SUPPLEMENTARY MATERIAL

METHODS

Biomarkers analysis

For biomarker analyses, additional citrated and EDTA blood samples were collected at designated timepoints. After centrifugation (2500 rpm, 20 min), plasma aliquots were stored at −80°C until batch analysis.

Protein C and plasminogen were measured using automated chromogenic assays, (HemosIL Protein C and HemosIL Plasminogen (Werfen, Bedford, MA)), and free protein S concentrations were obtained by an automated latex ligand immunoassay (HemosIL Free Protein S, Werfen); all tests were performed on an ACL TOP analyser (Werfen, Bedford, MA). PAI-1 activity was measured using the ELISA test Zymutest PAI-1 Activity (Hyphen BioMed, Neuville-sur-Oise, France).

Reference ranges:

- Protein C: 65-160 %
- Plasminogen: 80 - 130 %
- Protein S: 60-170 %
- PAI-1: 1-25 ng/mL

Classification of septic patients according to early ICU fibrinolysis measurements

Septic patients were classified according to fibrinolysis status using alternative time-window definitions. Using Day 0–Day 1 measurements, 58 of 89 patients (65.2%) were classified as having impaired fibrinolysis, increasing to 65 of 86 patients (75.6%) when the Day 0–Day 2 window was considered. When patients with missing ClotPro® measurements were

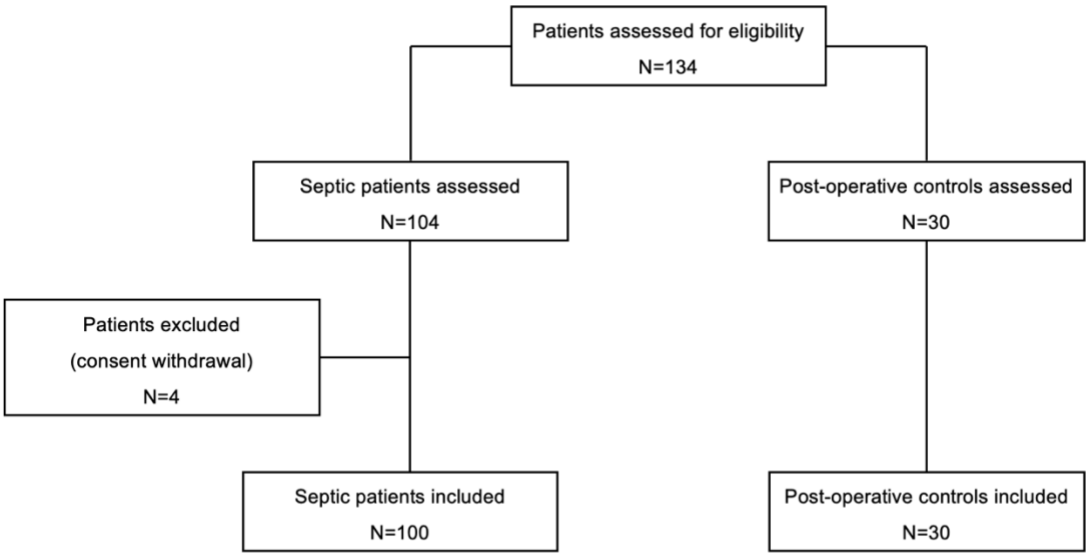
conservatively classified as having normal fibrinolysis (Group V2), the corresponding proportions were 58.6% (58/99) for Day 0–Day 1 and 65.7% (65/99) for Day 0–Day 2. The Day 0–Day 2 Group V2 definition was therefore used for the primary analysis, as it captured patients developing fibrinolysis impairment after the first 48 hours while assigning unavailable measurements to the normal category.

Classification of fibrinolysis status across alternative time-window definitions.

	Normal n (%)	Impaired n (%)
Group based on day 0 – day 1 [N=89]	31 (34.83)	58 (65.17)
Group based on day 0 – day 1 with missing inclusion (v2) [N=99]	41 (41.41)	58 (58.59)
Group based on day 0 – day 2 [N=86]	21 (24.42)	65 (75.58)
Group based on day 0 – day 2 with missing inclusion (v2) [N=99]	34 (34.34)	65 (65.66)

RESULTS

Supplementary Figure 1. Flow diagram of patients enrollment in the study.



Supplementary Figure 2. Estimated marginal means of total SOFA scores across ICU days in septic patients stratified by fibrinolysis group.

