Detection of Endoscopic and Histological Inflammation after an Attack of Colonic Diverticulitis is Associated with Higher Diverticulitis Recurrence

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ABSTRACT

Background & Aims: Colonic diverticulitis shows a high recurrence rate, but the factors associated with such recurrence are still unknown. The aim of our study was to investigate the role of endoscopic and histological inflammation as predictors for the recurrence of diverticulitis.

Methods: One hundred and thirty patients suffering from Acute Uncomplicated Diverticulitis (AUD) (81 males, 49 females, mean age 64.71 years, range 40-85) were prospectively assessed. All patients had AUD confirmed by computerized tomography (CT) and endoscopy. Clinical, endoscopic and histological follow-up was performed after 6, 12 and thereafter 24 months after diagnosis of AUD.

Results: Sixteen patients were lost to follow-up. Diverticulitis recurred in 18 patients (13.84%): 15 (13.15%) patients showed recurrence of AUD, whilst 3 (2.63%) showed recurrence of complicated diverticulitis. At the end of the follow-up period, endoscopic inflammation was still detected in 31 (27.67%) patients, and active histological inflammation in 41 patients (36.6%). Only detection of endoscopic and of histological inflammation during the follow-up was a predictor of diverticulitis recurrence (Log rank test, p = 0.0004).

Conclusions: Detection of endoscopic and histological inflammation after attack of AUD was identified as a predictor of diverticulitis recurrence.

Key words: diverticulitis – endoscopy – follow-up – histology – recurrence.
years, range 40-85). Diagnosis of AUD was made according to endoscopic and radiological criteria: macroscopic and microscopic inflammation involving the colon harbouring diverticula, and affecting bowel wall without complications, assessed by computerized tomography (CT) scan [3, 7] and colonoscopy with biopsies. All patients underwent both colonoscopy and abdominal CT.

Seventy three patients were originally diagnosed with AUD by abdominal CT scan because they had been admitted to the Emergency ward for acute abdominal pain. Thereafter, colonoscopy was performed at least 7 days after exclusion of complicated acute diverticulitis by CT scan.

Fifty seven patients were initially diagnosed with AUD by colonoscopy. Endoscopy was performed in outpatient care following complaints of abdominal symptoms, mainly due to a history of abdominal pain and/or constipation or diarrhea. Abdominal CT was performed immediately after colonoscopy.

Radiological appearance of AUD on CT at entry was assessed as mild (thickening of colonic wall ≥ 5 mm) or severe (thickening of colonic wall ≥ 5 mm with radiological signs of involvement of pericollic fat).

As no endoscopic classification is available, endoscopic severity of AUD at entry was arbitrarily graded as follows:
- mild: diverticular inflammation localized within and around diverticula;
- moderate: diverticular inflammation that affects both peri- and inter-diverticular mucosa;
- severe: inflammation that affects the overall colonic segment harbouring diverticula.

Following diagnosis, all patients were treated with mesalazine 3.2 g/day, rifaximin 800 mg/day, and metronidazole 1 g/day for 7 days. One hundred and seven patients (82.31%) obtained clinical remission, whilst 23 patients (17.69%) (9 suffering from mild AUD and 14 suffering from severe AUD) required further treatment with intravenous III generation cephalosporin for 7 days in order to achieve clinical remission. All patients went into remission, defined as absence of symptoms and normal values of fecal calprotectin (defined as undetection of calprotectin or detection of calprotectin concentration lower that 15 μg/g) within 30 days after ending of treatment, and none of them required surgery at that time.

**Follow-up**

After remission, patients were visited for endoscopic, histological, and clinical follow-up. Since the aim of this study was to assess whether the detection of macroscopic or microscopic inflammation could be predictive factors of the outcome of patients after an attack of AUD, patients were requested to undergo endoscopic and histological assessment during the follow-up period. For this reason, the study received prior approval of the Institutional Review Board, and all patients signed the informed consent form.

