Eosinophil decranulation as an allergy activation marker
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Chapter 9

Serum Eosinophil Cationic Protein in active and quiescent ulcerative colitis

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Abstract

Inflammatory bowel disorders are characterized by an accumulation of eosinophilic granulocytes, mast cells, lymphocytes and neutrophilic granulocytes in intestinal mucosa. The aim of this study was to examine the concentration of eosinophilic granulocytes in the blood of patients during active ulcerative colitis in comparison with patients during remission and apparently healthy control individuals. Besides the enumeration, the activity grade of eosinophilic granulocytes has been studied by estimation of their degranulation product, Eosinophil Cationic Protein, in serum. Patients with active ulcerative colitis could be distinguished from patients with quiescent ulcerative colitis by establishment of the Eosinophil Cationic Protein serum concentration, neutrophilic granulocyte blood count, Erythrocyte Sedimentation Rate, C-Reactive Protein and albumin concentration.

After two weeks of corticosteroid treatment, Eosinophil Cationic Protein serum concentrations and eosinophil counts in blood were significantly decreased. A decrease in blood eosinophil count was accompanied by a decrease in Eosinophil Cationic Protein concentrations in serum in most patients with ulcerative colitis. After twelve weeks of corticosteroids administration, serum albumin concentrations were significantly increased, whereas serum concentrations of C-reactive Protein were significantly decreased.

During treatment with corticosteroids, serum Eosinophil Cationic Protein concentrations and blood eosinophil counts are appropriate laboratory parameters to detect the effect of medication in the course of ulcerative colitis.
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**Introduction**

Ulcerative colitis (UC) is an inflammatory disorder of the gastrointestinal tract. The etiology of UC still remains unclear. The inflammatory process is characterized by oedema, congestion, spontaneous bleeding, erosion and ulcerations of colorectal mucosa. The damage caused to tissues during the inflammatory process results from involvement of inflammatory lymphocytes, neutrophils, eosinophils and mast cells (1). Proinflammatory cytokines, released in active UC, are able to activate eosinophilic granulocytes, resulting in the release of pivotal inflammatory proteins (1). Eosinophil Cationic Protein (ECP) is a cytotoxic protein localized in the matrix of eosinophil granules, which causes mucosal damage (2). Data concerning longitudinal follow-up of serum ECP concentrations in the course of UC are sparse. Several studies indicate that serum ECP is related to the activity grade of the inflammatory process (3).

The aim of this investigation was to examine the alterations of eosinophilic granulocytes during active phases of ulcerative colitis in comparison to UC patients in remission or apparently healthy control individuals. The activity grade of eosinophilic granulocytes was established by quantification of the degranulation product ECP in serum. Under standardized preanalytical conditions for blood sampling (4), ECP concentrations were determined in sera from UC patients and apparently healthy controls. Besides this parameter, the eosinophil blood counts, neutrophil blood counts, ESR, albumin and CRP concentration in serum were also determined.

**Patients and methods**

Fourteen patients with UC, referred to the gastroenterology department for endoscopy, were included in the study (2 women and 7 men with active UC (mean age 37 years, range 21-50) and 3 women and 2 men with quiescent UC (mean age 46 years, range 36 - 65)). Diagnosis of UC was documented on clinical, endoscopic and histopathologic
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criteria. Degree of severity of UC was assessed by using a patient score list (5), and an endoscopic score (6). Six patients had pancolitis, whereas three patients were diagnosed as having left-sided disease. Patients with an atopic constitution, tranexamic acid medication, thrombolytic therapy or treatment with coumarin derivatives, pregnancy, bacterial colitis or severe comorbidity were excluded from the study.

Patients with UC in remission were treated with 1.5 - 3 gram of mesalazine daily as a maintenance therapy. Patients with an exacerbation were newly diagnosed UC patients or known UC patients. These known UC patients were previously also treated with 1.5 - 3 gram of mesalazine daily. After exacerbation, patients were treated daily with a standard oral dose of 30 mg of prednisone or in severe pancolitis with 200 mg of hydrocortisone intravenously. Steroid dosage was tapered on signs of clinical symptoms. Following endoscopy, blood samples were drawn for determination of eosinophil and neutrophil counts, serum ECP, CRP and albumin concentration and ESR. Blood was taken at the first visit and 12 weeks later. From patients with active UC, blood samples were also taken two weeks after the start of corticosteroid treatment.

Besides patients with UC, 21 apparently healthy controls (11 women and 10 men (mean age 40 years, range 28 - 56) were included. The study was approved by the medical ethical committee of the hospital. All patients provided written informed consent.

