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AIRWAY BIOLOGY

Elevated Levels of Alveolar Nitric Oxide May Indicate Presence of Small Airway Inflammation in Patients with Inflammatory Bowel Disease

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Abstract

Introduction Pulmonary manifestations of inflammatory bowel disease (IBD), albeit not rare, are largely overlooked in clinical practice. The role of exhaled nitric oxide (eNO) as an established biological marker of airway inflammation compels us to use it as a tool to investigate the exact nature of these manifestations.

Methods Fractional eNO (FeNO) was measured in multiple flows, and with the use of a mathematical model, alveolar concentration of NO (C_ANO) and bronchial flux of NO (J_{aw}NO) were assessed in 27 patients with IBD [17 with Crohn's disease (CD) and 10 with ulcerative colitis (UC)] and in 39 healthy controls. Carefully selected criteria were used to exclude patients or healthy controls that presented factors considered to be correlated with eNO measurements. Disease activity was measured in Crohn's patients using the CD activity index (CDAI) score and in UC using the partial Mayo score.

Results C_ANO was significantly higher in the IBD group, compared to the control group ($p < 0.0001$). FeNO was significantly increased in patients with IBD ($p = 0.023$), while there was no statistical significance found regarding levels of J_{aw}NO in patients with IBD ($p = 0.106$), both compared to controls. There was no significant correlation between any eNO component and markers of disease activity.

Conclusions Alveolar concentration of NO is elevated in patients with IBD, regardless of disease activity. This may suggest that subclinical small airway inflammation is present in patients with IBD, even those with mild or inactive disease.

Keywords Inflammatory bowel disease · Exhaled nitric oxide · Small airway inflammation · Disease activity

Introduction

Inflammatory bowel diseases (IBD) are connected with many extraintestinal manifestations, observed in every single organ system of the human body. Among those, pulmonary manifestations are rarely observed and are systematically overlooked in clinical practice. However, there is a wide range of pulmonary disorders reported as possible extraintestinal manifestations of IBD.

Nitric oxide (NO) is a signaling molecule that contributes to many physiologic functions [1–4]. Studies have shown increased levels of NO in the serum [5] and colonic [6, 7] or rectal [8] mucosa of patients with active IBD. Fractional exhaled NO (FeNO) has been reported to correlate with airway hyperresponsiveness [9] and inflammation [10], resulting in it being used as standard clinical practice to diagnose asthma [11] and evaluate the effects of treatment in asthmatic patients [12]. eNO has been studied as a biomarker in various diseases of the lung [13–15] and utilized as a method of differentiating between them [16, 17]. Recent studies have demonstrated the reproducibility of eNO measurements, even in patients undergoing bronchodilation between them [18, 19].

Measuring eNO is far easier and cost-effective than measuring NO in blood, urine, or the gastrointestinal mucosa. The simplicity and cost-effectiveness of the method, as well as

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the already established link between IBD and NO, prompted researchers to evaluate eNO as a possible biomarker for pulmonary manifestations of IBD [20] and even disease activity [21–23]. Researchers have used FeNO to assess lung involvement in IBD. With the introduction of mathematical models regarding the concentration of NO in different parts of the lung [24], we are able to calculate alveolar concentration of NO (C_ANO), bronchial NO flux (J_{aw}NO), bronchial wall NO concentration (C_{aw}NO), and bronchial diffusing capacity of NO (D_{aw}NO) by measuring FeNO at multiple exhalation rates [25]. Notably, peripheral lung inflammation has been shown to correlate with the elevation of C_ANO levels, without simultaneous elevation of levels of J_{aw}NO, in various lung diseases [14, 26, 27].

The aim of this study was to investigate pulmonary manifestations in patients with IBD. Since pulmonary manifestations are rarely reported, we elected to use a marker indicative of airway inflammation, eNO. The findings of previous studies which have shown that eNO is elevated in patients with IBD, in conjunction with the ability to measure different components of eNO, each indicative of NO concentration in different parts of the lung, encouraged us to use these detailed eNO measurements to better understand lung pathophysiology in patients with IBD.

