Research Square

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Prospective Validation of Sepsis Subphenotypes Derived from Vital Sign Trajectories

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Abstract

Purpose

Sepsis is a heterogeneous syndrome, and prior retrospective work identified four clinically meaningful sepsis subphenotypes (A–D) using vital sign trajectories. This study prospectively validated this subphenotyping algorithm and evaluated associations with patient characteristics and clinical outcomes.

Methods

A prospective multicenter observational cohort study was conducted across six hospitals within Emory Healthcare (Atlanta, GA) September-December 2025. Adult emergency department patients hospitalized with sepsis defined by Sepsis-3 criteria were included. An Epic-integrated algorithm captured temperature, heart rate, respiratory rate, and blood pressure during the first 8 hours of hospitalization and assigned patients hourly to one of four predefined subphenotypes. The primary outcome was 30-day hospital mortality. Secondary outcomes included ICU admission, vasopressor use, renal replacement therapy, mechanical ventilation, inotrope use, and hospital length of stay. Multivariable regression adjusted for demographics and comorbidities compared outcomes across subphenotypes.

Results

Among 1,916 encounters, subphenotype distribution was Group A 16%, Group B 16%, Group C 27%, and Group D 41%. Subphenotypes demonstrated distinct physiologic patterns and differed in demographics and comorbidity burden. Similar to the retrospective model, thirty-day mortality ranged from 3.6% (Group B) to 9.2% (Group D) ($p = 0.034$). After adjustment, Group D had higher odds of vasopressor use (OR = 1.9, 95% CI 1.4–2.7) and 30-day mortality (OR = 1.6, 95% CI 1.0–2.5) versus Group C. Classification using only initial vital signs agreed with the 8-hour model in 59% of cases.

Conclusion

Real-time EHR-based classification of sepsis subphenotypes is feasible and reproducible, identifying groups with meaningful differences in outcomes, supporting future precision trial designs.

INTRODUCTION

Despite decades of clinical trials, the management of sepsis, a life-threatening syndrome caused by a dysregulated host response to infection, has largely followed a uniform, “one-size-fits-all” strategy, with limited success [1, 2]. More than 100 clinical trials evaluating over 30 candidate therapies have failed to demonstrate consistent benefit across the heterogeneous population of patients with sepsis [3, 4].

These outcomes highlight the substantial clinical and biological heterogeneity of sepsis and underscore the limitations of treating sepsis as a single entity [5, 6].

The limited success of identifying therapies through one-size-fits-all clinical trials has motivated a shift toward precision medicine, emphasizing the identification of clinically meaningful patient subgroups. A key component of this effort involves the use of unsupervised machine learning to define sepsis subphenotypes using routinely collected clinical data [7, 8]. Ideally, such models should identify subphenotypes that differ in underlying pathophysiology, clinical outcomes, and response to therapy, thereby supporting targeted treatment strategies and improving clinical trial design. In a retrospective study of over 12,000 hospitalized patients, four subphenotypes (A–D) were identified using group-based trajectory modeling of vital signs from the first eight hours following hospital arrival [9]. These subphenotypes demonstrated meaningful differences in baseline characteristics, organ dysfunction profiles, and clinical outcomes, highlighting the potential of data-driven approaches to capture clinically relevant heterogeneity in sepsis.

Although these retrospective findings represent an important initial step, prospective validation is essential for clinical translation. Models may work retrospectively but may fail prospectively, because real-time data are often incomplete, temporally misaligned due to delayed documentation, and subject to workflow constraints. To evaluate the clinical utility of these subphenotypes, a prospective, multicenter observational study was conducted using an electronic health record (EHR)-integrated tool to classify hospitalized patients into sepsis subphenotypes based on vital sign data, with the aim of characterizing their clinical characteristics and outcomes.

METHODS

Study Design and Setting

This prospective, multicenter observational cohort study was conducted to validate a previously developed sepsis subphenotyping algorithm across six hospitals within Emory Healthcare in Atlanta, Georgia. The study is registered on ClinicalTrials.gov (NCT05826223) and was approved by the Emory Institutional Review Board, which granted a waiver of informed consent as the study is observational, poses no more than minimal risk to patients, and does not alter clinical care.

