RESEARCH LETTER

CSTB and FABP5 Serum mRNA Differentiate Histologically Active and Inactive Patients With Eosinophilic Esophagitis

Eosinophilic esophagitis (EoE) patients undergo serial endoscopies to assess response to therapy.[1,2] Because of this burden, there is a need for noninvasive biomarkers of EoE, but none have been validated for use. As there is overlap between EoE and other atopic diseases, it may be challenging to identify noninvasive biomarkers specific to esophageal inflammation.[3] An accurate serum biomarker could reduce the number of endoscopies, leading to improved patient satisfaction and healthcare savings. A previously uninvestigated class of markers involves the esophageal squamous epithelium. In EoE, there are multiple histopathologic epithelial alterations in addition to eosinophilic infiltration, and epithelial and inflammatory components are hypothesized to leak into the serum. Therefore, we sought to determine whether a cell-free serum mRNA "liquid biopsy" panel, specific to the esophageal squamous epithelium, could be used to diagnose EoE and discriminate active histologic disease from remission.

To this end, we analyzed data and samples collected from incident EoE cases and non-EoE controls during a prospective study of adults undergoing outpatient endoscopy at the University of North Carolina, as previously described.[4] Cases were treated with topical corticosteroids per clinical protocol (budesonide slurry or swallowed fluticasone) for 8 weeks and had follow-up endoscopy.[5] None of the cases were proton pump inhibitor-responsive, and none were concurrently on biologic or dietary therapy during the study period. Prior to each procedure, a serum sample was obtained and stored at −80 °C. Only samples from EoE histologic responders to topical corticosteroids were selected to maximize power. This study was approved by the University of North Carolina Institutional Review Board; patients previously enrolled in the parent study provided informed consent for future sample use.

We used a previously described *in silico* bioinformatics technique to identify candidate tissue-specific biomarkers,[6] a method that has successfully been used in other gastrointestinal conditions.[6,7] This approach leverages the Human Protein Atlas and Galaxy-based Proteomics Research Environment bioinformatics methodology. We identified 20 candidate biomarkers based on the following criteria: 1) including genes specific to the esophageal squamous epithelium; 2) removing genes measured at mRNA levels <10 transcripts per million. The final candidate probes were: PPL, JUP, SerpinB13, CES2, KLK13, SerpinB3, CRNN, KRT4, SerpinB4, CSTB, SerpinB5, CYSRT-1, LYPD3, SFN, FABP5, MAL, SPRR1A, Galectin 3, MUC22, and TGM3.

Once candidate markers were identified, we retrieved archived serum samples, masked them to case/control status, and extracted RNA with the Quick-cfRNA Serum & Plasma Kit. Polymerase chain reaction amplification was performed with Fluidigm Biomark HD and the delta-delta count method was used to determine fold gene expression changes (Supplemental Methods), and a Wilcoxon test was performed with $P < .05$ used to determine significance between groups.[8]

To compare EoE cases to controls, we calculated the delta-delta count values relative to the average delta count value of all the controls. To compare EoE cases with active disease (pretreatment) and in histologic remission (defined as <15 eosinophils per high-power field [eos/hpf] posttreatment), we computed delta-delta count values relative to the pretreatment sample for each patient. Nonparametric testing was used to compare cases and controls, with paired nonparametric testing for EoE cases pre- and post-treatment. To assess discrimination, we constructed a generalized linear model using individual genes (and a panel of genes) to distinguish paired specimens with active disease (pretreatment) from histologic remission (posttreatment). Due to small sample size, we built the model using the whole dataset and then predicted response in the dataset itself. Genes found to be differentially expressed in serum samples were evaluated using similar analytic techniques in a separate cohort of EoE patients with whole blood total RNA sequence data available (n = 6 with histologic remission) from a prior study conducted by our group.[9]

We included 16 EoE cases (mean age 41 years; 62% male; 100% White; 69% atopic) and 15 controls (mean age 38 years; 53% male; 80% White; 80% atopic; 47% gastroesophageal reflux disease) (Table A1); posttreatment samples were available for all EoE cases. For cases, the EoE Endoscopic Reference Score decreased from 3.0 to 0.2 posttreatment; and peak eosinophil counts decreased from 178 eos/hpf to 1.

No genes were differentially expressed between cases and controls. Four of the 20 mRNA probes were reliably detectable in pre- and posttreatment samples of at least 9 of 16 EoE cases. Cystatin B (CSTB) serum mRNA expression trended toward increased expression in active disease compared to histologic remission with a median log 2-fold change of 1.312 ($P = .055$). Serum fatty acid binding protein 5 (FABP5) was significantly higher in histologic remission compared to active disease with a median log 2-fold change of 0.515 ($P = .0004$) (Figure 1A and B). For classifying paired specimens into active disease (pretreatment) and remission (posttreatment), area under the curve was 0.88 for CSTB and 0.93

