ORIGINAL ARTICLE

Improved Risk Stratification in Pediatric Septic Shock Using Both Protein and mRNA Biomarkers

PERSEVERE-XP

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Abstract

Rationale: We previously derived and validated the Pediatric Sepsis Biomarker Risk Model (PERSEVERE) to estimate baseline mortality risk in children with septic shock. The PERSEVERE biomarkers are serum proteins selected from among the proteins directly related to 80 mortality risk assessment genes. The initial approach to selecting the PERSEVERE biomarkers left 68 genes unconsidered.

Objectives: To determine if the 68 previously unconsidered genes can improve upon the performance of PERSEVERE and to provide biological information regarding the pathophysiology of septic shock.

Methods: We reduced the number of variables by determining the biological linkage of the 68 previously unconsidered genes. The genes identified through variable reduction were combined with the PERSEVERE-based mortality probability to derive a risk stratification model for 28-day mortality using classification and regression tree methodology (n = 307). The derived tree, PERSEVERE-XP, was then tested in a separate cohort (n = 77).

Measurements and Main Results: Variable reduction revealed a network consisting of 18 mortality risk assessment genes related to tumor protein 53 (*TP53*). In the derivation cohort, PERSEVERE-XP had an area under the receiver operating characteristic curve (AUC) of 0.90 (95% confidence interval, 0.85–0.95) for differentiating between survivors and nonsurvivors. In the test cohort, the AUC was 0.96 (95% confidence interval, 0.91–1.0). The AUC of PERSEVERE-XP was superior to that of PERSEVERE.

Conclusions: PERSEVERE-XP combines protein and mRNA biomarkers to provide mortality risk stratification with possible clinical utility. PERSEVERE-XP significantly improves on PERSEVERE and suggests a role for *TP53*-related cellular division, repair, and metabolism in the pathophysiology of septic shock.

Keywords: sepsis; mortality; stratification; biomarkers

(Received in original form January 10, 2017; accepted in final form March 20, 2017)

Supported by National Institutes of Health grants R01 GM099773 and R01 GM108025 (H.R.W.).

Author Contributions: H.R.W.: conceived of and developed the study, obtained funding for the study, conducted the analyses, and wrote the initial manuscript; N.Z.C., N.A., G.L.A., N.J.T., M.T.B., S.L.W., J.C.F., P.A.C., K.M., M.Q., M.H., R.G., R.J.F., J.N., S.S.R., S.G., and J.R.G.: enrolled subjects at the participating institutions, provided clinical data and biological samples, and provided critical review and approval of the manuscript; C.J.L.: collaborated with H.R.W. in conceiving of the study, data analysis, and editing of the manuscript.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 196, Iss 4, pp 494–501, Aug 15, 2017

Originally Published in Press as DOI: 10.1164/rccm.201701-0066OC on March 21, 2017
Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the Subject: Reliable risk stratification of patients with septic shock has numerous clinical applications, but it is a challenging task because of significant patient heterogeneity. We previously derived and validated the Pediatric Sepsis Biomarker Risk Model (PERSEVERE) for estimating baseline mortality risk in children with septic shock. The 12 biomarkers considered for PERSEVERE are serum proteins selected from among the proteins directly related to 80 genes having an association with mortality in pediatric septic shock. We posit that the remaining 68 genes have the potential to improve the accuracy of PERSEVERE for estimating baseline mortality probability, as well as for providing biological information regarding the pathophysiology of septic shock.

What This Study Adds to the Field: PERSEVERE-XP combines the PERSEVERE-based mortality risk with four mRNA biomarkers related to tumor protein 53 (*TP53*). PERSEVERE-XP unambiguously improves upon the performance of PERSEVERE and demonstrates possible clinical utility upon testing. Beyond improved mortality risk stratification, PERSEVERE-XP serves as a hypothesis generator regarding the biological pathways that drive poor outcome resulting from septic shock.

Reliable risk stratification has numerous clinical applications for critical care medicine. These include better-informed allocation of critical care resources, appropriate selection of patients for higher-risk and more costly therapies, and benchmarking of outcomes. In addition, risk stratification can serve as a prognostic enrichment tool to greatly enhance the efficiency of clinical trials (1). Reliable risk stratification of patients with septic shock remains a challenging task because of significant patient heterogeneity (2).

