Alimentary Tract

Faecal calprotectin assay after induction with anti-Tumour Necrosis Factor α agents in inflammatory bowel disease: Prediction of clinical response and mucosal healing at one year

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A B S T R A C T

Background: Faecal calprotectin levels correlate with inflammation in inflammatory bowel disease. We evaluated the role of faecal calprotectin after anti-Tumour Necrosis Factor α induction in inflammatory bowel disease patients to predict therapeutic effect at one year.

Methods: Faecal calprotectin levels were measured in stools of 63 patients before and after induction of anti-Tumour Necrosis Factor α therapy. Clinical activity, measured by clinical indices, was assessed before and after biologic treatment. Clinical responders after induction were included in the study and colonoscopy was performed before and after one year of treatment to assess mucosal healing.

Results: 63 patients (44 Crohn’s disease, 19 ulcerative colitis) were prospectively included (41.2% males, mean age at diagnosis 33 years). A sustained clinical response during the first year was observed in 57% of patients; median faecal calprotectin was 106 μg/g after induction versus 308 μg/g pre-induction (p<0.0001). Post-induction faecal calprotectin was significantly lower in responders versus non-responders (p<0.0002). Post-induction faecal calprotectin had 83% sensitivity and 74% specificity (cut-off ≤168 μg/g) for predicting a sustained clinical response at one year (p<0.0001); also, sensitivity was 79% and specificity 57% (cut-off ≤121 μg/g) for predicting mucosal healing (p<0.0001).

Conclusions: In inflammatory bowel disease faecal calprotectin assay after anti-Tumour Necrosis Factor α induction can be used as a marker to predict sustained clinical response and mucosal healing at one year.

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1. Introduction

Inflammatory bowel disease (IBD), which includes Crohn’s disease (CD) and ulcerative colitis (UC), is a chronic disorder characterized by fluctuating periods of remission and episodes of disease activity. The main endpoint of treatment in IBD is the induction and maintenance of disease remission. Biological therapies with anti-Tumour Necrosis Factor α (anti-TNFα) agents have substantially improved the IBD clinical course since they proved to be effective at inducing and maintaining remission. Mucosal healing is considered an additional highly significant therapeutic target for IBD [1].

Currently, the gold standard method for assessing mucosal inflammation is endoscopy with biopsy. However, endoscopy is a costly, invasive, time-consuming, and uncomfortable procedure for patients. To overcome these limitations and reduce the use of the current techniques, new markers detected with a simple, inexpensive, and non-invasive procedure are needed for measuring the response to biological therapy in IBD.

Faecal calprotectin (FC) is a reliable surrogate marker of bowel inflammation throughout the gastrointestinal tract [2] and is useful for discriminating between organic and non-organic bowel disease [3]. FC is a calcium-binding protein that is largely confined to the cytosol of neutrophil granulocytes and macrophages; it is extremely stable in the faeces and is released in biological fluids under inflammatory conditions. Several studies have led to

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growing interest in the value of FC for IBD monitoring. FC levels are correlated with active inflammation and have been used to predict disease relapse in CD and UC [4,5]. FC seems to be a useful surrogate marker to estimate IBD activity because it correlates with clinical assessment and endoscopic findings in both CD [6,7] and UC [8]. Data on FC as a surrogate marker of mucosal healing are also emerging in IBD patients [9,10].

Clinical remission and mucosal healing remain the main goals of therapy with anti-TNFα agents in IBD. A reliable and non-invasive marker for predicting clinical outcome and mucosal healing could provide clinicians with crucial information after the induction of anti-TNFα treatment in these diseases. However, previous studies have shown conflicting results concerning the predictive value of FC for the outcome of anti-TNFα treatment in IBD [11–13].

The aim of our study was to evaluate the predictive role of FC as a non-invasive marker of inflammation in IBD patients to be used for monitoring the clinical response within the first year of treatment. To this aim, we compared the FC levels after induction treatment with TNFα antagonists both in patients who subsequently exhibited sustained clinical responses and in those who did not. As a secondary outcome, we evaluated the predictive role of post-induction FC on mucosal healing evaluated at one year.

2. Patients and methods

During the period between February 2011 and June 2012, consecutive IBD patients who were found to require anti-TNFα treatment for active luminal disease were included in this prospective study. IBD diagnosis for all patients was established with endoscopic and histological criteria at least 6 months before inclusion in the study. Patients with contraindications to anti-TNFα treatment, absence of response to the induction course, pregnancy, ostomy, perianal fistulizing CD without luminal inflammation, and long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) were excluded.