Methods

Study Population

The study population consisted of two groups, patients with IBD and controls. Furthermore, patients with IBD were divided into patients with Crohn's disease (CD) and ulcerative colitis (UC). Patients with IBD had histologically confirmed diagnosis of IBD and were recruited from the IBD outpatient clinic of the hospital, while the healthy subjects were recruited from the hospital staff and students of the university. Exclusion criteria for both groups were habits, diseases, or pharmacological treatments that have been reported to affect eNO levels such as positive current smoking status, current use of corticosteroids, diagnosis of asthma, allergic rhinitis, or any other respiratory disease (including respiratory infection within the last 4 weeks) [11, 28–32]. Both groups were evaluated with spirometry, in order to exclude the presence of restrictive or obstructive pulmonary disease. Spirometry was conducted in accordance with international guidelines [33]. Additionally, the subjects were evaluated for the possibility of allergic rhinitis, using the allergic rhinitis and its impact on asthma (ARIA) guidelines. Subjects with at least one symptom suggestive of allergic rhinitis were excluded from the study. Age, gender, weight, and height of every participant were also recorded.

Exhaled Nitric Oxide Measurements

An ECO-MEDICS/CLD88sp (Switzerland) analyzer was used for the measurement of $FeNO_{50}$, with respect to ATS/ERS guidelines [34]. All subjects were fasting and had not undergone physical activity prior to the measurements. Twelve patients with IBD underwent the procedure twice, in order to access the reproducibility of the results. If there was no significant difference between the two measurements, the first measurement was taken into account for the statistical analysis. Four different flows were used to measure eNO [30, 50, 100, 300 milliliters/second (ml/s)]. J_{aw}NO, C_ANO, C_{aw}NO, and D_{aw}NO were calculated using a mathematical model by measuring eNO in three different flows (30, 100, 300 ml/s). Measurements of FeNO, C_ANO, and C_{aw}NO were in parts per billion (ppb), measurements of J_{aw}NO were in picolitres/second (pl/s), and measurements of D_{aw}NO were in ml/s.

Disease Activity Determination

CD activity index (CDAI) was used to assess disease activity in patients with CD, using a score of 150 as the cut-off between clinical remission and active disease [35]. Partial Mayo score was used to define UC disease activity (disease activity classified as clinical remission, mild activity, moderate activity, and severe activity) [36].

Statistical Analysis

Study data were analyzed with IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, NY, USA). We used Student's t test to compare numeric values, ANOVA to compare numeric values of multiple groups, χ^2 test to compare categorical values, and Pearson's correlation coefficient to investigate the correlation between variables. Reproducibility of eNO measurements was evaluated with paired t tests, with limits of agreement demonstrated with the 95% confidence intervals of the difference between the two measurements. Values are described as mean ± standard deviation. p values < 0.05 were considered as statistically significant.

Results

Demographics

The study population comprised 66 subjects. There were 27 patients with IBD, 17 with CD and 10 with UC, and 39 healthy subjects. Baseline characteristics of all groups are portrayed in Table 1. The mean CDAI of the CD group was

Table 1 Baseline characteristics of patients with inflammatory bowel disease (IBD) and controls

	Controls	CD	UC	*p* value
Number	39	17	10	
Gender (men/women)	20/19	5/12	5/5	0.304
Age (years)	38 ± 17	39 ± 16	48 ± 14	0.229
Height (cm)	170 ± 9	166 ± 12	169 ± 10	0.287
Weight (kg)	78 ± 19	70 ± 19	71 ± 18	0.315
BMI	27 ± 6	25 ± 5.6	25 ± 4.3	0.49
Disease activity (CDAI/partial Mayo)		99 ± 82	2.7 ± 2.5	
Regimen (5ASA/azathioprine/anti-TNF)		0/6/11	5/0/5	

Values are depicted as mean ± standard deviation

98 ± 81, with 5 (29.4%) subjects being in clinical relapse and 12 (70.6%) being in clinical remission. The mean partial Mayo score of the UC group was 2.7 ± 2.5, with 3 (30%) subjects being in clinical remission, 5 (50%) having mild disease activity, 1 (10%) having moderate disease activity, and 1 (10%) having severe disease activity. Regarding the baseline characteristics (gender, age, height, weight, BMI), there was no statistical difference between the control group and the IBD group as a whole or the UC and CD groups independently.

