Peripheral Eosinophilia in Patients With Inflammatory Bowel Disease Defines an Aggressive Disease Phenotype

Benjamin Click, MD[1], Alyce M. Anderson, PhD[1], Ioannis E. Koutroubakis, MD, PhD[1], Claudia Ramos Rivers, MD[1], Dmitriy Babichenko, MS[2], Jorge D. Machicado, MD[1], Douglas J. Hartman, MD[3], Jana G. Hashash, MD[1], Michael A. Dunn, MD[1], Marc Schwartz, MD[1], Jason Swoger, MD[1], Arthur Barrie III, MD, PhD[1], Sally E. Wenzel, MD[4], Miguel Regueiro, MD[1] and David G. Binion, MD[1]

OBJECTIVES: **Peripheral blood eosinophilia (PBE) in inflammatory bowel disease (IBD) is associated with ulcerative colitis (UC) and active disease. Little data exist on the long-term impact of PBE on disease course. We aimed to investigate the multi-year patterns of PBE and its impact on disease severity in a large IBD cohort.**

METHODS: **We performed a registry analysis of a consented, prospective, natural history IBD cohort at a tertiary center from 2009 to 2014. Demographics, comorbidities, disease activity, healthcare utilization, and time to hospitalization or surgical resection of patients who displayed PBE were compared to patients without PBE.**

RESULTS: **Of the 2,066 IBD patients, 19.2% developed PBE. PBE was significantly associated with UC ($P<0.001$), extensive colitis ($P<0.001$), and shorter disease duration ($P=0.03$). Over six years, PBE patients had more active disease (Harvey–Bradshaw Index $P=0.001$; ulcerative colitis activity index $P<0.001$), concurrent C-reactive protein elevation ($P<0.001$), healthcare utilization (hospitalization $P<0.001$, IBD surgery $P<0.001$), and more aggressive medical therapy (prednisone $P<0.001$, anti-TNF $P<0.001$). Patients with PBE had a significantly reduced time to hospitalization in both UC ($P<0.001$) and Crohn's disease (CD) ($P<0.001$) and reduced time to colectomy in UC ($P=0.003$). On multivariable modeling, PBE remained significantly associated with hospitalization and surgery in both CD and UC. New diagnosis of UC with PBE was associated with increased steroid ($P=0.007$) and anti-TNF ($P=0.001$) requirement.**

CONCLUSION: **This multi-year study of a large IBD cohort suggests that peripheral blood eosinophilia represents a biomarker of a distinct IBD subgroup, with a unique inflammatory signature, and at risk for worse clinical outcomes.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

Am J Gastroenterol advance online publication, 7 November 2017; doi:10.1038/ajg.2017.402

INTRODUCTION

Eosinophils are bone-marrow-derived granulocytic leukocytes that play a vital role in mucosal innate immunity, affected through a variety of cellular mechanisms. Eosinophils modulate immune system function via proinflammatory mediator prostaglandin, leukotrienes, and cytokines as well as antigen-presenting function. Inflammatory bowel disease (IBD) is a chronic, lifelong, inflammatory disorder of the gastrointestinal tract mediated by a variety of immune cells including neutrophils, plasma cells, and eosinophils. Associations between IBD and increased levels of circulating and mucosal eosinophils date back to the 1960s (1,2). However, the exact function of eosinophils in IBD is still debated,

[1]Division of Gastroenterology, Hepatology and Nutrition, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA; [2]School of Information Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [3]Department of Anatomic Pathology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA; [4]Asthma Institute, Division of Pulmonary, Allergy, and Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA.
Correspondence: David G. Binion, MD, Professor of Medicine, Division of Gastroenterology, Hepatology and Nutrition, University of Pittsburgh School of Medicine, 200 Lothrop Street Mezzanine Level C Wing, Pittsburgh, Pennsylvania 15213, USA. E-mail: binion@pitt.edu
Presented in part at Digestive Disease Week 2015, Washington, D.C.
Received 16 December 2015; accepted 20 September 2017

but may include inflammatory, regulatory, and/or tissue repair function.

In IBD, prior studies demonstrated that peripheral blood eosinophilia (PBE) was mainly associated with ulcerative colitis (UC), showing significant correlations with disease activity and the development of primary sclerosing cholangitis (PSC) (3,4). In a study of newly diagnosed pediatric IBD patients, Sadi *et al.* described a 40.4% incidence of PBE, and PBE was associated with disease activity indices and colonic eosinophilia on biopsy (5). Multi-year period prevalence and clinical impact of PBE in IBD patients is unknown. Thus, we aimed to characterize the nature and clinical significance of PBE in a large, adult IBD patient population. We hypothesized that a minority of patients would develop PBE and these patients would have more severe disease and worse long-term clinical outcomes compared to patients without this laboratory finding.

METHODS

Study population

The study population consisted of patients with IBD (Crohn's disease (CD), UC diagnosed using established criteria) who enrolled in a consented, prospective, natural history IBD registry maintained at UPMC from 2009 to 2014 (6). Patients who enrolled after 2009 but were seen previously in UPMC had their complete demographic, clinical, disease activity, and laboratory data set back-filled to 2009. Patients enrolled in the registry and who had a measurement of eosinophil count at any point during the years 2009–2014 were considered for inclusion. Patients were excluded if there was no eosinophil measurement during the study period, a diagnosis of IBD unclassified, hypereosinophilic syndrome, primary eosinophilic disorder of the gastrointestinal tract (e.g., eosinophilic esophagitis) or other organs, or demonstrated postoperative (≤14 days) PBE due to this relatively common postanesthetic laboratory finding.

PBE was defined as absolute eosinophil count greater than or equal to 0.4×10^9/l, (the upper limit of normal as defined by UPMC clinical laboratory) or an electronically flagged high value in the electronic medical record (if another reference range or units of measurement were used) at any point during the study period. Eosinophilia was categorized as mild ($0.41–1.5\times10^9$/l or 1–3 times upper limit normal (ULN)), moderate ($1.5–5\times10^9$/l or 3–10× ULN), severe ($>5\times10^9$/l or >10× ULN) (7,8), or unknown if the value was flagged as elevated, but no units or reference range were provided. Repeated complete blood counts were not systematically planned at defined intervals, but were obtained as clinically indicated during the course of routine care. Annual patterns of PBE were created by dichotomizing each year as any PBE or no PBE if a blood count with differential was obtained.

