Significance of granulomas in the outcomes of Crohn's disease patients

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Abstract

Background The presence of granulomas in the gastrointestinal (GI) tract is one of the characteristic histologic features of Crohn's disease (CD). The clinical significance of granulomas remains unclear. In this study, we aimed to determine whether the presence of granulomas on endoscopic pinch biopsy or surgical resection from the upper or lower GI tract is associated with worse outcomes among patients with CD.

Methods This was a retrospective chart review of patients with CD evaluated at a tertiary care center between 1996 and 2019. Patients were divided into 2 groups based on the presence or absence of granulomas on GI histology. Clinical and laboratory data, and outcomes of interest, were obtained from the electronic medical records. Patients' characteristics and outcomes were compared between the 2 groups.

Results A total of 237 patients were included in our study; 41 (17.3%) had granulomas on their biopsy/resection specimen. The presence of granulomas in the GI tract was significantly associated with the development of intra-abdominal abscesses and/or fistulas (P=0.037), greater utilization of immunomodulators (P=0.029), and greater use of immunosuppressive medications (immunomodulator and/or biologic therapy) (P=0.015). No significant differences were found between the 2 groups in terms of number of hospitalizations, presence of perianal disease, intestinal resection, mean age, mean age at initial diagnosis of CD, duration of disease, sex, or smoking history.

Conclusions The presence of granulomas in the GI tract of CD patients may serve as a prognostic biomarker of worse disease severity. Larger studies are needed to better validate this finding.

Keywords Crohn's disease, granuloma, prognosis, disease severity, immunosuppressive medications

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Conflict of Interest: None

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Introduction

Crohn's disease (CD) is a chronic inflammatory condition of the gastrointestinal (GI) tract characterized by a progressive and unpredictable relapsing/remitting course [1]. Patients are often diagnosed in their early adolescent years, with a second spike in their sixth decade of life [2]. The main goals of therapy are to achieve mucosal healing and stop the inflammatory process. In turn, control of inflammation improves patient's symptoms, improves quality of life and prevents complications [3]. Currently, decisions regarding CD management are driven by multiple factors, including disease activity, severity, and the presence of disease complications. Stratification of patients is based on endoscopic, clinical,

laboratory and molecular markers. This allows for treatment optimization according to disease profile and severity [4].

The presence of epithelioid non-caseating granulomas on histology has been described as the hallmark of CD. Granulomas are present in 10-40% of patients with CD and have been investigated as a marker of disease activity [5]. A granuloma is usually composed of epithelioid cells, multinucleated giant cells and lymphocytes, which mediate a Th1 response to activate the immune system [6]. Some histopathological classification schemes list the presence of granulomas as a definite criterion for the diagnosis of CD; nonetheless, the significance of granulomas in CD pathogenesis and prognosis remains unclear, with several studies leading to conflicting results [7].

Some studies have shown that granulomas found on random endoscopic biopsies were more localized to the ileocolonic region and were associated with penetrating and stricturing disease [8,9]. A large-scale cohort study revealed that the presence of ileal and colonic granulomas on histology was associated with a more aggressive disease phenotype, defined as higher rates of medication use (steroids, immunomodulators, biologics, and narcotics), higher rates of hospitalizations, and higher levels of serologic inflammatory markers [10]. Another study demonstrated that granulomas on pinch biopsies from endoscopic procedures were more evident in the colonic region and were associated with stricturing disease, whereas no association was found with perianal complications or extraintestinal manifestations [11]. In contrast, a recent study revealed that granulomas were associated with an increased risk of perianal fistula but not strictures [12]. A relatively older study, on the other hand, found no association between the presence of granulomas on biopsy or surgically resected specimens and the severity of the CD course [13].

The aim of our study was to identify the significance of granulomas in the histology of patients with CD, specifically defining their role as a predictive marker of outcomes of CD, as defined by the need for intestinal surgery, the frequency of hospitalizations, the presence of perianal, penetrating or stricturing disease, and the need for immunomodulator or biologic medications. We hypothesized that patients with CD who harbor granuloma on tissue from their GI tract would have a more aggressive phenotype, as reflected by noninvasive markers of inflammation (serologic markers), and would also show greater healthcare utilization (medications and hospitalizations).

