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PERSEVERE-II: Redefining the pediatric sepsis biomarker risk model with septic shock phenotype

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AUTHOR COMPETING INTERESTS

Dr. Wong and the Cincinnati Children's Hospital Research Foundation have submitted a provisional patent application for PERSEVERE.

Dr. Lindsell is named as a co-inventor in the above patent application.

The other authors have no competing interests to report.

AUTHOR CONTRIBUTIONS

Hector R. Wong: Conceived and developed the study, obtained funding for the study, directly took part in the analyses, and wrote the manuscript.

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Kelli Howard and Erin Frank: Maintained the clinical database and coordinated all inter-institutional research activity.

Kelli Harmon: Maintained the biological repository and processed all biological samples.

Patrick Lahni: Conducted all biomarker assays.

Kimberly Hart: Assisted with statistical analysis.

Trung Nguyen: Assisted in the concept and design of the study.

Christopher J. Lindsell: Developed the study, assisted with analysis, and edited the manuscript.

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Abstract

Objective—PERSEVERE, a pediatric sepsis risk model, uses biomarkers to estimate baseline mortality risk for pediatric septic shock. It is unknown how PERSEVERE performs within distinct septic shock phenotypes. We tested PERSEVERE in children with septic shock and thrombocytopenia-associated multiple organ failure (TAMOF), and in those without new onset thrombocytopenia but with multiple organ failure (MOF).

Design—PERSEVERE-based mortality risk was generated for each study subject ($n = 660$). *A priori*, we determined that if PERSEVERE did not perform well in both the TAMOF and MOF cohorts, we would revise PERSEVERE to incorporate admission platelet counts.

Setting—Multiple pediatric intensive care units in the United States.

Interventions—Standard care.

Measurements and Main Results—PERSEVERE performed well in the TAMOF cohort (AUC 0.84 [95% CI: 0.77 – 0.90]), but less well in the MOF cohort (AUC 0.71; [0.61 – 0.80]). PERSEVERE was revised using 424 subjects previously reported in the derivation phase.

PERSEVERE-II had an AUC of 0.89 (0.85 – 0.93) and performed equally well across TAMOF and MOF cohorts. PERSEVERE-II performed well when tested in 236 newly enrolled subjects. Sample size calculations for a clinical trial testing the efficacy of plasma exchange for children with septic shock and TAMOF indicated PERSEVERE-II-based stratification could substantially reduce the number of patients necessary, when compared to no stratification.

Conclusions—Testing PERSEVERE in the context of septic shock phenotypes prompted a revision incorporating platelet count. PERSEVERE-II performs well upon testing, independent of TAMOF or MOF status. PERSEVERE-II could potentially serve as a prognostic enrichment tool.

Keywords

sepsis; biomarkers; organ failure; stratification; enrichment; trial simulation

INTRODUCTION

A recently published roadmap for future research in the field of sepsis encourages incorporation of biomarkers and enrichment strategies for clinical trials [1]. Septic shock is highly heterogeneous, which translates to complex and challenging clinical decision making [1, 2]. Several programs of research are attempting to directly improve clinical care by providing tools to differentiate patients with septic shock on the basis of mortality risk, and on the basis of their clinical phenotype [3–5]. While differentiating between septic shock phenotypes might guide selection of treatments specific to the phenotypic characteristics, tools that inform clinicians in real time about mortality risk both within and across septic shock phenotypes can help determine who needs aggressive and potentially higher risk treatments and who does not.

The Pediatric Sepsis Biomarker Risk Model (PERSEVERE) estimates baseline 28-day mortality risk for children with septic shock [6, 7]. PERSEVERE was derived using Classification and Regression Tree (CART) methodology, and the model incorporates a panel of biomarkers and age. The PERSEVERE biomarkers were selected objectively, using discovery oriented transcriptomic studies [7, 8]. PERSEVERE performs well when tested in a heterogeneous septic shock cohort [9], but it is unknown how PERSEVERE performs when applied to distinct clinical phenotypes of septic shock.

Thrombocytopenia-associated multiple organ failure (TAMOF) has been proposed as an important clinical phenotype of septic shock, with high mortality that is potentially modifiable by plasma exchange [10–12]. TAMOF is defined by new onset multiple organ failure with new onset thrombocytopenia. The mechanistic link between thrombocytopenia and organ failure is thought to involve a form of microangiopathy analogous to thrombotic thrombocytopenic purpura (TTP), including decreased levels of ADAMTS-13 (A Disintegrin And Metalloprotease with ThromboSpondin motifs) and increased von Willebrand factor activity [12, 13]. ADAMTS-13 regulates microvascular thrombosis by cleaving large and ultra-large thrombogenic von Willebrand factor multimers into smaller, less thrombogenic forms. Preliminary experience suggests plasma exchange restores ADAMTS-13 levels and restores organ function in children with TAMOF [10], although an appropriately powered study has yet to be conducted.