Clinical data collection

Baseline patient demographic data, comorbid conditions known to be associated with PBE (asthma, allergic rhinitis, adrenal insufficiency, PSC), and rheumatologic diseases (rheumatoid arthritis, Sjogren's syndrome, systemic lupus erythematosus, dermatomyositis, systemic sclerosis, and connective tissue disorder), as well as general allergy and medication allergy data was collected using data captured by ICD-9 code and/or electronic medical record problem list. Baseline disease characteristics including disease type (CD, UC), duration of disease, disease location and behavior according to Montreal Classification (9) at initial endoscopic or radiographic encounter, and history of IBD-related surgery prior to 2009 were also recorded.

Disease activity was evaluated using biochemical inflammatory markers (high sensitivity C-reactive protein (hsCRP) and erythrocyte sedimentation rate (ESR)) and disease activity indices (Harvey–Bradshaw Index (HBI) for CD (10) and ulcerative colitis activity index (UCAI) for UC (11)) which were collected prospectively at each clinical encounter. Annual dichotomous patterns (elevated vs. normal) of biochemical inflammatory markers (hsCRP: ≥0.74 mg/dl; ESR: >20 mm/h per UPMC laboratory standards) as well as annual mean values for disease activity indices were created.

Healthcare utilization measures included emergency department use, IBD-related hospital admissions and IBD surgery (as verified by manual review of discharge summaries and operative reports), radiographic studies, endoscopies, and financial charges. Financial data included all healthcare charges for both inpatient and outpatient sources within the UPMC system (20 hospitals and >500 clinic sites), excluding pharmacy charges. Financial charges were inflated to base year 2014 using the Consumer Price Index (http://www.bls.gov/data/inflation_calculator.htm). IBD medication exposure was determined for immunomodulator agents (azathioprine, methotrexate, 6-mercaptopurine), prednisone (no other systemic glucocorticoids to avoid overlap with adrenal insufficiency treatment), enteric steroids (oral budesonide and per rectum corticosteroid preparations), anti-TNF therapy (infliximab, adalimumab, certolizumab pegol), and 5-ASA compounds (sulfasalazine, mesalamine, balsalazide, either orally or topically). Health-related quality of life was assessed using the published version of short IBD questionnaire (SIBDQ) (12,13) which was also prospectively collected at each visit.

Patients were followed through 2014 or last known clinical contact, which was determined by date of last telephone encounter or outpatient IBD clinic visit.

Subgroup analysis

To examine the impact of PBE in patients with recently diagnosed IBD, we performed a subgroup analysis of patients with a new IBD diagnosis within the study period (2009–2014) and a CBC with differential within the subsequent 12 months. We compared disease activity, IBD medication requirement, healthcare utilization, and total financial charges by presence of PBE within 1 year of IBD diagnosis.

Statistical analysis

Descriptive measures of centrality were calculated using median and interquartile range (IQR) for continuous variables. Kruskal–Wallis and Wilcoxon rank-sum tests were utilized to determine significant association for continuous parameters. Categorical variables were presented as proportions. Pearson χ^2 or Fisher's

exact tests were used to assess association between categorical variables. Spearman rank-order correlation was used for correlation analysis of both absolute eosinophil counts as well as number of years (range 0–6) with PBE with rates of healthcare utilization and disease activity metrics.

Logistic regression modeling was performed for outcomes of IBD-related hospitalization or surgical resection. Covariates tested included PBE, hsCRP elevation, baseline demographics, disease characteristics, prior IBD surgery, IBD medications, comorbid anxiety and/or depression, asthma, allergies, smoking status, prescription opiate use, adrenal insufficiency, and rheumatologic conditions. Univariate modeling was performed and covariates with univariate significance of $P \leq 0.10$ were included in the multivariate model. PBE, hsCRP, and asthma were included in the final model regardless of univariate significance to control for these important variables. Patients with PSC were excluded from regression modeling.

Time-to-event analysis was performed from the date of laboratory testing (date of PBE in the case of PBE patients and date of initial complete blood cell count in non-PBE patients) using end points of IBD-related hospitalization or surgical resection. If a patient developed PBE after normal laboratory evaluations, the date of PBE was used in time-to-event analysis.

All tests were 2-sided, with statistical significance considered at level $P \leq 0.05$, unless otherwise stated. Data were analyzed using Stata Statistical Software (Version 13; StataCorp LP, College Station, TX).

Ethical considerations

Enrollment in and use of the research registry (Protocol # 0309054) as well as the current study (Protocol # 15050080) was approved by University of Pittsburgh Institutional Review Board.

RESULTS

Study cohort

As of 31 December 2014 there were 2,564 patients enrolled in the IBD registry. There were 23,889 blood count laboratory evaluations in 2,066 distinct patients. Of the blood counts, 5.2% (n=1,243) demonstrated elevated eosinophil levels (1,164 mild eosinophilia, 48 moderate, 3 severe) in 396 (19.2%) distinct patients, defining the PBE group. Of the PBE patients, 106 (26.7%) had eosinophilia at the first visit during the 6-year period while 73.2% of PBE was detected during follow up.

PBE was significantly associated with younger age (P=0.02), comorbid asthma (P<0.0001), rheumatologic disease (P=0.045), adrenal insufficiency (P=0.02), and PSC (P<0.0001) (**Table 1**). Significantly more PBE patients had allergies (P<0.0001) and specifically medication allergies (P<0.0001).

PBE was significantly associated with UC (P<0.0001) and shorter disease duration (P=0.03) (**Table 1**). There was no significant difference between ileal and colonic CD (P=0.22) or complicated and non-complicated CD (P=0.14). UC patients with PBE had significantly more extensive disease (P<0.0001) compared to distal.