**Endoscopy**

All patients underwent the same standard endoscopic examination 6, 12, and therefore 24 months after obtaining remission. All patients received the same bowel preparation prescribed in our centres consisting of an oral polyethylene glycol solution to be taken in the evening. The following day, a pancolonoscopy (clean colon colonoscopy) was performed and six biopsy samples of colonic mucosa were collected in the sigmoid tract for histological examination. Colonoscopy was again performed at the time of the recurrence, if any.

Persistence of endoscopic inflammation was defined as detection of signs of inflammation (hyperemia, erosions, petechiae) around the diverticular opening.

**Histology**

Biopsies were taken from the mucosa around diverticula after 6, 12, and every 12 months after obtaining remission. The presence of inflammatory infiltrate was assessed by a semi-quantitative lymphocyte and neutrophil count on 10 colonic fields with HPF (high power field) technique at 40x magnification, assessed both at the bottom and on the whole crypts, as already validated [8]. Hematoxilin and Eosin (HE) staining was performed to assess histology of the sigmoid tract.

Count Lymphocyte Assay (CLA) for T-cells was performed by immunohistochemical detection of lymphocytes by anti-CD3 monoclonal antibodies. Lymphocyte infiltration was graded as follows: 3-5 cells: normal lymphocytic infiltrate (score: 0); 6-8 cells: mild (score: 1); 9-10 cells: moderate (score: 2); >10 cells: severe (score: 3). Neutrophilic infiltrate was also evaluated in order to assess active or non-active inflammation by using an arbitrary and semi-quantitative grading: non-active (absence of neutrophilic infiltrate, score=0); mild active (focal presence of neutrophils, score=1); moderate (presence of neutrophils intermediate between mild and severe, score=2); severe (diffuse neutrophilic infiltrate, score=3). Neutrophils were localized by myeloperoxidase staining as well as immunohistochemical reactivity using an anti-CD15 monoclonal antibodies.

Persistence of active histological inflammation was defined as neutrophilic score at least=1.

Histological assessment was again performed at the time of the recurrence.

**Clinical assessment**

A medical control visit was performed every 6 months after remission. Since there is no widely accepted definition of recurrent diverticulitis in the medical literature - some researchers defined it as recurrence of symptoms [4], others as CT evidence of diverticulitis for more than 30 days after the initial onset of the disease [9], we defined recurrence of diverticulitis when the patient returned to our office complaining of abdominal pain with or without other symptoms (constipation or diarrhea and/or fever), associated with detection of increased fecal calprotectin (FC) [10] (defined as detection of calprotectin concentration at least between 15 μg/g and 60 μg/g), more than 30 days after obtaining remission. In particular, FC was analysed using a quick and cheap test (CAL Detect®, Sofar SpA, Trezzano Rosa (MI), Italy). This semi-quantitative test was developed from the quantitative ELISA method which is regarded as the gold standard, showing sensitivity and specificity >95% and >70% respectively [11]. The result of this test is given as a 1 to 4 bands of colour, which indicate increasing calprotectin concentration as follows:
Inflammation as risk factor for diverticulitis recurrence

1. a single control line (C) in the results window indicates that the test has run correctly and calprotectin is undetectable;
2. the presence of two coloured bands (C and T1) inside the results window indicates a calprotectin concentration ≤ 15 μg/g confirming the absence of bowel inflammation;
3. the presence of three coloured bands (C, T1 and T2) inside the results window indicates a calprotectin concentration between 15 μg/g and 60 μg/g: a mild inflammatory process is in progress in the mucosa;
4. the presence of four coloured bands (C, T1, T2, and T3) inside the results window indicates a calprotectin concentration higher than 60 μg/g: a high grade inflammatory process is present in the mucosa.

Since radiological or clinical assessment may under- or over-estimate the real recurrence of the disease, we considered that the association of both clinical symptoms and the use of a simple, non invasive and sensitive test to assess intestinal inflammation was an efficient combination to diagnose the recurrence. In particular, we chose FC assessment because it proved effective in detecting colonic inflammation in DD [10]. Moreover, we previously demonstrated that FC was increased in all patients suffering from AUD and was subsequently decreased as response to therapy [10].

