Induced sputum eosinophilia in ulcerative colitis patients: The lung as a mirror image of intestine?

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Received 15 October 2008; accepted 15 January 2009

KEYWORDS
Eosinophils;
Induced sputum;
Ulcerative colitis

Summary
Background: Ulcerative colitis (UC) is a systemic disease of unknown etiology with extra-intestinal manifestation. Induced sputum (IS) non-invasively assesses extrapulmonary involvement in Crohn’s disease. We sought to determine whether there is a cellular marker of lung injury in UC patients detectable by IS.

Methods: Nineteen UC patients (mean age 46.4 ± 11.3 years, disease duration 8.6 ± 7.5 years [range 1–25 years] 68.4% males) were studied, 6 with active disease and 13 in remission. Eleven received 5-ASA, 5 received steroids and/or azathioprine and 3 patients were untreated. UC patients were compared with 27 healthy non-smoker controls. IS was recovered after 20 min inhalation of 3% saline with an ultrasonic nebulizer by the selecting plugs method, and 300 cells were differentially cell counted in cytospin Giemsa-stained slides. CD4/CD8 subsets were identified by FACS. Pulmonary function tests were performed by the Jaeger Masterlab spirometer.

Results: UC patients’ IS contained higher % eosinophils than controls (p = 0.05) and lower FEV1/FVC ratios (p = 0.001). Steroid- and/or azathioprine-treated patients had significantly lower FEV1/FVC ratios than only 5-ASA-treated patients (p = 0.019). Eosinophil infiltration in airways was high in 5-ASA-treated patients compared to those receiving steroids and/or azathioprine (p = 0.046) and those with less extensive disease (p = 0.05). Using a cutoff of 3% eosinophils, IS had a sensitivity of 67% and specificity of 73% to differentiate patients with a cutoff of 70 eosinophils/mm² in biopsy.

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0954-6111/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2009.01.016

Please cite this article in press as: Fireman E et al., Induced sputum eosinophilia in ulcerative colitis patients: The lung as a mirror image of intestine?, Respiratory Medicine (2009), doi:10.1016/j.rmed.2009.01.016
**Introduction**

Ulcerative colitis (UC) is a chronic inflammatory disorder of the gastrointestinal (GI) tract that involves the large bowel. The etiology of UC is presently unknown. Patients with UC commonly present with a wide range of systemic and extra-intestinal manifestations, most commonly involving the skin, eyes, mouth, joints, and liver\(^1\) and less commonly having clinically relevant bronchopulmonary manifestations.\(^2\) Several studies suggested that patients with inflammatory bowel diseases (IBDs) have altered pulmonary function tests (PFTs).\(^3,4\) It was recently shown that patients with UC have decreased functional parameters, especially the ones with active disease.\(^5\)

Inflammatory changes were shown in the bronchoalveolar lavage (BAL) fluid of IBD patients: alveolar lymphocytosis was evident in fluid from Crohn’s disease (CD) patients without respiratory symptoms,\(^6\) and mixed inflammatory cells were revealed in the BAL fluid of UC patients.\(^7\) BAL is an important tool for evaluating lung inflammatory processes in the lung is the examination of sputum.\(^9\)

We had previously shown that induced sputum (IS) was useful for identifying a high (65%) incidence of CD4 T lymphocyte infiltration in the airways of CD patients who had no respiratory symptoms.\(^10\) The IS technique has also revealed the presence of bronchial eosinophilic infiltration in CD patients.\(^11\)

We hypothesized that UC is a T-helper lymphocyte 2 (Th2) disease, while CD is mainly a T-helper lymphocyte 1 (Th1) disease.\(^12\) We originally designed the current study to non-invasively search for a cellular marker in the airways that can best reflect the clinical condition in patients with UC.

