Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Point Of Care Evaluation of Fibrinolysis In Sepsis: The POCEFIS study

Claudia Bonetti

bonetti.clo@gmail.com

Ospedale Papa Giovanni XXIII: Aziende Socio Sanitarie Territoriale Papa Giovanni XXIII
https://orcid.org/0009-0003-2049-7261

Ivan Silvestri

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico

Alessio Caccioppola

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico https://orcid.org/0000-0003-1936-3111

Andrea Meli

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico

Gabriele Zimei

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico

Fabiana Madotto

University of Milan: Universita degli Studi di Milano

Cristina Novembrino

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico

Massimo Boscolo Anzoletti

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico

Giacomo Grasselli

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico

Mauro Panigada

Fondazione IRCCS Policlinico San Matteo

Research Article

Keywords:

Posted Date: November 10th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11444559/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Point Of Care Evaluation of Fibrinolysis In Sepsis: The POCEFIS study

Claudia Bonetti[1,2], Ivan Silvestri[1,3], Alessio Caccioppola[3], Andrea Meli[3], Gabriele Zimei[1,3], Fabiana Madotto[1], Cristina Novembrino[6], Massimo Boscolo Anzoletti[6], Giacomo Grasselli[1,3], Mauro Panigada[4,5]

[1] Department of Pathophysiology and Transplantation, University of Milan, Milan (MI), Italy
[2] Emergency and Intensive Care Department, ASST Papa Giovanni XXIII, Bergamo, Italy
[3] Department of Anesthesiology, Critical Care and Emergency, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan (MI), Italy
[4] Department of Clinical-Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy
[5] General Intensive Care Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[6] A. Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Corresponding author:
Dr. Alessio Caccioppola
Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico,
Via F. Sforza 35, 20122 Milan, Italy
Tel.: +39 0255033258 E-mail: alessio.caccioppola@policlinico.mi.it

ORCID ID:
Claudia Bonetti: 0009-0003-2049-7261
Ivan Silvestri: 0000-0002-7650-5326
Alessio Caccioppola: 0000-0003-1936-3111
Andrea Meli: 0000-0002-5470-2978
Gabriele Zimei: 0009-0007-7377-8621
Fabiana Madotto: 0000-0002-8829-9160
Cristina Novembrino: 0000-0003-1450-9935
Massimo Boscolo Anzoletti 0009-0002-5536-5245
Mauro Panigada: 0000-0003-4841-9794
Giacomo Grasselli: 0000-0002-1735-1400

Abstract

Purpose: Sepsis-induced coagulopathy (SIC) involves a complex imbalance between coagulation activation and fibrinolysis inhibition, contributing to microvascular thrombosis and organ dysfunction. Conventional coagulation tests inadequately capture functional fibrinolytic alterations. The POCEFIS study used the ClotPro® tissue plasminogen activator (TPA) test to characterize fibrinolysis in septic patients and evaluate its clinical relevance and temporal evolution during the early phase of sepsis.

Methods: In a cohort of septic intensive care unit (ICU) patients, fibrinolysis was assessed using the ClotPro® TPA-test at enrollment and on day 1, 2, 3 and 7 after enrollment. Specific plasma biomarkers (PAI-1, plasminogen, protein C, and protein S) were analyzed at enrollment and on day 2 and 7. Septic patients were classified according to temporal evolution of fibrinolytic status during the first three days of observation (normal versus impaired fibrinolysis).

Results: At enrollment, impaired fibrinolysis was present in 38% of septic patients. However, considering the first three ICU days, 66% of patients exhibited fibrinolysis impairment. Impaired fibrinolysis was associated with higher lactate (4.2 mmol/L vs 2.9 mmol/L, p=0.0482), INR (1.5 vs 1.2, p=0.0156), and SOFA score (10.9 vs. 9.4, p=0.0282) at enrollment. Patients with impaired fibrinolysis showed persistently higher C-reactive protein and D-dimer values during the first week of observation (linear mixed-effects model, group effect p=0.0047 and p=0.0201).

Conclusions: Fibrinolysis impairment is frequent, dynamic, and clinically relevant in sepsis. The ClotPro® TPA-test provides a global point-of-care evaluation of fibrinolysis. Serial viscoelastic assessment may improve risk stratification and support the development of individualized therapeutic strategies targeting SIC.

Take-Home message

Fibrinolysis impairment is common in septic patients, highly dynamic, and inadequately detected by conventional coagulation tests. The fibrinolysis-enhanced viscoelastic test ClotPro® TPA identified fibrinolysis abnormalities in up to two-thirds of septic patients during the first 72 hours of ICU stay. Its repeatable point-of-care application allows real-time monitoring of the evolving coagulation–fibrinolysis balance in septic patients.

Key words

Sepsis-induced coagulopathy

Fibrinolysis

ClotPro® TPA

Viscoelastic testing

Point-of-care

Abbreviations

ASA – American Society of Anesthesiologists

AT – Antithrombin

BMI – Body Mass Index

CRP – C-Reactive Protein

CT – Clotting Time

DIC – Disseminated Intravascular Coagulation

ECMO – Extracorporeal Membrane Oxygenation

FEU – Fibrinogen Equivalent Units

FE-VET – Fibrinolysis-Enhanced Viscoelastic Testing

ICU – Intensive Care Unit

INR – International Normalized Ratio

IQR – Interquartile Range

ISTH – International Society on Thrombosis and Haemostasis

ICH-GCP - International Council for Harmonisation Good Clinical Practice

LOS – Length Of Stay

LT – Lysis Time

MAP – Mean Arterial Pressure

MCF – Maximum Clot Firmness

ML – Maximum Lysis

MV – Mechanical Ventilation

PAI-1 – Plasminogen Activator Inhibitor Type 1

SD – Standard Deviation

SIC – Sepsis-Induced Coagulopathy

SOFA – Sequential Organ Failure Assessment

STROBE - Strengthening the Reporting of Observational Studies in Epidemiology

TPA – Tissue Plasminogen Activator

UK-TEG – Urokinase-Modified Thromboelastography

1. BACKGROUND

Sepsis is associated with coagulation activation, suppression of natural anticoagulant pathways, and impairment of fibrinolysis, a triad that drives microvascular thrombosis, tissue hypoperfusion, and organ failure [1–3]. Fibrinolysis impairment, defined as a reduced capacity to dissolve fibrin despite ongoing coagulation activation, represents a clinically relevant but frequently neglected phenotype. It affects a subset of septic patients and is associated with organ dysfunction, tissue ischaemia, and worse outcomes [4–6]. Conventional coagulation tests and scoring systems such as the sepsis-induced coagulopathy (SIC) and overt disseminated intravascular coagulation (DIC) scores do not capture the functional status of fibrinolytic system [7].