Table 1. Study cohort demographics and disease characteristics by the presence of PBE (n=2066)

	PBE (n=396)	No PBE (n=1670)	P-value
%	19.2	80.8	—
Demographics			
Female, %	193 (48.7)	862 (51.6)	0.26
Age, median (IQR)	42 (24)	44 (24)	0.02
BMI, median (IQR)	25.4 (7.1)	25.5 (6.9)	0.34
Smoking, %			0.35
Never	287 (72.5)	1137 (68.1)	
Former	71 (17.9)	348 (20.8)	
Current	26 (6.6)	105 (6.3)	
Comorbidity, %			
Anxiety/depression	49 (12.4)	152 (9.1)	0.06
Hypertension	38 (9.6)	114 (6.8)	0.07
Hyperlipidemia	19 (4.8)	80 (4.8)	1.00
Diabetes mellitus	17 (4.3)	35 (2.1)	0.02
Asthma	22 (5.6)	24 (1.4)	<0.0001
Allergic rhinitis	13 (3.3)	44 (2.6)	0.50
Rheumatologic disease[a]	11 (2.8)	22 (1.3)	0.045
Adrenal insufficiency	5 (1.3)	4 (0.2)	0.02
PSC	32 (8.1)	55 (3.3)	<0.0001
Any allergy	303 (76.5)	1108 (66.3)	<0.0001
Medication allergies	267 (67.4)	925 (55.4)	<0.0001
IBD characteristics			
Disease, %			<0.0001
CD (n=1148)	171 (48.4)	977 (64.3)	
UC (n=725)	182 (51.6)	543 (35.7)	
CD Location,[b] %			0.03
Ileal (L1)	45 (26.3)	142 (17.3)	0.30
Colonic (L2)	40 (23.4)	175 (21.3)	1.00
Ileocolonic (L3)	80 (46.8)	462 (56.3)	0.003
Upper GI (L4)	6 (3.5)	41 (5.0)	0.35
CD Behavior,[c] %			0.14
Inflammatory (B1)	76 (49.0)	384 (42.6)	0.049
Stricturing (B2)	39 (25.2)	297 (32.9)	<0.0001
Penetrating (B3)	40 (25.8)	221 (24.5)	0.12
Perianal disease, (%)	33 (19.3)	173 (19.2)	0.30
UC Extent,[d] %			0.09
Proctitis (E1)	8 (4.7)	34 (7.2)	0.85
Left-Sided (E2)	50 (29.1)	171 (36.1)	0.17
Extensive (E3)	114 (66.3)	269 (56.8)	<0.0001
Duration (years, median (range))	14 (11)	15 (13)	0.03
History of IBD-surgery, %	109 (27.5)	495 (29.6)	0.61

BMI, body mass index; CD, Crohn's disease; IQR, interquartile range; PBE, peripheral blood eosinophilia; PSC, primary sclerosing cholangitis; UC, ulcerative colitis.

[a]Rheumatoid arthritis, Sjogren's, systemic lupus erythematosis, systemic sclerosis, polyangiitis with granulomatosis, connective tissue disorder not otherwise specified.

[b]Location data missing in 157 patients.

[c]Behavior data missing in 91 patients.

[d]Extent data missing in 79 patients.

Disease type (UC or CD) did not impact degree of PBE (P=0.25) or recurrence of PBE (P=0.41) (**Supplementary Table 1**).

Disease activity and healthcare utilization

Over the 6-year period for both UC and CD, PBE patients had significantly higher rates of elevated hsCRP (P<0.0001), more active disease on both UCAI (P<0.0001) and HBI (P=0.0001), emergency department use (P<0.0001), hospitalization (P<0.0001), and IBD-related surgery (P<0.0001) compared to patients without PBE (**Table 2**). PBE patients had significantly greater rates of prednisone use (P<0.0001) anti-TNF agents (P<0.0001), incurred higher median cumulative charges ($94,470 vs. $21,675, P<0.001) compared to patients without PBE in both UC and CD. The analysis was repeated after removal of patients with PSC and all significant parameters remained (**Supplementary Table 2**).

On multivariate modeling controlling for significant univariate variables (**Supplementary Table 3**) as well as elevated hsCRP and asthma, PBE remained significantly associated with IBD-related hospitalization in both CD (adjusted odds ratio (AOR) 1.60; 95% CI 1.07–2.39) and UC (AOR 2.35; 95% CI 1.48–3.74) (**Table 3**).

Table 2. Six-year rates of healthcare utilization, disease activity, and IBD medication exposure by presence of PBE

	PBE		No PBE				
	CD (n=171)	UC (n=182)	CD (n=977)	UC (n=543)	PBE vs. No PBE P-value	CD P-value	UC P-value
Healthcare utilization							
ED, %	108 (63.2)	112 (61.5)	461 (47.2)	237 (43.6)	<0.0001	<0.0001	<0.0001
Hospitalization, %	120 (70.2)	109 (59.9)	459 (47.0)	190 (35.0)	<0.0001	<0.0001	<0.0001
IBD Surgery, %	88 (51.5)	43 (23.6)	308 (31.5)	67 (12.3)	<0.0001	<0.0001	0.01
Radiology, %							
CT	105 (61.4)	105 (57.7)	554 (56.7)	303 (55.8)	0.47	0.27	0.67
MR	35 (20.5)	31 (17.0)	161 (16.5)	95 (17.5)	0.38	0.23	1.00
SBFT	21 (12.3)	13 (7.1)	87 (8.9)	32 (5.9)	0.26	0.16	0.59
Endoscopy, %[a]	151 (88.3)	156 (85.7)	842 (86.2)	473 (87.1)	0.7	0.54	0.62
Disease activity							
SIBDQ, median (IQR)	50.5 (17.4)	51.6 (14.7)	52.8 (17.0)	57.0 (13.7)	0.0001	0.01	<0.0001
HBI, median (IQR)	4.5 (5.7)	—	3.0 (4.7)	—	0.0001	0.0002	—
UCAI, median (IQR)	—	3.7 (4.3)	—	2.1 (4.4)	<0.00001	—	<0.0001
hsCRP elevation, %	114 (66.7)	117 (64.3)	432 (44.2)	194 (35.7)	<0.0001	<0.0001	<0.0001
ESR elevation, %	90 (52.6)	94 (51.6)	316 (32.3)	159 (29.3)	<0.0001	<0.0001	<0.0001
IBD medications							
Prednisone, %	109 (63.7)	137 (75.3)	450 (46.1)	277 (51.0)	<0.0001	<0.0001	<0.0001
Biologic, %							
anti-TNF	112 (65.5)	63 (34.6)	466 (47.7)	108 (19.9)	<0.0001	<0.0001	<0.0001
Other[b]	4 (2.3)	1 (0.5)	5 (0.5)	2 (0.4)	0.34	0.03	0.58
IM, %	97 (56.7)	93 (51.1)	590 (60.4)	202 (37.2)	0.32	0.40	0.001
5-ASA, %	56 (32.7)	120 (65.9)	309 (31.6)	326 (60.0)	0.004	0.79	0.16
Enteric steroids[c], %	44 (25.7)	16 (8.8)	179 (18.3)	42 (7.7)	0.36	0.03	0.64
Financial charges							
Total charges (USD), median (IQR)	$120,904 (307,140)	$72,630 (240,171)	$30,952 (133,370)	$15,728 (54,224)	<0.0001	<0.0001	<0.0001

CD, Crohn's disease; CT, computed tomography; ED, emergency department; ESR, erythrocyte sedimentation rate; HBI, Harvey–Bradshaw Index; hsCRP, high sensitivity C-reactive protein; IBD, inflammatory bowel disease; IM, immunomodulator; MRI, magnetic resonance imaging; PBE, peripheral blood eosinophilia; SBFT, small bowel follow through; SIBDQ, short inflammatory bowel disease questionnaire; UC, ulcerative colitis; UCAI, ulcerative colitis activity index; TNF, tumor necrosis factor; 5-ASA, 5-aminosalicylate.