Study Population

All adult patients (≥ 18 years) presenting to the emergency department (ED), hospitalized between September 18 and December 14, 2025, and with sepsis within 24 hours of ED arrival were included. Sepsis was defined per Sepsis-3 criteria: evidence of infection from body fluid cultures and antibiotics, plus an increase of ≥ 2 points in the Sequential Organ Failure Assessment (SOFA) score documented in the EHR.

Subphenotype Classification

The EHR-integrated algorithm automatically captured vital signs (temperature, heart rate, respiratory rate, and blood pressure) during the first eight hours of hospital presentation. Using this longitudinal data, patients were classified to one of four previously identified subphenotypes (A–D) based on a validated group-based trajectory model. In this model, each subphenotype is defined by a unique set of polynomial trajectory functions describing each vital sign as a function of time from hospital presentation (e.g., Temperature = $\beta 0 + \beta 1 \cdot \text{Time} + \beta 2 \cdot \text{Time}^2$) [9]. The algorithm calculates the patient's distance from the four reference trajectories at each hour and assigns the patients to the group where the patient is the smallest distance from. Subphenotype classifications were not visible to clinicians and did not influence patient care. Concurrently with this study, there was a separate randomized controlled trial enrolling Group D patients for fluid resuscitation [10].

EHR Implementation

The algorithm to compute these subphenotype classifications was built with Python code and uploaded to Epic's Software as a Service (SaaS) cloud environment, Nebula, where this custom model was stored, maintained, and executed on real-time patient data utilizing Nebula's infrastructure to generate these subphenotype classifications.

The configuration of this model involved the mapping of the features (vital signs) that are ingested by the model to generate a classification and the creation of a Reporting Workbench (RW) report that displays a predicted score corresponding to each patient's subphenotype classification. This report served a critical role in verifying appropriate feature utilization by the model and in facilitating real-time, reproducible access to prediction results. Finally, batch processes were configured to run in the background of the cloud-compute environment every hour, enabling repeated calculations on an hourly basis of subphenotype classification and structured storage for downstream analysis.

Data Collection and Outcomes

Patient demographics, comorbidities, vital signs, and clinical outcomes were extracted from the Epic Clarity database one month after study completion to ensure complete outcome capture. Comorbidities included congestive heart failure, pulmonary disease, hypertension, diabetes, chronic kidney disease, chronic liver disease, and cancer, and overall comorbidity burden was summarized using the Elixhauser Comorbidity Index (ECI). Baseline SOFA-1 scores were obtained; if unavailable, the sepsis-qualifying SOFA within eight hours of hospital arrival was used.

The primary outcome was 30-day hospital mortality, defined as death within 30 days of hospital arrival, discharge disposition of expired, or hospice within 30 days of hospital arrival. Secondary outcomes included total hospital length of stay, ICU admission, renal replacement therapy, mechanical ventilation, vasopressor use, and inotrope use.

Statistical Analysis

All analyses were conducted in R (version 4.4.0). Subphenotype stability and transitions over the first eight hours were visualized using an alluvial plot, with any unassigned hour carrying forward the patient's most recent subphenotype. The final subphenotype assignment after the 8-hour window for each patient was used for all subsequent analyses.

Descriptive statistics were summarized as frequencies and percentages for categorical variables and medians with interquartile ranges for continuous variables (age and length of stay). Group differences were assessed using chi-square tests for categorical variables and ANOVA for continuous variables, with two-sided p-values < 0.05 considered statistically significant.

Vital sign trajectories were aggregated into hourly bins. Mean values were calculated for each hour and subphenotype, and smoothed trajectories were generated using LOESS to visualize temporal patterns stratified by patients' final subphenotype.