Fibrinolysis-enhanced viscoelastic testing (FE-VET), obtained by adding an exogenous fibrinolytic activator to a whole-blood viscoelastic assay, provides a functional, point-of-care assessment of fibrinolytic capacity [8]. Our group first applied this concept to sepsis in 2015, using an in-house urokinase-modified thromboelastography (UK-TEG) that detected impaired fibrinolysis in 45% of patients with severe sepsis or septic shock and demonstrated its association with morbidity and mortality [4].

Subsequent studies using different FE-VET confirmed fibrinolysis resistance as a relevant phenotype in critically ill septic patients, linked to adverse outcomes [5,6,9,10]. However, these studies were limited to single time-point assessments, mixed or COVID-19-predominant populations, or lacked integration with plasma fibrinolytic biomarkers. A recent scoping review identified serial FE-VET assessment combined with biomarkers evaluation as methodological needs in the field [8].

The POCEFIS study (Point-of-Care Evaluation of Fibrinolysis in Sepsis) addresses these gaps, using the ClotPro® TPA-test (Enicor GmbH, a Haemonetics company, Munich, Germany), a standardized whole-blood assay with a fixed, pre-dosed recombinant tissue

plasminogen activator (TPA) challenge that reduces the operator dependence of our earlier urokinase method.

Aim of this study was to characterize the prevalence and temporal evolution of fibrinolysis impairment during the first week in a septic population, integrating serially repeated FE-VET measurements with plasma biomarkers.

2. METHODS

Study design and ethics

This prospective, monocentric, non-pharmacological study, was conducted at the Intensive Care Unit (ICU) of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. The study was registered on ClinicalTrials.gov (NCT06312488) and approved by the local Ethics Committee (n. 4097_S_P; November 13th, 2023). It was conducted according to the Declaration of Helsinki and the International Council for Harmonisation Good Clinical Practice (ICH-GCP) standards. Written informed consent was obtained from patients at enrollment or deferred upon recovery of consciousness when required.

Study population

The septic group included adult ICU patients with a diagnosis of sepsis or septic shock, defined by suspected or confirmed infection with acute cardiovascular failure requiring vasopressors to maintain mean arterial pressure (MAP) ≥65mmHg despite adequate volume resuscitation (cardiovascular Sequential Organ Failure Assessment (SOFA) score >2). Enrollment occurred within 12 hours of diagnosis. Exclusion criteria included known coagulation disorders, oral anticoagulant therapy, and extracorporeal membrane oxygenation (ECMO) support.

The post-operative control group included adult patients admitted to the ICU for post-operative monitoring following surgery. Exclusion criteria were: sepsis or septic shock, oral anticoagulant therapy, coagulation disorders, American Society of Anesthesiologists (ASA) status >3, and solid organ transplant surgery.

Sample size

Based on prior data showing impaired fibrinolysis in approximately 45% of septic patients [4], a sample size of 100 patients was calculated to ensure, with 80% probability, that the observed prevalence would not be lower than 38% [11]. A sample of 30 post-operative controls was planned.

Study procedures

In septic patients, blood samples were collected at enrollment (Day 0) and on Days 1, 2, 3, and 7. At each time point, standard laboratory tests and ClotPro® TPA-test were performed. Plasma biomarkers (plasminogen activator inhibitor type 1 (PAI-1), plasminogen, protein C and protein S), were measured at Day 0, 2, and 7. A detailed description of biomarkers assays used is provided in the Supplementary Material.

Clinical data, SOFA scores, DIC and SIC scores - calculated according to the International Society on Thrombosis and Haemostasis (ISTH) overt DIC scoring system [12] and as proposed by Iba et al. [13], respectively - were recorded daily. Follow-up continued until Day 28 or ICU discharge.

Controls underwent a single ClotPro® TPA-test and standard laboratory assessment at enrollment.

Viscoelastic testing

Fibrinolysis was assessed using the ClotPro® system (Enicor GmbH, a Haemonetics company, Munich, Germany), a viscoelastic hemostatic analyzer based on elastic motion thromboelastography. The TPA-test employs an active tip preloaded with dried reagents: coagulation is activated by recombinant tissue factor and $CaCl_2$, heparin effects are blocked by polybrene, and fibrinolysis is triggered by recombinant TPA at a concentration of 650 ng/mL. The following parameters were recorded: clotting time (CT, seconds), maximum clot firmness (MCF, mm), lysis time (LT, seconds, time from CT to 50% reduction of MCF), and maximum lysis (ML, %). 340 µL of citrated whole blood were pipetted into the test cup using the assay-specific syringe. Tests were performed within 30 minutes of sampling.

Definition of fibrinolysis impairment

Impaired fibrinolysis was defined as a TPA-test LT >393s, corresponding to the upper limit of the 95% confidence interval of the TPA-test LT measured in 60 healthy volunteers at Augsburg University Clinic [14]. Patients without an available TPA-test result on a given day

were classified as having normal fibrinolysis on that day for the primary analysis; patients with no TPA-test result during the whole D0-D2 period were not classified.

Outcomes

The primary outcome was the proportion of septic patients with impaired fibrinolysis (TPA-test LT >393s). We used the period from D0 to D2 to stratify septic patients into two groups: patients with impaired fibrinolysis (LT >393s in at least one measurement), and patients with normal fibrinolysis otherwise. This 72-hour window covers the acute phase of sepsis and includes patients in whom impairment first appeared on day 2; the shorter D0-D1 window was tested as a sensitivity analysis (see Supplementary Material).

Secondary outcomes included: the association between fibrinolysis impairment and standard coagulation parameters, plasma biomarkers, DIC/SIC scores, SOFA score, ICU length of stay, ICU mortality, and the longitudinal evolution of fibrinolysis status during the first week of ICU stay.

