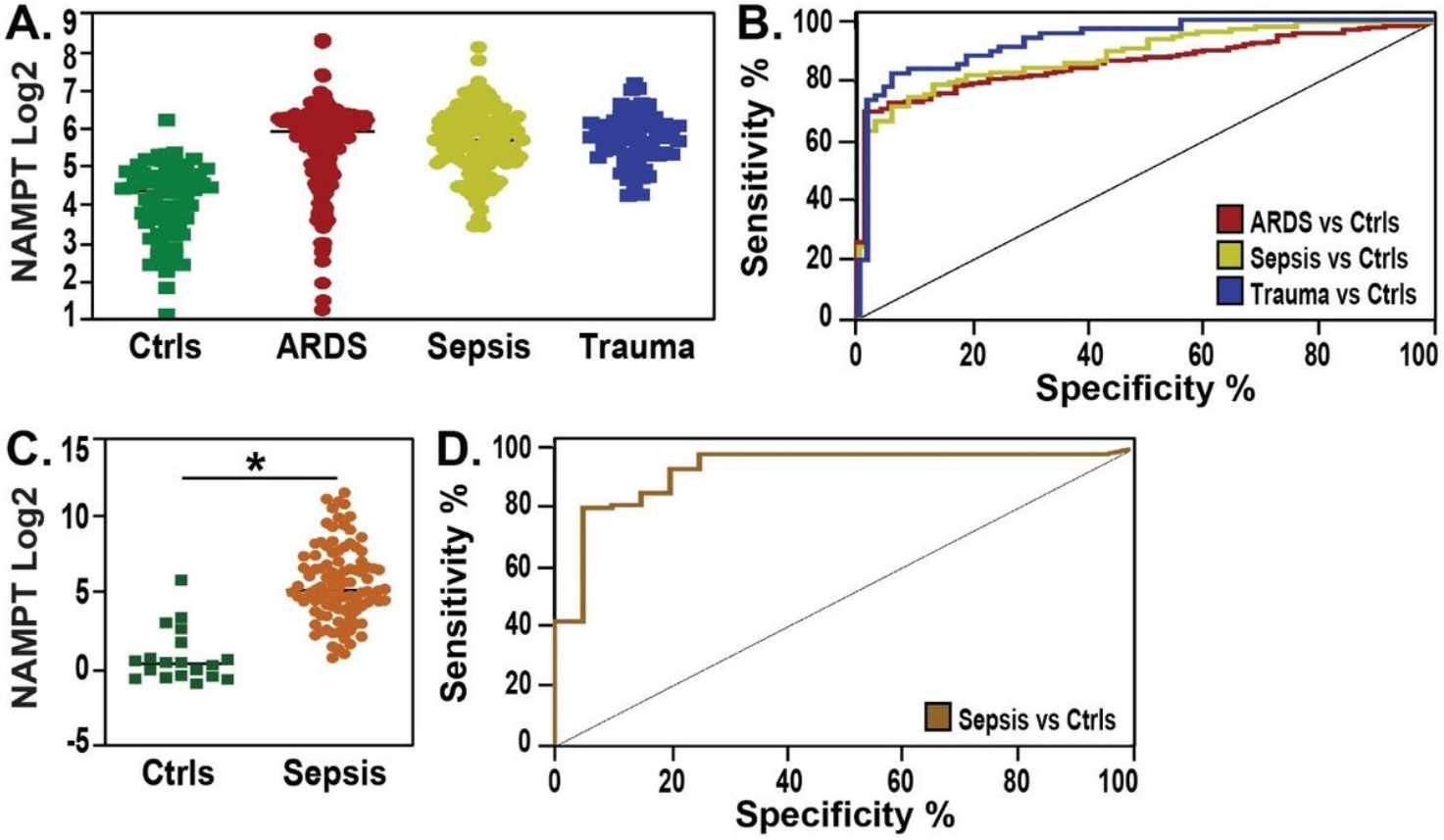

Figure 2

Discovery Cohort: Circulating eNAMPT elevated in ARDS, Sepsis and Trauma compared to healthy controls. Circulating eNAMPT levels are elevated in ARDS, Sepsis and Trauma compared to healthy controls. Data represented in Panels A and B reflect eNAMPT measurements in plasma samples and in Panels C and D reflect eNAMPT measurements in serum samples. In Panels A and C, the Y-axis represents of eNAMPT Log 2 transformed values; X-axis group comparison: healthy controls (green), ARDS (red), Sepsis (yellow or orange) and Trauma (blue). B. ROC plot and the corresponding AUC show that eNAMPT (at baseline), distinguishes: ARDS (red) from healthy controls (AUC=0.86, 95% confidence interval: 0.82-0.90, p< 0.001); sepsis (yellow) from healthy controls (AUC=0.89, 95% confidence interval: 0.85-0.93 p< 0.001) and trauma (blue) vs healthy controls (AUC=0.94, 95% confidence interval: 0.90-0.97 p< 0.001. D. ROC plot and AUC of eNAMPT in serum of septic subjects, AUC 0.93 95%CI 0.86-0.99, p<0.01.

