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Elevated serum globulin fraction as a biomarker of multiyear disease severity in inflammatory bowel disease

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Abstract

Background Serum protein reflects albumin and globulin levels, both of which can be altered in inflammatory bowel disease (IBD). The implications of a high globulin fraction in IBD are unknown. We hypothesized that a high globulin fraction may function independently of albumin as a biomarker of disease severity in IBD patients over a multiyear period.

Methods This was an observational study from a prospective IBD registry of a tertiary care center. High globulin fraction was defined as an elevated globulin level >4 g/dL. Data collected included patient demographics, medication exposures, quality-of-life scores, disease activity, emergency department visits, telephone calls, hospitalizations, and IBD-related surgeries over a 4-year period. Comparisons between patients with a high globulin fraction and those without were performed using Pearson's chi-squared, Student's and Mann-Whitney tests. Multivariate analyses were used to assess the relationship between high globulin fraction and healthcare utilization.

Results A total of 1767 IBD patients with a 4-year follow up were included: 53.5% female, mean age 48.4±15.1 years, and 65.4% with Crohn's disease. Of these patients, 446 (25.2%) presented with elevated globulin fraction. Patients with a high globulin fraction were more likely to be hospitalized during the study period. This result remained significant after multivariate analysis for both Crohn's disease patients and those with ulcerative colitis.

Conclusion A high globulin fraction is independently associated with greater disease severity and healthcare utilization in IBD patients, and may function as a routinely available biomarker of a more severe future disease trajectory.

Keywords Globulin, albumin, total protein, quality of life, inflammatory bowel disease

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Introduction

The major forms of inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are characterized by chronic inflammatory injury to the gastrointestinal tract, leading to cumulative damage and organ dysfunction. In addition to bowel damage, chronic inflammation will often result in changes to routine blood laboratory values; these can include anemia and altered levels of serum proteins, which may rise or fall in the course of the disease [1]. There is a growing body of evidence that albumin, particularly low levels of albumin, can function as a biomarker of gut inflammation severity, while also reflecting blood levels of antibody-based therapies, thus functioning as a proxy in therapeutic drug monitoring [2,3]. On the other hand, there has been less investigation regarding the elevation of certain serum proteins in IBD, specifically levels of immunoglobulin. Total serum protein (total protein) primarily reflects the cumulative sum of albumin and globulins, a readily available component of routine bloodwork used to monitor patients with IBD. The globulin fraction can be readily calculated by subtracting albumin from total protein and is an approximation of the quantitative globulin, which may not be as frequently assessed in IBD patient management.

Globulin fraction is not a single molecule, but instead, a combination of proteins, which include immunoglobulins as the major constituent [4]. A high globulin fraction may reflect

Conflict of Interest: None

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increased globulin production and/or increased humoral immune activity, which can be seen in cases of leukemia, multiple myeloma, autoimmune liver diseases, as well as autoimmune and chronic inflammatory illnesses including IBD. The clinical implications of a high globulin fraction in IBD are unknown.

Expansion of the globulin fraction as a component of serum total protein has been associated with chronic inflammatory conditions, and this has been best exemplified in the sera of patients with autoimmune hepatitis. In a study by Hennes *et al*, patients with autoimmune hepatitis were found to have median γ-globulin levels 1.53 times the upper limit of normal compared to controls (P<0.001) [5]. Similarly, in these autoimmune hepatitis patients, their median immunoglobulin G (IgG) levels were 1.44 times the upper limit of normal compared to controls (P<0.001) [5]. Both γ-globulin-levels and IgG levels were determined to be univariate discriminators for the diagnosis of autoimmune hepatitis [5].

Similar to those with autoimmune hepatitis, IBD patients have a dysregulated immune system, involving both innate and adaptive immune function [6,7]. Several studies have investigated levels of immunoglobulins and their subtypes in patients with IBD [8-10]. A recent study demonstrated a relationship between immunoglobulins and IBD-related surgeries [7]. However, these findings were derived from IgG subtype profiles and not total globulin fraction.

There are limited data investigating the clinical implications of an elevated globulin fraction in patients with IBD. Given the availability of total globulin fraction in routine laboratory evaluations, we investigated the role of elevated globulin fraction in IBD. We hypothesized that an elevated globulin fraction might function as a biomarker of disease severity and healthcare utilization in IBD patients followed over a multiyear period.

Patients and methods

Study population

The study population consisted of IBD patients consented and prospectively followed in a natural history IBD registry maintained at the University of Pittsburgh Medical Center (UPMC) [11]. Briefly, the UPMC IBD registry is a prospective natural history registry that systematically derives clinical data from the electronic medical record. All patients with IBD were diagnosed using established criteria. Patients enrolled in the IBD registry and tested for total serum protein (total protein) in the outpatient setting were included in the study. For patients with a normal globulin fraction throughout the study, the time point of inclusion was the patient's first laboratory assessment with a normal value in the prospective IBD registry. For patients with an elevated globulin fraction, the time point of study inclusion was the first time their globulin fraction was high. Having random time points for inclusion would make our results more generalizable and more applicable and would increase external validity (i.e., patients were not enrolled exclusively at the time of IBD diagnosis but instead during routine care). Patients were excluded if there was no total protein measurement during the study period or if they carried a diagnosis of IBD unclassified. An increased globulin fraction (>4 g/dL) was defined by subtracting same day albumin level from total protein level (the upper limit of normal of UPMC clinical laboratory) at the day of enrollment into the study. Thus, patients were stratified into 2 groups: initially elevated globulin fraction, or persistently normal globulin fraction. To better compare less frequent outcomes between the 2 groups (for example IBD-related surgeries), the 2 study groups were designed to have a comparable follow-up period. We chose to include patients with at least 4 years of clinical follow up (determined by date of most recent telephone encounter or physician clinic visit) from 2009-2019, as it would provide an effective sample size and a good long-term follow-up period.