**Methods**

**Selection of patients**

There were 19 UC patients (male 68.4%), of whom 13 were non-smokers and 6 were present or past smokers. Their mean age was 46.4 ± 11.3 years and the mean disease duration was 8.6 ± 7.5 years (range 1–25 years). Three had asymptomatic bronchial asthma (not treated at the time of recruitment), while none of the remaining 16 reported respiratory symptoms. They were all diagnosed by clinical, endoscopic and historical histological criteria.\(^13\) The activity of the disease index we used ranged from 0 to 12, with the higher total scores representing more severe disease. The index was based on the Ulcerative Colitis Disease Activity Index (UCDAI) of Truelove and Witts.\(^14\) In general, a patient is considered to be in remission if the UCDAI score is 2 and to have severe disease if the score is >10. Nine patients (47.4%) had UCDAI scores ≤ 2 (remission) and the other 10 patients had active diseases (UCDAI > 2), of whom 7 (36.8%) had proctitis. Twelve patients (63.2%) had extensive UC (3 of them had pancolitis). Sixteen patients were treated: 11 received 5-aminosalicylic acid (5-ASA) 2–4 g/day, 4 received prednisolone (mean 20 mg/day), 4 received betamethasone 5 mg/day by enema, 1 was taking azathioprine 100 mg/day and 3 were not taking any medications. Some of the patients were taking more than one medication (Table 1).

The control group comprised 27 apparently healthy non-smokers (mean age 35.4 ± 9.7 years) without any known respiratory disease, who were recruited from the hospital staff. All participants signed informed consent forms and the study was approved by the Institutional Review Board of the Tel-Aviv Sourasky Medical Center and Hillel Yaffe Medical Center.

**Study design**

Pulmonary function tests (PFTs; spirometry, lung volumes and diffusion capacity) were performed by a Masterlab (Masterlab E. Jaeger, Wurzburg, Germany). Measurement was carried out using standard protocols according to the American Thoracic Society guidelines.\(^15\)

Sputum induction was carried out by an aerosol of hypertonic saline generated by a DeVilbiss Aerosonic

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**Table 1 Clinical characteristics of the 19 ulcerative colitis (UC) study patients.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.4 ± 11.3</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>13/6</td>
</tr>
<tr>
<td>Smoking</td>
<td>6</td>
</tr>
<tr>
<td>Documented lung diseases</td>
<td>3</td>
</tr>
<tr>
<td><strong>UCDAI</strong></td>
<td></td>
</tr>
<tr>
<td>Remission(^a)</td>
<td>9 patients (47.4%)</td>
</tr>
<tr>
<td>Active disease(^b)</td>
<td>10 patients (52.6%)</td>
</tr>
<tr>
<td><strong>Treatment(^c)</strong></td>
<td></td>
</tr>
<tr>
<td>5-Aminosalicylic acid</td>
<td>11 patients</td>
</tr>
<tr>
<td>Prednisolone (oral)</td>
<td>4 patients</td>
</tr>
<tr>
<td>Betamethasone enema</td>
<td>4 patients</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>1 patient</td>
</tr>
<tr>
<td>No treatment</td>
<td>3 patients</td>
</tr>
<tr>
<td><strong>Extent of UC</strong></td>
<td></td>
</tr>
<tr>
<td>Proctitis</td>
<td>7 patients</td>
</tr>
<tr>
<td>Extensive</td>
<td>9 patients</td>
</tr>
<tr>
<td>Pancolitis</td>
<td>3 patients</td>
</tr>
<tr>
<td>Duration of disease</td>
<td>8.6 ± 7.5 years</td>
</tr>
</tbody>
</table>

\(^a\) UCDAI score ≤ 2.  
\(^b\) UCDAI score > 2.  
\(^c\) Some of the patients receive more than one medication.
Ultrasonic Nebulizer 5000D/5000I (DeVilbiss-Health Care Corporation Somerset, PA, USA) with an output of 0.5 ml/min and a particle size of <5 μm aerodynamic mass median diameter, using a slightly modified version of the method of Pin et al. Briefly, subjects inhaled nebulized 3% saline for up to 20 min delivered by an ultrasonic nebulizer through a mouthpiece without a valve or noseclip. Ten minutes after the start of nebulization and every 5 min thereafter, the subjects were asked to rinse their nose and mouths with water to minimize contamination of the nasal secretion with saliva. They were encouraged to cough and expectorate sputum into a sterile plastic container. The nebulization was stopped after 20 min had elapsed or earlier if the sputum sample was of sufficiently good quality. The sputum was collected prior to any treatment.

