Coagulation Phenotypes in Sepsis and Effects of Recombinant Human Thrombomodulin: An Analysis of Three Multicenter Observational Studies

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Research

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Abstract

Background: A recent randomized trial showed that recombinant thrombomodulin did not benefit patients who had sepsis with coagulopathy and organ dysfunction, suggesting that the effects of thrombomodulin are heterogeneous across sepsis phenotypes. We examined the latent phenotypes of sepsis with coagulopathy and the associations between thrombomodulin treatment and 28-day and in-hospital mortality for each phenotype.

Methods: This was a secondary analysis of multicenter registries containing data on adult patients (aged ≥16 years) who were admitted to intensive care units for severe sepsis or septic shock in Japan. Three multicenter registries were divided into derivation (two registries) and validation (one registry) cohorts. Phenotypes were derived using k-means with coagulation markers, platelet counts, prothrombin time/international normalized ratios, fibrinogen, fibrinogen/fibrin-degradation-products (FDP), D-dimer, and antithrombin activities. Associations between thrombomodulin treatment and survival outcomes (28-day and in-hospital mortality) were assessed in derived clusters using a generalized estimating equation.

Results: Four sepsis phenotypes were derived from 3,694 patients in the derivation cohort. Cluster dA (n = 323) had severe coagulopathy with high FDP and D-dimer levels, severe organ dysfunction, and high mortality. Cluster dB had severe disease with moderate coagulopathy. Clusters dC and dD had moderate and mild disease with and without coagulopathy, respectively. Thrombomodulin was associated with a lower 28-day (adjusted risk difference [RD]: −17.8% [95% CI −28.7% to −6.9%]) and in-hospital (adjusted RD: −17.7% [95% CI −27.6 to −7.8%]) mortality only in cluster dA. Sepsis phenotypes were similar in the validation cohort, and thrombomodulin treatment was also associated with lower 28-day (RD: −24.9% [95%CI −49.1% to −0.7%]) and in-hospital mortality (RD: −30.9% [95%CI −55.3 to −6.6%]).

Conclusions: We identified four coagulation marker-based sepsis phenotypes. The treatment effects of thrombomodulin varied across sepsis phenotypes. This finding will facilitate future trials of thrombomodulin, in which a sepsis phenotype with high FDP and D-dimer can be targeted.

Background

Sepsis is the leading cause of death in intensive care units (ICUs), accounting for 26% of deaths in high-income countries [1]. Recombinant human thrombomodulin (rhTM) has anti-inflammatory and anticoagulation activities [2], and it has been suggested as an adjunct therapy for patients with sepsis, particularly those with sepsis-induced coagulopathy [3]. Nevertheless, a recent phase III randomized controlled trial revealed no beneficial effect of rhTM in patients with sepsis-induced coagulopathy [4]. This result can be explained by the heterogeneity of patients with sepsis and inappropriate criteria of coagulopathy [5] using the prothrombin time/international normalized ratio (PT-INR) and a platelet count based on subgroup analysis of an international phase II trial of rhTM [6].

Sepsis is a highly heterogeneous syndrome with variable etiology and pathophysiology [7]. Thus, a specific therapy may benefit some, but not all, patients with sepsis. Several recent studies have classified

sepsis into several phenotypes with distinct characteristics using cluster analysis [8–10], an unsupervised machine learning method that can identify relatively homogenous groups in a heterogeneous population [11]. Furthermore, these studies indicated that specific therapies conferred benefits only in patients with specific phenotypes of sepsis [8–10].

Identifying optimal targets based on biomarker cut-offs or clinical criteria can be difficult. To address this issue, we examined latent sepsis phenotypes in terms of coagulopathy and identified which phenotypes would benefit from rhTM using machine learning approaches.

Methods

Study Design and Settings

Details of the methods and analytical processes in the present study are provided in the Supplemental Digital Content. This was a secondary analysis of the following multicenter registries: the Japan Septic Disseminated Intravascular Coagulation (JSEPTIC-DIC) study [12], Tohoku Sepsis Registry [13], and Focused Outcomes Research in Emergency Care for Acute Respiratory Distress Syndrome, Sepsis, and Trauma (FORECAST) sepsis study [14]. All three registries include information on consecutive adult patients admitted to ICUs for severe sepsis or septic shock [15, 16]. These studies were approved and the need for informed consent was waived by the institutional review boards at the participating hospitals.

Study Population

We included all adult patients (aged ≥ 16 years) who were admitted to the ICU for severe sepsis or septic shock as defined in the three registries according to the International Sepsis Definitions Conference criteria [15, 16].