Clinical data collection

The database is maintained from the prospective IBD registry (ClinicalTrials.gov NCT04243525). We retrospectively queried this database for the purposes of the study. Baseline patient demographic data and data pertaining to comorbid conditions known to be associated with increased globulin fraction—leukemia, multiple myeloma, monoclonal gammopathy of unknown significance, autoimmune hepatitis, and rheumatologic diseases—were collected using ICD-9 codes and from the electronic medical record problem lists. Baseline disease characteristics, including IBD subtype (CD or UC), duration of disease, disease location and behavior according to the Montreal Classification at initial endoscopic or radiographic encounter, and history of IBD-related surgery prior to 2009 were also recorded [12]. Disease activity was evaluated using patient-reported disease activity and biochemical inflammatory markers. Patient-reported disease activity included the Harvey-Bradshaw Index (HBI) for CD patients, and the UC activity index for UC patients. All patient-reported disease activity indices were collected prospectively at outpatient clinical encounters and annual mean values for disease activity indices were created. Health-related quality of life was assessed using a published version of the short-form IBD questionnaire (SIBDQ), which was prospectively collected at outpatient visits. We generated mean SIBDQ scores over the study period for each study participant.

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Biochemical inflammatory markers included high sensitivity C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). We evaluated annual dichotomous patterns (any elevated value vs. all normal values during a calendar year) of biochemical inflammatory markers (CRP ≥0.74 mg/dL; ESR >20 mm/h per UPMC laboratory standards). Index visit laboratory data were eligible for inclusion if obtained within 1 month of the index visit.

Healthcare utilization measures included the total number of outpatient clinic visits, total count of telephone encounters, number of radiographic studies, and number of endoscopic procedures. As for emergency department (ED) use, IBD-related hospital admissions, and IBD-related surgery, these were treated as binary (yes/no) variables.

IBD medication exposure was determined by annual prescriptions in the electronic medical record. We collected data on exposures to immunomodulators, biologics, steroids and 5-aminosalicylic acid compounds. Immunomodulator agents included azathioprine, 6-mercaptopurine and methotrexate. Steroid prescriptions included systemic prednisone and excluded other systemic glucocorticoids, to avoid prescriptions indicated for adrenal insufficiency. We also included enteric steroids (oral budesonide and per rectum corticosteroid preparations). Biologics included anti-tumor necrosis factor medications (infliximab, adalimumab, certolizumab pegol). Finally, 5-aminosalicylic acid compounds included sulfasalazine, mesalamine, balsalazide, either oral or topical. Use of oral vancomycin was also collected as a proxy for *Clostridioides difficile*.

Statistical analysis

The variables, collected prospectively during the 4-year follow-up period, were treated as outcomes. Some data to better characterize the patients at baseline were collected prior to the index date but they were not treated as outcomes in our study. Continuous variables, based on the distribution in the study population, were recorded either as mean and standard deviation (SD) or median and interquartile range (IQR) and analyzed with Student's *t*-test or the Mann-Whitney test, respectively. Categorical variables were presented as proportions. Pearson's chi-square or Fisher's exact tests were used to assess associations between categorical variables. We utilized univariate analysis to assess the relationship between high globulin fraction and markers of disease activity, such as exposure to IBD-related medications, biomarkers of disease severity and healthcare utilization, including need for hospitalization, ever using the ED, and requiring IBD-related surgery. Multivariate logistic regression was then used to control for potential confounding factors related to hospitalizations and IBD-related surgeries in relation to high globulin fraction. We controlled for the different disease activity of each patient, approximated by clinical biomarkers at baseline (within 30 days of index visit), and differences with significance P<0.1 in demographics and medication exposure. All tests were 2-sided, with statistical significance considered at level P=0.05. A Kaplan-Meier survival analysis, as a non-parametric univariate event analysis, and a Cox regression analysis were performed for the main outcomes: hospitalizations and IBD-related surgeries. Data were analyzed using SPSS Statistical software platform version 26 (Armonk, NY).

Ethical considerations

Enrollment in and use of the research registry (Protocol # 0309054) as well as the current study (Protocol #17040443) were approved by the University of Pittsburgh Institutional Review Board. The UPMC IBD Research Registry has been registered with clinicaltrials.gov (NCT04243525). All authors had access to the study data and reviewed and approved the final manuscript.