The method of sputum examination described by Popov et al. was used with some modifications. Sputum was processed as soon as possible, always within 2 h. It was poured onto a Petri dish and all portions with few or nonsquamous epithelial cells that were considered to originate from the lower respiratory tract were selected under an inverted microscope and placed in an Eppendorf tube, whereupon the weight was recorded. Dithiothreitol (DTT; Sputalysin, Calbiochem Corp., San Diego, CA, USA) was freshly prepared in a dilution of 1:10 with distilled water according to the manufacturer’s instructions. The volume added was twice the recorded weight of the plugs, and it was mixed mechanically with the sputum by aspiration in and out of a pipette about 20 times to ensure an adequate blend. The sample was then placed in a shaking water bath at 37 °C for 15 min to ensure complete homogenization. To stop the effect of DTT, the suspension was further diluted with phosphate-buffered solution (PBS) to a volume equal to the sputum plus DTT. The cell suspension was filtered through 52-μm nylon gauze (BNSH Thompson, Scarborough, Ontario, Canada) to remove debris and mucus, and the volume of the filtrate was recorded. The total cell count was measured by a hemocytometer (Neubauer chamber). The filtered cell suspension was centrifuged and the supernatants were stored at −80 °C until examination. The pellet was diluted with RPMI supplemented with 10% fetal calf serum (FCS, Biological Industries, Beit Haemek, Israel) to achieve a concentration of 10/μl. One drop was placed in each cytacentrifuge cup which is already in place in a Shandon III cytacentrifuge (Shandon Southern Instruments, Sewickley, PA, USA). Separate cytospin slides were stained by Giemsa. The cell counts were performed by scanning the cytospins, starting at the top left corner and continuing in an undulating manner from top to bottom while moving across the slide using high power (×100) magnification. Two-hundred nonsquamous cells were counted, and the results were expressed as a percentage of the total nonsquamous count.

For the evaluation of phenotype of sputum cells, flow cytometric analysis was performed on a dual FACS 440 equipped with an Ar+ and Kr laser (Becton–Dickinson). Data were collected and analyzed using the Consort VAX and Disp4 and Disp2D programs (Becton–Dickinson). The information was collected on a logarithmic scale. The selection of lymphocyte population was based on side scatter, and expressions of CD45 lymphocytic subsets were identified by monoclonal antibodies as follows: CD3 = total T-cells, CD4 = T-helper cells, and CD8 = T suppressor–cytotoxic cells. Monoclonal antibodies were directly conjugated to either phycoerythrin (RD1) or fluorescein isothiocyanate (FITC). The cells were incubated for 10 min with Epics Coulter Q-Prep and read either immediately or 24 h later.

For the evaluation of eosinophils in tissue biopsy, one section was separately stained with Vital New Red (VNR) according to the method of Li et al. Counts were carried out from 5 selected areas, and the results were averaged for each patient and converted to cells/mm². The VNR stain plus the counterstain allowed obtaining both the total lamina propria cell density (>10⁵/mm²) and the eosinophil density. Biopsy was performed at the time of diagnosis and was not repeated at the time of IS induction for ethical reasons.

Statistical analysis

Eosinophils and CD4/CD8 were analyzed as continuous parameters and also as dichotomous according to clinical relevance cutoffs: 3% for eosinophils and 2.5 for CD4/CD8. Comparisons between the study and control groups with regard to demographic parameters (sex, age), PFT results and differential cell count results were done using the Mann–Whitney non-parametric, Chi-square and Fisher’s exact tests. Spearman correlation coefficients were calculated in order to examine the relationships between all continuous parameters. The association between inflammatory and functional parameters and the type of treatment and extent of disease in the UC group was examined using the Mann–Whitney non-parametric test. Significance was set at a p value of less than 0.05 and the SPSS, for windows, software, version 14.0 (Chicago, IL) was used for the analysis.

Results

All study participants underwent sputum induction and PFTs. The IS samples of 2 UC patients contained >75% epithelial squamous cells and those samples were discarded. The IS samples of the remaining 17 UC patients displayed a significantly higher percentage of eosinophils than the control group (5.4 ± 9.6% vs 0.49 ± 1.0%, p = 0.0001, p = 0.02 adjusted to age) and a lower FEV₁/FVC ratio (77.6 ± 6.03 vs 85.6 ± 7.7%, p = 0.0001, p = 0.046 adjusted to age). No significant differences were found in the other examined parameters (Table 2). Ten of the UC patients had an FEV₁/FVC ratio < 80% but there was no reversion of the obstructive pattern after beta 2 agonist treatment.