Using discovery-oriented transcriptomic studies, we previously identified 80 genes as having an association with mortality in pediatric septic shock (3, 4). From among the proteins directly related to these genes, we selected 12 protein biomarkers using two criteria simultaneously. First, the gene should have a biologically plausible link to septic shock pathophysiology. Second, the protein transcribed from the gene can readily be measured in the blood compartment. Using these 12 protein biomarkers, we derived and validated the Pediatric Sepsis Biomarker Risk Model (PERSEVERE) for estimating baseline mortality risk in children with septic shock (4–7). PERSEVERE is based on 5 of the 12 candidate biomarkers and age. The biomarkers are measured in blood samples obtained during the first 24 hours of a septic shock diagnosis.

Although pragmatic, the selection criteria for the PERSEVERE biomarkers were limited by existing knowledge and paradigms of septic shock pathophysiology, as well as by technical considerations, leaving just 12 potential biomarkers for consideration. Consequently, 68 genes were left unconsidered, some of which might have the ability to improve on the ability of PERSEVERE to estimate baseline mortality risk and some of which might provide information about biological mechanisms associated with mortality in septic shock.

In the present study, we tested whether these previously unconsidered mortality risk assessment genes could improve the performance of PERSEVERE for estimating baseline mortality risk, and we explored whether there are previously unconsidered mechanistic pathways of importance in septic shock outcomes. We call the resulting risk stratification model "PERSEVERE-XP," reflecting the integration of PERSEVERE with gene expression data.

Methods

Study Subjects and Data Collection

Study subjects were participants in an observational cohort study ongoing at multiple pediatric intensive care units (PICUs) across the United States. The data collection protocol was approved by the local institutional review boards of each participating institution and was previously described in detail (8, 9). Briefly, children 10 years of age or younger admitted to the PICU and meeting pediatrics-specific consensus criteria for septic shock (10) were enrolled after informed consent was obtained from the children's parents or legal guardians. Blood samples were obtained within 24 hours of a septic shock diagnosis for isolation of serum and RNA. Total RNA was isolated from whole blood using the PAXGene Blood RNA System (PreAnalytiX/QIAGEN/BD, Hombrechtikon, Switzerland). Clinical and laboratory data were collected daily while participants were in the PICU. Mortality was tracked for 28 days after enrollment. Organ failure was tracked over the first 7 days after enrollment and defined using pediatrics-specific consensus criteria (10). Baseline illness severity was measured using Pediatric Risk of Mortality (PRISM)-III scores reflecting data from the first 24 hours of admission (11).

Potential subjects for the present study consisted of the group in which PERSEVERE was developed and validated (4–6) and another group of newly enrolled subjects. Among the 771 previously reported PERSEVERE subjects, there were 266 with an available corresponding RNA sample. The new group consisted of 118 subjects, generating a final cohort of 384 subjects with complete protein and mRNA data. From among these, we randomly selected 307 subjects (80%) for deriving PERSEVERE-XP and held back the remaining 77 subjects as an independent test cohort.

PERSEVERE protein biomarkers. PERSEVERE includes the following biomarkers: C-C chemokine ligand 3 (*CCL3*), IL-8 (*IL8*), heat shock protein 70 kD 1B (*HSPA1B*), granzyme B (*GZMB*), and matrix metallopeptidase 8 (*MMP8*) (4). Serum concentrations of these protein biomarkers were measured using a multiplex magnetic bead platform (MILLIPLEX MAP) designed for this project by the EMD Millipore Corporation (Billerica, MA) and a Luminex 100/200 System (Luminex Corporation, Austin, TX) according the manufacturers' specifications. Assay performance data were previously published (4).

Multiplex mRNA quantification. Table E1 in the online supplement provides a list of the 68 previously unconsidered mortality risk assessment genes. Gene expression was measured using the NanoString nCounter and a custom-made code set (NanoString Technologies, Seattle, WA). The technology is based on standard hybridization between the target gene and target-specific capture

and reporter probes to generate a digital readout of mRNA counts (12). All NanoString-based measurements were conducted at the University of Minnesota Genomics Center Core Facility. Four housekeeping genes were used to normalize the NanoString-derived expression data, as previously described (13): beta-2 microglobulin (*B2M*), folylpolyglutamate synthase (*FPGS*), 2,4-dienoyl-coenzyme A reductase 1 (*DECR1*), and peptidylprolyl isomerase B (*PPIB*). Expression values were normalized to the geometric mean of the housekeeping genes.

Variable reduction. For deriving PERSEVERE-XP, we planned to reduce the number of genes considered on the basis of biological linkage. We uploaded the 68 mortality risk assessment genes to the Ingenuity Pathway Analysis discovery platform (QIAGEN, Hilden, Germany) (8, 14, 15) to identify genes associated with any particular signaling pathways or gene networks. The network analysis was restricted to report only direct relationships between gene nodes. We proposed *a priori* to select the highest-scoring pathway or gene network resulting from this analysis for further consideration.

