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αIIb-integrin (CD41) associated with blood eosinophils is a potential biomarker for disease activity in eosinophilic esophagitis

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Capsule summary:

After EoE treatment, association of platelets with blood eosinophils, as reported by CD41, predicted esophageal eosinophil count. Percentage CD41+ circulating eosinophils is a potential non-invasive biomarker for EoE disease activity.

Keywords

Eosinophilic esophagitis; α_{IIb}-integrin; CD41; blood eosinophils; platelets; platelet-eosinophil association; peak eosinophil count; disease activity; biomarker; non-invasive

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Eosinophilic esophagitis (EoE), a chronic inflammatory disease characterized by symptoms of esophageal dysfunction and eosinophilic infiltration of the esophageal mucosa, has increased in incidence and prevalence the past two decades.[1, 2] The standard for assessing disease activity is performing endoscopy and pathological examination of esophageal biopsies. There is a critical need for a biomarker to replace such invasive monitoring. A number of potential tests have been evaluated but none have been incorporated into guideline recommendations or routine clinical practice.[3, 4]

We previously demonstrated by flow cytometry that the activation epitope for monoclonal antibody (mAb) N29 on the β_1 integrin subunit on circulating eosinophils correlates with and predicts decreased pulmonary function in patients with non-severe asthma.[5] Further, we linked N29 epitope appearance to eosinophil engagement by P-selectin (CD62P) on activated platelets and showed that P-selectin-stimulated eosinophil $\alpha_4\beta_1$ integrin activation causes enhanced adhesion to vascular cell adhesion molecule (VCAM)-1, which is induced on activated endothelium.[6] We hypothesized that a similar pathway occurs in EoE and undertook the present study to determine if eosinophil surface biomarkers correlate with and predict eosinophil count in esophageal biopsies.

Patients were recruited from the University of Wisconsin (UW) Health Gastroenterology Clinic after failing to respond for two months to proton-pump inhibitor (PPI) therapy and following endoscopy diagnostic of EoE. Informed written consent was obtained at Visit 1 (V1) within two weeks of release of the endoscopy results, according to a protocol approved by the UW-Madison Health Sciences Institutional Review Board (see Online Repository text and Table EI). Patients received standard of care EoE treatment for eight weeks followed by V2 and repeat endoscopy. N29, CD62P, CD41 (α_{IIb} integrin subunit, a reporter of platelet association), and 13 other surface markers (Table EII) were assayed by flow cytometry of eosinophils gated in whole blood. Peak eosinophil count (PEC) per high power field (HPF) was assessed on esophageal biopsy. Flow cytometry, clinical assessments, and statistical analysis are described in the Online Repository.

Twenty-five patients (Table I) completed V1 and V2. At V1, all patients had active disease, as specified by the enrollment criteria. Changes in PEC and disease scores from V1 to V2 are visualized in Fig E1. PEC (Table I) and scores decreased significantly, from means of 11.0 to 4.6 for EoEHSS (histology, $P = 0.001$), 31.6 to 19.0 for EEsAI (symptoms, $P < 0.001$), and 4.1 to 2.8 for EREFS (endoscopy, $P < 0.001$). At V2, eleven patients (44%) had PEC < 6/HPF and three others, for a total of 14 (56%) patients, had PEC < 15/HPF (Fig. E1, *A*). Correlations among the three scores and PEC at V1 or V2 are shown in Fig E2. The strongest correlation was between EoEHSS and PEC, whereas the other correlations were weaker.

Flow cytometry histogram examples for patients with low or high level activated β_1 integrin (N29 signal) or low or high degree α_{IIb} (CD41) positivity are displayed in Fig E3. N29 intensity and levels of and percentage eosinophils positive for P-selectin (CD62P) and CD41 correlated among each other (Fig E4). CD62P and CD41 positivity correlated with PEC at V2, whereas N29 intensity did not (Fig E5). Adjusting for the RCAT (Rhinitis Control Assessment Test) score, allergy and asthma, or treatment including steroid, did not affect

these correlations (Table EIII). In addition, patients on steroids did not have significantly different expression of the markers compared to patients not on steroids (Table EIV). None of the other 13 markers correlated significantly with PEC at V2 (Table III). N29 intensity or CD62P or CD41 positivity did not correlate significantly with the other measures of EoE disease activity (Table EV). However, CD41 positivity correlated with eosinophil inflammation and lamina propria fibrosis EoEHSS subscores (Table EVI).

We performed receiver operating characteristic (ROC) analysis to investigate the ability of these potential biomarkers to predict PEC below pre-specified cutoffs. CD62P or CD41 significantly predicted PEC < 6/HPF; CD41 was best with an area under curve (AUC) of 0.84, 93% specificity and 82% sensitivity for the statistically optimal criterion < 22.9% CD41-positive cells (Fig 1, *A*; Table EVII). CD41 also significantly predicted PEC < 15/HPF (Fig E6 legend). Dividing patients according to median CD41 positivity (26.7%), CD41-low patients had median PEC = 0/HPF and CD41-high patients significantly higher median PEC = 31/HPF (Fig 1, *B*). Alternatively, categorizing the patients according to PEC < or ≥ 6 or 15/HPF, PEC-low patients had significantly fewer CD41-positive blood eosinophils than PEC-high patients (Fig E6).