[a]Colonoscopy, enteroscopy, esophagogastroduodenoscopy, pouchoscopy.

[b]Natalizumab, vedolizumab, golimumab, rituximab.

[c]Oral budesonide, per rectum corticosteroid preparations.

Table 3. Multivariate logistic regression of hospitalization and IBD surgery for patients with CD (*n*=1,111) and UC (*n*=677) after exclusion of patients with primary sclerosing cholangitis

Hospitalization				
	Crohn's Disease		**Ulcerative Colitis**	
	AOR (95% CI)	***P*-value**	**AOR (95% CI)**	***P*-value**
PBE	1.60 (1.07–2.39)	0.02	2.35 (1.48–3.74)	<0.0001
Elevated hsCRP	2.39 (1.77–3.23)	<0.0001	1.49 (0.97–2.31)	0.07
Age	—	—	1.01 (1.00–1.02)	0.12
Anxiety and/or Depression	0.82 (0.51–1.32)	0.42	1.18 (0.58–2.37)	0.65
Asthma	0.71 (0.27–1.92)	0.50	0.86 (0.17–2.22)	0.46
Allergies	1.13 (0.66–1.93)	0.65	1.23 (0.38–1.49)	0.57
Medication Allergies	0.85 (0.52–1.40)	0.52	0.75 (0.38–1.49)	0.41
Smoking Status	1.15 (0.92–1.44)	0.22	1.18 (0.78–1.78)	0.43
IBD Surgery prior to 2009	1.39 (1.03–1.87)	0.03	1.89 (1.08–3.33)	0.03
Behavior	1.14 (0.95–1.37)	0.16	—	—
Extent	—	—	1.21 (0.85–1.73)	0.29
anti-TNF	0.99 (0.74–1.34)	0.97	1.11 (0.69–1.81)	0.64
Prednisone	2.05 (1.51–2.79)	<0.0001	1.27 (0.80–2.01)	0.32
IMs	1.12 (0.82–1.52)	0.48	—	—
Opiates	2.53 (1.88–3.41)	<0.0001	3.66 (2.34–5.73)	<0.0001
IBD Surgery				
	Crohn's Disease		**Ulcerative Colitis**	
	AOR	***P*-value**	**AOR**	***P*-value**
PBE	1.77 (1.04–3.01)	0.03	1.76 (0.99–3.15)	0.05
CRP	2.33 (1.57–3.48)	<0.0001	1.42 (0.79–2.55)	0.24
BMI	0.97 (0.94–1.00)	0.04	—	—
Asthma	0.34 (0.08–1.38)	0.13	0.16 (0.02–1.39)	0.10
Rheumatoid Arthritis	—	—	6.9 (0.52–90.93)	0.14
Adrenal Insufficiency	2.31 (0.08–66.29)	0.62	—	—
Allergies	1.26 (0.64–2.48)	0.50	1.03 (0.38–2.81)	0.95
Medication Allergies	0.75 (0.40–1.41)	0.37	0.71 (0.27–1.84)	0.48
Smoking Status	—	—	1.07 (0.64–1.80)	0.79
IBD Surgery prior to 2009	—	—	3.57 (1.80–7.09)	<0.0001
Location	1.17 (0.87–1.58)	0.30	—	—
Behavior	1.71 (1.34–2.18)	<0.0001	—	—
Extent	—	—	1.09 (0.67–1.77)	0.72
Duration	0.97 (0.95–0.99)	0.01	—	—
anti-TNF	1.83 (1.22–2.73)	0.003	1.81 (0.99–3.33)	0.05
Prednisone	1.06 (0.70–1.61)	0.77	1.79 (0.93–3.45)	0.08
IMs	1.32 (0.88–1.99)	0.18	—	—
Opiates	3.25 (2.16–4.88)	<0.0001	5.77 (3.22–10.36)	<0.0001

AOR, adjusted odds ratio; anti-TNF, anti-tumor necrosis factor; BMI, body mass index; CD, Crohn's disease; CRP, C-reactive protein; PBE, peripheral blood eosinophilia; IM, immunomodulator; UC, ulcerative colitis.

Variables were selected for inclusion in multivariate models based on univariate significance with $P<0.10$ (**Supplementary Table 4**).