Procedures for Deriving PERSEVERE-XP

Consistent with our previous approach to deriving PERSEVERE, we used classification and regression tree methodology to derive PERSEVERE-XP (Salford Predictive Modeler version 8.0; Salford Systems, San Diego, CA) (4, 6, 16, 17). The primary outcome variable for the modeling procedures was 28-day mortality. All deaths in this cohort resulted from sepsis-associated multiple organ failure. Predictor variables included the baseline 28-day mortality probability calculated using the previously validated PERSEVERE model (4) and the genes identified through variable reduction. We pruned terminal nodes (TNs) having less than 5% of the subjects in the root node, as well as TNs that did not improve classification, using the class probability method. Weighting of cases and costs for misclassification were not used.

Other Statistical Analyses

Descriptive data are reported using medians, interquartile ranges, frequencies, and percentages. For comparisons between groups, we used the Mann-Whitney *U* test, the chi-square test, or Fisher's exact test, as appropriate. For descriptive statistics and comparisons, we used SigmaStat software (Systat Software, Inc., San Jose, CA). PERSEVERE-XP performance is reported using diagnostic test statistics with 95% confidence intervals (CIs) computed using the score method as implemented by the VassarStats Website for Statistical Computation (18). Areas under the receiver operating characteristic curves (AUCs) were compared using the method of Hanley and McNeil for nonindependent samples (19).

Results

Table 1 shows the demographic and clinical data for the two cohorts. There were no differences between the two cohorts. For both cohorts, nonsurvivors had higher baseline illness severity as measured by the PRISM-III score, as well as a higher PERSEVERE-based mortality probability, than the survivors. In the derivation cohort, no other differences were noted between the nonsurvivors and the survivors. In the test cohort, nonsurvivors had a higher rate of infection secondary to a gram-positive bacteria than the survivors. No other differences were noted.

Ingenuity Pathway Analysis of the 68 mortality risk assessment genes revealed a gene network with tumor protein 53 (*TP53*, *p53*) as a highly connected central node. Eighteen of the 68 mortality risk assessment genes are represented in this network (Table E2). Figure 1 shows the network with the 18 gene nodes corresponding to the mortality risk assessment genes colored in blue to depict increased gene expression in the nonsurvivors relative to the survivors or in yellow to depict decreased gene expression in the nonsurvivors relative to the survivors. Functionally, the gene network

Table 1. Clinical and Demographic Data for the Derivation and Test Cohorts According to 28-Day Mortality

	Derivation Cohort		Test Cohort	
	Survivors	Nonsurvivors	Survivors	Nonsurvivors
n (%)	278 (91)	29 (9)	69 (90)	8 (10)
Age, yr, median (IQR)	2.5 (1.0–6.5)	1.4 (0.4–5.9)	2.0 (0.6–5.5)	0.9 (0.2–3.6)
Male sex, n (%)	163 (59)	29 (62)	35 (51)	4 (50)
PRISM-III score, median (IQR)	11 (6–17)	19 (13–29)*	12 (8–17)	19 (10–22)*
Mortality probability† (95% CI)	0.08 (0.06–0.10)	0.28 (0.20–0.36)‡	0.05 (0.03–0.07)	0.30 (0.18–0.42)‡
Gram-positive bacteria, n (%)	53 (19)	7 (24)	16 (23)	5 (63)§
Gram-negative bacteria, n (%)	62 (22)	8 (28)	18 (26)	1 (13)
Other organism‖, n (%)	36 (13)	6 (21)	10 (14)	0 (0)
Culture negative, n (%)	127 (46)	8 (28)	25 (36)	2 (25)
Comorbidity, n (%)	121 (44)	13 (45)	28 (41)	1 (13)
Malignancy, n (%)	18 (6)	0 (0)	6 (9)	0 (0)
Immunosuppression, n (%)	26 (9)	3 (10)	8 (12)	0 (0)
Bone marrow transplant, n (%)	12 (4)	0 (0)	2 (3)	0 (0)

Definition of abbreviations: CI = confidence interval; IQR = interquartile range; PRISM-III = Pediatric Risk of Mortality-III.
*$P < 0.05$ versus respective survivors by chi-square test.
†Based on Pediatric Sepsis Biomarker Risk Model.
‡$P < 0.05$ versus respective survivors by *t* test.
§$P < 0.05$ versus respective survivors by Fisher's exact test.
‖Refers to viral, fungal, or mixed infections.