Whether PERSEVERE is useful for risk stratifying patients with TAMOF is unknown. In the current study we tested the performance of PERSEVERE in children with septic shock and TAMOF, in those without new onset thrombocytopenia but with multiple organ failure (MOF), and in those without MOF. Since PERSEVERE has potential as a prognostic enrichment tool, we then estimated the sample sizes required to conduct a clinical trial testing the efficacy of plasma exchange in children with TAMOF, with and without PERSEVERE-based stratification.

METHODS

Study Subjects and Data Collection

The study cohort included 660 subjects with septic shock. There were 424 subjects with available platelet data previously reported in the derivation and validation of PERSEVERE [6, 9]. An additional 236 new subjects newly enrolled since the derivation and validation of PERSEVERE were also included. No subjects received plasma exchange for TAMOF.

The protocol for collection and use of biological specimens and clinical data was approved by the Institutional Review Boards of each of the 18 participating institutions. Children 18 years of age admitted to the pediatric intensive care unit (PICU) and meeting pediatric-specific consensus criteria for septic shock were eligible for enrollment [14, 15]. There were no exclusion criteria, other than the inability to obtain informed consent, which was obtained from parents or legal guardians prior to any data or sample collection.

Serum samples were obtained within 24 hours of first meeting the criteria for septic shock in the PICU, which was typically at presentation to the PICU. Clinical and laboratory data were collected daily while in the PICU. Organ failure data were tracked up to day 7 of septic shock using previously published criteria [14]. Mortality was tracked for 28 days after enrollment. Complicated course was defined as the persistence of two or more organ failures at day seven of septic shock or 28-day mortality [4]. Illness severity was estimated using PRISM scores [16].

Definition of TAMOF and MOF

TAMOF was defined as new onset thrombocytopenia (platelet count < 100,000/ μ L) and two or more organ failures [11, 12]. MOF was defined as new onset of two or more organ failures but with either platelet counts $\geq$ 100,000/ μ L or with a known pre-existing condition resulting in thrombocytopenia. All other subjects were classified in the No MOF group.

PERSEVERE Biomarkers

PERSEVERE includes C-C chemokine ligand 3 (CCL3), interleukin 8 (IL8), heat shock protein 70 kDa 1B (HSPA1B), granzyme B (GZMB), and matrix metalloproteinase 8 (MMP8) [6]. Serum concentrations of these biomarkers were measured using a multi-plex magnetic bead platform (MILLIPLEX™ MAP) designed for this project by the EMD Millipore Corporation (Billerica, MA). Biomarker concentrations were measured in a Luminex® 100/200 System (Luminex Corporation, Austin, TX), according to the manufacturers' specifications. Assay performance data were previously published [6].

Statistical Analysis

Initially, data are described using medians, interquartile ranges, frequencies, and percentages. Comparisons between groups used the Mann-Whitney U-test, Chi-square, or Fisher's Exact tests as appropriate. Descriptive statistics and comparisons used SigmaStat Software (Systat Software, Inc., San Jose, CA).

Each study subject was assigned a 28 day mortality probability using the previously published PERSEVERE model [6]. PERSEVERE performance is reported using diagnostic test statistics with 95% confidence intervals computed using the score method as implemented by the VassarStats Website for Statistical Computation [17].

A priori, we determined that if PERSEVERE did not fit equally well in patients with TAMOF as in patients without TAMOF, we would revise PERSEVERE to incorporate variables associated with thrombocytopenia. Specifically, we considered the presence of malignancy, immune suppression, bone marrow transplantation, and new onset thrombocytopenia as dichotomous predictor variables. Continuous predictor variables included the PERSEVERE biomarkers, age, and admission platelet count. To revise PERSEVERE, we used CART methodology (Salford Predictive Modeler v6.6, Salford Systems, San Diego, CA) [18, 19]. The primary outcome variable was 28-day mortality. Weighting of cases and the addition of cost for misclassification were not used in the modeling procedures. The code and data used to generate the model is available from the authors. The revised tree (PERSEVERE-II) was derived using the original 424 subjects used to develop PERSEVERE, and was subsequently internally validated in the 236 newly enrolled subjects.

