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REPORTS OF ORIGINAL INVESTIGATIONS

White blood cell count trajectory and mortality in septic shock: a historical cohort study

Évolution de la numération leucocytaire et mortalité en cas de choc septique : une étude de cohorte historique

Emily Rimmer, MD, MSc · Allan Garland, MD, MA · Anand Kumar, MD · Steve Doucette, MSc · Brett L. Houston, MD · Chantalle E. Menard, MD · Murdoch Leeies, MD · Alexis F. Turgeon, MD, MSc · Salah Mahmud, MD, PhD · Donald S. Houston, MD, PhD · Ryan Zarychanski, MD, MSc

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Abstract

Purpose *Septic shock is associated with a mortality of 20–40%. The white blood cell count (WBC) at hospital admission correlates with prognosis in septic shock. Here, we explore whether the trajectory of WBC after admission provides further information about outcomes. We aimed to identify groups of patients with different WBC trajectories and the association of WBC trajectory with mortality.*

Methods *We included adult patients with septic shock in two academic intensive care units (ICU) in Winnipeg, MB, Canada between 2006 and 2012. We used group-based trajectory analysis to group patients according to their WBC patterns over the first seven days in the ICU. Our primary analysis was the association of WBC trajectory group on 30-day mortality using multivariable Cox proportional hazards regression.*

Results *We included 917 patients with septic shock. The final model identified seven distinct WBC trajectories. The rising WBC trajectory was independently associated with increased mortality (hazard ratio, 3.41; 95% confidence interval, 1.86 to 6.26; $P < 0.001$) compared with the stable WBC trajectory.*

Conclusion *In patients with septic shock, distinct and clinically relevant groups can be identified by analyzing WBC trajectories. A rising WBC trajectory was associated with higher mortality.*

Résumé

Objectif *Le choc septique est associé à une mortalité de 20 à 40 %. La numération leucocytaire à l'admission à l'hôpital est corrélée au pronostic en cas de choc septique. Dans ce manuscrit, nous tentons de déterminer si l'évolution de la numération leucocytaire après*

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E. Rimmer, MD, MSc (✉)
Department of Community Health Sciences, University of Manitoba, Winnipeg, MB, Canada
e-mail: erimmer@cancercare.mb.ca

Department of Internal Medicine, Section of Medical Oncology/Hematology, University of Manitoba, Winnipeg, MB, Canada

Department of Medical Oncology and Haematology, CancerCare Manitoba, 675 McDermot Ave, Winnipeg, MB R3E 0V9, Canada

A. Garland, MD, MA · M. Leeies, MD
Department of Community Health Sciences, University of Manitoba, Winnipeg, MB, Canada

Department of Internal Medicine, Section of Critical Care Medicine, University of Manitoba, Winnipeg, MB, Canada

A. Kumar, MD
Department of Internal Medicine, Section of Critical Care Medicine, University of Manitoba, Winnipeg, MB, Canada

Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, MB, Canada

S. Doucette, MSc
Capital District Health Authority, Dalhousie University, Halifax, NS, Canada

l'admission fournit plus d'informations sur les devenirs. Nous avons cherché à identifier des groupes de patients présentant différentes trajectoires d'évolution de numération leucocytaire et l'association entre l'évolution de la numération et la mortalité.

Méthode *Nous avons inclus des patients adultes atteints d'un choc septique dans deux unités de soins intensifs (USI) universitaires à Winnipeg, Manitoba, Canada entre 2006 et 2012. Nous avons utilisé une analyse de l'évolution basée sur le groupe pour regrouper les patients en fonction du type d'évolution de la numération leucocytaire au cours des sept premiers jours à l'USI. Notre analyse principale consistait à déterminer l'association entre le groupe d'évolution de numération leucocytaire et la mortalité à 30 jours en utilisant une régression multivariable à risque proportionnel de Cox.*

Résultats *Nous avons inclus 917 patients atteints de choc septique. Le modèle final a identifié sept types de trajectoire d'évolution de numération leucocytaire distincts. Une évolution ascendante de la numération leucocytaire était indépendamment associée à une augmentation de la mortalité (rapport de risque, 3,41; intervalle de confiance à 95 %, 1,86 à 6,26; P < 0,001) par rapport à une évolution de numération leucocytaire stable.*

Conclusion *Chez les patients atteints de choc septique, des groupes distincts et cliniquement pertinents peuvent être identifiés en analysant les trajectoires d'évolution de la numération leucocytaire. Une évolution ascendante de la numération leucocytaire était associée à une mortalité plus élevée.*

Keywords Septic shock · trajectory analysis · white blood cell count

Infection is the third leading cause of mortality worldwide.[1] The incidence of sepsis in adults is approximately three per 1,000 per year and the mortality of septic shock ranges from 20% to 40%.[2, 3] Abnormal white blood cell counts (WBCs) are common in critically ill patients with infection, and correlate with disease severity in patients admitted to an intensive care unit (ICU).[5] The WBC integrates many parameters that are pertinent to the host response to infection.[6] Although derangements in the WBC are associated with increased mortality in septic shock, the impact of individual variation in the WBC over time has not been well described. Prior studies evaluating the evolution of WBC in infected patients have assessed average values over time;[7–9] however, mean values can obscure the actual WBC trajectories. As such, no individual may have a trajectory described by the mean WBC and therefore methods, such as group-based trajectory modeling (GBTM), which account for repeated measures of a variable over time, may be helpful.[10]