In summary, our results indicate that a pathway of platelet activation and platelet-eosinophil association, likely also P-selectin-triggered $\alpha_4\beta_1$ integrin activation and $\alpha_4\beta_1$-mediated eosinophil arrest on VCAM-1 on activated endothelium in the esophagus, occurs in EoE, as suggested in the lung in asthma.[5, 6] Such a scenario is consistent with evidence for platelet activation and platelet-eosinophil interactions, as visualized by immunofluorescence microscopy *in vitro* of α_{IIb}/CD41-positive eosinophils,[7] which we have also observed *in vivo* in a patient with EoE (Fig. E7), leading to eosinophil recruitment in allergic diseases.[8] It is also consistent with upregulated VCAM-1 in the esophagus in EoE and association between response to treatment and decreased VCAM-1.[9]

PEC was our primary measure of disease status. PEC correlated strongly with the histology score, whereas the other correlations among scores were more modest. This is consistent with the struggles to identify an ideal metric for EoE disease activity, as there is a loose relationship among symptoms, pathology, and visualization of the esophagus. To date, PEC remains the primary metric for EoE diagnosis and monitoring. Limitations of the present study include the relatively small sample size, exclusion of children, and that subjects were recruited by EoE guidelines at the time of study design, i.e., subjects with PPI-responsive esophageal eosinophilia were excluded. To validate our findings and address these and other limitations, we plan to examine a larger cohort including patients with PPI-responsive esophageal eosinophilia and pediatric patients.

In conclusion, we demonstrate that α_{IIb} integrin (CD41) associated with circulating eosinophils is a potential non-invasive biomarker for residual esophageal eosinophilic inflammation, after a period of recommended standard of care treatment. (Word count 1000)

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

1. Collins MH, Capocelli K, Yang GY. Eosinophilic gastrointestinal disorders pathology. Front Med (Lausanne) 2017; 4:261. [PubMed: 29379785]
2. Dellon ES, Hirano I. Epidemiology and natural history of eosinophilic esophagitis. Gastroenterology 2018; 154:319–32 e3. [PubMed: 28774845]
3. Metcalfe DD, Pawankar R, Ackerman SJ, Akin C, Clayton F, Falcone FH, et al. Biomarkers of the involvement of mast cells, basophils and eosinophils in asthma and allergic diseases. World Allergy Organ J 2016; 9:7. [PubMed: 26904159]
4. Hines BT, Rank MA, Wright BL, Marks LA, Hagan JB, Straumann A, et al. Minimally invasive biomarker studies in eosinophilic esophagitis: A systematic review. Ann Allergy Asthma Immunol 2018; 121:218–28. [PubMed: 29753832]
5. Johansson MW. Eosinophil activation status in separate compartments and association with asthma. Front Med (Lausanne) 2017; 4:75. [PubMed: 28660189]
6. Johansson MW, Mosher DF. Integrin activation states and eosinophil recruitment in asthma. Front Pharmacol 2013; 4:33. [PubMed: 23554594]
7. Johansson MW, Mosher DF. Activation of beta1 integrins on blood eosinophils by P-selectin. Am J Respir Cell Mol Biol 2011; 45:889–97. [PubMed: 21441381]
8. Shah SA, Page CP, Pitchford SC. Platelet-eosinophil interactions as a potential therapeutic target in allergic inflammation and asthma. Front Med (Lausanne) 2017; 4:129. [PubMed: 28848732]
9. Nhu QM, Aceves SS. Tissue remodeling in chronic eosinophilic esophageal inflammation: Parallels in asthma and therapeutic perspectives. Front Med (Lausanne) 2017; 4:128. [PubMed: 28831387]

