



OPEN Plasma glutamic acid predicts myelosuppression and mortality in septic patients using machine learning

Alessio Nunnari^{1,5}✉, Filippo Mearelli^{1,5}✉, Francesca Spagnol¹, Filippo Giorgio Di Girolamo², Nicola Fiotti^{1,3}, Erik Roman Pognuz³, Verena Zerbato⁴, Stefano Di Bella^{3,4} & Gianni Biolo^{1,3}

Myelosuppression is a common secondary manifestation of sepsis and is associated with increased morbidity and mortality. Recent evidence suggests that amino acid metabolism, particularly that of glutamic acid, may influence hematopoietic function and inflammatory responses¹. Using a machine learning technique, we aimed to evaluate whether plasma amino acid levels at hospital admission are associated with myelosuppression and 30-day mortality in septic patients, with a focus on glutamic acid as a potential early biomarker. We conducted a prospective observational study on 390 adult patients hospitalized with sepsis at the University Hospital of Trieste. Patients were stratified into two groups on the basis of peripheral blood counts: myelosuppressed and non-myelosuppressed. Plasma levels of 13 amino acids were quantified at admission via high-performance liquid chromatography. Inflammatory markers, clinical outcomes, and 30-day mortality were recorded. Predictive models for myelosuppression were developed via Random Forest and interpreted via SHapley Additive exPlanations (SHAP) analysis. While nutritional intake and longitudinal amino acid trends were not available, we accounted for major comorbidities in the analysis. Myelosuppression was identified in 152 patients (39%) and was associated with significantly higher levels of inflammatory markers (e.g., C-reactive protein (CRP) and procalcitonin), an increased incidence of bacteremia, and increased 30-day mortality (20% vs. 12%, $p = 0.03$). Patients experiencing myelosuppression had lower median glutamic acid concentrations (134 vs. 154 $\mu\text{mol/L}$, $p = 0.05$). Among the 44 clinical and metabolic features, glutamic acid was the strongest predictor according to SHAP analysis. Furthermore, glutamic acid concentrations less than 134 $\mu\text{mol/L}$ were associated with reduced survival according to Kaplan Meier analysis ($p = 0.013$). Reduced plasma glutamic acid levels at admission are independently associated with myelosuppression and worse prognosis in septic patients. Although causality cannot be established and nutritional/temporal factors were not captured, glutamic acid may represent an early biomarker for risk stratification and deserves further investigation in longitudinal and interventional studies.

Keywords Sepsis, Myelosuppression, Amino acids, Machine learning

Abbreviations

ALA	Alanine
AMI	Acute myocardial infarction
Any cancer	Sarcomas
BUN	Blood Urea Nitrogen
CHF	Chronic Heart Failure
Clinical	Clinically documented sepsis
COPD	Chronic obstructive pulmonary disease
Crea	Creatinine
CRP	C-reactive protein

¹Clinica Medica Unit, University of Trieste, Trieste, Italy. ²Pharmacy Unit, University of Trieste, Trieste, Italy. ³Clinical Department of Medical, Surgical and Health Sciences, Trieste, Italy. ⁴Infectious Diseases Unit, University of Trieste, Trieste, Italy. ⁵ These authors contributed equally: Alessio Nunnari and Filippo Mearelli ✉email: alessio.nunnari@asugi.sanita.fvg.it; filippo.mearelli@asugi.sanita.fvg.it

CVDs	Cardiovascular diseases
CYS	Cysteine
GLU	Glutamic acid
GLN	Glutamine
GLY	Glycine
High-GLU	Glutamic acid $\geq 134.5 \mu\text{mol/L}$
LACT	Lactate
LEU	Leucine
Low-GLU	Glutamic acid $< 134.5 \mu\text{mol/L}$
MET	Methionine
Micro	Microbiologically documented sepsis
Myelo	Myelosuppressed
Non-myelo	Not myelosuppressed
Only respiratory	Pneumonia without other site of infection
Only urinary	Urosepsis without other site of infection
PCA	5-Oxoproline
PAD	Peripheral Artery Disease
PCT	Procalcitonin
PHE	Phenylalanine
PRO	Proline
SER	Serine
THR	Threonine
TYR	Tyrosine

Background

At present, approximately two-thirds of patients with sepsis are admitted to internal medicine departments, with in-hospital mortality exceeding 30%². As defined by the Third International Consensus Definitions for Sepsis and Septic Shock, “Sepsis is a potentially fatal organ dysfunction caused by a dysregulated host response to infection.”³, p. 9].

In the absence of pathognomonic biomarkers for sepsis, research into both its prognosis and diagnosis has relied on the integration of clinical variables with widely used biomarkers such as CRP and procalcitonin⁴. This approach underscores the inherent difficulty of conducting a comprehensive, system-wide analysis of septic syndrome, thereby highlighting the need for deeper investigations into the specific effects of sepsis on individual organ systems. Among the various systems affected during the systemic inflammatory response to sepsis, the hematolymphopoietic system plays a pivotal role, significantly influencing both patient prognosis and the host’s capacity to mount an effective immune response. Although the diagnostic criteria remain debated, in clinical practice, myelosuppression is usually identified by the concomitant reduction of two or more hematological lineages⁵, a pragmatic definition that was adopted in our study. In the context of sepsis, the combination of leukopenia, anemia, and thrombocytopenia not only increases the risk of secondary infections, hemorrhagic events, and organ failure but also significantly constrains therapeutic options.

The pathogenesis of sepsis-associated myelosuppression is complex and multifactorial, arising from intricate interactions between the infectious agent and the host immune response. These interactions involve a wide spectrum of pathogenic mechanisms, including immune dysregulation⁶ and the hyperactivation of some proinflammatory cytokines and chemokines, such as interleukin-6 (IL-6), chemokine ligand 1 (CXCL1/KC), macrophage inflammatory protein-1 alpha (CCL3/MIP-1 α) and monocyte chemoattractant protein-1 (CCL2/MCP-1)⁷. Disruption of the bone marrow microenvironment, alongside increased peripheral consumption of blood cells, further exacerbates hematopoietic impairment⁸. Additionally, the use of myelotoxic agents, including certain antibiotics and immunosuppressants, may compound bone marrow suppression and worsen clinical outcomes⁹.

Early identification of myelosuppression in septic patients is critical for optimizing clinical management. Comprehensive hematological evaluation, coupled with tailored therapeutic strategies—including transfusion support, administration of hematopoietic growth factors, and modulation of the inflammatory response—has the potential to improve survival. However, particularly among elderly patients, therapeutic interventions remain limited, and clinical responses are often transient.

Historically, attention has also been directed toward the potential role of nutritional support in improving outcomes in sepsis patients, both through the provision of adequate caloric intake¹⁰ and the enhancement of immune competence¹¹.

Previous studies in critically ill patients suggest that alterations in amino acid metabolism may influence the inflammatory response and prognosis, but data in the context of sepsis and myelosuppression are lacking¹².

We therefore hypothesized that specific amino acids measured at hospital admission might be associated with bone marrow suppression and prognosis in septic patients. In this work, we aimed to integrate plasma metabolomic profiling with machine learning approaches to identify metabolic predictors of myelosuppression and mortality.