Pragmatic strategies to distinguish and segregate patients with dissimilar biological processes are needed. Essential to this paradigm is the ability to recognize a patient's clinical course so that appropriate treatment strategies can be considered. In the setting of septic shock, trajectory analysis may be an important tool for identifying unique phenotypic clusters. Used extensively in the social sciences,[10] trajectory analysis has been employed in medicine to describe differences within a population to characterize disease biology, describe healthcare utilization, or identify patients who may have different outcomes or may benefit from additional therapies.[11–16]

The primary objectives of our study were to identify distinct groups of patients with different WBC trajectories and to evaluate the association of WBC trajectory with mortality in patients with septic shock. Secondary objectives were to identify patient and illness factors associated with different WBC trajectories.

B. L. Houston, MD · C. E. Menard, MD · D. S. Houston, MD, PhD
Department of Internal Medicine, Section of Medical Oncology/Hematology, University of Manitoba, Winnipeg, MB, Canada

Department of Medical Oncology and Haematology, CancerCare Manitoba, 675 McDermot Ave, Winnipeg, MB R3E 0V9, Canada

A. F. Turgeon, MD, MSc
CHU de Québec – Université Laval Research Centre, Population Health and Optimal Health Practices Research Unit, Trauma – Emergency – Critical Care Medicine, Université Laval, Québec City, QC, Canada

Department of Anesthesiology and Critical Care Medicine, Division of Critical Care Medicine, Faculty of Medicine, Université Laval, Québec City, QC, Canada

S. Mahmud, MD, PhD
Department of Community Health Sciences, University of Manitoba, Winnipeg, MB, Canada

R. Zarychanski, MD, MSc
Department of Community Health Sciences, University of Manitoba, Winnipeg, MB, Canada

Department of Internal Medicine, Section of Medical Oncology/Hematology, University of Manitoba, Winnipeg, MB, Canada

Department of Medical Oncology and Haematology, CancerCare Manitoba, 675 McDermot Ave, Winnipeg, MB R3E 0V9, Canada

Department of Internal Medicine, Section of Critical Care Medicine, University of Manitoba, Winnipeg, MB, Canada

Methods

Research design and study population

We conducted a historical cohort study of patients ≥ 18 yr of age with septic shock who were admitted to an ICU at either the Health Sciences Center or Saint Boniface Hospital in Winnipeg, MB, Canada between 2006 and 2012. This study was approved by the University of Manitoba Health Research Ethics Board (HS21067, H2017:270). Patients were identified using the Cooperative Antimicrobial Therapy of Septic Shock (CATSS) database.[17,18] The data for this study were developed by linking the CATSS database and laboratory variables from the Laboratory Information System at both participating hospitals. White blood cell count measurements were obtained from Sysmex XE-2100 automated hematology analyzers (Sysmex Canada, Mississauga, ON, Canada). Septic shock is defined in this database by the 1991 Society for Critical Care Medicine/ American College of Chest Physicians Consensus Statement on Septic Shock, and remained consistent throughout the study period.[19] Data were collected on each case up to time of death, to hospital discharge, or to a maximum of 30 days in hospital.

Seeking to minimize WBC abnormalities unrelated to septic shock, we excluded patients with malignancy, hepatic cirrhosis, human immunodeficiency virus (HIV), or immunosuppression (including cytotoxic chemotherapy). To allow sufficient elapsed time to generate a WBC trajectory and to reduce the potential for survival bias, we limited our analysis to those patients who had an ICU length of stay of at least 48 hr and had at least three serial WBC measurements to determine the trajectory. For participants with more than one eligible ICU admission during the study period, only the first admission was included.

Study variables

Variables in the CATSS database include age, sex, geographic location, Acute Physiology and Chronic Health Evaluation (APACHE) II score,[5] number of new organ failures on day one of ICU admission, type of organ failure (cardiovascular, renal, respiratory, metabolic, or hematologic), comorbid conditions (liver failure, chronic obstructive pulmonary disease, diabetes, New York Heart Association Class IV heart failure, dialysis dependency, malignancy, HIV infection or acquired immunodeficiency syndrome), immunosuppression, documented or presumed site and source of infection, supportive treatments (e.g., mechanical ventilation, vasopressors/inotropes, renal replacement therapy), and the time to first appropriate antimicrobial therapy. Appropriate antimicrobial therapy was defined by the administration of antimicrobials with *in vitro* activity for the isolated pathogen, or appropriate for the clinical syndrome in cases where no pathogen was isolated.[20] Organ failures were defined as follows: cardiovascular = systolic blood pressure < 90 mm Hg or more than 40 mm Hg drop from normal OR mean arterial pressure < 65 mm Hg for at least one hour despite adequate fluid resuscitation (2 L saline or equivalent) OR the use of vasopressors; renal = elevation of baseline creatinine to $> 1.5 \times$ normal value; respiratory = invasive mechanical ventilation required; hematologic = platelet count $< 80 \times 10^9 \cdot L^{-1}$; and metabolic = lactate > 3 mM·L^{-1}. Endpoints obtained from the CATSS database included ICU survival, survival to 30 days or hospital discharge alive, and ICU length of stay. Laboratory variables collected included WBC; hemoglobin level; platelet count; international normalized ratio; and bilirubin, creatinine, and lactate levels.