Statistical analysis

Categorical variables are reported as frequencies and percentages; continuous variables as median [1st–3rd quartile] or mean ± standard deviation, as appropriate.

Group comparisons were performed using the Student's t-test or Wilcoxon rank-sum test for continuous variables and Fisher’s exact test or chi-square test for categorical variables.

Longitudinal changes within the septic group were analyzed using linear mixed-effects models with group, time, and their interaction as fixed effects, a random intercept for each patient, and an unstructured covariance matrix. Overall effects were assessed using Type III F-tests; when the overall effect of group or the group × time interaction was statistically significant, post-hoc pairwise comparisons between groups at individual timepoints were performed using Bonferroni-adjusted p-values. A two-sided p-value <0.05 was considered statistically significant.

Analyses were performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA) and R software (version 4.5.2; R Foundation for Statistical Computing, Vienna, Austria).

The results of the study are reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement.

3. RESULTS

Characteristics of study population

From January 2024 to November 2025, 104 septic patients and 30 post-operative controls were enrolled. Four septic patients withdrew consent and were therefore excluded (Supplementary Figure 1).

Demographic characteristics of septic patients are listed in Table 1. No statistical differences in age, sex, and BMI were observed between the two groups. Patients with impaired fibrinolysis had higher SOFA scores during the first three days but not at day 7. To study the overall effects of time, group, and their interaction, model-estimated SOFA scores were calculated (Supplementary Figure 2). In this model, neither the overall group effect (p=0.12) nor the group-by-time interaction (p=0.16) was statistically significant.

At enrollment a higher proportion of patients with impaired fibrinolysis had a SIC score ≥4 (80.7% vs 50.0%, p=0.002), while the overt DIC score did not differ between groups (39.3% vs 26.7%, p=0.234).

	Overall	Normal	Impaired	p-value
N	100 (100.0)	34 (34.34)	65 (65.66)	-
Males, n (%)	65 (65.00)	22 (64.71)	42 (64.62)	0.9929
Age (years), mean ± SD	63.6 ± 15.8	65.1 ± 14.9	62.8 ± 16.4	0.6199
Weight (kg), mean ± SD	70.1 ± 15.8	68.4 ± 15.6	70.7 ± 16.1	0.7489
Height (cm), mean ± SD	168.9 ± 9.6	168.3 ± 8.0	169.2 ± 10.5	0.6500
BMI (kg/m^2), mean ± SD	24.4 ± 4.5	24.0 ± 4.6	24.6 ± 4.5	0.7879
Etiology, n (%)				0.0563
Gastrointestinal	47 (48.45)	16 (51.61)	31 (47.69)	
Respiratory	19 (19.59)	5 (16.13)	13 (20.00)	
Urinary tract	15 (15.4)	4 (12.90)	11 (16.92)	
Bone/Joints	6 (6.19)	5 (16.13)	1 (1.54)	
Other	10 (10.31)	1 (3.23)	9 (13.85)	
Comorbidities, n (%)				
Cardiovascular	60 (60.00)	25 (73.53)	34 (52.31)	**0.0410**
Respiratory	22 (22.00)	2 (5.88)	19 (29.23)	**0.0070**
Neurologic	21 (21.00)	9 (26.47)	11 (16.93)	0.2612
Gastro/Liver	28 (28.00)	8 (23.53)	19 (29.23)	0.5453
Kidney	19 (19.00)	4 (11.76)	14 (21.54)	0.2312
Haematologic	16 (16.00)	5 (14.71)	11 (16.92)	0.7760
Neoplastic	23 (23.00)	11 (32.35)	12 (18.46)	0.1202
Metabolic	33 (33.33)	15 (44.12)	17 (26.56)	0.0777
Other	27 (27.00)	8 (23.53)	19 (29.23)	0.5453
Use of drugs, n (%)				
Antiplatelet	18 (18.00)	8 (23.53)	9 (13.85)	0.2251
SOFA, mean ± SD				
Day 0 (n=34+65)	10.4 ± 3.1	9.4 ± 2.9	10.9 ± 3.1	**0.0282**
Day 1 (n=30+64)	9.4 ± 4.4	7.6 ± 4.4	10.1 ± 4.2	**0.0177**
Day 2 (n=24+57)	8.1 ± 3.9	6.4 ± 4.4	8.6 ± 3.6	**0.0434**
Day 3 (n=16+43)	8.8 ± 3.8	8.8 ± 4.7	8.7 ± 3.6	0.9457
Day 7 (n=9+23)	5.4 ± 3.6	6.3 ± 4.6	5.1 ± 3.6	0.9577
SIC ≥4 D0, n (%)	66 (69.5)	16 (50.0)	50 (80.7)	**0.002**
DIC ≥5 D0, n (%)	32 (34.8)	8 (26.7)	24 (39.3)	0.234
Lactate > 2 mmol/L, n (%)	79 (81.44)	23 (69.70)	55 (87.30)	0.0368
Outcomes				
ICU Mortality, n (%)	15 (15.00)	5 (14.71)	10 (15.38)	0.9287
LOS ICU (days), median [IQR]	5 [3 – 8]	3 [2 – 8]	5 [3 – 8]	0.0510
Duration MV (days), median [IQR]	2 [1 – 4]	2 [1 – 4]	2 [1 – 3]	0.7905

Table 1. Characteristics of septic patients. One patient could not be classified as no Clot Pro results were available during the period D0-D2. No adjustment for baseline characteristics was performed between groups. Percentages are calculated on patients with available data; etiology was missing for three patients with normal fibrinolysis.

Figure 1 shows the temporal evolution of the fibrinolytic status of septic patients during the first week of ICU stay.

