

A Continuous Metric to Measure Endotype and Corticosteroid Interaction in Septic Shock

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Abstract

The benefit of corticosteroids for septic shock remains unclear, reflecting heterogeneity of treatment effect. We previously reported a binary septic shock endotype classification based on expression of 100 genes. This binary classification provides information regarding prognosis and corticosteroid responsiveness but does not capture the endotypes continuum. Here, we describe a continuous metric for endotype assignment, the Z value, and its relationship with septic shock outcomes and corticosteroid responsiveness. In a derivation cohort, a lower Z value was independently associated with increased odds of mortality. When the Z value was fit using cubic splines, the odds of mortality increased considerably below a Z value of 15 among patients exposed to corticosteroids. A similar interaction between Z value and corticosteroid exposure was observed when the model was applied to pediatric and adult validation cohorts. The Z value provides more detailed evidence of patients with septic shock in whom corticosteroids are harmful.

Introduction

Endotypes represent subgroups of otherwise heterogenous clinical syndromes that share underlying biological commonalities (1–3). Septic shock is the quintessential heterogenous clinical syndrome among critically ill patients. Three research programs independently leveraged the discovery potential of transcriptomics to propose the existence of endotypes among adults with septic shock, defined by shared gene expression patterns broadly corresponding to immunity, inflammation, and coagulation (4–8). The endotypes reported by the respective programs are characterized by differences in clinical features such as illness severity, organ failure burden, and mortality. More importantly, the respective endotype-defining gene expression signatures imply differential responses to treatment, including responsiveness to adjunctive corticosteroids among patients with septic shock (9, 10).

Studies reported by us have focused on defining and validating gene expression-based endotypes among children with septic shock (11–15). The strategy relies on a self-organizing map algorithm to generate gene expression mosaics (16, 17). Mosaics from individual patients are compared to reference mosaics using computer assisted image analysis. The 100 genes included in the mosaics reflect adaptive immune function and glucocorticoid receptor signaling, which are highly relevant to the pathobiology of septic shock. The Z value computed when comparing the individual mosaics to the references was used as a binary classifier. A negative value defined endotype A, while a positive value defined endotype B.

This binary classification has direct potential for precision medicine by broadly identifying likely treatment response to adjunctive corticosteroids. Pediatric endotype A patients are characterized by a higher mortality rate and greater organ failure burden when compared to endotype B patients, and corticosteroid prescription is associated with increased risk of mortality among endotype A patients after adjusting for illness severity (14). Conversely, among the subset of endotype B patients who are at intermediate to high baseline risk of mortality, as measured by the Pediatric Sepsis Biomarker Risk Model

(18), corticosteroid prescription is associated with decreased risk of poor outcome after adjusting for illness severity (19).

The current pediatric endotyping strategy assumes a dichotomy but given the complexity and heterogeneity of critical illness it is expected that pediatric septic shock endotypes exist along a biological continuum. To develop a more complete understanding of the relationships between endotype assignment and response to corticosteroids, and possibly further inform treatment decisions, we evaluated the endotype Z value as a continuous metric. We reasoned that a continuous metric could more accurately capture the endotype continuum and, if modeled flexibly without forcing a linear association, non-arbitrary cut points of the Z value might be evident. The goals of this study were to describe the relationship between septic shock outcomes and endotype Z value, and to ascertain the role of the Z value in further describing differential treatment response to corticosteroids among children with septic shock.

Methods

Study Subjects and Data Collection

The study subjects for deriving a model to describe the interaction between the Z value and corticosteroid prescription were previously reported (14, 19, 20), whereas the study subjects for testing the model were newly enrolled for this study. Briefly, children ≤ 18 years of age admitted to the pediatric intensive care unit (PICU) and meeting pediatric-specific criteria for septic shock were enrolled after informed consent from parents or legal guardians (21). Blood samples were obtained within 24 hours of initial presentation to the PICU with septic shock. Total RNA was isolated from whole blood using the PaxGene™ Blood RNA System (PreAnalytiX, Qiagen/Becton Dickson, Valencia, CA). Clinical and laboratory data were collected daily while in the PICU. Mortality and organ failure were tracked for 28 days after enrollment. Complicated course was defined as death by 28 days or persistence of 2 or more organ failures at day 7 of septic shock (19). Illness severity was measured using PRISM scores (22).

