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Validation of diagnostic gene sets to identify critically ill patients with sepsis

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ABSTRACT

Purpose: Gene expression diagnostics have been proposed to identify critically ill patients with sepsis. Three expression-based scores have been developed, but have not been compared in a prospective validation. We sought to validate these scores using an independent dataset and analysis.

Methods: We generated gene expression profiles from 61 critically ill patients. We validated the performance of 3 expression-based sepsis scores including 1) the Sepsis MetaScore (SMS); 2) the SeptiCyte™ Lab; and 3) the FAIM3:PLAC8 ratio. Sepsis was identified as the presence of definite, probable, or possible infection in the setting of organ dysfunction (SOFA score ≥ 2).

Results: For all 3 models, scores were significantly different between patients with and without sepsis. Discrimination was highest for the SMS (area under the receiver operating characteristics curve [AUROC 0.80 [95% CI 0.67–0.92]), with greater confidence in the presence of infection resulting in better model performance (max AUROC 0.93 [0.87–1.0]).

Conclusions: All three scores distinguished septic from non-septic ICU patients, with the SMS showing the best performance overall in our cohort. Our results suggest that models developed from the co-analysis of multiple cohorts are more generalizable. Further work is needed to identify expression-based biomarkers of response to specific therapies.

1. Introduction

The search for biomarkers of sepsis has long been a focus of critical care research, with the number of candidate markers now numbering in the hundreds [1]. Traditionally, biomarkers have been investigated with the aim of distinguishing patients with either sterile inflammation or uncomplicated infection from those with sepsis, as the latter may benefit from early antibiotics, fluid resuscitation, close monitoring, and other supportive measures. Biomarker discovery is increasingly focused on identifying features that portend a positive response to a particular treatment. This objective aligns with recent trends in precision medicine, an emerging area of focus in critical care research [2-4].

Recently, sepsis biomarker research has turned to whole genome platforms such as microarray-based gene expression profiling, in order to better account for the complexities of the septic response at the cellular and molecular level [5,6]. As one example, The Molecular Diagnosis and Risk Stratification of Sepsis consortium (MARS) has developed the SeptiCyte™ Lab, a four-gene classifier (CEACAM4, LAMP1, PLA2G7, and PLAC8) that was derived from a cohort in Australia, and validated in a separate Dutch cohort using quantitative PCR [7]. SeptiCyte™ Lab was found to accurately differentiate patients with sepsis from non-septic controls, with an area under the receiver operator characteristics curve (AUROC) of between 0.89 and 0.95. MARS investigators have also described the ratio between two genes (FAIM3:PLAC8) as being useful in distinguishing ICU patients with community acquired pneumonia (CAP) from those with common CAP mimics (mostly aspiration, exacerbation of COPD, asthma, and heart failure), with an AUROC of 0.845 [8].

A third expression-based biomarker, the Sepsis MetaScore (SMS), was derived using publicly available data from a number of different

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studies, in order to address the potential for overfitting of predictive models derived from smaller datasets [9]. The 11-gene SMS was shown to differentiate critically ill patients with sepsis, from those with sterile inflammation, as defined by SIRS criteria. Similar methods were subsequently used to identify a 7-gene expression signature differentiating viral from bacterial infection [10].

A recent prospective validation of the SeptiCyte™ score conducted by researchers involved in its development showed that it performed well in an independent cohort (AUROC 0.82–0.89) [11]. All 3 scores were recently validated in a total of 39 legacy datasets from publicly available sources [12]. Some of the validation datasets used in this study were also used to derive the scores themselves, although the study's authors also separately reported score performance as determined using only the datasets that were not used in their derivation. Gene expression data derived from microarrays are highly complex, and gene expression studies based on these may lack reproducibility. In fact, one analysis aimed at reproducing microarray-based studies failed to do so in more than half of the 18 cases studied [13]. These above considerations may limit the generalizability of these results, and suggest that prospective, independent validation would be useful in this regard. External validation – an important step in the development of any biomarker of diagnostic strategy – is therefore particularly important with data of this nature.

In this study, we used gene expression data collected prospectively in the context of a randomized controlled trial in an ICU, to perform an independent validation and comparison of these 3 scores using a novel microarray platform, and to assess their performance with the newer Sepsis-3 definitions of sepsis [14].