Statistical analyses

We used group-based trajectory modelling (GBTM) to segregate patients according to the trajectory of their total WBC. Group-based trajectory modelling is a statistical method used to describe the trend of a variable over time.[21] PROC TRAJ is a procedure in SAS (SAS Institute, Cary, NC, USA) that identifies groups of individuals with similar characteristics over time using group-based modelling.[21–23] Rather than prespecifying groups within a population, GBTM allows for different groups with different trajectories to emerge from the data.

We included up to two WBC measurements per day; if more than two were available, the first within a 12-hr period was included. We anchored the start of the trajectory analysis to the time of the first WBC drawn, as most patients had this done at or shortly after ICU admission. We included measurements for the first seven days after ICU admission to capture the WBC trajectory attributable to septic shock.

We followed a previously described procedure for model building using PROC TRAJ.[22] We started with a one-group model and increased up to ten-group model. Trajectories were fitted to third-order polynomials to capture nonlinear patterns over time. The optimal number of trajectories was selected using the standard method of evaluating the Bayesian Information Criterion (BIC),[21] augmented by clinical interpretation and sensibility. Clinical interpretation was based on expert opinion reviewed at regular meetings with senior critical care physicians and included an assessment of whether the trajectories were as expected for patients with septic shock. Model fit statistics were performed on the final model. The

first model diagnostic assesses the ratio between the probability of group membership (pj) to the actual group membership. The probability of group membership measures the proportion of the population that belongs to a certain group. The ratio for each group should be close to 1. The second model diagnostic test is the average posterior probability (AvePP) for each group. The posterior probability is the probability that an individual with a specific pattern belongs to a specific trajectory group and is calculated for each individual in PROC TRAJ. The AvePP is calculated by taking the average of the posterior probabilities for each trajectory group. It is recommended that each group should have an AvePP of greater than 0.7.[23] The third diagnostic test is the Odds of Correct Classification (OCC) and is calculated by taking the ratio of the AvePP odds (AvePP/1-AvePP) to the pj odds (pj/1-pj). The higher the number, the better the odds of being correctly classified are. It is recommended that the OCC should be > 5 for each group. A minimum sample size of 100 participants with at least three data points for each participant is recommended for GBTM.[23]

We summarized the baseline characteristics of the entire cohort and compared baseline characteristics across the identified trajectory groups using the Kruskal–Wallis or Chi square test. Summary statistics are presented as means with standard deviation (SD) or medians with interquartile range [IQR] for continuous variables, and frequencies and proportions for categorical variables.

To investigate the independent association of WBC trajectories on 30-day mortality, we constructed a multivariable Cox proportional hazard model adjusted for variables known or thought to be associated with mortality, including patient variables (age, sex), illness variables (APACHE II score [modified to exclude the WBC component: APACHE-WBC]), number of organ failures on day 1 of ICU admission, pathogen variables (bacteremia, culture positive or negative) and cointervention variables (time from onset of hypotension to first antibiotic, the provision of appropriate or combination antibiotics). Although not well recognized, *P* values derived from multivariable regression are not adjusted for inherent multiple comparisons,[24] which increases type I errors. To adjust for this, we used Benjamini–Hochberg's (Simes') step-up procedure to control the false discovery rate at 5%.[25]

All statistical analyses were performed using SAS software version 9.4. P values < 0.05 were considered statistically significant.

Results

We identified 1,758 patients with septic shock. We excluded 323 patients with malignancy, 55 receiving chemotherapy or immunosuppression, three with pre-existing neutropenia, 84 with cirrhosis, and 19 with mixed-shock states defined as a treating physician determination that there was a substantial contribution ($> 50\%$) of another form of shock. Finally, we excluded one readmission, 152 records with unlinkable data, and 205 patients with fewer than three WBC measurements. The final cohort comprised 917 patients.

Half (454/917) of the included patients were male, the mean (SD) age was was 61 (17) yr, and the mean APACHE II score was 24 (7). Median [IQR] time to first antibiotics was 2 [0–6] hr and 816/917 (89%) patients received appropriate antibiotics. Of the 917 included patients, 605 (66%) had a positive culture, and 214 (23%) of these had bacteremia. Gram-positive bacterial infections were identified in 254 (28%) patients, gram negative bacteria in 258 (28%) patients, and fungal infections in 35 (4%) patients (Table 1).

Description of WBC trajectories

Our final trajectory solution consisted of seven trajectory groups representing distinct WBC patterns in septic shock (Fig. 1). This solution provided the lowest BIC value, as well as clinical sensibility. There were different WBC patterns. In group 1, the WBC started off normal and remained so over time; in group 2, the WBC started at a normal level and rose over time; in group 3, the WBC was moderately higher than normal at the start ($\sim 15 \times 10^9 \cdot L^{-1}$) and gradually decreased over time; in group 4, the WBC was high at the start ($> 20 \times 10^9 \cdot L^{-1}$) and gradually decreased; in group 5, the WBC was high at the start and continued to rise over time; in group 6, the WBC was high at the start and then rapidly decreased; and in group 7, the WBC was extremely elevated ($> 50 \times 10^9 \cdot L^{-1}$) at the start. Although we had planned *a priori* to have at least 5% of the population in each group, a small group of participants emerged with fewer than 2% of the population starting at the three-group stage of model development, which was present up to and including the final model. For this reason, the final seven-group model included groups with a small number of participants.