Building on previous evidence regarding amino acid supplementation in critically ill patients, as well as the documented effects of leucine on lymphocytogenesis, we hypothesized that specific amino acids may play a supportive role in hematopoiesis during sepsis. To test this hypothesis, we analyzed amino acid profiles in relation to systemic inflammatory markers and indicators of bone marrow suppression.

Aims and objectives

To investigate the mechanisms underlying hematopoietic alterations in septic patients, we measured the plasma concentrations of 13 amino acids—glutamic acid, alanine, cysteine, glycine, glutamine, leucine, methionine, 5-oxoproline, phenylalanine, proline, serine, tryptophan, and tyrosine—using high-performance liquid chromatography. These concentrations were subsequently compared with key clinical markers of inflammation, including body temperature, CRP, and complete blood counts (red blood cells, white blood cells, lymphocytes, and platelets).

While these clinical parameters are routinely employed as indicators of systemic inflammation, their prognostic utility in sepsis may be significantly enhanced when they are integrated with metabolic biomarkers to better assess the risk of myelosuppression. Upon hospital admission, blood samples were collected from all patients with suspected sepsis and immediately stored at $-80\text{ }^{\circ}\text{C}$. The plasma amino acid concentrations were subsequently quantified via an Agilent 6890 N gas chromatograph coupled with an Agilent 5973 mass spectrometer.

To evaluate clinical outcomes, 30-day mortality data were systematically collected and analyzed in relation to variables such as comorbidities, immune status, and infection severity. This longitudinal follow-up was crucial for characterizing the trajectory of sepsis-associated immune dysfunction.

By integrating clinical and metabolic data, this approach provides critical insights into the biological mechanisms underlying hematopoietic dysregulation in septic patients. Furthermore, this study contributes valuable information for the optimization of patient management strategies in the hospital setting, where timely and targeted interventions are essential for improving survival in critically ill populations.

Methods

Study design and ethical approval

This study is a sub-analysis of data derived from an Italian multicentre prospective cohort study on the diagnosis of sepsis entitled “Need Speed.” The research was conducted in accordance with the ethical principles of the Declaration of Helsinki and received approval from the Institutional Review Board of the University of Trieste (Report No. 39; approval date: 16 April 2012). Informed consent was obtained from all participants prior to enrollment.

Study population

Between 1 January 2013 and 1 June 2015, a total of 443 patients admitted to the Clinica Medica (Internal Medicine Unit) of the University Hospital of Trieste with suspected sepsis were enrolled. All participants were monitored throughout their hospital stay in the internal medicine ward. Inclusion criteria were: (1) age over 18 years, (2) ability to provide informed consent, (3) blood culture collection, (4) initiation of empirical antibiotic therapy, and (5) a baseline Sequential Organ Failure Assessment (SOFA) score ≥ 2 .

Data collection and clinical parameters

For each patient, initial clinical characteristics were recorded using a dedicated case report form. On admission, relevant clinical parameters related to inflammation (body temperature, white blood cell count, and serum C-reactive protein levels) and immune status (lymphocyte count) were collected. Acute liver failure was defined as an international normalized ratio ≥ 1.5 accompanied by signs of hepatic encephalopathy. Chronic immunodeficiency conditions that could affect lymphocyte count—including HIV infection, lymphoma, leukemia, myeloma, and long-term corticosteroid or immunosuppressive therapy—were also documented.

Classification of infections

Infections were categorized as either clinically documented (sepsis without microbiological confirmation, indicated by negative Gram stain or culture) or microbiologically documented (confirmed by positive microbiological testing). Microbiologically documented infections were further classified into bacterial and non-bacterial groups based on the pathogen identified in sterile biological samples or other diagnostic investigations.

Bacterial and Non-bacterial sepsis

Cases of bacterial sepsis were subdivided into polymicrobial sepsis, involving multiple bacterial pathogens, and monomicrobial sepsis, involving a single pathogen. All infections caused by viruses, protozoa, or fungi were classified as non-bacterial sepsis. A bloodstream infection was defined as the presence of a viable pathogen in the bloodstream; however, a single positive blood culture for organisms commonly considered skin contaminants—such as coagulase-negative staphylococci, corynebacteria, or alpha-hemolytic streptococci—was classified as contamination rather than true infection.

Participants

From the initial population of 443 individuals, we excluded all patients with leukemia, lymphoma, or multiple myeloma or those who were receiving steroid and/or immunosuppressive therapy (53 subjects). The remaining population of 390 individuals was then divided into two groups (Fig. 1):

1. **Patients with myelosuppression (152 patients):** This group included all patients with a reduced peripheral blood cell count affecting at least two cell lines. The cutoff values considered were as follows:
 - Lymphocytes $< 1000/\text{mL}$.
 - White blood cells $< 4000/\text{mL}$.
 - Red blood cells $< 4.5 \times 10^6/\text{mL}$.

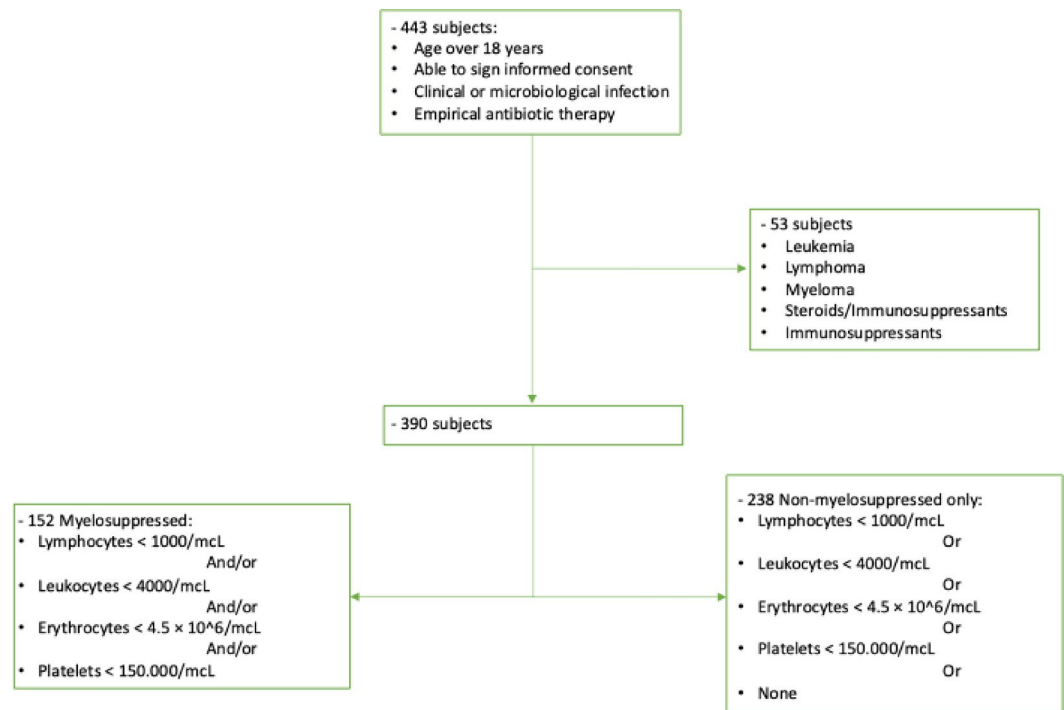


Fig. 1. Population CONSORT.