Results

Study cohort

There were 1767 IBD patients with 4 years of clinical follow up who had an assessment of serum globulin and these patients formed the study population. The median number of times a globulin fraction was checked per patient was 7 (IQR 3-14). Amongst these IBD patients, the majority of participants were female (53.5%) and the mean age was 48.4±15.1 years. Over half of the participants (65.4%) had CD, while 34.5% had UC. Among the study participants, 446 (25.2%) patients had an elevated globulin fraction at the start of the defined study time period and these patients formed the high (i.e., elevated) globulin fraction group, while the remaining patients comprised the normal globulin fraction group.

High globulin fraction group

IBD patients in the high globulin fraction group were more likely to be female (P=0.004) and to have undergone prior IBD-related surgery (P<0.001), as demonstrated in Table 1. At the time of the initial test, there was no difference in disease duration between patients with a high globulin fraction and those with a normal globulin fraction. Regarding other biomarkers of disease activity checked on the initial visit, patients with a high globulin fraction had significantly higher CRP, ESR, anemia, and hypoalbuminemia than the patients without globulin elevation. Table 1 displays the differences in patient demographics and disease characteristics between patients with a high globulin fraction and their controls. Supplementary Table 1 shows the disease location in these patients, using the Montreal classification.

Table 1 Demographics and disease characteristics of the overall cohort, comparing patients with high and normal globulin fraction from their index visit

Characteristics	Total study population n=1767	Globulin fraction category		P-value
		High globulin n=446	Normal globulin n=1321	
Age (mean years±SD)*	48.4±15.1	48.1±15.1	48.5±15.2	0.984
Female sex, n (%)	945 (53.5)	265 (59.4%)	680 (51.5%)	0.004
Tobacco smoking, (n, %)				
Never	1302 (73.7%)	322 (72.2%)	980 (74.2%)	0.499
Former smoker	187 (10.6%)	46 (10.3%)	141 (10.7%)	
Current smoker	278 (15.7%)	78 (17.5%)	200 (15.1%)	
Disease characteristics				
Ulcerative colitis	611 (34.5%)	138 (30.9%)	473 (35.8%)	0.062
Crohn's disease	1156 (65.4%)	308 (69.1%)	848 (64.2%)	
Years of disease (mean years±SD)*	10.7±9.8	11.8±10.4	10.3±9.6	0.259
History of IBD-related surgery	232 (13.1%)	90 (20.2%)	142 (10.7%)	<0.001
Biomarkers at index visit				
Albumin, median (IQR)	4.0 (0.7)	3.7 (0.6)	4.1 (0.6)	<0.001
Hypoalbuminemia, n (%)	813 (46.0%)	341 (76.5%)	472 (35.7%)	<0.001
C-reactive protein, median (IQR)	0.6 (1.2)	1.4 (2.9)	0.5 (0.7)	<0.001
Elevated C-reactive protein, n (%)	343 (19.4%)	117 (26.2%)	226 (17.1%)	<0.001
Erythrocyte sedimentation rate, median (IQR)	15.0 (21.1)	27.3 (31.8)	13.2 (17.3)	<0.001
Elevated erythrocyte sedimentation rate, n (%)	263 (14.9%)	114 (25.6%)	149 (11.3%)	<0.001
Hemoglobin, median (IQR)	13.4 (2.3)	12.5 (2.4)	13.6 (2.1)	<0.001
Anemia, n (%)	396 (22.4%)	175 (39.2%)	221 (16.7%)	<0.001

SD, standard deviation; IQR, interquartile range

Table 2 Four-year disease outcomes between patients with ulcerative colitis and high vs. normal globulin fraction

Outcomes	Total (n = 611)	Globulin faction category		P-value
		High globulin (n = 138)	Normal globulin (n = 473)	
Medication use (n, %)†				
Immunomodulators	241 (39.4%)	61 (44.2%)	180 (38.1%)	0.200
Biologics	149 (24.4%)	50 (36.2%)	99 (20.1%)	<0.001
Systemic steroids	295 (48.3%)	76 (55.1%)	219 (46.3%)	0.081
5-aminosalicylic acids	425 (69.6%)	81 (58.7%)	344 (72.7%)	0.002
Vancomycin (CDI antibiotic)	98 (16.0%)	37 (26.8%)	61 (12.9%)	<0.001
Average total SIBDQ (median, [IQR]), n = 314	55.8 [14.5]	53.9 [15.4]	56.3 [13.6]	0.093
Disease activity metrics (median, [IQR])				
UCAI, n = 389	2.5 [4.0]	3.0 [4.8]	2.4 [3.9]	0.135
Biomarkers of severity				
C-reactive protein, median (IQR)	0.5 (0.7)	0.8 (2.3)	0.4 (0.6)	<0.001
Elevated C-reactive protein, n (%)	210 (34.4%)	72 (52.1%)	138 (29.2%)	<0.001
Erythrocyte sedimentation rate, median (IQR)	13.0 (16.6)	19.7 (24.8)	12.0 (12.8)	<0.001
Elevated erythrocyte sedimentation rate, n (%)	152 (24.9%)	63 (45.7%)	89 (18.8%)	<0.001
Hemoglobin, median (IQR)	13.8 (2.0)	13.0 (2.2)	13.9 (1.9)	0.003
Anemia, n (%)	255 (41.7%)	85 (61.6%)	170 (35.9%)	<0.001
Albumin, median (IQR)	4.2 (0.6)	4.1 (0.6)	4.3 (0.5)	<0.001
Hypoalbuminemia, n (%)	145 (23.7%)	50 (36.2%)	95 (20.1%)	<0.001
Healthcare utilization				
Emergency room visit, (n, %)	245 (40.1%)	87 (63%)	158 (33.5%)	<0.001
Hospitalization, (n, %)	201 (32.9%)	66 (47.8%)	135 (28.5%)	<0.001
IBD-related surgery, (n, %)	75 (12.3%)	26 (18.8%)	49 (10.4%)	0.012
Clinic visits, (median, [IQR])	4 [4]	3 [5]	4 [4]	0.164
Telephone encounters, (median, [IQR])	8 [12]	9 [14]	8 [11]	0.371

