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

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# METHODS

## Patients

In a protocol approved by the University of Wisconsin (UW)-Madison Health Sciences Institutional Review Board, adult patients (> 18 years of age) with established eosinophilic esophagitis (EoE) were recruited from the UW Health Gastroenterology Clinic following a diagnostic endoscopy for esophageal dysfunction. Patients with esophageal biopsy showing ≥ 15 eosinophils/high power field (HPF), with one patient enrolled with a peak eosinophil count (PEC) of 11/HPF who remained symptomatic, despite use of high dose proton pump inhibitor (PPI, equivalent of omeprazole ≥ 40 mg daily) for at least two months (with exception for one patient who had contraindication to PPI use), were invited to participate and enroll within two weeks of receiving the endoscopy results. Histology staining was used to identify eosinophils. The PEC was derived from either the proximal or distal esophagus, as the greatest value. A total of 28 patients were recruited for a 9-week prospective observational study between 2016 and 2018. A summary of the visits is shown in Table EI. Informed written consent was obtained from each subject prior to participation in the study at visit (V) 1. Exclusion criteria included major health problem such as autoimmune disease, heart disease, type I or II diabetes, uncontrolled hypertension, or lung disease other than asthma; or pregnancy, lactating, or a planned pregnancy during the course of the study. Twenty-five patients completed V1 and V2 and are included in the analyses.

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## Study visits and assessments

A complete medical history including other diagnoses, medications, and scoring of symptoms was performed at V1. Symptom scoring used the eosinophilic esophagitis activity

index (EEsAI), a validated seven-day recall tool of seven questions developed to quantify EoE symptoms.[E1] Patients with concomitant asthma had spirometry performed and completed the Asthma Control Questionnaire-7.[E2] Patients with concomitant allergic rhinitis completed the Rhinitis Control Assessment Test (RCAT).[E3] Updates to the medical history, medication changes, and symptom scores for EoE, asthma, and allergic rhinitis were recorded at V2. Allergy skin prick testing for aeroallergens and foods was performed at V1. A 55 ml blood draw was performed at V1 and V2. Ten ml was aliquoted into vacuum tubes containing CTAD (citrate, theophylline, adenosine, and dipyridamole) anticoagulant solution (BD Vacutainer Systems, Franklin Lakes, NJ), to minimize artifactual platelet activation,[E4-6] and were used for flow cytometry (see below).

Standard of care EoE therapy, specifically recommendations of either swallowed steroid or food elimination were offered. For the food elimination option, in patients with no positive food tests, the recommendation was a six-food elimination diet (milk, egg, peanut, wheat, soy, and shellfish). Patients with positive food tests were offered the option of six-food elimination or restricted elimination of foods to which they tested positive. Patients then received this standard of care treatment, appropriate to each patient, for eight weeks, followed by V2 (Table EI).

Endoscopies were performed at the UW Digestive Health Center on an outpatient basis and not during acute food impaction nor during acute respiratory infection or sinusitis. The validated Endoscopic Reference Score (EREFS) for endoscopic assessment of EoE was used to uniformly score the endoscopic signs of EoE for the endoscopy report.[E7] Biopsies of the esophageal epithelium were obtained using standard protocol: A 3 mm cup biopsy forceps was used to obtain four to six biopsies of the distal esophagus and a second sampling of four to six biopsies were performed of the proximal esophagus. Esophageal mucosal samples were assessed

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by the UW Pathology Department to determine PEC/HPF and provide assessment of the EoE Histological Scoring System (EoEHSS).[E8]

**Antibodies and flow cytometry**

Primary mAbs used in this study included clones used previously for flow cytometry on eosinophils in whole blood from patients with asthma (by us) or EoE (by others).[E4, 5, 9-14] The primary mAbs and isotype controls are listed in Table EII. Notably, for $\beta_1$ integrin activation, P-selectin (CD62P), and $\alpha_{IIb}$ integrin (CD41), the following clones were used: N29 (Millipore Sigma, Burlington, MA), AC1.2 (BD Biosciences, San Jose, CA), and HIP8 (BD). Phycoerythrin (PE)-conjugated anti-mouse and anti-rat immunoglobulin (Ig) G secondary antibodies, and fluorescein isothiocyanate (FITC)-conjugated anti-CD14 mAb clone M5E2 and anti-CD16 3G8[E6] were from BD.

Whole unfractionated CTAD blood from V1 and V2 was directly processed (100 μl per flow cytometry tube) for flow cytometry, and data were acquired at the UW Comprehensive Cancer Center Flow Cytometry Facility as previously described.[E4, 5, 10, 11] Data were collected, analyzed, and expressed as specific geometric channel fluorescence (gMCF).[E5, 6, 11] P-selectin (CD62P) and $\alpha_{IIb}$ integrin (CD41), which have been observed to have heterogeneous distributions, were also expressed as percentage positive cells.[E11] This is in contrast to other markers, such as N29, which have homogeneous distributions and for which expression level is a more representative way to present data, as previously discussed.[E10] FITC-labeled anti-CD14 and anti-CD16 were used to gate eosinophils based on scatter and lack of FITC staining, as done previously.[E4, 5, 10, 11] Mid-range one-peak rainbow fluorescent beads (Spherotech, Lake Forest, IL) were run at setup to set the sensitivity of the detectors in a standardized manner, thus

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maximizing reproducibility and optimizing data comparisons among subjects and visits. Compensation was performed with unlabeled, FITC-labeled, and PE-labeled Calibrite (BD) beads. Data analysis was performed using FlowJo software (Ashland, OR).

