Balkan Med J 2023;40:244-51

Content of this journal is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Sepsis-induced Coagulopathy Subphenotype Identification by Latent Class Analysis

Dan Cai[1], Massimiliano Greco[2,3], Qin Wu[1], Yisong Cheng[1]

[1]Department of Critical Care Medicine, West China Hospital, Sichuan University, Sichuan, China
[2]Biomedical Sciences, Humanitas University, Pieve Emanuele, Italy
[3] Department of Anesthesia and Intensive Care, IRCCS Humanitas Research Hospital, Milan, Italy

Background: Recent studies have shown that anticoagulant therapy has heterogeneous treatment effects on patients with sepsis-induced coagulopathy (SIC).

Aims: To identify the latent phenotypes of patients with SIC.

Study design: Retrospective cohort study.

Methods: We obtained data of patients with SIC from the Medical Information Mart for Intensive Care IV database. SIC subphenotypes were identified by latent class analysis (LCA) and K-means clustering. Clinical and laboratory variables were obtained in patients who met the diagnostic criteria for SIC. The baseline characteristics of the patients and the association between the heterogeneity of anticoagulant therapy and clinical outcomes (28-day and in-hospital mortality) were compared between the subphenotypes.

Results: We identified 4,993 patients with SIC. The LCA and K-means clustering analysis robustly identified three subphenotypes of SIC. Class 1 patients (n = 1,808) had the lowest blood cell counts (leukocytes, erythrocytes, and platelets). Class 2 patients (n = 1,157) had severe coagulopathy with a high prothrombin time and international normalized ratio, multiple-organ dysfunction, high lactate, sequential organ failure assessment score, and mortality. Class 3 (n = 2,028) were older, had more comorbidities, a higher fibrinogen concentration, and lower plasma and platelet infusion rates. After variable adjustments, heparin therapy reduced the 28-day mortality (odds ratio [OR] 0.39, 0.30-0.49, $p < 0.001$) and in-hospital mortality (OR 0.42, 0.33-0.53, $p < 0.001$) only in class 2.

Conclusion: Three SIC subphenotypes were defined using clinical findings and laboratory variables. The effects of heparin treatment differ between the subphenotypes. This finding will facilitate the identification of target patients with SIC who should receive anticoagulant therapy.

INTRODUCTION

Sepsis is the leading cause of mortality and morbidity and remains a remarkable adversary to the intensive care unit (ICU).[1,2] Coagulation disorder is a major manifestation of sepsis induced by infection and acute systemic inflammatory response that results in endothelial injury.[3,4] Sepsis-induced coagulopathy (SIC) is the coagulation disturbance of sepsis and is defined by the prothrombin time (PT)/international normalized ratio (INR) as well as platelet count, together with the sequential organ failure assessment (SOFA) score.[5] Retrieving coagulation abnormalities in patients with SIC is important; however, current evidence reveals that the effects of anticoagulation therapy are controversial. Moreover, the Surviving Sepsis Campaign does not provide any specific anticoagulation recommendations.[1]

Sepsis is a highly heterogeneous syndrome with different etiologies and pathophysiologies.[6] The effectiveness of anticoagulant therapy in patients with SIC is controversial. Some therapies may benefit certain phenotypes; however, other phenotypes might be affected by the intervention, resulting in a neutral effect in all patients. Several studies have reported that anticoagulant therapy may improve outcomes in patients with SIC.[7,8] Another study showed that only the high-risk group benefit but not low-to-moderate-risk subgroups.[9] However, a phase III randomized controlled clinical trial revealed that recombinant human thrombomodulin (rhTM) did

Corresponding author: Qin Wu, Department of Critical Care Medicine, West China Hospital, Sichuan University, Chengdu, China
E-mail: qinwu0221@gmail.com
Yisong Cheng, Critical Care Medicine, West China Hospital, Sichuan University, Sichuan, China
e-mail: yisongcheng01@163.com

Received: April 05, 2023 Accepted: May 23, 2023 Available Online Date: July 12, 2023 • DOI: 10.4274/balkanmedj.galenos.2023.2023-4-6

Available at www.balkanmedicaljournal.org

ORCID iDs of the authors: D.C. 0000-0002-5183-0587; M.G. 0000-0003-1003-4637; Q.W. 0000-0002-6585-941X; Y.C. 0000-0002-5183-0587.

Cite this article as:
Cai D, Greco M, Wu Q, Cheng Y. Sepsis-induced Coagulopathy Subphenotype Identification by Latent Class Analysis. *Balkan Med J.*; 2023; 40(4):244-51.

Copyright@Author(s) - Available online at http://balkanmedicaljournal.org/

not significantly reduce the 28-day mortality rate in patients with sepsis.[10] In a multicenter registry study, 3,195 patients with severe sepsis or septic shock were classified into four phenotypes. rhTM was only associated with lower 28-day mortality and in-hospital mortality rates in one of the phenotypes.[11] Thus, identifying the distinct therapeutic phenotypes of SIC is essential for targeted anticoagulant therapy.

This study aimed to identify the subphenotypes of SIC based on clinical and laboratory variables. Thus, we applied unsupervised consensus clustering and examined which subphenotype of SIC would benefit most from anticoagulant therapy using retrospective data from a large public database.

MATERIALS AND METHODS

Data Source

The Medical Information Mart for Intensive Care IV (MIMIC-IV) database was used to identify patients with SIC. MIMIC-IV is a longitudinal single-center public free database that includes data of more than 40,000 patients admitted to the ICU and 11,263 patients with sepsis (Sepsis-3 definition) at the Beth Israel Deaconess Medical Center from 2008 to 2019.[12] All patients remained anonymous, and informed consent was approved by original ethical committee (Massachusetts Institute of Technology, No. 0403000206; Beth Israel Deaconess Medical Center, 2001P001699).