- Platelets < 150,000/mcL.

2. **Patients with adequate hematopoietic response (238 patients):** This group included patients who did not meet the criteria listed above or had only one isolated abnormal parameter.

Statistical analysis

All the statistical analyses were performed via SPSS 26.0 and R Studio 2024.12.0 + 467. Missing data were eliminated using Listwise deletion for descriptive statistics to ensure the greatest homogeneity between groups in the demographic and outcome studies, whereas all other analyses were performed using mean imputation. Continuous variables are expressed as medians and interquartile ranges, whereas categorical variables are reported as absolute numbers and intragroup percentages. Correlations were assessed using Spearman's analysis for categorical variables and Pearson's analysis for continuous variables.

The ability of variables to predict bone marrow suppression was assessed via Random Forest, with the number of variables per split determined through Tune Random Forest for optimal number of variables (mtry) and node size parameters (randomForestSRC package). Internal validation was performed by partitioning the cohort, with 70% used for model training and the remaining 30% reserved for validation. The predictive value of each variable and its importance within the random forest model were evaluated via SHapley Additive exPlanations (SHAP).

Mortality was analyzed via Kaplan–Meier survival curves and the log-rank test. Cut off was identified via Youden index.

Results

Our population consisted of 390 patients with clinically documented sepsis, with onset from the lower respiratory tract in 57% of patients. Among them, 53% were men with a median age of 82 years, and 48% had heart disease. At the time of admission, patients had a median white blood cell count of 13,000/ μ L, CRP level of 185 mg/L, and a median blood urea nitrogen level of 46 mg/dL. At 30 days, we recorded a mortality rate of 15% (Table 1).

A total of 152 patients were identified as myelosuppressed, exhibiting higher procalcitonin levels (0.82 vs. 0.39 ng/mL) and a higher rate of bacteremia (26% vs. 14%) compared to the rest of the population. Additionally, the myelosuppressed group had a higher mortality rate (20% vs. 12%) and lower levels of plasma glutamic acid (134 vs. 154 μ mol/L) and leucine (116 vs. 120 μ mol/L) (Table 2).

Compared with the non-myelosuppressed group, in the myelosuppressed group, we observed an inverse correlation between CRP and lymphocytes, leucine did not show a statistically significant correlation with glutamic acid ($\rho = 0.082$, $p = 0.318$). (Fig. 2).

In the SHAP analysis of the Random Forest model used to classify myelosuppressed patients, 5-oxoproline, leucine, lactate, and glutamic acid showed the highest Shapley values in predicting an adequate hematopoietic response (Fig. 3).

Population	N° 390
Female	182 (47)
Median age (years)	82 (75-88)
Comorbidity	
Myocardial Infarction	117 (30)
Heart Failure	120 (31)
Peripheral Artery Disease	72 (19)
Cardiovascular diseases	186 (48)
Chronic Obstructive Pulmonary Disease	119 (31)
Connective Tissue Disease	7 (2)
Diabetes	128 (33)
Home Antibiotic Therapy	136 (35)
Solid Cancer	26 (7)
Cancer of unknown primary	34 (9)
Chronic Liver Disease	27 (7)
Chronic Kidney Disease	94 (24)
Laboratory Variables	
Median Platelets (10 ³ cell/ mL)	234 (169-296)
Median White blood cells (10 ³ cell/ mL)	13 (9.3-17)
Median C-reactive protein (mg/L)	185(100-279)
Median Lymphocytes (10 ³ cell/ mL)	0.87 (0.56-1.27)
Median Procalcitonin (ng/mL)	0.47 (0.18-3.4)
Median body temperature (C°)	37(36-38)
Median BUN (mg/dL)	46 (32-72)
Median Lactate (mg/dL)	14 (10-19)
Median Creatinine (mg/dL)	1.10 (0.86-1.72)
Amino acids	
Median ALA (μmol/L)	253 (198-321)
Median GLY (μmol/L)	134 (106-164)
Median LEU (μmol/L)	117 (93-141)
Median PHE (μmol/L)	81 (70-98)
Median GLN (μmol/L)	415 (325-516)
Median GLU (μmol/L)	148 (104-211)
Median PCA (μmol/L)	106 (78-135)
Median TYR (μmol/L)	55 (46-69)
Median THR (μmol/L)	70 (54-85)
Median SER (μmol/L)	69 (56-85)
Median PRO (μmol/L)	141 (111-183)
Median CYS (μmol/L)	69 (40-105)
Median MET (μmol/L)	17 (12-23)
Source	
Multiple	28 (7)
Single:	
-LRTI (Lower Respiratory Tract Infection)	224 (57)
-Urinary	57 (15)
-Bones and Skin	29 (7)
-Abdomen	43 (11)
-Other	9 (3)
Clinically documented	226 (58)
Microbiologically documented	164 (42)
Polymicrobial	34 (8)
Monomicrobial	130 (33)
Etiology	
-Gram +	69 (18)
-Gram -	97 (25)
-Non-bacterial	22 (6)
-Atypical	2 (0.5)
Continued	

Population	N° 390
Bloodstream infections	73 (19)
Myelosuppressed	152 (39)
Mortality 30 days	60 (15)

Table 1. Demographic table. The values in parentheses are percentages for categorical variables and interquartile ranges for continuous variables. All abbreviations and acronyms are listed at the end of the text.

The five most important variables according to SHAP were in order: glutamic acid, serine, plasma urea nitrogen, alanine, and non-bacterial sepsis (Fig. 4).

According to the Kaplan–Meier analyses, patients with myelosuppression and those with plasma glutamic acid concentrations below 134 $\mu\text{mol/L}$, cut-off identified via Youden index, presented increased mortality, with log-rank test p values of 0.03 and 0.013, respectively (Figs. 5 and 6).

When comparing Kaplan–Meier curves among myelosuppressed patients and those with glutamic acid concentrations below the cut-off, mortality in the non-myelosuppressed group did not vary significantly with glutamic acid levels ($p=0.262$), whereas the difference was significant in the myelosuppressed group ($p=0.035$), with an overall p -value of 0.0086 (Fig. 7).

Discussion

In this study, we identified glutamic acid as the amino acid most strongly associated with the distinction between myelosuppressed and non-myelosuppressed septic patients. Among the 44 clinical and metabolic variables analyzed, glutamic acid consistently emerged as the feature with the highest predictive value according to SHAP analysis, underscoring its potential relevance in the pathophysiology of sepsis-associated bone marrow dysfunction.

Interest in the relationship between amino acids and hematopoietic function has longstanding roots, dating back to early *in vitro* studies in the 1950s that examined leucine incorporation into normal and leukemic white cells¹³. Subsequently, a series of investigations were launched to evaluate the effects of amino acids on hematopoiesis, oxidative stress, and mortality in critically ill patients^{14,15}.

