Simplification of a Septic Shock Endotyping Strategy for Clinical Application

To the Editor:

We previously identified pediatric septic shock endotypes by computer-assisted image analysis of gene expression mosaics representing the expression patterns of 100 genes (1–4). The endotypes differ with respect to outcome and treatment response. Because the endotype-defining genes reflect adaptive immunity and glucocorticoid receptor signaling, assigning patients to an endotype might enable precision critical care medicine. Using the expression pattern for 100 genes to assign endotype is currently impractical for time-sensitive decision-making concerning critically ill patients with septic shock (5). A strategy that uses a much smaller number of genes would more readily translate into a rapid clinical test amenable to decision-making for this patient population. We aimed to reduce the existing 100 endotype-defining genes into a minimum subset needed to accurately differentiate endotypes.

Methods

Using classification and regression tree methodology (Salford Predictive Modeler, version 7.0; Salford Systems, San Diego, CA), we developed a decision tree to accurately predict assignment to endotype A or endotype B, using the smallest possible subset of genes from among the original 100. We derived the tree using data from the 300 subjects enrolled in the prior study (1) and tested the tree in 43 newly enrolled subjects. RNA was derived from whole blood collected within 24 hours of a septic shock diagnosis, and gene expression was measured with the NanoString nCounter (NanoString Technologies, Seattle, WA) and a custom-made code set as previously detailed (1).

The primary outcome was allocation to either endotype A or endotype B. The modeling procedure considered all 100 genes as candidate predictor variables and used the class probability method. We pruned terminal nodes having less than 5% of the subjects in the root node and terminal nodes that did not improve classification. Weighting of cases and costs for misclassification were not used. Tenfold cross validation was used to estimate model performance. The code and data used to generate the model are available from the authors.

Results and Discussion

The clinical characteristics and demographics of the 300 derivation subjects are described elsewhere (1). There were 120 endotype A subjects (40%) and 180 endotype B subjects. Figure 1 shows the derived decision tree, consisting of four genes. Using our original endotype classification strategy as the criterion standard, the area under the receiver operating curve (AUROC) of the decision tree was 0.97 (95% confidence interval [CI], 0.95–0.99) for differentiating between endotypes A and B. The tenfold cross-validation procedure yielded an AUROC of 0.90. Subjects allocated to terminal nodes 1, 2, and 4 had a higher probability (57.1–97.4%) of being an endotype A, whereas subjects allocated to terminal nodes 3, 5, and 6 had a lower probability (0.0–22.2%) of being an endotype A. On the basis of allocation to these terminal nodes, 19 subjects allocated to endotype A were originally endotype B, and 7 subjects allocated to endotype B were originally endotype A. This results in the following diagnostic test characteristics for identifying endotype A subjects: sensitivity, 94% (95% CI, 88–97%); specificity, 89% (84–93%); positive predictive value, 86% (78–91%); negative predictive value, 96% (91–98%); positive likelihood ratio, 8.9 (5.8–13.7); and negative likelihood ratio, 0.07 (0.03–0.13).

Using the original endotyping strategy, there were 14 endotype A subjects and 29 endotype B subjects in the test cohort. When these subjects were classified according to the derived four-gene tree, the AUROC was 0.97 (95% CI, 0.93–1.00). The sensitivity and specificity for identifying endotype A were 100% (95% CI, 73–100%) and 79% (95% CI, 60–91%), respectively.

Using the 100 gene mosaics, we previously showed that endotype A subjects had worse outcomes compared with endotype B subjects, and corticosteroid prescription was associated with increased mortality risk among endotype A subjects (1). To determine whether reclassification modified these observations, we combined the derivation and test cohorts ($n = 343$), and compared the clinical characteristics of the endotype A and B subjects, as defined by the four-gene decision tree. Table 1 shows that endotype A patients had a higher mortality rate and a higher rate of complicated course compared with endotype B subjects. Using logistic regression to adjust for age and illness severity (Pediatric Risk of Mortality score), we found that allocation to endotype A was associated with increased odds of mortality (odds ratio [OR], 2.3; 95% CI, 1.1–4.9; $P = 0.022$) and complicated course (OR, 2.1; 95% CI, 1.3–3.6; $P = 0.004$). Among endotype A subjects, corticosteroid prescription was associated with increased odds of mortality (OR, 3.7; 95% CI, 1.4–9.8; $P = 0.008$).

These data suggest that we successfully reduced our septic shock endotyping strategy to a decision tree consisting of just four genes. The decision tree has excellent test characteristics for distinguishing endotype A from endotype B in both the derivation and test cohorts, although there were some reclassifications of subjects relative to the original, reference criterion endotyping strategy. Despite these reclassifications, allocation to endotype A remained independently associated with increased odds of poor outcome, and corticosteroid prescription remained independently associated with increased odds of mortality among endotype A subjects.