The patients were requested to avoid the use of non steroid anti-inflammatory drugs (NSAIDs) during the treatment and during the follow-up period, and were invited to a control visit whenever they felt it necessary. Finally, CT was performed if considered necessary (namely in case of suspected complicated diverticulitis).

Statistics

The collection and analysis of data were performed by using the SPSS® Release 13.0 (SPSS, Inc., Chicago, IL). Statistical analysis was performed by chi-squared test with Yates’ correction for continuity for categorical data and the Student’s t-test for continuous data. Time to recurrence of diverticulitis was calculated by the Kaplan-Meier method.

Adjusted odds ratios (aORs) with 95% confidence intervals (CI) for recurrence were calculated with a logistic regression model that was controlled by severity of abdominal CT at entry, severity of endoscopic inflammation at entry, and severity of histological inflammation at entry.

P<0.05 was considered significant.

RESULTS

Diverticular diseases was diagnosed for the first time in 93 patients, whilst 37 patients referred had a past history of DD. The characteristics of the patients are described in Table I.

The patients were followed up for 24 months. One hundred and fourteen patients were available for the final evaluation, and 16 patients were lost to follow-up.

At entry, 59/130 patients (45.38%) showed signs of mild AUD at abdominal CT evaluation, while 71/130 patients (54.62%) showed signs of severe AUD at abdominal CT evaluation.

Regarding the endoscopic appearance of diverticular inflammation at enrolment, 77/130 patients (59.23%) showed signs of mild inflammation, 42/130 (32.30%) showed moderate inflammation, and 11/130 (8.47%) showed signs of severe endoscopic inflammation.

All patients showed active histological inflammation at enrolment. All patients had a lymphocytic score=3, while the neutrophilic score was 3 in 62/130 (47.69%) patients, score 2 in 47/130 (36.15%) patients, score 1 in 21/130 (16.16%) patients. There was no major difference in the parameters assessed at entry between patients with a first diagnosis of DD and patients referring history of DD, except for the histological activity which was less severe in the former group (Table I).

Table I. Characteristics of patients at entry.

<table>
<thead>
<tr>
<th></th>
<th>First DD</th>
<th>Previous DD</th>
<th>p</th>
<th>Colonoscopy first</th>
<th>CT first</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>43</td>
<td>38</td>
<td>0.4</td>
<td>35</td>
<td>39</td>
<td>0.5</td>
</tr>
<tr>
<td>Females</td>
<td>21</td>
<td>28</td>
<td>0.5</td>
<td>22</td>
<td>34</td>
<td>0.2</td>
</tr>
<tr>
<td>CT scan severity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>27</td>
<td>22</td>
<td>0.1</td>
<td>30</td>
<td>37</td>
<td>0.9</td>
</tr>
<tr>
<td>Severe</td>
<td>27</td>
<td>34</td>
<td>0.2</td>
<td>27</td>
<td>36</td>
<td>0.9</td>
</tr>
<tr>
<td>Endoscopic severity</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>37</td>
<td>40</td>
<td>0.3</td>
<td>32</td>
<td>39</td>
<td>0.9</td>
</tr>
<tr>
<td>Moderate</td>
<td>19</td>
<td>23</td>
<td>0.4</td>
<td>15</td>
<td>21</td>
<td>0.9</td>
</tr>
<tr>
<td>Severe</td>
<td>7</td>
<td>7</td>
<td>0.1</td>
<td>10</td>
<td>13</td>
<td>0.8</td>
</tr>
<tr>
<td>Histological severity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild (neutrophil score: 1)</td>
<td>39</td>
<td>23</td>
<td>0.05</td>
<td>29</td>
<td>38</td>
<td>0.9</td>
</tr>
<tr>
<td>Moderate (neutrophil score: 2)</td>
<td>21</td>
<td>26</td>
<td>0.1</td>
<td>14</td>
<td>23</td>
<td>0.5</td>
</tr>
<tr>
<td>Severe (neutrophil score: 3)</td>
<td>3</td>
<td>8</td>
<td>0.05</td>
<td>14</td>
<td>12</td>
<td>0.3</td>
</tr>
<tr>
<td>WBC count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10⁶</td>
<td>39</td>
<td>33</td>
<td>0.6</td>
<td>26</td>
<td>40</td>
<td>0.4</td>
</tr>
<tr>
<td>&gt;10⁶&lt;15 x 10⁶</td>
<td>16</td>
<td>20</td>
<td>0.7</td>
<td>16</td>
<td>18</td>
<td>0.8</td>
</tr>
<tr>
<td>&gt;15 x 10⁶&lt;20 x 10⁶</td>
<td>9</td>
<td>13</td>
<td>0.6</td>
<td>15</td>
<td>15</td>
<td>0.6</td>
</tr>
<tr>
<td>Body mass index &gt; 30 kg/m²</td>
<td>21</td>
<td>30</td>
<td>0.1</td>
<td>17</td>
<td>34</td>
<td>0.1</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>15</td>
<td>19</td>
<td>0.5</td>
<td>12</td>
<td>22</td>
<td>0.3</td>
</tr>
<tr>
<td>Previous acute diverticulitis</td>
<td>5</td>
<td>7</td>
<td>0.8</td>
<td>3</td>
<td>9</td>
<td>0.3</td>
</tr>
</tbody>
</table>