The model was also tested using a publicly available transcriptomic data set from the Vasopressin vs. Norepinephrine as Initial Therapy for Septic Shock (VANISH) trial (9, 23). The VANISH trial compared the efficacy of vasopressin to norepinephrine as initial vasopressor therapy for septic shock among adults. Patients who reached a prespecified dose of either vasopressor were further randomized to receive hydrocortisone or placebo. Details of the procedures for assigning the cohort randomized to hydrocortisone or placebo to pediatric endotypes A or B were previously described (10, 15).

Multiplex mRNA Quantification and Z value Calculation

A custom NanoString nCounter™ codeset (NanoString Technologies, Seattle, WA) was generated for the 100 endotype-defining genes, as previously described (14). Gene expression mosaics representing the expression patterns of the 100 genes were generated using the Gene Expression Dynamics Inspector, as

previously described (15–17). The gene expression mosaics from individual patients were compared to endotype A and B reference mosaics using ImageJ. Z values were calculated using the equation

$$Z = | \text{Ref}_A - \text{Patient}_i | - | \text{Ref}_B - \text{Patient}_i |$$

Where Ref_A is the reference mosaic for endotype A, Ref_B is the reference mosaic for endotype B, and Patient_i is the reference mosaic for an individual patient.

Statistical Analyses

Demographic and clinical characteristics are summarized using medians, interquartile ranges, frequencies, and percentages. In the derivation dataset a logistic regression model was fit to predict 28-day mortality. Covariates considered were the Z values, which was modeled using restricted cubic splines with 3 knots, PRISM to adjust for severity, and corticosteroid use. Age and comorbidity burden were also considered as possible predictors of mortality. The interaction between corticosteroid use and Z values was evaluated to discern possible differential treatment effects. We subsequently repeated this analysis in the two test datasets using the same model specification to confirm our findings. Analyses used SPSS 27.0 (IBM Corporation, Armonk, NY) and R (package rms) (24, 25).

Results

Table 1 shows the demographic and clinical data for the derivation cohort (n = 425). Forty-eight subjects (11%) did not survive to 28 days. The median Z value for the derivation cohort was 16, with an interquartile range of -29 to 45. To characterize the association between Z value and outcomes, we fit a logistic regression model to predict 28-day mortality. Lower Z value, higher PRISM score, and corticosteroid exposure were each associated with increased odds of 28-day mortality in univariable models, whereas age and comorbidity burden were not (**Table 2**). A lower Z value remained associated with increased odds of 28-day mortality after adjusting for the PRISM score and corticosteroid exposure, consistent with previously reported associations between binary endotype assignment and 28-day mortality.

Relationship between the Z value and response to corticosteroids

To characterize whether the association between corticosteroids and outcomes from septic shock differ based on Z value, we used logistic regression to model the interaction between Z value and receipt of corticosteroids. Rather than assume a linear association, the Z value was fit using cubic splines. **Figure 1A** shows the association between Z values and outcomes, and the potential for effect modification of corticosteroids. While the interaction term was not statistically significant, among subjects exposed to corticosteroids the odds of mortality start to increase considerably as the Z value decreases below

approximately 15. In contrast, among those not exposed to corticosteroids the odds of mortality gradually increase as the Z value decreases, but throughout the entire range of Z values.

Validation in a pediatric cohort

The above observations were made using the same cohort in which the association between corticosteroids and mortality among endotype A patients was originally reported (14). To determine whether the current findings are reproducible, we analyzed data from a new cohort of previously unreported pediatric patients. **Table 1** shows the demographic and clinical characteristics of the validation cohort (n = 230). Compared to the derivation cohort, the subjects in the validation cohort were older, had lower median PRISM scores, and a greater proportion had a comorbidity. No other differences were noted.

The associations between Z value, corticosteroids, and outcomes in the validation cohort are shown in **Figure 1B**. The model shows strong similarities with the original for patients exposed to corticosteroids, with the odds of 28-day mortality considerably increasing below a Z value of approximately 15. Unlike the original model, however, the Z value was not associated with mortality among those not exposed to corticosteroids. There were only 6 deaths among validation cohort patients not exposed to corticosteroids, and among these only one had a negative Z value, resulting in imprecise estimates of treatment effects at low Z values. Nonetheless, the model yielded significant interaction effects, even after adjusting for illness severity using PRISM (p=0.008 for the main effect, p=0.08 for the non-linear term)

