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Comprehensive Validation of the *FAIM3*:*PLAC8* Ratio in Time-matched Public Gene Expression Data

To the Editor:

Scicluna and colleagues recently reported the gene expression ratio of Fas apoptotic inhibitory molecule 3 (*FAIM3*) to placenta-specific 8 (*PLAC8*) as a new sepsis diagnostic biomarker (1). Accurate sepsis diagnosis is critical, as the mortality rate of sepsis increases for each hour that antibiotics are not administered (2). The *FAIM3*:*PLAC8* ratio was discovered in a cohort comparing critically ill patients within 24 hours of admission for community-acquired pneumonia with noninfected patients. Scicluna and colleagues found that the *FAIM3*:*PLAC8* ratio had areas under the receiver operating characteristic curve (AUCs) of 0.845 and 0.784 for diagnosing community-acquired pneumonia in their discovery and validation cohorts, respectively (1).

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Of paramount importance in the further study of any proposed biomarker is its validation in independent heterogeneous cohorts, as estimates in discovery cohorts are often overfit. As we recently reported, there are multiple public gene expression datasets that compare noninfected systemic inflammatory response syndrome (SIRS)/trauma patients with patients with sepsis from various sources; we comprehensively organized all publicly available cohorts into 13 time-matched subcohorts and performed gene expression meta-analysis to derive an 11-gene set diagnostic for sepsis compared with sterile SIRS/trauma (3). These public datasets can be reanalyzed to test the diagnostic power of new gene expression biomarkers as well.

We thus tested the *FAIM3*:*PLAC8* ratio in the previously described time-matched gene expression cohorts (four intensive care unit admission cohorts, nine Glue Grant trauma subcohorts; total n = 881). In intensive care unit admission cohorts, the *FAIM3*:*PLAC8* mean AUC was 0.72 (range, 0.60–0.88; Figure 1A). In the Glue Grant trauma cohorts (4), which were split into time-matched subcohorts comparing patients within $\pm$24 hours of diagnosis of infection with time-matched trauma patients who were never infected, the mean AUC was 0.83 (range, 0.62–0.98; Figure 1B) and generally increased over time since injury. In comparison, our 11-gene set had mean AUCs of 0.82 and 0.87 in the intensive care unit admission and Glue Grant cohorts, respectively. Finally, we tested our 11-gene set for diagnostic power in the 134 discovery samples from the study of Scicluna and colleagues (GEO dataset GSE65682; AUC = 0.79).

Perhaps the greatest surprise is poor performance of the *FAIM3*:*PLAC8* ratio in GSE40012 (5), another dataset that specifically studied community-acquired pneumonia. However, both GSE40012 and GSE32707 (6) were run on Illumina platforms and performed worse than almost all other tested cohorts; technical differences may thus be contributing to the observed decrease in AUC. In addition, the performance of *FAIM3*:*PLAC8* in the Glue Grant data (mean AUC, 0.83) was nearly as good as in the original discovery cohort.

As noted by Scicluna and colleagues, biomarkers often perform better in combination than alone. In the cohorts tested here, the *FAIM3*:*PLAC8* ratio was generally highly correlated to our sepsis diagnostic 11-gene set (3) (mean Spearman correlation, 0.68; SD, 0.10), and combining the two separate scores into a single diagnostic did not yield a synergistic increase in AUC (not shown).

We wish to congratulate Scicluna and colleagues for a well-performed study and for their derivation of the *FAIM3*:*PLAC8* sepsis diagnostic biomarker. The AUCs of the *FAIM3*:*PLAC8* ratio may not be high enough to bring it into practice in sole use; however, the team demonstrated superiority to procalcitonin in their cohort. Thus, it may be worthy of further study in combination with other infection markers. In addition, the use of a simple gene expression ratio (similar to other popular methods such as differences of arithmetic or geometric means) allows for easy testing and comparison without constructing a complicated model. Overall, given the relative ease with which researchers can now test a gene expression biomarker in multiple public cohorts, we recommend that further gene expression biomarker studies should include these comparisons as a basic benchmark. ■