†Immunomodulators include 6-mercaptopurine, azathioprine, methotrexate. Biologics include anti-tumor necrosis factor agents (infliximab, adalimumab, and certolizumab), anti-integrin therapy (natalizumab)

SD, standard deviation; SIBDQ, short inflammatory bowel disease questionnaire; IBD, inflammatory bowel disease; GI, gastrointestinal; IQR, interquartile range; UCAI, ulcerative colitis activity index; CDI, Clostridioides difficile infection

During the study period, high globulin fraction patients required more biologics than the normal globulin fraction patients, regardless of IBD subtype. Furthermore, high globulin fraction IBD patients had associated biomarkers of disease severity at the index visit (Table 1), including higher CRP, ESR, anemia and hypoalbuminemia, and during the 4-year follow up, as shown in Tables 2 and 3. UC and CD patients with a high globulin fraction had higher healthcare utilization in the form of ED visits (P<0.001), hospitalizations (P<0.001), and IBD-related surgeries, compared with patients with normal globulin (Tables 2 and 3). Additionally, CD patients with a high globulin fraction had more telephone encounters. In univariate Kaplan-Meier survival analysis curves, elevated globulin fraction patients, whether with CD or UC, demonstrated significantly worse outcomes in hospital admissions (CD: P<0.001, UC: P<0.001) and IBD-related surgeries (CD: P<0.001, UC: P=0.013), compared to the normal globulin patients (Fig. 1). Finally, in Cox regression survival analysis, after controlling for differences in age, sex, history of IBD-surgery, and presence of anemia, elevated CRP and hypoalbuminemia on the day of for globulin fraction testing between the 2 groups, patients with an elevated globulin fraction had significantly more hospital admissions (CD: P=0.037, UC: P=0.030), while IBD-related surgery was significantly associated only with CD (CD: P=0.012, UC: P=0.538) (Fig. 2).

Multivariate analysis

On multivariate modeling, after controlling for significant baseline differences in demographics, clinical characteristics and medication exposure, a high globulin ratio remained significantly associated with higher rates of hospitalization in both CD and UC patients (Table 4). On the other hand, the association of elevated globulin fraction with increased need for IBD surgery remained significant only for the CD patients, but not the UC patients (Table 4).

Comorbidities associated with elevated globulin fraction

We conducted an additional analysis to determine the rates of comorbidities associated with the difference in serum globulins between the elevated globulin and normal globulin IBD patient cohorts. We analyzed referral patterns to specialist clinics in hepatology, hematology/oncology

Table 3 Four-year disease outcomes between patients with Crohn's disease and high vs. normal globulin fraction

Outcomes	Total (n = 1156)	Globulin faction category		P-value
		High globulin (n = 308)	Normal globulin (n = 848)	
Medication use (n, %)†				
Immunomodulators	646 (55.9%)	176 (57.1%)	470 (55.4%)	0.639
Biologics	572 (49.5%)	179 (58.1%)	393 (46.3%)	<0.001
Systemic steroids	495 (42.8%)	153 (49.7%)	342 (40.3%)	0.005
5-aminosalicylic acids	343 (29.7%)	84 (27.3%)	259 (30.5%)	0.308
Vancomycin (CDI antibiotic)	162 (14.0%)	66 (21.4%)	96 (11.3%)	<0.001
Average total SIBDQ (median, [IQR]), n = 553	53.6 [16.9]	52.0 [17.1]	54.2 [16.9]	0.025
Disease activity metrics (median, [IQR])				
Harvey-Bradshaw Index, n = 649	3.2 [4.6]	3.9 [4.8]	2.9 [4.4]	0.003
Biomarkers of severity				
C-reactive protein, median (IQR)	0.5 (0.8)	0.8 (1.5)	0.5 (0.6)	<0.001
Elevated C-reactive protein, n (%)	463 (40.1%)	161 (52.3%)	302 (35.6%)	<0.001
Erythrocyte sedimentation rate, median (IQR)	14.1 (18.0)	22.4 (22.9)	13.0 (15.2)	<0.001
Elevated erythrocyte sedimentation rate, n (%)	344 (29.8%)	146 (47.4%)	198 (23.3%)	<0.001
Hemoglobin, median (IQR)	13.5 (1.9)	12.9 (2.0)	13.6 (1.9)	<0.001
Anemia, n (%)	567 (49.0%)	212 (68.8%)	355 (41.8%)	<0.001
Albumin, median (IQR)	4.2 (0.6)	3.9 (0.5)	4.2 (0.6)	<0.001
Hypoalbuminemia, n (%)	302 (26.1%)	134 (43.5%)	168 (19.8%)	<0.001
Healthcare utilization				
Emergency room visit, (n, %)	556 (48.1%)	200 (64.9%)	356 (42.0%)	<0.001
Hospitalization, (n, %)	483 (41.8%)	172 (55.8%)	311 (36.7%)	<0.001
Surgeries, (n, %)	192 (16.6%)	68 (22.1%)	124 (14.6%)	0.003
Clinic visits, (median, [IQR])	4 [6]	5 [6]	4 [5]	0.476
Telephone encounters, (median, [IQR])	11 [16]	12 [18]	10 [15]	0.016