Study Population

Initially, the study enrolled patients with sepsis who were admitted to the ICU. Sepsis was defined as suspected or confirmed infection plus an increase in the SOFA score of ≥ 2.[13] Then, we enrolled patients with SIC by calculating the PT, platelet count, and SOFA score after ICU admission for each patient, and a total score of ≥ 4 was used to diagnose SIC (Supplemental Table S1).[5] Patients who met the following criteria were excluded: pregnancy, age of < 18 years, ICU stay of < 24 h, and $\geq 20\%$ missing values.

Variables

Clinical and laboratory variables were collected after the diagnosis of SIC. Baseline and demographic variables included age, sex, weight, comorbidities, time of ICU admission, and length of ICU stay. Vital signs including heart rate, body temperature, respiratory rate, blood pressure, and blood oxygen saturation were measured. Laboratory indicators included pH, PO_2, PCO_2, HCO_3, $PaCO_2$, base excess, lactate, hemoglobin, hematocrit (HCT), red blood cells (RBCs), white blood cells (WBCs), RBC, distribution width, mean corpuscular hemoglobin (MCH), platelets, lymphocyte, albumin, alanine aminotransferase (ALT), creatinine, blood urea nitrogen (BUN), and electrolytes. Coagulation variables included fibrinogen, PT, INR, and partial thromboplastin time (PTT). Risk scores, including the SOFA score and the simplified acute physiology score (SAP III), were calculated the day after diagnosis of SIC. Anticoagulant therapy included anticoagulants, heparin, plasma infusion, and platelet infusion. Other treatment and prognosis data were also obtained from the database.

Missing values were imputed by first applying the next observation carried backward (NOCB) method, followed by the last observation carried forward (LOCF) method.[14] Briefly, we preferentially used the observations after the timepoint when SIC was diagnosed, and if the observation was still missing, it was imputed with the last observation value before that timepoint. If the missing value was not available from the database, the missing value was imputed by multiple imputations using the MICE package of R (Supplemental Table 2S).[15]

SIC Subphenotypes

SIC subphenotypes were explored by the latent class analysis (LCA) and K-means clustering. Clinical and laboratory variables representing key pathophysiological domains were evaluated as class-defining variables, including baseline characteristics (age and heart rate), organ dysfunction severity (SOFA and SAP III scores), blood gas analysis (pH, PO_2, and lactate), coagulation indicators (fibrinogen, INR, and PTT), hematology (WBCs, RBCs, hemoglobin, MCH, and platelets), and liver and renal functions (ALT, BUN, and creatinine). Correlations between variables were evaluated by Pearson's correlation analysis, and highly correlated variables (> 0.7) were excluded, including PT, AST concentration, and total bilirubin (TBIL) concentration (Supplemental Figure 1S).

LCA is one of the probabilistic finite-mixture modeling algorithms that allows the determination of unmeasured or unobserved groups within the population.[16] During model training, the parameters were estimated based on maximum likelihood estimation. For the LCA, the basic approach was to select the model with the fewest classes that best fitted the data. A lower Akaike information criterion (AIC), sample size adjusted Bayesian information criterion (SABIC), and higher entropy were considered a good fit. In addition, the bootstrapped likelihood ratio test was conducted to compare whether the *k* class was better than the *k-1* class.[17]

We also determined the optimal number of clusters using a consensus K-means clustering approach. With K-means clustering, the separation of consensus matrix heatmaps was evaluated using the cumulative distribution function of the elbow method and cluster consensus plots. Statistical indices such as the Calinski-Harabasz (CH) index, Hartigan index, cubic clustering criterion (CCC), Scott index, Davies and Bouldin (DB) index; and the Rubin and Beale index were reported using the NbClust package.[18] Visual clustering was also performed using t-distributed stochastic neighbor embedding (t-SNE) to reduce the dimensions and visualize in the lower dimensional space.[19] The number of clusters was determined using the elbow and matrix heatmaps.[20]

Statistical Analysis

We described and compared the frequency and clinical characteristics of each class using the analysis of variance or Kruskal-Wallis test for numeric variables and the chi-square test or Fisher's exact test for categorical variables. Thereafter, in each class, the relationship between anticoagulant therapy (anticoagulants, heparin, plasma, and platelet infusion) and clinical outcomes (28 days and in-hospital mortality) was explored using

the logistic regression analysis. The adjusted variables were age, heart rate, systolic blood pressure, hypertension, diabetes mellitus, SOFA score, fibrinogen, INR, hemoglobin, platelets, WBCs, creatinine, and lactate.

A *p*-value of < 0.05 was considered statistically significant. All analyses were performed using Stata version 14.1 (StataCorp., College Station, TX, USA) and R version 3.6.2 (R Foundation, Vienna, Austria).

RESULTS

Patients' Baseline Characteristics

Of the 11,263 patients with sepsis who were admitted to the ICU, SIC developed in 4,993, who were enrolled in this study. The mean age was 67 years, and 58.7% were male. The rates of hypertension, diabetes mellitus, chronic obstructive pulmonary disease (COPD), and cancer were 32.4%, 34.8%, 26.6%, and 19.4%, respectively. Overall, anticoagulants and heparin were administered to 14.1% and 51.5% of the patients, respectively, and the plasma and platelet infusion rates were 25.2% and 16.5%, respectively. The in-hospital mortality and 28-day mortality rates were 23.3% and 31.0%, respectively (Table 1).

SIC Subphenotypes

Overall, 18 features (age, heart rate, SOFA score, APS III score, fibrinogen, INR, PTT, platelets, hemoglobin, MCH, WBCs, RBCs, BUN, creatinine, ALT, pH, PO_2, and lactate) were included in the subphenotype analysis (correlation analysis in Supplemental Figure 1S). Generally, the AIC and SABIC values declined from class 2 to class 9; however, class 3 had the highest entropy value (0.92) among all classes in the LCA model (Table 2). Similar results were observed for the K-means clustering analysis. The elbow method also showed that the decline in the slope of the sum of the square errors was the greatest in class 3 (Figure 1a). The matrix heatmaps of K-means clustering showed the overall samples divided into three classes (Figure 1b). The three-class clusters were also confirmed by K-means clustering, such as the CH index, Hartigan index, CCC index, and DB index (Supplemental Figure 2S), as well as hierarchical clustering (Supplemental Figure 3S). Thus, the three-class model was considered the best model by the LCA and K-means clustering. Then, we used t-SNE to reduce the dimensionality of the features and visualize the outputs. Each dot represents a patient that displayed clusters within the dimensionally reduced and scaled down feature space of the autoencoder embedding (Figure 2).