Pulmonary Function Tests

None of the patients of both groups had an FEV_1/FVC ratio lower than 70% or FEV_1 lower than 70% predicted. Patients with IBD had mean predicted FEV_1 of 102.96 ± 13.81%, mean predicted FVC of 99.22 ± 15.69%, and mean predicted $FEF_{25–75}$ of 106.52 ± 23.24%. Controls had a mean predicted FEV_1 of 103.46 ± 11.02%, mean predicted FVC of 105.03 ± 10.9%, and mean predicted $FEF_{25–75}$ of 108 ± 11.81%. There was no statistically significant difference between the FEV_1, FVC, and $FEF_{25–75}$ measurements of the two groups ($p = 0.071$, 0.103, and 0.762, respectively). None of the healthy controls had $FEF_{25–75} < 80\%$ predicted, while five patients with IBD had < 80% predicted (4 with CD, 1 with UC) and 1 had < 70% predicted.

eNO Measurements

Regarding the reproducibility results from the 12 patients by whom C_ANO, J_{aw}NO, and FeNO were assessed twice, there was no statistical difference between the eNO parameters. C_ANO, J_{aw}NO, and FeNO had a 0.883, 0.949, and 0.892 correlation coefficient with the respective second measurements. The limits of agreement were − 1.25 to 0.85 ($p = 0.683$), − 173.2 to 578.2 ($p = 0.26$), and − 4.54 to 7.32 ($p = 0.616$) for C_ANO, J_{aw}NO, and FeNO, respectively. eNO measurements for all groups are depicted in Table 2.

FeNO levels were significantly higher in the IBD group, compared to the control group ($p = 0.023$, Fig. 1). More importantly, patients with IBD had significantly elevated levels of C_ANO compared to the control group ($p < 0.0001$, Fig. 2). There was no statistically significant difference observed between the levels of J_{aw}NO in the IBD and control groups ($p = 0.106$, Fig. 3).

Table 2 Nitric oxide measurements of patients with inflammatory bowel disease (IBD) and controls

	Controls	IBD	CD	UC
FeNO (ppb)	24 ± 12	34 ± 19	31 ± 19	40 ± 18
C_ANO (ppb)	1.9 ± 1	5 ± 3.2	4.8 ± 3.6	5.3 ± 2.4
J_{aw}NO (pl/s)	1708 ± 1061	2283 ± 1571	2142 ± 1723	2523 ± 1324
C_{aw}NO (ppb)	60 ± 55	79 ± 63	73 ± 69	88 ± 53
D_{aw}NO (ml/s)	45 ± 30	49 ± 41	54 ± 47	41 ± 29

Values are depicted as mean ± standard deviation

FeNO and J_{aw}NO levels were significantly higher in the UC group, compared to the control group ($p = 0.003$ and 0.046, respectively). There was no statistically significant difference in the levels of D_{aw}NO or C_{aw}NO between the three groups. Additionally, there was no significant difference observed between the levels of FeNO and J_{aw}NO in the CD and control groups.

C_ANO in both IBD groups was significantly higher than in the control group even when the smaller groups were independently analyzed ($p = 0.005$ in CD group, $p = 0.001$ in UC group). Even when we compared C_ANO between controls and patients with CD being in remission (CDAI < 150, $n = 12$) or patients with UC that had inactive or mild disease (partial Mayo ranking of 0 or 1, $n = 8$), the C_ANO levels were significantly higher in both IBD groups ($p = 0.024$ and 0.005, respectively). Regarding disease activity, no correlation was observed between any eNO parameter and CDAI, partial Mayo score, or C-reactive protein. There was a significant inverse correlation between C_ANO and $FEF_{25–75}$ measurements in patients with IBD ($p = 0.003$). Furthermore, no correlation was observed between the type of therapeutic regimen and all eNO parameters.