2. Materials & methods

We collected whole blood samples for gene expression profiling on a subset of patients enrolled in the PREVAIL study (NCT 01996579), a multicenter randomized controlled trial examining the use of bovine lactoferrin to prevent nosocomial infection among critically ill patients [15]. The study recruited patients within 48 h of ICU admission who were expected to require at least 72 h of invasive mechanical ventilation at the time of enrollment (total sample size = 214). Patients were followed for the duration of their ICU and hospital stay. In addition to basic demographic data, severity of illness data, primary diagnosis, and ICU interventions, we collected information on baseline infections and any infections and antibiotic use over the course of the ICU stay. The study was approved by the Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board. Informed consent – including for the use of genomic data – was obtained from each study participant or their substitute decision maker before any study procedures were conducted.

For each of the included patients, the presence of infection was adjudicated at the end of the study by a reviewer blinded to the treatment arm, using clinical and microbiological data. Infection was categorized as definite, probable, or possible using standardized definitions [15,16]. To best reflect contemporary sepsis definitions, we classified as septic any patient with definite, probable, or possible infection and a SOFA score ≥ 2 at the time of ICU admission, with the assumption in the absence of pre-ICU SOFA scores that most patients were free of significant organ failure prior to ICU admission. Between January 2014 and February 2016, we enrolled 75 consecutive patients in the genomics sub-study, all from the same 33-bed mixed medical/surgical/neurocritical/trauma ICU at a tertiary academic medical center. In addition, we collected samples from 23 volunteers who served as healthy controls. These were included to facilitate co-analysis of our expression data with data generated on other platforms [10].

We collected whole blood samples in PAXgene tubes, and analyzed these using the Affymetrix PrimeView microarray platform. For the enrolled patients, we collected samples at up to six different time points, including days 1, 3, 7, 14, 21, and 28. Samples were only collected if patients were in the ICU at the sample time point. Samples yielding low quality RNA were excluded, as were gene expression profiles that were identified as outliers. Details on this and the remainder of the bioinformatics workflow are provided in the Supplemental Digital Content. Gene expression data are available from the Gene Expression Omnibus (GSE118657).

We generated scores for the SMS, SeptiCyte™ Lab, and FAIM3:PLAC8 using the methods described in the original manuscripts (see Supplementary Digital Content). These calculations returned a single number expressing the likelihood of a diagnosis of sepsis. We compared the performance of the various gene sets at the time of ICU admission using the area under the receiver operator characteristics curve (AUROC). We compared ICU patients with adjudicated infections (definite, probable, or possible) against non-infected ICU patients, and performed sensitivity analyses to asses the effect of the certainty of infection. In addition to validation at the time of admission, we examined the score trajectory of patients over time using samples from the first three weeks of their ICU stay (day 3, day 7, day 14 and day 21). We also used log-transformed procalcitonin levels (Bio-Rad Laboratories, Inc.) as a general comparator of a more conventional biomarker for infection. We used generalized linear models to examine the association between sepsis diagnosis and the three diagnostic scores, as well as procalcitonin, with adjustment for potential confounding by age, sex, and severity of illness.

3. Results

The clinical characteristics of the patients included in the sub-study are shown in Table 1. After preprocessing, the gene expression dataset included 209 samples from 69 patients (at various time points), and 21 controls (Supplementary Digital Content). Baseline gene expression data was available for 61 patients. Of these, infection adjudication showed that at the time of ICU admission, 5 patients had definite infection, 9 patients had probable infection, and 9 patients had possible infection. At the time of ICU admission, SOFA scores for patients classified as septic ranged from 4 to 13, with a median score of 7.

For each of the SMS, SeptiCyte™ Lab, and FAIM3:PLAC8, we found statistically significant differences between patients classified as septic, and those who were critically ill but classified as not septic (Wilcoxon $P < .0001$ for SMS, $P < .05$ for FAIM3:PLAC8, and $P < .05$ for SeptiCyte™ Lab, Fig. 1). Overall accuracy for the classification of sepsis, as measured by the area under the receiver operating characteristics curve (AUROC), was highest for the SMS (0.80 [95% CI 0.67–0.92]), followed by the FAIM3:PLAC8 ratio (0.69 [0.53–0.85]), and the SeptiCyte™ score

Table 1
Patient characteristics for the cohort from the PREVAIL trial included in the study.