Areas under the receiver operating characteristic curves (AUC) were compared using the method of Hanley and McNeil for non-independent samples [20]. The net reclassification improvement (NRI) was used to estimate the incremental predictive ability of PERSEVERE-II compared to PERSEVERE [21]. The NRI was computed using the R-package Hmisc [22].

Finally, we estimated the sample size needed for conducting a clinical trial comparing plasma exchange to no plasma exchange in children with TAMOF. We considered using PERSEVERE-II as an enrichment strategy to target patients most likely to benefit from plasma exchange. We assumed independence of study arms would be tested using a continuity corrected Chi-square test with power $(1-\beta)$ set to 0.8 and $\alpha=0.05$. Calculations were conducted using nQuery Advisor v 7.0 (Statistical Solutions Ltd., Cork, Ireland).

RESULTS

PERSEVERE performance in subjects with TAMOF and MOF

Table 1 shows the demographics and clinical characteristics of the TAMOF, MOF and No MOF cohorts. Subjects with TAMOF ($n = 209$) had a higher mortality, a higher rate of complicated course, and a higher median PRISM score when compared to the MOF ($n = 290$) and No MOF ($n = 161$) subjects. A lower proportion of subjects with TAMOF had no causative pathogen isolated, and a lower proportion had comorbidities when compared to the

MOF and No MOF subjects. The subjects with No MOF were older than the TAMOF and MOF subjects. No other differences were noted.

Table 2 shows the test characteristics of PERSEVERE for estimating the probability of 28-day mortality in all subjects and for subjects in the TAMOF, MOF, and no MOF groups. PERSEVERE had very good performance in the TAMOF cohort, with an AUC of 0.84 (95% CI: 0.77 – 0.90), a sensitivity of 91% (95% CI: 79 – 97), and a negative likelihood ratio of 0.1 (95% CI: 0.01 – 0.3). In contrast, PERSEVERE performed less well in the MOF group, with an AUC of just 0.71 (0.61 – 0.80).

Derivation of PERSEVERE-II

Because PERSEVERE did not perform equally well in the TAMOF group and the MOF group, we proceeded to revise PERSEVERE taking into account variables associated with thrombocytopenia. Figure 1 shows PERSEVERE-II. The top node of the decision tree, the root node, provides the total number of subjects as well as the number and proportion of survivors and nonsurvivors. Subjects in the root node are subsequently allocated to daughter nodes based on the results of binary recursive partitioning. Each daughter node provides the criterion for deciding subsequent partitions, along with the number and proportion of survivors and nonsurvivors. Terminal nodes reflect the final assignment of risk to an individual case. We have annotated the tree to number the terminal nodes; the numbers appear in bold above each terminal node in the tree. All five PERSEVERE biomarkers (CCL3, IL8, GZMB, HSPA1B, and MMP8) contributed to the predictive capacity of PERSEVERE-II. Admission platelet count was found to augment predictive accuracy, but age and comorbidity burden did not.

PERSEVERE-II had five low risk terminal nodes (1.9% risk of death; nodes 1, 2, 5, 8, and 9), four intermediate risk terminal nodes (16.7% to 33.3% risk of death; nodes 4, 6, 7, and 10), and two high risk terminal nodes (44.4% risk of death; nodes 3 and 11). Among the 273 subjects classified as low risk, two (0.7%) died by 28 days. Among the 151 subjects classified as intermediate or high risk, 47 (31.1%) died by 28 days. Table 3 shows the test characteristics of PERSEVERE-II in the derivation cohort.

Testing PERSEVERE-II

We tested the performance of PERSEVERE-II using the 236 subjects newly enrolled since the initial derivation of PERSEVERE. Supplementary Figure 1 shows how the 236 test subjects were classified. Among the 141 test subjects classified as low risk, three (2.1%) died by 28 days. Among the 95 test subjects classified as intermediate or high risk, 34 (35.8%) died by 28 days. Table 3 shows the test characteristics of PERSEVERE-II in the test cohort.