Data in previous asthma studies, and the initial phase and larger part of this study were acquired using a FACSCalibur cytometer (BD Biosciences, San Jose, CA) as before.[E4-6, 9-11] During the course of the current study, the Flow Cytometry Facility discontinued the Calibur cytometers and replaced them with Attune cytometers (ThermoFisher, Waltham, MA). In order to transition the study from the Calibur to an Attune cytometer, transform new Attune data into Calibur equivalents, and compare the Attune data to data generated with the Calibur, the following measures were taken, after consultation with Flow Facility staff. From a whole blood sample, 100 µl blood was analyzed in each tube using the primary mAb or isotype controls listed in Table EII, or buffer only, giving a total of 22 tubes (16 primary mAbs, 4 isotype controls, 2 tubes with buffer plus anti-mouse or anti-rat secondary antibody). These tubes were processed according to the standard flow cytometry protocol (see above). At the end of the protocol, cells in each tube were resuspended in 500 µl FACS buffer. This volume was divided in 2 x 250 µl aliquots. Data from one set of aliquots, i.e., 22 aliquots, were acquired in the Calibur as before. The other set of aliquots was diluted with 250 µl FACS buffer to a total volume of 500 µl. Data from these aliquots, i.e., also 22 aliquots, were acquired in the Attune. The same type of rainbow beads, to set the sensitivity of the detectors in a standardized manner, and the same type of Calibrite beads for compensation (see above) were used with the two instruments. With the Calibur data, eosinophils were gated as before (see above), i.e., first by a region within a side scatter-height (SSC-H) versus forward scatter (FSC)-H plot, then by gating this region in a SSC-H versus FITC-H (CD14/CD16) plot. With the Attune data, eosinophils were similarly first gated

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by SSC-H versus FSC-H, then for singlets by FSC-H versus FSC-area (A)[E6] and SSC-H versus SSC-A, and then, similarly as for the Calibur data, by SSC-H versus the BL1 (FITC)-H channel. After analysis of the data sets from the two instruments as obtained after rainbow-bead-guided detector settings, linear regression was performed to compare the Attune-generated to the Calibur-generated data. This yielded the equation $y = 0.07404x + 18.34$ with $r^2 = 0.9998$ ($n$ = 22), where y = Calibur geometric mean and x = Attune geometric mean fluorescent intensities. As the goodness of fit was excellent, this equation was used from then on to transform new Attune data to Calibur equivalents. Thus, the data presented in the manuscript are based on fluorescence intensities obtained on the Calibur cytometer or as would have been obtained on the Calibur cytometer.

**Immunohistochemistry**

Slides with formalin-fixed, paraffin-embedded esophageal tissue biopsy sections from an EoE patient were stained with hematoxylin and eosin as well as immunohistochemistry for platelets using mouse mAb clone 2f2 against $\beta_3$ integrin (CD61) (Cell Marque, Rocklin, CA), the $\beta$ subunit that heterodimerizes with the $\alpha_{IIb}$ integrin subunit (CD41) to form the platelet $\alpha_{IIb}\beta_3$ integrin (CD41/CD61), and the UltraView Universal 3,3'-diaminobenzidine (DAB) Detection Kit containing a horseradish peroxidase-conjugated anti-mouse secondary antibody, according to the manufacturer's instructions (Ventana Medical Systems, Tucson, AZ).

**Statistical analysis**

Study data were collected and managed using REDCap (Research Electronic Data Capture) tools, hosted at the UW-Madison Department of Medicine, for data downloads to common statistical packages.[E15] Mann-Whitney *U* test or Kruskal-Wallis rank sum comparison

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test was used to compare data between two groups of subjects. Wilcoxon matched-pairs signed-rank test was used to compare data between two visits by the same subjects. Spearman rank test was used to analyze correlations. A level of probability ($P$) $\leq 0.05$ was considered significant. Group data are reported as mean ± standard deviation (SD) if the variable was normally distributed or median with $25^{th}$ and $75^{th}$ percentiles if the variable was not normally distributed. Receiver operating characteristic (ROC) curve analysis was done to evaluate the ability of blood eosinophil-surface protein expression data to predict PEC. Analyses were performed using Prism (GraphPad, San Diego, CA), SAS (Cary, NC), or R software (Vienna, Austria).

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## TABLES

**TABLE EI.** Timeline and procedure chart

| | **Pre-recruitment** | **V1** | **V2** |
|---|---|---|---|
| Time | - ≤ 2 wks | 0 | 8 wks |
| Informed consent | | x | |
| Procedures: | | | |
| Medical history/symptoms | | x | x |
| Food allergy testing | | x | |
| Blood draw | | x | x |
| Endoscopy with biopsy | x | | x |

*V*, visit; *wk*, week.

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**TABLE EII.** Primary mAbs used for flow cytometry

| Antigen | Clone | Isotype | Company |
|---|---|---|---|
| $\beta_1$ integrin (CD29) | MAR4 | mouse $IgG_1$ | BD |
| Activated $\beta_1$ integrin | N29 | mouse $IgG_1$ | Millipore Sigma |
| $\beta_2$ integrin (CD18) | L130 | mouse $IgG_1$ | BD |
| $\alpha_L$ integrin (CD11a) | HI111 | mouse $IgG_1$ | BD |
| $\alpha_M$ integrin (CD11b) | LT11 | mouse $IgG_1$ | MyBioSource |
| $\alpha_X$ integrin (CD11c) | B-ly6 | mouse $IgG_1$ | BD |
| ICAM1 (CD54) | HCD54 | mouse $IgG_1$ | BioLegend |
| FcεRII (CD23) | M-L233 | mouse $IgG_1$ | BD |
| CD40 | 5C3 | mouse $IgG_1$ | BD |
| CD44 | BJ18 | mouse $IgG_1$ | BioLegend |
| PSGL-1 (CD162) | KPL-1 | mouse $IgG_1$ | BioLegend |
| $\alpha_{IIb}$ integrin (CD41) | HIP8 | mouse $IgG_1$ | BD |
| P-selectin (CD62P) | AC1.2 | mouse $IgG_1$ | BD |
| CCR3 (CD193) | 5E8 | mouse $IgG_{2b}$ | BioLegend |
| CEACAM8 (CD66b) | G10FS | mouse $Ig_M$ | BD |
| CRTH2 (CD294) | BM16 | rat $IgG_{2a}$ | BD |
| Isotype control | MOPC-21 | mouse $IgG_1$ | BD |
| Isotype control | 27-35 | mouse $IgG_{2b}$ | BD |
| Isotype control | G155-228 | mouse $Ig_M$ | BD |