Recognizing that a binary cut point facilitates clinical decision making, and that in both the derivation and validation datasets a Z value of 15 is a threshold below which the odds of death start to increase among those exposed to corticosteroids, we determined the association between corticosteroid use and outcomes considering a high Z value (≥ 15 , n=112) compared with a low value (< 15 , n=118). Among those with a low Z value and exposed to corticosteroids (n = 51), there were 11 deaths (22%), and 21 subjects (41%) had a complicated course. In contrast, among validation cohort subjects with a low Z value and not exposed to corticosteroids, there were 2 deaths (3%, p = 0.004) and 10 subjects (15%, p = 0.003) had a complicated course. Corticosteroids were associated with increased odds of death among subjects with a low Z value, even after adjustment for illness severity based on the PRISM score (**Table 3**). Corticosteroids were also associated with increased odds of complicated course among subjects with a low Z value, but this association no longer persisted after adjustment for illness severity and age (**Table 4**). Corticosteroids were not associated with 28-day mortality nor complicated course among subjects with a high Z value (data not shown).

Validation in an independent adult cohort

We further validated our findings above using a subset of adult subjects from the VANISH trial who were randomized to placebo or hydrocortisone, and for whom there existed publicly available transcriptomic data (n = 97). Fitting the same model in this cohort resulted in the associations shown in **Figure 1C**. Briefly, patients with no hydrocortisone exposure and lower Z values tended to have lower odds of death at 28 days, compared to those with hydrocortisone exposure and lower Z values, after controlling for illness severity using the APACHE score. The model supports that separation between groups treated with hydrocortisone and those treated with placebo occurs below a threshold Z value of 15, although the effect was not statistically significant.

Discussion

The role of adjunctive corticosteroids in septic shock has been intensely debated since the 1960s (26). The rationale for prescribing corticosteroids for patients with septic shock reflects their pluripotent effects on inflammation, immunity, and the cardiovascular system. Across multiple, large, randomized clinical trials testing the efficacy of adjunctive corticosteroids among adults with septic shock, there is the consistent observation that corticosteroids reduce vasopressor requirements, but there has not been a consistent demonstration of a survival benefit (27–30). Among children with septic shock, the efficacy of adjunctive corticosteroids is less clear given the lack of comparable data from large, randomized trials (31).

It has been suggested that the inability of corticosteroids to consistently confer a survival benefit in septic shock reflects heterogeneity of treatment effect among subgroups of patients enrolled in clinical trials, such that any potential survival benefit is attenuated by the inclusion of patients in whom corticosteroids are of no benefit or harmful (1, 3, 32). Further, it has been suggested that defining sepsis endotypes provides an opportunity to identify which subgroups of patients with septic shock are more likely to respond favorably, or unfavorably, to adjunctive corticosteroids. This latter concept is well supported by observational studies that employed a binary endotyping strategy based on whole blood-derived gene expression patterns (9, 10, 14).

In the current study we build on our previous work by evaluating a continuous metric to better understand heterogeneity of treatment effect with respect to adjunctive corticosteroids and septic shock. The rationale for this approach is that complex critical illnesses, such as septic shock, likely reflect a continuum of underlying biology that cannot be fully captured by a binary designation of, for example, endotype A vs. endotype B. We further reasoned that a measure capturing this continuum might enable more precise treatment decisions.

The Z value is a measure of how different an individual's gene expression mosaic is from a reference, and it can be used to approximate where an individual patient resides along the continuum of endotype A to endotype B. In our original endotyping strategy any negative Z value would have yielded an endotype A assignment, and endotype A patients had increased odds of mortality, independent of baseline illness severity and corticosteroid exposure. Consistent with this previous observation, lower Z values are

independently associated with increased odds of mortality. Among patients in the derivation cohort who were exposed to corticosteroids, the odds of mortality substantially increased as the Z value decreased below 15, whereas the odds of mortality were relatively constant with Z values greater than 15. We corroborated this association in a new, previously unreported cohort of children with septic shock, and in a cohort of adults who were enrolled in the VANISH trial. By allowing the Z values to have a non-linear relationship with outcomes, we further show that if a binary cut point were to be used, then a value of 15 would be the threshold suggested by the data.