In the late 1980s, the first murine animal model studies demonstrated that the cytokine cascade could alter bone marrow responses during sepsis¹⁶, leading to permanent modifications in the gene expression of hematopoietic stem and progenitor cells (HSPCs)¹⁷. These changes promote the production of myeloid-derived suppressor cells (MDSCs)¹⁸, which inhibit the proliferation of all myeloid lineages and result in the release of immature myeloid elements into the circulation.

An observational study revealed that although these cells are present from birth, they are overexpressed in certain individuals, who, upon severe infectious stimulation, exhibit a cellular response characterized by a high number of circulating immature granulocytes, reduced immune reactivity, and blunted lymphocytic activation¹⁹.

To date, the pathogenesis of this altered cellular response remains unclear. The observation of elevated levels of interleukin-6 and free radicals led the authors to hypothesize that a specific population is predisposed to oxidative stress due to excessive production of type-I interferons and other proinflammatory cytokines (IL-6, IL-12, and TNF- α), which in turn stimulate the bone marrow proliferation of HSPCs^{20–21}.

On the basis of these findings, we hypothesized that the presence of myelosuppression—defined as the reduction of at least two hematological lineages in the peripheral blood—could be associated with alterations in the amino acid profile.

Glutamic acid is one of the main components of glutathione, and the conversion of gamma-glutamylcysteine to glutathione results in the release of a molecule of 5-oxoproline (Fig. 8).

Glutamic acid and 5-oxoproline are interconvertible under basal conditions; therefore, a decrease in the former should lead to a reduction in the latter (Fig. 9).

As shown in Fig. 1; Table 2, the myelosuppressed population exhibited an inverse correlation between lymphocytes and CRP, along with lower median concentrations of glutamic acid (134 vs. 154 $\mu\text{mol/L}$, $p=0.05$), despite identical levels of 5-oxoproline. Although leucine participates in glutamic acid turnover through transamination reactions and was identified as a relevant feature in both classical statistics and machine learning, its levels did not correlate with glutamic acid in myelosuppressed patients. Combined with the observation of higher procalcitonin levels in this group, these findings indicate that the inflammatory response may disrupt amino acid homeostasis in ways not solely dependent on precursor–product relationships.

Our findings align with experimental evidence showing that alterations in glutamate metabolism may influence hematopoietic stem cell activity and immune responses.

Additional support for a link between glutamate metabolism and hematopoietic activity comes from recent work in multiple myeloma patients, in whom higher glutamic acid concentrations were directly correlated with the proliferation of osteoclasts and CD14⁺ bone marrow cells. Inhibition of EAAT1 (Excitatory Amino Acid Transporter 1), a component of one glutamate-responsive pathway, reduced the proliferation of these cells²². Although these findings suggest functional interactions between glutamate signaling and hematopoietic differentiation, our observational design does not permit causal inference. Given the diverse roles of glutamic acid in neurotransmission, energy metabolism, and ammonia detoxification, the associations we observed may reflect broader metabolic alterations rather than a specific mechanistic pathway.

A notable strength of our study lies in its focus on amino acid concentrations at hospital admission—a time point that reflects real-world clinical decision-making. Early risk stratification is essential in sepsis, and,

Population	Myelosuppressed	Non-myelosuppressed	p ^a
	N° 152	N° 238	
Female	74 (41)	108 (45)	NS ^b
Median age (years)	82 (76-89)	82 (75-88)	NS
Comorbidity			
Myocardial Infarction	39 (26)	78 (33)	NS
Heart Failure	48 (18)	72 (18)	NS
Peripheral Artery Disease	28 (39)	44 (61)	NS
Cardiovascular diseases	70 (46)	116 (48)	NS
Chronic Obstructive Pulmonary Disease	43 (28)	76 (33)	NS
Connective Tissue Disease	1 (1)	6 (3)	NS
Diabetes	49 (32)	79 (33)	NS
Home Antibiotic Therapy	48 (32)	88 (37)	NS
Solid Cancer	14 (9)	12 (5)	NS
Cancer of unknown primary	17 (11)	17 (7)	NS
Chronic Liver Disease	13 (9)	14 (6)	NS
Chronic Kidney Disease	44 (29)	50 (21)	NS
Laboratory Variables			
Median C-reactive protein (mg/L)	130 (44-195)	90 (31-182)	NS
Median Procalcitonin (ng/mL)	0.82 (0.20-6)	0.39 (0.16-1.9)	0.01
Median body temperature (C°)	37.5 (36.5-38)	37.3 (36.5-38)	NS
Median BUN (mg/dL)	47 (33-79)	45 (32-69)	NS
Median Lactate (mg/dL)	14 (11-20)	14 (10-18)	NS
Median Creatinine (mg/dL)	1.21 (0.87-1.83)	1.08 (0.85-1.66)	NS
Amino acids			
Median ALA (μmol/L)	242(193-304)	261 (203-325)	NS
Median GLY (μmol/L)	128(106-168)	137(109-164)	NS
Median LEU (μmol/L)	116(91-137)	120(98-149)	0.03
Median PHE (μmol/L)	84(68-105)	81(71-96)	NS
Median GLU (μmol/L)	134(92-212)	154(109-211)	0.05
Median GLN (μmol/L)	425(321-521)	409(329-514)	NS
Median PCA (μmol/L)	105(72-138)	106 (80-133)	NS
Median TYR (μmol/L)	55 (46-70)	55 (46-67)	NS
Median THR (μmol/L)	69 (52-84)	70 (56-87)	NS
Median SER (μmol/L)	67 (55-82)	70 (56-87)	NS
Median PRO (μmol/L)	140(106-181)	141(114-184)	NS
Median CYS (μmol/L)	75 (41-107)	64(40-103)	NS
Median MET (μmol/L)	16(12-22)	17(12-23)	NS
Source			
Multiple	12 (8)	16 (7)	NS
Single:			
-LRTI (Lower Respiratory Tract Infection)	83 (55)	141 (60)	NS
-Urinary	21 (14)	36 (15)	NS
-Bones and Skin	15 (10)	14 (6)	NS
-Abdomen	17 (11)	26 (11)	NS
Clinically documented	82 (54)	144 (61)	NS
Microbiologically	70 (46)	94 (40)	NS
Etiology			
-Gram +	30 (20)	39 (16)	NS
Continued			

Population	Myelosuppressed	Non-myelosuppressed	<i>p</i> ^a
	N° 152	N° 238	
-Gram -	37 (24)	60 (25)	NS
-Non-bacterial	13 (9)	9 (4)	NS
-Atypical	0 (0)	2 (1)	NS
Bloodstream infections	40 (26)	33 (14)	0.02
30 days non survival	31 (20)	29 (12)	0.03

Table 2. Myelosuppressed patients vs. non-myelosuppressed patients. The values in parentheses are percentages for categorical variables and interquartile ranges for continuous variables. a) Two-tailed statistical significance; b) not significant. All abbreviations and acronyms are listed at the end of the text.