Comparison of PERSEVERE and PERSEVERE-II

Supplementary Figure 2 shows the PERSEVERE and PERSEVERE-II receiver operating characteristic curves for all subjects. For estimating the risk of 28-day mortality, the AUC for PERSEVERE-II (0.87; 95% CI 0.84 – 0.90) was superior to that of PERSEVERE (0.80; 95% CI 0.75 – 0.86; $p = 0.031$). When risk stratifying using PERSEVERE-II compared to

PERSEVERE, the NRI was 0.50 (95% CI: 0.28 – 0.72; $p < 0.0001$), indicating improved classification of subjects. Admission platelet count alone had an AUC of 0.73 (95% C.I. 0.68 – 0.78) for estimating the risk of 28-day mortality.

Designing a clinical trial to test the efficacy of plasma exchange using PERSEVERE-II as a prognostic enrichment strategy

One practical application of PERSEVERE is as a prognostic enrichment tool to inform patient selection for clinical trials. For example, PERSEVERE-II could allow for exclusion of patients having a low baseline mortality probability with standard care and who would unlikely benefit from an experimental intervention. Exclusion of such patients could enrich the study population with patients having a higher baseline mortality probability, and consequently decrease the number of patients required for an interventional trial. As an example, one recent trial used the approach of restricting enrollment to patients with three or more organ failures as a means of enriching the study population with more severely ill patients (clinicaltrials.gov; NCT00118664).

In our study, 108 of the TAMOF subjects had at least three organ failures and could therefore have theoretically met the enrollment criteria for NCT00118664. We used data from these 108 subjects enrolled in our study to estimate the sample size required to conduct a clinical trial testing the efficacy of plasma exchange in children with TAMOF, with and without PERSEVERE-based stratification.

Among these 108 subjects there were 41 deaths (38% mortality), and PERSEVERE-II had an AUC of 0.82 (95% CI: 0.74 – 0.90) for estimating 28-day mortality. In comparison, PRISM had an AUC of 0.60 (95% CI: 0.49 – 0.72) for estimating 28-day mortality in these subjects. PERSEVERE-II correctly predicted 28-day survival for 32 subjects (true negatives) and incorrectly predicted 28-day survival for 2 subjects (false negatives). Using PERSEVERE-II-based stratification, these true and false negative subjects would be excluded from a clinical trial, leaving 39 true positive and 35 false positive subjects for inclusion. Among the true and false positive subjects, the 28-day mortality was 53%.

We calculated the number of un-stratified patients (38% mortality) and PERSEVERE-II-stratified patients (53% mortality) required in a trial randomizing patients with TAMOF and at least 3 organ failures to standard care or plasma exchange. We used a range of assumptions for the relative mortality reduction attributable to plasma exchange (10% to 50%), and we set power ($1-\beta$) to 0.8 and α to 0.05. Table 4 shows the results. In each scenario, PERSEVERE-II-based stratification reduced the number of subjects needed in each study arm by between 39% and 44%.

DISCUSSION

Given the clinical and research interest in TAMOF as a distinct clinical phenotype of septic shock, we tested the performance of PERSEVERE in children with septic shock and TAMOF and found it had acceptable performance. In contrast, PERSEVERE did not perform well in children with septic shock and MOF in the absence of new onset thrombocytopenia. We revised PERSEVERE to have broader applicability across septic

shock phenotypes. PERSEVERE-II incorporates admission platelet count into the risk stratification, and performs well in both the TAMOF and MOF phenotypes. This feature of PERSEVERE-II is biologically plausible because new onset thrombocytopenia is the primary determinant when distinguishing TAMOF from MOF [11, 12]. PERSEVERE-II continued to perform well when tested in a separate cohort. Our data demonstrate that PERSEVERE-II outperforms PERSEVERE, as measured by comparisons of the receiver operating characteristic curves and the NRI.

The current study supports the concept that TAMOF is a distinct clinical phenotype of septic shock [11]. Patients with septic shock and TAMOF had significantly higher rates of mortality and complicated course compared to patients with MOF in the absence of new onset thrombocytopenia. Thus, the design of therapies specifically targeting the TAMOF phenotype seems warranted.

Plasma exchange may be a potential therapy for TAMOF, but it has not been tested in a randomized clinical trial [1, 11]. The American Society of Apheresis classifies the use of plasma exchange for “sepsis with multiple organ failure” as being supported with only level III evidence, and they provide a grade 2B recommendation [23]. This states that the role of plasma exchange in this condition is not truly established, and that the supporting evidence is considered weak and of moderate quality. Because plasma exchange carries more than minimal risk given the need for large caliber central venous access, exposure to blood products, and need for an extracorporeal circuit, a rational strategy for using plasma exchange as a therapeutic strategy for TAMOF should optimize the risk to benefit ratio.