†Immunomodulators include 6-mercaptopurine, azathioprine, methotrexate. Biologics include anti-tumor necrosis factor agents (infliximab, adalimumab, and certolizumab), anti-integrin therapy (natalizumab)

SD, standard deviation; SIBDQ, short inflammatory bowel disease questionnaire; IBD, inflammatory bowel disease; GI, gastrointestinal; IQR, interquartile range; UCAI, ulcerative colitis activity index; CDI, Clostridioides difficile infection

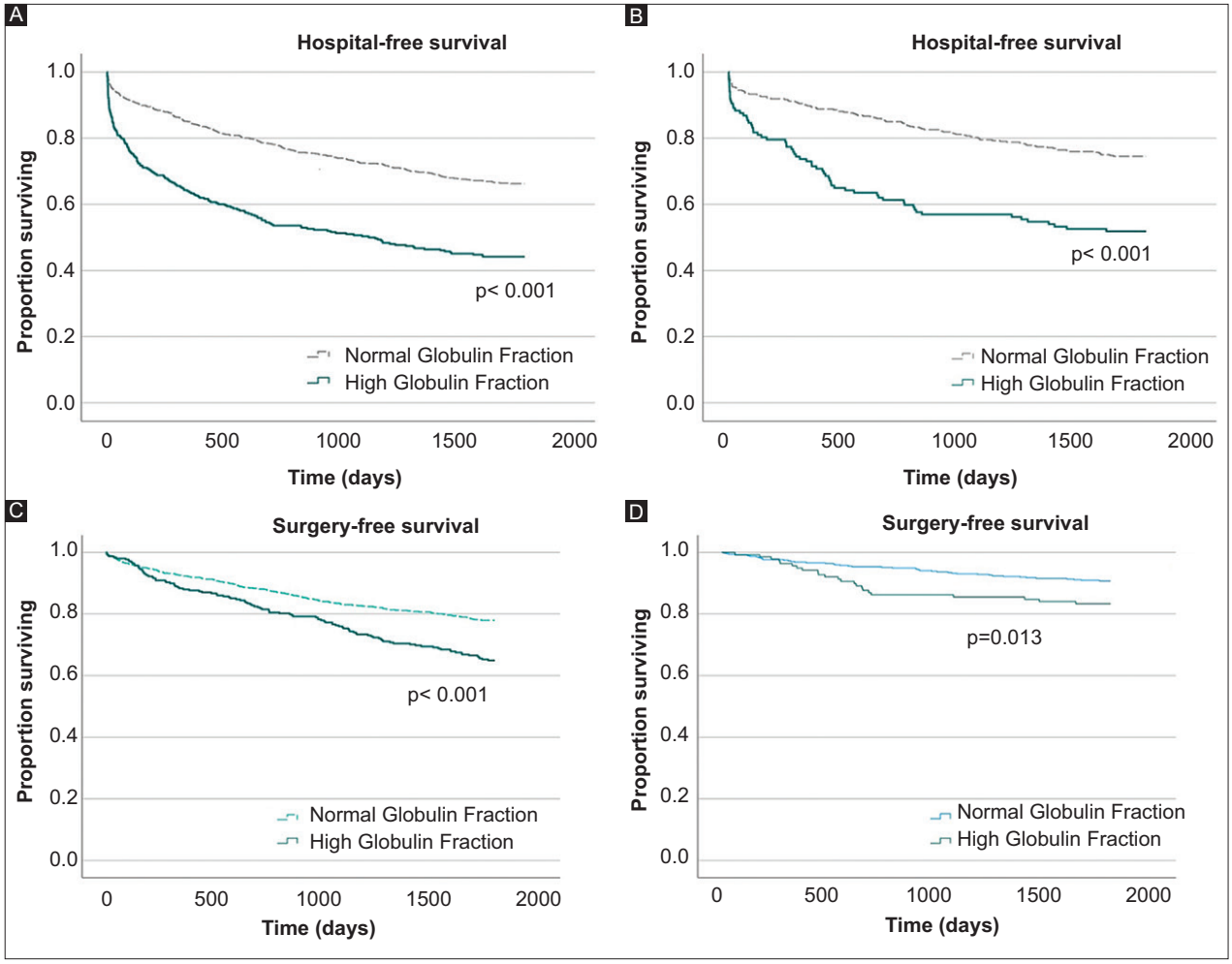

Figure 1 Kaplan-Meier curve for hospitalization and inflammatory bowel disease (IBD)-related surgeries amongst IBD patients with high globulin fraction vs. normal globulin fraction: (A) hospitalization in Crohn's disease patients, (B) hospitalization in ulcerative colitis patients, (C) IBD-related surgeries in Crohn's disease patients, (D) IBD-related surgeries in ulcerative colitis patients