Clinical outcomes were analyzed using regression models adjusted for demographics and comorbidities, with Group C serving as the reference group, consistent with the prior retrospective study. Logistic regression was used for dichotomous outcomes to estimate odds ratios with 95% confidence intervals, and linear regression was applied to LOS with coefficients and 95% CI reported. Additionally, a likelihood ratio test was performed to evaluate whether including subphenotype in the mortality model improved model fit compared with a model containing only demographics, comorbidities, and SOFA score.

RESULTS

Distinct Physiologic Patterns Define Each Subphenotype

Among 1,916 patient encounters with sepsis, the four subphenotypes represented distinct physiological patterns: Group A patients (n = 306, 16%) demonstrated higher temperatures, tachycardia, and tachypnea, and were relatively hypotensive compared with other groups (Fig. 1**)**. Group B patients (n = 309, 16%) also showed elevated temperature, heart rate, and respiratory rate, though less pronounced than Group A, and were distinctly hypertensive with the highest systolic and diastolic blood pressures. In contrast, Groups C (n = 509, 27%) and D (n = 792, 41%) had lower temperatures, heart rates, and respiratory rates overall, with Group C demonstrating intermediate blood pressures and Group D representing the most hypotensive subphenotype.

Subphenotypes Differ by Demographics and Comorbidity Burden

The subphenotypes differed in demographics, comorbidity burden, and markers of acute illness (Table 1**)**. Group B was the youngest (median 60 years), while Groups C and D were oldest (median 69–70 years, $p < 0.0001$). Males were more common in Groups B and D (56% and 53%, $p = 0.038$), and the

proportion of Black patients was higher in Groups A and B (59% and 65%, $p < 0.0001$). Key comorbidities varied: hypertension was most frequent in Groups B and C, chronic kidney disease and congestive heart failure were higher in Groups C and D, and cancer was most prevalent in Group D. Differences in pulmonary disease, diabetes, and liver disease were non-significant. Indicators of acute illness severity also differed, with ICU admission more frequent in Groups B and D (46% and 44%; $p = 0.0008$), vasopressor use highest in Group D (21%; $p < 0.0001$), and 30-day mortality ranging from 3.6% in Group B to 9.2% in Group D ($p = 0.034$), highlighting variation in clinical risk across the subphenotypes.

Table 1
Comparison of Clinical Characteristics Between Sub-phenotypes of Patients with Sepsis

Group, n (%)	**A** **n = 306 (16%)**	**B** **n = 309 (16%)**	**C** **n = 509 (27%)**	**D** **n = 792 (41%)**	**p-value**
Demographics					
Age, years	62 (42–73)	60 (47–72)	69 (56–79)	70 (58–79)	< .0001
Sex, male	143 (47%)	173 (56%)	242 (48%)	416 (53%)	0.038
Race					< .0001
Black	179 (59%)	202 (65%)	279 (55%)	372 (47%)	
White	84 (27%)	76 (25%)	176 (35%)	339 (43%)	
Other	43 (14%)	31 (10%)	54 (11%)	81 (10%)	
Ethnicity					0.61
Hispanic	13 (4.3%)	11 (3.6%)	27 (5.3%)	47 (5.9%)	
Non-Hispanic	279 (91%)	285 (92%)	466 (92%)	718 (91%)	
Unknown	14 (4.6%)	13 (4.2%)	16 (3.1%)	27 (3.4%)	
SOFA	2 (0–3)	2 (0–3)	2 (0–3)	2 (0–3)	0.51
Comorbidities					
ECI	5 (0–9)	5 (0–11)	5 (0–12)	6 (0–13)	< .0001
CHF	89 (29%)	110 (36%)	200 (39%)	313 (40%)	0.008
Pulmonary	127 (42%)	155 (50%)	253 (50%)	408 (52%)	0.028
Hypertension	202 (66%)	258 (84%)	412 (81%)	612 (77%)	< .0001
DM	124 (41%)	162 (52%)	248 (49%)	368 (47%)	0.023
CKD	112 (37%)	147 (48%)	272 (53%)	418 (53%)	< .0001
Liver	104 (34%)	89 (29%)	156 (31%)	269 (34%)	0.29

Presented are the comparison of demographics, comorbidities, and outcomes between subphenotypes A-D. Age and LOS are presented as median (IQR), and all other values are presented as frequencies (%). p values signify the results of comparisons between subphenotypes through chi-square or ANOVA testing.