The local ethics committee approved the protocol, and all patients provided written informed consent. The study was performed at the IBD Unit of the Complesso Integrato Columbus, Catholic University, in Rome.

Patients provided samples for the FC assay before and after the induction course of anti-TNFα therapy. For the induction, CD patients received infliximab 5 mg/kg at 0, 2, and 6 weeks, adalimumab 160/80/40 mg every 2 weeks, or certolizumab pegol 400 mg initially, followed by 400 mg in weeks 2 and 4. For maintenance treatment, patients received infliximab 5–10 mg/kg every 8 weeks, adalimumab 40 mg every 2 weeks, or certolizumab pegol 400 mg every 4 weeks. UC patients received infliximab induction (5 mg/kg at 0, 2, and 6 weeks) and maintenance with 5–10 mg/kg every 8 weeks. In some cases the biological therapy was combined with immunosuppressants (azathioprine 1–2.5 mg/kg or 6-mercaptopurine 1.5 mg/kg).

Clinical disease activity was assessed using clinical indices: the CDAI (Crohn's Disease Activity Index) [14] for CD patients, and the CAI (Colitis Activity Index) [15] for UC patients. CDAI and CAI values were calculated at baseline, after the induction treatment, and at one year. We defined as “clinical response” a decrease in the CDAI of more than 100 points in CD patients or a decrease in the CAI of 4 or more points in UC patients. All patients who exhibited clinical responses after the induction course were included in the study. We defined as “loss of clinical response” during the first year of treatment the requirement for an anti-TNFα dose escalation, a course of steroid treatment, or surgery. We defined patients as having a “sustained clinical response” if they had a clinical response both after the anti-TNFα induction and at the end of the first year of treatment.

The endoscopic findings were scored according to the CDEIS (Cronh's Disease Index of Severity) [16] in CD patients, the Rutgeerts score [17] in CD patients who were previously operated, and the Mayo score [18] in UC patients. The endoscopic assessment of disease activity was performed before and after one year of anti-TNFα treatment. We defined mucosal healing as an endoscopic CDEIS <3, a Rutgeerts score = ii, and a Mayo endoscopic score = 0.

The levels of C-reactive protein (CRP, normal value <5 mg/L) were measured at inclusion, after induction, and at one year.

2.1. Faecal calprotectin assay

Stool samples were collected in a plastic container and placed into a disposable screw-cap tube with extraction solution (2.5 ml). After a 30–60-s agitation on a mixer, followed by homogenization for 20 min (3000 rpm on a shaker), the supernatants were collected and analyzed immediately or frozen at −20 °C for later analysis. The FC levels were measured in the supernatants using enzyme-linked immunosorbent assay (ELISA, Calprest, Eurospital s.p.a., Trieste, Italy). The results are expressed as μg/g.

2.2. Statistical analysis

The sample size was calculated after hypothesizing a loss of response rate of 50% and a 30% difference in FC levels after anti-TNFα induction among the patients who exhibited a sustained clinical response and those who did not, with an α level of 0.05 and a power of 0.80. The calculated sample size was 58 patients. Continuous variables are presented as medians and interquartile ranges (IQRs). The Mann–Whitney test was used to evaluate the differences between the independent samples, and the Wilcoxon test was used for paired samples. Differences between frequencies were assessed using the Fisher's exact test. The FC cut-off level predicting the outcomes was established using a receiver operating characteristic (ROC) curve analysis, with the best combination of sensitivity and specificity. Time-to-relapse curves were obtained using Kaplan–Meier survival curves. In addition, multivariate analysis with the stepwise multiple logistic regression model was performed. Statistical significance was set at p < 0.05. The MedCalc, version 9.2.1.0, software (MedCalc Software bvba, Ostende, Belgium) was used for data analysis.

3. Results

Overall, 63 IBD patients (44 with CD and 19 with UC) were prospectively included in the study. The mean age at IBD diagnosis was 33 years (IQR 21.5–47 years). The baseline characteristics of the enrolled patients are shown in Table 1. Forty-two patients were treated with infliximab, 18 with adalimumab, and 3 with certolizumab pegol.