Inflammatory and functional parameters in the UC patients were correlated with the treatment and the extent of disease. Both the non-treated patients and those receiving 5-ASA (n = 11) had significantly higher percentages of eosinophils and lymphocytes than those treated with steroids or azathioprine (n = 8, 6.7 ± 10.7% vs 1.1 ± 1.9, p = 0.046 and 19.2 ± 3.4 vs 11.6 ± 4.8, p = 0.044, respectively). An inverse pattern was observed in biopsies: eosinophils were lower in the patients treated with 5-ASA and in the patients not receiving treatment than
in the patients treated with steroids or azathioprine (59.7 ± 37 vs 142.6 ± 10, p = 0.044). UC patients with limited proctitis showed higher percentages of eosinophils than those with extensive proctitis and pancolitis (10.4 ± 13.8% vs 1.9 ± 2.4%, p = 0.05). The same inverse pattern was observed in tissue biopsies of proctitis patients with fewer eosinophils than in those of patients with pancolitis (61.2 ± 42.1 in proctitis vs 96.2 ± 85.7 in pancolitis), but the difference did not reach a level of significance (p = 0.44). As for PFT results, the patients with extensive UC who were treated with steroids or azathioprine had lower FEV₁/FVC ratios and forced expiratory flow at 50% vital capacity (FEF₅₀) than non-treated patients and proctitis patients receiving 5-ASA (79.7 ± 4.8 vs 71.8 ± 5.6, p = 0.019 and 79.1 ± 23.9 vs 51.5 ± 14.4, p = 0.015, respectively), but lower FEV₁/FVC ratios and lower FEF₅₀ levels (80.3 ± 3.2 vs 75.6 ± 6.9, p = 0.19 and 86.7 ± 6.8 vs 62.1 ± 28.4, p = 0.015, respectively, Table 3). Fig. 1 displays a representative patient with abundant eosinophils in biopsy but not in his IS, and Fig. 2 shows a representative patient with scarce eosinophils in biopsy and abundant in sputum.

The patients and controls were divided into 4 groups according to the cutoffs of eosinophils and T-cell subsets which are known to have clinical relevance, i.e., ≤3% eosinophils, >3% eosinophils, ≤2.5 CD4/CD8 and >2.5 CD4/CD8 (Fig. 3). Similar number of patients were found to have higher CD4/CD8 ratios compared to the control group (9/17 vs 12/24, respectively, p = 1.0), while more patients with UC had percentage of eosinophils > 3% than controls (7/17 vs 1/24, respectively, p = 0.005, Fig. 1). Moreover, the UC patients with eosinophils > 3% in the sputum differential counts were the ones with proctitis but not the ones with extensive disease (p = 0.05). These high levels of eosinophils were not correlated to any decreases in FEV₁% (94.3 ± 13.8%): only 3 patients reported suffering from

### Table 2
Demographic characteristics and results of the pulmonary function tests and differential cell counts of the study and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Ulcerative colitis (N = 19)</th>
<th>Controls (N = 27)</th>
<th>p Valuea</th>
<th>p Valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.4 ± 11.3</td>
<td>35.4 ± 9.7</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>41.4 ± 23.2</td>
<td>46.9 ± 21.5</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>5.4 ± 9.6</td>
<td>0.49 ± 1.0</td>
<td>0.0001</td>
<td>0.02</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>12.9 ± 5.4</td>
<td>14.2 ± 7.14</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>40.2 ± 22.2</td>
<td>38.4 ± 20.2</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>CD4/CD8</td>
<td>2.8 ± 1.76</td>
<td>2.6 ± 1.25</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>FEV₁ (%)</td>
<td>91.5 ± 14.7</td>
<td>96.6 ± 12.2</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>FVC (%)</td>
<td>97.5 ± 13.8</td>
<td>96.6 ± 12.2</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>77.6 ± 6.03</td>
<td>85.6 ± 7.7</td>
<td>0.0001</td>
<td>0.046</td>
</tr>
<tr>
<td>DLCO (%)</td>
<td>81.9 ± 17.7</td>
<td>84.7 ± 9.38</td>
<td>0.96</td>
<td></td>
</tr>
</tbody>
</table>