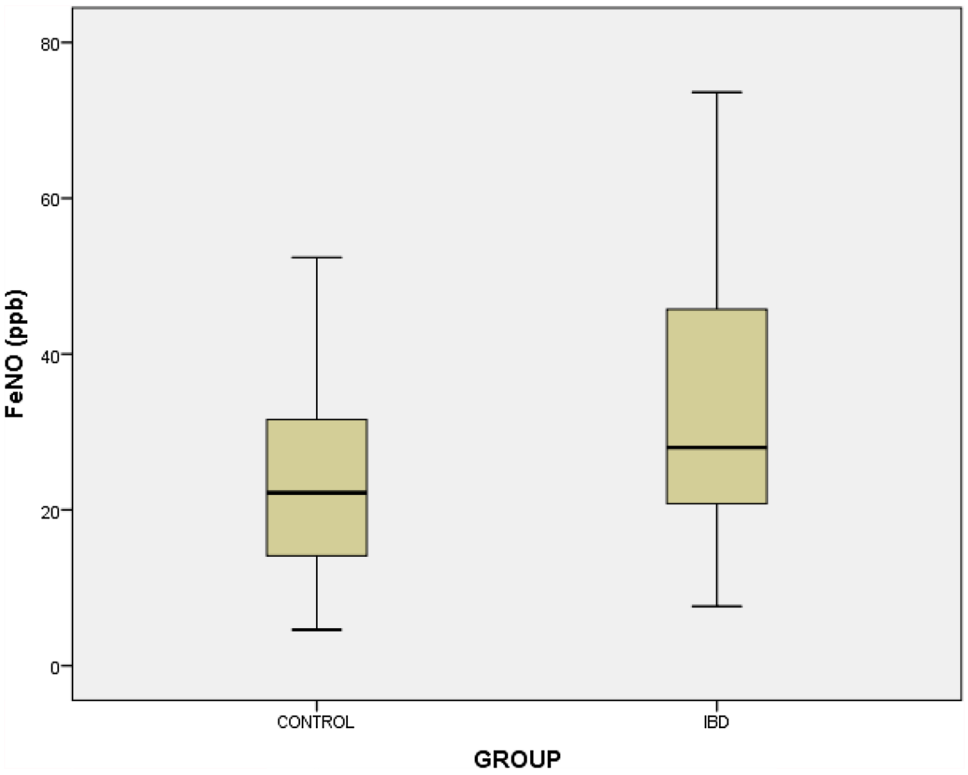

Fig. 1 $FeNO_{50}$ values. There is a statistically significant difference observed between $FeNO_{50}$ levels of the IBD and control groups ($p = 0.023$)

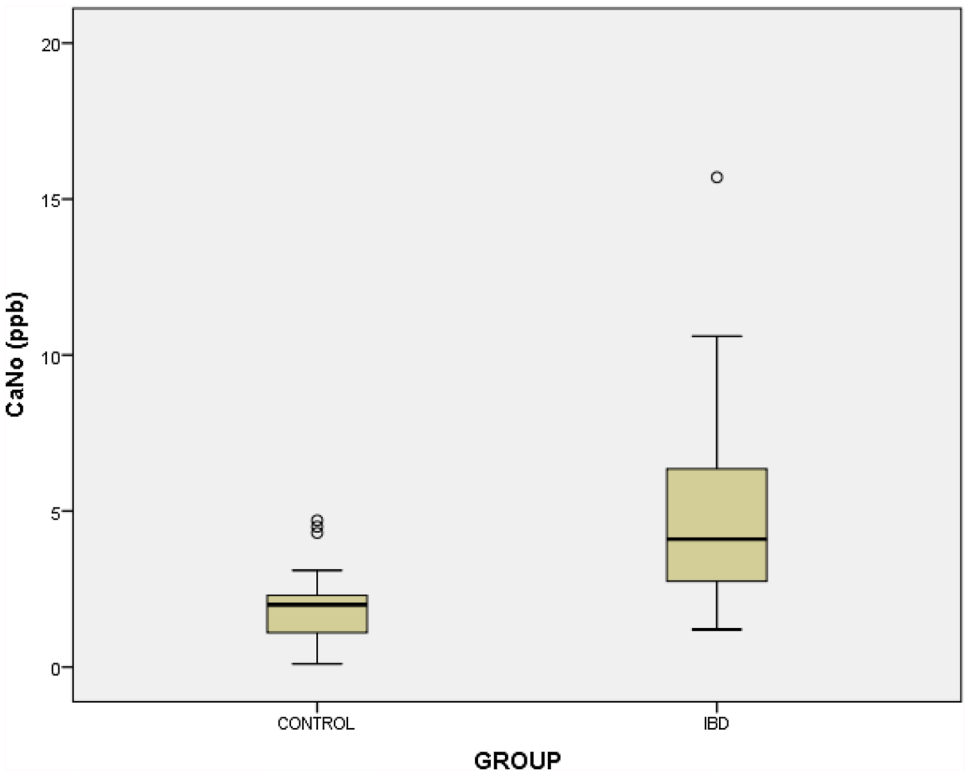

Fig. 2 $C_A NO$ values. There is a highly statistically significant difference observed between $C_A NO$ levels of the IBD and control groups ($p < 0.0001$)