CT: computerized tomography; WBC: white blood cells.
Fecal calprotectin was increased in all patients at enrolment with a concentration of >60 µg/g in 88/130 patients (67.7%), and between 15 µg/g and 60 µg/g in 42/130 patients (32.3%).

**Follow-up**

During the follow-up, diverticulitis recurred in 18 of the 114 (15.78%) evaluated patients. Fifteen (13.15%) patients showed recurrence of AUD, whilst 3 (2.63%) showed recurrence of complicated diverticulitis (confirmed by CT scan). All three latter cases of complicated diverticulitis presented small pericolic abscesses, and were treated as in-patients. Two patients had a recurrence within 6 months of the first episode of AUD. They were therefore excluded from the final evaluation because they did not undergo the first endoscopic/histological follow-up at 6 months from original remission. It is noteworthy that diverticulitis recurred more frequently within 11 and 14 months after the first episode (the timing of recurrence is described in Fig. 1).

At the end of follow-up, endoscopic inflammation was still detected in 31/112 (27.67%). All patients with endoscopic inflammation showed also histological inflammation, whilst 10 patients showed histological inflammation but not endoscopic inflammation. At the end of the follow-up period, active inflammation was thus detected in 41/112 patients (36.60%).

Looking at the correlation between the detection of endoscopic/histological inflammation during the follow-up visits and recurrence of diverticulitis, we found that the disease recurred more frequently in patients with detected endoscopic/histological inflammation than in patients with no detected inflammation (Fig. 2). In particular, at the end of the follow-up period, diverticulitis recurred in 12/41 (29.27%) of patients with detected endoscopic/histological inflammation and only in 4/71 (5.63%) patients with no detected endoscopic/histological inflammation (p<0.0121).

**Multivariate analysis**

Neither CT appearance nor severity of the endoscopic appearance, nor severity of histological inflammation at entry, nor smoking habit reached statistical significance as predictors of AUD recurrence (Table II). On the contrary, body mass index >30 and previous episodes of acute diverticulitis were predictors of AUD recurrence (Table II).