FEV₁ – flow expiratory volume in 1 s; FVC – forced vital capacity; DLCO – diffusion lung carbon monoxide.

a Mann–Whitney non-parametric test.
b Adjusted to age.
c Percent of 300 cells counted as described in Methods.
d Percent prediction.

### Table 3
Inflammatory and functional parameters according to the treatment and extent of ulcerative colitis disease (19 patients).

<table>
<thead>
<tr>
<th></th>
<th>EOS-IS (IS) (%)a</th>
<th>EOS-biopsy (cells/mm²)a</th>
<th>LY (%)b</th>
<th>FEV₁/FVC</th>
<th>FEF₅₀%c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated with 5-aminosalicylic acid or not treated (N = 14)</td>
<td>6.7 ± 10.7</td>
<td>59.7 ± 37</td>
<td>19.2 ± 3.4</td>
<td>79.7 ± 4.8</td>
<td>79.1 ± 23.9</td>
</tr>
<tr>
<td>Treated with steroids or azathioprine (N = 5)</td>
<td>1.1 ± 1.9</td>
<td>142.6 ± 110</td>
<td>11.6 ± 4.8</td>
<td>71.8 ± 5.6</td>
<td>51.5 ± 14.4</td>
</tr>
<tr>
<td>Proctitis (N = 7)</td>
<td>10.4 ± 13.8</td>
<td>61.2 ± 42.1</td>
<td>13.2 ± 5.2</td>
<td>80.3 ± 3.2</td>
<td>86.7 ± 6.8</td>
</tr>
<tr>
<td>Extensive UC (N = 12)</td>
<td>1.9 ± 2.4</td>
<td>96.2 ± 85.7</td>
<td>12.8 ± 5.9</td>
<td>75.6 ± 6.9</td>
<td>62.1 ± 28.4</td>
</tr>
</tbody>
</table>

All p values are obtained by the Mann–Whitney non-parametric test.

EOS-IS (IS) – eosinophils in induced sputum; EOS-biopsy – eosinophils in biopsy; LY – lymphocytes; UC – ulcerative colitis; FEV₁ – flow expiratory volume in 1 s; FVC – forced vital capacity; FEF – flow expiratory flow 50%.

a Counts undertaken from 5 randomly chosen area (Methods).
b Percent of 300 cells counted (cells/mm²).
c Percent prediction.
d Some also received 5-aminosalicylic acid.

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bronchial asthma and they had decreased FEV₁% (75.9 ± 9.1%) (Table 4).

The sensitivity, specificity and positive likelihood ratio were the best when a cutoff of 3% of eosinophils in sputum was used to differentiate high/low density of eosinophils in tissue (67%, 73% and 2.44, respectively) (at a cutoff of 70 cells/mm²; Table 5).

There was no correlation between UCDAI and any of the functional and inflammatory parameters.

**Discussion**

Having had demonstrated a link between IS results and Crohn’s disease, we designed the current study to see whether the same connection exists between IS and another IBD, UC. We were not at all surprised to confirm that such a relationship does indeed exist. What was entirely unexpected was that bronchial eosinophilic infiltration in the IS of patients with UC was more pronounced in mildly treated patients with less extensive disease. Moreover, there was an inverse eosinophil accumulation in

![Figure 1](image1.png)  
(A) Sputum sample with a low content of eosinophils. (B) Biopsy of the same patient’s intestine showing a high content of eosinophils.

![Figure 2](image2.png)  
(A) Sputum sample with a high content of eosinophils. (B) Biopsy of the same patient’s intestine showing a low content of eosinophils.

![Figure 3](image3.png)  
Comparison between eosinophils and T-cell lymphocytes in the patients with ulcerative colitis compared to the controls. CD4 — cluster of differentiation 4; CD8 — cluster of differentiation 8; EOS — eosinophils.