Of the 63 patients, 36 maintained sustained clinical responses within the first year of anti-TNFα therapy (57%, 12 UC and 24 CD), whereas 27 did not (43%, 7 UC and 20 CD). At one year, ileocolonoscopies were performed in all patients. Mucosal healing was achieved in 9/44 (20%) CD patients and in 5/19 (26%) UC patients.

Table 2 shows that in the cohort of patients with sustained clinical responses at one year the median FC value was reduced from 308 μg/g (IQR 128–500) to 106 μg/g (IQR 30–140) after induction therapy (p < 0.0001). In contrast, in patients who did not achieve sustained clinical responses, the difference in FC values – from 398 μg/g (IQR 199–495) to 300 μg/g (IQR 142–475) – was not significant. At baseline, the FC values did not differ between patients who achieved sustained clinical responses and those who did not, whereas after anti-TNFα induction, the latter group had significantly higher median FC values compared to the former group (p = 0.0002). In addition, the median CRP levels significantly
decreased after anti-TNFα induction in the group of patients with sustained clinical response at one year ($p = 0.0054$).

Based on the ROC curve analyses, the FC level after anti-TNFα induction was able to predict a sustained clinical response within the first year with a cut-off of $\leq 168 \mu g/g$ (area under the curve [AUC] 0.77, $p = 0.0001$), with a sensitivity of 83% and a specificity of 74%, in the entire group of IBD patients (Fig. 1a). When considering only the 50 patients with UC and colonic or ileal-colonic CD, the FC value of $\leq 168 \mu g/g$ was optimized as the best cut-off predicting a sustained clinical response at one year, with a sensitivity of 80% and a specificity of 85% (AUC 0.80, $p = 0.0001$) (Fig. 1b).

A post-induction FC reduction of 45%, compared to baseline, generated the maximum sums of sensitivity and specificity for the prediction of sustained clinical response within the first year: respectively 72% and 85% considering all IBD patients (AUC 0.78, 95% confidence interval – CI: 0.66–0.88, $p = 0.0001$, positive predictive value – PPV: 87%, negative predictive value – NPV: 70%), and 67% and 90% considering the group of patients with UC and colonic or ileal-colonic CD (AUC 0.76, 95% CI 0.62–0.87, $p = 0.0001$, PPV 91%, NPV 64%).

The survival analysis showed a significantly higher probability of loss of response within the first year for patients with FC values $> 168 \mu g/g$ after anti-TNFα induction (hazard ratio [HR] 5.20, $p < 0.0001$, Fig. 2).

Based on the ROC curve analysis, the capacity of the post-induction FC values to predict mucosal healing after one year (Fig. 3a) exhibited a sensitivity of 79% and a specificity of 57%, with a cut-off of $\leq 121 \mu g/g$ ($p = 0.038$). Regarding the group of UC patients and colonic and ileal-colonic CD patients, the FC levels after anti-TNFα induction with a cut-off of $\leq 121 \mu g/g$ exhibited a sensitivity and specificity both of 70% for predicting mucosal healing ($p = 0.03$, Fig. 3b).

Levels of FC equal to or lower than the cut-off of 168 $\mu g/g$ were observed post-induction in 81% of the sustained responders versus 26% of those who lost the response ($p = 0.000024$ with the Fisher’s test, Supplementary Figure S1a). Based on the endoscopic findings, 79% of patients achieving mucosal healing at one year had post-induction FC values less than the cut-off of 121 $\mu g/g$, compared to 43% of the patients with signs of disease activity at follow-up endoscopy ($p = 0.031$ with the Fisher’s test, Supplementary Figure S1b).

Table 1
Baseline characteristics of enrolled patients.

<table>
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<tr>
<td>N</td>
<td>63</td>
<td></td>
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</tr>
<tr>
<td>Crohn’s disease</td>
<td>44</td>
<td>70%</td>
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<tr>
<td>Ulcerative colitis</td>
<td>19</td>
<td>30%</td>
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<tr>
<td>Male gender</td>
<td>26</td>
<td>41%</td>
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<td>Median age at diagnosis (years)</td>
<td>33</td>
<td>(IQR 21.5–47)</td>
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<tr>
<td>Anti-TNFα agents</td>
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<tr>
<td>Infliximab</td>
<td>42</td>
<td>67%</td>
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<tr>
<td>Adalimumab</td>
<td>18</td>
<td>28%</td>
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<tr>
<td>Certolizumab</td>
<td>3</td>
<td>5%</td>
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<tr>
<td>Phenotype (Montreal)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Crohn’s disease</td>
<td></td>
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</tr>
<tr>
<td>Behaviour: B1, B2, B3, p</td>
<td>32</td>
<td>(73%), 9 (20%), 3 (7%), 8 (18%)</td>
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<tr>
<td>Location: L1, L2, L3</td>
<td>13</td>
<td>(29%), 7 (16%), 24 (55%)</td>
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<tr>
<td>UC</td>
<td></td>
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<tr>
<td>Previous surgery</td>
<td>13</td>
<td>21%</td>
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</table>

TNF, Tumour Necrosis Factor; UC, ulcerative colitis; CD, Crohn’s disease.