A potential strategy for optimizing the risk to benefit ratio when employing higher risk experimental therapies is to stratify patients based on baseline mortality risk. This concept is known as prognostic enrichment [24]. By using a tool to estimate outcome risk, prognostic enrichment selects patients with a greater event rate. Because sample size for an event-based study is inversely proportional to effect size and directly proportional to event rate, prognostic enrichment can allow for a smaller sample size. Importantly, prognostic enrichment does not affect relative risk reduction, but will increase the absolute effect size of an experimental therapy.

A recent publication opined that severity scores such as Acute Physiology and Chronic Health Evaluation (APACHE) and PRISM should not be used as entry criteria for clinical trials and provided several reasons in support [25]. The publication further called for the development of biomarker-based stratification strategies as a means to enhance selection criteria for clinical trials. The utility of PERSEVERE as an enrichment strategy is consistent with this recommendation.

We tested the concept of prognostic enrichment in the design of a trial of plasma exchange for patients meeting TAMOF criteria, based on entry criteria from a recent observational study. We demonstrate that when eligible patients are stratified for baseline mortality risk using PERSEVERE-II, the sample size needed to demonstrate efficacy is substantially reduced when compared to no stratification. The simulation demonstrates how almost one third of patients with septic shock and TAMOF could theoretically be spared exposure to

plasma exchange based on a reliable estimation of low baseline mortality risk with standard care.

We note three main limitations of our study. First, in the absence of pre-PICU admission platelet count data we considered thrombocytopenia to be of new onset when the study subject had an admission platelet count $< 100,000/\mu\text{l}$ and did not have a comorbidity associated with thrombocytopenia. Conversely, in subjects with a comorbidity associated with thrombocytopenia, any thrombocytopenia event was considered related to the comorbidity, rather than being of new onset. This could have led to misclassification of some subjects. Second, the test cohort used for internal validation was a convenience sample representing subjects newly enrolled since the derivation and validation of PERSEVERE. We are in the process of enrolling a prospective cohort in which to validate the performance of PERSEVERE-II. Finally, because PERSEVERE is designed to assign a baseline mortality probability, we only considered admission platelet counts in the modeling process. This precludes analysis of how temporal changes in platelet counts are associated with changing risk. We have developed a temporal version of PERSEVERE and will pursue the opportunity to consider how changes in platelet counts reflect changing risk over time.

In conclusion, testing the accuracy of PERSEVERE in the context of organ failure phenotypes of septic shock prompted a revision of PERSEVERE incorporating admission platelet count information. PERSEVERE-II performs well upon testing, independent of TAMOF or MOF status. Tools such as PERSEVERE-II have the potential to provide prognostic enrichment for a trial of plasma exchange in children with septic shock and TAMOF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. The PERSEVERE-II classification tree

The classification tree includes C-C chemokine ligand 3 (CCL3), interleukin 8 (IL8), heat shock protein 70 kDa 1B (HSPA1B), granzyme B (GZMB), matrix metalloproteinase 8 (MMP8), and admission platelet counts. The biomarker concentrations are shown in ng/ml and platelet counts are shown in number/ μ l. The root node provides the total number of patients in the derivation cohort, and the number of survivors and non-survivors, with the respective rates. Each daughter node provides the respective decision rule criterion and the number of survivors and non-survivors, with the respective rates. Terminal nodes (TN) TN1, TN 2, TN 5, TN8, and TN9 are low risk terminal nodes (1.9% risk of death). TN4, TN6, TN7, and TN10 are intermediate risk terminal nodes (16.7% to 33.3% risk of death). TN3 and TN11 are high risk terminal nodes (44.4% risk of death).

Table 1

Demographics and clinical characteristics of the study cohorts.

	TAMOF	MOF	No MOF
N (%)	209 (32)	290 (44)	161 (24)
Median Age, Years (IQR)	2.1 (0.7 – 6.5)	3.0 (1.0 – 7.1)	7.0 (2.6 – 13.0) ¹
Males, # (%)	122 (58)	158 (54)	83 (52)
28-day mortality, # (%)	47 (22) ²	39 (13) ³	0 (0)
Complicated course, # (%)	99 (47) ²	87 (30) ³	0 (0)
Median PRISM score (IQR)	17 (11 – 24) ²	11 (7 – 17) ³	9 (5 – 12)
# with gram negative bacteria (%)	60 (29)	61 (21)	32 (20)
# with gram positive bacteria (%)	57 (27)	56 (19)	29 (18)
# with other pathogen isolated (%)	18 (9)	25 (9)	13 (8)
# with no pathogen identified (%)	74 (35) ²	148 (51)	87 (54)
# with comorbidity (%)	79 (38) ³	148 (51)	76 (47)
# with malignancy (%)	0 (0) ²	53 (18)	28 (17)
# with immune suppression (%)	12 (6) ²	67 (23)	34 (21)
# with bone marrow transplantation (%)	0 (0) ²	25 (9)	13 (8)

¹ p < 0.05 vs. TAMOF and MOF.² p < 0.05 vs. MOF and No MOF.³ p < 0.05 vs. MOF.