Phenotyping Variables

We measured the following coagulation markers upon admission to the ICU for phenotyping: platelet counts, PT-INR, fibrinogen, fibrinogen/fibrin degradation products (FDP), D-dimer, and antithrombin activities.

Exposure

Patients were exposed to rhTM.

Outcomes

The outcomes were 28-day and in-hospital mortality.

Statistical Analysis

Analytical Cohorts

We derived sepsis phenotypes using the JSEPTIC-DIC study (n = 3195) and Tohoku Sepsis Registry (n = 499) and validated the phenotypes utilizing the FORECAST sepsis study (n = 1,184).

Cluster Derivation

We initially assessed the distribution and missingness in phenotyping variables (**Table S1**). Non-normal data were log-transformed and scaled. Patients without 28-day mortality information were excluded. Missing data were imputed using the random forest method for each study cohort with the *missForest* package [17]. Random forest imputation is a nonparametric algorithm that accommodates nonlinearities and interactions and does not require the specification of a specific parametric model [18]. This approach generated single-point estimates by random draws from independent normal distributions centered on conditional means predicted by random forest. Random forest applies bootstrap aggregation of multiple regression trees to reduce the risk of overfitting and combines estimates from many trees [17]. Missingness was imputed using patient characteristics, laboratory data, outcomes, and other covariates, including in-hospital management.

We applied k-means with Euclid distance, which is a basic and widely used machine learning-based clustering approach, to derive sepsis phenotypes [9, 11]. We then determined the optimal number of clusters using a consensus clustering approach that provides quantitative and visual stability evidence to estimate the number of unsupervised classes in a dataset by inducing sampling variability with sub-sampling [19]. In consensus clustering, we evaluated the separation of consensus matrix heatmaps, the elbow method, cumulative distribution function, and cluster-consensus plots. We also visually evaluated clustering using t-distributed stochastic neighbor embedding (t-SNE) to reduce dimensionality and visualize high-dimensional datasets [20]. We also derived phenotypes using a divisive hierarchical clustering approach as an alternative to k-means to confirm the cluster consistency. The number of clusters was decided using dendrogram and elbow and gap statistic methods [21].

Evaluation of RhTM Effects in Derived Phenotypes

We examined the associations between rhTM and clinical outcomes in each derived cluster using a generalized estimating equation to adjust for hospital-level variance. Although patients within a derived cluster presumably had similar characteristics in terms of rhTM treatment, we analyzed the associations after adjusting for the potential confounders of age, sex, comorbidities, and sequential organ failure assessment (SOFA) scores. We did not adjust for pre- or co-existing in-hospital management in the derivation cohort, as information on when each management was initiated was not available. We examined the cluster-level effect modification of rhTM by including the interaction term rhTM use –x– cluster in the model. A significant interaction term indicated different effects of rhTM across clusters. Further, to confirm the robustness of the association of interest, we applied a Bayesian regression model to assess the associations between rhTM and clinical outcomes for each derived cluster based on k-means in the derivation cohort [21, 22]. Bayesian regression was achieved by using a Markov chain Monte Carlo procedure with four chains of 2,000 iterations per chain. The results are shown as the beta

coefficients with 95% credible intervals and displayed as odds ratios with 95% credible intervals for simplicity.

Cluster Validation and Evaluation of RhTM Effects

We predicted patient phenotypes in the FORECAST sepsis study as external data based on coagulation markers of clusters in the derivation cohort (derived from JSEPTIC-DIC and Tohoku Sepsis Registry). Predictions arose from the Euclidean distance from each patient to the centroid of each FORECAST phenotype. In each predicted cluster in the FORECAST sepsis study, we first described the frequency and clinical characteristics of the clusters. Thereafter, we used a generalized estimating equation to account for patient clustering within hospitals to assess associations between rhTM and clinical outcomes in each predicted cluster in the external data. The adjusted variables were age, sex, comorbidities, SOFA scores, and in-hospital management, including renal replacement therapy, and treatment with steroids, intravenous immunoglobulin, antithrombin, and vasopressors. Because the FORECAST sepsis data included information on the time of management, we included pre- or co-existing management as a covariate to estimate the effects of rhTM on clinical outcomes. As in the derivation cohort, we analyzed the association by Bayesian regression with a Markov chain Monte Carlo procedure with four chains.

Values with $p < 0.05$ were considered as statistically significant. All data were analyzed by using Stata version 14.1 (StataCorp, College Station, TX, USA) and R version 3.4.1 package for t-SNE (https://cran.r-project.org/web/packages/tsne/tsne.pdf) (R Foundation, Vienna, Austria).