Table 2
Faecal calprotectin and C reactive protein levels at baseline and after anti-TNFα induction.

<table>
<thead>
<tr>
<th></th>
<th>Baseline faecal calprotectin level (µg/g)</th>
<th>Faecal calprotectin level after anti-Tumour Necrosis Factor α induction (µg/g)</th>
<th>$p$</th>
<th>Baseline C reactive protein level (mg/dl)</th>
<th>C reactive protein level after anti-Tumour Necrosis Factor α induction (mg/dl)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sustained clinical response</td>
<td>308 (128–500)</td>
<td>106 (30–140)</td>
<td>&lt;0.0001</td>
<td>9.8 (2–37.7)</td>
<td>2.1 (0.6–6.9)</td>
<td>0.0054</td>
</tr>
<tr>
<td>Loss of response</td>
<td>398 (199–495)</td>
<td>300 (142–475)</td>
<td>0.27</td>
<td>6.4 (1.9–18.6)</td>
<td>5.5 (1.8–20)</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Median (interquartile range). TNF, Tumour Necrosis Factor. * By Wilcoxon test.

$p$ value of faecal calprotectin level after anti-TNFα induction for “sustained clinical response” versus “loss of response” groups = 0.0002 by Mann–Whitney test.
Multiple logistic regression analysis was also performed for patients with both "sustained clinical response" and "mucosal healing", and the variables included were age of disease onset, gender, type of IBD, type of chosen anti-TNFα, combination therapy with immunosuppressants, CRP normalization after anti-TNFα induction, and FC levels after anti-TNFα induction; the analysis indicated that the only variable retained in the models was the FC level.

4. Discussion

The IBD clinical course is generally unpredictable. The identification of patients at significant risk of clinical relapse of disease could facilitate targeted treatment selection. Several studies have investigated the use of the FC assay to predict or monitor the response to medical treatment in IBD. Our data demonstrated that the FC levels measured after an anti-TNFα induction course can be used as a surrogate marker to estimate the efficacy of anti-TNFα agents in IBD and to predict the clinical response and mucosal healing at one year.

Many authors have observed that IBD patients in clinical remission exhibit low FC levels, whereas patients with high levels of this biomarker are at increased risk of relapsing, suggesting that the FC level might be used not only as a marker of clinical remission but also to predict relapse [19–21]. The cut-off values identified in these studies differ according to the specific assay used; however, direct comparisons are possible only if the same assay is used.

To monitor the response to biological therapy in IBD patients, we propose a level of FC ≤ 168 µg/g after anti-TNFα induction to predict sustained clinical response within the first year because this value offered the best combination of specificity and sensitivity. Our results are consistent with the previously published data in this field: in a study including 60 IBD patients, Molander et al. [12], demonstrated that the FC level is a useful marker for predicting clinical outcome after anti-TNFα treatment. At one year, 84% of patients with FC < 100 µg/g after induction were in clinical remission versus 38% of those with a post-induction FC > 100 µg/g; the authors identified a cut-off of 139 µg/g with a better sensitivity and specificity for predicting a risk of clinically active disease after one year. In contrast, in a study on CD patients achieving clinical remission with infliximab induction, a role for FC in predicting relapse was not established [13]. However, the patients in the above-mentioned study were not on maintenance treatment with anti-TNFα, as those in our population, but were treated with azathioprine after a successful induction course of infliximab (the so-called bridge therapy).