SOFA Sequential Organ Failure Assessment, *ECI* Elixhauser Comorbidity Index, *CHF* Congestive Heart Failure, *Pulmonary* Chronic Pulmonary Disease, *DM* Diabetes Mellitus, *CKD* Chronic Kidney Disease, *Liver* Chronic Liver Disease, *Cancer* Metastatic Cancer, *LOS* Hospital Length of Stay, *ICU* Intense Care Unit

Group, n (%)	A n = 306 (16%)	B n = 309 (16%)	C n = 509 (27%)	D n = 792 (41%)	p-value
Demographics					
Cancer	101 (33%)	73 (24%)	155 (30%)	290 (37%)	0.0004
Outcomes					
LOS	5.8 (3.7–9.4)	5.5 (3.6–9.9)	5.3 (3.3–8.9)	5.8 (3.5–10.3)	0.41
ICU	129 (42%)	141 (46%)	173 (34%)	351 (44%)	0.0008
Dialysis	2 (0.65%)	12 (3.9%)	25 (4.9%)	20 (2.5%)	0.004
Ventilator	22 (7.2%)	46 (15%)	49 (9.6%)	88 (11%)	0.016
Vasopressors	44 (14%)	38 (12%)	61 (12%)	166 (21%)	< .0001
Inotropes	9 (2.9%)	4 (1.3%)	5 (0.98%)	23 (2.9%)	0.059
30-day Mortality	18 (5.9%)	10 (3.3%)	31 (6.1%)	72 (9.1%)	0.004

Presented are the comparison of demographics, comorbidities, and outcomes between subphenotypes A-D. Age and LOS are presented as median (IQR), and all other values are presented as frequencies (%). p values signify the results of comparisons between subphenotypes through chi-square or ANOVA testing.

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Subphenotypes Show Variable ICU, Vasopressor, Inotrope Use, and 30-Day Mortality

After adjustment for demographics, comorbidities, and SOFA score, subphenotypes demonstrated distinct patterns of critical care utilization (Fig. 2). Compared with Group C, Groups A, B, and D had higher odds of ICU admission (OR 1.6 for each). Odds of renal replacement therapy were lower in Groups A (OR 0.12), B (OR 0.46), and D (OR 0.52). Vasopressor use was highest in Group D (OR 1.9, 95% CI 1.4–2.7), while inotrope use was elevated in Groups A (OR 4.3, 95% CI 1.4–14.7) and D (OR 3.2, 95% CI 1.3–9.6). Group D also had higher odds of 30-day mortality (OR 1.6, 95% CI 1.0–2.5). In addition, a likelihood ratio test comparing models with and without subphenotype assignment was significant (p = 0.028), indicating that subphenotype classification contributed additional explanatory value for 30-day mortality beyond established clinical risk factors. Length of stay and ventilator use did not significantly differ between groups.

Early Vital Signs Predict Subphenotype Stability

Subphenotype assignment was tracked over the first eight hours of hospital presentation (Fig. 3). When comparing the subphenotype classification based on the initial set of vital signs at hour 1 to classifications after the full 8 hours, 59% of patients never switched subphenotypes. Stability was similar across groups, with 62% of Group A, 58% of Group B, 56% of Group C, and 61% of Group D patients remaining in the same subphenotype throughout the observation period.

DISCUSSION

Major Findings

In this multicenter study, we prospectively validated the previously described sepsis subphenotypes using trajectories of vital signs from the first 8 hours of presentation. Demographic and comorbidity distributions were consistent with those observed in the retrospective study, supporting the reproducibility of the subphenotypes. The sepsis subphenotypes also had distinct clinical outcomes, including ICU admission, shock, renal replacement therapy, and mortality. To our knowledge, this represents the first prospective evaluation of a sepsis subphenotyping model deployed in real-time, extending prior work that has largely been retrospective or applied post-hoc to completed trial datasets.

