Tumour Necrosis Factor-Alpha Expression in Segmental Colitis Associated with Diverticulosis Down-Regulates After Treatment

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Abstract

Background & Aims: Tumour Necrosis Factor-α (TNF-α) expression may be increased in Segmental Colitis Associated with Diverticulosis (SCAD). Our aim was to assess TNF-α expression in SCAD in relationship to the treatment. Methods: 10 patients affected by severe (type B and D) SCAD were studied (6 males, 4 females, mean age 60.54 years, range 43-85 years). All patients were treated with beclomethasone dipropionate 10 mg/day plus a probiotic preparation VSL#3 for 8 weeks. At that time, clinical, endoscopic and histological reassessment was performed. Controls were 5 patients with active ulcerative colitis (UC). Results: After treatment, all SCAD B and no SCAD D patients were in remission. The TNF-α expression dropped from 42.7% (±7.58) to 15.7% (±2.6) in SCAD B patients (p=0.001), and from 40% (±5.9) to 28.6% (±5.3) in SCAD D patients (p=0.005). In UC patients, the TNF-α expression dropped from 45.5% (±5.09) to 22.5% (±2.5) (p=0.001). Neither SCAD B nor SCAD D patients showed a significant difference in TNF-α expression compared to UC after treatment. Finally, TNF-α was significantly overexpressed in SCAD D than in SCAD B at the end of treatment (p=0.048). Conclusions: TNF-α expression in SCAD down regulates after treatment, and seems to be related to the clinical response to therapy. This behaviour, similar to that of Inflammatory Bowel Diseases (IBD), confirms that this disease should be considered as a subtype of IBD.

Key words


Introduction

Segmental colitis associated with diverticulosis (SCAD) is defined as a chronic colitis that is confined to the diverticular segment in individuals with otherwise uncomplicated diverticular disease [1, 2]. By definition, the rectum and proximal colon are endoscopically and histologically normal [3]. These inflammatory manifestations differ therefore from conventional colonic diverticulitis, which is essentially a pericolic inflammatory process that originates within diverticula and extends into the surrounding tissues, but spares the nondiverticular colonic mucosa [4, 5].

Tumour necrosis factor alpha (TNF-α) – a 17-kDa polypeptide produced by macrophages, lymphocytes and natural killer cells – has been shown to play a major role in the inflammatory process, with high levels being found in patients with inflammatory bowel disease (IBD) [6-8]. Although this cytokine has been primarily implicated in the pathogenesis of type 1 helper T (Th1) cell-mediated disorders, such as Crohn’s disease (CD) and rheumatoid arthritis [9, 10], high levels of TNF-α have also been shown in the blood, stool and intestinal tissues of patients with ulcerative colitis (UC), suggesting a possible role even in type 2 helper T (Th2) cell-mediated diseases [11, 12]. The role of anti-TNF-α drugs (mainly infliximab and adalimumab) in inducing and maintaining a clinical and endoscopic remission in patients with moderate to severe CD and UC has been demonstrated [13-16]. Furthermore, a profound down-regulation of TNF-α in the affected mucosa has been associated with a dramatic improvement, as assessed both histologically and clinically, after infliximab therapy, thereby providing additional support for the importance of this cytokine in IBD pathogenesis [12, 17].

Conversely, only limited data are available regarding the role of TNF-α on SCAD pathogenesis. Some recent studies found an increased expression of TNF-α in patients affected by SCAD [18-20]. Moreover, as in IBD, TNF-α expression in SCAD may correlate with the severity of the disease, suggesting a possible role for TNF-α also in the pathogenesis of SCAD [20, 21].

In light of these patho-physiological hypotheses, and
considering that a profound TNF-α expression down-regulation appears to be strictly associated with a dramatic regression of the inflammation in patients UC [12], the aim of this study was therefore: to assess the TNF-α expression in SCAD after treatment, and to associate such values with both the clinical response, and the endoscopic and histological activity.

Patients and methods

SCAD patients

We enrolled in this study 10 patients (6 males, 4 females, mean age 58.87 years, range 43-85 years) who had undergone a total colonoscopy, affected by different degrees of SCAD and in whom SCAD was diagnosed for the first time between January 2009 and January 2011.