Three-model diagnostics were performed to assess the fit of the seven-group trajectory model. The ratio between the predicted (probability of group membership) and the actual group membership for each group ranged from 0.82 to 1.0 with most groups having a ratio > 0.95. The AvePP for each group ranged from 0.82 (group 3) to 0.99 (group 7). All AvePP were greater than 0.7. Finally, all OCC

Table 1 Baseline characteristics of the entire cohort and each WBC trajectory group

	Entire cohort	WBC trajectory groups						
		1 Normal, flat	2 Normal, rising	3 Moderate, decreasing	4 High, decreasing	5 High, rising	6 High, rapid decrease	7 Significantly elevated
	N = 917	*N* = 325	*N* = 57	*N* = 329	*N* = 130	*N* = 27	*N* = 42	*N* = 7
General demographics								
Male sex, *n*/total *N* (%)	454/917 (49.5%)	178/325 (54.8%)	31/57 (54.4%)	163/329 (49.5%)	55/130 (42.3%)	9/27 (33.3%)	17/42 (40.5%)	1/7 (14.3%)
Age (yr), mean (SD)	60.9/917 (16.5)	60.7 (16.5)	57.1 (17.1)	62.3 (16.6)	60.2 (15.5)	58.4 (15.3)	60.3 (17.8)	53.1 (21.1)
Duration of hospitalization before shock (days), median [IQR]	1 [0–2]	1 [0–3]	1 [0–2]	0.82 [0–2]	0.95 [0–2]	0.89 [0–2]	1 [0–4]	0 [0–3]
APACHE II score, mean (SD)	23.7 (7.2)	22.7 (7)	24.5 (8.1)	23.2 (6.9)	25.3 (7.2)	26.7 (7.5)	26 (7.7)	27.7 (5.8)
Time to 1st antibiotic (hr), median [IQR]	2 [0.03–6]	2.6 [0.4–6.3]	1.2 [0–5.7]	1.6 [0.02–5.6]	1.3 [0–4.2]	2.8 [0–6.4]	1.4 [0.08–4]	16.4 [0–71.2]
Appropriate antibiotic use, *n*/total *N* (%)	816/917 (89%)	286/325 (88%)	50/57 (87.7%)	296/329 (90%)	114/130 (87.7%)	24/27 (88.9%)	41/42 (97.6%)	5/7 (71.4%)
Geographic distribution								
Center 1, *n*/total *N* (%)	526/917 (57.4%)	192/325 (59.1%)	31/57 (54.4%)	186/329 (56.5%)	77/130 (59.2%)	13/27 (48.1%)	23/42 (54.8%)	4/7 (57.1%)
Center 2, *n*/total *N* (%)	391/917 (42.6%)	133/325 (40.9%)	26/57 (45.6%)	143/329 (43.5%)	53/130 (40.8%)	14/27 (51.9%)	19/42 (45.2%)	3/7 (42.9%)
Pre-existing medical conditions, *n*/total *N* (%)								
Chronic obstructive pulmonary disease	141/917 (15.4%)	44/325 (13.5%)	11/57 (19.3%)	54/329 (16.4%)	24/130 (18.5%)	0/27 (0%)	8/42 (19%)	0/7 (0%)
Diabetes mellitus	366/917 (39.9%)	124/325 (38.2%)	17/57 (29.8%)	129/329 (39.2%)	67/130 (51.5%)	10/27 (37%)	18/42 (42.9%)	1/7 (14.3%)
Chronic kidney disease	138/917 (15)	55/325 (16.9%)	7/57 (12.3%)	44/329 (13.4%)	19/130 (14.6%)	4/27 (14.8%)	8/42 (19%)	1/7 (14.3%)
Dialysis dependence	114/917 (12.4%)	45/325 (13.8%)	5/57 (8.8%)	40/329 (12.2%)	15/130 (11.5%)	1/27 (3.7%)	7/42 (16.7%)	1/7 (14.3%)
NYHA class IV heart failure	6/917 (0.7%)	5/325 (1.5%)	0/57 (0%)	1/329 (0.3%)	0/130 (%)	0/27 (0%)	0/42 (0%)	0/7 (0%)
Physiologic and laboratory variables on admission, median [IQR]								
Platelets ($\times 10^9 \cdot L^{-1}$)	183	165	164	186.5	216	186.5	213	272
	[118–259]	[106–224]	[107–245]	[128–266.5]	[159–313]	[87–312]	[122–339]	[154–311]
Serum creatinine ($\mu Mol \cdot L^{-1}$)	175	164	200	170	192	206	206	160
	[98–329]	[90–338.5]	[117–262]	[101–311]	[98–351]	[113–294]	[105–314]	[94–356]
INR	1.4 [1.2–1.7]	1.4 [1.2–1.7]	1.4 [1.2–1.6]	1.4 [1.2–1.7]	1.4 [1.2–1.7]	1.4 [1.3–1.9]	1.4 [1.2–2]	1.8 [1.4–1.9]
Bilirubin ($\mu Mol \cdot L^{-1}$)	11.4 [7–23]	11 [7–22]	15.5 [8–24]	12 [6–24]	12 [7–32]	10 [7–23]	9 [7–17]	10 [5–41]
HCO_3 ($mEq \cdot L^{-1}$)	19 [16–23]	19.5 [16–23]	20 [16–22]	19 [16–23]	18.8 [15.9–22]	19 [17–22]	18 [14–19.5]	20 [12–21]
Source of sepsis, *n*/total *N* (%)								
Culture positive	605/917 (65.9%)	198/325 (60.9%)	45/57 (78.9%)	214/329 (65%)	94/130 (72.3%)	15/27 (55.5%)	32/42 (76.2%)	7/7 (100%)
Bacteremia	214/917 (23.3%)	70/325 (21.5%)	13/57 (22.8%)	83/329 (25.2%)	27/130 (20.8%)	8/27 (29.6%)	12/42 (28.6%)	1/7 (14.3%)
Catheter-related infection	16/917 (1.7%)	8/325 (2.5%)	0/57 (0%)	6/329 (1.8%)	1/130 (0.8%)	1/27 (3.7%)	0/42 (0%)	0/7 (0%)
Respiratory	324/917 (35.3%)	121/325 (37.2%)	22/57 (38.6%)	105/329 (31.9%)	52/130 (40%)	12/27 (44.4%)	9/42 (21.4%)	3/7 (42.9%)
Urinary tract	96/917 (10.5%)	22/325 (6.8%)	1/57 (1.8%)	47/329 (14.3%)	11/130 (8.5%)	5/27 (18.5%)	10/42 (23.8%)	0/7 (0%)
Intra-abdominal	290/917 (31.6%)	102/325 (31.4%)	23/57 (40.4%)	111/329 (33.7%)	30/130 (23.1%)	5/27 (18.5%)	16/42 (38.1%)	3/7 (42.9%)
CNS	10/917 (1.1%)	1/325 (0.3%)	0/57 (0%)	3/329 (0.9%)	5/130 (3.8%)	1/27 (3.7%)	0/42 (0%)	0/7 (0%)
Skin and soft tissue	124/917 (13.5%)	43/325 (13.2%)	8/57 (14%)	40/329 (12.2%)	24/130 (18.5%)	2/27 (7.4%)	6/42 (14.3%)	1/7 (14.3%)
Surgical site	8/917 (0.9%)	5/325 (1.5%)	1/57 (1.8%)	0/329 (0%)	2/130 (1.5%)	0/27 (0%)	0/42 (0%)	0/7 (0%)
Cardiac/pericardial	3/917 (0.3%)	1/325 (0.3%)	0/57 (0%)	2/329 (0.6%)	0/130 (0%)	0/27 (0%)	0/42 (0%)	0/7 (0%)
Other	19/917 (2.1%)	9/325 (2.8%)	2/57 (3.5%)	3/329 (0.9%)	4/130 (3.1%)	1/27 (3.7%)	0/42 (0%)	0/7 (0%)