There has now been almost a decade of research on identifying sepsis subphenotypes. Prior studies have identified between two to six subphenotypes, based on different data sources including transcriptomics, biomarkers, and clinical data [11, 12]. Seymour et al. found four subphenotypes by examining laboratory and vital signs data, with the subphenotypes correlating with distinct host-response patterns and clinical outcomes [13]. Shankar-Hari et al. found two subphenotypes in the VANISH study and three in the LeoPARDS research, using both clinical and biomarker data [11]. They observed a hyperinflammatory subphenotype correlated with increased mortality, but without different responses to treatments. In the PROWESS Shock Study, Gardlund et al. examined baseline acute and chronic clinical categories and found six septic shock subphenotypes [15]. While there could be countless ways to slice the data and thus countless potential sepsis subphenotypes, there is a significant scarcity of implementation and prospective validation of these subphenotypes.

Clinical Implications and Potential Applications

The study demonstrates that prospective real-time subphenotype classification is feasible. However, the prospective model performance does not perfectly replicate retrospective performance. For instance, the distribution across the subphenotypes was different from the retrospective data, in which Group A represented 28% of the sepsis population compared to 16% in this study. The discrepancies noted between the retrospective and prospective performance could be due to several potential reasons. First, retrospective data can include measurements documented retroactively. For instance, if a patient is acutely deteriorating, vital signs may not be documented in real-time for several hours until the patient is stabilized. Retrospective data would include retroactively documented measurements during the deterioration period, while the prospective data would not include these measurements. Second, the cohort of interest itself (i.e., patients with sepsis) is logistically simpler to identify retrospectively rather

than in real-time. For instance, retrospective studies could use surveillance-based criteria like Acute Sepsis Event definitions or ICD-10 codes for sepsis, which are not available in real-time. Third, time zero is logistically simpler to identify retrospectively. In our model, time zero is the time of patient's presentation to the emergency room. Prospectively, the location of the patient may not be updated till later, while retrospectively, the location could be backfilled to have a more exact time zero. These limitations underscore the necessity of prospective testing of any validated sepsis model. Despite these limitations, we found that the subphenotypes had similar patterns of clinical characteristics and outcomes that were observed in the retrospective studies. The similarities between the retrospective and current prospective study included demographics (Group A and B were younger, while C and D were older; Group B had relatively higher percentage of males) and comorbidities (Group B had higher prevalence of hypertension and diabetes mellitus; Group D had higher prevalence of CHF). Importantly, subphenotypes were associated with distinct outcomes similar to the findings in retrospective studies, with Group D having higher incidence of vasopressor use and higher mortality rate. One of the important questions that remain is whether these Groups actually respond differently to different treatments, which would pave the way for a precision medicine approach to sepsis. Future clinical trials could integrate a priori subphenotype stratification to evaluate for heterogeneity of treatment effect.

Strengths and Limitations

The multicenter design included six hospitals with diverse patient populations, enhancing generalizability. The algorithm was prospectively integrated into the clinical workflow, allowing for automated, real-time subphenotype classification, which strengthens the feasibility and potential clinical usability. Limitations include the lack of biological data such as immune markers that could further elucidate whether these subphenotypes represent distinct biological entities. Also, we did not explore any heterogeneity of treatment effects, which limits the immediate clinical applicability of these subphenotypes. Finally, there was a simultaneous clinical trial that enrolled patients who were Group D based on the initial set of vital signs, and the trial intervention may have an impact on Group D outcomes.

CONCLUSIONS AND FUTURE DIRECTIONS

This study demonstrates that subphenotypes identified by vital sign trajectories can be reliably reproduced in a prospective, multicenter setting. Future work will focus on integrating these subphenotypes into clinical trials to evaluate the heterogeneity of treatment effects.

Declarations

COMPETING INTERESTS

The authors have no competing interests to report.