Characteristic	All patients ($n = 61$)	Septic ($n = 23$)	Non-septic ($n = 38$)
Age, median (IQR)	64 (56–75)	70 (60–77.5)	64 (55–73.5)
Male sex, n (%)	36 (59%)	13 (56.5%)	23 (60.5%)
APACHE II score, median (IQR)	24 (20–29)	26 (22.5–32)	22 (16–26.5)
ICU length of stay in days, median (IQR)	9 (7–16)	9 (5.5–15.5)	9.5 (7–15.75)
ICU mortality, n (%)	21 (34.4%)	8 (13.1%)	13 (34.2%)
SOFA at admission, median (IQR)	6 (5–8)	7 (4–9.5)	5 (4–7)
Procalcitonin at admission, median (pg/mL), median (IQR)	9045 (4332–15,789)	12,439 (8272–22,834)	7589 (3649–12,902)
Primary diagnosis categories			
Cardiovascular/vascular	9 (15%)	4 (17.4%)	5 (13.2%)
Gastrointestinal	1 (2%)	1 (4.3%)	0 (0%)
Neurologic	16 (26%)	2 (8.7%)	14 (36.8%)
Respiratory	10 (16%)	4 (17.4%)	6 (15.8%)
Trauma	10 (16%)	0 (0%)	10 (26.3%)
Sepsis	10 (16%)	9 (39.1%)	1 (2.6%)
Other	5 (8%)	3 (13.0%)	2 (5.3%)

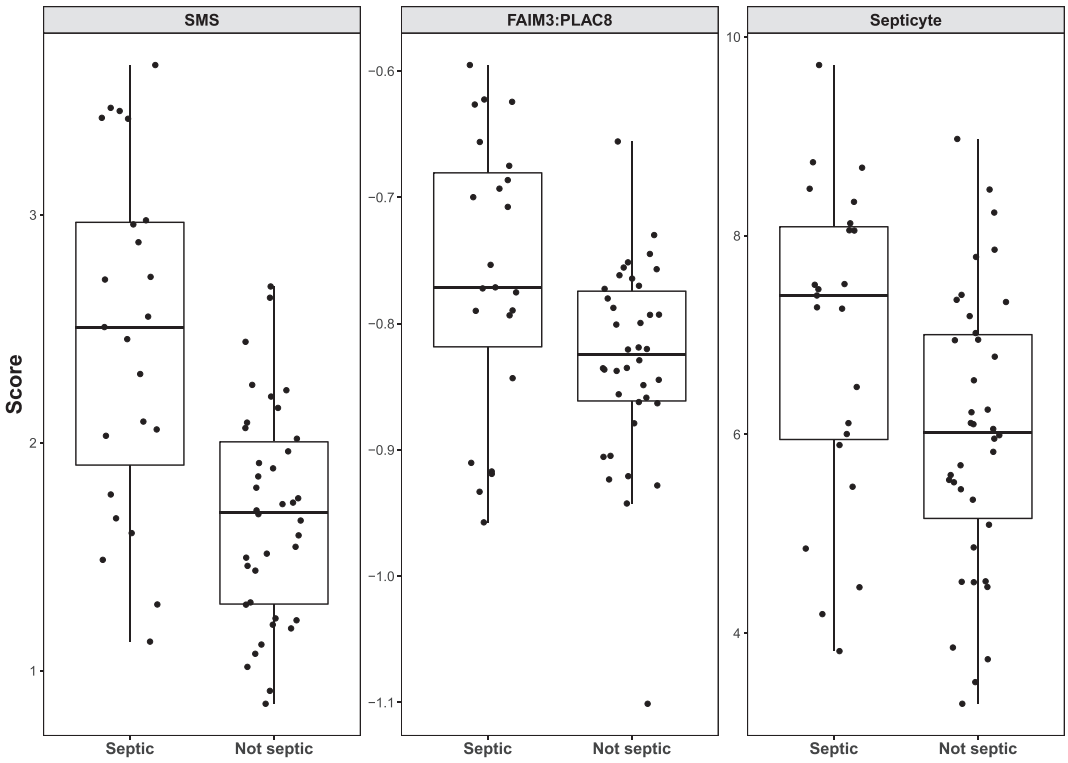

Fig. 1. Boxplots showing differences in admission scores. Statistically significant differences were observed between patients classified as septic vs non-septic for each of the three diagnostic scores (Wilcoxon $P < .00001$ for SMS, $P < .05$ for FAIM3:PLAC8 ratio, and $P < .05$ for SeptiCyte™ Lab).