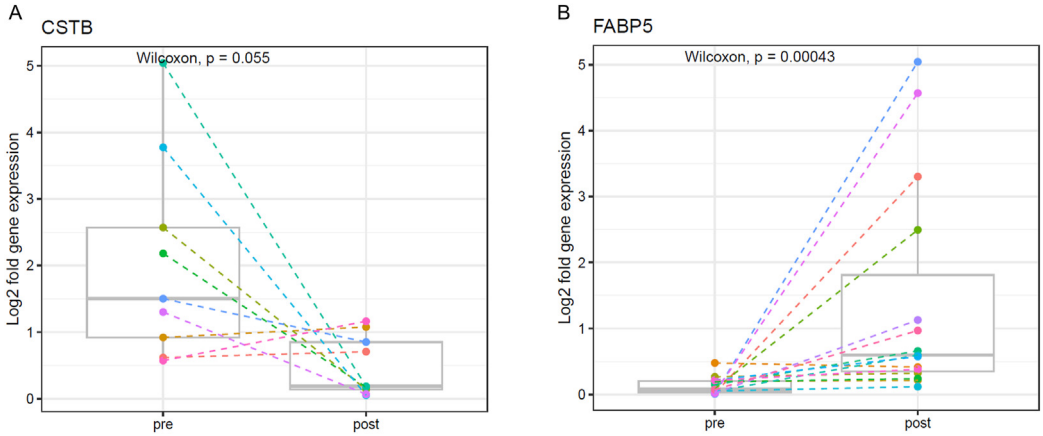

Figure 1. Log2 gene expression changes of (A) CSTB and (B) FABP5 mRNA pretreatment and posttreatment for those patients achieving histologic remission.

for FABP5 (Figure 2A and B). A model including both genes had an area under the curve of 0.96 (Figure 2C). There was no correlation between gene fold change and changes in Endoscopic Reference Score or eosinophil counts. In the separate 6-patient whole blood RNA seq cohort, observed trends were similar with higher median CSTB expression pretreatment and lower FABP5 expression pretreatment, although these findings were limited by small sample size (not statistically significant; Figure A1).

We found that CSTB and FABP5 serum mRNA expression tracked with histologic treatment response in EoE patients, but not between cases and controls. We hypothesize that the delta-delta reference method for monitoring before/after therapy of an individual patient was able to account for many potential confounding factors such as sex, age, medications, and body mass index. Both CSTB and FABP5 expression have been shown to be differentially expressed in esophageal biopsies of EoE patients, further lending plausibility to our findings.[10] Our study is limited by small sample size and lacks a serum validation cohort. However, the fact that CSTB and FABP5 expression trends were observed to a lesser degree in whole blood suggests that these expression differences are driven by the serum rather than leukocyte fraction. Planned future studies will aim to replicate these findings in larger cohorts of patients and other blood compartments. If validated, these findings could serve as a foundation for an esophagus-specific mRNA liquid biopsy to monitor EoE disease activity noninvasively.

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Supplementary Materials

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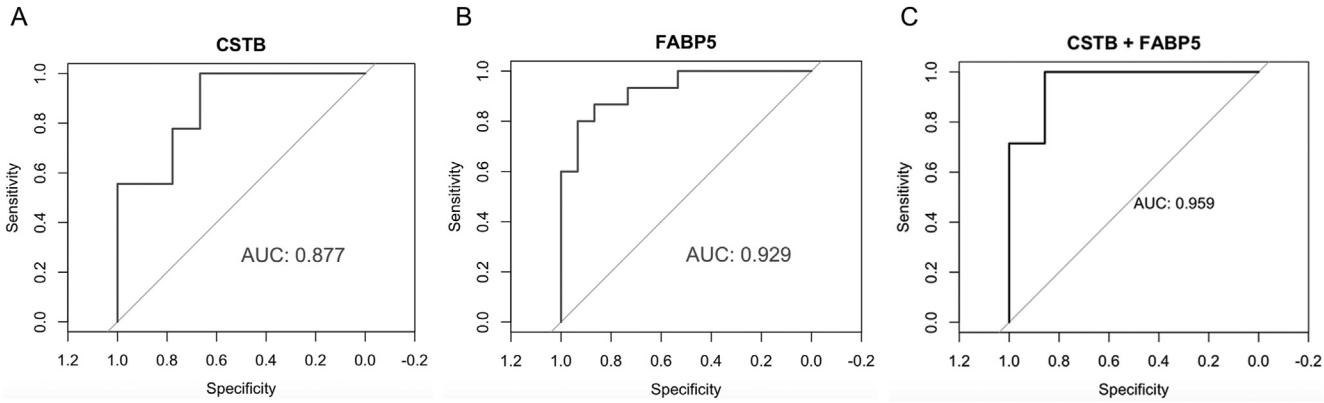

Figure 2. The AUC of the threshold model for (A) CSTB, (B) FABP5, and (C) CSTB + FABP in distinguishing a patient with active disease from histologic remission. AUC, area under the curve.

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Abbreviations used in this paper: **EoE, eosinophilic esophagitis; GERD, gastroesophageal reflux disease**

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Ethical Statement:
The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Data Transparency Statement:
Data may be shared with qualified researcher upon reasonable request to the corresponding author.

Reporting Guidelines:
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