After decades of study, the role of adjunctive corticosteroids in septic shock remains controversial (6, 7). It is relatively unclear which patients stand to gain the most benefit from adjunctive corticosteroids (8, 9). The septic shock endotypes we report might provide an opportunity to estimate corticosteroid responsiveness and therefore inform clinical trial design and perhaps clinical care. In another recent *post hoc* analysis, we demonstrated that by combining mortality risk stratification with endotype assignment, it might be possible to identify a subgroup of patients most likely to benefit from corticosteroids (10).

In summary, we have simplified our septic shock endotyping strategy to a four-gene decision tree. The simplified strategy is amenable to translation to the bedside of critically ill patients and therefore warrants further evaluation. ■

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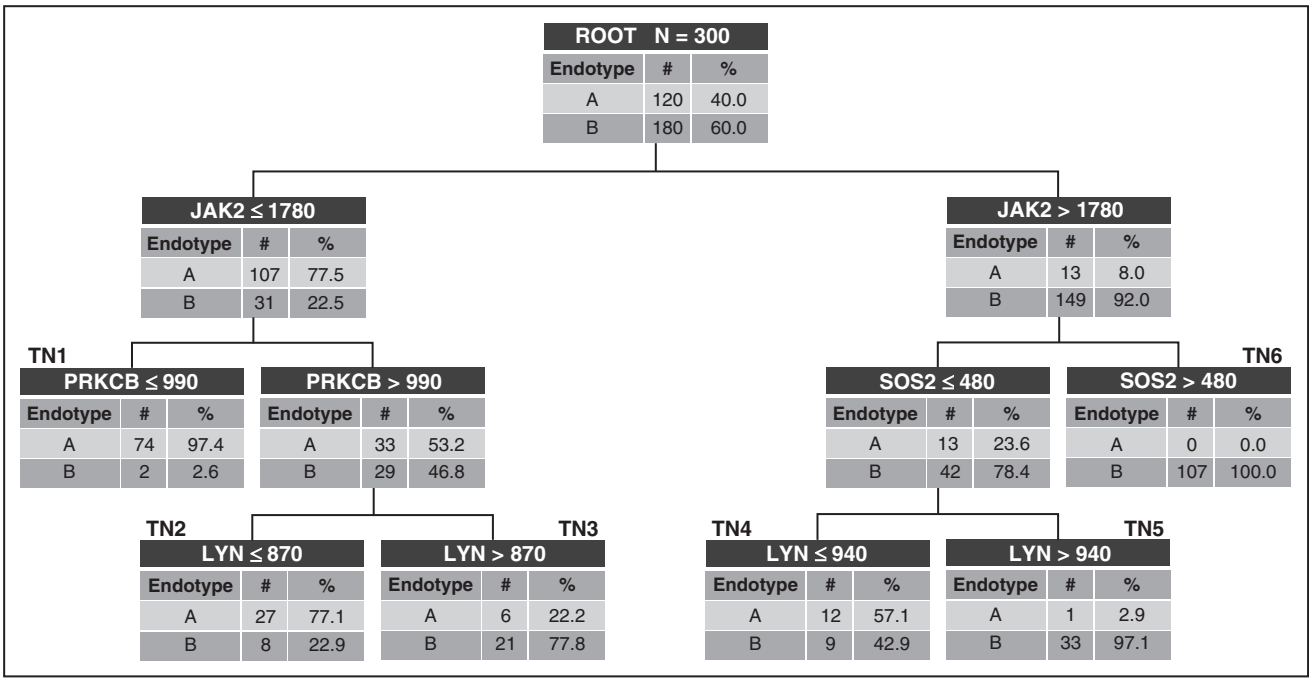

Figure 1. The derived decision tree. The decision tree includes Janus kinase 2 (*JAK2*), protein kinase C, β (*PRKCB*), SOS Ras/Rho guanine nucleotide exchange factor 2 (*SOS2*), and LYN proto-oncogene, Src family tyrosine kinase (*LYN*). The gene expression values are provided in arbitrary units of mRNA counts, as generated by the NanoString nCounter platform and normalized to four housekeeping genes. The root node provides the total number of subjects originally allocated to endotypes A and B, and their respective rates. Each daughter node provides the respective decision rule criterion based on a gene expression level, and the number of endotype A and B subjects, with the respective rates. Terminal nodes (TN) TN1, TN2, and TN4 contained subjects having a higher probability of being an endotype A (57.1–97.4%), whereas TN3, TN5, and TN6 contained subjects having a higher probability of being an endotype B (77.8–100%).