To measure serum ECP, a standardized blood clotting procedure was applied by blood clotting for two hours at 37°C. Serum samples were drawn with the Vacutainer® system (ref. 367783 with addition of SST gel and clot activator, Becton Dickinson, Plymouth, UK). Serum samples were stored at -20°C until analysis was performed. ECP concentrations were measured with an immunoassay kit (ECP FEIA; Kabi Parmacia, Uppsala, Sweden). CRP and albumin serum concentrations were determined by use of nephelometry (Dade Behring, Marburg, Germany). For measuring the leukocyte count and differentiation, blood samples were taken in Vacutainer® tubes with K₃EDTA as an anticoagulant (ref. 367652, Becton Dickinson, Plymouth, UK). Blood samples were
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counted on a Sysmex NE-8000 haematology analyser (Charles Goffin Medical Systems BV, Tiel, The Netherlands).

To calculate sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV), reference ranges for laboratory parameters were indicated as follows: eosinophil count < 0.5 x 10⁹/l, serum ECP concentration 12-99 µg/l (7), ECP/eosinophil ratio 61-367 µg/10⁹ (7) and CRP < 5 mg/l.

Statistics

Results of analyses are expressed as mean values with standard deviations. Application of ANOVA and (paired) T-test was performed when appropriate. The level of statistical significance was set at 0.05. All calculations were performed with SPSS 6.1. Sensitivity, specificity, positive and negative predictive values are calculated as described by Shultz (8).

Results

ECP concentrations in serum were found to be significantly higher in individuals with active UC than in either patients with quiescent UC or apparently healthy controls (table I). However, significant differences were not established for eosinophil blood counts between active and quiescent UC patients or apparently healthy controls (table I). In the active phase of UC, neutrophil blood counts, CRP and ESR, as markers of inflammation were significantly higher than in quiescent UC patients or apparently healthy controls. Serum albumin concentrations were decreased in active UC patients when compared with UC patients in remission or apparently healthy adults (table I). To discriminate between patients with active or quiescent UC (UC in exacerbation or in remission) and apparently healthy individuals, indicators concerning sensitivity and specificity of
laboratory parameters were calculated. Results referring to negative and positive 
predictive values of laboratory parameters are listed in table II.
During 2 weeks of corticosteroid treatment, serum ECP and blood eosinophil counts 
significantly decreased to 30.9 µg/l and 0.11 x 10⁹/l, respectively.
After 12 weeks of corticosteroid therapy, mean values of serum albumin concentrations 
increased to 42.4 g/l and CRP concentrations decreased to 5.6 mg/l. The mean values for 
ESR showed a tendency to decrease. However, this shift towards lower values was not 
statistically significant.
The increased neutrophilic granulocyte count at baseline showed a further increase until 
peak values after two weeks of treatment with glucocorticoids. The propensity to release 
granule proteins, measured by the serum ECP per eosinophil ratio, remained constant, 
except in one patient (table I). In patients with quiescent UC and in apparently healthy 
controls, similar results were measured at the first visit and 12 weeks later.
Overall, a positive correlation was found between eosinophil blood counts and serum 
ECP concentration in the active UC population (r = 0.67, p<0.0001).
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Table I: Blood parameters [mean values and (standard deviations)] in patients with UC and apparently healthy individuals. A statistically significant deviation in comparison with UC patients in exacerbation during the first visit. ● statistically significant differences between UC patients in remission and apparently healthy individuals.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>UC; at exacerbation (n = 9)</th>
<th>UC; in remission (n = 5)</th>
<th>apparently healthy individuals (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t = 0</td>
<td>t = 2 weeks</td>
<td>t = 12 weeks</td>
</tr>
<tr>
<td>eosinophil count (10^9/l)</td>
<td>0.34 (0.27)</td>
<td>0.11 (0.08)^a</td>
<td>0.09 (0.06)^a</td>
</tr>
<tr>
<td>ECP (μg/l)</td>
<td>90 (49)^•</td>
<td>31 (14)^a</td>
<td>23 (10)^a</td>
</tr>
<tr>
<td>ECP/eos (μg/10^9)</td>
<td>204 (93)</td>
<td>191 (70)</td>
<td>180 (82)</td>
</tr>
<tr>
<td>neutrophil count (10^9/l)</td>
<td>6.9 (2.8)^•</td>
<td>10.6 (5.3)</td>
<td>6.7 (4.2)</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>32 (12)^•</td>
<td>35 (6)</td>
<td>42 (6)^a</td>
</tr>
<tr>
<td>ESR (mm/hour)</td>
<td>27 (18)^•</td>
<td>24 (21)</td>
<td>18 (18)</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>19 (13)^•</td>
<td>14 (22)</td>
<td>6 (3)^a</td>
</tr>
</tbody>
</table>

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Table II: Efficacy of various laboratory parameters and predictive values to establish a diseased or healthy state.