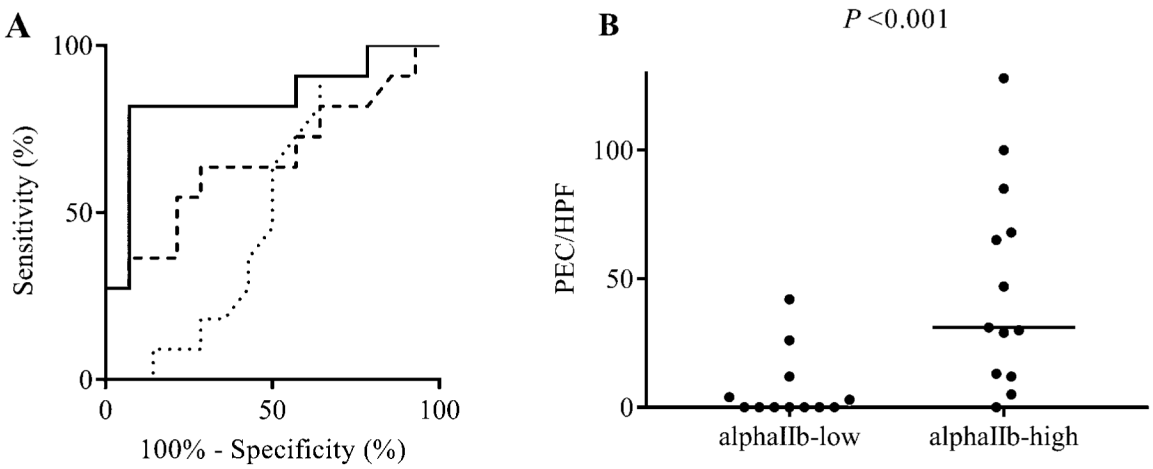

FIG 1.

ROC curve for the ability of blood eosinophil activated β_1 integrin (mAb N29), P-selectin (CD62P)- or α_{IIb} integrin (CD41)-positive blood eosinophils to predict PEC at V2, and PEC at V2 in α_{IIb}-low or –high patients. **A,** Dotted line, N29; dashed line, P-selectin (CD62P); solid line, α_{IIb} (CD41). To predict PEC < 6/HPF, area under curve (AUC) = 0.52 for N29, 0.66 for P-selectin, and 0.84 for α_{IIb} ($P < 0.001$); for criterion < 22.9% α_{IIb}-positive cells, specificity = 93% and sensitivity = 82%. **B,** Patients were divided according to median α_{IIb}-positive cells (26.7%) at V2; Bar, median PEC in each group (median in the α_{IIb}-low group = 0).

TABLE I.

Patient characteristics

Variable	Value
n	25
Age (years) (mean ± SD)	38 ±14
Sex (*n* [percentage] male)	19 (76%)
Race/ethnicity (white non-Hispanic) (*n* [percentage])	24 (96%) *
Time since symptom onset (years) (median [25th, 75th percentiles])	5 (2, 10)
V1 blood eosinophils (per μl) (median [25th, 75th percentiles])	194 (70,299)
V2 blood eosinophils (per μl) (median [25th, 75th percentiles])	158 (114,273)
Allergic rhinitis diagnosis or symptoms (*n* [percentage])	20 (80%)
VI RCAT score (mean ± SD) (*n* = 21)	24.1 ± 4.3
V2 RCAT score (mean ± SD) (*n* = 22)	23.3 ± 4.4
Asthma diagnosis (*n* [percentage])	2 (8%)
Treatment from V1 to V2 (*n*)	12 topical steroid
	7 food elimination
	5 both
	1 other
Reported use of NSAID(s) (including aspirin) (*n*)	13
EoE severity:	
Required dilations (*n* [percentage])	12 (48%)
Required ED visits for EoE (*n* [percentage])	13 (52%)
Allergic sensitivities:	
Trees (*n* [percentage])	17 (68%)
Grass (*n* [percentage])	12 (48%)
Ragweed/late fall pollen (n [percentage])	10 (40%)
Mold (*n* [percentage])	5 (20%)
Dust mite (*n* [percentage])	10 (40%)
Cat or dog (*n* [percentage])	9 (36%)
Food sensitivities:	
Egg (*n* [percentage])	9 (36%)
	2 (8%)
Peanut (*n* [percentage])	6 (24%)
Shrimp (*n* [percentage])	5 (20%)
Soy (*n* [percentage])	1 (4%)
Wheat (*n* [percentage])	3 (12%)
Any above food (*n* [percentage])	13 (52%)
V1 PEC (per HPF) (median [25th, 75th percentiles])	42 (28, 68)
(percentage ≥ 6/HPF or ≥ 15/HPF)	100% ≥ 6, 96% ≥ 15
V2 PEC (per HPF) (median [25th, 75th percentiles])	12 (0, 44) †
(percentage ≥ 6/HPF or ≥ 15/HPF)	56% ≥ 6, 44% ≥ 15

ED, emergency department; *EoE*, eosinophilic esophagitis; *HPF*, high power field; *n*, number of subjects; *NSAID*, non-steroidal anti-inflammatory drug; *PEC*, peak eosinophil count; *RCAT*, rhinitis control assessment test; *V*, visit.

Data are presented as mean ± SD (if data are normally distributed) or median (25^{th}, 75^{th} percentiles) (if data are not normally distributed).

*One patient was white and American Indian/Alaska Native.

†$P = 0.02$ versus V1.

For blood eosinophils, $P = 0.96$ between V2 and V1.

Allergic and food sensitivities are based on skin test.

Note: Information on NSAID use was captured as part of the information on medication but not captured in relation to study visits.