SIC Characteristics Among Subphenotypes

Figure 3 shows the characteristics of the three classes, and Table 1 presents the statistical comparisons. Class 1 (n = 1,808) had the lowest proportion of men (54.9%) and the highest rate of cancer (28.4%) and lowest body mass index, WBCs, RBCs, hemoglobin, HCT, platelets, INR, PT, TBIL, and creatinine. Class 2 (n = 1,157) was characterized by severe coagulopathy and multiple-organ dysfunction, had the highest INR, PT, PTT, TBIL, creatinine, lactate, and SOFA and SAP III scores, and had the highest rates of CRRT, vasopressin, and anticoagulant use; however, it still had the highest in-hospital mortality (48.1%) and 28-day mortality (55.9%). Class 3 was the largest (n = 2,028) and was characterized by older age and higher rates of comorbidities (hypertension, diabetes, and COPD), highest RBC and platelet counts, highest fibrinogen concentration, and lowest plasma and platelet infusion rates.

Effect of Anticoagulant Therapy in Subphenotypes with Outcomes

Class 2 had significantly higher 28-day mortality and in-hospital mortality rates than the other classes. Moreover, class 2 received anticoagulants and plasma and platelet infusion more than the other classes. As shown in Table 3, in the unadjusted analysis, most anticoagulant therapies (anticoagulants, heparin, and plasma and platelet infusions) were risk factors for 28-day mortality and in-hospital mortality (odds radio [OR] > 1, $p < 0.05$) in all three classes, except for heparin therapy in classes 1 and 2, which was associated with a reduced risk of 28-day and in-hospital mortality

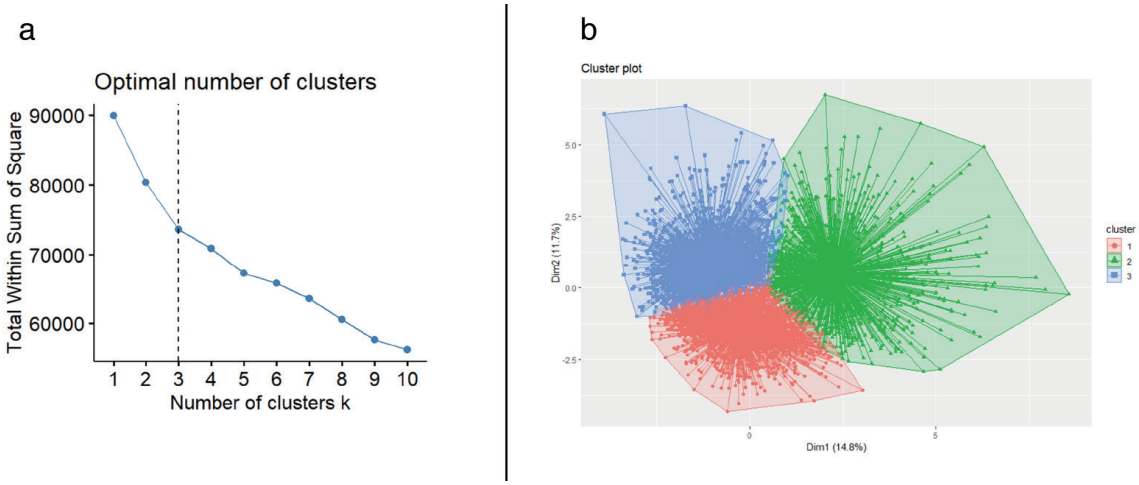

FIG. 1. Elbow methods (a) and matrix heatmaps (b) of K-means clustering. (1a) The sum of the squared errors decreases sharply when k = 3, representing 3 is the best clusters. (1b) Distance from the overall samples to the cluster center.

TABLE 1. Baseline Characteristics of Clinical and Laboratory According Class.