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|---|---|---|---|
| Isotype control | R35-95 | rat $IgG_{2a}$ | BD |

*CD*, cluster of differentiation; *CCR*, C-C type chemokine receptor; *CEACAM*, carcinoembryonic antigen-related cell adhesion molecule; *CRTH2*, chemoattractant receptor-homologous molecule expressed on Th2 cells (or prostaglandin $D_2$ receptor 2); *Fc*, fragment, crystallizable (of immunoglobulin); *ICAM*, intercellular adhesion molecule; *Ig*, immunoglobulin; *mAb*, monoclonal antibody; *PSGL*, P-selectin glycoprotein ligand; *R*, receptor; *Th2*, T helper (cell) type 2.

For antigens, conventional names and CD numbers are given.

Companies: BD Biosciences, San Jose, CA; BioLegend, San Diego, CA; Millipore Sigma, Burlington, MA; MyBioSource, San Diego, CA.

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**TABLE EIII.** Correlations between potential blood eosinophil surface biomarkers and PEC at V2.

| | $r_s$ | *P* | $r_s$ | *P* | $r_s$ | *P* | $r_s$ | *P* |
|---|---|---|---|---|---|---|---|---|
| **Correlation adjusted for:** | **Not adjusted** | | **RCAT** | | **Allergy** | | **Treatment group** | |
| **Antigen** | | | | | **and asthma** | | **and steroid** | |
| $\beta_1$ integrin (CD29) | -0.32 | 0.12 | -0.27 | 0.24 | -0.38 | 0.07 | -0.31 | 0.15 |
| Activated $\beta_1$ integrin/N29 | -0.09 | 0.66 | -0.09 | 0.69 | -0.10 | 0.65 | -0.13 | 0.55 |
| $\beta_2$ integrin (CD18) | -0.21 | 0.31 | -0.20 | 0.38 | -0.15 | 0.50 | -0.24 | 0.26 |
| $\alpha_L$ integrin (CD11a) | -0.05 | 0.80 | 0.09 | 0.71 | 0.02 | 0.91 | -0.10 | 0.64 |
| $\alpha_M$ integrin (CD11b) | -0.08 | 0.69 | -0.05 | 0.83 | -0.04 | 0.86 | -0.11 | 0.63 |
| $\alpha_X$ integrin (CD11c) | 0.02 | 0.91 | 0.03 | 0.91 | -0.06 | 0.79 | -0.08 | 0.70 |
| ICAM1 (CD54) | 0.08 | 0.72 | 0.06 | 0.78 | 0.02 | 0.95 | 0.08 | 0.71 |
| FcεRII (CD23) | 0.34 | 0.10 | 0.28 | 0.22 | 0.28 | 0.19 | 0.28 | 0.19 |
| CD40 | -0.31 | 0.14 | -0.38 | 0.08 | -0.28 | 0.19 | -0.32 | 0.14 |
| CD44 | -0.34 | 0.10 | -0.22 | 0.34 | -0.30 | 0.16 | -0.32 | 0.13 |
| PSGL-1 (CD162) | -0.24 | 0.24 | -0.21 | 0.36 | -0.26 | 0.23 | -0.29 | 0.18 |
| $\alpha_{IIb}$ integrin (CD41): level | 0.39 | 0.05 | 0.42 | 0.06 | 0.47 | 0.02 | 0.35 | 0.10 |
| % positive cells | 0.60 | 0.002 | 0.56 | 0.009 | 0.55 | 0.006 | 0.61 | 0.002 |
| P-selectin (CD62P): level | 0.25 | 0.24 | 0.33 | 0.14 | 0.32 | 0.14 | 0.24 | 0.27 |
| % positive cells | 0.47 | 0.02 | 0.52 | 0.02 | 0.55 | 0.007 | 0.51 | 0.01 |
| CCR3 (CD193) | -0.29 | 0.15 | -0.25 | 0.27 | -0.31 | 0.15 | -0.28 | 0.20 |

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|---|---|---|---|---|---|---|---|---|
| CEACAM8 (CD66b) | -0.08 | 0.71 | -0.04 | 0.86 | -0.11 | 0.63 | -0.13 | 0.55 |
| CRTH2 (CD294) | 0.01 | 0.98 | 0.06 | 0.79 | 0.04 | 0.85 | -0.06 | 0.77 |

*P*, probability; *RCAT,* Rhinitis Control Assessment Test; $r_s$, Spearman rank correlation coefficient; *V*, visit.

For abbreviations of antigens, see Table EII legend.

Data for all antigens are based on expression level, except for $\alpha_{IIb}$ integrin (CD41) and P-selectin (CD62P) based on expression level or percentage positive cells.