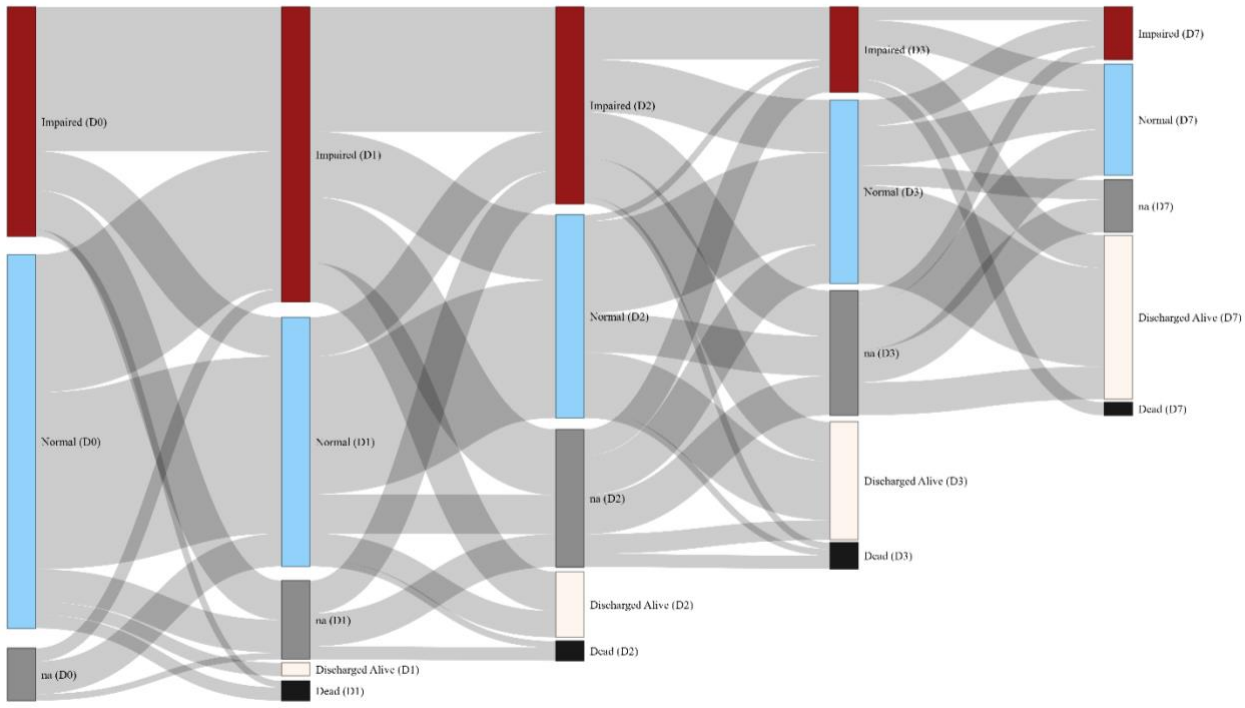


Figure 1. Sankey diagram. Temporal evolution of fibrinolytic status of septic patients during the first week of ICU stay.

At enrollment 38% of septic patients had impaired fibrinolysis. Point prevalence rose to 54% on day 1 and 49% on day 2, before declining on days 3 and 7. This later decrease parallelled a reduction in the number of evaluable patients due to death or discharge.

Considering the first three days, 66% of patients (65/99) met the criterion for impaired fibrinolysis at least once. The proportion of patients with impaired fibrinolysis varied over the week (global day effect, p=0.0159). For LT and ML values, see Supplementary Table 1.

Standard laboratory tests

At enrollment, patients with impaired fibrinolysis had higher lactate levels compared with those with normal fibrinolysis (4.2 mmol/L vs 2.9 mmol/L, p=0.0482).

Fibrinogen levels (4.61 g/L vs 3.75 g/L, p=0.0879) and platelet count (132.5 10^3/mm³ vs 152.0 × 10^3/mm³, p=0.5809) were comparable between groups. Longitudinal mixed-effects models showed a significant effect of time, with no significant overall group effect or group-by-time interaction, indicating similar temporal patterns between groups (Figure 2).

At enrollment, patients with impaired fibrinolysis showed higher D-dimer levels (7,536.0 ng/mL vs 3,838.0 ng/mL, p=0.0427) and higher INR values (1.5 vs 1.2, p=0.0156) compared with those with normal fibrinolysis. During follow-up, D-dimer levels remained higher in patients with impaired fibrinolysis.

C-reactive protein (CRP) levels were significantly higher in patients with impaired fibrinolysis during the first week of ICU stay, with a significant overall group effect (p=0.0047).