and rheumatology, and additionally analyzed rates of specific conditions, including liver cirrhosis, hepatitis C, autoimmune hepatitis, monoclonal gammopathy, rheumatoid arthritis, systemic lupus erythematosus, and Sjögren's syndrome, between the 2 IBD patient groups (Supplementary Table 2). Comorbidities associated with an elevated globulin fraction were more common in the IBD cohort with an elevated globulin fraction. However, more granular analysis suggested that these comorbidities occurred in small subgroups of the elevated globulin IBD patients and did not account for the majority of these patients. Specific diagnoses, such as monoclonal gammopathy, were rare (1 individual), and rheumatologic processes were found in 5% of these IBD patients with elevated globulin fraction. The IBD elevated globulin fraction patients were overall sicker, with higher rates of anemia and liver conditions associated with elevated globulin fraction. Thus, our analysis demonstrates that elevated globulin fraction continues to be a biomarker of clinical severity in IBD, which reflects the impact of both the underlying disease and possible comorbidities.

Discussion

Predicting the multiyear clinical course of IBD with the use of biomarkers remains an elusive, yet important goal. This has been a challenge, not only because of the lack of candidate biomarkers, but also given the lack of a uniformly accepted strategy for gauging multiyear disease severity in IBD. In this study, we addressed both of these challenges by utilizing a prospective natural history registry to assess the performance of increased globulin fraction, a routinely available serum lab test, as a candidate biomarker of multiyear IBD severity. Increased globulin fraction, either reported

Table 4 Multivariate analysis: ulcerative colitis and Crohn's disease

Factors	Hospital admissions			
	Crohn's disease		Ulcerative colitis	
	AOR (95% CI)	P-value	AOR (95% CI)	P-value
Elevated fraction	1.413 (1.033-1.934)	0.031	1.799 (1.104-2.931)	0.018
Age	1.006 (0.992-1.015)	0.153	0.991 (0.977-1.004)	0.172
Female sex	1.027 (0.799-1.320)	0.834	0.954 (0.629-1.446)	0.824
Biologic use	0.935 (0.726-1.203)	0.599	0.720 (0.440-1.178)	0.191
History of IBD-surgery	1.585 (1.150-1.387)	0.004	2.571 (1.057-6.256)	0.037
CRP elevation (index visit)	1.219 (0.868-1.711)	0.253	1.318 (0.687-2.527)	0.406
Anemia (index visit)	1.318 (0.970-1.791)	0.078	1.870 (1.132-3.088)	0.015
Hypoalbuminemia (index visit)	3.197 (2.436-4.219)	<0.001	3.587 (2.670-4.683)	<0.001
	IBD-related surgery			
	Crohn's disease		Ulcerative colitis	
	AOR (95%CI)	P-value	AOR (95%CI)	P-value
Elevated fraction	1.559 (1.334-2.134)	0.006	1.229 (0.683-2.211)	0.492
Age	0.994 (0.985-1.004)	0.238	0.977 (0.959-0.995)	0.014
Female sex	1.031 (0.782-1.360)	0.829	0.806 (0.465-1.398)	0.443
Biologic use	0.972 (0.739-1.278)	0.839	1.081 (0.603-1.937)	0.795
History of IBD-surgery	1.113 (0.843-)	0.838	0.797 (0.296-2.143)	0.652
CRP elevation (index visit)	0.984 (0.677-1.430)	0.932	1.115 (0.501-2.481)	0.790
Anemia (index visit)	0.936 (0.668-1.311)	0.700	1.633 (0.894-2.981)	0.110
Hypoalbuminemia (index visit)	1.650 (1.218-2.235)	0.001	2.143 (1.347-2.974)	<0.001

AOR, adjusted odds ratio; CI, confidence interval; IBD, inflammatory bowel disease; CRP, C-reactive protein

directly or calculated as the difference between the serum total protein and serum albumin, was associated with higher levels of inflammatory biomarkers (CRP) and higher rates of healthcare utilization (including hospitalization) over the 4-year study period. The association between hospitalization and high globulin fraction remained significant on multivariable logistic regression analysis amongst CD and UC patients. These data suggest that increased globulin fraction may identify a subgroup of patients at risk for worse clinical trajectories, who may benefit from closer clinical monitoring and treatment.

Over the past several years, multiple studies have examined the role of biomarkers in diagnosing and predicting the behavior of IBD (including CRP, fecal calprotectin, and anemia) [13-17]. A number of studies evaluated the correlation between albumin levels and IBD status [13,14,18,19], but none have assessed globulin fraction as a biomarker in IBD.