The location of the inflammation may influence the accuracy of FC determination. In previous studies, the accuracy of FC for the prediction of IBD relapse increased when only the patients with colonic disease were included. The role of FC in the small bowel of patients with CD remains to be clarified: a significant correlation emerged between a positive FC test and the probability of relapse in UC patients; conversely, among CD patients, only cases of colonic CD showed a significant correlation between a positive FC test and the probability of relapse [5]. Most of the studies confirmed that the capacity of predicting relapses was lower in patients with ileal disease [22]. Although our study was not designed to explore a different behaviour of FC in ileal versus colonic IBD, our results are consistent with those previously published. In fact, the sensitivity and specificity of the best cut-off value for FC post-induction predicting sustained clinical response at one year were higher in the group of patients affected by UC and colonic or ileal–colonic CD than that obtained for all IBD patients. However, in some reports, FC was
equally sensitive in colonic and small bowel CD [23]. A recent meta-analysis considering 672 IBD patients underscored the capacity of FC to predict relapse, which was comparable between UC and CD patients; furthermore, FC was more accurate for ileal-colonic and colonic CD patients. In the small bowel of patients with CD, a predictive value could not be assessed because of the scarce amount of available data [24].

Several subsequent studies have documented that FC concentrations in IBD show a stronger correlation with endoscopic disease activity than with clinical indices [8,25].

In a cohort of CD patients, Sipponen et al. found that the FC value could discriminate mild, moderate, or highly active disease according to the Simple Endoscopic Score for Crohn’s Disease (SES-CD) classification [26,27]. These results are consistent with the findings obtained from another larger cohort of CD patients, demonstrating that the correlation between SES-CD and FC was closer than that with CRP, blood leucocytes, and the CDAI. The overall accuracies for active disease detection at endoscopy were 87% for FC (cut-off 70 µg/g), 66% for elevated CRP, 54% for blood leukocytosis, and 40% for CDAI scores > 150 [7]. Comparable results were obtained in another study in which the authors used the CDEIS score to demonstrate that FC and lactoferrin are more sensitive surrogate markers than the CDAI score and CRP for endoscopic CD activity [6].

Moreover, a significant correlation between FC values and endoscopic disease scores (CDEIS or SES-CD and Mayo scores, respectively) was recently demonstrated in both CD and UC for predicting endoscopic remission [10].

One of the first studies on the clinical significance of faecal markers during anti-TNFα treatment showed that both FC and lactoferrin correlated closely with endoscopic activity at 3 months after the beginning of anti-TNFα induction [28]; this time point is close to the one used in our study for the post-induction determination. A cohort of 53 patients affected by UC had decreased FC levels after the first infliximab infusion; at week 10, an FC value < 50 mg/kg was a very good predictor of concomitant mucosal healing [11].

In our study, by using the CDEIS, Rutgeerts, and Mayo scores to evaluate the endoscopic findings at baseline and after one year of biological treatment, we also described a role for FC measured after anti-TNFα induction in predicting subsequent endoscopic activity. We found a sensitivity of 79% and a specificity of 57% for predicting mucosal healing at one year with a cut-off of 121 µg/g in all IBD patients.

In summary, FC can be used in daily clinical practice for predicting clinical remission and mucosal healing during biological treatment. In clinical practice, we suggest an FC cut-off of 168 µg/g after anti-TNFα induction for predicting clinical response and a cut-off of 121 µg/g for predicting endoscopic remission at one year.

A limitation of our study is the small number of patients that did not allow for a separate analysis of UC and CD patients; such analysis could indeed produce different cut-off values that may be specific for each disease. Other limitations are the inclusion of patients treated with three different anti-TNFα agents, sometimes in combination with conventional immunosuppressants, and the focus on the modification of the FC levels only after anti-TNFα induction, thus not accounting for FC variability during the disease course. Due to these limitations, our results require further validation with larger and more homogeneous populations.

FC is increasingly being employed as a biomarker in clinical IBD practice and, particularly, in screening for possible diagnoses, monitoring disease activity during follow-up, predicting the future disease course, assessing the loss of response to treatments (mainly to biologics), and as a surrogate marker of endoscopy findings. Our results confirm and extend these applications, indicating an important role for the calprotectin assay after the induction course of anti-TNFαs in responding patients. The persistence of high FC levels could imply the absence of mucosal healing and a high probability of disease reactivation during maintenance treatment.

Specifically designed clinical studies could confirm the hypothesis that modifying the therapeutic strategy (e.g., increasing the dose of the anti-TNFαs agent or adding an immunosuppressant) according to the FC levels measured post-induction, or during the maintenance treatment, could improve clinical outcomes in IBD patients.

Conflict of interest
None declared.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdd.2014.07.013.

References