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Figures

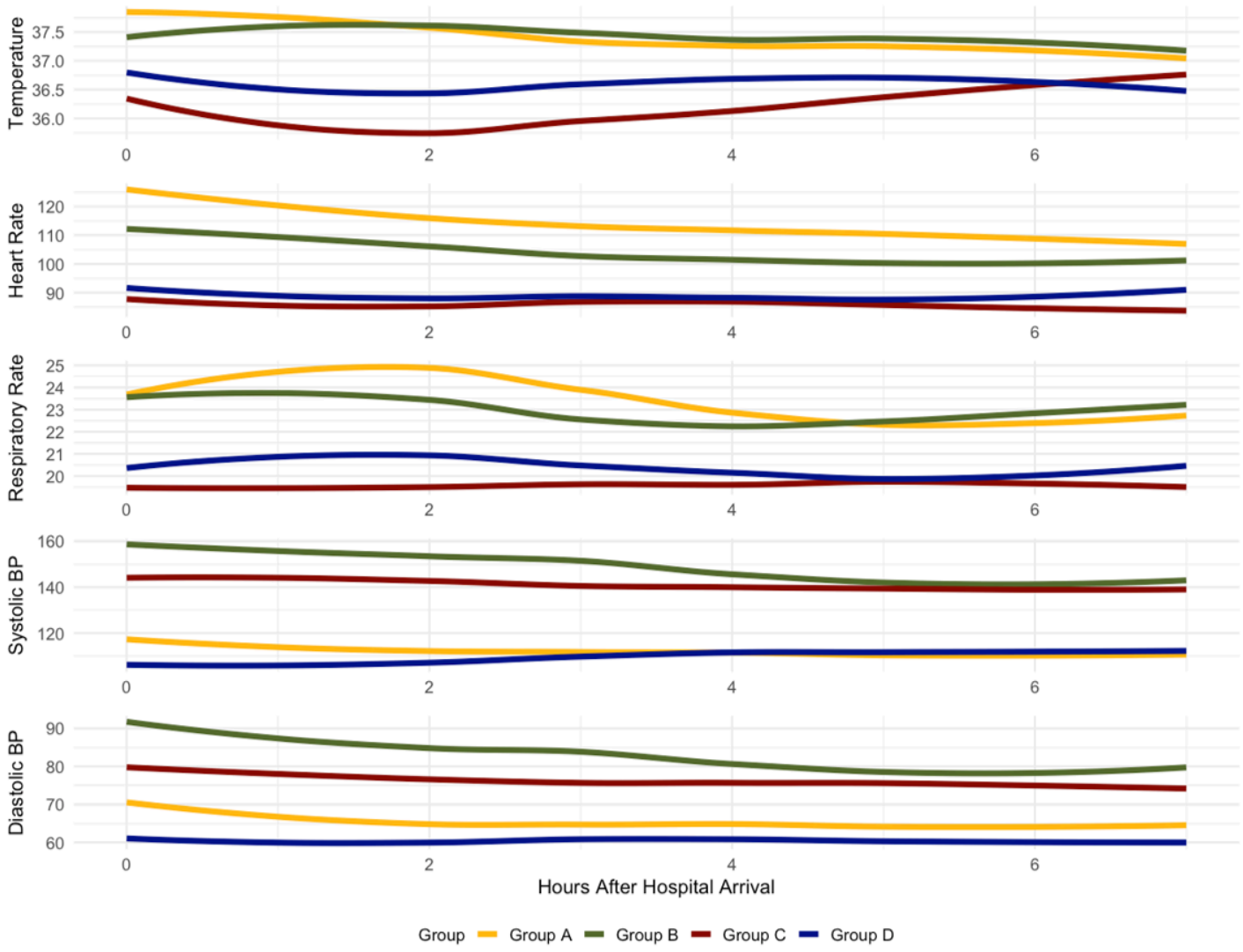


Figure 1

Group-based trajectory modeling of vital signs. Four subphenotypes of vital sign trajectories were identified using group-based trajectory modeling. Each patient was assigned to a final subphenotype group, which is displayed in the figure.