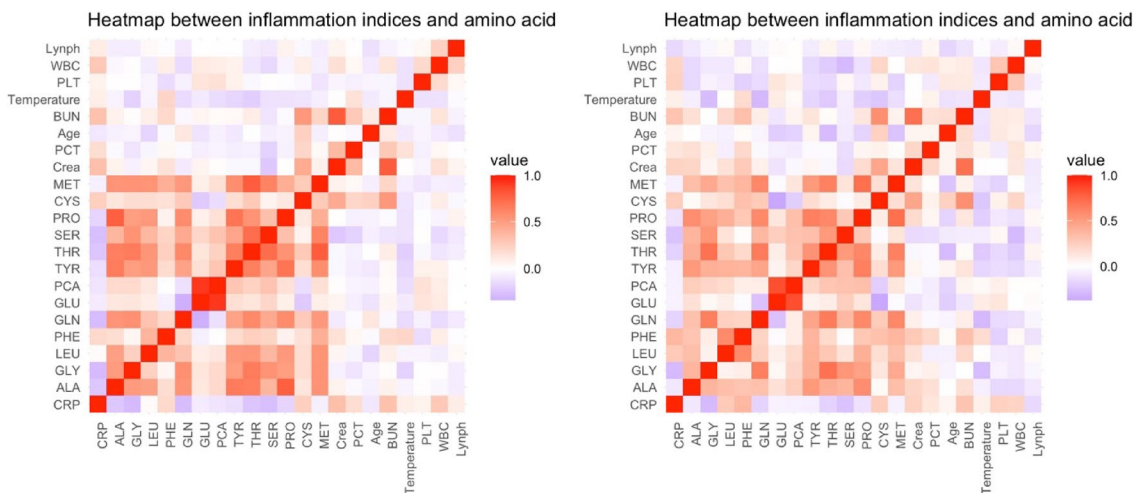


Fig. 2. Left figure: Heatmap of non-myelosuppressed patients. Right figure: Heatmap of myelosuppressed patients. Squares shaded in red indicate a positive correlation coefficient, whereas those shaded in purple indicate a negative correlation coefficient. The heatmaps were generated using R Studio version 2024.12.0 + 467 (<https://www.r-project.org>), with the corrrplot package version 0.95 (<https://cran.r-project.org/package=corrrplot>) and ggplot2 version 4.0.1 (<https://ggplot2.tidyverse.org>). All abbreviations and acronyms are listed at the end of the text.

to the best of our knowledge, no prior study has examined the relationship between amino acid profiles and myelosuppression in this population. Our findings therefore provide an initial framework for integrating metabolic biomarkers into the hematologic evaluation of septic patients.

Ultimately, although reduced plasma glutamic acid cannot yet be regarded as a causal factor in sepsis-related myelosuppression, the observation of lower glutamic acid concentrations in myelosuppressed patients, together with the association of higher mortality with comparable blood levels, in contrast to trends reported in previous studies¹² and in line with more recent studies that have focused specifically on this amino acid's ability to promote cellular differentiation²², suggests that this pattern represents a distinctive feature of high mortality myelosuppressed patients. We therefore hypothesize that glutamic acid may serve as an early biomarker for identifying patients at high risk of mortality. This insight advances our understanding of the metabolic-immune interplay in sepsis and underscores the need for further translational research.

Our study has several limitations, including a relatively small sample size, lack of randomization, and reliance on a single plasma amino acid measurement. In our cohort, major comorbidities were systematically recorded and accounted for in the analysis; however, we did not capture detailed nutritional intake or serial amino acid measurements. These limitations prevent us from determining whether reduced glutamic acid levels reflect dietary deficiency, increased metabolic consumption, or impaired synthesis. Notably, the cutoff for glutamic acid corresponds to the value independently identified for myelosuppression.

Although the internal validation process used in our machine learning model may have mitigated concerns related to sample size and randomization, there remains a clear need to validate our findings in a randomized clinical trial, with amino acid levels measured at multiple time points, collected at standardized intervals, and complemented by information on each patient's metabolic and nutritional status.

Conclusion

In this prospective cohort of septic patients, reduced plasma glutamic acid levels at hospital admission were significantly associated with the presence of myelosuppression and increased 30-day mortality.

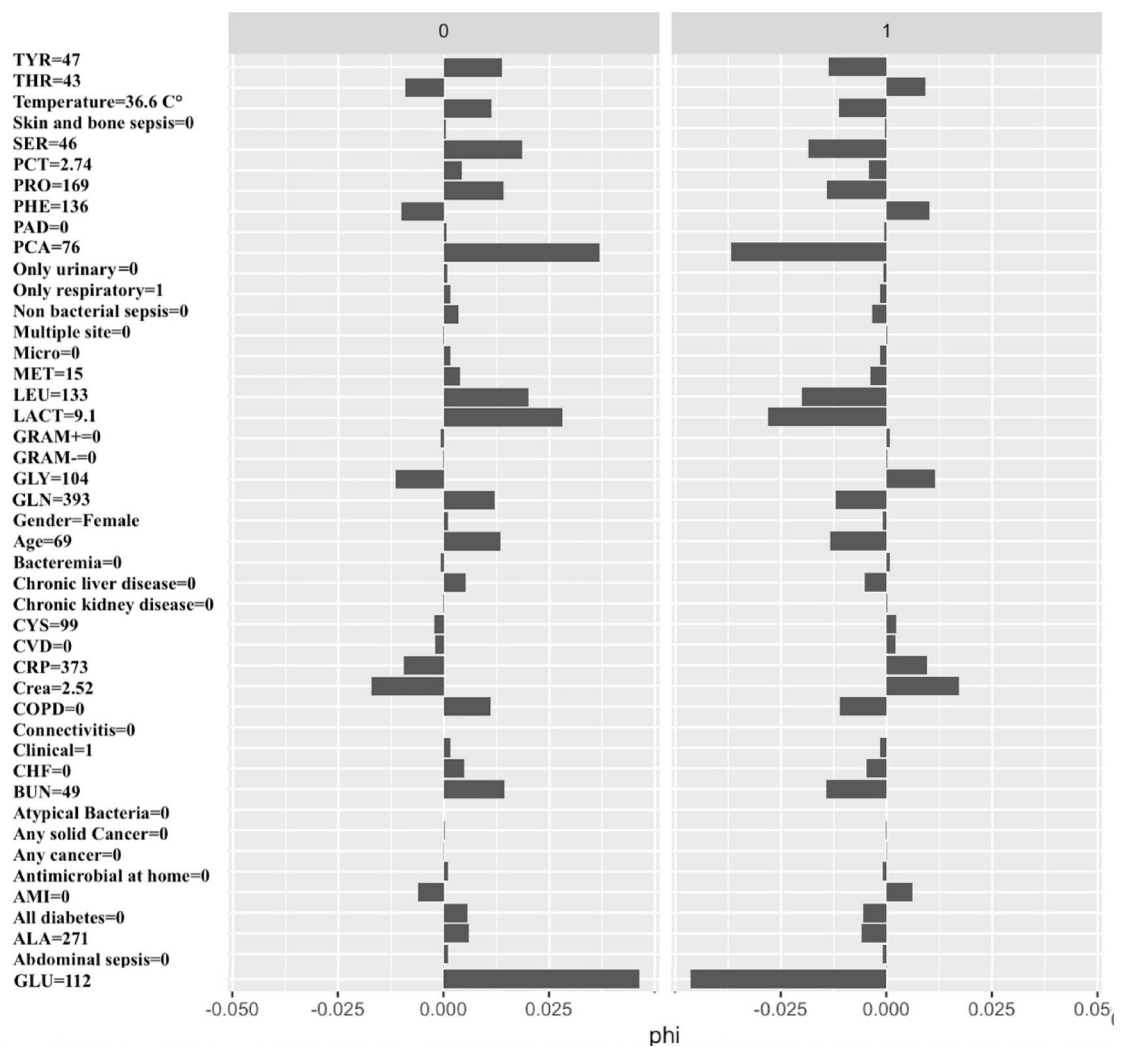


Fig. 3. SHAP of Random Forest. 0 indicates the absence of myelosuppression, whereas 1 indicates its presence. The values shown next to each variable correspond to those for which the reported Phi coefficient was calculated, as indicated in the graph. Phi represents the extent to which a given variable contributes to the deviation of the model's prediction from its baseline (mean) value. All abbreviations and acronyms are listed at the end of the text.