Table 1 continued

	Entire cohort	WBC trajectory groups						
		1 Normal, flat	2 Normal, rising	3 Moderate, decreasing	4 High, decreasing	5 High, rising	6 High, rapid decrease	7 Significantly elevated
	N = 917	*N* = 325	*N* = 57	*N* = 329	*N* = 130	*N* = 27	*N* = 42	*N* = 7
Type of infection, *n*/total *N* (%)								
Gram-positive organism	254/917 (28.3%)	76/325 (23.8%)	25/57 (44.6%)	93/329 (28.7%)	41/130 (33.1%)	9/27 (33.3%)	8/42 (19%)	2/7 (28.6%)
Gram-negative organism	258/917 (28.7%)	90/325 (28.2%)	13/57 (23.2%)	95/329 (29.3%)	34/130 (27.4%)	4/27 (14.8%)	20/42 (47.6%)	2/7 (28.6%)
Fungal	35/917 (3.9%)	12/325 (3.8%)	5/57 (8.9%)	10/329 (3.1%)	5/130 (4%)	1/27 (3.7%)	1/42 (2.4%)	1/7 (14.3%)
Other	40/917 (4.4%)	14/325 (4.4%)	1/57 (1.8%)	11/329 (3.4%)	8/130 (6.5%)	1/27 (3.7%)	3/42 (7.1%)	2/7 (28.6%)
New organ failures* on admission, *n*/total *N* (%)								
Renal failure	509/917 (55.5%)	171/325 (52.6%)	39/57 (68.4%)	173/329 (52.6%)	74/130 (56.9%)	19/27 (70.4%)	28/42 (66.7%)	5/7 (71.4%)
Hematologic failure	113/917 (12.3%)	49/325 (15.1%)	9/57 (15.8%)	32/329 (9.7%)	13/130 (10%)	5/27 (18.5%)	4/42 (9.5%)	1/7 (14.3%)
Hepatic failure	145/917 (15.8%)	43/325 (13.2%)	10/57 (17.5%)	54/329 (16.4%)	29/130 (22.3%)	5/27 (18.5%)	3/42 (7.1%)	1/7 (14.3%)
Respiratory failure	733/917 (79.9%)	268/325 (82.5%)	52/57 (91.2%)	253/329 (76.9%)	100/130 (76.9%)	22/27 (81.5%)	33/42 (78.6%)	5/7 (71.4%)
CNS failure	292/917 (31.8%)	111/325 (34.2%)	15/57 (26.3%)	100/329 (30.4%)	44/130 (33.8%)	10/27 (37%)	10/42 (23.8%)	2/7 (28.6%)
Coagulation failure	315/917 (34.4%)	106/325 (32.6%)	17/57 (29.8%)	106/329 (32.2%)	53/130 (40.8%)	11/27 (40.7%)	18/42 (42.9%)	4/7 (57.1%)
Metabolic failure	391/917 (42.6%)	122/325 (37.5%)	31/57 (54.4%)	137/329 (41.6%)	57/130 (43.8%)	14/27 (51.9%)	27/42 (64.3%)	3/7 (42.9%)
Total number of day one organ failures, mean (SD)	3.7 (1.4)	3.7 (1.4)	4 (1.2)	3.6 (1.5)	3.8 (1.5)	4.2 (1.5)	3.9 (1.5)	4 (1.9)