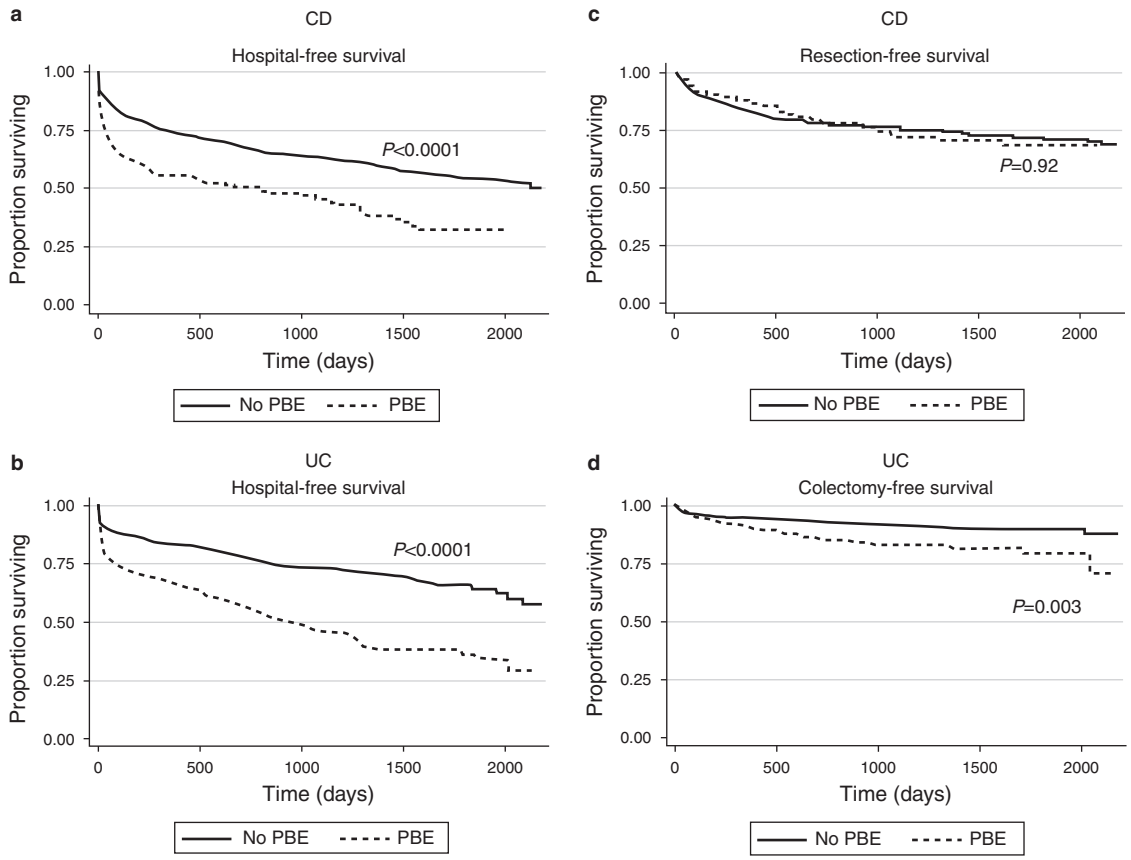

Figure 1. Time-to-event analysis by presence of peripheral blood eosinophilia (PBE) (n=396) compared to patients without PBE (n=1670). (**a**) Hospitalization in CD (n=1148). (**b**) Hospitalization in UC (n=725). (**c**) Surgical resection in CD. (**d**) Colectomy in UC.

Similarly, PBE remained significantly associated with IBD surgery in both CD (AOR 1.77; 95% CI 1.04–3.01) and UC (AOR 1.76; 95% CI 0.99–3.15).

On time-to-event analysis, PBE patients had significantly more rapid hospitalization in both UC (P<0.00001) and CD (P<0.00001) compared to IBD patients without PBE (**Figure 1**). Furthermore, patients with UC and PBE had a significantly decreased time to surgical resection (P=0.003), while there was no difference in CD (P= 0.92).

Multi-year patterns and correlation analysis

The majority (59.8%) of PBE patients had their eosinophilic episode(s) in 1 year, while 20.1% had PBE in 2 years, 13.0% in 3 years, and 7.1% between 4 and 6 years. The number of years with PBE was significantly associated with comorbid PSC (P<0.001). Consequently, we excluded patients with PSC from further analysis.

The number of years with PBE correlated with number of hospitalizations (CD: ρ=0.81 P<0.00001; UC: ρ=0.72, P<0.00001), emergency department visits, (CD: ρ=0.21, P<0.00001; UC: ρ=0.21, P=0.0001), and IBD surgeries (CD: ρ=0.17, P<0.00001; UC: ρ=0.13, P=0.0004) (**Supplementary Table 4**). With regards to disease activity, number of years with PBE also significantly correlated with elevated hsCRP rates (CD: ρ=0.19, P<0.00001; UC: ρ=0.30, P<0.00001), UCAI (ρ=0.19, P<0.00001), HBI (ρ=0.12, P=0.0001), and total financial charges (CD: ρ=0.21, P<0.00001; UC: ρ=0.27, P<0.00001). Number of years with PBE was inversely correlated with quality of life (CD: ρ=−0.09, P=0.005; UC: ρ=−0.16, P=0.0001).

Compared to PBE in 1 year only, patients with multiple years of PBE had a trend toward faster hospitalization (P=0.06) and surgical resection (P=0.06) in CD, but did not meet statistical significance. In UC, there was no difference between 1 year and multiple years of PBE in time to hospitalization (P=0.59) or time to resection (P=0.17).

Using absolute eosinophil counts in 376 PBE patients (94.9% all PBE patients) with the same reference range and units of measurement, absolute eosinophil counts did not significantly correlate with either hospitalization or IBD surgery in either UC or CD.

Association with hsCRP

To further clarify the association between PBE and hsCRP, we analyzed patients with same-day hsCRP and eosinophil measurements. There were 1,256 patients who had concurrent laboratory measurements (54.4% of PBE patients and 59.0% of no PBE patients). Of these, 33.0% demonstrated elevated hsCRP,

Table 4. Healthcare utilization, disease activity, and IBD medication exposure by the presence of PBE and simultaneous hsCRP elevation compared to hsCRP elevation alone

	PBE+hsCRP (*n*=98)	hsCRP (*n*=303)	*P*-value
Healthcare utilization			
ED, %	66 (67.3)	142 (46.9)	<0.0001
Hospitalization, %	62 (63.3)	159 (52.5)	0.02
IBD Surgery, %	31 (31.6)	104 (34.3)	0.9
Disease activity and quality of life			
HBI, median (IQR)	4.0 (4.7)	3.4 (4.6)	0.57
UCAI, median (IQR)	4.5 (5.3)	3.8 (6.3)	0.51
SIBDQ, median (IQR)	52.0 (16.3)	50.1 (21.1)	0.32
IBD medications			
Prednisone, %	69 (70.4)	168 (55.4)	0.002
Biologic, %			
Anti-TNF	48 (49.0)	136 (44.9)	0.34
Other[a]	2 (2.0)	0	0.06
Immunomodulator, %	57 (58.2)	196 (64.7)	0.54
5-ASA, %	49 (50.0)	122 (40.3)	0.04
Enteric steroids,[b] %	14 (14.3)	72 (23.8)	0.08
Financial charges			
Total charges (USD), median (IQR)	$78,726 (254,031)	$43,247 (134,572)	0.001

ED, emergency department; HBI, Harvey–Bradshaw Index; hsCRP, high sensitivity C-reactive protein; IBD, inflammatory bowel disease; PBE, peripheral blood eosinophilia; SIBDQ, short inflammatory bowel disease questionnaire; UC, ulcerative colitis; UCAI, ulcerative colitis activity index; anti-TNF, anti-tumor necrosis factor; 5-ASA, 5-aminosalicylate.

[a]Natalizumab, vedolizumab, golimumab, rituximab.