Results

Patients in the Derivation Cohort

We excluded 117 patients without 28-day mortality information in the derivation cohort from the two multicenter registries, leaving 3,694 patients who were eligible for analysis (3,195 from JSEPTIC-DIC and 499 from the Tohoku Sepsis Registry). Table 1 summarizes the patients' characteristics. The median age was 72 years, and 40% of patients were female. Overall, rhTM was administered to 26.2% of patients. The in-hospital mortality and 28-day mortality rates were 32.1% and 20.4%, respectively.

Table 1
Characteristics of patients in the derivation cohort according to clusters.

	Overall	Cluster dA	Cluster dB	Cluster dC	Cluster dD	*P**
Variables	**n = 3694**	**n = 323**	**n = 629**	**n = 1147**	**n = 1595**	
Age, median (IQR)	72.0 (62.0, 81.0)	72.0 (58.0, 80.0)	72.0 (63.0, 81.0)	73.0 (63.0, 81.0)	72.0 (62.0, 80.0)	0.25
Sex, female	1468 (39.7%)	164 (50.8%)	268 (42.6%)	483 (42.1%)	553 (34.7%)	< 0.001
Body weight kg, median (IQR)	54.7 (46.6, 64.2)	55.0 (47.5, 64.0)	52.0 (45.0, 61.1)	55.0 (46.8, 64.3)	55.0 (47.0, 65.0)	< 0.001
Comorbidity						
Liver	149 (4.0%)	28 (8.7%)	73 (11.6%)	30 (2.6%)	18 (1.1%)	< 0.001
Respiratory	141 (3.8%)	8 (2.5%)	23 (3.7%)	43 (3.7%)	67 (4.2%)	0.52
Cardiovascular	316 (8.6%)	20 (6.2%)	49 (7.8%)	97 (8.5%)	150 (9.4%)	0.23
Renal	306 (8.3%)	24 (7.4%)	50 (7.9%)	111 (9.7%)	121 (7.6%)	0.23
Immunodeficiency	709 (19.2%)	62 (19.2%)	119 (18.9%)	233 (20.3%)	295 (18.5%)	0.69
Infection site						< 0.001
Unknown	218 (6.8%)	32 (11.0%)	49 (8.4%)	64 (6.4%)	73 (5.5%)	
Catheter-related	44 (1.4%)	1 (0.3%)	6 (1.0%)	19 (1.9%)	18 (1.4%)	
Bone/soft tissue	374 (11.7%)	20 (6.9%)	60 (10.3%)	108 (10.9%)	186 (14.0%)	
Cardiovascular	68 (2.1%)	13 (4.5%)	4 (0.7%)	26 (2.6%)	25 (1.9%)	

Six coagulation markers (bold font) were used for clustering. Variables (red font) were potential confounders that were adjusted in a generalized estimating

equation. **P* between clusters. Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; DIC, disseminated intravascular coagulation;

FDP, fibrinogen/fibrin degradation product; IQR, interquartile range; PT-INR, prothrombin time-international normalized ratio; SIRS, Systemic Inflammatory

Response Syndrome; SOFA, Sequential Organ Failure Assessment; WBC, white blood cells.

	Overall	Cluster dA	Cluster dB	Cluster dC	Cluster dD	*P**
Variables	**n = 3694**	**n = 323**	**n = 629**	**n = 1147**	**n = 1595**	
Central nervous system	63 (2.0%)	14 (4.8%)	1 (0.2%)	26 (2.6%)	22 (1.7%)	
Urinary tract	509 (15.9%)	71 (24.5%)	40 (6.8%)	210 (21.1%)	188 (14.2%)	
Lung/thoracic	827 (25.9%)	38 (13.1%)	117 (20.0%)	243 (24.4%)	429 (32.3%)	
Abdomen	1032 (32.3%)	94 (32.4%)	294 (50.3%)	279 (28.1%)	365 (27.5%)	
Other	60 (1.9%)	7 (2.4%)	13 (2.2%)	19 (1.9%)	21 (1.6%)	
APACHE2, median (IQR)	22.0 (17.0, 28.0)	26.0 (20.0, 33.0)	26.0 (19.0, 32.0)	23.0 (17.0, 29.0)	20.0 (15.0, 26.0)	< 0.001
SIRS score, median (IQR)	3.0 (2.0, 4.0)	3.0 (3.0, 4.0)	3.0 (2.0, 4.0)	3.0 (2.0, 4.0)	3.0 (2.0, 4.0)	< 0.001
SOFA scores	9.0 (6.0, 12.0)	13.0 (10.0, 16.0)	11.0 (9.0, 14.0)	10.0 (7.0, 12.0)	7.0 (5.0, 10.0)	< 0.001
Lab data						
White blood cell (10^3/μL), median (IQR)	11.3 (4.8, 17.8)	12.2 (4.6, 19.7)	7.8 (2.2, 15.5)	11.7 (6.0, 18.6)	11.6 (6.0, 17.5)	< 0.001
Platelet (10^3/μL), median (IQR)	122.0 (65.0, 194.0)	59.5 (32.0, 92.0)	78.0 (46.5, 128.0)	103.0 (54.0, 162.0)	178.0 (121.0, 252.0)	< 0.001
PT-INR, median (IQR)	1.3 (1.2, 1.6)	1.6 (1.4, 2.1)	1.7 (1.5, 2.2)	1.3 (1.2, 1.5)	1.2 (1.1, 1.4)	< 0.001
Fibrinogen (mg/mL), median (IQR)	421.0 (296.0, 528.9)	231.0 (151.0, 311.0)	245.3 (157.0, 350.0)	452.0 (367.0, 563.0)	476.9 (395.3, 576.0)	< 0.001
FDP (μg/mL), median (IQR)	17.6 (10.1, 36.2)	120.2 (79.2, 266.0)	16.0 (10.4, 24.0)	34.3 (22.8, 55.1)	10.0 (7.6, 13.8)	< 0.001