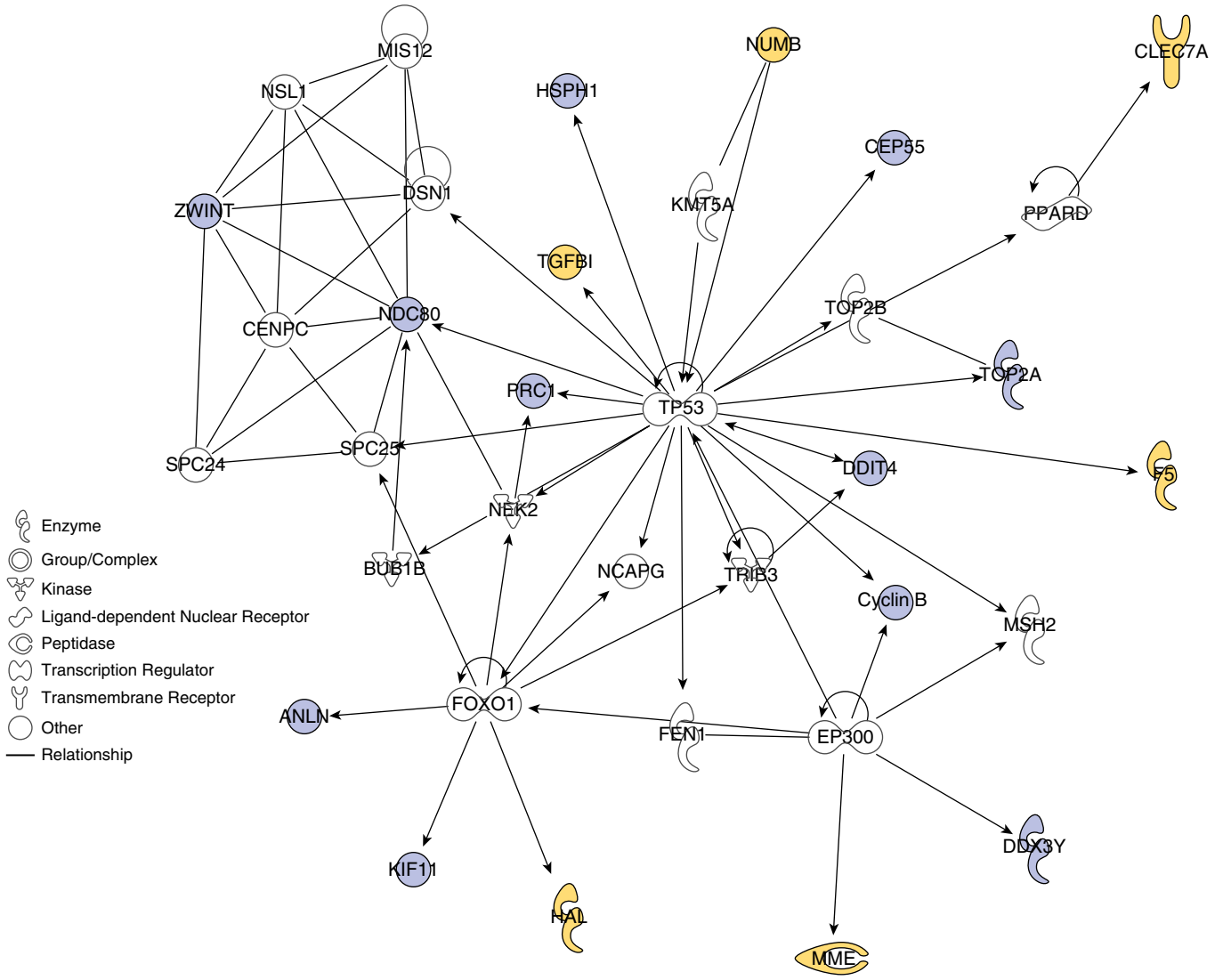

Figure 1. Gene network containing 18 of the 68 mortality risk assessment genes. The gene network was generated by uploading the 68 mortality risk assessment genes to the Ingenuity Pathway Analysis discovery platform with the restriction of generating gene networks having direct relationships only. This was the highest-scoring network resulting from the analysis (network score, 30). The score reflects the probability of generating the network based on random input of 35 network-eligible genes. The score is generated by the equation: $-\log(P \text{ value by Fisher's exact test})$, meaning that $P = 1 \times 10^{-30}$. The gene network is centered on a *TP53* gene node (p53) that was not part of the 68 mortality risk assessment genes. The 18 genes are shown in Table E2. *Blue nodes* depict increased gene expression in nonsurvivors relative to the survivors, whereas *yellow nodes* depict decreased gene expression in the nonsurvivors relative to the survivors. *CCNB1* and *CCNB2* are depicted as "cyclin B" in the network. *Relationship lines with arrows* indicate a direct modification (e.g., activation, transcription, phosphorylation, or cleavage). *Relationship lines without arrows* indicate a direct interaction (e.g., protein–protein, correlation, or RNA–RNA).

corresponds to cell cycle; cell-cycle arrest; cellular assembly and organization; and DNA replication, recombination, and repair.