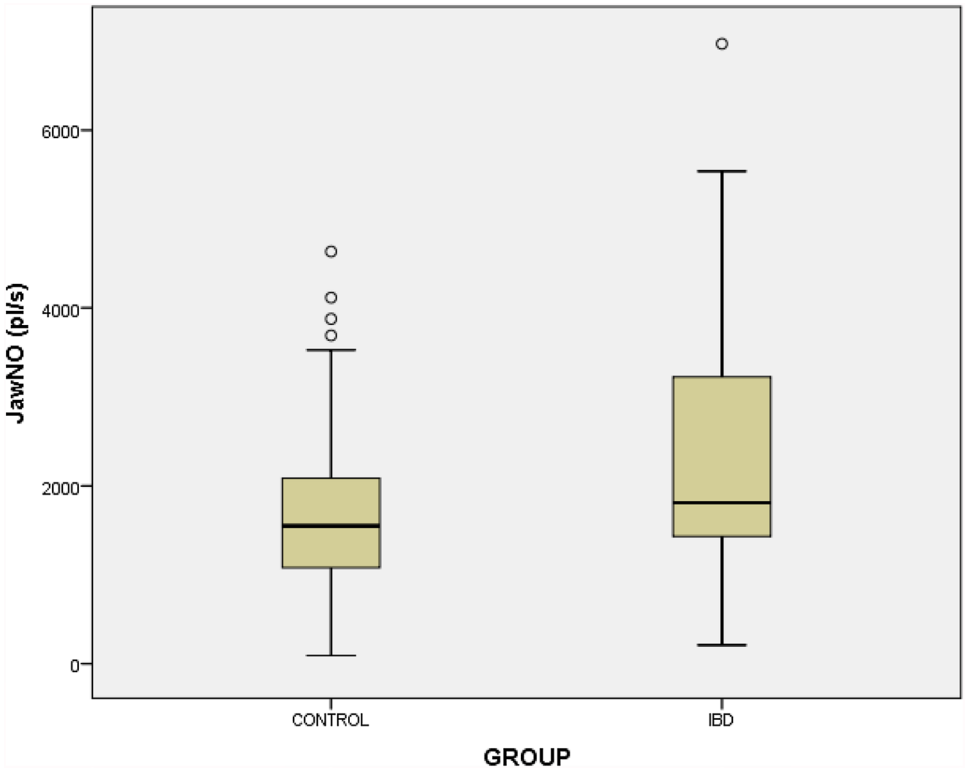

Fig. 3 J_{aw}NO values. There was no statistically significant difference observed between J_{aw}NO levels of the control and IBD groups ($p = 0.106$)

Discussion

The main outcome of this study was that alveolar NO levels are significantly elevated in both CD and UC. Even in patients considered as being in clinical remission or with mild disease, C_ANO was significantly higher than in healthy controls.

Small airway involvement in IBD has been reported in the past. Tzanakis et al. [37] showed that certain pulmonary function tests (PFTs), which are indicative of small airway function, are negatively affected in patients with IBD. Yilmaz et al. [38] demonstrated that FEF_{25-75}, a marker of small airway function, was lower in patients with IBD compared to controls. Two studies obtained bronchoalveolar lavage from patients with CD and found evidence of subclinical alveolitis in most patients [39, 40].

C_ANO is considered a marker of small airway inflammation and it has been thoroughly studied in asthma [10, 26, 27, 41, 42] and other pulmonary diseases [14, 41, 43]. In our study, the fact that even the patients that were in clinical remission had significantly higher C_ANO levels than controls may indicate that subclinical small airway inflammation is present even in the absence of disease activity, something that has only been proposed for large airway involvement in past studies [44, 45]. The vast difference ($p < 0.0001$) observed between patients and healthy subjects regarding alveolar concentrations of NO reinforces the suggestion that subclinical small airway inflammation exists in patients with IBD.

The exact pathophysiologic mechanism responsible for the occurrence of pulmonary manifestations of IBD has not been adequately described yet. Most theories revolve around common links between intestinal and pulmonary mucosa. Specifically, the intestinal and pulmonary mucosa share a common embryological origin, as they both arise from the primitive foregut [46]. Additionally, both epitheliums contain lymphoid tissues (gut-associated lymphoid tissue and bronchus-associated lymphoid tissue) that are components of the mucosa-associated lymphoid tissue, playing a major role in the immune response of both organs. These commonalities between the mucosal layers of the two organs have given rise to theories that inflammatory mediators and other luminal contents that can cause systemic inflammation are absorbed by intestinal lumen, enter the systemic circulation, and induce pulmonary inflammation by binding to receptor molecules that are found in both organs [47].