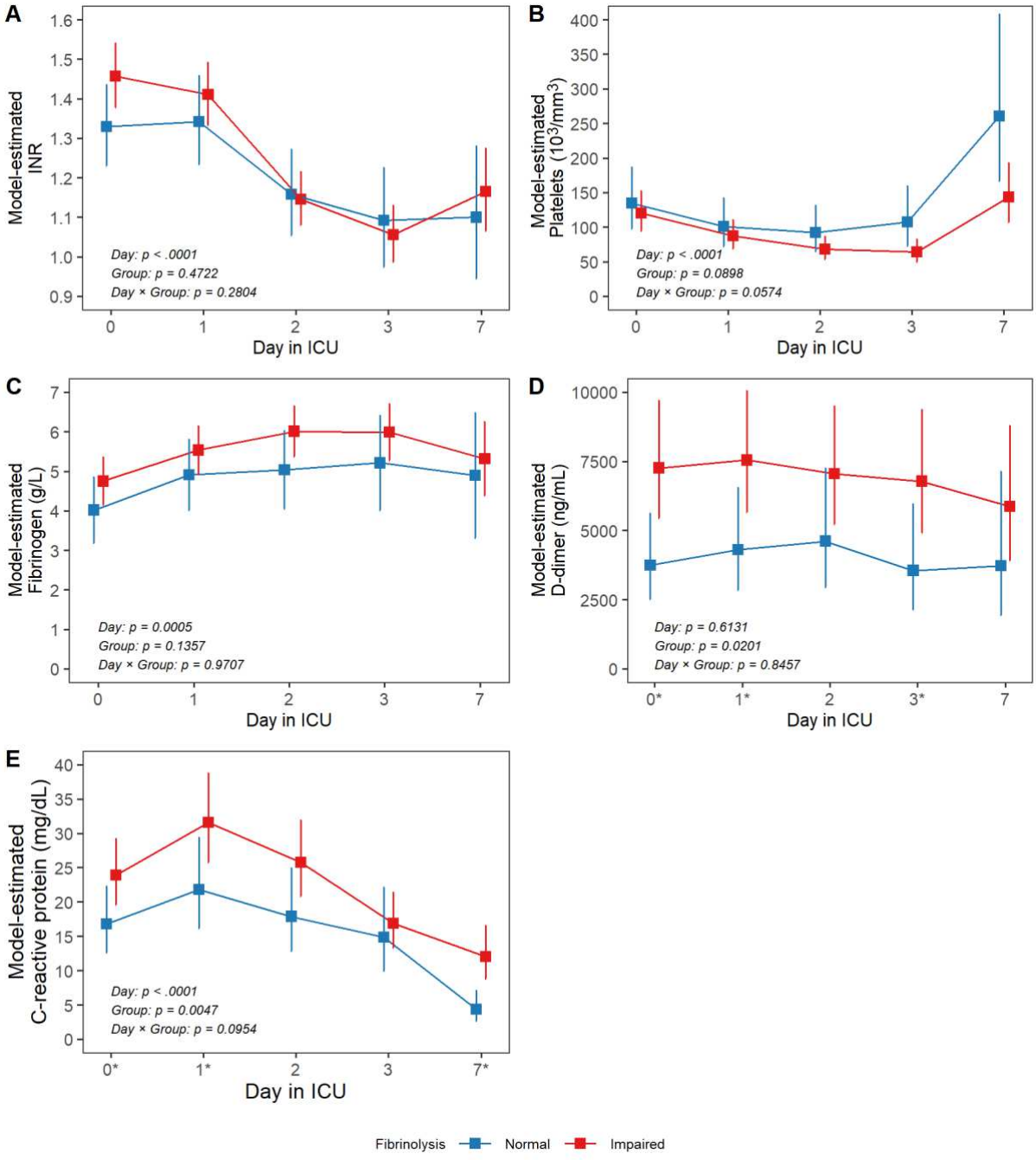


Figure 2. Estimated marginal means of laboratory tests across ICU days in septic patients stratified by fibrinolysis group. (A) INR; (B) platelets; (C) fibrinogen; (D) D-dimer; (E) C-reactive protein.

Estimated marginal means were derived from linear mixed-effects models with time (categorical: days 0, 1, 2, 3, and 7), fibrinolysis group, and their interaction as fixed effects, and a random intercept per patient with an unstructured covariance matrix.
Variables violating normality assumptions were log-transformed prior to analysis (INR, platelets, D-dimer, CRP); back-transformed least-squares means with 95% confidence intervals are presented for these variables. The omnibus effects of time, group, and their interaction were assessed via Type III F-tests. Post-hoc pairwise comparisons between groups at individual timepoints were performed only when the corresponding omnibus test for the group effect or group × day interaction was statistically significant (* $p<0.05$). For detailed temporal evolution of standard laboratory test during the first week of observation, see Supplementary Table 2 and Supplementary Figure 3.

Plasma biomarkers

Plasma biomarkers were assessed on day 0, 2, and 7 (Table 2, Figure 3).

	Overall	Normal	Impaired	p-value
PAI-1 (ng/mL)				
Day 0	22.4 [12.2 – 26.2]	22.7 [16.5 – 24.7]	22.4 [11.0 – 26.8]	0.5495
Day 2	1.1 [0.5 – 6.1]	0.9 [0.4 – 1.0]	1.5 [0.6 – 6.5]	**0.0028**
Day 7	1.4 [0.9 – 3.7]	5.8 [1.3 – 12.9]	1.4 [0.8 – 1.8]	**0.0477**
Plasminogen (%)				
Day 0	38.0 [30.0 – 48.0]	42.0 [32.0 – 56.0]	38.0 [29.0 – 46.0]	0.1854
Day 2	45.5 [36.0 – 58.0]	61.0 [35.0 – 81.0]	45.0 [37.0 – 55.0]	0.0850
Day 7	75.0 [54.0 – 95.0]	58.5 [49.0 – 112.0]	80.5 [57.5 – 91.5]	0.7997
C protein (%)				
Day 0	46.0 [34.0 – 61.0]	50.0 [37.0 – 63.0]	44.0 [32.0 – 61.0]	0.2277
Day 2	60.5 [45.0 – 99.0]	82.0 [44.5 – 112.0]	60.0 [47.0 – 89.0]	0.2314
Day 7	103.0 [83.0 – 113.0]	91.5 [72.0 – 113.0]	106.0 [90.0 – 116.0]	0.5622
S protein (%)				
Day 0	48.0 [35.8 – 60.0]	50.0 [36.0 – 67.5]	46.0 [35.7 – 57.9]	0.3401
Day 2	58.0 [39.0 – 66.0]	57.1 [47.3 – 65.8]	58.0 [39.0 – 66.0]	0.7948
Day 7	77.1 [53.0 – 103.0]	75.2 [71.0 – 84.5]	79.3 [52.5 – 103.5]	0.4944

Table 2. Plasma biomarkers in septic patients, stratified by group. All values are reported as median [IQR]. In the overall population, measurements were available for 93/100 (93.0%), 46/82 (56.1%), and 26/33 (78.8%) patients at days 0, 2, and 7, respectively. Corresponding values were 29/34 (85.3%), 8/24 (33.3%), and 6/9 (66.7%) in the Normal group, and 63/65 (96.9%), 37/57 (64.9%), and 20/23 (87.0%) in the Impaired group. Denominators at days 2 and 7 refer to patients present in the ICU. At day 7, PAI-1 was measured in 25 patients overall and protein S in 5 patients in the Normal group.

PAI-1 exhibited a distinct temporal pattern according to fibrinolysis status, with a significant group-by-time interaction (p=0.0040). Although PAI-1 levels decreased in both groups, patients with impaired fibrinolysis had higher levels at day 2 and lower levels at day 7, suggesting a different modulation of PAI-1 over time. No significant differences in longitudinal trajectories between groups were observed for plasminogen, protein C, or protein S.