(0.68 [0.53–0.83]) (Fig. 2). Pairwise comparisons of ROC curves for the various scores showed that discrimination for the SMS was likely higher than for the FAIM3:PLAC8 ratio (DeLong's test $P = .05$). The difference between septic and non-septic patients was marginal for procalcitonin (Wicoxon $P = .05$), as was its discrimination (AUROC 0.66 [0.51–0.82].

The trajectories of scores for septic patients over the first three weeks of ICU admission are shown in Fig. 3. There was a monotonic decrease in the SMS score for patients with sepsis over the first 3 weeks of their ICU stay. Both FAIM3:PLAC8 and SeptiCyte™ scores showed a decreasing trend overall. Individual patient score trajectories are shown in Supplemental Figs. 1–3. Among septic patients, the median change in SMS from baseline to Day 3 was +1% among non-survivors, and − 20% among survivors. Changes from baseline were less pronounced for the FAIM3:PLAC8 and SeptiCyte™ scores (Supplementary Fig. 4).

We created logistic regression models using the gene expression scores and procalcitonin levels as predictors of infection, with age, sex, and severity of illness as covariates. While 9 patients had missing procalcitonin data, a logistic model using procalcitonin missingness as the outcome and the other observed data as predictors failed to show a statistically significant effect on the odds of missingness at a $p < .05$ level.

Univariate analysis of the gene scores and log(procalcitonin) showed that each was associated with an increased odds of infection (Supplementary Table 1), and that the SMS had the largest odd ratio (OR 6.18, 95% CI 2.23–22.04, $p = .002$). Each of the other gene scores, as well as log(procalcitonin), had a statistically significant odds ratio at the $p < .05$ level. As missing values might be a potential source of bias, the univariate models for the gene scores were refit using all patients. Again, all gene scores were associated with increasing odds of infection, with the SMS yielding the highest OR (8.50, 95% CI 3.09–30.73, $p < .001$). Multivariable analyses incorporated age, sex, and APACHE II physiology score as they are plausible confounding factors. Only the SMS remained statistically significant in multivariable analysis (OR 5.43, 95% CI 1.68–24.30, $p = .01$; Table 2).

Given that overall performance seemed to be best for the SMS, we plotted SMS scores in descending order for all patients, as well as the healthy controls (Fig. 4). This shows that healthy controls and ICU patients without infection are concentrated in the lower half of the SMS range, while patients with probable and definite infection cluster within the higher range.

3.1. Sensitivity analyses

As a sensitivity analysis for the infection adjudication in the study cohort, we evaluated the performance of the transcriptomic scores with stricter definitions of infection (Fig. 5). When only patients with probable or definite infection were classified as having sepsis, the performance of all three scores improved (AUROCs of 0.88 [0.77–1.0], 0.74 [0.56–0.92], and 0.68 [0.49–0.88] for SMS, FAIM3:PLAC8 ratio, and SeptiCyte™ Lab, respectively). Discrimination was even further enhanced when only patients with definite infection were classified as septic (AUROCs of 0.93 [0.87–1.0], 0.88 [0.73–1.0], 0.92 [0.85–0.99] for SMS, FAIM3:PLAC8 ratio, and SeptiCyte™ Lab, respectively).

4. Discussion

In this study, we used an independent, prospectively collected dataset to carry out a fully independent validation of three previously published gene expression-based classifiers for sepsis. To our knowledge, ours is the first validation of this group of classifiers that is entirely independent of the derivation cohorts. It is also the first validation against the newly updated sepsis definitions (Sepsis-3). In our cohort, the SMS achieved the best classification performance with AUROCs ranging from 0.80 to 0.93, depending on the stringency of infection attribution used in the sepsis classification. This result is comparable to that achieved in the original SMS derivation study (AUROC 0.87) [9]. We also found that with increasing certainty around infection status,