### Table II. Predictive value of diverticulitis recurrence of different parameters assessed at entry.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adjusted OR (95%CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT scan severity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>1.10 (0.65-1.91)</td>
<td>0.6</td>
</tr>
<tr>
<td>Severe</td>
<td>1.08 (0.68-1.98)</td>
<td>0.3</td>
</tr>
<tr>
<td>Endoscopic severity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>1.10 (0.72-1.62)</td>
<td>0.9</td>
</tr>
<tr>
<td>Moderate</td>
<td>1.08 (0.64-2.07)</td>
<td>0.8</td>
</tr>
<tr>
<td>Severe</td>
<td>1.27 (0.46-2.7)</td>
<td>0.4</td>
</tr>
<tr>
<td>Body mass index &gt;30</td>
<td>1.92 (0.78-2.10)</td>
<td>0.03</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>1.01 (0.59-1.48)</td>
<td>0.7</td>
</tr>
<tr>
<td>Previous acute diverticulitis</td>
<td>2.01 (0.85-2.15)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Diverticulitis represents a significant economic and clinical burden to the western Health Care Systems and its patients [12]. Recent studies focused on identifying prognostic factors for its recurrence and on identifying the best treatment to maintain longer remission.

We currently know that CT scan severity at entry is a good prognostic factor [13]. In particular, CT scan showing inflammation confined to the colonic/pericolic wall (Hinchey modified stage Ia-Ib) [13] indicates a lower risk of diverticulitis recurrence than a CT scan showing presence of retroperitoneal abscesses or peritoneal free fluids (Hinchey modified stage II-IV) [13]. The prognostic role of CT has been recently confirmed by Etzioni et al, who showed that women with a very high WBC count (> 18,000 mm³) and patients with free fluid at CT scan appeared to be at a higher risk of diverticulitis recurrence or treatment failure [4]. Other prognostic factors have been investigated (age, race, antibiotic type and duration), but none of them reached statistical significance.

We tried to identify other factors which may affect diverticulitis recurrence. First of all, we tried to see whether CT scan appearance of only bowel thickening (defined as “mild AUD”) or bowel thickening associated with pericolic...
fat involvement (defined as “severe AUD”) were risk factors of diverticulitis recurrence. Multivariate analysis found that none of them was a risk factor of diverticulitis recurrence.

We investigated other less classical risk factors. In particular, we investigated the role of detecting endoscopic and histological damage after clinical remission. This hypothesis is related to the new pathophysiological findings of diverticular disease, which seems to share several characteristics with Inflammatory Bowel Diseases (IBD). As in IBD [14], protein C reactive seems to be predictive of histological damage [15] and of severity of the disease [16]. As in IBD [17], TNF-alpha is related to the severity of diverticulitis [18].

We found that detection of both endoscopic and histological inflammation after an attack of AUD is predictive of diverticulitis recurrence. It is important to note that it is not the severity of the endoscopic damage at entry, but the persistence of endoscopic inflammation during the follow-up that represents a predictive factor of the recurrence. This is probably related to the type of disease, in which a single fecolith blocking the diverticular neck is able to develop the disease. The endoscopic behaviour of AUD seems to be similar to that of IBD, in which persistence of endoscopic damage during the follow-up is indeed a predictor of the course of the disease [19-21].

Detection of histological inflammation during the follow-up is also a predictor of the recurrence of the disease. And again this histological behaviour of AUD seems to be surprisingly similar to that occurring in IBD, in which persistence of histological damage during the follow-up is predictor of the recurrence [22, 23].

We found also that higher BMI (>30) and previous episodes of diverticulitis are risk factors for diverticulitis recurrence, confirming what has been previously reported [6]. Both of these findings reinforce the hypothesis of the persisting inflammation as a risk factor for diverticulitis recurrence. In fact we know that symptoms may persist also after surgical treatment of the disease [24], probably due to persistence of microscopic inflammation similarly to that which occurs in IBD [23]. Moreover, we know that overweight in diverticular disease may release various mediators including adipokines and chemokines, which play a pro-inflammatory role [25]. So, both previous episodes of diverticulitis and overweight may be considered two pro-inflammatory situations at higher risk of diverticulitis recurrence.