Six coagulation markers (bold font) were used for clustering. Variables (red font) were potential confounders that were adjusted in a generalized estimating

equation. **P* between clusters. Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; DIC, disseminated intravascular coagulation;

FDP, fibrinogen/fibrin degradation product; IQR, interquartile range; PT-INR, prothrombin time-international normalized ratio; SIRS, Systemic Inflammatory

Response Syndrome; SOFA, Sequential Organ Failure Assessment; WBC, white blood cells.

	Overall	Cluster dA	Cluster dB	Cluster dC	Cluster dD	*P**
Variables	**n = 3694**	**n = 323**	**n = 629**	**n = 1147**	**n = 1595**	
D-dimer (μg/mL), median (IQR)	7.8 (3.9, 17.2)	51.9 (35.2, 113.0)	7.7 (4.8, 11.7)	15.4 (10.5, 25.0)	3.8 (2.7, 5.6)	< 0.001
Antithrombin (%), median (IQR)	60.0 (50.8, 69.0)	52.0 (42.4, 60.5)	42.6 (33.0, 50.4)	60.1 (54.0, 68.0)	66.0 (59.0, 73.7)	< 0.001
Lactate (mmol/L), median (IQR)	2.9 (1.7, 5.7)	5.3 (2.9, 10.1)	4.3 (2.3, 8.0)	2.7 (1.5, 5.4)	2.3 (1.4, 4.1)	< 0.001
ISTH DIC score						< 0.001
0	685 (18.7%)	0 (0.0%)	15 (2.4%)	25 (2.2%)	645 (41.1%)	
1	239 (6.5%)	2 (0.6%)	17 (2.7%)	15 (1.3%)	205 (13.0%)	
2	701 (19.2%)	3 (0.9%)	104 (16.6%)	116 (10.2%)	478 (30.4%)	
3	592 (16.2%)	25 (7.8%)	99 (15.8%)	327 (28.8%)	141 (9.0%)	
4	530 (14.5%)	42 (13.0%)	143 (22.8%)	261 (23.0%)	84 (5.3%)	
5	441 (12.1%)	79 (24.5%)	105 (16.7%)	240 (21.1%)	17 (1.1%)	
6	250 (6.8%)	78 (24.2%)	83 (13.2%)	88 (7.7%)	1 (0.1%)	
7	169 (4.6%)	72 (22.4%)	38 (6.1%)	59 (5.2%)	0 (0.0%)	
8	40 (1.1%)	20 (6.2%)	15 (2.4%)	5 (0.4%)	0 (0.0%)	
ISTH DIC score ≥ 5	1430 (39.2%)	291 (90.7%)	384 (62.0%)	653 (57.5%)	102 (6.5%)	
Managements						

Six coagulation markers (bold font) were used for clustering. Variables (red font) were potential confounders that were adjusted in a generalized estimating

equation. **P* between clusters. Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; DIC, disseminated intravascular coagulation;

FDP, fibrinogen/fibrin degradation product; IQR, interquartile range; PT-INR, prothrombin time-international normalized ratio; SIRS, Systemic Inflammatory

Response Syndrome; SOFA, Sequential Organ Failure Assessment; WBC, white blood cells.