Variables	Total (n = 4,993)	Class 1 (n = 1,808)	Class 2 (n = 1,157)	Class 3 (n = 2,028)	P*
Age, years	67.57 ± 15.69	64.75 ± 15.68	64.06 ± 15.17	72.11 ± 14.88	< 0.001
Male, n (%)	2,930 (58.7)	992 (54.9)	708 (61.2)	1,230 (58.7)	< 0.001
BMI, kg/m^2	29.47 ± 8.89	28.40 ± 7.90	30.44 ± 9.19	29.68 ± 9.35	< 0.001
Heart rate, bpm	95.13 ± 21.00	95.26 ± 20.04	97.65 ± 22.05	93.60 ± 21.11	< 0.001
SBP, mmHg	106.56 ± 19.55	106.41 ± 18.84	104.51 ± 20.38	107.90 ± 19.58	0.002
DBP, mmHg	58.29 ± 14.23	58.27 ± 13.06	56.74 ± 15.09	59.22 ± 14.64	0.002
Comorbidity, n (%)					
Hypertension	1,618 (32.4)	557 (30.8)	291 (25.2)	770 (38.0)	< 0.001
Diabetes	1,737 (34.8)	561 (31.0)	427 (36.9)	749 (36.9)	< 0.001
COPD	1,327 (26.6)	461 (25.5)	278 (24.0)	588 (29.0)	0.004
Malignant cancer	969 (19.4)	513 (28.4)	173 (15.0)	283 (14.0)	< 0.001
WBC, *10^9/l	12.40 (7.50 - 19.00)	9.10 (5.20 - 14.55)	13.95 (8.10 - 21.70)	14.70 (10.10 - 20.60)	< 0.001
RBC, *10^9/l	3.28 ± 0.73	2.75 ± 0.44	3.19 ± 0.69	3.79 ± 0.59	< 0.001
Hemoglobin, g/dl	9.76 ± 2.03	8.46 ± 1.34	9.69 ± 2.00	10.93 ± 1.84	< 0.001
Hematocrit, %	30.16 ± 6.16	25.92 ± 3.84	30.04 ± 6.13	33.97 ± 5.31	< 0.001
RDW, %	16.58 ± 2.71	16.93 ± 2.82	17.13 ± 2.78	15.96 ± 2.44	< 0.001
MCH, pg	30.03 ± 3.12	30.90 ± 2.94	30.67 ± 3.15	28.90 ± 2.90	< 0.001
MCV, fl	92.87 ± 8.60	94.79 ± 8.52	95.12 ± 8.91	89.92 ± 7.57	< 0.001
Platelet, *10^9/l	131.00 (81.00 - 215.00)	101.00 (61.00 - 149.00)	107 (66.00 - 173.50)	185.00 (123.00 - 288.00)	< 0.001
Fibrinogen, IU/l	299.00 (165.00 - 474.00)	294.00 (168.25 - 443.75)	194.00 (129.00 - 345.00)	464.00 (302.75 - 623.50)	< 0.001
INR	2.17 ± 1.32	1.82 ± 0.74	2.67 ± 1.84	2.18 ± 1.27	< 0.001
PT, s	18.70 (16.00 - 25.20)	17.30 (15.10 - 21.50)	22.30 (17.50 - 31.18)	18.60 (16.30 - 24.70)	< 0.001
PTT, s	36.90 (31.40 - 47.900)	35.70 (30.60 - 44.30)	44.50 (35.10 - 60.50)	35.50 (30.90 - 43.2)	< 0.001
Total bilirubin, K/µL	1.20 (0.60 - 3.10)	1.30 (0.50 - 3.20)	2.40 (0.90-7.30)	0.90 (0.50 - 1.90)	< 0.001
ALT, IU/l	34.00 (18.00 - 91.00)	29.00 (16.00 - 60.00)	55.00 (23.75 - 256.00)	32.00 (17.00 - 87.00)	< 0.001
AST, IU/l	54.00 (27.00 - 133.00)	44.00 (25.00 - 90.00)	113.00 (47.00 - 467.00)	46.00 (24.00 - 111.00)	< 0.001
BUN, mg/dl	30.00 (19.00 - 49.00)	24.00 (15.00 - 39.00)	53.00 (35.00 - 78.00)	28.00 (18.00 - 43.00)	
Creatinine, mg/dl	1.40 (0.90 - 2.40)	1.10 (0.70 - 1.70)	2.90 (1.90 - 4.50)	1.30 (0.90 - 1.90)	< 0.001
Albumin, mEq/l	2.70 ± 0.63	2.61 ± 0.62	2.72 ± 0.75	2.76 ± 0.53	0.002
CK-MB, mg/dl	5.00 (2.00 - 11.00)	3.00 (2.00 - 7.00)	7.00 (4.00 - 20.00)	4.00 (2.00 - 9.00)	< 0.001
Troponin t, mg/dl	0.10 (0.04 - 0.28)	0.09 (0.04 - 0.22)	0.14 (0.06 - 0.41)	0.09 (0.04 - 0.25)	< 0.001
CRP	109.50 (54.05 - 186.15)	109.55 (51.68 - 182.38)	102.35 (50.43 - 175.15)	113.10 (58.80 - 211.85)	0.261
PH	7.33 ± 0.11	7.38 ± 0.08	7.25 ± 0.12	7.34 ± 0.09	< 0.001
PO_2, mmHg	74.00 (44.00 - 118.00)	71.00 (43.00 - 114.50)	80.00 (48.00 - 129.00)	73.00 (43.00 - 113.00)	< 0.001
PCO_2, mmHg	39.00 (33.00 - 46.00)	38.00 (32.25 - 45.00)	39.00 (32.00 - 47.00)	41.00 (35.00 - 48.00)	< 0.001
Lactate, mmol/l	2.10 (1.40 - 3.60)	1.80 (1.20 - 2.80)	3.80 (2.20 - 7.00)	1.90 (1.40 - 2.90)	< 0.001
Sodium, mmol/l	137.99 ± 6.30	137.78 ± 5.78	137.25 ± 7.30	138.59 ± 6.08	< 0.001
Potassium, mmol/l	4.19 ± 0.80	3.99 ± 0.67	4.55 ± 0.95	4.16 ± 0.75	< 0.001
CRRT, n (%)	716 (14.3)	140 (7.7%)	427 (36.9)	149 (7.3)	< 0.001
Vasopressin, n (%)	3462 (69.3)	1120 (61.9)	1006 (86.9)	1336 (65.9)	< 0.001
Anticoagulants, n (%)	703 (14.1)	152 (8.4)	330 (28.5)	221 (10.9)	< 0.001
Heparin, n (%)	2570 (51.5)	866 (47.9)	552 (47.7)	1152 (56.8)	< 0.001
Plasma infusion, n (%)	1259 (25.2)	383 (21.2)	525 (45.4)	351 (17.3)	< 0.001
Platelet infusion, n (%)	824 (16.5)	373 (20.6)	354 (30.6)	97 (4.8)	< 0.001
APSIII	70.00 (53.00 - 94.00)	63.00 (49.00 - 82.00)	101.00 (82.00 - 120.00)	62.00 (48.00 - 81.00)	< 0.001
SOFA score	4.00 (3.00 - 6.00)	4.00 (3.00 - 5.00)	6.00 (4.00 - 9.00)	3.00 (2.00 - 4.00)	< 0.001
Hospital length, day	10.22 (5.83 - 19.32)	11.21 (6.79 - 21.47)	10.54 (3.05 - 21.39)	9.59 (5.74 - 16.89)	< 0.001
ICU length, day	3.45 (1.83 - 7.75)	3.18 (1.82 - 6.86)	4.65 (1.90 - 10.02)	3.20 (1.82 - 6.95)	< 0.001
In-hospital mortality	1,161 (23.3)	296 (16.4)	556 (48.1)	309 (15.2)	< 0.001
28-day mortality	1,546 (31.0)	457 (25.3)	647 (55.9)	442 (21.8)	< 0.001
90-day mortality	1,766 (35.4)	542 (30.0)	695 (60.1)	529 (26.1)	< 0.001