*Organ failure definitions (cardiovascular = systolic blood pressure < 90 mm Hg or more than 40 mm Hg drop from normal OR MAP < 65 mm Hg for at least one hour despite adequate fluid resuscitation [2 L saline or equivalent] OR the use of vasopressors; renal = elevation of normal baseline creatinine to $> 1.5 \times$ normal value; respiratory = ventilation required; hematologic = platelet count $< 80 \times 10^9 \cdot L^{-1}$; metabolic = lactate > 3 mMol$\cdot L^{-1}$)

APACHE II = acute physiology and chronic health evaluation II; CNS = central nervous system; HCO_3 = bicarbonate; INR = international normalized ratio; IQR = interquartile range; MAP = mean arterial pressure; NYHA = New York Heart Association; SD = standard deviation; WBC = white blood cell count

values for groups 1–7 were greater than 5. Thus, the model diagnostics all support the correct group assignment for the seven-group model.

For the entire cohort, unadjusted ICU mortality was 27% and 30-day mortality was 26%. The median [IQR] ICU length of stay for the entire cohort was 8 days [4–14]. Mechanical ventilation was required by 84% of patients (771/917) and 20% (186/917) required acute renal replacement therapy. Intensive care unit mortality (see Electronic Supplementary Material, eTable) ranged from a low of 25% (80/325) in group 1 (normal, flat) to a high of 67% (18/27) in group 5 (high, rising). Likewise, 30-day mortality ranged from 23% (76/325) in group 1 (normal, flat) to 63% (17/27) in group 5 (high, rising). Groups 2 and 5 had a rising WBC trajectory and a higher mortality.

Cox proportional hazard model assessing the effect of trajectory group on mortality over 30 days

After adjusting for variables known to be associated with survival in the ICU, a multivariable Cox proportional hazard model (Table 2) showed that group 5 (high, rising) was independently associated with an increased mortality at 30 days (hazard ratio, 3.41; 95% confidence interval, 1.86 to 6.26; $P < 0.001$) compared with group 1 (normal; flat). Unadjusted Kaplan–Meier survival curves (Fig. 2) illustrate differences in mortality among the seven trajectory groups.

Discussion

In this historical cohort study of patients with septic shock, we used group-based trajectory analyses to segregate patients into distinct and clinically relevant WBC trajectories. These groups have different starting values and unique evolutionary patterns of the WBC over time. A high and rising WBC was independently associated with an increased risk of death at 30 days; a similar mortality trend was observed in patients with an initially normal but rising WBC.

Prognosis in critical care is customarily founded on baseline characteristics, such as the APACHE II score.[5] More recently, the importance of temporal trends in predicting prognosis has been recognized, as reflected by the change in Sequential Organ Failure Assessment (SOFA) score.[26] Nevertheless, studies evaluating temporal trends typically fail to assess differences between patient subgroups, and instead assess the entire group in aggregate, such as the evaluation of the mean platelet count over time in critical illness[27] and septic shock.[28] Trajectory analysis is a method of assessing data in medicine, which is greatly facilitated by the use of electronic medical records and large data repositories. A recent study evaluating the temperature trajectory of hospitalized patients with infection showed four subphenotypes of sepsis using a similar methodology to our study.[16] The hyperthermic group was associated with more frequent abnormalities of the WBC and had higher levels of inflammatory biomarkers including erythrocyte sedimentation rate and C-reactive protein than the normothermic and hypothermic groups did. Furthermore, there were significant differences in mortality between the groups.[16] A limitation of that study was the inclusion of all patients presenting to an emergency room with suspected infection (defined as having a blood culture drawn and four days of antibiotics) and there were no data specific to septic shock. Our findings suggest there are subphenotypes of septic shock that are based on WBC trajectory, highlighting the heterogeneity of septic shock.

Intensive care physicians have historically valued tools to discern heterogeneity in clinical syndromes. It is conceivable that a rising WBC trajectory could alert clinicians to patients at increased risk for a poor outcome and potentially inform real-time patient-level decision-making. Alternatively, unique WBC trajectories may provide insight into phenotypic or genotypic differences that reflect host biologic variability in septic shock.