	Overall	Cluster dA	Cluster dB	Cluster dC	Cluster dD	*P**
Variables	**n = 3694**	**n = 323**	**n = 629**	**n = 1147**	**n = 1595**	
rhTM	969 (29.3%)	128 (44.1%)	184 (31.5%)	334 (33.6%)	210 (15.8%)	< 0.001
Vasopressor use	2789 (75.5%)	289 (89.5%)	558 (88.7%)	882 (76.9%)	1060 (66.5%)	< 0.001
Renal replacement therapy	971 (26.3%)	135 (41.8%)	220 (35.0%)	339 (29.6%)	277 (17.4%)	< 0.001
Steroids	894 (24.2%)	112 (34.7%)	214 (34.1%)	285 (24.8%)	283 (17.7%)	< 0.001
Intravenous immunoglobulin	1088 (29.5%)	116 (35.9%)	239 (38.0%)	362 (31.6%)	371 (23.3%)	< 0.001
Antithrombin	1092 (29.6%)	161 (49.8%)	296 (47.1%)	367 (32.0%)	268 (16.8%)	< 0.001
Outcomes						
28-day death	753 (20.4%)	117 (36.2%)	198 (31.5%)	200 (17.4%)	238 (14.9%)	< 0.001
In-hospital death	1186 (32.1%)	151 (46.8%)	301 (47.9%)	358 (31.0%)	376 (23.6%)	< 0.001
Six coagulation markers (bold font) were used for clustering. Variables (red font) were potential confounders that were adjusted in a generalized estimating						
equation. **P* between clusters. Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; DIC, disseminated intravascular coagulation;						
FDP, fibrinogen/fibrin degradation product; IQR, interquartile range; PT-INR, prothrombin time-international normalized ratio; SIRS, Systemic Inflammatory						
Response Syndrome; SOFA, Sequential Organ Failure Assessment; WBC, white blood cells.						

Derivation of Clinical Sepsis Phenotypes

We assessed the distributions and missingness among phenotyping variables (**Table S1**). According to clustering using k-means, a four-class model including the phenotype clusters derivation dA, dB, dC, and dD ("d" represents "derivation") may be an optimal fit. The heatmap matrix (**Figure S1**), cumulative distribution function curve (**Figure S2**), and elbow method (**Figure S3**) indicated that the four-class model was optimal, whereas the cluster-consensus plot suggested that two, three, or four clusters were optimal (**Figure S4**). The four-class model was supported by the t-SNE plot with clear separation (Fig. 1). **Figure S5** shows a cluster dendrogram obtained using a divisive hierarchical clustering approach. The elbow method showed that a two- or four-cluster model is optimal (**Figure S6**), whereas the gap statistic method [21] showed that the four-cluster model was optimal (**Figure S7**).

Patients in cluster dA were likely to have a severe physiological status and organ dysfunction (high APACHE II and SOFA scores), coagulopathy (low platelet counts, prolonged PT-INR, low fibrinogen, and extremely high FDP and D-dimer levels), high lactate levels, and high mortality (Table 1). Approximately 90% of patients in this cluster required vasopressors. The characteristics of patients in cluster dB were similar to those in cluster dA in terms of severity but likely to have abdominal infection with normal white blood cell counts, moderate coagulopathy with moderate FDP and D-dimer levels, and low antithrombin activity. Patients in clusters dC and dD had moderate and mild disease, respectively. Although patients in cluster dC had coagulopathy with high FDP and D-dimer levels, those in cluster dD were likely to have respiratory infection without coagulopathy. The phenotypes were similar according to four-cluster hierarchical clustering (**Table S2)**.

Evaluation of RhTM Effects in the Derivation Cohort

Recombinant human thrombomodulin was administered to 128 (44.1%), 184 (31.5%), 334 (33.6%), and 210 (15.5%) patients in clusters dA, dB, dC, and dD, respectively. Clinical outcomes in cluster dA were better with than in those without rhTM (adjusted risk difference [RD], − 17.8% [95% CI, − 28.7% to − 6.9%] for 28-day mortality; RD, − 17.7% [95% CI − 27.6% to − 7.8%] for in-hospital mortality; Table 2). In contrast, rhTM was not associated with better outcomes in other clusters except for in-hospital death in cluster dC. Analysis of the rhTM effect modification across clusters using cluster dA as the reference showed that the effects of rhTM differed across clusters (all, $p < 0.05$), except for in-hospital mortality in cluster dB ($p = 0.31$). The associations were similar according to four-cluster hierarchical clustering (**Table S3**). Furthermore, rhTM treatment was associated with better clinical outcomes in cluster dA according to Bayesian regression (**Table S4**).

Table 2
Unadjusted and adjusted associations between recombinant thrombomodulin use and outcomes.