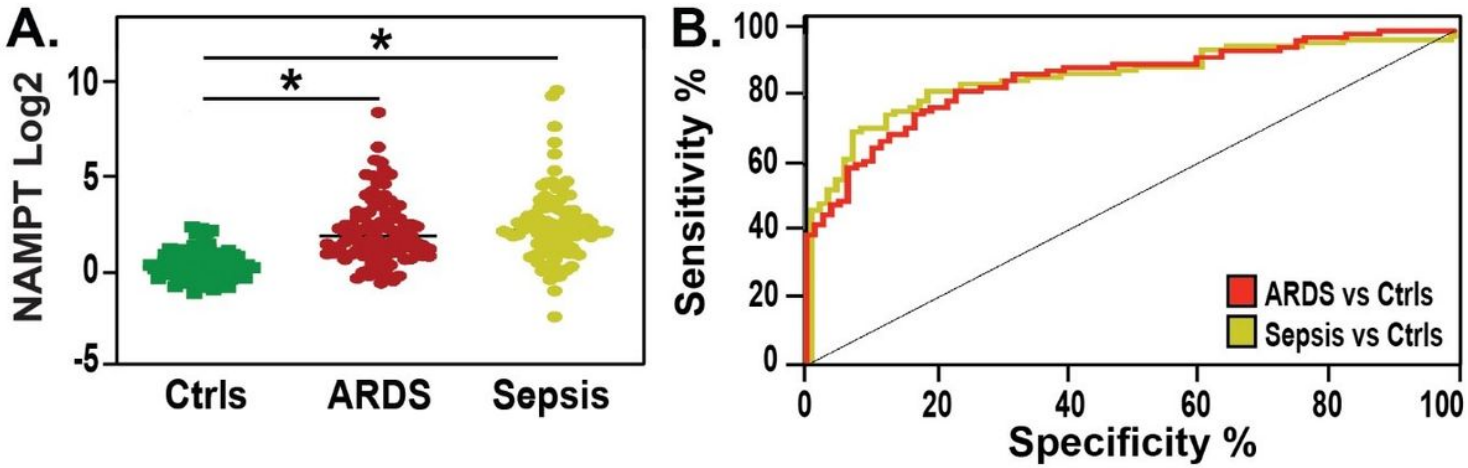

Figure 3

Validation Cohort: Circulating eNAMPT levels significantly elevated in Sepsis and ARDS compared to healthy controls. Validation cohort: Circulating eNAMPT levels significantly elevated in Sepsis and ARDS compared to healthy controls. In Panel A Y-axis represents of eNAMPT Log 2 transformed values; X-axis group comparison: healthy controls (green), ARDS (red), Sepsis (yellow). Median plasma eNAMPT values were significantly higher in ARDS and Sepsis compared to healthy controls - 3.78 ng/ml, 4.7 ng/ml and 1.2 ng/ml respectively; p<0.01. B. ROC Curve. Plasma eNAMPT levels accurately distinguished healthy controls from ARDS (red), AUC 0.85 (95% CI 0.8-0.9, p value <0.0001), and healthy controls from Sepsis (yellow), AUC 0.87 (95% IC 0.82-0.92 p value <0.0001). No significant differences between ARDS and Sepsis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Sup.T1.docx

- Sup.T2.docx
- Sup.T3.docx
- Sup.T4.docx