These 18 genes and the PERSEVERE-based mortality probability were used as predictor variables to derive a model, PERSEVERE-XP, estimating the risk of 28-day mortality. Figure 2 shows the derived PERSEVERE-XP decision tree, which had an AUC of 0.90 (95% CI, 0.85–0.95) for differentiating between survivors and nonsurvivors. The PERSEVERE-based mortality probability occupied the first-level decision rule. *DDIT4*, *HAL*, *PRC1*, and *ZWINT* contributed to the subsequent decision rules. None of the other mortality risk assessment genes in the network contributed to the decision tree. Subjects allocated to TN1, TN3, and TN5 had a low probability of mortality (0.000–0.027), whereas subjects allocated to TN2, TN4, TN6, and TN7 had a higher probability of mortality (0.176–0.406). Among the 227 subjects allocated to the low-risk TNs, 2 (1%) died by 28 days. Conversely, among the 80 subjects allocated to the higher-risk TNs, 27 (34%) died by 28 days.

Figure E1 shows the classification of the test cohort subjects according to PERSEVERE-XP. This classification yielded an AUC of 0.96 (95% CI, 0.91–1.0). Among the 56 test cohort subjects allocated to

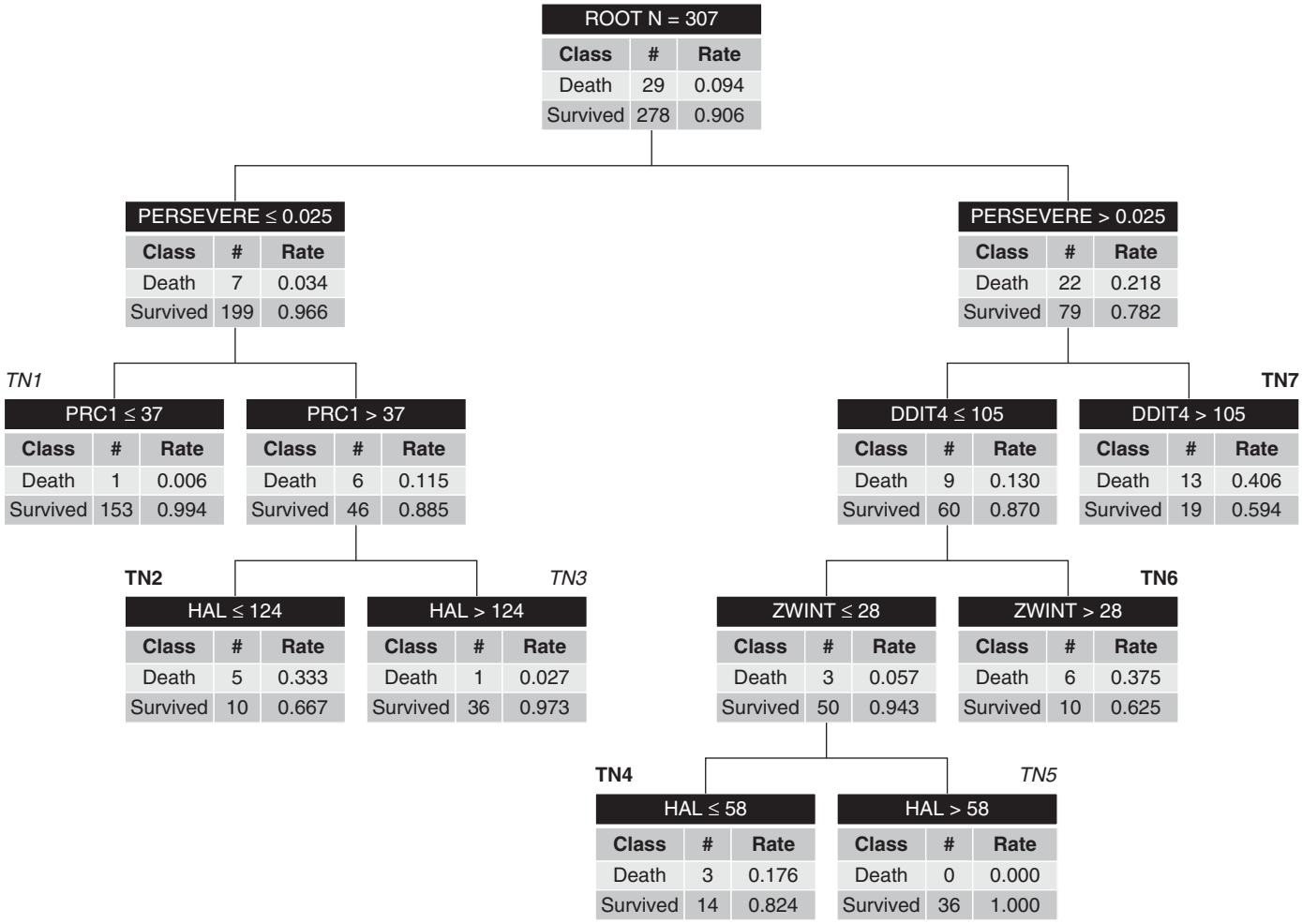

Figure 2. The derived Pediatric Sepsis Biomarker Risk Model (PERSEVERE)-XP decision tree. The decision tree contains the PERSEVERE-based mortality probability, protein regulator of cytokinesis 1 (*PRC1*), histidine ammonia-lyase (*HAL*), DNA damage-inducible transcript 4 (*DDIT4*), and ZW10 interacting kinetochore protein (*ZWINT*). 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 nonsurvivors and survivors in the derivation cohort, as well as the respective rates. Each daughter node provides the respective decision rule criterion based on either the PERSEVERE-based mortality probability or a gene expression level, as well as the number of nonsurvivors and survivors with the respective rates. Terminal nodes (TNs) TN1, TN3, and TN5 (highlighted in *italic type*) contain subjects having a low probability of mortality (0.000–0.027), whereas TN2, TN4, TN6, and TN7 (highlighted by *boldface type*) contain subjects having a higher probability of mortality (0.176–0.406).