Outcomes	Cluster dA	*p*-value	Cluster dB	*p*-value	Cluster dC	*p*-value	Cluster dD	*p*-value
Associations in the derivation cohorts, risk difference, % (95%CI)								
Unadjusted association (vs. non rhTM use)								
28-Day death	-10.8 (-21.5 to -0.1)	0.047	3.5 (-4.6 to 11.5)	0.40	-1.9 (-6.9 to 3.2)	0.47	2.2 (-2.8 to 7.1)	0.39
In-hospital death	-10.9 (-20.8 to -1.1)	0.03	1.6 (-7.2 to 10.3)	0.73	-8.0 (-13.8 to -2.3)	0.01	0.8 (-5.2 to 6.8)	0.78
Adjusted association (vs. non rhTM use)								
28-Day death	-17.8 (-28.7 to -6.9)	0.001	0.7 (-7.1 to 8.6)	0.85	-3.1 (-8.3 to 2.1)	0.24	-0.7 (-4.5 to 6.0)	0.79
In-hospital death	-17.7 (-27.6 to -7.8)	< 0.001	0.2 (-7.9 to 8.3)	0.97	-10.2 (-15.9 to -4.6)	< 0.001	-1.3 (-7.6 to 4.9)	0.67
	Cluster vA	*p*-value	Cluster vB	*p*-value	Cluster vC	*p*-value	Cluster vD	*p*-value
Associations in the validation cohorts, risk difference, % (95%CI)								
Unadjusted association (vs. non rhTM use)								
28-Day death	-15.0 (-32.2 to 2.2)	0.09	3.2 (-12.5 to 18.87)	0.69	4.4 (-5 to 13.83)	0.36	7.1 (-2.4 to 16.56)	0.14
In-hospital death	-22.2 (-39.6 to -4.93)	0.01	8.8 (-7.3 to 24.82)	0.29	5.7 (-4.9 to 16.26)	0.3	14.2 (3.8 to 24.65)	0.008
Adjusted association (vs. non rhTM use)								
28-Day death	-24.9 (-49.1 to -0.7)	0.04	-5.7 (-29.9 to 18.5)	0.64	1.4 (-12.8 to 15.7)	0.84	-6.7 (-19.4 to 6.0)	0.3
In-hospital death	-30.9 (-55.3 to -6.6)	0.01	-3.7(-27.9 to 20.5)	0.77	-0.5 (-16.1 to 15.1)	0.95	0.7 (-13.0 to 14.5)	0.92
Abbreviations: rhTM, recombinant human thrombomodulin								

Characteristics of Phenotypes in the Validation Cohort

Table S5 shows the patients' characteristics in each cluster in the validation cohort. The median age was 73 years, 40% of the patients were women, and rhTM was administered to 21.2% of patients. In-hospital

and 28-day mortality rates were 23.4% and 19.0%, respectively. These characteristics were similar to those in the derivation cohort but the rate of rhTM treatment and mortality were relatively lower.

We used only coagulation markers to predict clusters in the validation cohort, and the characteristics were similar to those in the derivation cohort ("v" represents "validation"). Similar to the patients in cluster dA, those in cluster vA were likely to have a severe physiological status and organ dysfunction (high APACHE II and SOFA scores), coagulopathy (low platelet counts, prolonged PT-INR, low fibrinogen, and extremely high FDP and D-dimer levels), high lactate levels, and moderately high mortality. Patients in cluster vB had a high mortality rate with moderate coagulopathy and moderate FDP and D-dimer levels. Patients in clusters vC and vD had moderate and mild disease, respectively. Patients in cluster vC had coagulopathy with high FDP and D-dimer levels, whereas those in cluster vD did not have coagulopathy.

Evaluation of the Effect of rhTM in the Validation Cohort

All 1,184 patients in the FORECAST sepsis study dataset were analyzed for validation. Recombinant human thrombomodulin was administered to 44 (44.4%), 54 (31.2%), 98 (26.3%), and 46 (9.3%) patients in clusters vA, vB, vC, and vD, respectively. Clinical outcomes in cluster vA were better than in those without rhTM (adjusted RD, − 24.9% [95%CI − 49.1% to − 0.7%] for 28-day mortality; RD − 30.9% [95%CI − 55.3% to − 6.6%] for in-hospital mortality; Table 2). In contrast, rhTM was not associated with better outcomes in the other clusters. These associations were consistent with the findings of the Bayesian regression analysis (**Table S4** and **Figure S8**).

Discussion

This secondary analysis of the sepsis registries identified four phenotypes with various coagulation features among patients with severe sepsis. Treatment with rhTM was associated with lower in-hospital mortality rates only in the phenotype with severe coagulopathy characterized by low platelet counts, extremely high levels of FDP and D-dimer (phenotype clusters dA and vA), and severe organ dysfunction. These results were not identified in the other phenotypes.