The criteria for SCAD included endoscopic lesions within the sigmoid-descending colon harbouring diverticula, combined with endoscopic and histological rectal sparing and sparing of the proximal colon [3]. The patients were divided according to the recent endoscopic classification of SCAD (see below in the further paragraph): 6 patients affected by type B SCAD and 4 patients affected by type D SCAD (Table I).

Since there is no clear definition of remission in SCAD, we defined remission as the disappearance of symptoms and healing of endoscopic lesions after 8 weeks of treatment.

Control group

The control group comprised 5 patients affected by moderate-to-severe active ulcerative colitis (UC) (6 males, 4 females, mean age 38.27 years, range 23-48 years) already diagnosed and who had undergone a total colonoscopy for recurrence of symptoms (diarrhea, abdominal pain, rectal bleeding) despite the fact that they were under continuous treatment with mesalazine 2.4 g/day. The UC severity was assessed by Disease Activity Index (DAI) [22]: all patients showed a DAI score >8.

We chose UC as a control group because the clinical (diarrhea and rectal bleeding), endoscopic (erosion and ulcers in the sigmoid region), and histological (polymorphonuclear infiltration of the epithelium and lamina propria, crypt abscesses, loss of glandular parallelism) features of SCAD type B and D are similar to those of UC. At the same time, the endoscopic and histological inflammation differs, because it affects both the sigmoid and rectum in UC, whilst, by definition, the rectum is spared from inflammation in SCAD. These characteristics influence also the classification of the disease activity, which is based on DAI in UC, but it is still lacking for SCAD. Thus, these two diseases are similar but different at the same time [23].

Endoscopic procedures

All patients underwent the same standard bowel preparation prescribed in our centres consisting of an oral polyethylene glycol solution to be taken in the evening. The day after a pancolonoscopy (clean colon colonoscopy) was performed and six colonic biopsies were collected both in the sigmoid tract and in the rectum for histological examination. In SCAD, biopsies are taken from the inter-diverticular mucosa which, by definition, is affected by the disease [1].

The endoscopic classification of SCAD was based on a recent score formulated prospectively by the authors [1]. This endoscopic classification is not based on the progressive severity of the lesions, but on the character of the endoscopic pattern, as follows:

Type B. “Mild-to-moderate ulcerative colitis-like” pattern. A moderate severity disease, with diffuse loss of vascular pattern, mucosal edema and hyperemia and diffuse erosions. Also in these cases diverticular orifices are always spared.

Type D. “Severe ulcerative colitis-like” pattern - severe degree of disease, with diffuse loss of vascular pattern, diffuse hyperemia, and easy bleeding at contact with the colonoscope. Edema in the mucosa with ulcerations is marked, sometimes with reduction of the lumen. The diverticular orifices are not always easy to recognize, but they may be visible, spared by inflammation, at maximal air inflation. Sometimes inflammation involves also the diverticular orifices: in these cases, differential diagnosis with UC together with diverticulosis is based on SCAD rectal sparing.

Endoscopy was performed in SCAD patients at entry and after 8 weeks of treatment.

In UC patients, endoscopic activity was assessed by

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<th>Table I. Characteristics of the SCAD patients at entry</th>
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Abbreviations: SCAD: Segmental colitis associated with diverticulosis; TNF: Tumour Necrosis Factor.

* Mean inflammation was scored from 0 (normal) to 3 (1, mild; 2, moderate; 3, severe), according to the total number of neutrophils detected in the lamina propria; ** TNF-α expression was defined as the percentage of positively stained stromal cells compared with the total number of lamina propria mononuclear cells.
the Mayo Subscore for Endoscopy [24] at entry and after 8 weeks.

**Histological assessment**

The haematoxylin and eosin (H&E)-stained specimens of the inflamed colonic tract were evaluated by two methods.

The first aimed to assess the whole mucosal damage and the second the activity of the inflammation. Mucosal damage was reflected by a score representing the mean value of the single scores of the following histological characteristics: polymorphonuclear infiltration of the epithelium and lamina propria, crypt abscesses, loss of glandular parallelism, crypt shortening and/or ramification, mucus epithelial depletion, involvement of muscularis mucosae and/or submucosa [25]. Each histology score ranged from 0 (normal) to 3 (1, mild; 2, moderate; 3, severe). The activity of inflammation was expressed by the total number of neutrophils in the lamina propria, according to a method we have already described [12]. In detail, cells were counted in five high-power fields (original magnification 400x), selected on the basis of high cellular density, for each specimen. The value was expressed as the number of cells/mm² using the LEICA Q WIN software program (Leica Microsystems Imaging Solutions, Cambridge, UK). The activity of the inflammation was considered to be mild at a value ≥5 and <10 cells/mm²; moderate at a value ≥10 and <15 cells/mm²; severe at a value ≥15 cells/mm².