Estimated marginal means were obtained from a linear mixed-effects model with total SOFA score as the dependent variable, including time (categorical), fibrinolysis group, and their interaction as fixed effects, and a random intercept for each patient. Error bars represent 95% confidence intervals. The overall effects of time, group, and their interaction were statistically evaluated. Pairwise comparisons between groups at each ICU day were derived from the model-based estimated marginal means and adjusted for multiple testing.

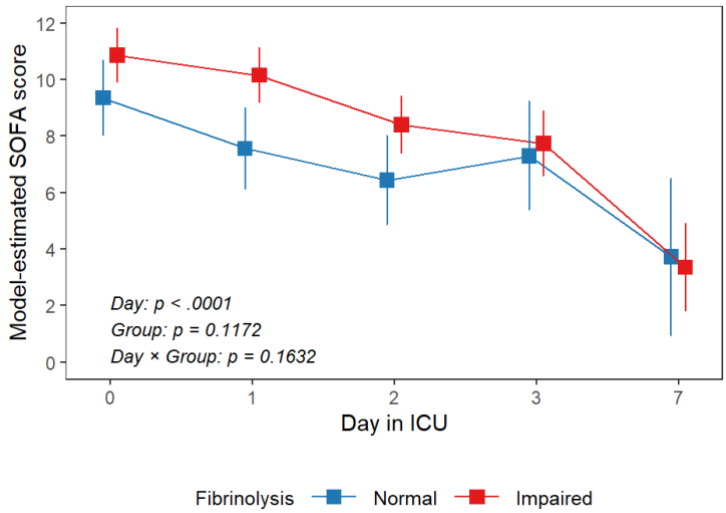


Table for Supplementary Figure 2

	SOFA score	
Fixed effect		
	Statistic F°	**p-value**
Time	23.36	<.0001
Group	2.47	0.1172
Time × Group	1.65	0.1632
Estimated marginal means		
	Normal LS Mean (95%CI)	**Impaired LS Mean (95%CI)**
Day 0	9.35 (8.03 – 10.67)	10.86 (9.91 – 11.81)
Day 1	7.56 (6.14 – 8.97)	10.15 (9.19 – 11.11)
Day 2	6.44 (4.86 – 8.02)	8.40 (7.40 – 9.40)
Day 3	7.30 (5.37 – 9.23)	7.74 (6.61 – 8.86)
Day 7	3.72 (0.94 – 6.50)	3.35 (1.81 – 4.89)

° F-tests for the fixed effects of time (categorical) were obtained from a linear mixed-effects model with a random intercept for each patient and an unstructured covariance matrix. Dependent variable was modeled longitudinally across days 0 to 7. Pairwise comparisons between time points were performed using Bonferroni-adjusted tests.

Abbreviations: CI = confidence interval; LS = least-squares.

Supplementary Table 1. ClotPro TPA parameters of fibrinolysis over the first 7 days in the ICU in septic patients.

	Day 0	Day 1	Day 2	Day 3	Day 7
TPA Lysis time (sec)	323 [241–2401]	410 [283–2401]	383 [315–2401]	303 [257–408]	335 [288–428]
TPA Maximum lysis (%)	94 [42–96]	74 [2–95]	89 [7–95]	95 [88–96]	97 [96–97]
Impaired* n (%)	35 (38.0)	45 (54.2)	30 (49.2)	13 (31.7)	8 (32.0)
Normal* n (%)	57 (62.0)	38 (45.8)	31 (50.8)	28 (68.3)	17 (68.0)

Abbreviations: TPA = tissue plasminogen activator
* Impaired: TPA Lysis time >393 sec; Normal: TPA Lysis time ≤ 393 sec.
Continuous data are reported as median [1st quartile – 3rd quartile].

Supplementary Table 2. Laboratory parameters over the first 7 days in the ICU in septic patients, stratified by group (normal, impaired).