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Eosinophils are involved in several chronic inflammatory diseases that affect organ systems normally exposed to antigens, such as the respiratory tract and the skin, and increasing evidence suggests that they may be involved in the pathogenesis of IBD. We performed IS in patients with historical biopsy-proven UC and compared their differential cell counts with those cells obtained from an allegedly healthy control group. The question we sought to answer is whether the sputum eosinophilia seen in the UC patients in our study might reflect the GI tract infiltration of eosinophils.

We had previously demonstrated that 65% of the CD patients in spite of them being asymptomatic had an accumulation of CD4+ cells in their IS specimens. These findings were similar to those reported by Pabst and Tschering in BAL samples (54% with an abnormal CD4/CD8 ratio), demonstrating that there is a migration of intestinal lymphocytes through the general circulation to the common mucosal immune system. Rutgers et al. showed that eosinophil content was comparable in samples from IS and BAL samples in asthmatics, and Walleart et al. demonstrated a mucosal eosinophilic inflammation associated with a Th2 cytokine expression in the gut of asthmatic patients, suggesting that while asthma is clinically expressed in the airways, the immune abnormalities are present throughout the mucosae.

The IS samples from our UC patients contained higher levels of eosinophils than the samples of the control group. These findings are supported by those of previous studies that showed that although these cells are normally present in the intestinal mucosa and participate in host defense, their numbers are highly increased in patients with UC. Moreover, increased levels of eosinophil granule proteins have been detected in intestinal perfusion fluid and in feces from patients with UC.

The functional parameters which were tested by PFT demonstrated an obstructive pattern with a significantly decreased FEV1/FVC ratio compared to the control group. Our findings were similar to those recently reported by others, by showing mostly obstructive lung diseases, although no restrictive pattern was apparent among our patients. The presence of eosinophils was not correlated with any decrease in FEV1 or with the percentage of change in FEV1 and FEV1/FVC after bronchodilators, indicating that the eosinophil infiltration is not correlated to an asthmatic pattern with hyperreactivity of airways but rather to an eosinophilic bronchitis pattern in which these two pathways are unrelated. Eosinophilic bronchitis is recognized as being a common but not a consistent feature of asthma and one that can occur when symptoms are absent and the FEV1 is normal.

Dividing IS samples into 4 groups according to the cutoffs of eosinophils and T-cell subsets showed that the threshold of 3% eosinophils plays a major role in differentiating UC patients with proctitis from the UC patients with extensive pancolitis and from controls in a manner similar to that in asthma and eosinophilic bronchitis. This cutoff is the best for sensitivity, specificity and positive likelihood ratio to differentiate low/high density of eosinophils in the tissue biopsy. In contrast, there were no differences when we used a 2.5 CD4/CD8 cutoff of T lymphocytes as we had done earlier in patients with CD, sarcoidosis and other granulomatous diseases. This emphasizes the fact that these profiles are associated with a Th2 pattern in UC driven by the production of IL-13 and eosinophils recruitment and

| Lung disease | Yes | 0 | 3 | 0.051 | 75.9 ± 9.1 | 0.047 |
| Extent of UC | Proctitis | 0 | 7 | 0.001 | 97.9 ± 12.1 | 0.07 |
| | Extensive | 10 | 0 | 86.9 ± 15.2 |

The article also presents tables that detail the correlation between eosinophil levels and functional parameters according to the extent of ulcerative colitis disease and lung involvement.

**Table 4** Correlation between eosinophil levels and functional parameters according to the extent of ulcerative colitis disease and lung involvement.

<table>
<thead>
<tr>
<th>Eosinophils in biopsy (cells/mm²)</th>
<th>Eosinophils in sputum (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>≤3%</td>
<td>&gt;3%</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>42.9</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>66.7</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>85.7</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>20</td>
</tr>
<tr>
<td>LR (+)</td>
<td>1.29</td>
</tr>
</tbody>
</table>

**Table 5** Sensitivity, specificity and positive likelihood ratio of induced sputum (3%) to differentiate high/low density of eosinophils in tissue.

PPV — positive predictive value; NPV — negative predictive value; LR (+) — positive likelihood ratio.

* Counts undertaken from 5 randomly chosen areas (Methods).