<table>
<thead>
<tr>
<th></th>
<th>eos pos</th>
<th>eos neg</th>
<th>ECP pos</th>
<th>ECP neg</th>
<th>ECP/Eos pos</th>
<th>ECP/Eos neg</th>
<th>CRP pos</th>
<th>CRP neg</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC active (n = 9)</td>
<td>1</td>
<td>8</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>9</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>sensitivity</td>
<td>11%</td>
<td>44%</td>
<td>0%</td>
<td>89%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC quiescent (n = 5)</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>specificity</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>apparently healthy controls (n = 21)</td>
<td>0</td>
<td>21</td>
<td>0</td>
<td>21</td>
<td>1</td>
<td>20</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>specificity</td>
<td>100%</td>
<td>100%</td>
<td>95%</td>
<td>89%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>100%</td>
<td>100%</td>
<td>0%</td>
<td>80%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPV</td>
<td>62%</td>
<td>68%</td>
<td>59%</td>
<td>76%</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Discussion & conclusions

In this study increased serum ECP levels were observed in patients with active UC in comparison with patients with quiescent UC and apparently healthy controls. A statistically significant deviation in eosinophil blood counts could not be established. Therefore, establishment of serum ECP concentration, and not eosinophil count, is useful to distinguish a group of patients with active UC from patients with UC in remission. It should be noticed that for all mentioned parameters a large overlap of the results between the studied groups was found. To establish diagnostic efficacy and clinical value in discriminating active UC from quiescent UC or a healthy state at the individual level, the most favourable results were found for CRP concentration. To detect an active stage of disease, eosinophil blood counts and serum ECP concentration revealed comparable results; the positive predictive value of both tests amounted to 100%. To separate subjects with UC from a healthy
subject group, CRP was demonstrated to be the most sensitive test, with a negative predictive value of 76%.

Treatment with corticosteroids resulted in decreased values in serum ECP concentrations and blood eosinophil counts. Within 2 weeks of corticosteroids administration, a decrease in blood eosinophil count was accompanied by a simultaneous decrease in serum ECP concentration. The propensity to release granule proteins, measured by the ECP per eosinophil ratio remained unaltered. After 12 weeks of therapy, serum albumin concentrations increased significantly when compared with the baseline measurement during active UC. CRP concentration, being an inflammation marker, remained significantly decreased after 12 weeks of corticosteroid treatment. This observation implicates that in the first stage of corticosteroid treatment the allergic inflammation process is resolved to a considerable extent, as can be concluded from the decreased eosinophil count and serum ECP concentration. Involvement of the gastrointestinal tract can be evaluated for the long term by the interpretation of deviations with respect to serum albumin and CRP concentrations.

Initially, the neutrophilic granulocyte blood counts increased immediately after starting corticosteroid treatment. Only at a later stage, i.e. after 2 weeks, the neutrophil blood counts decreased. Nittoh et al. (9) showed that glucocorticoids enhance eosinophil apoptosis but inhibit neutrophil apoptosis. This corticosteroid-induced effect on apoptosis has been described previously also by others (10, 11, 12). These findings accentuate the contrasting effects of corticosteroids on eosinophil and neutrophil concentrations. After two weeks, the inflammation activity diminished, and effects on the cell production may have caused the decrease in the neutrophilic granulocyte blood counts. ESR, a less specific parameter for inflammation, did not change significantly during steroid treatment of 12 weeks but showed higher values in comparison to patients with UC in a remission state and apparently healthy subjects.
Therefore, we conclude that patients with active UC need a longer period of treatment to reach a final remission state.

During corticosteroid treatment, eosinophil blood counts and serum ECP concentrations were low compared to UC patients in remission and apparently healthy controls, while ECP release per eosinophil of UC patients in a remission state was lower than in apparently healthy controls and UC patients who were treated with corticosteroids for 12 weeks. These observations are in agreement with previous results (7). Release of ECP per eosinophil is negatively correlated with eosinophil blood counts. Therefore, a bivariate reference area for serum ECP per eosinophil with respect to the eosinophil count has been calculated (7). Usually, steroid therapy will induce downregulation of cytokine release from monocytes, macrophages and T cells, resulting in a decrease in eosinophil count and eosinophil activation (13). The present study showed that the activation grade (expressed as serum ECP/eosinophil ratio) of eosinophils treated with corticosteroids did not differ from eosinophils of apparently healthy individuals (7), irrespective of their eosinophil counts. Other methods to detect eosinophil activation have also been described in literature. Some investigators (14, 15) proposed that the method of discontinuous Percoll density gradient centrifugation might be another possibility. However, this is not one of the available ready-to-use laboratory tests. In conclusion, serum ECP concentration can be used to distinguish groups of patients with UC and apparently healthy subjects. However, to diagnose UC at the individual level, neither ECP nor eosinophil blood count is a useful parameter, because the overlap between both groups is too large. After starting treatment with corticosteroids in patients with active UC, serum ECP concentrations and blood eosinophil counts are the most sensitive laboratory parameters to detect the change during the course of UC. It must be emphasized that results concerning peripheral blood cells or constituents do not necessarily illustrate processes in the large intestine, specifically the colonic mucosa. For diagnosing UC or monitoring the effect of medication, biopsies from the mucosa should be studied.
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Therefore, histological and biochemical data of mucosal biopsies will be combined with laboratory parameters in a follow-up study.
References


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