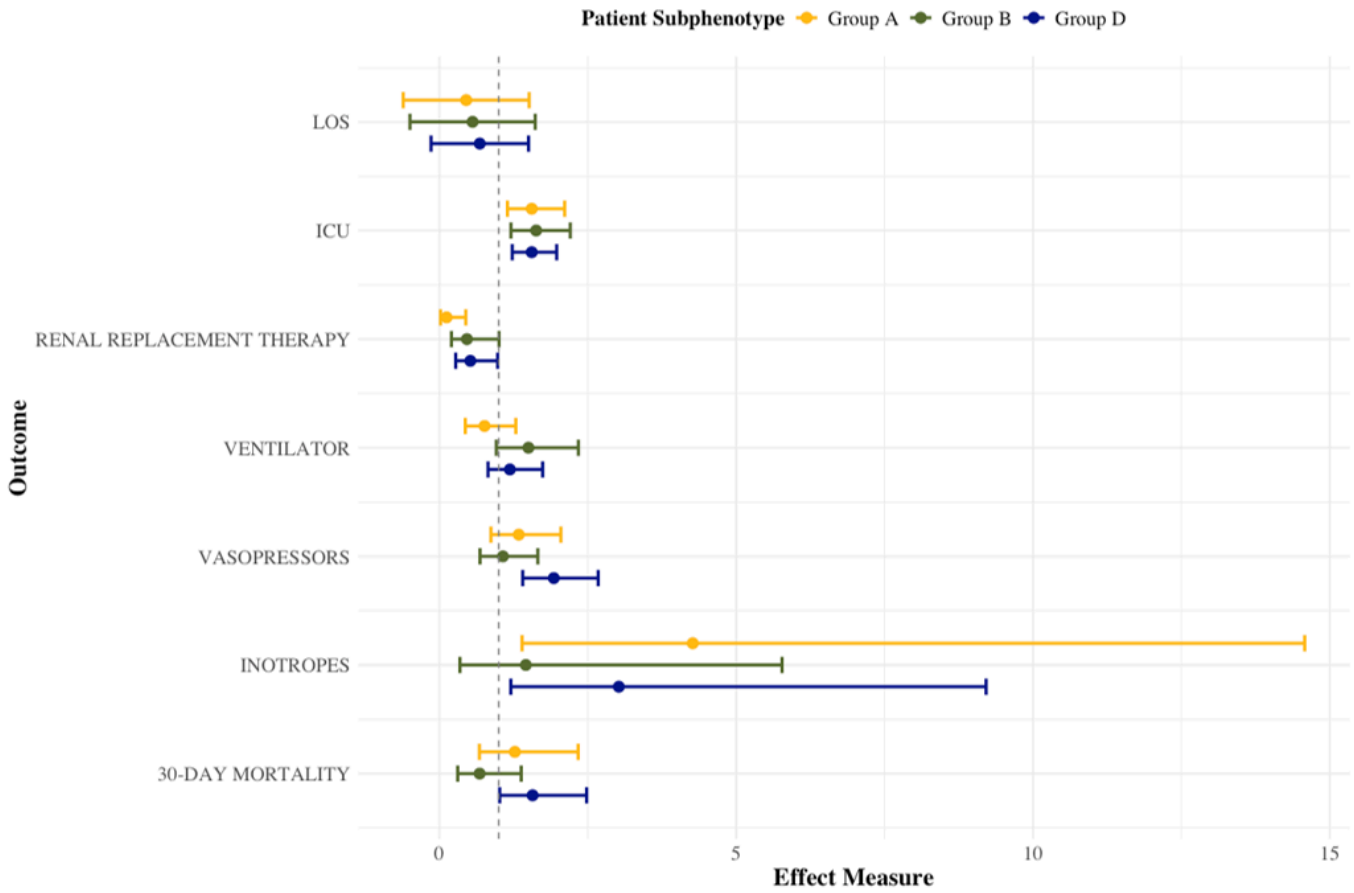


Figure 2

Association of Patient Subphenotype with Outcomes. Logistic regression was performed adjusting for demographics and comorbidities. Odds ratios were presented for all outcomes, with the exception of LOS, a continuous measure, presented as the β coefficient.