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Patients and methods

We carried out a retrospective chart review of patients with CD who underwent a surgical resection or endoscopy with pinch biopsy at the American University of Beirut Medical Center (AUBMC) between January 1996 and January 2019. During this 23-year period, each patient was followed-up from their date of inclusion until January 2019. Patients were identified by screening the Department of Pathology database using the search key terms "CD", "ileitis", "colitis", "ileal CD", "CD colitis", "inflammatory bowel disease", and "granuloma". The medical records of these patients were reviewed and those with confirmed CD were included in the study. Patients were then divided into 2 groups based on the presence or absence of granulomas on their histology. Granulomas associated with foreign bodies (suture material or food material) and those associated with infectious organisms, such as mycobacteria or fungi, were excluded from the study. The American University of Beirut Institutional Review Board has approved this study design (BIO-2018-0624).

Data were collected through an elaborate retrospective chart review using the electronic medical record system of the hospital. The data collected included patient demographics; CD details pertaining to areas affected by CD; complications of CD, including perianal disease; intra-abdominal abscess and/or fistula; intestinal strictures; number of CD-related hospitalizations; CD-related surgeries; and medication use (immunomodulators or biologics). Laboratory data was also obtained from the charts, including the highest value recorded during the study period for C-reactive protein, erythrocyte sedimentation rate, white blood cell count, differential, and platelet count. The lowest values recorded during the study period for hemoglobin (Hb) and vitamin D levels were also recorded.

Statistical analysis

The data were analyzed to assess whether there were associations between the presence of granuloma on biopsy specimens and the previously predefined disease severity parameters. The association between granulomas and other categorical variables was assessed using the chi-square test, while Student's *t*-test was used for the association with continuous variables.

Results

A total of 237 patients with CD were included in our study, of whom 41 (17.3%) had granulomas on their surgical or endoscopic specimens. The average age at diagnosis was 36.7 years, 151 patients (63.7%) were male, and patients had a mean duration of disease of 6.8 years. Ninety-five patients (47.3%) were active smokers. The majority of patients included in the study had ileocolonic CD as per the Montreal

classification (89.3%). Forty-seven patients (19.83%) had upper GI involvement. Of the patients included in the analysis, 14.34% had a history of perianal disease, 9.7% had intra-abdominal abscess and/or fistula, and 17% had intestinal strictures. Granulomas were mainly found in tissue from the lower GI tract (80.5%), while only 6 patients had upper GI tract granulomas (14.63%) in their gastric and/or duodenal tissue (Table 1). Table 2 displays the demographic and disease characteristics of the patients included in the study and a summary of their laboratory tests.

Comparison of patients with CD with and without granulomas

Patients with granuloma were compared to those without. Significant associations were noted between the presence of granulomas and the development of intra-abdominal abscesses and/or fistulas (P=0.037), greater utilization of immunomodulators (P=0.029), and greater use of immunosuppressive medications (immunomodulator and/or biologic therapy) (P=0.015). When comparing patients receiving combination therapy (immunomodulator and biologic agent), no difference was found between patients who had granulomas and those who did not. Table 3 shows the differences between the CD patients with granuloma and those without.

In contrast, no difference was found between patients with and without granuloma with regard to mean age, mean age at initial diagnosis of CD, duration of disease, sex, active smoking, laboratory values, number of hospitalizations, presence of perianal disease, intestinal resection, or use of steroids or biologic agents.

Discussion

The exact etiology and pathogenesis of CD remain unclear. Current understanding is that CD is the product of complex interactions between environmental risk factors, genetic susceptibility and the innate gut microbiome, which result in a dysregulated immune response. One of the distinguishing factors of CD is the presence of epithelioid granulomas. As defined by Dalziel in 1913, a granulomatous response is a collection of macrophages, lymphocytes and epithelioid cells. It is known that multiple infectious organisms can cause granulomas, such as *Mycobacterium* tuberculosis, *Salmonella* and *Yersinia*, yet there has not been a consistent detection of organisms within CD granulomas [14]. Despite being a hallmark of CD, granulomas are neither specific nor sensitive for CD.

In this analysis of a large, retrospective, multiyear observational cohort, we characterized the relationship between epithelioid granulomas and disease severity in patients with CD. We found that the presence of granulomas on histology from either endoscopic pinch biopsy or surgical resection specimen of patients with CD was associated with

Table 1 Location of granulomas and how they were diagnosed

Location of the granulomas	Value
Upper GI tract	6 (14.63%)
Lower GI tract	33 (80.46%)
Upper and Lower GI tract	2 (0.48%)
Granulomas found in surgery vs. biopsy	
Surgery	6 (14.3%)
Biopsy	35 (85.6%)

GI, gastrointestinal

Table 2 Characteristics of patients, treatments and outcomes (N=237)