ALT, alanine aminotransferase A; APSIII, acute physiological score; BMI, body mass index; BUN, blood urea nitrogen; CK-MB, creatine kinase isoenzyme; COPD, chronic obstructive pulmonary disease; CRP, C-reaction protein; CRRT, continuous renal replacement therapy; DBP, diastolic blood pressure; ICU, intensive care unit; INR, international normalized ratio; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; PT, prothrombin time; PTT, partial thromboplastin time; RBC, red blood cell; RDW, erythrocyte distribution width; SBP, systolic blood pressure; SOFA, sequential organ failure assessment; ST, aspartate aminotransferase; WBC, white blood cell. *P between the clusters.

(OR < 1, $p < 0.05$). However, after variable adjustment, only class 2 benefited from heparin therapy, which reduced 28-day mortality (OR 0.39, 0.30-0.49, $p < 0.001$) and in-hospital mortality (OR 0.42, 0.33-0.53, $p < 0.001$).

DISCUSSION

In this study, we identified three SIC subphenotypes that showed distinct clinical and laboratory characteristics by the LCA, and the results were also confirmed by K-means clustering. The effects of anticoagulants varied by treatment and subphenotypes. Only class 2 benefited from heparin therapy, which reduced 28-day mortality and in-hospital mortality. These findings have important implications to understand the heterogeneity of SIC and inform future works to promote optimal anticoagulant therapy across subphenotypes.

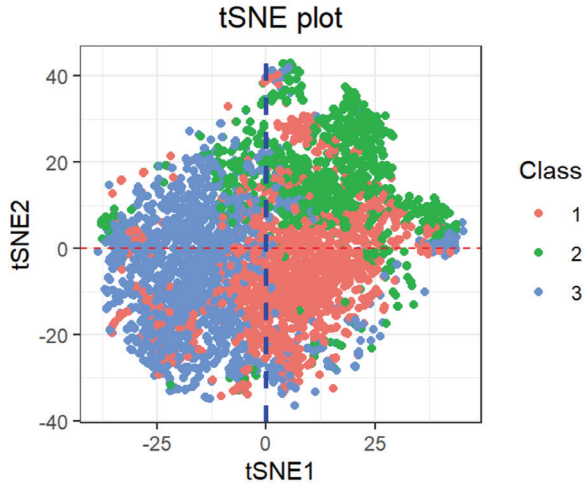

FIG. 2. t-distributed stochastic neighbor embedding (t-SNE) plot. The t-SNE is a dimensionality reduction for graphically simplifying dataset. Each dot represents a patient who displayed clusters within the dimensionally reduced and scaled down feature space of the autoencoder embedding (red, Class 1; green, Class 2; blue, Class 3).

The present study confirms previous findings that specific therapies confer benefits only in patients with specific sepsis phenotypes.[11,21,22] For example, Joseph et al. identified four sepsis phenotypes with different anti-inflammatory responses using 25 bedside variables. They analyzed heterogeneous treatment interactions and mortality risks among these phenotypes and found that one phenotype had a lower mortality rate than other phenotypes when treated with combined immunoglobulin G and methylprednisolone.[23] Activated protein C, a toll-like receptor 3 antagonist, and fluid input had different effects on each phenotype.[24] In clinical practice, the goal of precision medicine is to choose the optimal therapy for each patient, for which machine learning-based clustering for optimal therapy is an effective method.[25,26] Although the present study does not fully address the biological or pathophysiological mechanism-defined endotype of coagulation in sepsis, the findings improve the understanding of SIC subphenotypes.

The classification appears to be stable in the present study because both the LCA and K-means clustering obtained the same optimal number of classes, and the minimum and maximum class membership probabilities were 0.89 and 0.98, respectively. Our results have some similarities and differences with those of a previous study showing that sepsis can be classified into four phenotypes only with coagulation features and that rhTM therapy is associated with better outcomes only in the phenotype characterized by a low platelet count, high fibrin degradation product and D-dimer concentrations, and severe dysfunction.[11] This study also showed that only the phenotype with severe coagulopathy and organ dysfunction (Class 2) benefited from heparin therapy; however, we aimed to identify the key and common variables of the underlying latent phenotypes of SIC using clinical and laboratory