**References**

1. E. Fireman et al., Induced sputum eosinophilia in ulcerative colitis patients: The lung as a mirror image of intestine?, Respiratory Medicine (2009), doi:10.1016/j.rmed.2009.01.016
a Th1 pattern in CD driven by the production of IL-12/IFN-γ and recruitment of lymphocytes.12

The eosinophil count in the lung was in contrast to the percentage of eosinophils present in the lamina propria being a mirror image of the intestine. We found a higher percentage of IS eosinophils (>3%) in untreated UC patients as well as in proctitis patients treated with 5-ASA compared with patients who were treated with steroids or azathioprine and UC patients who had extensive pancolitis. A unique feature of eosinophils is that they largely reside in the tissues, instead of staying in the blood circulation as neutrophils do. There is trafficking into the gastrointestinal tract and, under homeostatic conditions, eosinophils home into the thymus, mammary gland, and uterus. The accumulation in the respiratory mucosa is selectively activated by eosinophil-selective cytokine IL-5 and the eotaxin subfamily of chemokines.33 The inverse differential accumulation in gastrointestinal and airways mucosa is similar to that seen in peripheral lymphopenia, with high accumulation of activated lung lymphocytes in sarcoidosis.34

It should be borne in mind that treatment with 5-ASA itself could also be responsible for pulmonary eosinophilia.35 Although we cannot rule out an effect of 5-ASA in some of our UC patients, the fact that patients who were not receiving 5-ASA also clearly displayed sputum eosinophilia indicates that factors other than a reaction to this drug must be involved in these patients. In contrast, the percentage of eosinophils dropped to normal levels in the UC patients treated with steroids or azathioprine. These findings are not unexpected since sputum eosinophilia may be reduced by systemic corticosteroids.36 This systemic antiinflammatory treatment, however, apparently does not affect the airways of the UC patients since it did not reverse their airway obstruction.

These findings raise the intriguing question about the role of eosinophils in UC which appears not to be cut.37,38 Clinical investigations have been performed to assess an association between eosinophil infiltration and disease activity. Sarin et al.39 showed rectal eosinophilic cell counts to be significantly higher in active UC than in quiescent UC and healthy controls, but there was no correlation between the eosinophil cell counts and disease severity. Jezierska et al.40 reported an increased number of eosinophils only in active IBD and much fewer in chronic quiescent disease. In contrast, Korelitz and Sommers41 described low mucosal eosinophil counts in active untreated UC and no correlation between the number of eosinophils in the lamina propria and treatment response to sulfasalazine. Those authors demonstrated that the presence of tissue eosinophils corresponded to the general tendency for the condition to deteriorate and require intense medical therapy. Lampinen et al.42 further established that the activity of eosinophils was high in active disease. During the inactive phase of UC, the patients had a higher number of eosinophils while being asymptomatic both clinically and histopathologically. These observations have sparked even more controversy, implying that eosinophils might also be involved in the resolution of inflammation and repair of damaged tissue, which has also been elicited in allergy and asthma.43

There are a number of limitations to this study. The first is the relatively small population of UC patients in the study group. The second is that all of the UC patients were a priori diagnosed: in other words, sputum induction had not been done at the time of intestinal biopsy and tissue immunohistochemistry which were performed for diagnosing infiltration of eosinophils in the mucosa by gastroenterologists.

In conclusion, we propose that eosinophil cutoff points in the IS samples from UC patients can be used as a noninvasive approach for evaluating GI mucosa for monitoring the extent of disease and assessing treatment.

Conflict of interest

none whatsoever.

Current controlled trials number, NCT004990048 [controlled-trials.com].

Acknowledgment

Esther Eshkol is thanked for editorial assistance.

All the authors declare no potential conflict of interest related to the article or the research described.

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Dr. Elizabeth Fireman: manuscript preparation and head of the pulmonary laboratory.

Dr. Masarwy: recruitment of patients.

Dr. Groissman: histology assessment.

Dr. Shtark: sputum induction and processing.

Dr. Kopelman: colonoscopy procedures.

Dr. Kivity: preparation of Helsinki proof documents.

Dr. Fireman: clinical assessment and head of the gastroenterology department.

References