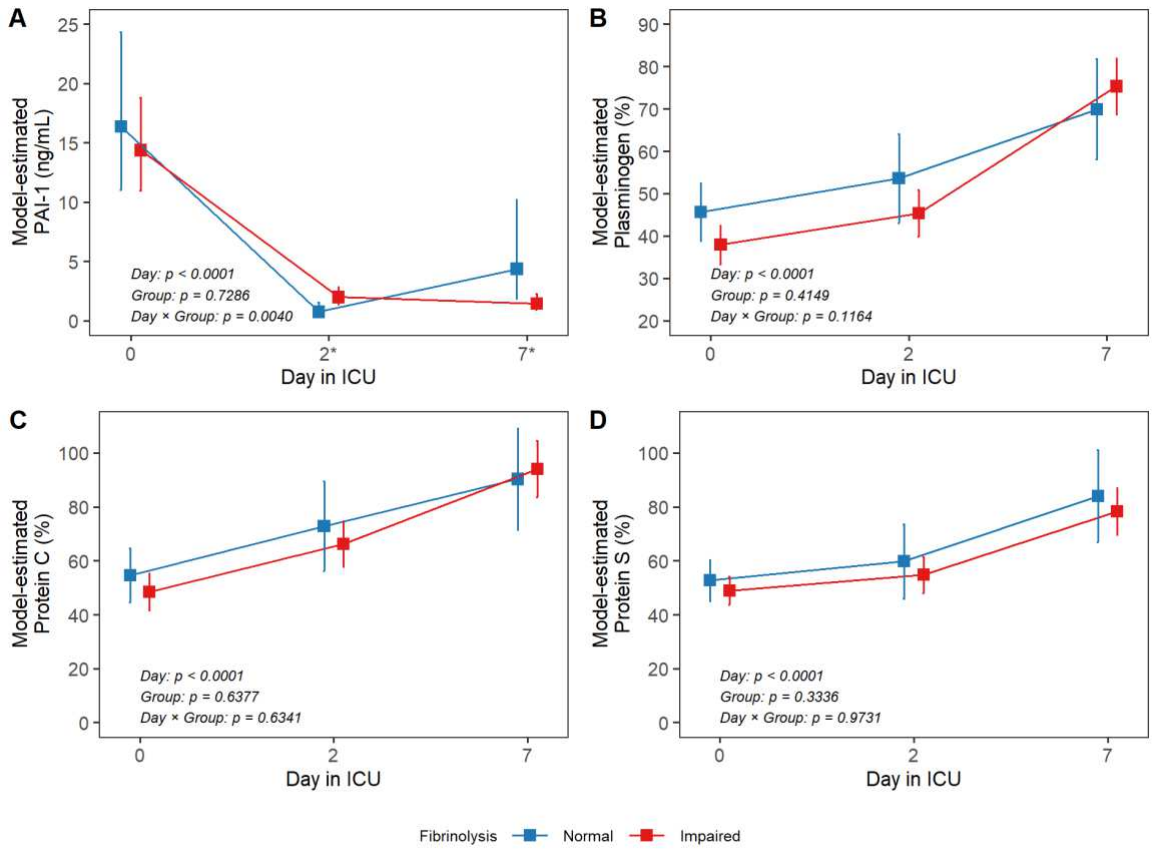


Figure 3. Estimated marginal means of biomarkers across ICU days in septic patients stratified by fibrinolysis group. (A) PAI-1; (B) Plasminogen; (C) Protein C; (D) Protein S.

Estimated marginal means were derived from linear mixed-effects models with time (categorical: days 0, 2, and 7), fibrinolysis group, and their interaction as fixed effects, and a random intercept per patient with an unstructured covariance matrix.
Variables violating normality assumptions were log-transformed prior to analysis (PAI-1); back-transformed least-squares means with 95% confidence intervals are presented for these variables. The omnibus effects of time, group, and their interaction were assessed via Type III F-tests. Post-hoc pairwise comparisons between groups at individual timepoints were performed only when the corresponding omnibus test for the group effect or group × day interaction was statistically significant (* $p<0.05$).

Control patients

ClotPro® TPA-test data at ICU admission were available for 29 of 30 enrolled postoperative patients. The median LT was 207s [175-230], and the median ML was 95% [94-96]. 27 patients (93.1%) showed normal fibrinolysis at ICU admission. Only two patients had a ClotPro® TPA-test LT >393s and were therefore classified as patients with impaired fibrinolysis; in these cases, intraoperative bleeding requiring tranexamic acid administration occurred. For detailed demographic characteristics of post-operative controls, see Supplementary Table 3.

4. DISCUSSION

In the POCEFIS study, we followed fibrinolysis with a dedicated viscoelastic test through the first ICU week, and the picture that emerges is a moving one rather than a fixed portrait. At diagnosis, impaired fibrinolysis was present in 38% of patients, a proportion in line with earlier reports [4,15]. However, when the first three days were considered, impaired fibrinolysis was detected in nearly two thirds of the cohort, a burden that a single baseline sample does not capture.

Patients with impaired fibrinolysis carried higher SOFA scores through the first three days, a gradient consistent with earlier work linking organ dysfunction to reduced fibrinolytic capacity [4,6]. Lactate at enrollment was higher in the impaired group, consistent with more severe tissue hypoperfusion.

At enrollment, 69.5% of patients met the SIC criteria, with a higher prevalence among those with impaired fibrinolysis (80%). This fits the purpose of the score, which is to catch SIC at an early stage and to flag patients likely to progress toward overt DIC [13]. However, the score cannot capture the fibrinolysis function or its dynamic changes, a limitation overcome by the TPA-test. The two therefore are complementary: the score identifies coagulopathic patients, while the functional test shows whether fibrinolysis is impaired and its changes over time.

Persistently higher CRP levels in patients with impaired fibrinolysis support previous findings suggesting SIC and fibrinolysis impairment are driven by a greater degree of systemic inflammation [14,16,17].

D-dimer was consistently higher in the impaired group, with a significant group effect ($p=0.0201$) and separation at days 0, 1, and 3. Since D-dimer reflects both coagulation activation and fibrin degradation, its interpretation in isolation is challenging. In the ALBIOS cohort, D-dimer levels alone were not associated with mortality; however, D-dimer corrected for thrombin and plasmin generation showed a J-shaped relationship with mortality [18]. In

the setting of impaired fibrinolysis, elevated D-dimer is more likely to identify intense clot formation with inadequate clearance rather than potentially protective lysis.