	Normal	Impaired
AT (%)		
Day 0 *(n=34+65)*	49.0 [41.0 – 63.0]	45.0 [35.0 – 62.0]
Day 1 *(n=30+64)*	44.0 [37.0 – 65.0]	41.0 [34.0 – 57.0]
Day 2 *(n=24+57)*	48.0 [36.0 – 77.0]	46.0 [34.0 – 58.0]
Day 3 *(n=16+43)*	59.0 [50.0 – 85.0]	53.0 [41.0 – 70.0]
Day 7 *(n=9+23)*	69.5 [45.0 – 94.0]	91.0 [77.5 – 98.5]
Bilirubin (mg/dL)		
Day 0	0.9 [0.6 – 1.3]	1.4 [0.8 – 2.4]
Day 1	0.9 [0.5 – 1.7]	1.1 [0.7 – 2.1]
Day 2	0.6 [0.4 – 1.4]	0.9 [0.6 – 1.8]
Day 3	0.4 [0.4 – 3.2]	1.1 [0.6 – 2.0]
Day 7	0.9 [0.3 – 4.0]	0.7 [0.5 – 1.1]
Creatinine (mg/dL)		
Day 0	1.2 [1.0 – 1.8]	1.5 [0.9 – 2.8]
Day 1	1.0 [0.9 – 1.4]	1.5 [1.0 – 2.3]
Day 2	1.0 [0.8 – 1.4]	1.2 [0.9 – 1.9]
Day 3	0.9 [0.6 – 1.8]	1.1 [0.9 – 1.9]
Day 7	0.7 [0.6 – 1.3]	1.0 [0.7 – 2.0]
D-dimer (ng/mL)		
Day 0	3,838.0 [2,039.0 – 7,206.0]	7,536.0 [2,141.0 – 20,385.0]
Day 1	4,339.0 [1,605.0 – 12,404.0]	6,071.0 [2,954.0 – 21,414.0]
Day 2	4,710.5 [1,875.0 – 10,972.0]	5,731.0 [2,853.0 – 19,805.0]
Day 3	2,059.5 [1,512.0 – 4,952.5]	5,986.0 [2,829.0 – 15,820.0]
Day 7	2,239.5 [1,888.0 – 13,212.0]	4,173.0 [2,846.0 – 11,117.0]
Fibrinogen (g/L)		
Day 0	3.75 [2.73 – 5.40]	4.61 [3.31 – 6.15]
Day 1	4.51 [3.27 – 6.11]	5.78 [3.40 – 7.42]
Day 2	4.66 [3.38 – 6.69]	5.91 [3.56 – 8.06]
Day 3	5.11 [3.63 – 6.60]	6.19 [4.02 – 8.19]

	Normal	Impaired
Day 7	5.30 [3.03 – 5.99]	5.16 [4.02 – 8.19]
INR		
Day 0	1.2 [1.1 – 1.4]	1.5 [1.3 – 1.6]
Day 1	1.3 [1.1 – 1.5]	1.3 [1.1 – 1.7]
Day 2	1.1 [1.0 – 1.2]	1.1 [1.0 – 1.3]
Day 3	1.1 [1.0 – 1.2]	1.0 [0.9 – 1.1]
Day 7	1.1 [1.1 – 1.1]	1.1 [1.1 – 1.2]
Lactate (mmol/L)		
Day 0	2.9 [2.0 – 5.2]	4.2 [2.5 – 7.0]
Day 1	1.8 [1.0 – 2.4]	2.0 [1.2 – 3.4]
Day 2	1.1 [0.7 – 2.2]	1.2 [1.1 – 1.7]
Day 3	1.2 [0.8 – 2.1]	1.2 [1.0 – 1.6]
Day 7	1.3 [1.1 – 1.4]	1.2 [1.0 – 1.4]
C-reactive protein (mg/dL)		
Day 0	19.6 [12.1 – 36.2]	25.5 [16.0 – 32.5]
Day 1	20.6 [14.4 – 31.2]	31.9 [18.3 – 41.1]
Day 2	16.9 [9.3 – 30.2]	27.6 [16.5 – 34.7]
Day 3	15.4 [8.6 – 26.1]	17.3 [12.6 – 30.1]
Day 7	5.7 [1.4 – 12.6]	10.1 [6.6 – 27.8]
Platelets (10^3/mm^3)		
Day 0	152.0 [99.0 – 244.0]	132.5 [76.0 – 229.0]
Day 1	118.0 [37.0 – 231.0]	110.0 [48.0 – 159.0]
Day 2	124.0 [44.5 – 204.5]	83.0 [41.0 – 122.0]
Day 3	162.5 [54.0 – 235.5]	68.0 [30.0 – 122.0]
Day 7	219.0 [90.0 – 461.0]	145.0 [102.0 – 294.0]

All values are reported as median [1st quartile – 3rd quartile]. Reference ranges provided by the local laboratory were as follows: antithrombin (AT) 82–112%; total bilirubin 0.12–1.10 mg/dL; creatinine 0.72–1.18 mg/dL; D-dimer <500 ng/mL (FEU); fibrinogen 1.65–3.50 g/L; INR 0.84–1.20; lactate (not routinely standardized; reference range typically 0.5–2.0 mmol/L); C-reactive protein <5.0 mg/L; platelets 130–400 $\times 10^9$/L.