Characteristics	Total N=237
Age at diagnosis, mean±SD (years)	36.75±17.5
Duration of disease (years)	6.82±5
Sex	
Male	151 (63.71%)
Female	86 (36.28%)
Active smoking	95 (47.26%)
History of Crohn's disease related surgery	31 (13.08%)
Highest ESR, mean±SD (mm/h)	35.76±28.31
Highest CRP, mean±SD (mg/L)	59.65±83.24
Highest WBC, mean±SD (/μL)	12580.85±6058.72
Highest platelets, mean±SD (/μL)	394125.58
Lowest hemoglobin, mean±SD (g/dL)	11.30±2.28
Highest eosinophils, mean±SD (%)	4.42±3.50
Lowest vitamin D, mean±SD (ng/mL)	18.02±10.60
Steroid use	96 (41.20%)
Immunomodulators use	104 (44.44%)
Methotrexate use	19 (8.08%)
Thiopurine use	88 (37.77%)
Biologic use	120 (51.28%)
Vedolizumab use	11 (4.71%)
Adalimumab use	67 (28.64%)
Infliximab use	63 (26.80%)
Ustekinumab use	22 (9.44%)
Certolizumab use	2 (0.86%)
Number of hospitalizations, mean±SD	0.71±1.32
Location as per Montreal classification for CD	
L1 (Ileal)	78 (33.3%)
L2 (Colon)	112 (47.9%)
L3 (Ileocolonic)	209 (89.3%)
L4 (Upper GI)	47 (19.83%)
Behavior as per Montreal classification for CD	
B1 (Non structuring, non penetrating)	174 (73.41%)
B2 (Stricturing)	40 (16.88%)
B3 (Penetrating)	23 (9.71%)
p (Perianal disease)	34 (14.34%)

SD, standard deviation; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; WBC, white blood cells; CD, Crohn's disease

multiple markers of disease activity and severity. The markers of disease severity included disease complications, such as

Table 3 Comparison of patients with CD who had GI granulomas and those without granulomas

Granuloma	No	Yes	P-value
	N=196	N=41	
Patient characteristics			
Mean age at diagnosis±SD (years)	37.2±17.8	34.8±16.2	0.421
Duration of disease (years)	6.6±5.1	7.7±4.2	0.238
Sex			
Male	128 (65.3%)	23 (56.1%)	0.265
Female	68 (34.7%)	18 (43.9%)	0.265
Active smoking	77 (46.1%)	18 (52.9%)	0.467
Laboratory values			
Highest ESR, mean±SD (mm/h)	35.3±28.6	37.7±27. 2	0.689
Highest CRP, mean±SD (mg/L)	58.6±85.2	64.4±74.3	0.714
Highest WBC, mean±SD (/μL)	12,582±6172.2	12,571±5573. 1	0.992
Highest platelets, mean±SD (/μL)	395,623±317,019	386,919±114,046	0.869
Lowest hemoglobin, mean±SD (g/dL)	11.4±2.3	10.9±2.2	0.199
Highest eosinophils, mean±SD (%)	4.3±3.5	4.9±3.7	0.342
Lowest vitamin D, mean±SD (ng/mL)	17.8±9.9	19.1±13.8	0.552
Outcomes			
CD-related intestinal surgery	23 (11.7%)	8 (19.5%)	0.179
Number of CD-related hospitalizations, mean±SD*	0.65±1.31	0.98±1.35	0.151
Perianal disease	26 (13.5%)	8 (19.5%)	0.319
Intra-abdominal abscess and/or fistula	15 (7.7%)	8 (19.5%)	0.037
Intestinal stricture	30 (15.5%)	10 (24.4%)	0.167
Medication use*			
Number of patients using steroids	83 (43.2%)	13 (31.7%)	0.174
Methotrexate use	12 (6.2%)	7 (17.1%)	0.052
Thiopurine (azathioprine or 6-mercaptopurine) use	69 (35.9%)	19 (46.3%)	0.212
Any Immunomodulator use (methotrexate or thiopurine)	76 (38.7%)	24 (58.5%)	0.019
Infliximab use	51 (26.3%)	12 (29.3%)	0.696
Adalimumab use	56 (29%)	11 (26.8%)	0.779
Certolizumab use	2 (1%)	0 (0%)	>0.99
Vedolizumab use	10 (5.2%)	1 (2.4%)	0.694
Ustekinumab use	16 (8.3%)	6 (14.6%)	0.237
Any anti-TNF use	102 (52%)	21 (51.2%)	0.923
Any biologic use	96 (49.7%)	24 (58.5%)	0.306
Need for 2 or more biologics	26 (13.2%)	5 (12.1%)	0.853
Need for 3 or more biologics	9 (4.5%)	1 (2.4%)	0.532
Use of any immunosuppressive medication (biologic and/or immunomodulator)	108 (55.1%)	31 (75.6%)	0.015