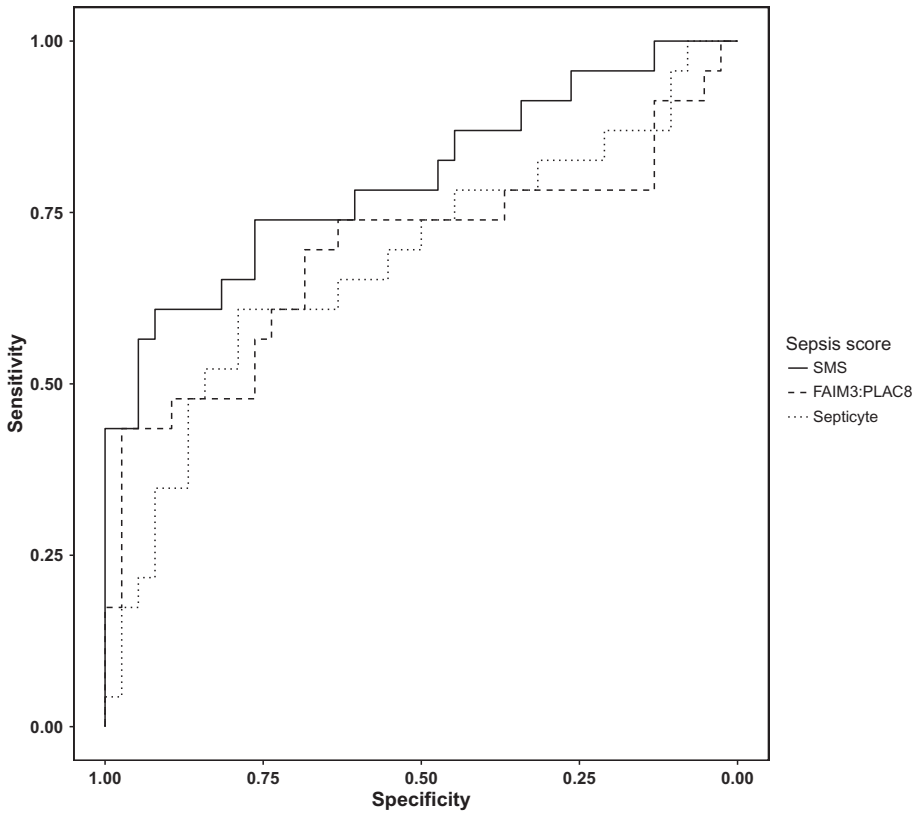

Fig. 2. Receiver operator characteristics (ROC) curves showing the performance of each diagnostic score for identifying septic patients. AUROC was highest for the SMS (0.80 [95% CI 0.67–0.92]), followed by the FAIM3:PLAC8 ratio (0.69 [0.53–0.85]), and then by SeptiCyte™ score (0.68 [0.53–0.83]).

the SMS showed both persistent gains in performance, and narrower confidence intervals for the AUROC values. While the original studies deriving the FAIM3:PLAC8 ratio and SeptiCyte™ lab report AUROC values ranging from 0.84 to 0.95 [7,8], these scores achieved AUROCs ranging from 0.68 to 0.92 in our cohort. All scores showed better discrimination than procalcitonin levels.

Our results resemble those of an earlier validation study in which these 3 scores were compared in a variety of publicly available sepsis gene expression datasets [12]. In that study, the SMS again performed best (AUROC 0.82), with the FAIM3:PLAC8 and SeptiCyte™ lab showing slightly less accuracy in discriminating patients with sepsis from those without (AUROCs 0.78 and 0.73 respectively). Another validation study, carried out by the team that developed the SeptiCyte™ score, showed that among hospitalized patients with acute respiratory failure, the SeptiCyte™ score performed well among patients with confirmed infection, but was less useful among patients for whom infection was ruled out [17]. A more recent study also showed that SeptiCyte™ performed well, this time in a prospective cohort of nearly 500 patients