This is the first study to assess J_{aw}NO in patients with IBD. The demonstrated elevation of C_ANO in these patients along with the absence of elevation of J_{aw}NO is very important, since it draws the line between the pathology of the large and the small airway, indicating that only the latter is involved as a result of extraintestinal manifestations of IBD, at least in people with mild disease. However, we must not overlook the fact that the patients in the group of UC had also significantly higher J_{aw}NO compared to the controls.

The small group of patients and the modest level of significance ($p = 0.046$) indicate that these results should be replicated in a bigger cohort of patients in order to establish their validity. Nonetheless, a possible explanation could be hiding behind the fact that there are studies that have reported a higher incidence of large airway manifestations in patients with UC compared to patients with CD [48, 49].

There have been a group of heterogeneous studies that have studied eNO in IBD. Koek et al. demonstrated that patients with IBD have elevated levels of FeNO and found a correlation between eNO levels and disease activity [21]. Ozyilmaz et al. displayed that FeNO is elevated in patients with IBD and showed that FeNO levels are higher in patients with confirmed pulmonary involvement [20]. Malerba et al. published findings that concurred with the findings of the previous studies regarding FeNO levels and showed that C_ANO is elevated in CD [23]. Quenon et al. argued that FeNO is elevated in CD patients with active disease, but decreased in CD patients in remission, compared to healthy controls [22].

Compared to previous studies, there were some differences in the outcomes, the most important of which is not being able to correlate eNO levels with disease activity. This can be attributed to differences in patient recruitment methods, since our patients were recruited from an outpatient clinic and most of them were in clinical remission or had mild symptoms at the time of the study. Our study agrees with the findings of previous studies regarding the elevation of eNO in patients with IBD compared to healthy controls, but also demonstrates for the first time that C_ANO and not J_{aw}NO is responsible for that elevation. $FEF_{25–75}$ was within normal values for most patients with IBD, something that can be attributed to the careful selection of patients and the presence of multiple exclusion criteria. However, the inverse correlation between C_ANO and $FEF_{25–75}$ indicates a possible link between small airway inflammation and small airway function in patients with IBD.

Our study has both advantages and limitations. We studied all components of eNO by using different flow rates. We comprehensively evaluated our patients to eliminate the possibility of co-existing pulmonary disorders or possible confounders. We acquired detailed medical history (smoking status, corticosteroid use, previous diagnosis of pulmonary disease, etc.), tested for allergic rhinitis, and conducted spirometry to evaluate PFTs. Our control group's baseline characteristics were matched with the baseline characteristics of both IBD groups. Additionally, by measuring eNO twice in some patients, we confirmed the reproducibility of our method. The major limitation of our study was the small study population. Since small airway function parameters such as $FEF_{25–75}$ provide different information compared with eNO, which is an inflammatory marker, high-resolution computed tomography of the lung seems to be ideal in determining the degree of small airway disease [50–52] in these patients. Nevertheless, considering the high levels of radiation and the fact that our study consisted of patients without known respiratory disease, we could not perform it to our patients for ethical reasons.

On the surface, the fact that our study was comprised mostly of patients that did not have active disease at the time seems to be a limitation. In reality, that is a blessing in disguise, since most studies examining pulmonary manifestations of IBD focus on patients with active disease, overlooking patients being in clinical remission. Additionally, clinical remission does not necessarily mean complete remission of the disease, with studies showing that mucosal inflammation is present in a significant number of patients with IBD considered to be in clinical remission [53, 54]. Perhaps studies that will directly investigate the correlation between intestinal mucosal inflammation and pulmonary involvement can be more accurate in evaluating the link between intestinal and lung inflammation. Nevertheless, it seems that C_ANO assessment could be utilized as a marker of severity of the patients with IBD exhibiting alveolitis who may need treatment for their lung involvement.

To conclude, our study suggests that subclinical inflammation is present in the small airways of patients with IBD, regardless of disease activity. Larger, prospective studies must be conducted in order to assess the probability of this subclinical entity progressing to clinical pulmonary disease.

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Compliance with Ethical Standards

Conflicts of interest None.

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