The severity of coagulopathy is defined by the DIC scoring systems, such as the International Society on Thrombosis and Haemostasis (ISTH) scoring system for diagnosing overt DIC [23] and Japanese Association for Acute Medicine DIC scoring system [24], both of which have been applied in many studies. The difference between these systems and machine learning-based clustering is the use of a trivial cut-off. Tables 2, **S2**, and **S5** show that each phenotype cluster included patients with various ISTH DIC scores without a clear cut-off that overlapped with the other clusters. This suggests that clustering based on machine learning can detect novel phenotypes that cannot be identified with conventional scoring systems.

Recombinant human thrombomodulin has anticoagulation effects and was shown to be beneficial for patients with sepsis and coagulopathy in observational studies and in a subgroup analysis of a phase II trial [3, 6]. The latest phase III trial focused on patients with sepsis with cardiovascular or respiratory

dysfunction as well as coagulopathy according to subgroup analysis of the phase II trial [4]. However, the phase III trial did not identify a positive effect of rhTM on survival, suggesting that differentiating a subgroup that may benefit from rhTM is difficult using conventional methods with clear cut-offs. In our study, despite overlapping characteristics, various DIC scores, and differences in severity among clusters, cluster dA (vA) was the only phenotype in which rhTM was associated with better survival outcomes. This suggests that machine learning clustering can identify optimal clinical phenotypes for rhTM treatment. Additionally, the machine learning clustering described herein used only six variables, all of which are general markers that can be measured in most hospitals. Additionally, the results can be available soon after admission before deciding to administer rhTM in an emergency room or ICU.

Other studies using machine learning clustering for patients with sepsis also suggested that several specific therapies have beneficial effects only for patients with specific phenotypes. A Toll-like receptor 4 antagonist, protocol-based resuscitation, activated protein C, and fluid input affected each phenotype differently [9, 10]. The effectiveness of rhTM also varied across phenotypes in our study. Therefore, selecting an optimal clinical phenotype may be key to the success of specific therapy for patients with sepsis. Including entire populations with sepsis may explain why previous randomized trials found no beneficial effects of adjunctive therapies [25–28]. The goal of precision/tailored medicine is to select the optimal therapy for patients, for which machine learning-based clustering can be effective. Although our study does not fully address the definite endotypes of coagulation in sepsis biologically or pathophysiologically, our findings improve the understanding of the true endotypes of sepsis with coagulation.

Limitations

This study had several limitations. We used three registries that included different variables. Therefore, unmeasured confounders and a lack of information such as the timing of rhTM administration may have biased our findings. Nevertheless, the data included detailed clinical information that is generally used for adjustment, and the results in the validation cohort accounted for pre- and co-existing in-hospital management. Although missing data imputation using the random forest approach is considered valid, missingness may have limited our findings. However, valid imputation reduces bias, even when the proportion of missingness is high [29]. Our data did not include the duration of rhTM administration, which was presumably 6 days according to Japanese medical insurance guidelines. We could not evaluate whether the phenotypes and efficacy of rhTM are consistent with patients with sepsis defined by the Sepsis-3 criteria [30], as three observational studies enrolled patients with sepsis using the Sepsis-2 definition [16], and the datasets did not include SOFA scores before admission. Finally, our data were derived from Japanese hospitals; thus, the generalizability of the results may be limited.

Conclusions

The findings derived using machine learning clustering indicated that rhTM can benefit only patients with a severe coagulopathy phenotype. Identifying patients for whom a therapy will have a beneficial effect

can lead to precision/tailored medicine in critical care. To achieve this goal, the accuracy of phenotyping should be increased by analyzing more patients and through further validation. A randomized trial focusing on suitable phenotypes determined by effective phenotyping is warranted.

Abbreviations

FDP, fibrinogen/fibrin degradation product; FORECAST, Focused Outcomes Research in Emergency Care for Acute Respiratory Distress Syndrome, Sepsis, and Trauma; ICU, intensive care unit; ISTH, International Society on Thrombosis and Haemostasis; JSEPTIC-DIC, Japan Septic Disseminated Intravascular Coagulation; PT-INR, prothrombin time/international normalized ratio; RD, risk difference; rhTM, human thrombomodulin; SOFA, sequential organ failure assessment

Declarations

Ethics approval and consent to participate: Three original studies were approved, and the need for informed consent was waived by the institutional review boards at the participating hospitals.

Consent for publication: Not applicable

Availability of data and materials:

The datasets generated and/or analyzed during the original studies are available in the Scientific data, https://www.nature.com/articles/sdata2018243 (J-SEPTIC DIC study), and Mendeley Data, https://data.mendeley.com/datasets/vvv89kw3k5/1 (Tohoku Sepsis Registry). The dataset of the FORECAST sepsis study is not publicly available based on the decision by the Japanese Association for Acute Medicine.