The primary limitation of the current study is that in the two pediatric cohorts corticosteroid prescription was at the discretion of the clinical team caring for the patient. Accordingly, corticosteroid prescription was neither random, nor standard, as would occur in a clinical trial. This has the potential to introduce a variety of known and unknown confounders. One major known confounder involves illness severity, wherein it is possible that corticosteroid prescription could be more likely among patients having greater illness severity. We accounted for this potential confounder, in part, by including PRISM scores in our logistic regression model. While the findings in the VANISH cohort showed a similar pattern for adults as for pediatrics, and in the context of standard and randomized corticosteroid prescription, the strength of association did not meet statistical significance due to a relatively small sample size.

Collectively, these data provide further and more detailed evidence that there likely exist patients with septic shock in whom adjunctive corticosteroids are more likely to cause harm. This contingency is potentially a major impediment to the conduct of clinical trials focused on adjunctive corticosteroids for septic shock and must be taken into consideration in the design of future trials. Gene expression-based endotyping strategies, such as the one in the current study, provide a potential strategy for addressing this challenge. Whether a continuous metric enables more precise treatment decisions, compared to a binary classifier, warrants further evaluation. Of note, two recently launched trials of corticosteroids among children and adults with septic shock are incorporating gene expression-based endotyping strategies into their respective study designs as a means of identifying which patients with septic shock will benefit from adjunctive corticosteroids (NCT03401398 and NCT04280497; clinicaltrials.gov).

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Tables

Table 1: Demographic and clinical data for the pediatric derivation and validation cohorts.

	Derivation Cohort	Validation Cohort
N	425	230
Median age, years	2.5 (1.0 – 6.2)	3.8 (1.3 – 7.0) ¹
Females, n (%)	177 (42)	110 (48)
PRISM	12 (8 – 19)	10 (5 – 15) ¹
Mortality, n (%)	48 (11)	20 (9)
Complicated course, n (%)	124 (29)	51 (22)
Maximum Organ Failures, median	2 (2 – 3)	2 (2 – 3)
Median PICU Length of Stay, days	7 (3 – 14)	8 (4 – 14)
Median PICU Free Days	19 (8 – 24)	19 (9 – 24)
Received Corticosteroids, n (%)	220 (52)	110 (48)
Comorbidity, n (%)	176 (41)	127 (55) ²
Malignancy, n (%)	32 (8)	16 (7)
Immune suppression, n (%)	43 (10)	24 (10)
Bone marrow transplantation, n (%)	17 (4)	17 (7)
Gram negative infection, n (%)	84 (20)	61 (27)
Gram positive infection, n (%)	88 (21)	62 (27)
Fungal infection, n (%)	5 (1)	2 (1)
Viral infection, n (%)	36 (8)	28 (12)
Negative cultures, n (%)	147 (35)	83 (36)
Median Z value	16 (-29 – 45)	13.4 (-21.2 – 38.3)

¹p < 0.05, Rank Sum test.

²p < 0.05, Chi-square test.

Table 2: Univariable and multivariable regression for variables associated with 28-day mortality in the derivation cohort.

Variable	Univariable Regression			Multivariable Regression		
	OR	95% C.I.	P value	OR	95% C.I.	P value
Z value	0.990	0.981 - 0.998	0.013	0.989	0.981 - 0.998	0.015
PRISM	1.096	1.061 - 1.113	<0.001	1.095	1.058 - 1.133	<0.001
Corticosteroids	2.2	1.2 - 4.3	0.014	2.3	1.2 - 4.5	0.018
Co-morbidity	0.7	0.4 - 1.3	0.230	--	--	--
Age	0.9	0.8 - 1.0	0.178	--	--	--

Table 3: Univariable and multivariable regression for variables associated with 28-day mortality among validation cohort patients with a low Z value.

Variable	Univariable Regression			Multivariable Regression		
	OR	95% C.I.	P value	OR	95% C.I.	P value
Corticosteroids	8.9	1.9 - 42.4	0.006	6.1	1.1 - 32.4	0.034
PRISM	1.2	1.1 - 1.2	<0.001	1.1	1.1 - 1.2	<0.001
Co-morbidity	0.9	0.3 - 3.0	0.924	--	--	--
Age	0.8	0.7 - 1.1	0.153	--	--	--

Table 4: Univariable and multivariable regression for variables associated with complicated course among validation cohort patients with a low Z value.