[b]Oral budesonide, per rectum corticosteroid preparations.

18.0% PBE, and 8.0% both hsCRP elevation and PBE. Concurrent hsCRP elevation and PBE was significantly associated with UC (*P*<0.0001) (**Supplementary Figure 1**). Absolute hsCRP level was not significantly correlated with absolute eosinophil count in patients with concurrent hsCRP elevation and PBE (*P*=0.39).

After exclusion of comorbid PSC, simultaneous PBE and hsCRP elevation was significantly associated with more ED visits (*P*<0.0001), hospitalization (*P*=0.02), prednisone use (*P*=0.002), and higher median cumulative financial charges ($78,726 vs. $43,247, *P*=0.001) compared to patients with hsCRP elevation alone over the 6-year period (**Table 4**).

In time-to-event analysis, UC patients with both PBE and hsCRP elevation had more rapid time to hospitalization (*P*<0.001) (**Supplementary Figure 2**) compared to patients with hsCRP elevation alone. There was no difference in time to hospitalization (*P*=0.13) in CD or time to surgery for either UC (*P*=0.11) or CD (*P*=0.74) when comparing subjects with both PBE and hsCRP to those with hsCRP elevation alone.

New IBD diagnosis subgroup

There were 188 subjects with IBD diagnosed between 2009 and 2014. Of these, 131 (CD *n*=62, UC *n*=69) had a CBC with differential within 12 months following diagnosis and 30 (22.9%) had PBE. The majority (70%) of subjects with new IBD diagnosis and PBE had UC.

Subjects with PBE within 1 year of IBD diagnosis had nearly two times the rate of hsCRP elevation (CD: 100 vs. 54.5%, *P*=0.009; UC: 76.2 vs. 37.0%, *P*=0.004), but there was no significant difference in disease activity scores (HBI *P*=0.68; UCAI *P*=0.06) by presence of PBE (**Table 5**). In UC, patients with PBE were significantly more likely to require corticosteroids (95.2 vs. 63.0%, *P*=0.007) and anti-TNF therapy (61.9 vs. 17.4%, *P*=0.001) compared to new IBD patients without PBE. There was a trend toward more immunomodulator use (71.4 vs. 50.0%), but this did not reach statistical significance (*P*=0.12). Medication exposure was not significantly different in CD.

PBE within 1 year of IBD diagnosis was significantly associated with higher ED use in UC (71.4 vs. 43.5%, *P*=0.04), but not CD (*P*=0.15). Nearly half (49.6%, *n*=64) of all new IBD diagnosis patients were hospitalized and 24.8% (*n*=25) required IBD-related surgery within the study timeframe. On time-to-event analysis, there was no significant difference between subjects with PBE and those without for either hospitalization (*P*=0.25) or IBD surgery (*P*=0.87). This did not differ by disease subtype.

DISCUSSION

In this prospective registry analysis of PBE in a large IBD cohort followed over a multi-year period, a minority of IBD patients displayed PBE, oftentimes recurrent, and associated with concurrent inflammation. After controlling for inflammation, PBE remained significantly associated with more severe disease with negative clinical outcomes compared to patients without this laboratory finding. Furthermore, an incident cohort of recently diagnosed IBD patients with PBE displayed similar associations as the entire PBE cohort. These findings suggest that PBE identifies a unique at-risk subgroup of IBD patients that require further pathophysiologic mechanistic investigation and potentially targeted therapies.

The prevalence of PBE in our study is consistent with recent reports (3) albeit lower than initial studies in the 1960s as well as a recent pediatric study, which reported prevalence rates between 30–40% in IBD patients (1,2,5). This historical prevalence difference may be explained by study design, or more effective medical therapies currently, thus controlling inflammation and reducing the eosinophilic signal. The lower adult prevalence of PBE may be related to age-mediated differences in propensity toward PBE in IBD. In this study, PBE was associated with other comorbidities known to display peripheral eosinophilia such as asthma, rheumatologic diseases, and allergies, which is similar to other population-based studies (14). This may suggest an altered genetic predisposition to eosinophilic-mediated process systemically in these individuals.

PBE was associated with concurrent hsCRP elevation suggesting that PBE may be a marker of an active inflammatory cascade

Table 5. Healthcare utilization, disease activity, medication requirements, and healthcare charges in subjects with new diagnosis of IBD between 2009 and 2014 with a complete blood cell count with differential within a year after diagnosis by presence of PBE

	PBE (*n*=30)		No PBE (*n*=101)				
	CD (*n*=9)	UC (*n*=21)	CD (*n*=55)	UC (*n*=46)	PBE vs. No PBE *P*-value	CD *P*-value	UC *P*-value
Healthcare utilization							
ED, %	7 (77.8)	15 (71.4)	26 (47.3)	20 (43.5)	0.01	0.15	0.04
Hospitalization, %	6 (66.7)	10 (47.6)	21 (38.2)	14 (30.4)	0.09	0.15	0.27
IBD-surgery, %	6 (66.7)	2 (9.5)	22 (40.0)	3 (6.5)	0.82	0.16	0.65
Disease activity							
HBI, median (IQR)	2.8 (3.4)	—	2.6 (4.2)	—	0.63	0.93	—
UCAI, median (IQR)	—	4.0 (3.5)	—	2.3 (3.2)	0.10	—	0.11
hsCRP elevation, %	9 (100)	16 (76.2)	30 (54.5)	17 (37.0)	<0.0001	0.009	0.004
ESR elevation, %	5 (55.5)	11 (52.4)	20 (36.4)	12 (26.1)	0.05	0.29	0.05
SIBDQ, median (IQR)	55.4 (19.1)	52.3 (10.3)	53.3 (20.1)	54.8 (10.7)	0.62	0.75	0.42
IBD medications							
Prednisone, %	5 (55.5)	20 (95.2)	30 (54.5)	29 (63.0)	0.02	1.00	0.007
anti-TNF, %	3 (33.3)	13 (61.9)	27 (49.1)	8 (17.4)	0.09	0.48	0.001
IM, %	8 (88.9)	15 (71.4)	41 (74.5)	23 (50.0)	0.20	0.67	0.12
5-ASA, %	6 (66.7)	18 (85.7)	31 (56.4)	38 (82.6)	0.26	0.72	1.00
Financial charges							
Total charges (USD), median (IQR)	$164,162 (201,758)	$47,104 (199,207)	$42,454 (123,994)	$14,266 (53,996)	0.004	0.01	0.03