Our findings suggest that glutamic acid may serve as an early biomarker for risk stratification in sepsis, complementing conventional clinical and laboratory parameters. However, owing to the lack of nutritional assessment, the absence of serial amino acid measurements, and the multifactorial pathogenesis of sepsis, causality cannot be established.

Future studies should incorporate longitudinal sampling and detailed nutritional data to clarify whether low glutamic acid reflects increased metabolic demand, impaired synthesis, or dietary deficiency. Interventional trials exploring the modulation of glutamic acid metabolism may also help determine its therapeutic potential in myelosuppressed septic patient.

In summary, while preliminary, our findings highlight glutamic acid as a promising early marker of bone marrow suppression and prognosis in sepsis, warranting validation in larger and multicenter cohorts. Moreover, the possibility that this amino acid influences hematopoietic responses raises the need for further translational research aimed at understanding its role within the complex metabolic-immune interactions that characterize sepsis.

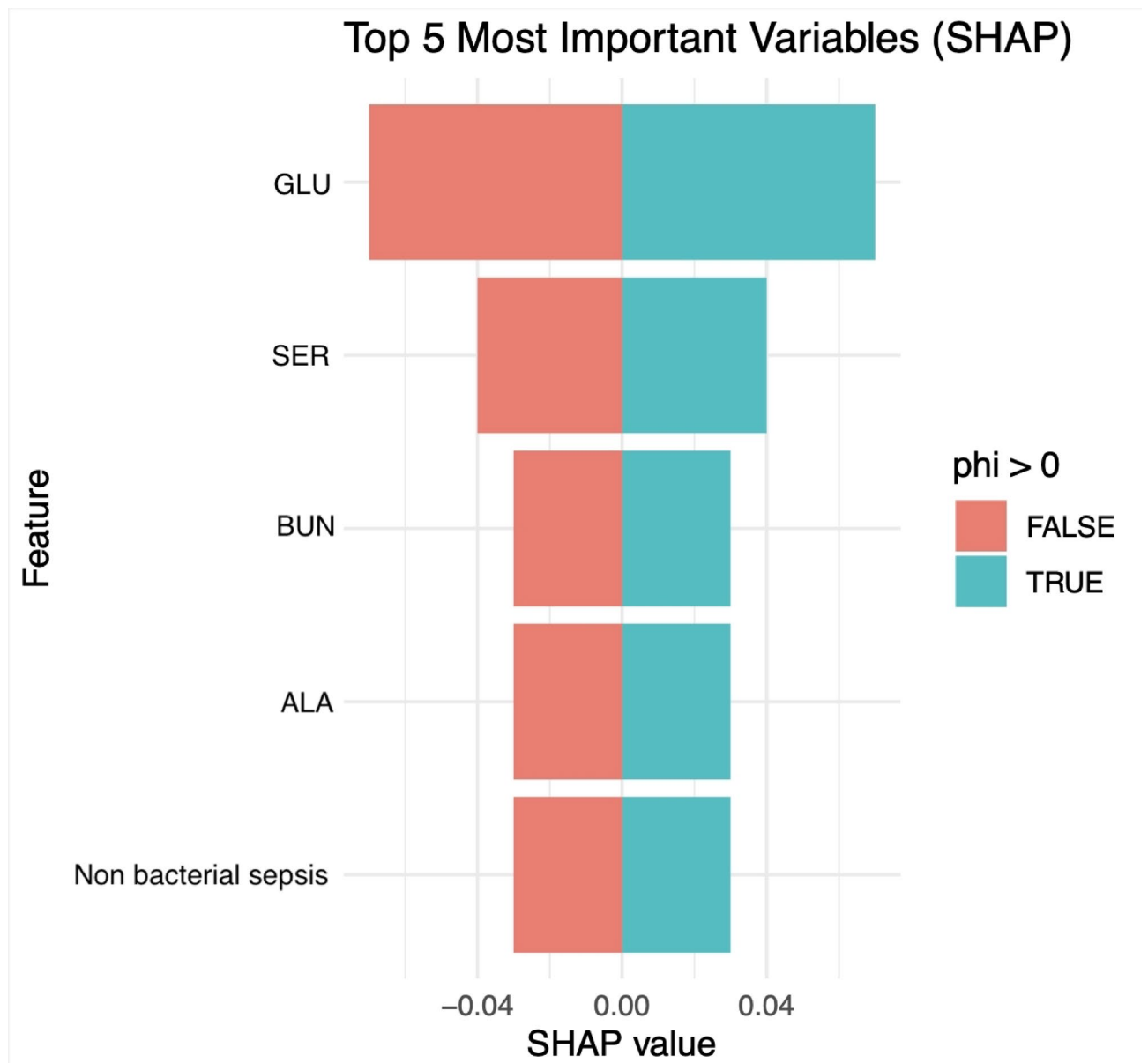


Fig. 4. SHAP importance. Phi represents the extent to which a given variable contributes to the deviation of the model's prediction from its baseline (mean) value. The SHAP value is a numerical value that quantifies the extent to which a specific feature has influenced (positively or negatively) a particular model prediction. All abbreviations and acronyms are listed at the end of the text.