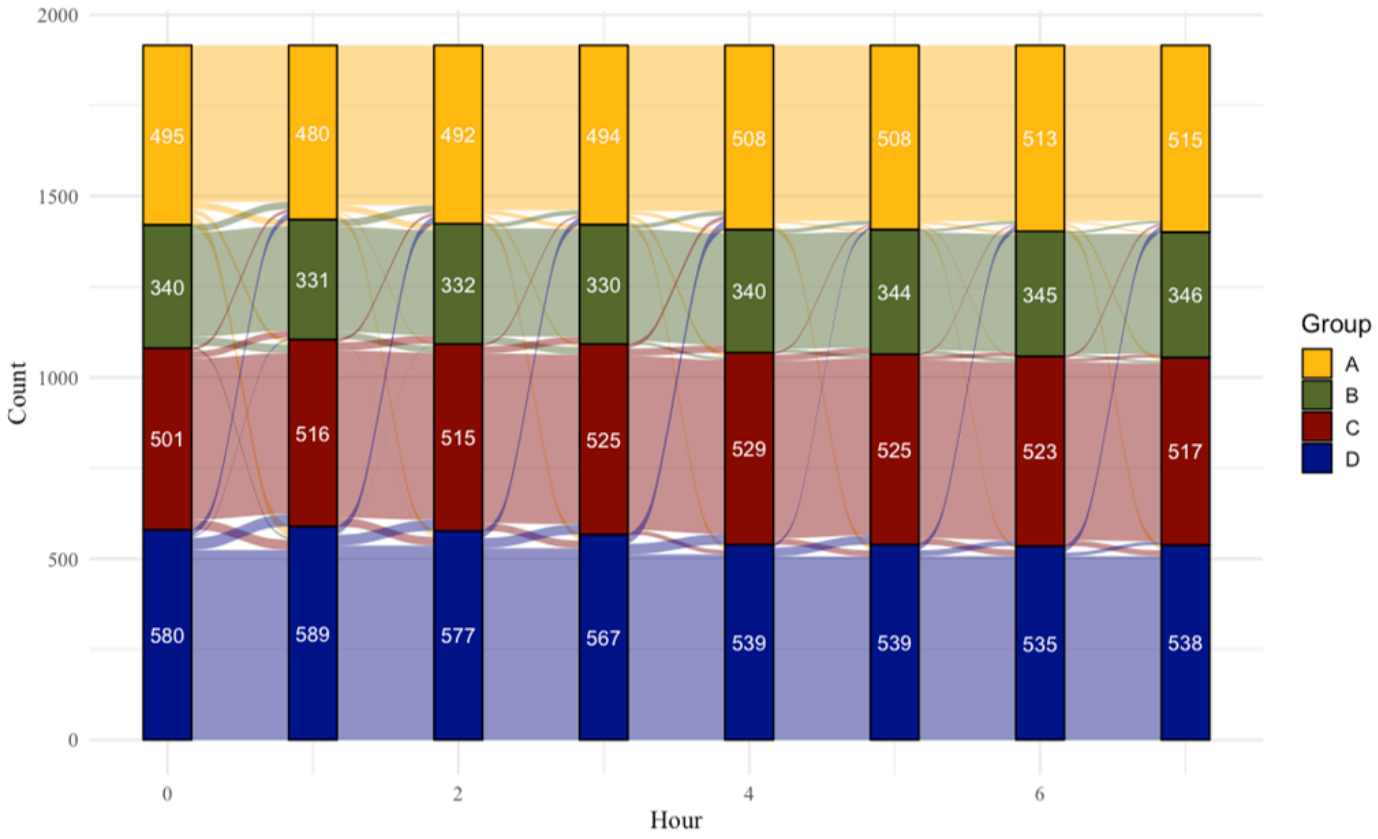


Figure 3

Hourly subphenotype over the first 8 hours of hospital presentation. The alluvial plot illustrates patient movement between the 4 sepsis subphenotypes each hour. Width of flows corresponds to the number of patients transitioning between groups, highlighting the stability and changes in subphenotype assignment over time.

Supplementary Files

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

- SchwinneSTROBEchecklist.docx