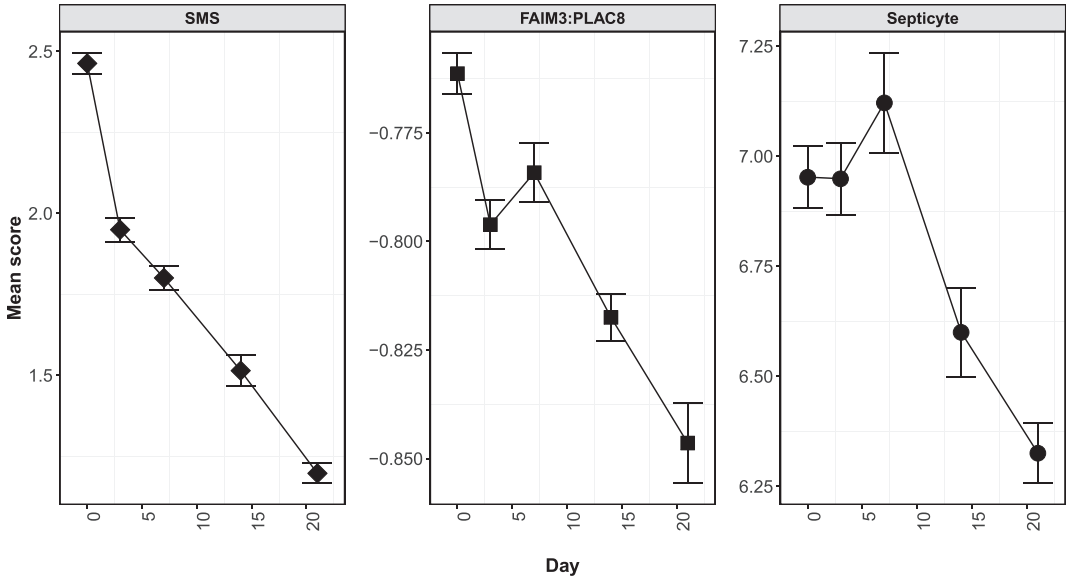

Fig. 3. Line plots showing the trajectories in diagnostic scores over time for septic patients. Bold points indicate median values and horizontal bars indicate standard error.

Table 2
Results of multivariate logistic regression modeling.

	Characteristic	Multivariate model ($n = 53$)			
			OR 95% CI		
		OR	Lower	Upper	*p*-Value
SMS models	Age (Years)	1.02	0.98	1.07	0.43
	Male (Reference: Female)	1.13	0.28	4.68	0.86
	APACHE II score (Points)	1.00	0.91	1.11	0.98
	log(Procalcitonin)	1.40	0.58	3.68	0.46
	SMS score (Points)	5.43	1.68	24.30	0.01
FAIM3:PLAC8 models	Age (Years)	1.01	0.97	1.05	0.69
	Male (Reference: Female)	0.63	0.17	2.21	0.47
	APACHE II score (Points)	1.04	0.95	1.15	0.39
	log(Procalcitonin)	1.52	0.66	3.82	0.34
	FAIM score (/0.1 Point)	1.55	0.78	3.36	0.23
SeptiCyte models	Age (Years)	1.01	0.97	1.06	0.53
	Male (Reference: Female)	0.70	0.19	2.49	0.59
	APACHE II score (Points)	1.04	0.95	1.15	0.44
	log(Procalcitonin)	1.54	0.66	3.85	0.33
	SeptiCyte score (Points)	1.31	0.87	2.06	0.21
No gene score	Age (Years)	1.01	0.97	1.05	0.68
	Male (Reference: Female)	0.66	0.18	2.27	0.51
	APACHE II score (Points)	1.05	0.96	1.16	0.32
	log(Procalcitonin)	1.74	0.78	4.27	0.19

from the U.S. and the Netherlands (AUROC 0.82–0.89) [11]. Our sensitivity analysis showed that diagnostic performance increased with increasing certainty around the diagnosis of sepsis. This finding is in keeping with results from the original SeptiCyte™ lab study, which found a decrement in score performance among more heterogeneous populations with less certainty around the diagnosis of sepsis [7].