The processes that result in critical illness are diverse in their etiology, severity, and clinical trajectory over time. In the response to the poor progress of trials evaluating new interventions in critically ill patients, Laffey and Kavanagh reminded us that "sepsis and ARDS are biologically heterogeneous syndromes: they are not diseases per se, with singular mechanisms that are plausibly amenable to singular interventions."[29] Many potential sources of heterogeneity exist in sepsis and include patient, illness, and treatment specific factors. Databases that include high-quality clinical, laboratory, and therapeutic data, and possibly biomarkers and genetic information will be required to comprehensively understand which factors contribute to WBC trajectory group assignment.

A strength of this study was the use of GBTM, which allows exploration of variability according to unique phenotypic groupings instead of population summary estimates. To allow the data to show different trajectories, we did not prespecify groups, but allowed the data to guide the results through an algorithmic classification procedure. To minimize selection bias, we included all consecutive patients admitted to the ICUs with septic shock. Further strengths included the exclusion of patients with alternative explanations for WBC abnormalities, which enabled us to assess the WBC trajectory attributable to septic shock.

The process of arriving at the final trajectory model was iterative and involved both clinical judgment and

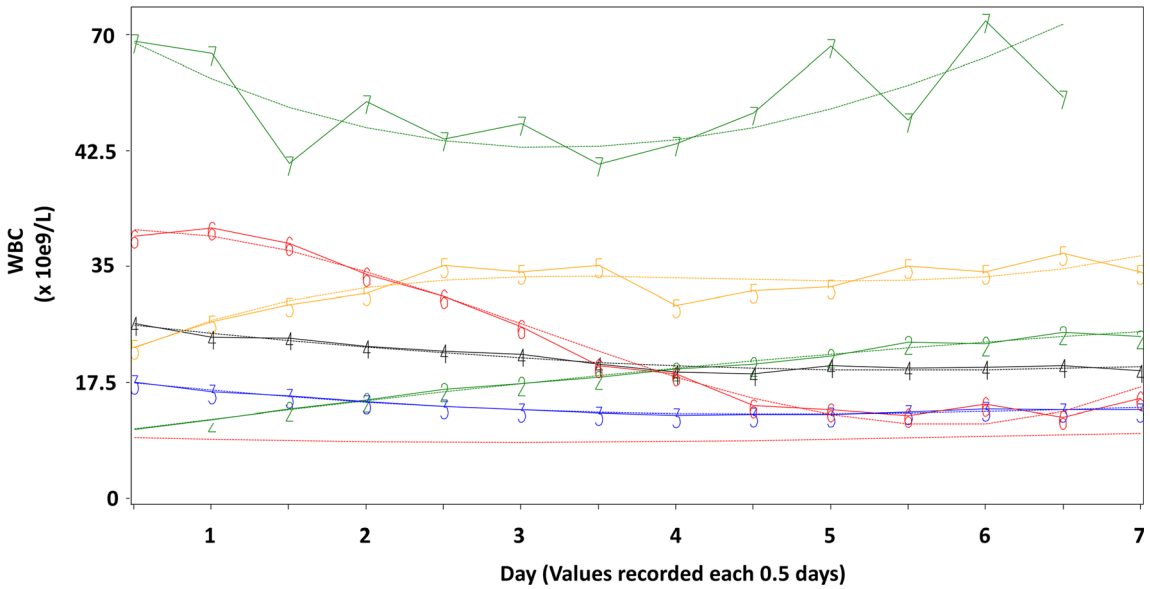

Fig. 1 Graphical representation of the seven-group model of white blood cell count (WBC) in septic shock for seven days after intensive care unit admission

Table 2 Results of multivariable Cox proportional hazard model assessing the effect of WBC trajectory group on 30-day mortality

WBC trajectory group	Hazard ratio* (95% CI)	*P* value	Adjusted *P* value†
Group 1 (ref) normal, flat	ref	ref	ref
Group 2 normal, rising	1.45 (0.83 to2.53)	0.19	0.56
Group 3 moderate, decreasing	1.13 (0.81 to 1.59)	0.46	0.56
Group 4 high, decreasing	0.83 (0.5 to 1.37)	0.47	0.56
Group 5 high, rising	3.41 (1.86 to 6.26)	< 0.001	< 0.001
Group 6 high, rapid decrease	1.42 (0.69 to 2.91)	0.34	0.56
Group 7 significantly elevated	1.46 (0.35 to 6.12)	0.60	0.60

*Adjusted for age, sex, APACHE II score, baseline comorbidities, type of infection, site, antibiotic timing and type

† Adjusted for false discovery rate using Simes' step-up procedure

APACHE II = acute physiology and chronic health evaluation II; CI = confidence interval; WBC = white blood cell count

mathematical computation, which may be seen as a potential limitation.[30] The choice of a seven-group model was made based on statistical fit (BIC)[23] and clinical assessment. We acknowledge that this analysis requires validation in other septic shock populations. We do not have complete clinical data on the use of corticosteroids in our data set and therefore cannot account for their effect on the WBC count. The use of corticosteroids to treat septic shock, however, was not routinely recommended during the study period.[31] The analysis was limited by the small numbers of patients in some of the WBC trajectory groups, so we could not draw firm conclusions from certain groups with low absolute numbers. Because of the historical nature of this study, the influence of unmeasured confounders cannot be excluded. Less tangible patient factors such as frailty, differences in the host immune response to infection,[32] and genomic variation are difficult to identify and evaluate in a retrospective analysis. Finally, it is worth acknowledging that it is possible that participants with a rising WBC had the wrong diagnosis or that the rising WBC was related to underlying patient factors.