Table 2

PERSEVERE test characteristics across the study cohorts.

	ALL SUBJECTS	TAMOF	MOF	NO MOF
N	660	209	290	161
False Positive	151	57	77	17
True Positive	72	43	29	0
True Negative	424	105	175	144
False Negative	13	4	9	0
Sensitivity	85 (75 – 91)	91 (79 – 97)	76 (59 – 88)	--
Specificity	74 (70 – 77)	65 (57 – 72)	69 (58 – 69)	--
PPV	32 (26 – 39)	43 (33 – 53)	27 (19 – 37)	--
NPV	97 (95 – 98)	96 (90 – 99)	95 (91 – 98)	--
+LR	3.2 (2.7 – 3.8)	2.6 (2.1 – 3.3)	2.5 (1.9 – 3.2)	--
–LR	0.2 (0.1 – 0.3)	0.1 (0.05 – 0.3)	0.3 (0.2 – 0.6)	--
AUC	0.80 (0.75 – 0.86)	0.84 (0.77 – 0.90)	0.71 (0.61 – 0.80)	--

Table 3

PERSEVERE-II test characteristics in the derivation and test cohorts.

	ALL SUBJECTS	TAMOF	MOF	NO MOF
Derivation cohort, N=424				
N	424	159	155	110
False Positive	104	57	26	21
True Positive	47	34	13	--
True Negative	271	66	116	89
False Negative	2	2	0	--
Sensitivity	96 (85 – 99)	94 (80 – 99)	100 (72 – 100)	--
Specificity	72 (67 – 77)	54 (44 – 63)	82 (74 – 87)	--
PPV	36 (31 – 40)	37 (28 – 48)	33 (20 – 50)	--
NPV	99 (97 – 100)	97 (89 – 99)	100 (96 – 100)	--
+LR	3.5 (2.9 – 4.1)	2.0 (1.7 – 2.5)	5.5 (3.9 – 7.7)	--
–LR	0.06 (0.01 – 0.2)	0.1 (0.03 – 0.4)	--	--
AUC	0.89 (0.85 – 0.93)	0.82 (0.75 – 0.88)	0.93 (0.88 – 0.97)	--
Test cohort, N=236				
N	236	50	135	51
False Positive	61	17	36	8
True Positive	34	11	23	--
True Negative	138	22	73	43
False Negative	3	0	3	--
Sensitivity	92 (77 – 98)	100 (68 – 100)	88 (69 – 97)	--
Specificity	69 (62 – 76)	56 (40 – 72)	67 (57 – 76)	--
PPV	36 (26 – 46)	39 (22 – 59)	39 (27 – 53)	--
NPV	98 (93 – 99)	100 (82 – 100)	96 (88 – 99)	--
+LR	3.0 (2.4 – 3.8)	2.3 (1.6 – 3.3)	2.7 (2.0 – 3.6)	--
–LR	0.1 (0.04 – 0.3)	--	0.2 (0.1 – 0.5)	--
AUC	0.84 (0.78 – 0.90)	0.92 (0.84 – 1.00)	0.79 (0.71 – 0.88)	--

Table 4

Number of un-stratified and PERSEVERE-II-based stratified subjects required in each study arm in a trial of plasma exchange for TAMOF (assuming a continuity corrected chi-square test to compare two equally sized groups with power $(1-\beta)$ set to 0.8 and $\alpha=0.05$).

Projected relative reduction in mortality with plasma exchange	No. of un-stratified patients required in each study arm (% absolute mortality reduction)	No. of stratified patients required in each study arm (% absolute mortality reduction)
10%	2,559 (3.8)	1,434 (5.3)
20%	637 (7.6)	366 (10.6)
30%	281 (11.4)	165 (15.9)
40%	156 (15.2)	94 (21.2)
50%	98 (19.0)	60 (26.5)