Plasma biomarkers showed the expected pattern of SIC, namely preserved PAI-1 and reduced plasminogen, protein C, and protein S at diagnosis, with progressive normalization by day 7.

None of the baseline values discriminated between patients with impaired or normal fibrinolysis; only the PAI-1 trajectory differed between groups (day-by-group interaction, p=0.0040). This likely reflects the nature of the FE-VET, which captures the integrated response to a fibrinolytic challenge, incorporating substrate availability, inhibitors and clot architecture. Consistently, the exogenous TPA concentration used in the assay (650 ng/mL, ~10 nmol/L) greatly exceeds measured PAI-1 levels (~22 ng/mL, ~0.4 nmol/L), suggesting that the added TPA overwhelms the inhibitor, and baseline PAI-1 alone can hardly determine the assay result.

This may also explain why the correlation between LT and PAI-1 reported in a small series of patients with COVID-19 [10] is not confirmed in our cohort, where baseline PAI-1 did not differ between groups; fibrinolytic patterns also differ between sepsis and COVID-19.

Plasminogen represents an interesting case. It was reduced in both groups and unable to discriminate between them, indicating that substrate levels alone cannot account for the observed phenotype. Nevertheless, plasminogen is not a mere bystander. It is consumed and degraded in septic coagulopathy, and its restoration re-establishes fibrinolytic capacity as observed both in the recent trial by Tschirhart [19], and in ex vivo experiments on resistant whole blood [5]. Scarlatescu reached a similar conclusion: in a mixed population of septic patients and healthy donors, plasminogen levels were lower in patients with fibrinolysis resistance, while PAI-1 levels did not differ from patients with preserved fibrinolytic function [20]. Taken together, these findings support the hypothesis that TPA-test is a functional,

proof-of-concept assay that captures an integrated fibrinolytic deficit not reflected by individual biomarkers and points to substrate availability as potentially modifiable target.

The main strength of our study is its serial design. Indeed, previous studies measured fibrinolysis only at admission [8]. By repeating the test, we found that fibrinolytic status changes significantly over the first week: the same patient moves in and out of the impaired range, as the Sankey diagram shows.

Post-operative controls provide a reference for comparison. Despite surgery and the associated inflammatory response, 93% of them maintained a normal fibrinolytic profile, suggesting that the impairment observed in sepsis is related more to the dysregulated host response to infection than to the nonspecific stress of surgery or ICU admission. The two patients with impaired fibrinolysis experienced intraoperative bleeding requiring tranexamic acid administration, which may have represented a potential confounding factor for TPA-test LT result.

We found no association between impaired fibrinolysis and either mortality or length of stay, but our study was not designed to establish prognostic associations, as it was powered on the prevalence of the phenotype and not on survival. In any case, current literature provides inconsistent evidence regarding the association between fibrinolysis impairment and patient outcomes. In a mixed ICU population, Brewer reported an adjusted odds ratio for 30-day mortality of 2.26, but with a confidence interval (0.83 to 6.69) including no effect [6]; Schmitt described lysis indices that separated survivors almost perfectly, a finding difficult to generalize from a pilot study of 30 septic patients [9]; and our UK-TEG study from 2015 showed a mortality difference that this larger and technically different cohort has not confirmed [4]. These results suggest that the prognostic value attributed to a single fibrinolytic snapshot has been overstated, as the real contribution of functional testing is to describe a dynamic phenotype rather than to forecast mortality.

Our study has limitations. This is a single-center study performed in a Unit where coagulation is monitored routinely, which may limit generalizability to other settings. When the test was unavailable on a given day, we counted the patient as normal. This conservative approach likely reduced between-group differences and may have underestimated phenotype prevalence. Without a gold standard for fibrinolysis resistance, the TPA-test cannot be validated against a reference, while the variety of activators, doses, and run times used across laboratories still limit direct comparability. These considerations support the interpretation of the TPA-test as a functional, proof-of-concept tool, and highlight the need for standardization and outcome-linked studies.

These findings have practical implications. If fibrinolytic status shifts from one day to another, and a single baseline sample misses nearly one-third of affected patients, then any treatment directed at the fibrinolytic system will need serial, point-of-care guidance to reach the right patient at the right time. Two emerging strategies support this approach: the restoration of fibrinolytic capacity by plasminogen supplementation in sepsis-induced DIC [19], and low-dose fibrinolytic therapy steered by the TPA-test, now under prospective evaluation [5]. In this framework, POCEFIS describes the dynamics of fibrinolysis impairment in septic patients, mapping its prevalence and time course. It also leaves an unanswered question: whether LT is the readout that matters most, or whether other fibrinolytic parameters taken from the same viscoelastic tracing describe these patients more precisely.

5. CONCLUSION

The POCEFIS study demonstrates that the ClotPro® TPA-test is a feasible point-of-care tool for identifying fibrinolysis impairment in septic patients. Fibrinolysis impairment affects over one-third of septic patients at diagnosis and nearly two-thirds during the first three days of ICU stay. Serial FE-VET may improve risk stratification by identifying persistent fibrinolytic dysfunction and support future individualized therapeutic strategies for SIC.

Author contributions

CB, IS, AC, AM, MP, and GG conceived the study. CB, IS and GZ collected the data. FM performed the statistical analysis. CN and MBA performed biomarker analysis. CB, AC, AM and MP wrote the manuscript. All authors contributed to data interpretation, have read and approved the final version of the manuscript.

Funding

This study was partially funded by Italian Ministry of Health (Current Research IRCCS).

Data availability

The data sets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

The study was approved by the local Ethics Committee (n. 4097_S_P, November 13, 2023) and registered on ClinicalTrials.gov (NCT06312488).

Consent to participate

Written informed consent was obtained from all patients at enrollment or, when required, deferred until recovery of consciousness.

Previous presentation

Preliminary results of this study were presented at ESICM LIVES 2024 (Barcelona, Spain). The results were also presented at the International Expert Meeting on Coagulation & Beyond 2026 (Obergurgl, Austria) and at SMART 2026 (Milan, Italy).

Conflict of interest

MP received personal honoraria for lectures and support for attending meetings and travel by Werfen Company, Barcelona, Spain.