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

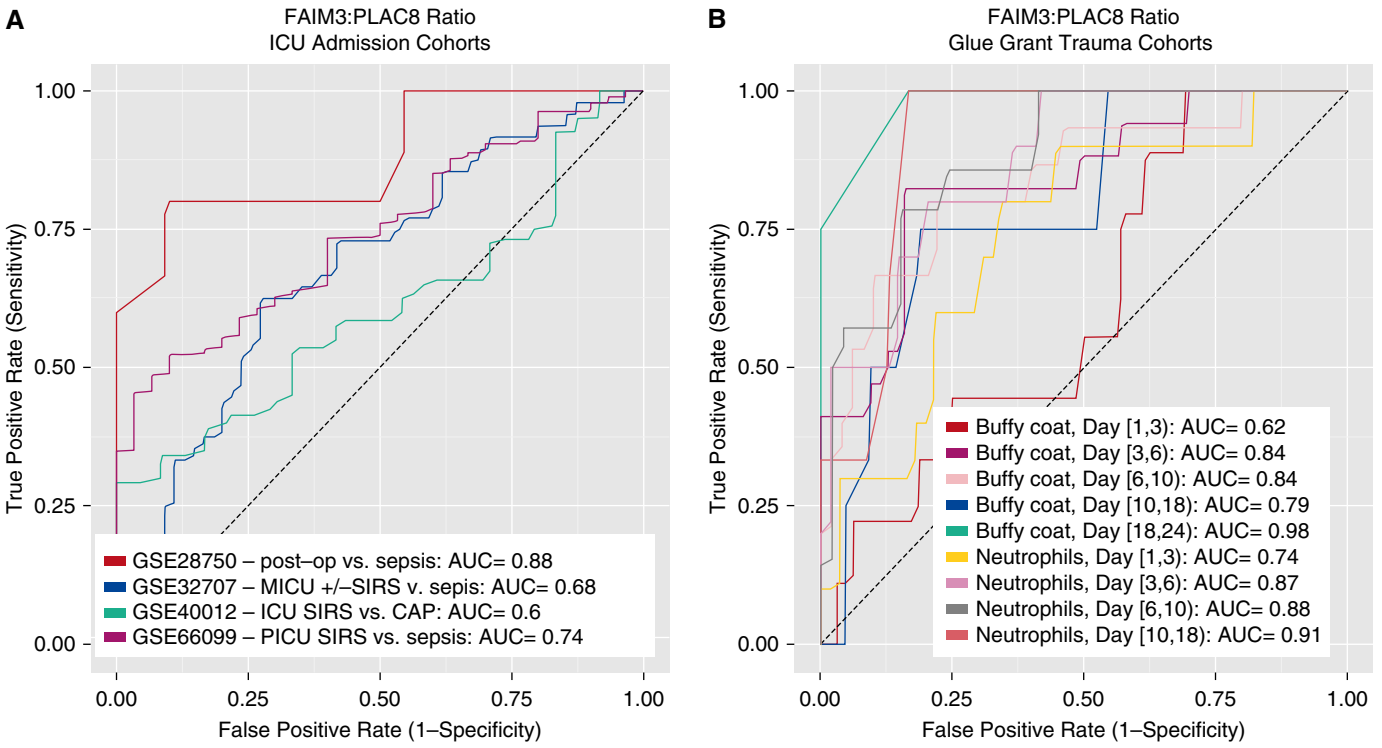

Figure 1. Receiver operating characteristic curves showing the diagnostic power of sterile SIRS/trauma patients versus patients with sepsis in multiple public gene expression datasets. (*A*) Datasets comparing patients at intensive care unit admission. (*B*) Subcohorts of time-matched trauma patients, split out by days since initial injury. Buffy coat and neutrophil data were from separate patient cohorts. AUC = area under the receiver operating characteristic curve; CAP = community-acquired pneumonia; FAIM3 = Fas apoptotic inhibitory molecule 3; ICU = intensive care unit; MICU = medical ICU; PICU = pediatric ICU; PLAC8 = placenta-specific 8; SIRS = systemic inflammatory response syndrome.

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Reply

From the Authors:

We are thoroughly grateful to Dr. Sweeney and Dr. Khatri for their positive comments regarding our study on the *FAIM3:PLAC8* gene expression biomarker for the rapid diagnosis of community-acquired pneumonia (CAP) at intensive care unit (ICU) admission (1). Moreover, the additional analyses across different cohorts certainly add value to our proposed biomarker. We definitely concur with the authors in making use of publicly available datasets for further validation of proposed biomarkers. However, we would also like to point out that contrary to our study, which was centered on a specific population of ICU patients with suspected