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**TABLE EIV.** Expression of the potential blood eosinophil surface biomarkers and PEC at V2 in patients on or off steroid

| **Antigen or PEC** | **On steroid** (n = 17) **Median (quartiles)** | **Off steroid** (n = 8) **Median (quartiles)** | ***P*** |
|---|---|---|---|
| PEC (per HPF) | 4 (0, 47) | 28 (12, 30) | 0.42 |
| $\beta_1$ integrin (CD29) | 320 (180, 400) | 290 (240, 390) | 0.93 |
| Activated $\beta_1$ integrin/N29 | 140 (80, 180) | 130 (70, 190) | 0.95 |
| $\beta_2$ integrin (CD18) | 1020 (690, 1060) | 960 (770, 1140) | 1.00 |
| $\alpha_L$ integrin (CD11a) | 810 (730, 1010) | 770 (650, 1030) | 0.67 |
| $\alpha_M$ integrin (CD11b) | 1130 (900, 1230) | 1120 (830, 1170) | 0.59 |
| $\alpha_X$ integrin (CD11c) | 210 (120, 300) | 320 (250, 380) | 0.11 |
| ICAM1 (CD54) | 30 (10, 40) | 30 (10, 60) | 0.77 |
| FcεRII (CD23) | 0 (0, 10) | 5 (0, 40) | 0.47 |
| CD40 | 0 (0, 20) | 6 (0, 30) | 0.45 |
| CD44 | 350 (240, 480) | 330 (190, 430) | 0.67 |
| PSGL-1 (CD162) | 1440 (1060, 1560) | 1370 (1330, 1470) | 0.84 |
| $\alpha_{IIb}$ integrin (CD41): level | 230 (170, 420) | 460 (150, 670) | 0.32 |
| % positive cells | 24.2 (15.7, 33.9) | 38.8 (23.6, 56.7) | 0.12 |
| P-selectin (CD62P): level | 80 (20, 90) | 70 (50, 130) | 0.55 |
| % positive cells | 3.5 (2.5, 5.5) | 2.8 (2.0, 5.7) | 0.73 |
| CCR3 (CD193) | 1560 (1370, 1770) | 1600 (1410, 1680) | 0.59 |

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|---|---|---|---|
| CEACAM8 (CD66b) | 680 (450, 750) | 710 (630, 800) | 0.38 |
| CRTH2 (CD294) | 860 (730, 1040) | 820 (710, 940) | 0.55 |

*HPF,* high power field*; P*, probability; *PEC,* peak eosinophil count; *V,* visit.

For abbreviations of antigens, see Table EII legend.

Data for all antigens are based on expression level as specific geometric mean channel fluorescence, except for $\alpha_{IIb}$ integrin (CD41) and P-selectin (CD62P) based on expression level or percentage positive cells.

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**TABLE EV.** Correlations between blood eosinophil $\beta_1$ integrin activation (mAb N29), P-selectin (CD62P)- or $\alpha_{IIb}$ integrin (CD41)-positive blood eosinophils and disease activity scores at V2.

| **Antigen** | **Score** | | | | | |
|---|---|---|---|---|---|---|
| | **EoEHSS** | | **EEsAI** | | **EREFS** | |
| | $\mathbf{r_s}$ | ***P*** | $\mathbf{r_s}$ | ***P*** | $\mathbf{r_s}$ | ***P*** |
| Activated $\beta_1$ integrin/N29: | | | | | | |
| expression level | -0.21 | 0.36 | 0.12 | 0.60 | -0.36 | 0.10 |
| $\alpha_{IIb}$ integrin (CD41): | | | | | | |
| % positive cells | 0.23 | 0.31 | 0.20 | 0.36 | 0.09 | 0.70 |
| P-selectin (CD62P): | | | | | | |
| % positive cells | 0.23 | 0.31 | -0.35 | 0.11 | -0.17 | 0.46 |

*EEsAI*, Eosinophilic Esophagitis Activity Index; *EoEHSS*, EoE Histological Scoring System; *EREFS*, Endoscopic Reference Score; *P*, probability; $r_s$, Spearman rank correlation coefficient; *V*, visit.

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**TABLE EVI.** Correlations between blood eosinophil $\alpha_{IIb}$ integrin (CD41)-positive blood eosinophils and EoEHSS subscores at V2.

| **Subscore** | $\mathbf{r_s}$ | ***P*** |
|---|---|---|
| EI (eosinophil inflammation) | 0.43 | 0.03 |
| BZH (basal zone hyperplasia) | 0.26 | 0.20 |
| EA (eosinophil abscess) | 0.26 | 0.20 |
| SL (eosinophil surface layering) | 0.18 | 0.40 |
| DIS (dilated intracellular space) | 0.16 | 0.46 |
| SEA (surface epithelial alteration) | 0.14 | 0.49 |
| DEC (dyskeratotic epithelial cells) | 0.03 | 0.87 |
| LPF (lamina propria fibrosis) | 0.40 | 0.05 (0.046) |

*EoEHSS,* EoE Histological Scoring System; *P*, probability; $r_s$, Spearman rank correlation coefficient; *V,* visit.