the low-risk TNs, none died by 28 days. Conversely, among the 21 test cohort subjects allocated to the higher-risk TNs, 8 (38%) died by 28 days.

PERSEVERE-XP is best interpreted as a risk continuum, but the classifications are convertible to a binary format for calculating diagnostic test characteristics. Table E3 provides the diagnostic test characteristics for both the derivation and test cohorts (n = 384) when classifying subjects with a PERSEVERE-XP mortality probability less than or equal to 0.027 as survivors and those with a PERSEVERE-XP mortality probability greater than or equal to 0.176 as nonsurvivors. We recognize that a binary classification results in some information loss, but we posit that the decision tree clearly identifies a group of subjects that can be considered to be at low risk of mortality.

Table E4 provides the clinical and demographic data for the low- and higher-risk subjects in the combined derivation and test cohorts. Compared with the low-risk subjects, the higher-risk subjects were younger, a higher proportion had a gram-positive bacterial infection, and a lower proportion had culture-negative sepsis.

We next combined the derivation and test cohorts and compared the performance of PERSEVERE-XP with that of PERSEVERE for differentiating between survivors and nonsurvivors. Figure 3 shows that the AUC of PERSEVERE-XP (0.91; 95% CI, 0.87–0.95) was superior to that of PERSEVERE (0.78; 95% CI, 0.68–0.87; $P = 0.002$). Table 2 compares the diagnostic test characteristics of PERSEVERE-XP and PERSEVERE in the combined derivation and test cohorts on the basis of the binary classification outlined above.

We also compared the performance of PERSEVERE-XP with the maximum serum lactate concentration obtained within 24 hours of a septic shock diagnosis. Among the 257 subjects who had available serum lactate data, the AUC of PERSEVERE-XP