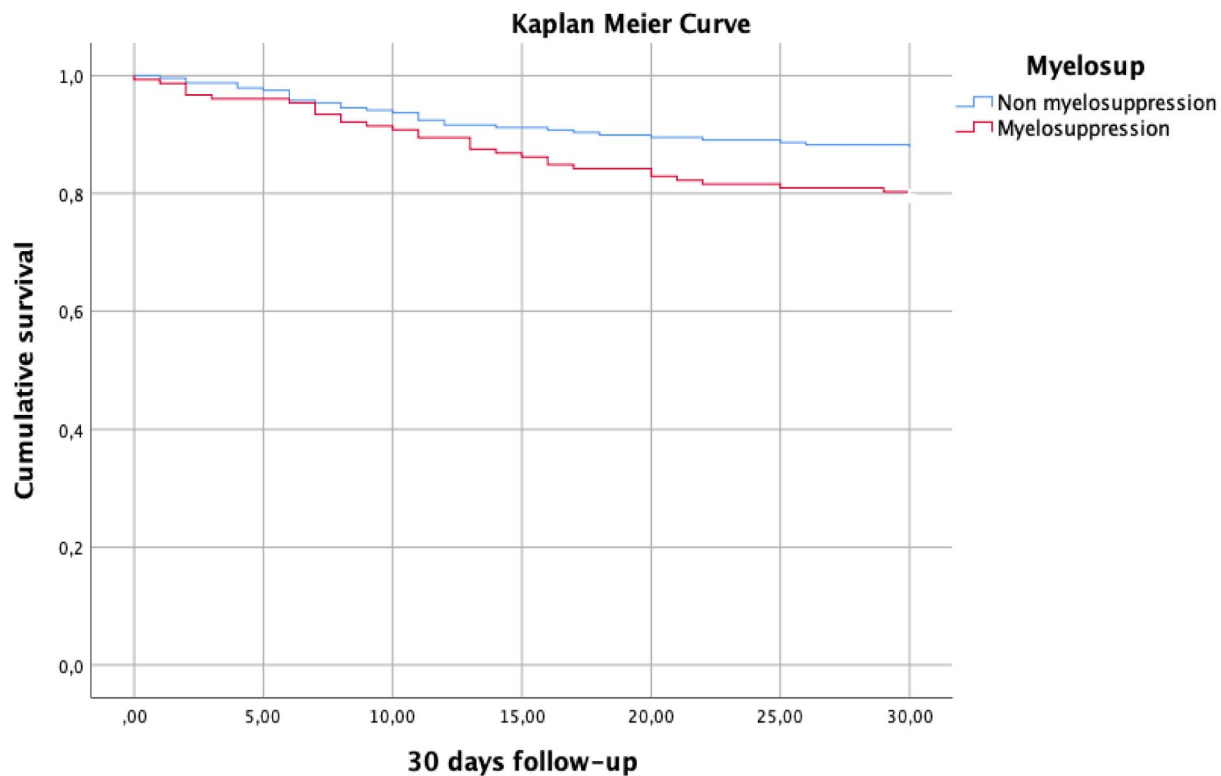


Fig. 5. Kaplan–Meier survival curves for mortality between myelosuppressed and non-myelosuppressed patients ($p=0.03$).

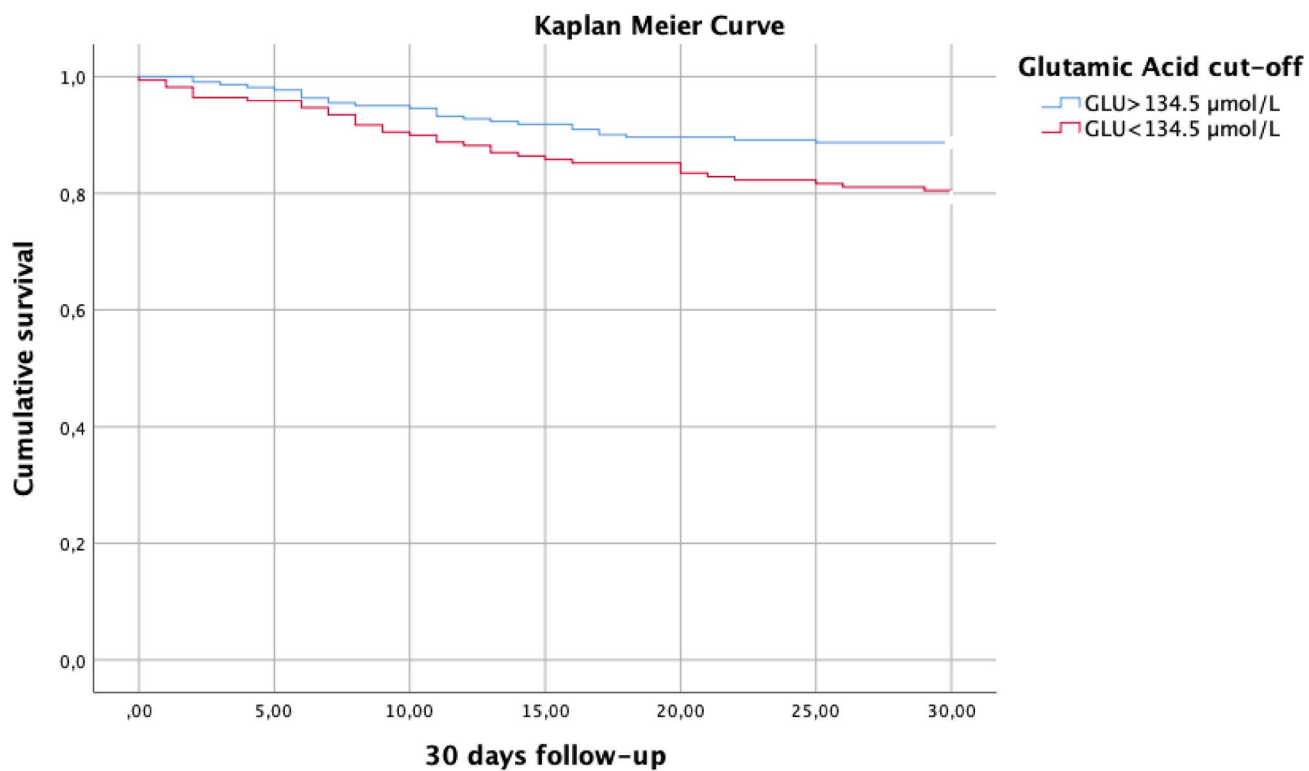


Fig. 6. Kaplan–Meier survival curves for mortality stratified by glutamic acid levels below and above 134.5 µmol/L ($p=0.013$).