We chose to analyze the total WBC count for familiarity among treating clinicians and generalizability to knowledge-users. Perhaps the total neutrophil count or lymphocyte count would be more instructive for exploring the biologic variability in septic shock or for determining the prognosis of patients in real time. Finally, it is unknown if a *single* variable trajectory is the best model to take forward to understand the biologic differences among the groups; perhaps a multiple trajectory model including both

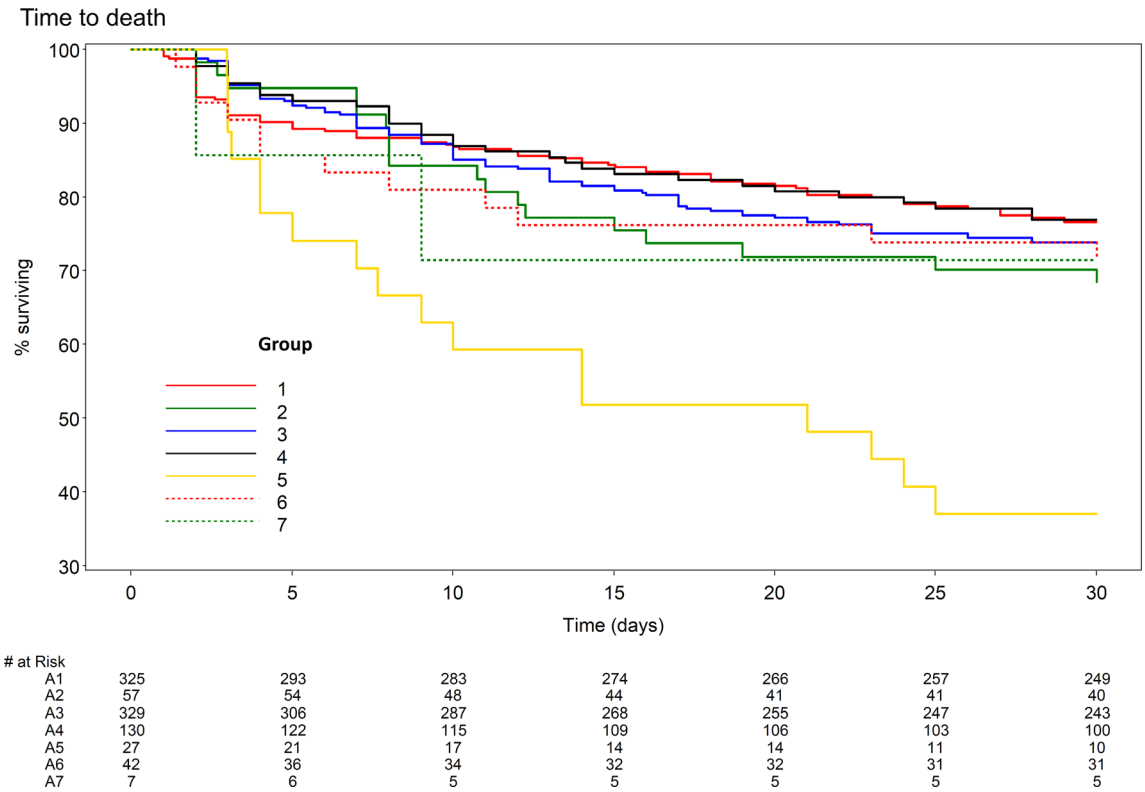

Fig. 2 Kaplan–Meier curve showing time to death among different WBC trajectory groups to a maximum of 30 days. Group 5 (high, rising) has the worst survival compared with other groups ($P < 0.01$). WBC = white blood cell count

platelet and WBC count plus other physiologic and laboratory variables such as temperature, creatinine and/or bilirubin, or genomic information would give more instructive and predictive trajectories.

Conclusions

In patients with septic shock, distinct and clinically relevant groups can be identified by analyzing WBC trajectories. Rising WBC trajectories are associated with increased mortality. Further studies are required to understand the clinical characteristics and prognosis associated with distinct WBC trajectories and whether this information, alone or in combination with other trajectory data, can inform care decisions and treatment responses.

Authors contributions *Emily Rimmer* and *Ryan Zarychanski* conceived the study. *Emily Rimmer*, *Allan Garland*, and *Ryan Zarychanski* designed the study. *Steve Doucette* performed the statistical analysis. *Emily Rimmer* interpreted the data and wrote the manuscript. All authors provided intellectual contributions to the interpretation of the data and read and reviewed the final manuscript.

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Data availability statement The data that support the findings of this study are available from Dr. Anand Kumar but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are, however,– available from the authors upon reasonable request and with permission of Dr. Anand Kumar.

Ethics approval and consent to participate This study was approved by the University of Manitoba Health Research Ethics Board (HS21067, H2017:270).

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