The globulin fraction may play a crucial role in IBD, as excessive immune cell recruitment and activation has been detected in multiple immune cell subsets [19]. Globulin fraction seems to vary amongst our IBD patients; therefore, it could be used as a biomarker to stratify patients based on this indication of increased humoral immune activity. The patients with an elevated globulin fraction on initial visit had associated markers of biochemical severity at that initial visit. This finding supports the use of a high globulin fraction as a prognostic indicator for worse disease severity. CD patients with a high globulin fraction also had worse clinical outcomes, as reflected by a lower quality of life, higher disease activity and more healthcare utilization than IBD patients with a normal globulin fraction. UC patients with high globulin fraction, however, did not have a significantly lower quality of life or higher disease activity, probably because of the lower numbers of UC patients included.

In our study, after controlling for confounding factors, including hypoalbuminemia, increased globulin fraction remained an important, independent predictor of increased hospitalization for CD and UC patients, as well as IBD-related surgery for CD patients. This routinely available, low-cost laboratory test is a well-recognized biomarker in autoimmune

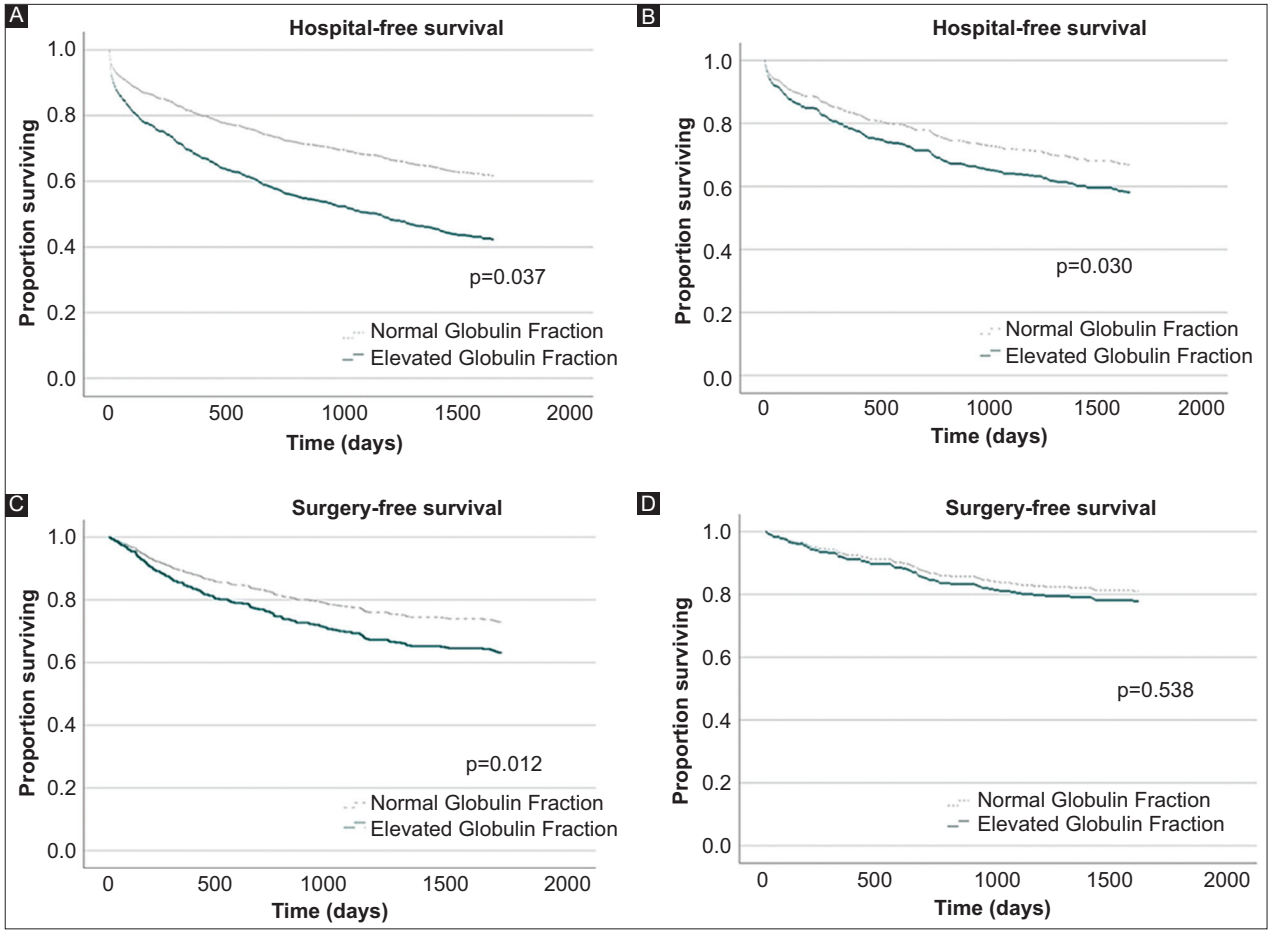

Figure 2 Cox regression survival analysis for hospitalization and inflammatory bowel disease (IBD)-related surgeries amongst patients with a high globulin fraction vs. normal globulin fraction. (A) Crohn's disease patients and hospitalization, (B) ulcerative colitis patients and hospitalization, (C) Crohn's disease patients and IBD-related surgeries, and (D) ulcerative colitis patients and history of IBD-related surgeries. All of these results are after controlling for differences in age, sex, history of IBD-surgery, presence of anemia, elevated C-reactive protein and hypoalbuminemia on the day of globulin fraction testing