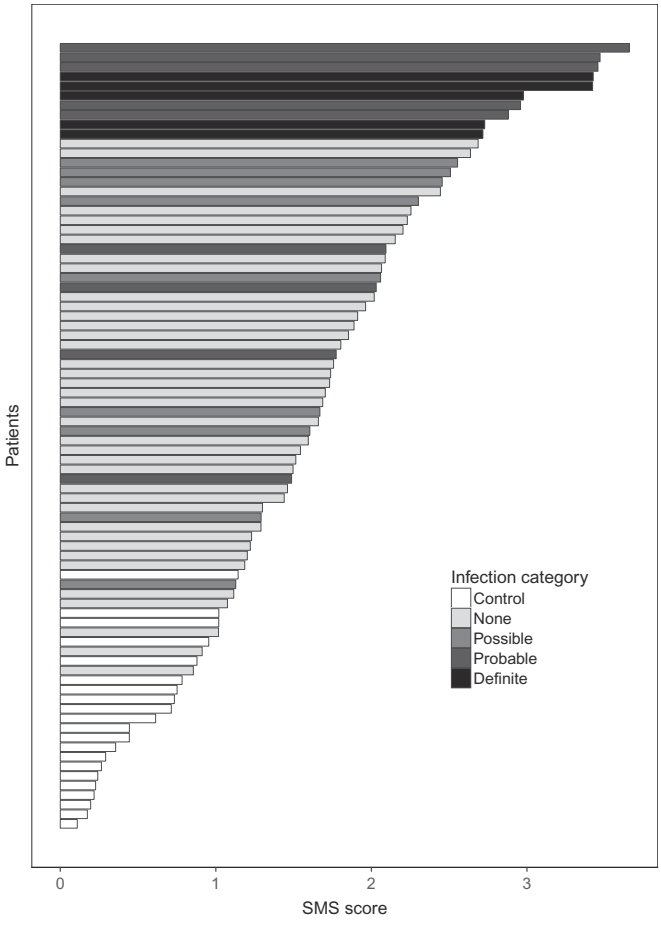

Fig. 4. SMS scores for all patients at the time of admission, as well as for 21 healthy controls. Scores are listed in descending order. Shading indicates infection labels (control, none, possible, probable, definite). Septic patients with definite or probable infection cluster at the high range of the SMS scores, while healthy controls cluster towards the bottom of the range.

We reasoned that discriminant scores would decrease over time with treatment among septic patients, as was seen in the original SMS derivation study [9]. We found that the SMS decreased more uniformly than both the FAIM3:PLAC8 and SeptiCyte™ score, and that septic patients who survived their ICU stay had a median 20% drop in their SMS, compared to no change among non-survivors. Response to treatment patterns was less evident for the other scores.

Our findings suggest that gene expression data from critically ill patients can be reliably generated and analyzed across a range of platforms and using a variety of bioinformatics techniques. This lends credibility to the increasing use of whole genome technologies in critical care research, and suggests that studies leveraging these modalities may yield durable insights. The SMS was developed using gene expression data from multiple studies, and seemed to yield the best diagnostic performance. This suggests there may be a benefit to pooled analyses of multiple gene expression studies in sepsis, as a means of enhancing generalizability and reproducibility. Unlike the FAIM3:PLAC8 ratio and SeptiCyte™ Lab – which were generated using a small number of patient cohorts – the SMS was developed based on data from 27 different datasets generated by a number of different investigators among a variety of different patient populations, and using a range of different assay techniques. By combining data from such diverse sources, the SMS may be better insulated against the problem of overfitting to any one dataset. This design strategy may maximize the generalizability of this score, and may account for some of the improved performance seen in our validation study.

Our study has a number of key strengths. We used a well annotated dataset, with biologic samples linked to high quality clinical data collected in the setting of a randomized controlled trial. We also used a microarray platform not previously used in the studies that generated the original scores. Our validation cohort included a diverse mix of critically ill patients, sampled at various time points throughout the course of their ICU stay.

Our study has some limitations as well. First, it is unknown if or how the study drug used in the PREVAIL trial may have influenced expression values for the genes included in the sepsis scores, however most of the analysis was based on samples drawn at admission, prior to the administration of the first dose. Second, the method by which patients were labeled as having sepsis may have lacked sensitivity or specificity. However, given that infection classification was based on formal adjudication using the entire clinical record, rather than culture results, antibiotics administered, or ICD codes, we believe this method of sepsis attribution is robust, and in keeping with the most recent consensus definitions of sepsis [14]. Furthermore, we provide a sensitivity analysis assessing classification performance under different schemas for assigning sepsis labels. Third, roughly half of the patients without sepsis had neurologic or trauma admission diagnoses, a group for whom the molecular determination of sepsis may be less clinically relevant. Fourth, gene expression profiling is a time consuming process, with turnaround times that preclude its timely use in a clinical environment. However, useful expression profiles can inform the development of rapid multiplex diagnostics that measure only the most contributory expression signals [18]. Lastly, the biological interpretation of the gene expression scores we validated requires further study, as does the optimal deployment of this tool in the clinical environment.

The independent replication of results serves an important function in biomedical research, especially where newer methodologies are concerned, such as gene expression profiling [13,19]. The results of our study increase our confidence in both the whole genome expression data used in critical care research, as well as the complex bioinformatics analyses used to analyze them. The diagnostic gene sets used in this case could be identified in data derived from a variety of different microarray platforms, each of which use different technologies, annotations, and analysis pipelines.