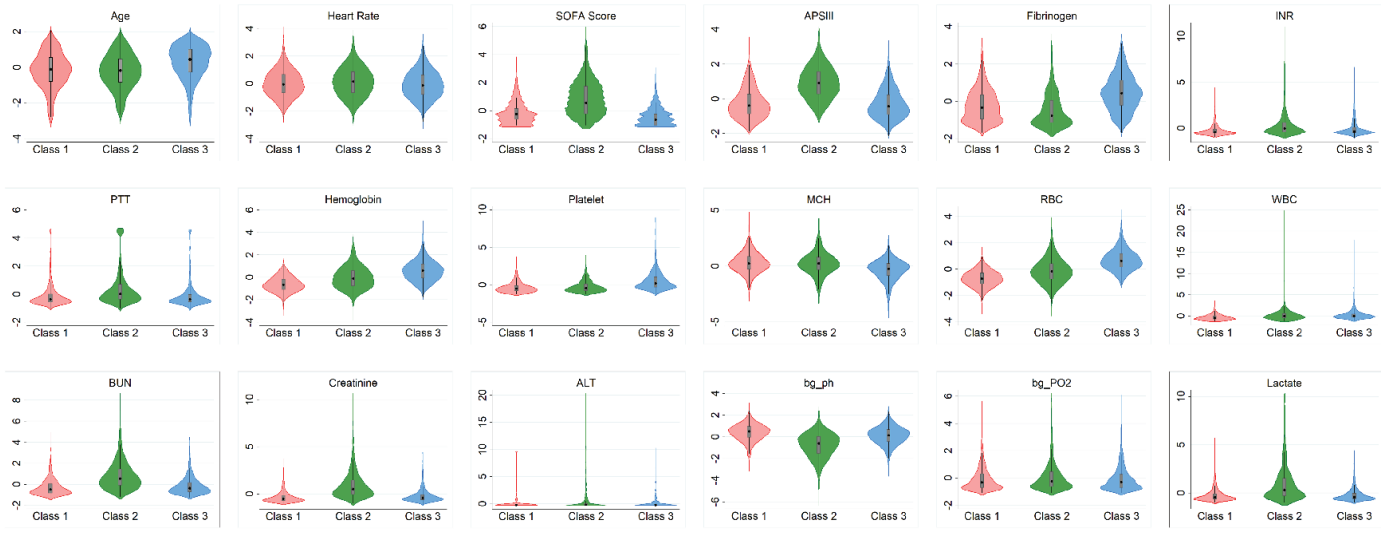

FIG. 3. Violin plots for the median value of 18 features enrolled in the clustering approach. Top 18 features with the largest differences within the three subphenotypes. Class 1 is represented in pink; Class 2, green; and Class 3, blue.

TABLE 2. Best Number of Classes by Latent Class Analysis.

Classes	AIC	SABIC	Entropy	prob_min	prob_max	n_min	n_max	BLRT_p
2	250533.9	250892.3	0.83	0.87	0.97	0.2	0.8	0.01
3	246806.7	247288.9	0.92	0.89	0.98	0.07	0.78	0.01
4	244608.6	245214.5	0.82	0.87	0.97	0.05	0.48	0.01
5	242673.7	243403.5	0.84	0.85	0.95	0.06	0.43	0.01
6	238154.4	239008	0.85	0.85	1	0.01	0.4	0.01
7	240189.2	241166.6	0.84	0.82	0.94	0.03	0.38	0.01
8	234370.6	235471.7	0.85	0.8	1	0.01	0.34	0.01
9	232528.4	233753.3	0.87	0.82	1	0.01	0.42	0.01
10	233624.4	234973.1	0.85	0.8	0.99	0.01	0.29	0.01

AIC, Akaike information criteria; BLTR, bootstrapped likelihood ratio test; SABIC, sample size adjusted Bayesian information criteria.

TABLE 3. Associations Between Anticoagulation and Procoagulation Treatment with in-Hospital and 28-Day Mortality.

	Class 1		Class 2		Class 3	
28-day mortality	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Anticoagulants						
Unadjusted	2.41 (1.71 - 3.39)	< 0.001	0.86 (0.69 - 1.12)	0.263	2.27 (1.68 - 3.01)	< 0.001
Adjusted	5.28 (1.41 - 19.71)	0.013	0.72 (0.37 - 1.37)	0.313	5.61 (0.89 - 35.4)	0.067
Heparin						
Unadjusted	0.62 (0.50 - 0.77)	< 0.001	0.39 (0.30 - 0.49)	< 0.001	1.28 (1.04 - 1.59)	0.023
Adjusted	0.62 (0.28 - 1.37)	0.237	0.21 (0.10 - 0.43)	< 0.001	1.06 (0.34 - 3.26)	0.926
Plasma infusion						
Unadjusted	2.14 (1.68 - 2.73)	< 0.001	1.81 (1.43 - 2.29)	< 0.001	1.79 (1.38 - 2.31)	< 0.001
Adjusted	1.08 (0.45 - 2.58)	0.960	1.62 (0.80 - 3.29)	0.185	0.63 (1.29 - 2.15)	0.460
Platelet infusion						
Unadjusted	2.25 (1.77 - 2.88)	< 0.001	1.28 (1.00 - 1.66)	0.053	2.22 (1.45 - 3.34)	< 0.001
Adjusted	0.49 (0.21 - 1.15)	0.100	0.89 (0.44 - 1.78)	0.732	0.90 (0.22 - 3.66)	0.882
	Class 1		Class 2		Class 3	
In-hospital mortality	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Anticoagulants						
Unadjusted	4.19 (2.95 - 5.95)	< 0.001	1.01 (0.78 - 1.30)	0.957	3.91 (2.88 - 5.32)	< 0.001
Adjusted	11.06 (2.79 - 43.82)	0.001	0.67 (0.35 - 1.29)	0.230	15.94 (2.14 - 118.85)	0.007
Heparin						
Unadjusted	0.70 (0.54 - 0.90)	0.006	0.42 (0.33 - 0.53)	< 0.001	1.58 (1.23 - 2.04)	< 0.001
Adjusted	0.78 (0.34 - 1.76)	0.538	0.21 (0.10 - 0.43)	< 0.001	2.14 (0.52 - 8.78)	0.291
Plasma infusion						
Unadjusted	3.06 (2.34 - 4.00)	< 0.001	2.10 (1.66 - 2.66)	< 0.001	2.59 (1.97 - 3.41)	< 0.001
Adjusted	1.37 (0.55 - 3.38)	0.501	1.49 (0.74 - 3.00)	0.267	0.69 (0.18 - 2.72)	0.598
Platelet infusion						
Unadjusted	3.09 (2.36 - 4.04)	< 0.001	1.43 (1.11 - 1.84)	0.005	3.25 (2.10 - 5.03)	< 0.001
Adjusted	0.89 (0.37 - 2.15)	0.802	0.85 (0.43 - 1.70)	0.650	1.43 (0.31 - 6.66)	0.653

Adjusted by age, heart rate, systolic blood pressure, hypertension, diabetes SOFA score, fibrinogen, INR, hemoglobin, platelets, white blood cells, creatinine, lactate, CI, confidence interval.
Anticoagulants include argatroban, bivalirudin, and warfarin.
Heparin therapies include heparin, enoxaparin, and fondaparinux.
Plasma infusions include fresh frozen plasma and cryoprecipitate.
Platelet infusions include platelets and thrombocyte suspension.

variables because we believe that SIC classification does not rely solely on coagulation features. This supports that machine-learning clustering is effective in identifying the optimal subphenotypes for anticoagulant therapy.

