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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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ABSTRACT

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.

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 2–2.5 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 (Crohn'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 = i0, 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