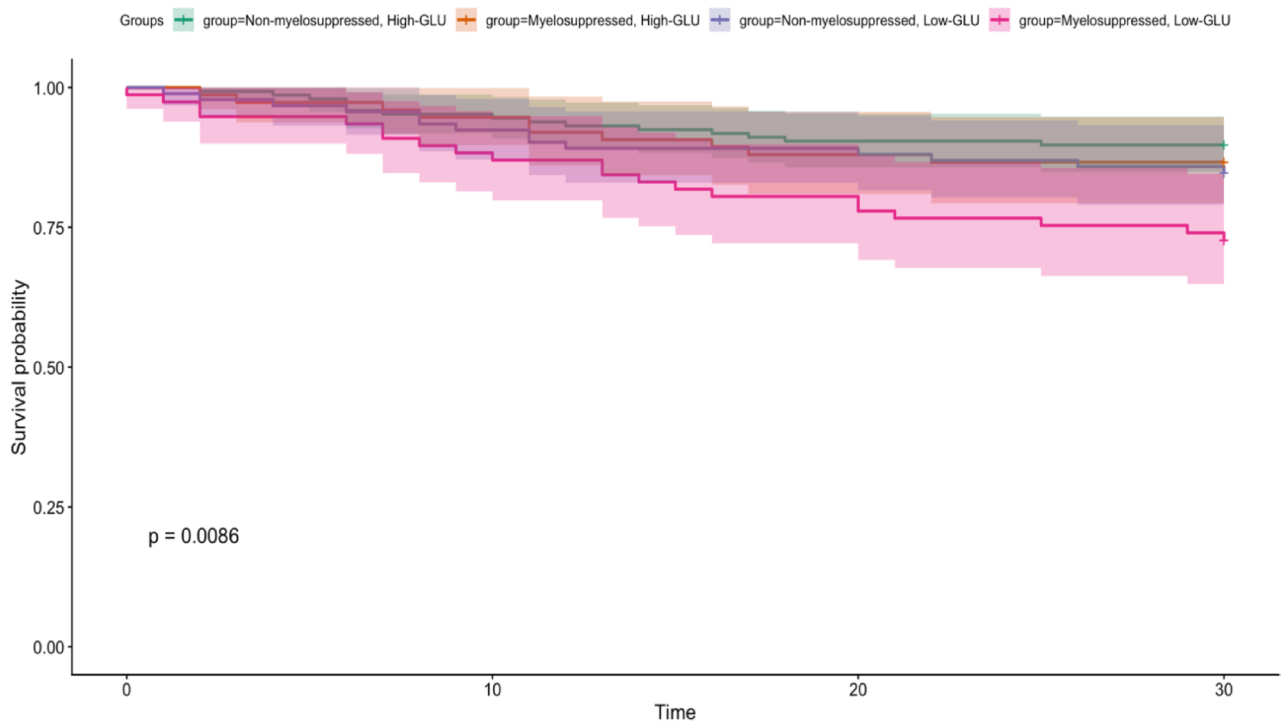


Fig. 7. Comparison of Kaplan–Meier curves among myelosuppressed and non-myelosuppressed patients, stratified by glutamic acid concentrations below and above the cut-off. All abbreviations and acronyms are listed at the end of the text.

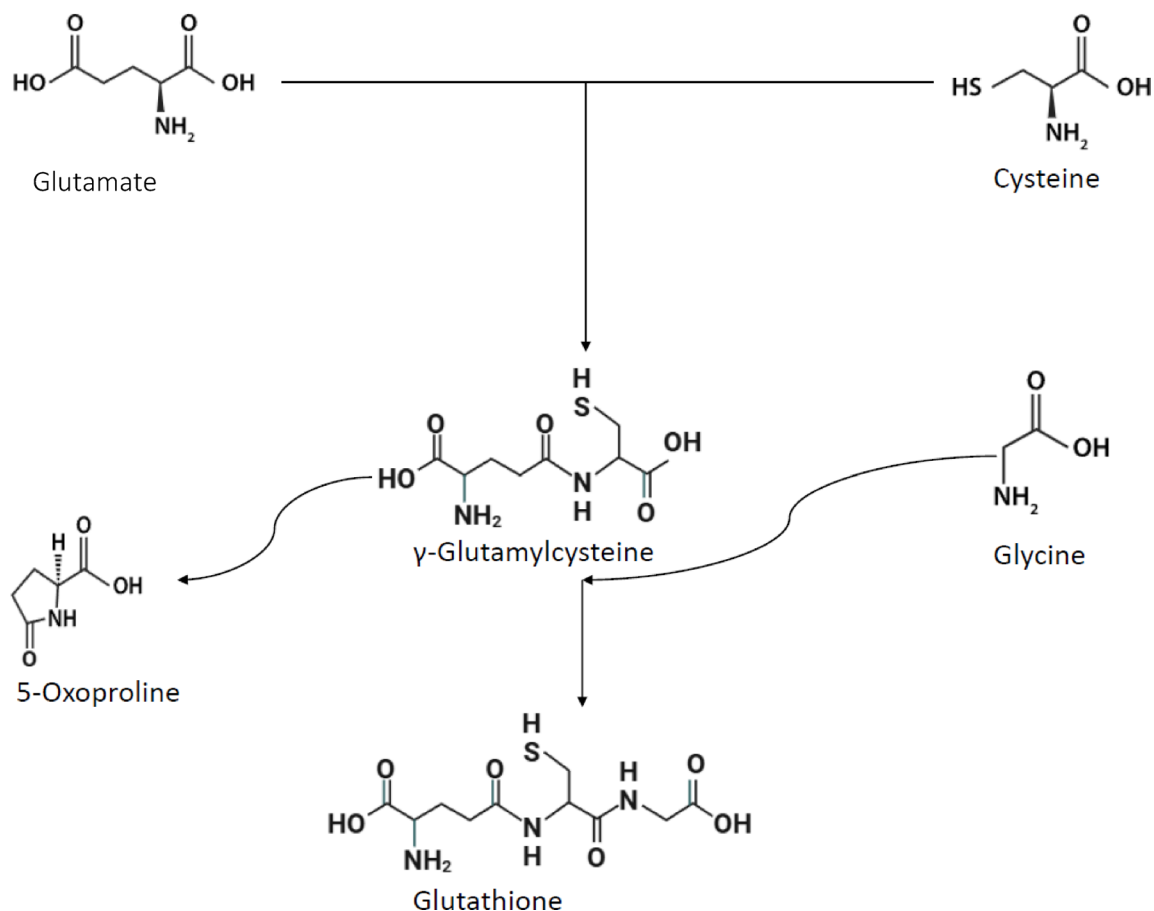


Fig. 8. Glutathione cycle. Created with BioRender.com.

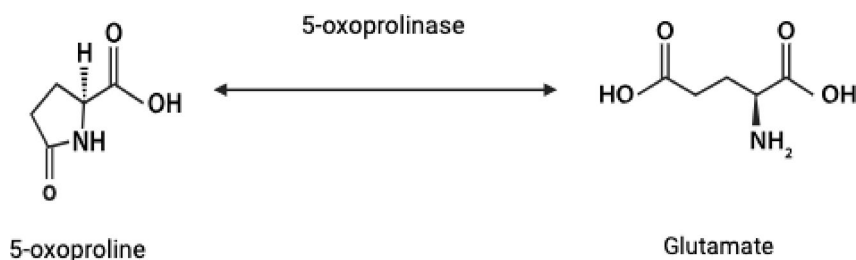


Fig. 9. Conversion of 5-oxoproline into glutamic acid by the enzyme 5-oxoprolinase. Created with BioRender.com.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Acknowledgements

Mariella Sturma, Clinica Medica, Strada di Fiume n° 447, Trieste, Italy. Giuseppe Ragusa, Clinica Medica, Strada di Fiume n° 447, Trieste, Italy.

Author contributions

A.N., F.M.: conceptualization, writing—original draft, N.F.: formal analysis, writing—review and editing, methodology, supervision. F.S.: investigation, data curation, writing—original draft. V.Z.: formal analysis, writing—review and editing; S.D.B.: formal analysis, writing—review and editing. E.R.P.: formal analysis, writing—review and editing. F.G.D.G.: methodology, writing—review and editing. G.B.: supervision, validation, writing—review and editing. All the authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

The study was carried out in accordance with the ethical standards of the Declaration of Helsinki. The study was approved by the Institutional Review Board of the University of Trieste (Report n°39; approval date 16 April 2012).

Informed consent

Informed consent was obtained from all the subjects involved in the study. Written informed consent was obtained from the patients to publish this paper. All patients received specific information about the potential use of their personal data for research purposes and were given the opportunity to refuse it.

Additional information

Correspondence and requests for materials should be addressed to A.N. or F.M.

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