Competing interests: D.K., M.H., and S.K. received personal fees from Asahi Kasei Pharma Corporation. The other authors have no conflicts of interest to declare.

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Authors' contributions: All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by DK, TG, RU, MH, TA, and AS. Statistical analysis was reviewed by RU, TA, and AS. The first draft of the manuscript was written by DK and TG. The manuscript was reviewed and edited by KY, TA, AS, and SK, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Funding acquisition: DK; supervision: SK.

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Figures

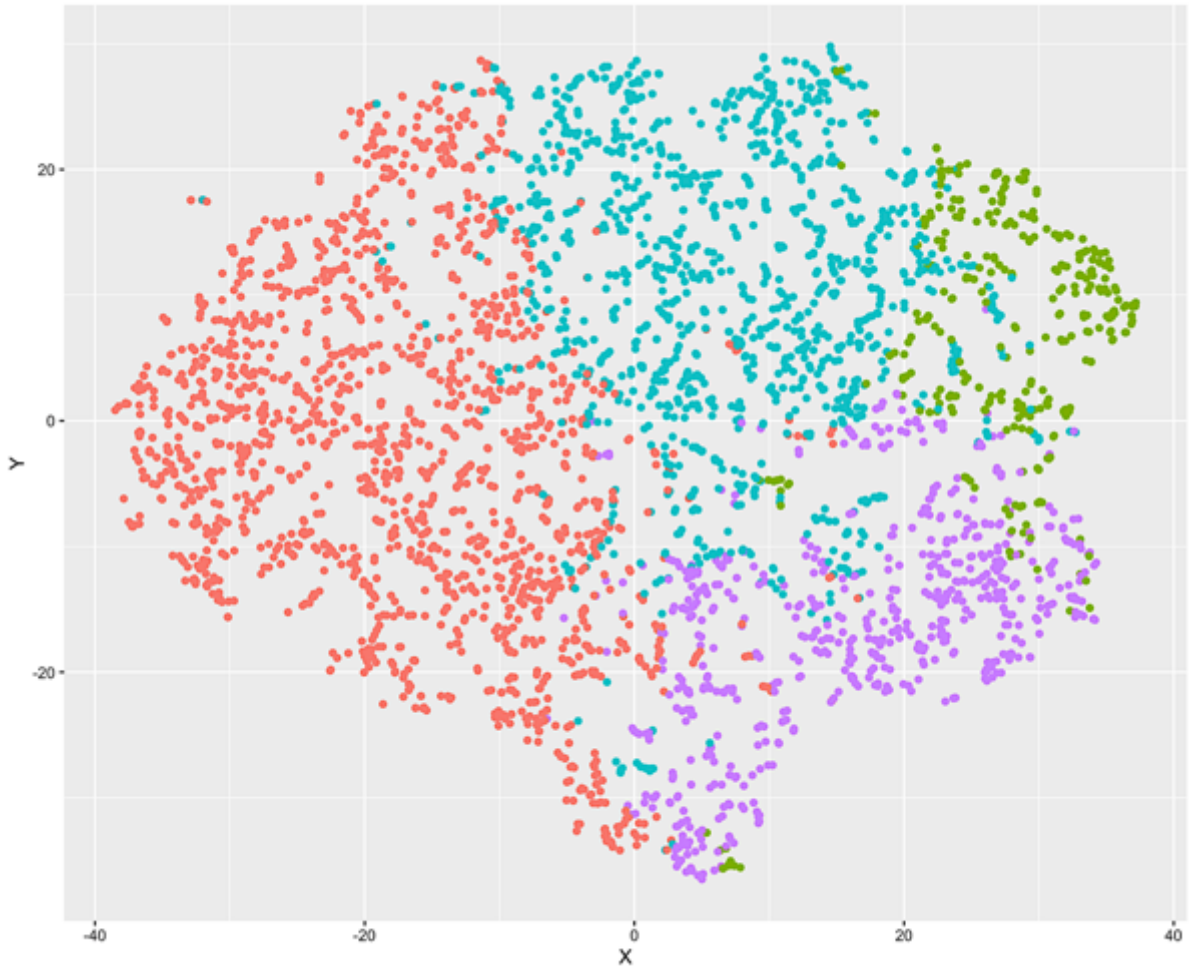

Figure 1

t-SNE plot This t-distributed stochastic neighbor embedding (t-SNE) plot is a dimensionality reduction technique for graphically simplifying extensive datasets. Four clusters are in plotted, and some patients are on the borderlines between clusters. Circles represent individual patients (green, cluster dA; purple, cluster dB; blue, cluster dC; red, cluster dD)

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