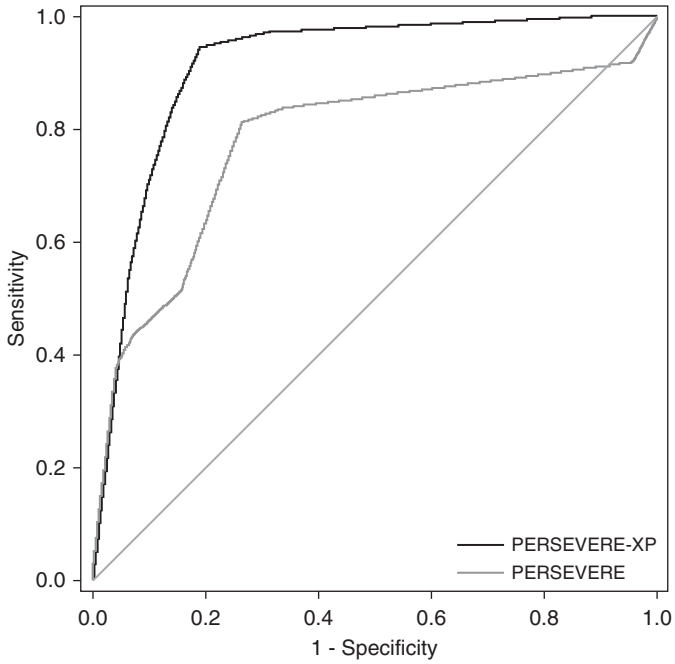

Figure 3. Comparison of the receiver operating characteristic curves for Pediatric Sepsis Biomarker Risk Model (PERSEVERE)-XP and PERSEVERE. The respective curves reflect the combined derivation and test cohorts (n = 384). The area under the receiver operating characteristic curve of PERSEVERE-XP (0.91; 95% confidence interval, 0.87–0.95) was superior to that of PERSEVERE (0.78; 95% confidence interval, 0.68–0.87; $P = 0.002$).

(0.92; 95% CI, 0.88–0.95) was superior to that of lactate (0.79; 96% CI, 0.69–0.89; $P = 0.016$) for differentiating between survivors and nonsurvivors.

To further evaluate the performance of PERSEVERE-XP, we generated summary AUCs for all subjects in our database with PERSEVERE data (n = 771) and PERSEVERE-II data (n = 660). The latter incorporates admission platelet data with the PERSEVERE biomarkers to estimate baseline mortality probability (6). The summary AUC of PERSEVERE was 0.80 (95% CI, 0.75–0.86), and that of PERSEVERE-II was 0.87 (95% CI, 0.84–0.90).

To understand reasons for misclassification by PERSEVERE-XP, we compared those predicted to be nonsurvivors but who survived (false positives) with those who were predicted to survive and did so (true negatives). Compared with the 281 subjects with true-negative results, the 66 subjects with false-positive results had a higher PRISM-III score, a greater number of days in the PICU, a greater maximum number of organ failures, and a greater proportion had persistent multiple organ failure at Day 7 of septic shock (Table E5).

There were only two subjects with false-negative results, so a similar analysis was not performed to compare false negatives and true positives. One of the subjects with a false-negative result had previously been healthy but presented with idiopathic fulminant liver failure and developed septic shock secondary to *Pseudomonas aeruginosa*. The other subject with a false-negative result had aplastic anemia and developed septic shock in association with disseminated cytomegalovirus.

Table 2. Diagnostic Test Characteristics of Pediatric Sepsis Biomarker Risk Model-XP and Pediatric Sepsis Biomarker Risk Model in the Combined Derivation and Test Cohorts

	PEREVERE-XP		PERSEVERE	
Variable	**Value**	**95% CI**	**Value**	**95% CI**
True positives, n	35	—	30	—
True negatives, n	281	—	256	—
False positives, n	66	—	91	—
False negatives, n	2	—	7	—
Sensitivity	95%	80–99	81%	64–91
Specificity	81%	76–85	74%	69–78
Positive predictive value	35%	26–45	25%	18–34
Negative predictive value	99%	97–100	97%	94–99
Positive likelihood ratio	5.0	4.0–6.3	3.1	2.4–3.9
Negative likelihood ratio	0.07	0.02–0.36	0.3	0.1–0.5

Definition of abbreviations: CI = confidence interval; PERSEVERE = Pediatric Sepsis Biomarker Risk Model.

Discussion

We combined PERSEVERE with previously unconsidered genes having predictive capacity for mortality to improve our risk stratification tool for pediatric septic shock. Upon testing, PERSEVERE-XP demonstrated performance that could be viewed as having clinical utility. This assertion is based on the observed likelihood ratios generated by PERSEVERE-XP. Negative and positive likelihood ratios less than or equal to 0.1 and greater than or equal to 10, respectively, are considered to be definitely clinically useful, whereas values less than or equal to 0.2 and greater than or equal to 5 are considered modestly useful, particularly when the pretest probability of the parameter being predicted is relatively uncertain (20, 21). In the case of PERSEVERE-XP, the negative likelihood ratio (0.07; 95% CI, 0.02–0.36) falls in the definitely clinically useful range, whereas the positive likelihood ratio (5.0; 95% CI, 4.0–6.3) falls in the modestly useful range.

Direct comparisons of PERSEVERE-XP and PERSEVERE among subjects with paired data showed unambiguous improvements in the ability to accurately estimate baseline mortality risk. We note that even when PERSEVERE-XP incorrectly classified a subject as a nonsurvivor, it

nonetheless identified subjects with greater illness severity based on PRISM-III score, organ failure burden, and duration of intensive care unit admission. We speculate that this could reflect subjects who were indeed at higher risk of mortality but in whom the risk was modified by clinical interventions.