liver diseases, as well as other autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis, but has not yet become the focus of dedicated investigation in IBD [5,20]. More recently, several studies have characterized immunoglobulin levels in patients with IBD [6]. Elevated immunoglobulin levels have been reported in conditions such as autoimmune hepatitis. Furthermore, several studies have suggested that increased immune reactivity and B-cell dysregulation play an important part in the pathogenesis of IBD [21,22]. A recent study by Wang *et al* suggested that circulating memory B-cells and plasmablasts are associated with the level of immunoglobulins and disease activity in UC patients [23]. Our findings are further strengthened by Shiraishi *et al*, who demonstrated a similar clinical signal in 277 UC patients, where an elevated globulin fraction was associated with lower rates of mucosal healing [24].

There are several important limitations to this proof-of-concept study that aims to show that globulin fraction can be used as a biomarker of disease activity in IBD patients. This was an observational study that was carried out at a tertiary referral center, whose patients may not be reflective of the IBD patients followed in the general community. The patients were included during different stages of their disease. To control for these potential limitations, patients with a 4-year follow-up period were included and multivariate analyses were conducted to control for age, sex, and other baseline characteristics of patients. Additionally, our structure for assessing multiyear patterns of disease severity has not been widely established, although it has been used for a number of published studies. We did not fully characterize the immunologic mechanisms which were associated with the increased globulin fraction in our IBD patient cohort, but as in other conditions with increased globulin fraction, we feel that this will be most likely linked to increased humoral immune activity in this patient population. Our goal in the present study was to determine whether this biomarker was found in a subset of patients and was potentially linked to a unique, more severe clinical trajectory. Once this first step was accomplished, then a more detailed investigation of these mechanistic questions could be further developed. Prospective studies are needed to confirm our findings.

In conclusion, our study demonstrates that increased globulin fraction is a readily available biomarker that is associated with a more severe clinical course in a subgroup of patients with IBD and is independent of low albumin. On multivariate analysis, elevated globulin fraction was associated with hospitalization amongst CD and UC patients as well as IBD-related surgeries in CD patients, after controlling for hypoalbuminemia. The use of globulin fraction adds no extra cost or effort in the evaluation of IBD patients as this is a routinely ordered test. The mechanism behind the increased globulin fraction remains unclear and the durability of this biomarker of severity requires further study, but it could be related to immune dysregulation and plasmablasts.

Summary Box

What is already known:

- The clinical implications of a high globulin fraction in multiyear inflammatory bowel disease (IBD) severity are unknown
- Globulin fraction is associated with endoscopic disease severity in ulcerative colitis
- Globulin fraction is a readily available component of routine bloodwork

What the new findings are:

- IBD patients with a high globulin fraction had higher rates of C-reactive protein elevation and anemia during a multiyear follow up
- IBD patients with a high globulin fraction had higher rates of biologic use during a multiyear follow up
- IBD patients with a high globulin fraction had higher rates of healthcare utilization during a multiyear follow up

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Supplementary material

Supplementary Table 1 Location characteristics using the Montreal classification

CD				
	Total	High globulin	Normal globulin	P-value
Total	644	183	461	
L1 (Ileal)	144 (22.4%)	32 (17.5%)	112 (24.3%)	0.518
L2 (colonic)	138 (21.4%)	45 (24.6%)	93 (20.2%)	0.242
L3 (Ileocolonic)	343 (53.2%)	100 (54.6%)	243 (52.7%)	0.663
L4 (isolated upper GI disease)	19 (3.0%)	6 (3.3%)	13 (2.8%)	0.797
UC				
	Total	High globulin	Normal globulin	
Total	256	57	199	
E1 (ulcerative proctitis)	14 (5.5%)	0 (0)	14 (7.0%)	0.083
E2 (left sided UC, distal)	76 (29.7%)	16 (28.1%)	60 (30.2%)	0.870
E3 (extensive UC, pancolitis)	166 (64.8%)	41 (71.9%)	125 (62.8%)	0.271

GI, gastrointestinal; CD, Crohn's disease; UC, ulcerative colitis

Supplementary Table 2 Distribution of comorbidities associated with increased serum globulins in IBD patients with and without elevated serum globulin fraction

Clinic/comorbidities	Elevated globulin fraction	Normal globulin fraction	Total N	P-value
Hepatology clinic	28.30%	12.90%	100	0.001
Hematology/ Oncology clinic	9.40%	2.70%	26	0.002
Rheumatology clinic	18.80%	12.10%	83	0.48
Autoimmune hepatitis/hepatitis C/cirrhosis	7.20%	1.70%	18	0.002
RA/lupus/Sjögren's	5.10%	2.70%	20	0.18

RA, rheumatoid arthritis