Class 1 was characterized by lower blood cell counts (WBCs, RBCs, platelets, and hemoglobin) and can be clinically characterized by blood loss rather than by coagulation disorder. However, the proportion of class 1 patients undergoing anticoagulant therapy was no lower than in those of other two classes. Furthermore, after adjustment, anticoagulant therapy was associated with increased 28-day and in-hospital mortality in Class 1.

Class 2 was characterized by severe coagulopathy and multiple-organ dysfunction. Moreover, class 2 had the highest INR, PT, PTT, TBIL concentrations, creatinine, lactate, and SOFA and SAP III scores and the highest rate of CRRT, vasopressin, and anticoagulant therapy; however, this class still had the highest mortality rate. This subphenotype resembles cluster dA phenotype in the JSEPTIC-DIC trial[11] and the δ phenotype in the SENECA trial,[24] is more likely to have a severe coagulopathy status and organ dysfunction, and could benefit from anticoagulant therapy.

Class 3 was the largest class and was characterized by older age and a higher rate of comorbidities, similar to the β-phenotype in the SENECA trial.[24] The fibrinogen concentration was the highest in Class 3. Fibrinogen is a positive acute-phase protein that increases in response to systemic inflammation, tissue damage, and various cancers.[27] Hyperfibrinogenemia during sepsis is due to increased fibrinogen and has been recognized as the cause of thrombosis and vascular damage.[28]

Heparin is a mammalian polysaccharide widely used in the treatment of thrombotic disorders in patients with sepsis. Heparin exerts anticoagulant effects by binding to lysine residue in antithrombin, resulting in irreversible conformational change at the arginine-reactive site.[29] Previous animal experiments and a meta-analysis of clinical trials have demonstrated that heparin decreases 28-day mortality when compared with placebo in sepsis.[30,31] Another meta-analysis revealed that the risk ratio for death was 1.30 when heparin was compared with other anticoagulants. However, the overall effect of heparin remains uncertain.[32] These studies were performed in patients with sepsis, but not consistently in patients with SIC. Our study showed that heparin therapy was better than other anticoagulant therapies and was associated with reduced mortality only in Class 2. This finding may facilitate the identification of patients with SIC who are optimal for anticoagulant therapy.

This study has several limitations. First, the subphenotypes were derived from the large retrospective MIMIC-IV database; thus, the unsupervised clustering approach should be validated in an independent population. Second, other coagulation indicators (such as D-dimer and thrombin concentrations) and regimens (such as procoagulants and antiplatelet drugs) were excluded from the analysis because of the high rates of missing data in the database. In this study, missing data were also common for some of the variables; thus, NOCB, LOCF, and multiple imputations were used in the primary analysis. Third, although we tried to classify patients with SIC into three subphenotypes, granularity may not be sufficiently high enough to support individualized anticoagulant therapy. Sepsis is a highly heterogeneous syndrome, and specific phenotypes require different anticoagulation regimens. However, high granularity would reduce the interpretability and application in clinical practice.

We used data-driven unsupervised machine-learning approaches to identify three SIC subphenotypes. In this study, heparin therapy only benefited patients with severe coagulopathy and organ dysfunction. Thus, identifying distinct phenotypes and determining optimal treatments in future trials is warranted.

Ethics Committee Approval: The MIMIC-IV database was approved by the Massachusetts Institute of Technology (Cambridge, MA) and Beth Israel Deaconess Medical Center (Boston, MA).

Informed Consent: The consent of all patients was obtained for the original data collection.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authorship Contributions: Design- Q.W., Y.C., M.G.; Writing- G.F.

Conflict of Interest: No conflict of interest was declared by the authors.

Funding: The work was supported by grants from the China National Key Research and Development Program (no. 2022YFC2504500 and 2020AAA0105005), Natural Science Foundation of Sichuan (2023NSFSC1471), and National Natural Science Foundation of China (81901998).

Acknowledgement: We appreciate the guidance from the ESICM mentorship program.

Supplementary: http://balkanmedicaljournal.org/uploads/pdf/2023-4-6-suplement.pdf

REFERENCES

1. Evans L, Rhodes A, Alhazzani W, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. *Intensive Care Med.* 2021;47:1181-1247. [CrossRef]
2. Fleischmann-Struzek C, Mellhammar L, Rose N, et al. Incidence and mortality of hospital- and ICU-treated sepsis: results from an updated and expanded systematic review and meta-analysis. *Intensive Care Med.* 2020;46:1552-1562. [CrossRef]
3. van der Poll T, van de Veerdonk FL, Scicluna BP, Netea MG. The immunopathology of sepsis and potential therapeutic targets. *Nat Rev Immunol.* 2017;17:407-420. [CrossRef]
4. McDonald B, Dunbar M. Platelets and Intravascular Immunity: Guardians of the Vascular Space During Bloodstream Infections and Sepsis. *Front Immunol.* 2019;10:2400. [CrossRef]
5. Iba T, Nisio MD, 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;7:017046. [CrossRef]
6. Cecconi M, Evans L, Levy M, Rhodes A. Sepsis and septic shock. *Lancet.* 2018;392:75-87. [CrossRef]