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**TABLE EVII.** Area under curve (AUC) values for the potential blood eosinophil surface biomarkers to predict PEC ≤ 6 or 15/HPF at V2.

| **Antigen** | **AUC** PEC ≤ 6/HPF | **AUC** PEC ≤ 15/HPF |
|---|---|---|
| $\beta_1$ integrin (CD29) | 0.60 | 0.62 |
| Activated $\beta_1$ integrin/N29 | 0.52 | 0.54 |
| $\beta_2$ integrin (CD18) | 0.47 | 0.59 |
| $\alpha_L$ integrin (CD11a) | 0.53 | 0.52 |
| $\alpha_M$ integrin (CD11b) | 0.53 | 0.47 |
| $\alpha_X$ integrin (CD11c) | 0.60 | 0.53 |
| ICAM1 (CD54) | 0.53 | 0.49 |
| FcεRII (CD23) | 0.70 | 0.62 |
| CD40 | 0.55 | 0.64 |
| CD44 | 0.66 | 0.62 |
| PSGL-1 (CD162) | 0.56 | 0.59 |
| $\alpha_{IIb}$ integrin (CD41): level | 0.68 | 0.73 |
| % positive cells | 0.84 | 0.79 |
| P-selectin (CD62P): level | 0.60 | 0.62 |
| % positive cells | 0.66 | 0.72 |
| CCR3 (CD193) | 0.60 | 0.61 |
| CEACAM8 (CD66b) | 0.50 | 0.51 |

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| | | |
|---|---|---|
| CRTH2 (CD294) | 0.58 | 0.57 |

*AUC*, area under curve (by receiver operating characteristic curve analysis); *HPF*, high power field; *PEC*, peak eosinophil count; *V*, visit.

For abbreviations of antigens, see Table EII legend.

Data for all antigens are based on expression level, except for $\alpha_{IIb}$ integrin (CD41) and P-selectin (CD62P) based on expression level or percentage positive cells.

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**FIGURE LEGENDS**

**FIG E1.** Changes in PEC and scores from V1 to V2. Changes in PEC/HPF (**A**), histological score (**B**), symptom score (**C**), and endoscopic score (**D**). *EEsAI,* Eosinophilic Esophagitis Activity Index; *EoEHSS,* EoE Histological Scoring System; *EREFS,* Endoscopic Reference Score; *HPF,* high power field; *PEC,* peak eosinophil count. Dashed and dotted lines in **A** represent PEC = 6 and 15/HPF, respectively.

**FIG E2.** Correlations among PEC and scores at V1 or V2. Plots with visualization of correlation between each pair of variables, i.e, a total of six correlations at each visit. Stronger correlations are represented by darker color. Numbers represent $r_s$ (Spearman rank correlation coefficient) values. For example, the correlation between EoEHSS and PEC at V1 has $r_s = 0.81$ and is represented by a dark blue box. *EEsAI,* Eosinophilic Esophagitis Activity Index; *EoEHSS,* EoE Histological Scoring System; *EREFS,* Endoscopic Reference Score; *PEC,* peak eosinophil count.

**FIG E3.** Examples of flow cytometry histograms for blood eosinophil activated $\beta_1$ integrin (mAb N29) and surface $\alpha_{IIb}$ integrin (CD41) at V2. Low (**A**) or high (**B**) N29 reactivity patient (subject No. 1 and 10, respectively); and $\alpha_{IIb}$ integrin-low (**C**), with 0.1% positive blood eosinophils and PEC = 0/HPF, or high (**D**) patient, with 69.4% positive cells and PEC = 85/HPF (subject No. 8 and 13). Red = specific mAb, blue = isotype control. Note in **B** that the entire N29 distribution is to the right of the isotype and is monophasic or homogeneous. Thus, this distribution compared to the control is best described with a value based on fluorescent intensity. Note in **D** that the CD41 distribution is biphasic or heterogeneous, with one peak or

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subpopulation overlapping with the isotype and the second one to the right. Thus, this distribution is best described as percentage CD41-positive cells.

**FIG E4.** Correlations among blood eosinophil activated $\beta_1$ integrin (mAb N29), blood eosinophil surface P-selectin (CD62P) and $\alpha_{IIb}$ integrin (CD41) at V1 or V2. Correlation plots as in Fig E2. *MFI*, based on specific geometric mean fluorescence intensity as geometric mean channel fluorescence; *pp*, as percentage positive cells.

**FIG E5.** Relationships between blood eosinophil activated $\beta_1$ integrin (mAb N29), P-selectin (CD62P)- or $\alpha_{IIb}$ integrin (CD41)-positive blood eosinophils and PEC at V2. N29 reactivity (**A**); eosinophil-surface P-selectin (CD62P), as percentage positive cells (**B**); or eosinophil-surface $\alpha_{IIb}$ integrin (CD41), as percentage positive cells (**C**), versus PEC/HPF. *gMCF*, Geometric mean channel fluorescence; *P*, probability; $r_s$, Spearman rank correlation coefficient; line, linear regression.

**FIG E6.** $\alpha_{IIb}$ integrin (CD41) in patients with PEC < or > 6 or 15/HPF. Patients were divided according to PEC < or > 6 (**A**) or 15 (**B**) /HPF. Bar, median in each group. Dotted line indicates the cutoff of < 22.9% (**A**) or 26.7% (**B**) $\alpha_{IIb}$ integrin-positive blood eosinophils, respectively, to predict PEC < 6/HPF (**A**, see Fig 1) or < 15/HPF (**B**), the latter with AUC (area under curve) = 0.79 ($P < 0.01$), specificity = 82%, and sensitivity = 71%.

**FIG E7.** EoE esophageal tissue biopsy stained with hematoxylin and eosin (**A**) and immunohistochemistry staining for platelets using anti-$\beta_3$ integrin (CD61) (**B**). Please note group

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of intravascular, vessel-, or vessel wall-associated eosinophils (arrow in **A**) and group of platelets in the same area (arrow in **B**), which appear to interact or be associated with the eosinophils. Also note that biopsies from normal subjects usually have no or only an occasional vascular eosinophil (not shown). Arrowhead in **A**, eosinophil in tissue apparently not associated with platelets. These observations are compatible with a scenario in which, in EoE, eosinophils with associated platelets accumulate in vessels in esophageal tissue, extravasate, and at some point during or after extravasation the platelets dissociate from the eosinophils. Esophageal lumen at bottom. Bar, 20 μm. This patient had PEC = 25/HPF.