Table 1. Clinical and Demographic Data for Combined Derivation and Test Cohort Subjects Allocated to Endotypes A and B, Using the Four-Gene Decision Tree

Variable	Endotype A	Endotype B	*P* Value
n	152	191	—
Males, n (%)	77 (58)	96 (57)	0.929
Age, yr, median (IQR)	1.6 (0.6–4.7)	3.2 (1.4–6.6)	<0.001
PRISM score, median (IQR)	13 (8–20)	11 (8–17)	0.163
Mortality, n (%)	27 (18)	15 (8)	0.009
Complicated course,* n (%)	60 (39)	41 (21)	<0.001

Definition of abbreviations: IQR = interquartile range; PRISM = Pediatric Risk of Mortality.
*Defined as persistence of two or more organ failures on Day 7 of septic shock or 28-day mortality (1).

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Toward Predicting Individual Risk in Asthma Using Daily Home Monitoring of Resistance

To the Editor:

Background

The main goal of asthma management is achieving asthma control (1) and minimizing future risk of adverse asthma outcomes, including exacerbations. At present, asthma control and, consequently, treatment decisions are mostly assessed using symptoms. However, symptom reporting is susceptible to patient recall bias and poor perception (2). Objective measures, such as lung function, are recommended by guidelines (1); however, spirometry requires clinic visits, and assessment over multiple weeks does not necessarily reflect actual symptom frequency/history. Meanwhile, evidence that monitoring patients at home by peak expiratory flow (PEF) can substantially modify asthma management is still lacking (3). Thus, better tools are still needed that reflect asthma control over time and predict future risk of exacerbations before they manifest by symptoms.

Previously, Thamrin and colleagues (4) analyzed past variations in daily PEF to calculate the probability of a future asthma exacerbation in an individual. This individual conditional probability (ICP) method extended a seminal proof-of-concept study (5) in which probabilities were calculated from the past number of occurrences of sudden drops in PEF, given PEF measured on any given day. The method has been highlighted in recent guidelines on severe asthma management (6).

In a separate study, Gulotta and colleagues (7) tested the original conditional probability concept (5) in daily home recordings of airway resistance measured by the forced oscillation technique (FOT). Compared with PEF, FOT is relatively easy to perform, especially for patients with severe airflow obstruction, and is promising to be a more sensitive measure of airway caliber (8), making it ideal for home monitoring (9, 10). However, the measures of risk obtained from FOT were based on averaged characteristics of the group (termed average conditional probability [ACP]), not the individual. We hypothesized that combining the two approaches (i.e., applying ICP analysis to daily FOT measurements) would improve and personalize our estimation of risk.

Methods

We studied the same group of 10 nonsmoking patients with mild asthma, and 10 nonsmoking, age-matched, healthy control subjects (7). Patients self-measured prebronchodilator FOT data at 5 Hz daily at home in the morning for 6 consecutive months. After removal of artifacts, the average inspiratory resistance (R_{insp}) for each recording was calculated and transmitted to a central server for further analysis.

We adapted the ICP method for FOT as follows (Figure 1). Future asthma "events" were defined as increases of R_{insp} above twice the subject's predicted value (7) on any day within the upcoming week. For any given day, the probability of occurrence of such events was quantified, taking into account the day-to-day variability in R_{insp} over the past 8 days and a risk profile (i.e., the conditional probability curve [4]) based on the statistical and correlation properties of the past 2 months of recordings of R_{insp} (the observation period).[1] This curve is unique for each subject and allows one to "look up" the probability for an asthma "event" occurring within 1 week given FOT recordings obtained in the past 8 days (i.e., given today's day-to-day variability in R_{insp} value). Once the initial observation period has passed, the 2-month window could be moved progressively, daily over the patient's time series, and calculations repeated to obtain updated risk profiles. We then compared accuracy, sensitivity, specificity, and positive and negative predictive values between the ICP and ACP (7) methods in their ability to predict an event within the next 7 days (testing period) after the observation period window, for all overlapping windows. Receiver operating characteristic curves were constructed on the basis of varying cutoff points for the probabilities obtained from ICP and ACP, pooling the results separately from all 10 subjects with asthma and 10 healthy subjects.

Because the ICP approach requires at least 2 months of recording to obtain the first risk profile for the patient, we also

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[1]On any day, the length of the observation period and the R_{insp} data in it determine the individual risk profile. A longer interval may provide more stable estimates from a technical/statistical point of view; however, it lessens the chances of obtaining a stable observation period from a physiological/clinical point of view, particularly for individuals with asthma.