Supplementary Figure 3. Estimated marginal means of laboratory tests across ICU days in septic patients stratified by fibrinolysis group. (A) AT; (B) bilirubin; (C) creatinine; (D) lactate.

Estimated marginal means were derived from linear mixed-effects models with time (categorical: days 0, 1, 2, 3, and 7), fibrinolysis group (Group V2 d0–d2), 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 (bilirubin, creatinine, lactate); 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. Pairwise between-group comparisons at each timepoint were performed only when the main effect of group or the group × time interaction was statistically significant (*p<.05).

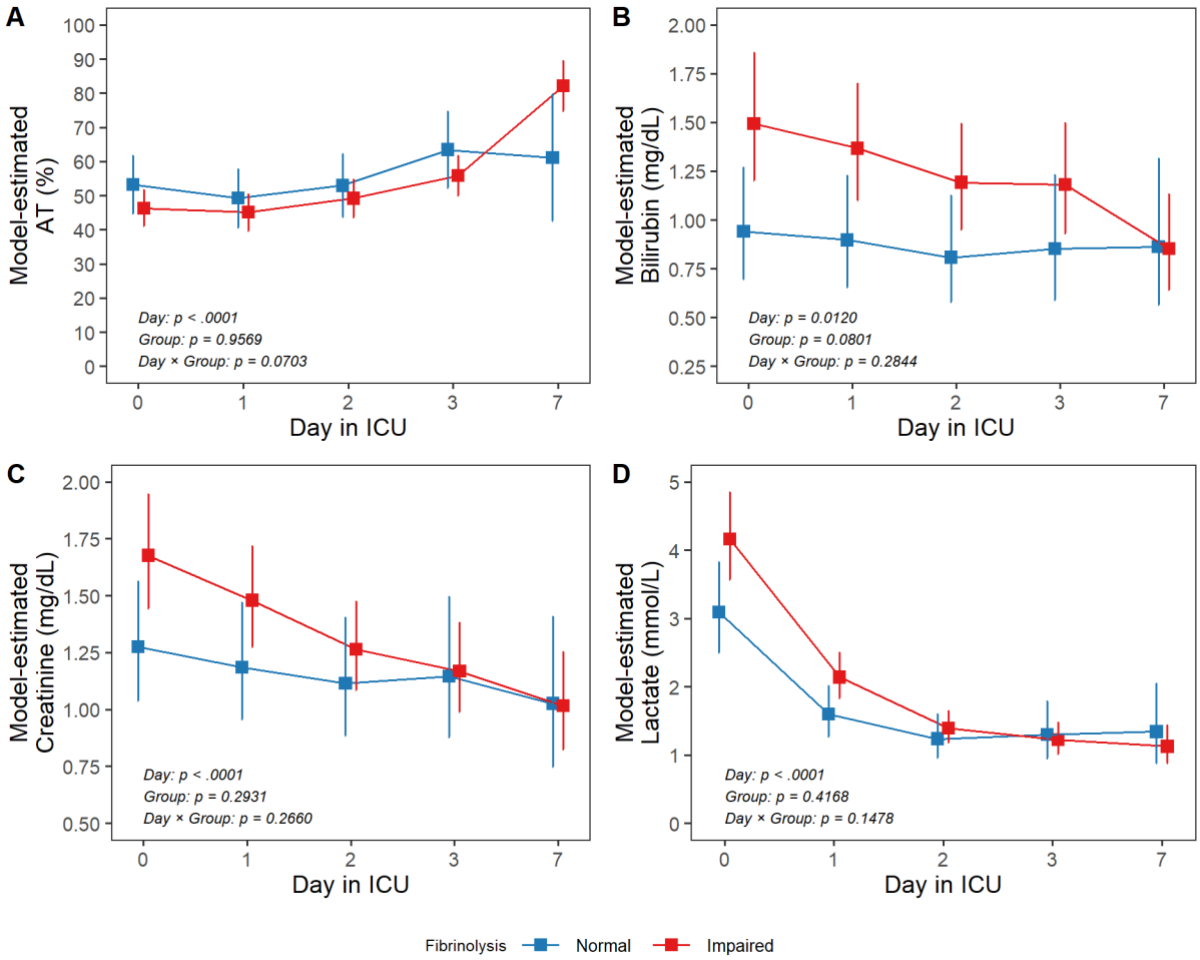


Table for Supplementary Figure 3 and Figure 2

	AT (%)		Bilirubin (mg/dL)	
Fixed effect				
	Statistic F°	**p-value**	**Statistic F°**	**p-value**
Time	9.02	<.0001	3.29	0.0120
Group	0.00	0.9569	3.09	0.0801
Time × Group	2.22	0.0703	1.27	0.2844
Estimated marginal means				
	Normal LS Mean (95%CI)	**Impaired LS Mean (95%CI)**	**Normal LS Mean (95%CI)**	**Impaired LS Mean (95%CI)**
Day 0	53.20 (44.81 – 61.59)	46.31 (40.93 – 51.70)	0.94 (0.70 – 1.27)	1.49 (1.20 – 1.86)
Day 1	49.21 (40.62 – 57.79)	45.07 (39.72 – 50.42)	0.90 (0.66 – 1.23)	1.37 (1.10 – 1.70)
Day 2	52.92 (43.64 – 62.20)	49.16 (43.59 – 54.73)	0.81 (0.58 – 1.12)	1.19 (0.95 – 1.49)
Day 3	63.42 (52.31 – 74.53)	55.89 (50.05 – 61.73)	0.85 (0.59 – 1.23)	1.18 (0.93 – 1.50)
Day 7	61.15 (42.58 – 79.71)	82.17 (74.81 – 89.53)	0.86 (0.56 – 1.32)	0.85 (0.64 – 1.13)

	Creatinine (mg/dL)		D-dimer (ng/mL)	