6. REFERENCES

1. Iba T, Levy JH. Sepsis-induced Coagulopathy and Disseminated Intravascular Coagulation. Anesthesiology. 2020;132(5):1238–45. doi:10.1097/ALN.0000000000003122 PubMed PMID: 32044801.
2. Semeraro N, Ammollo CT, Semeraro F, Colucci M. Sepsis, thrombosis and organ dysfunction. Thromb Res. 2012 Mar 1;129(3):290–5. doi:10.1016/j.thromres.2011.10.013
3. Gando S. Role of fibrinolysis in sepsis. Semin Thromb Hemost. 2013;39(4):392–9. doi:10.1055/s-0033-1334140 PubMed PMID: 23446914.
4. Panigada M, Zacchetti L, L'Acqua C, Cressoni M, Anzoletti MB, Bader R, et al. Assessment of fibrinolysis in sepsis patients with urokinase modified thromboelastography. PLoS One. 2015;10(8):e0136463. doi:10.1371/journal.pone.0136463 PubMed PMID: 26308340.
5. Coupland LA, Rabbolini DJ, Schoenecker JG, Crispin PJ, Miller JJ, Ghent T, et al. Point-of-care diagnosis and monitoring of fibrinolysis resistance in the critically ill: results from a feasibility study. Crit Care. 2023;27(1):55. doi:10.1186/s13054-023-04329-5 PubMed PMID: 36765421.
6. Brewer JS, Hvas CL, Hvas AM, Larsen JB. Impaired Whole-Blood Fibrinolysis is a Predictor of Mortality in Intensive Care Patients. TH Open. 2024;8(1):e164–74. doi:10.1055/a-2270-7673
7. Iba T, Helms J, Levy JH. Sepsis-induced coagulopathy (SIC) in the management of sepsis. Ann Intensive Care. 2024 Sep;14(1):148. doi:10.1186/s13613-024-01380-5 PubMed PMID: 39302568.
8. Self M, Coupland LA, Aneman A. Utility of fibrinolysis enhanced viscoelastic assays to evaluate fibrinolysis disorders in critically ill adults with severe infection: a scoping review. Ann Intensive Care. 2025;15:110. doi:10.1186/s13613-025-01528-x
9. Schmitt FCF, Manolov V, Morgenstern J, Fleming T, Heitmeier S, Uhle F, et al. Acute fibrinolysis shutdown occurs early in septic shock and is associated with increased morbidity and mortality: results of an observational pilot study. Ann Intensive Care. 2019;9(1):19. doi:10.1186/s13613-019-0499-6
10. Heinz C, Miesbach W, Herrmann E, Sonntagbauer M, Raimann FJ, Zacharowski K, et al. Greater Fibrinolysis Resistance but No Greater Platelet Aggregation in Critically Ill COVID-19 Patients. Anesthesiology. 2021 Mar 1;134(3):457–67. doi:10.1097/ALN.0000000000003685 PubMed PMID: 33417674.
11. Cocks K, Torgerson DJ. Sample size calculations for pilot randomized trials: A confidence interval approach. J Clin Epidemiol. 2013 Feb;66(2):197–201. doi:10.1016/j.jclinepi.2012.09.002 PubMed PMID: 23195919.
12. Taylor FB Jr, Toh CH, Hoots WK, Wada H, Levi M. Scientific Subcommittee on Disseminated Intravascular Coagulation (DIC) of the International Society on Thrombosis and Haemostasis (ISTH). Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. Thromb Haemost. 2001;86(5):1327–30. doi:10.1055/S-0037-1616068 PubMed PMID: 11816725.
13. Iba T, Di Nisio M, Levy JH, Kitamura N, Thachil J. New criteria for sepsis-induced coagulopathy (SIC) following the revised sepsis definition: A retrospective analysis of a nationwide survey. BMJ Open. 2017;7(9):e017046. doi:10.1136/bmjopen-2017-017046 PubMed PMID: 28963294.
14. Bachler M, Bösch J, Stürzel DP, Hell T, Giebl A, Ströhle M, et al. Impaired fibrinolysis in critically ill COVID-19 patients. Br J Anaesth. 2021;126(3):590–8. doi:10.1016/j.bja.2020.12.010 PubMed PMID: 33422287.
15. Savioli M, Cugno M, Polli F, Taccone P, Bellani G, Spanu P, et al. Tight glycemic control may favor fibrinolysis in patients with sepsis. Crit Care Med. 2009;37(2):424–31. doi:10.1097/CCM.0b013e31819542da PubMed PMID: 19114908.
16. Iba T, Helms J, Maier CL, Roberts I. Redefining Fibrinolytic Insufficiency in Sepsis-Associated DIC. Semin Thromb Hemost. 2026;52(7):858–68. doi:10.1055/a-2840-5585 PubMed PMID: 41956121.

17. Wilhelm G, Mertowska P, Mertowski S, Przysucha A, Strużyna J, Grywalska E, et al. The Crossroads of the Coagulation System and the Immune System: Interactions and Connections. Int J Mol Sci. 2023;24(16):12563. doi:10.3390/ijms241612563
18. Semeraro F, Ammollo CT, Caironi P, Masson S, Latini R, Panigada M, et al. D-dimer corrected for thrombin and plasmin generation is a strong predictor of mortality in patients with sepsis. Blood Transfus. 2020;18(4):304–11. doi:10.2450/2019.0175-19
19. Tschirhart M, Curtiaud A, Abou Rjeily R, Merdji H, Demiselle J, Severac F, et al. Plasminogen supplementation reverses fibrinolytic insufficiency in sepsis-induced disseminated intravascular coagulation: a pilot study. Intensive Care Med. 2026;52(3):456–65. doi:10.1007/s00134-026-08338-0
20. Scarlatescu E, Kim PY, Marchenko SP, Tomescu DR. Validation of the time to attain maximal clot amplitude after reaching maximal clot formation velocity parameter as a measure of fibrinolysis using rotational thromboelastometry and its application in the assessment of fibrinolytic resistance in septic patients: a prospective observational study: communication from the ISTH SSC Subcommittee on Fibrinolysis. J Thromb Haemost. 2024;22(4):1223–35. doi:10.1016/j.jtha.2023.12.012

Supplementary Files

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

- STROBEchecklist.pdf
- Supplementarymaterial.pdf