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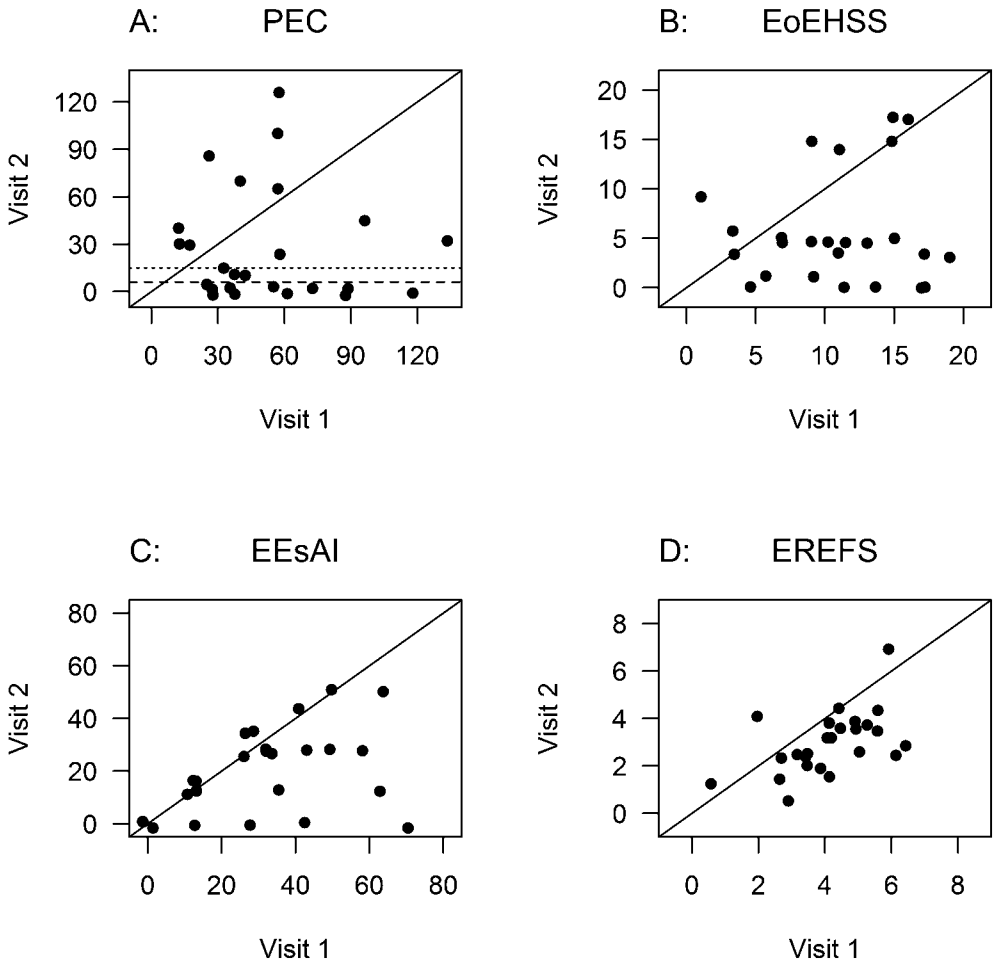
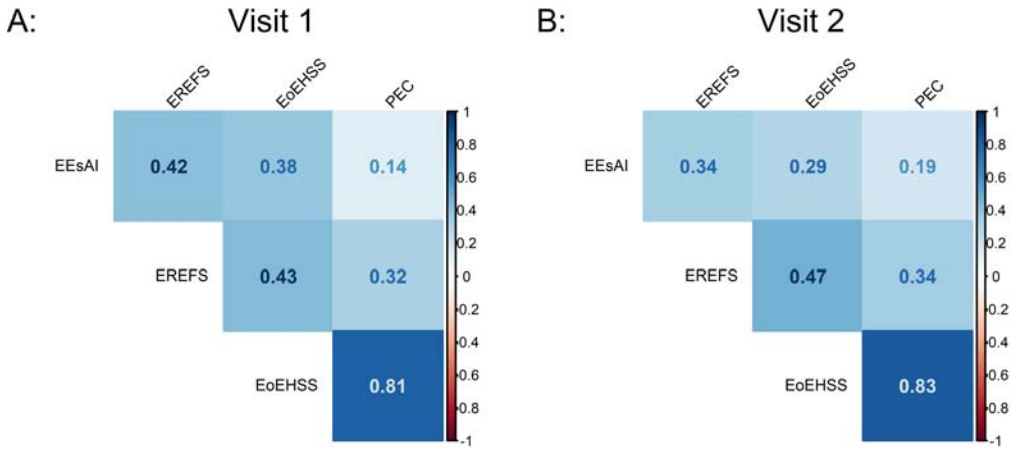

A: PEC
Visit 2
Visit 1
0
30
60
90
120
B: EoEHSS
Visit 2
Visit 1
0
5
10
15
20
C: EEsAI
Visit 2
Visit 1
0
20
40
60
80
D: EREFS
Visit 2
Visit 1
0
2
4
6
8