Some may criticize our endoscopic approach to study the disease. Performing colonoscopy in acute diverticulitis is still controversial, because acute inflammation of the diverticula may be at risk of perforation or bleeding [26, 27]. But recent literature data found that early colonoscopy (within 3 to 11 days after the admission) is as safe and effective as late colonoscopy (within 6 to 19 weeks following admission), without any complication in both approaches. We performed colonoscopy within 7 days after exclusion of complicated diverticulitis [which is considered the only parameter which would recommend avoiding early colonoscopy] [28], and we did not record any complications. Somebody may argue that CT colonography (CTC) may have a better diagnostic potential for imaging of diverticular disease-specific findings, when compared with colonoscopy. Moreover, CTC is less uncomfortable and may be preferred by a majority of patients [29]. However, we cannot forget that DD often shows circular muscle hypertrophy, that leads to a thickened colonic wall. This condition needs a correct differential diagnosis, since colorectal adenocarcinoma or new diagnosis of IBD (namely SCAD or UCD) can be established [30]. On this basis, colonoscopy may be performed in patients with AUD after careful clinical evaluation and after exclusion of free perforation under abdominal CT. However, it is important that colonoscopy be performed with caution in these patients, because the risk of perforation cannot be completely avoided.

Another possible source of error is that some patients were enrolled when at least 7 days passed between the CT scan and the time of mucosal assessment by colonoscopy whereas some patients (n=57) were enrolled with the two procedures done on the same day. We know that this different approach may lead to a different endoscopic/histological appearance at the time of the assessment. However, we thought that a preventive 7-day course of treatment may reduce but not cancel endoscopic/histological inflammation. Since our aim was to determine whether persistence of inflammation could be predictor of diverticulitis recurrence, we thought that a 7-day course might avoid the risk of complication under colonoscopy without affecting significantly the endoscopic/histological appearance of inflammation. The results of the study seem to confirm this hypothesis: the detection of inflammation during the follow-up visits, but not its severity at entry, was the predictor of diverticulitis recurrence.

The results of this study are very important for clinical practice, because they may influence the choice of the treatment in preventing recurrence of the disease. A large, long-term follow-up study on patients who were initially hospitalized for acute diverticulitis and subsequently followed up found that 19% of patients underwent emergency colectomy, and 13% had at least one recurrence during a 9-year mean follow-up period [31]. Similar results were described in a larger study [32]. Moreover, we must not forget that new data confirmed that recurrence rate of diverticulitis is 19–54% at 5 years [18, 33].

Antibiotics have been considered the mainstay in treating uncomplicated diverticulitis [18]. Hospital-based treatment is still an option for uncomplicated diverticulitis [18], even if the large majority of patients may be managed safely and effectively as outpatients, according to practice guidelines from the American Society of Colon and Rectal Surgeons [34]. This approach is particularly effective for mild diverticulitis, as also confirmed by more recent data [35, 36]. However, the main topic in daily practice is how we can prevent recurrence of the disease. In Italy, the most popular treatment to prevent the recurrence of diverticulitis is an antibiotic strategy, based on a monthly cyclic treatment with rifaximin (a broad spectrum non-absorbable antibiotic) [37, 38]. However, a recent position paper criticizes this approach due to high costs for the National Health System and the arguable results in effectively preventing diverticulitis [39].

A different therapeutic approach in DD, based on mesalazine has been investigated over the past years. Some studies have assessed that mesalazine (alone or in combination with antibiotics) seems to be a promising tool in treating and preventing recurrence of this disease [40]. There are no data
available comparing mesalazine and antibiotics in preventing recurrence of the disease, but clinical studies are currently ongoing.

The results of our study throw light on some mechanisms of the recurrence of the disease. In particular, the presence of histological and endoscopic inflammatory inflammation after an attack of AUD is a high pro-inflammatory intestinal milieu, and provides a further rationale for the use of anti-inflammatory drugs in patients suffering from DD.

CONCLUSION

Detection of endoscopic and histological inflammation after an attack of AUD was found to be a predictive factor of recurrence in acute diverticulitis. These findings are surprisingly similar to IBD, and lead us to consider diverticulitis as a chronic disease requiring long-term treatment in order to prevent recurrence and complications.

Conflicts of interest: none to declare.

REFERENCES