CD, Crohn's disease; ED, emergency department; ESR, erythrocyte sedimentation rate; HBI, Harvey–Bradshaw Index; hsCRP, high sensitivity C-reactive protein; IBD, inflammatory bowel disease; IM, immunomodulator; PBE, peripheral blood eosinophilia; SIBDQ, short inflammatory bowel disease questionnaire; UC, ulcerative colitis; UCAI, ulcerative colitis activity index; TNF, tumor necrosis factor; 5-ASA, 5-aminosalicylate.

in these individuals. Compared to patients with hsCRP elevation alone, simultaneous PBE and hsCRP elevation was associated with increased hospitalization and prednisone requirement, suggesting a potential additive risk of the two biomarkers. Histologic studies have demonstrated an increased number of mucosal eosinophils in IBD samples (15,16) and an increase in eosinophil-derived products (EDPs) in serum, pathologic, and fecal samples of actively inflamed IBD subjects compared to quiescent IBD controls (17–25). EDPs have been shown to diminish epithelial barrier function (26) and murine models with depleted EDPs have attenuated experimental colitis (25,27–29). Treatment with corticosteroids reduces EDPs (17,22) and may help explain the higher rates of steroid requirement in the current study. Together these findings implicate eosinophils as key figures in the disposition to, development of, and propagation of the inflammatory cascade in IBD. However, even after controlling for active inflammation and medication requirements in multivariate modeling, PBE remained associated with negative outcomes, signifying an unmet need in the care of PBE patients.

Absolute eosinophil counts did not significantly correlate with outcomes. Similarly, patients with multiple years of PBE compared to only 1 year of PBE displayed no difference in time to event analysis. This suggests that it is not the absolute increase in eosinophil number or necessarily the number of PBE occurrences, but rather the signature itself that predisposes the individual to more difficult disease.

Since the initial association of eosinophils and UC, their exact role has been debated. It was initially postulated that the etiology of UC was an allergic phenomenon, thus explaining the eosinophil signature (30). This idea was strengthened by the subsequent identification of Th2 cellular network and relevant cytokine pathways including interleukin (IL)-4, IL-5, IL-10, and IL-13. Eosinophil growth, chemotaxis, and activation are predominantly mediated by IL-5 with a synergistic chemotaxic effect of eotaxin and IL-13 (31). In the current study, PBE was associated with UC and higher rates of adverse outcomes in the UC PBE population compared to CD PBE. This may be explained by differences in chemotactic abilities, adhesion properties, and degranulation activity between UC and CD peripheral eosinophils (32), with potentially a more toxic effect of the eosinophil in UC compared to CD.

There are several limitations to this study. This was an observational study and thus prone to selection bias, information bias, and confounding variables. More patients with PBE were hospitalized thus predisposing to more laboratory tests and the opportunity to observe PBE. Only the initial laboratory values obtained in a hospitalization were used for this study. Of all patients in the

IBD registry, only 80.6% had a complete blood count with differential during the 6-year period. If serologic evaluation was only performed in patients suspected of active disease, this could skew the results. Endoscopic and histologic data on these patients would have been useful to correlate active inflammation and evaluate the presence of mucosal eosinophils. However, endoscopic and histologic data at the time of their laboratory evaluation was not available. Finally, we attempted to assess and control for potential confounding by conducting multiple subgroup analyses and using multivariable modeling including many potential confounding covariates.

There are also strengths of this study. This IBD cohort represents a large, adult IBD patient population followed longitudinally for 6 years with highly detailed phenotypic information, prospectively collected disease activity metrics, extensive clinical parameters, and healthcare utilization parameters. This level of detail allows for identifying unique patient subgroups and retaining sufficient population sizes to be able to conduct significant subgroup analyses. Furthermore, the observational nature of this study provides a real-world view of patterns in a heterogenous IBD population.

In conclusion, PBE occurs in a minority of IBD patients and is associated with worse clinical outcomes in a multi-year longitudinal analysis. This laboratory finding represents a biomarker for a unique inflammatory process that may not be addressed by conventional IBD maintenance therapies. Further research into the exact mechanism of eosinophil function in IBD is needed, but this subgroup of patients may be candidates for targeted therapy in future studies.

ACKNOWLEDGMENTS

We thank Leonard Baidoo, MD for his early guidance and assistance in data design and registry enrollment.

CONFLICT OF INTEREST

Guarantor of the article: David Binion, MD.

Specific author contributions: B.C.: study conception, data collection, performing analysis, interpreting data, drafting of manuscript, approval of final manuscript. A.M.: study conception, interpreting analysis, drafting of manuscript, approval of final manuscript. I.K.: study conception, critical review of manuscript, approval of final manuscript. C.R.: data collection, critical review of manuscript, approval of final manuscript. D.B.: data collection, critical review of manuscript, approval of final manuscript. J.M.: performing analysis, critical review of manuscript, approval of final manuscript. D.H.: study conception, critical review of manuscript, approval of final manuscript. J.H.: data collection, critical review of manuscript, approval of final manuscript. M.D.: provided study funding, critical review of manuscript, approval of final manuscript. M.S.: data collection, critical review of manuscript, approval of final manuscript. J.S.: data collection, critical review of manuscript, approval of final manuscript. A.B.: data collection, critical review of manuscript, approval of final manuscript. S.W.: study conception, critical review of manuscript, approval of final manuscript. M.R.: study conception, critical review of manuscript, approval of final manuscript. D.B.: study conception, interpreting data, critical review of manuscript, approval of final manuscript.

Financial support: This work was supported by the National Institutes of Health (5T32DK063922-12 to BC; PI: David Whitcomb, MD, PhD); sabbatical salary of Medical Faculty University of Crete, Greece (to IEK); the University of Pittsburgh Clinical and Translational Science Institute (5TL1TR000145-09 to AMA; PI: Steven Reiss, MD); and the United States Army Medical Research and Materiel Command (W81XWH-11-2-0133 to DGB and MAD).

Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Peripheral blood eosinophilia (PBE) in inflammatory bowel disease (IBD) is associated with UC and active disease.
- ✓ Little data exists on the long-term impact of PBE on disease course.

WHAT IS NEW HERE

- ✓ Nearly 20% of patients demonstrate PBE over a multi-year period in a large IBD cohort.
- ✓ PBE patients had increased healthcare utilization, more active disease, and required more aggressive medical therapy compared to IBD patients without PBE.
- ✓ PBE represents a biomarker of a distinct IBD subgroup, with a unique inflammatory signature, and at risk for worse clinical outcomes.