7. Wada H, Matsumoto T, Aota T, Imai H, Suzuki K, Katayama N. Efficacy and safety of anticoagulant therapy in three specific populations with sepsis: a meta-analysis of randomized controlled trials: comment. *J Thromb Haemost*. 2016;14:2308-2309. **[CrossRef]**
8. Yoshimura J, Yamakawa K, Ogura H, et al. Benefit profile of recombinant human soluble thrombomodulin in sepsis-induced disseminated intravascular coagulation: a multicenter propensity score analysis. *Crit Care*. 2015;19:78. **[CrossRef]**
9. Yamakawa K, Umemura Y, Hayakawa M, et al. Benefit profile of anticoagulant therapy in sepsis: a nationwide multicentre registry in Japan. *Crit Care*. 2016;20:229. **[CrossRef]**
10. Vincent JL, Francois B, Zabolotskikh I, et al. Effect of a Recombinant Human Soluble Thrombomodulin on Mortality in Patients With Sepsis-Associated Coagulopathy: The SCARLET Randomized Clinical Trial. *JAMA*. 2019;321:1993-2002. **[CrossRef]**
11. Kudo D, Goto T, Uchimido R, et al. Coagulation phenotypes in sepsis and effects of recombinant human thrombomodulin: an analysis of three multicentre observational studies. *Crit Care*. 2021;25:114. **[CrossRef]**
12. Goldberger AL, Amaral LA, Glass L, et al. PhysioBank, PhysioToolkit, and PhysioNet: components of a new research resource for complex physiologic signals. *Circulation*. 2000;101:215-220. **[CrossRef]**
13. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. *Intensive Care Med*. 2017;43:304-377. **[CrossRef]**
14. Zhang Z. Missing data imputation: focusing on single imputation. *Ann Transl Med*. 2016;4:9. **[CrossRef]**
15. Blazek K, van Zwieten A, Saglimbene V, Teixeira-Pinto A. A practical guide to multiple imputation of missing data in nephrology. *Kidney Int*. 2021;99:68-74. **[CrossRef]**
16. Sinha P, Calfee CS, Delucchi KL. Practitioner's Guide to Latent Class Analysis: Methodological Considerations and Common Pitfalls. *Crit Care Med*. 2021;49:63-79. **[CrossRef]**
17. Tein JY, Coxe S, Cham H. Statistical Power to Detect the Correct Number of Classes in Latent Profile Analysis. *Struct Equ Modeling*. 2013;20:640-657. **[CrossRef]**
18. Charrad M, Ghazzali N, Boiteau V, Niknafs A. NbClust: An R Package for Determining the Relevant Number of Clusters in a Data Set. *Journal of Statistical Software*. 2014;61:1-36. **[CrossRef]**
19. Linderman GC, Rachh M, Hoskins JG, Steinerberger S, Kluger Y. Fast interpolation-based t-SNE for improved visualization of single-cell RNA-seq data. *Nat Methods*. 2019;16:243-245. **[CrossRef]**
20. Ferreira A, Bressan C, Hardy SV, Saghatelyan A. Deciphering heterogeneous populations of migrating cells based on the computational assessment of their dynamic properties. *Stem Cell Reports*. 2022;17:911-923. **[CrossRef]**
21. Ma P, Liu J, Shen F, et al. Individualized resuscitation strategy for septic shock formalized by finite mixture modeling and dynamic treatment regimen. *Crit Care*. 2021;25:243. **[CrossRef]**
22. Bhavani SV, Carey KA, Gilbert ER, Afshar M, Verhoef PA, Churpek MM. Identifying Novel Sepsis Subphenotypes Using Temperature Trajectories. *Am J Respir Crit Care Med*. 2019;200:327-335. **[CrossRef]**
23. Qin Y, Kernan KF, Fan Z, et al. Machine learning derivation of four computable 24-h pediatric sepsis phenotypes to facilitate enrollment in early personalized anti-inflammatory clinical trials. *Crit Care*. 2022;26:128. **[CrossRef]**
24. Seymour CW, Kennedy JN, Wang S, et al. Derivation, Validation, and Potential Treatment Implications of Novel Clinical Phenotypes for Sepsis. *JAMA*. 2019;321:2003-2017. **[CrossRef]**
25. Geri G, Vignon P, Aubry A, et al. Cardiovascular clusters in septic shock combining clinical and echocardiographic parameters: a post hoc analysis. *Intensive Care Med*. 2019;45:657-667. **[CrossRef]**
26. Guirgis FW, Black LP, Henson M, et al. A hypolipoprotein sepsis phenotype indicates reduced lipoprotein antioxidant capacity, increased endothelial dysfunction and organ failure, and worse clinical outcomes. *Crit Care*. 2021;25:341. **[CrossRef]**
27. Davalos D, Akassoglou K. Fibrinogen as a key regulator of inflammation in disease. *Semin Immunopathol*. 2012;34:43-62. **[CrossRef]**
28. Omiya K, Sato H, Sato T, et al. Albumin and fibrinogen kinetics in sepsis: a prospective observational study. *Crit Care*. 2021;25:436. **[CrossRef]**
29. Rezaie AR, Yang L, Manithody C. Mutagenesis studies toward understanding the mechanism of differential reactivity of factor Xa with the native and heparin-activated antithrombin. *Biochemistry*. 2004;43:2898-2905. **[CrossRef]**
30. Wang C, Chi C, Guo L, et al. Heparin therapy reduces 28-day mortality in adult severe sepsis patients: a systematic review and meta-analysis. *Crit Care*. 2014;18:563. **[CrossRef]**
31. Tang Y, Wang X, Li Z, et al. Heparin prevents caspase-11-dependent septic lethality independent of anticoagulant properties. *Immunity*. 2021;54:454-467.
32. Zarychanski R, Abou-Setta AM, Kanji S, et al. The efficacy and safety of heparin in patients with sepsis: a systematic review and metaanalysis. *Crit Care Med*. 2015;43:511-518. **[CrossRef]**