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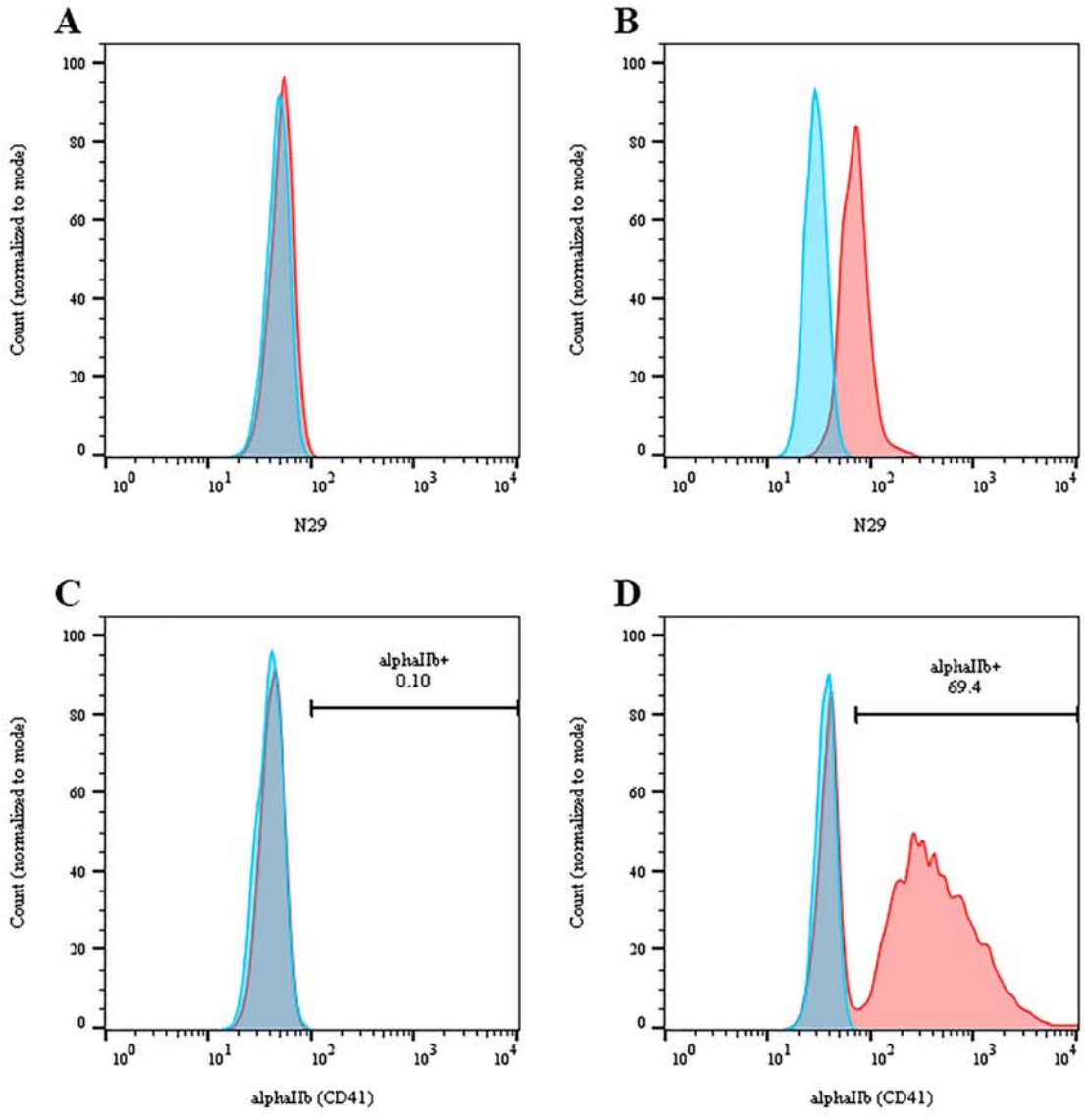
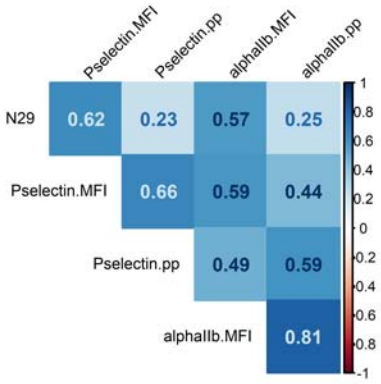
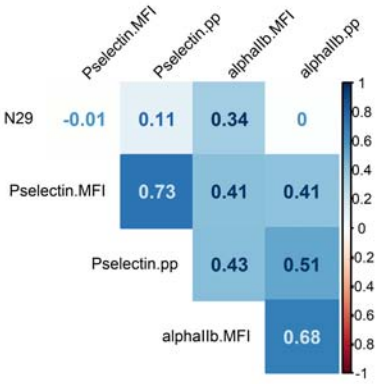

A
B
C
D
Count (normalized to mode)
N29
alphaIIb (CD41)
alphaIIb+
0.10
alphaIIb+
69.4