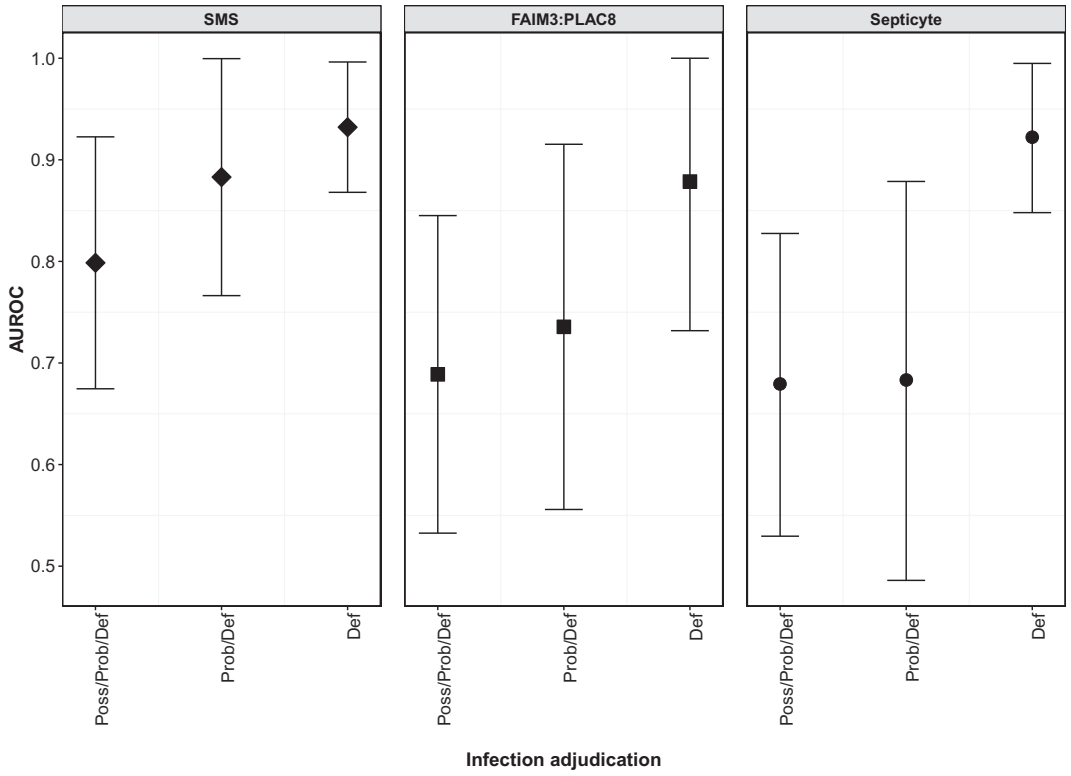

Fig. 5. Change in score performance according to stringency of infection definition. The AUROC for classification of patients as septic is shown, along with 95% confidence intervals represented by vertical error bars. As the criteria for infection progress from more lax (possible, probable, or definite), to more strict (definite), the performance of the scores improves.

As a research tool, gene expression profiling has begun to yield useful insights into the molecular dynamics of sepsis, and the host response to infection [6]. The clinical utility of this technology, however, remains underdeveloped. Barriers to the widespread deployment of current gene expression profiling technologies include high costs, long assay turnaround times, and uncertainty regarding the optimal timing of sampling to yield actionable results. Gene expression has been shown to shift quickly and radically in the face of physiologic insults such as endotoxin challenge [20], and trauma [21]. Better understanding of these changes is needed in order to differentiate adaptive from pathological responses to injury, and to better understand the gene expression dynamics of individual patients.

Ultimately the clinical utility of whole genome-derived biomarkers will be less in the stratification of patients according to diagnostic category, and more in predicting whether or not a given patient will respond to a specific therapy, at a particular time in the course of their critical illness. This objective is central to the development of precision critical care, and should form the basis of future studies in this area.

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Contributions

DMM – study design, data collection, data analysis, drafting of the manuscript, revising the manuscript, interpretation of results; TS – study design, data analysis, drafting of the manuscript, revising the manuscript, interpretation of results; KT – study design, revising manuscript, interpretation of results; RV – analysis of gene expression data (quality); linear regression modeling; revising manuscript, interpretation of results; JCM – study design, revising manuscript, interpretation of results; JM – study design, data collection, infection adjudication, revising the manuscript, interpretation of results.

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Competing interests

The authors have no conflicts of interest (financial or otherwise) to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcrc.2018.10.028.

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