Fixed effect				
	Statistic F°	**p-value**	**Statistic F°**	**p-value**
Time	6.56	<.0001	0.67	0.6131
Group	1.11	0.2931	5.49	0.0201
Time × Group	1.31	0.2660	0.35	0.8457
Estimated marginal means				
	Normal LS Mean (95%CI)	**Impaired LS Mean (95%CI)**	**Normal LS Mean (95%CI)**	**Impaired LS Mean (95%CI)**
Day 0	1.27 (1.04 – 1.56)	1.68 (1.45 – 1.94)	3,747.94 (2,498.69 – 5,621.79)	7,262.48 (5,443.51 – 9,689.27)*
Day 1	1.19 (0.96 – 1.47)	1.48 (1.27 – 1.72)	4,314.08 (2,838.74 – 6,556.18)	7,545.07 (5,656.91 – 10,063.00)*
Day 2	1.11 (0.88 – 1.40)	1.26 (1.08 – 1.47)	4,615.72 (2,939.84 – 7,246.97)	7,052.27 (5,237.28 – 9,496.26)
Day 3	1.14 (0.88 – 1.49)	1.17 (0.99 – 1.38)	3,553.93 (2,115.30 – 5,970.97)	6,781.77 (4,908.18 – 9,370.57)*
Day 7	1.03 (0.75 – 1.41)	1.02 (0.83 – 1.25)	3,723.12 (1,942.40 – 7,136.35)	5,865.58 (3,913.63 – 8,791.07)

	Fibrinogen (g/L)		INR	
Fixed effect				
	Statistic F°	**p-value**	**Statistic F°**	**p-value**
Time	5.21	0.0005	24.68	<.0001
Group	2.24	0.1357	0.52	0.4722
Time × Group	0.13	0.9707	1.28	0.2804
Estimated marginal means				
	Normal LS Mean (95%CI)	**Impaired LS Mean (95%CI)**	**Normal LS Mean (95%CI)**	**Impaired LS Mean (95%CI)**
Day 0	4.02 (3.18–4.85)	4.75 (4.15–5.35)	1.33 (1.23 – 1.44)	1.46 (1.38 – 1.54)
Day 1	4.91 (4.01–5.81)	5.53 (4.92–6.14)	1.34 (1.23 – 1.46)	1.41 (1.33 – 1.49)
Day 2	5.04 (4.03–6.04)	6.01 (5.37–6.65)	1.16 (1.05 – 1.27)	1.15 (1.08 – 1.22)
Day 3	5.21 (4.01–6.42)	5.99 (5.27–6.71)	1.09 (0.97 – 1.22)	1.06 (0.99 – 1.13)
Day 7	4.90 (3.31–6.48)	5.31 (4.37–6.26)	1.10 (0.94 – 1.28)	1.17 (1.07 – 1.27)