REFERENCES

1. Wright R, Truelove SC. Circulating and tissue eosinophils in ulcerative colitis. Am J Dig Dis 1966;11:831–46.
2. Riis P, Anthonisen P. Eosinophilia in peripheral blood and inflammatory exudate in non-specific proctocolitis. Acta Med Scand 1964;175:85–9.
3. Barrie A, Mourabet ME, Weyant K *et al.* Recurrent blood eosinophilia in ulcerative colitis is associated with severe disease and primary sclerosing cholangitis. Dig Dis Sci 2013;58:222–8.
4. Benfield GF, Asquith P. Blood eosinophilia and ulcerative colitis--influence of ethnic origin. Postgrad Med J 1986;62:1101–5.
5. Sadi G, Yang Q, Dufault B *et al.* Prevalence of peripheral eosinophilia at diagnosis in children with inflammatory bowel disease. J Pediatr Gastroenterol Nutr 2016;62:573–6.
6. Anderson AJ, Click B, Ramos-Rivers C *et al.* Development of an inflammatory bowel disease research registry derived from observational electronic health record data for comprehensive clinical phenotyping. Dig Dis Sci 2016;61:3236–45.
7. Roufosse F, Weller PF. Practical approach to the patient with hypereosinophilia. J Allergy Clin Immunol 2010;126:39–44.
8. Tefferi A. Blood eosinophilia: a new paradigm in disease classification, diagnosis, and treatment. Mayo Clin Proc 2005;80:75–83.
9. Silverberg MS, Satsangi J, Ahmad T *et al.* Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can J Gastroenterol 2005;19(Suppl A):5a–36a.
10. Harvey RF, Bradshaw JM. A simple index of Crohn's-disease activity. Lancet 1980;1:514.
11. Kozarek RA, Patterson DJ, Gelfand MD *et al.* Methotrexate induces clinical and histologic remission in patients with refractory inflammatory bowel disease. Ann Intern Med 1989;110:353–6.
12. Irvine EJ, Zhou Q, Thompson AK. The short inflammatory bowel disease questionnaire: a quality of life instrument for community physicians managing inflammatory bowel disease. CCRPT Investigators. Canadian Crohn's Relapse Prevention Trial. Am J Gastroenterol 1996;91:1571–8.
13. Jowett SL, Seal CJ, Barton JR *et al.* The short inflammatory bowel disease questionnaire is reliable and responsive to clinically important change in ulcerative colitis. Am J Gastroenterol 2001;96:2921–8.

14. Bernstein CN, Wajda A, Blanchard JF. The clustering of other chronic inflammatory diseases in inflammatory bowel disease: a population-based study. Gastroenterology 2005;129:827–36.
15. Protheroe C, Woodruff SA, de Petris G *et al.* A novel histologic scoring system to evaluate mucosal biopsies from patients with eosinophilic esophagitis. Clin Gastroenterol Hepatol 2009;7:749–755.e11.
16. Wedemeyer J, Vosskuhl K. Role of gastrointestinal eosinophils in inflammatory bowel disease and intestinal tumours. Best Pract Res Clin Gastroenterol 2008;22:537–49.
17. Luck W, Becker M, Niggemann B *et al. In vitro* release of eosinophil cationic protein from peripheral eosinophils reflects disease activity in childhood Crohn disease and ulcerative colitis. Eur J Pediatr 1997;156:921–4.
18. Berstad A, Borkje B, Riedel B *et al.* Increased fecal eosinophil cationic protein in inflammatory bowel disease. Hepatogastroenterology 1993;40:276–8.
19. Bischoff SC, Grabowsky J, Manns MP. Quantification of inflammatory mediators in stool samples of patients with inflammatory bowel disorders and controls. Dig Dis Sci 1997;42:394–403.
20. Saitoh O, Kojima K, Sugi K *et al.* Fecal eosinophil granule-derived proteins reflect disease activity in inflammatory bowel disease. Am J Gastroenterol 1999;94:3513–20.
21. Dubucquoi S, Janin A, Klein O *et al.* Activated eosinophils and interleukin 5 expression in early recurrence of Crohn's disease. Gut 1995;37:242–6.
22. Peterson CG, Sangfelt P, Wagner M *et al.* Fecal levels of leukocyte markers reflect disease activity in patients with ulcerative colitis. Scand J Clin Lab Invest 2007;67:810–20.
23. Chen W, Paulus B, Shu D *et al.* Increased serum levels of eotaxin in patients with inflammatory bowel disease. Scand J Gastroenterol 2001;36:515–20.
24. Mir A, Minguez M, Tatay J *et al.* Elevated serum eotaxin levels in patients with inflammatory bowel disease. Am J Gastroenterol 2002;97:1452–7.
25. Ahrens R, Waddell A, Seidu L *et al.* Intestinal macrophage/epithelial cell-derived CCL11/eotaxin-1 mediates eosinophil recruitment and function in pediatric ulcerative colitis. J Immunol 2008;181:7390–9.
26. Furuta GT, Nieuwenhuis EE, Karhausen J *et al.* Eosinophils alter colonic epithelial barrier function: role for major basic protein. Am J Physiol Gastrointest Liver Physiol 2005;289:G890–G897.
27. Forbes E, Hulett M, Ahrens R *et al.* ICAM-1-dependent pathways regulate colonic eosinophilic inflammation. J Leukoc Biol 2006;80:330–41.
28. Forbes E, Murase T, Yang M *et al.* Immunopathogenesis of experimental ulcerative colitis is mediated by eosinophil peroxidase. J Immunol 2004;172:5664–75.
29. Vieira AT, Fagundes CT, Alessandri AL *et al.* Treatment with a novel chemokine-binding protein or eosinophil lineage-ablation protects mice from experimental colitis. Am J Pathol 2009;175:2382–91.
30. Rowe AH. Chronic ulcerative colitis, an allergic disease. Ann Allergy 1949;7:727–51.
31. Adar T, Shteingart S, Ben Ya'acov A *et al.* From airway inflammation to inflammatory bowel disease: eotaxin-1, a key regulator of intestinal inflammation. Clin Immunol 2014;153:199–208.
32. Coppi LC, Thomazzi SM, de Ayrizono ML *et al.* Comparative study of eosinophil chemotaxis, adhesion, and degranulation *in vitro* in ulcerative colitis and Crohn's disease. Inflamm Bowel Dis 2007;13:211–8.