A: Visit 1




B: Visit 2

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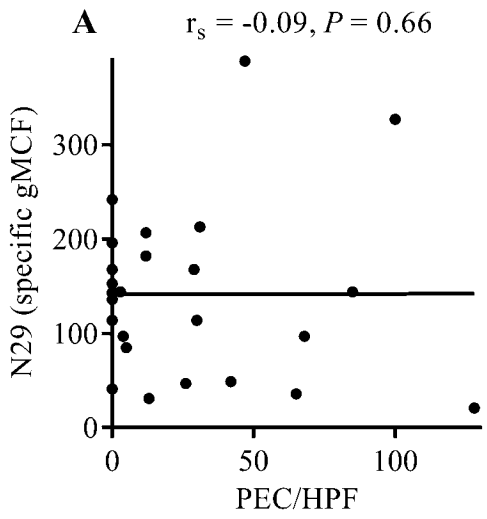
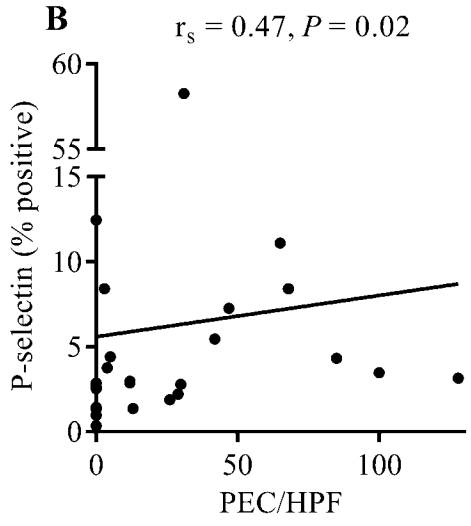
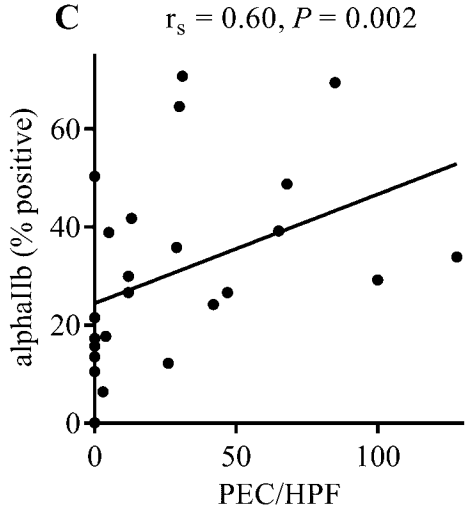
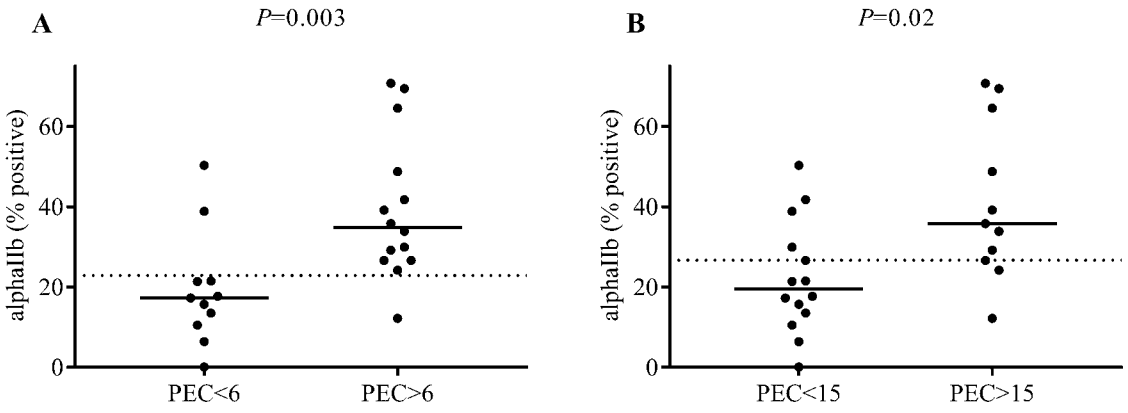

A
$r_s$ = -0.09, $P$ = 0.66
N29 (specific gMCF)
300
200
100
0
0
50
100
PEC/HPF


B
$r_s$ = 0.47, $P$ = 0.02
P-selectin (% positive)
60
55
15
10
5
0
0
50
100
PEC/HPF


C
$r_s$ = 0.60, $P$ = 0.002
alphaIIb (% positive)
60
40
20
0
0
50
100
PEC/HPF

e-proof

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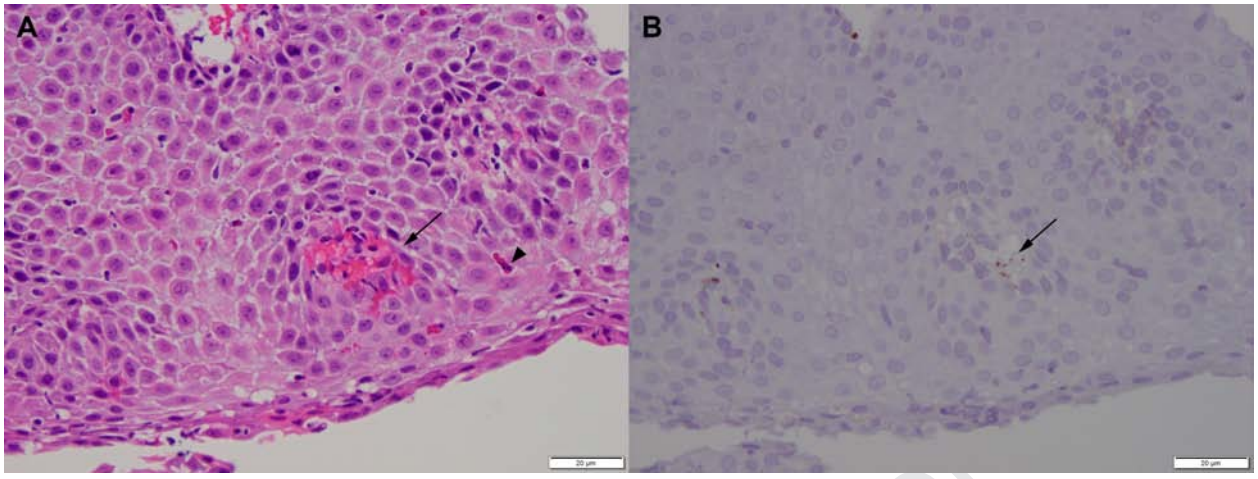


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