Variable	Uni-variable Regression			Multi-variable Regression		
	OR	95% C.I.	P value	OR	95% C.I.	P value
Corticosteroids	4.0	1.7 - 9.6	0.002	2.6	1.0 - 6.7	0.057
PRISM	1.1	1.1 - 1.2	<0.001	1.1	1.0 - 1.2	<0.001
Co-morbidity	1.2	0.5 - 2.7	0.698	--	--	--
Age	0.8	0.7 - 1.0	0.028	0.9	0.7 - 1.1	0.164

Declarations

Authors' Contributions:

Study concept and design: H.R.W., T.E.S., C.J.L. Acquisition of data: N.Z.C., S.L.W., J.C.F., M.T.B., P.N.J., A.S., R.L., J.N., G.L.A., N.J.T., J.R.G., T.B., M.Q., B.H., T.E.S., H.R.W. Analysis and interpretation of data: H.R.W., K.W.H., C.J.L. Drafting of the manuscript: H.R.W., C.J.L. Critical revision of the manuscript for important intellectual content: N.L.S., E.K.S., N.Z.C., S.L.W., J.C.F., M.T.B., P.N.J., A.S., R.L., J.N., G.L.A., N.J.T., J.R.G., T.B., M.Q., B.H., T.E.S. Statistical analysis: H.R.W., K.W.H., C.J.L.

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H.R.W. and the Cincinnati Children's Hospital Research Foundation hold U.S. patents for the endotyping strategy described in the manuscript. C.J.L. is a co-inventor on the patents. T.E.S. is a cofounder and CEO of Inflammatrix, Inc.

The study was approved by the Institutional Review Board of Cincinnati Children's Hospital Medical Center.

Figures

Figure 1

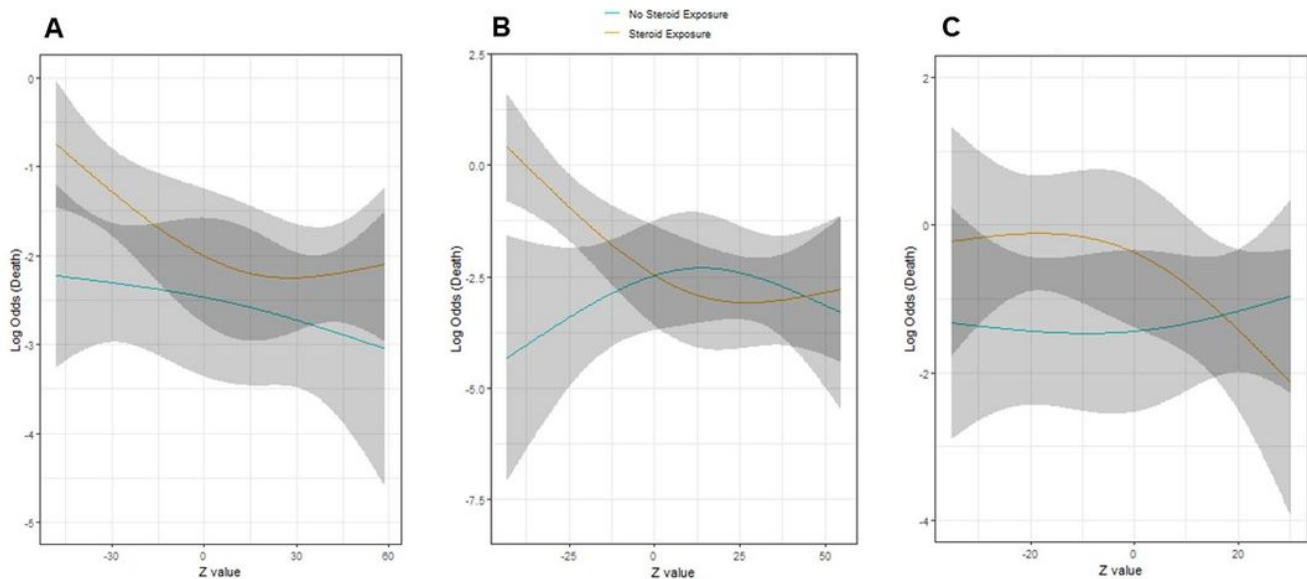


Figure 1

Logistic regression models depicting 28-day mortality as a function of the interaction between corticosteroids and the Z value. For all three models, the Z value was fit using cubic splines with three knots. The x-axis depicts the Z value as a continuous variable, and the y-axis depicts the log₁₀ of the

odds of 28-day mortality. A, patients in the pediatric derivation cohort (n = 425); B, patients in the pediatric validation cohort (n = 230); and C, patients in the adult validation cohort (VANISH cohort, n = 97).