*Duration of study period

CD, Crohn's disease; GI, gastrointestinal; SD, standard deviation; TNF, tumor necrosis factor; WBC, white blood cells; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein

intra-abdominal abscesses and/or fistulas, and the degree of utilization of immunomodulators or immunosuppressive medications. The data support the hypothesis that histologic evidence of granulomas may be used as a prognostic biomarker of disease severity and can predict the need for more aggressive therapy in patients with CD. However, it remains unclear why the presence of granuloma is associated with a more severe phenotype. It is plausible that the granuloma is the site where antigen speciation and Th1 cellular differentiation occurs, resulting in an innate host response. Whether there is an exact etiological agent residing in the granuloma remains to be determined [15,16].

The overall percentage of patients found to have granulomas in this study was 17.3%. Although most studies have previously reported rates within the range of 20-50% [17-19], some had rates up to 66% [20]. In a similar study utilizing a large inflammatory bowel disease database that included 1466 patients with CD, 187 patients (12.8%) had granulomas identified [10]. Population studies have shown that patients of Asian descent had higher rates of granulomas [11], and it should be noted that the population in this study was predominantly of Middle Eastern descent. We speculate that there may be certain genetic polymorphisms that play a role in granuloma formation among different ethnicities. It is also possible that the lower detection rate in this study's population may be related to the number of biopsies taken during endoscopic evaluation. In addition, the decision to obtain a biopsy from a particular location is operator-

dependent and could have affected the yield of granuloma detection.

In this study, patients with granulomas were found to have greater need and use of immunomodulators. This pattern of medication use points to a more aggressive disease phenotype. Interestingly, there was no significant difference in the use of biologics among the 2 groups. There was also no significant difference in the degree of elevation of inflammatory biomarkers. To our knowledge, this has been evaluated in only one study by Johnson *et al*, which found a greater degree of utilization of both immunosuppressant and immunomodulator medications, including biologics, and in the degree of elevation of inflammatory markers among patients with granulomas [10].

Disease behavior was also analyzed in this study, revealing that patients with granulomas were more likely to have intra-abdominal complications and a more penetrating phenotype. This is in concordance with other studies that found that patients with granulomas have a more damaging disease. However, there was no significant association in this study between the presence of granulomas and perianal disease, in contrast to what has been previously reported [10,13].

One of the interesting findings of this study was the inverse relationship between smoking and the presence of granulomas. It has been noted that smoking has a negative association on granulomatous diseases such as sarcoidosis [21]. Tobacco glycoprotein (TGP) is an immunostimulatory substance that has been isolated from cigarette smoke and is responsible for lymphocyte proliferation and cytokine production [17,18]. There has been speculation that granulomas may have a decreased TGP-induced lymphocyte proliferation, hence lower rates of granuloma formation in the smoking population [22].

This study has several limitations. First, the retrospective nature of the study limited our ability to elucidate a cause–effect relationship between the different variables. Another limitation was the inconsistency in the number of years of patient follow up. Lack of standardization in the endoscopic (i.e., simple endoscopic score for CD) and pathology reports made it difficult to further analyze associations between the degree of histologic disease activity and the presence of granuloma. Additionally, our study included a combination of endoscopic and surgical specimens; this may have introduced a detection bias, as surgical specimens tend to have higher granuloma detection rates, given the larger surface area resected, and patients undergoing surgery are generally more ill. More studies including only pinch biopsies are needed to uncover such differences. The strengths of this study include the relatively large number of patients from a single institution.

Our study shows that the presence of granulomas in the GI tract of CD patients could be associated with a more aggressive disease phenotype. Even though a cause–effect relationship could not be established, the authors of this study believe that identifying granulomas on endoscopic and/or surgical biopsies should be considered when determining prognosis and used in the risk stratification of CD patients.

Summary Box

What is already known:

- Crohn's disease (CD) has an unpredictable relapsing and remitting course
- Non-caseating granulomas are a hallmark histologic finding for the diagnosis of CD
- The significance of granulomas in tissue of patients with CD remains controversial in terms of disease prognosis and disease severity

What the new findings are:

- The presence of granulomas was significantly associated with the development of intra-abdominal abscesses and/or fistulas
- The presence of granulomas was significantly associated with a greater utilization of immunomodulators and immunosuppressive medications
- Granulomas may serve as a prognostic biomarker for predicting disease severity in patients with CD

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