	Lactate (mmol/L)		C-reactive protein (mg/dL)	
Fixed effect				
	Statistic F°	**p-value**	**Statistic F°**	**p-value**
Time	55.44	<.0001	20.45	<.0001
Group	0.66	0.4168	8.14	0.0047
Time × Group	1.71	0.1478	2.00	0.0954
Estimated marginal means				
	Normal LS Mean (95%CI)	**Impaired LS Mean (95%CI)**	**Normal LS Mean (95%CI)**	**Impaired LS Mean (95%CI)**
Day 0	3.09 (2.50 – 3.82)	4.16 (3.57 – 4.85)	16.76 (12.62 – 22.28)	23.87 (19.51 – 29.22)*
Day 1	1.60 (1.27 – 2.01)	2.14 (1.83 – 2.50)	21.78 (16.14 – 29.40)	31.59 (25.75 – 38.75)*
Day 2	1.24 (0.95 – 1.60)	1.39 (1.18 – 1.64)	17.88 (12.82 – 24.94)	25.78 (20.82 – 31.93)
Day 3	1.30 (0.95 – 1.78)	1.22 (1.02 – 1.47)	14.85 (9.96 – 22.13)	16.85 (13.28 – 21.37)
Day 7	1.34 (0.88 – 2.05)	1.12 (0.88 – 1.44)	4.37 (2.67 – 7.14)	12.05 (8.76 – 16.59)*

	Platelets (10^3/mm^3)	
Fixed effect		
	Statistic F°	***p-value***
Time	*25.30*	*<.0001*
Group	*2.90*	*0.0898*
Time × Group	*2.32*	*0.0574*
Estimated marginal means		
	Normal LS Mean (95%CI)	***Impaired LS Mean (95%CI)***
Day 0	*134.94 (97.38 – 186.99)*	*120.24 (94.87 – 152.39)*
Day 1	*101.29 (72.22 – 142.08)*	*87.42 (68.93 – 110.86)*
Day 2	*92.09 (64.53 – 131.40)*	*68.13 (53.42 – 86.89)*
Day 3	*107.63 (72.70 – 159.34)*	*64.00 (49.51 – 82.72)*
Day 7	*260.84 (166.78 – 407.96)*	*143.46 (106.61 – 193.04)*

° F-tests for the fixed effects of time (categorical) were obtained from a linear mixed-effects model with a random intercept for each patient and an unstructured covariance matrix. Each dependent variable was modeled longitudinally across days 0, 1, 2, 3, and 7. Variables violating normality assumptions were log-transformed prior to analysis (bilirubin, creatinine, D-dimer, INR, lactate, C-reactive protein, platelets), and back-transformed least-squares means with 95% CIs are presented. Pairwise comparisons between groups at each timepoint were performed when Group effect or interaction term was statistically significant; *p<.05.
Abbreviations: CI = confidence interval; LS = least-squares.

Supplementary Table 3. Characteristics of control patients.

	Postoperative Controls
N (%)	30 (100.00)
Males, n (%)	12 (40.00)
Age (years), mean ± SD	69.2 ± 12.1
Weight (kg), mean ± SD	67.6 ± 16.9
Height (cm), mean ± SD	164.3 ± 9.2
BMI (kg/m^2), mean ± SD	24.9 ± 5.6
Type of surgery, n (%)	
Elective	25 (83.33)
Urgent / Emergent	5 (16.67)
Diseases, n (%)	
Cardiovascular	16 (53.33)
Respiratory	10 (33.33)
Neurologic	2 (6.67)
Gastro/Liver	5 (16.67)
Kidney	4 (13.33)
Haematologic	2 (6.67)
Neoplastic	5 (16.67)
Metabolic	14 (46.67)
Other	12 (40.00)
Use of drugs, n (%)	
Antiplatelet	8 (26.67)
ASA class, n (%)	
1	1 (3.57)
2	14 (50.00)
3	13 (46.43)
4	0 (0.00)
SOFA day 0, mean ± SD	1.9 ± 2.0
Outcomes	
Mortality, n (%)	0 (0.00)
LOS ICU (days), median [IQR]	1 [1 – 1]
Duration MV (days), median [IQR]	1 [1 – 1.5]

Abbreviations. ASA = American Society of Anesthesiologists physical status classification; BMI = body mass index; ICU = intensive care unit; IQR = interquartile range [1st quartile – 3rd quartile]; LOS = length of stay; MV = mechanical ventilation; SD = standard deviation; SOFA = sequential organ failure assessment. ASA class was not available for two patients; percentages refer to the 28 patients with available data.