Our biological approach to variable reduction led us to consider a group of *TP53*-related genes for the derivation of PERSEVERE-XP. TP53 is a pleiotropic transcription factor best known to function as a tumor suppressor because it is induced by DNA damage and subsequently orchestrates cell-cycle arrest, thus enabling cells with the opportunity to repair the damage (22). Alternatively, when the damage is irreparable, TP53 can drive a cell toward apoptosis. The result in either scenario is preventing the generation and persistence of cells with genomic damage. It is now apparent that TP53 modulates cellular fate and function well beyond oncogenesis and cell-cycle arrest. For example, TP53 can modulate cellular metabolism (23), autophagy (24), redox homeostasis (25–27), cross talk with the nuclear factor-κB pathway (22, 23, 28), inflammation (22, 29), and lymphocyte apoptosis during experimental sepsis (30). In a murine model of LPS-induced lung injury, administration of a pharmacologic inducer of TP53 stabilization reduced the severity of lung injury (31). TP53 also interacts directly with the glucocorticoid receptor (32). All of these biological processes are relevant to sepsis pathophysiology.

PERSEVERE-XP contains four genes directly related to *TP53*: *DDIT4*, *HAL*, *PRC1*, and *ZWINT*. *PRC1* has an important role in organizing the central spindle essential for cytokinesis (33, 34). In a functionally related manner, *ZWINT* plays a role in mitosis and the mitotic checkpoint through its interactions with the kinetochore (35, 36). *HAL* catalyzes the first step in histidine metabolism, and mutations of *HAL* lead to the metabolic disease histidemia, characterized by high systemic levels of histidine (37). In contrast to the other three genes contributing to PERSEVERE-XP, *HAL* expression is decreased in the nonsurvivors relative to the survivors. Consistent with this observation, histidine has been shown to be one of the metabolites significantly increased in the serum compartment of children with septic shock relative to healthy control subjects and critically ill children without sepsis (38). *DDIT4*, also known as *REDD1* (regulated in development and DNA damage 1), plays an important role in energy homeostasis by serving as a modulator of insulin action (39) and of skeletal muscle metabolism (40). How these genes contribute to sepsis pathophysiology is a subject of ongoing studies.

The PERSEVERE biomarkers are generally associated with inflammation and cellular injury. These associations are well aligned with existing paradigms of poor outcome resulting from septic shock (7). However, therapies based on these paradigms have not led to clear improvements in patient outcomes, highlighting the need to expand knowledge of septic shock pathophysiology (2). Accordingly, PERSEVERE-XP serves as a hypothesis generator regarding the biological pathways that drive poor outcome resulting from septic shock. PERSEVERE-XP suggests that dysfunctional *TP53*-related cellular division, repair, and metabolism might also contribute to the pathophysiology of septic shock, in conjunction with dysfunctional inflammation. These concepts are biologically plausible and experimentally testable.

We note that the assay platforms we used in this study are analytically reliable but are not amenable to rapid data generation. The ideal risk stratification tool for septic shock should generate reliable biomarker data within a few hours to meet the time-sensitive demands of decision making in this patient population. Thus, clinical application of PERSEVERE-XP will require the development of rapid analytical platforms for both protein and mRNA biomarkers. The necessary technologies for developing such platforms are available.

PERSEVERE-XP must be interpreted in the context of the dynamic nature of sepsis. Consistent with clinical practice, our study protocol does not reliably capture precisely when sepsis begins. Rather, subjects are captured within 24 hours of PICU encounter, reflecting a critical decision-making period during a patient's course of care. We note that even within this 24-hour period, it is possible that gene expression can vary widely in a given subject, and this cannot be captured by a single measurement. Nonetheless, our approach represents a pragmatic and clinically relevant time point for stratification. Elsewhere, we have derived and validated a temporal version of PERSEVERE that considers how the biomarkers change over the first 3 days of illness and how those changes associate with outcome (41, 42).

In summary, we have derived and successfully tested a pediatric septic shock risk stratification tool based a combination of protein and mRNA biomarkers. PERSEVERE-XP adds significant predictive information to PERSEVERE and has possible clinical utility for identifying children with septic shock at both low and high risk of mortality. PERSEVERE-XP also suggests a potential link of *TP53* and related genes to the biology of poor outcome in septic shock. Clinical testing of PERSEVERE-XP in an independent cohort and testing of these novel hypotheses are warranted. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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