The expression of TNF-α was assessed by immunohistochemistry, as previously reported [12, 26]. The cytokine was stained using a polyclonal rabbit antibody (PromoCell, Heidelberg, Germany), and the reaction was revealed using a peroxidase/anti-peroxidase (PAP) technique with goat anti-rabbit immunoglobulins (Dako, Copenhagen, Denmark) and a complex of rabbit antibodies and horseradish peroxidase (Dako, Copenhagen, Denmark). A positive reaction was revealed by diamobenzidine and counterstained by H&E. For each staining procedure, we used positive and negative controls. In the immunoperoxidase-stained biopsy specimens, the TNF-α labelling index was defined as the percentage of positively stained stromal cells compared with the total number of lamina propria mononuclear cells, counted in five randomly selected high-power fields (original magnification 400x, at least 1000 total counted cells). All histological and immunohistochemical assessments were performed by a single expert gastrointestinal pathologist (E.I.), blinded regarding the degree of the endoscopic damage of the patients.

Histological assessment was performed at entry and after 8 weeks of treatment.

**Treatment**

**SCAD patients**

All patients underwent an 8-week therapeutic trial with beclomethasone dipropionate (BDP) (Clipper®, Chiesi S.p.A., Parma, Italy) 10 milligrams/day plus high-potency probiotic preparation VSL#3 (VSL Pharmaceuticals, Inc., Townson, MD, USA) 450 billion/day, consisting of sachets each containing 450 billion viable lyophilized bacteria, comprising 4 strains of lactobacilli (L. paracasei, L. plantarum, L. acidophilus and L. delbrueckii subsp. bulgaricus), 3 strains of bifidobacteria (B. longum, B. breve and B. infantis), and 1 strain of Streptococcus thermophilus. The choice of this treatment was based on the results of a previous study on diverticular colitis. At present, specific guidelines regarding the treatment of SCAD are not available. Beclomethasone dipropionate is a new glucocorticosteroid displaying a prompt and potent topical anti-inflammatory activity associated to limited systemic activity and effectiveness in treating UC [22]. VSL#3 has been proven to be effective in obtaining remission of mild to moderate UC when associated to low-dose of balsalazide [27]. Since SCAD is considered an atypical form of IBD and not a complication of diverticular disease [23], and since the association with BDP/VSL#3 has been shown to be effective in the treatment of diverticular colitis in a previous pilot study [28], we treated the patients of this study with the same therapeutic association (BDP plus VSL#3).

Since there is no clear definition of remission in SCAD, we defined remission as the disappearance of symptoms and healing of endoscopic lesions after 8 weeks of treatment.

**UC patients**

In relation to the severity of the disease, all these patients were treated with infliximab 5 mg/kg/i.v. at time 0, 2 and 6 weeks. Remission was defined as DAI score <3, and clinical response was defined as reduction of DAI score ≥50% of baseline value. Patients were re-evaluated after the third infusion: only patients obtaining remission or significant clinical reduction of DAI at 8th week continued to a scheduled treatment with infliximab 5 mg/kg/i.v. every 8 weeks in order to maintain remission.

**Statistics**

The means of TNF-α labelling index of the different groups underwent statistical evaluation. Statistical evaluation was carried out by using Wilcoxon’s test with Yate’s correction for small numbers and Mann-Whitney two samples U-test, as appropriate. Statistically significant difference was considered positive when p≤ 0.05. Also 95% confidence intervals (95% C.I.) for TNF-α expression were assessed.

**Ethics approval**

This study was approved by the Institutional Review Board and each subject gave prior written informed consent to participate in the study.

**Results**

After treatment, all SCAD B patients were in remission. On the contrary, none of the SCAD D patients was in remission. All SCAD D patients improved their symptoms and the endoscopic appearance of the disease, but they were not in remission. All UC patients were in remission (DAI ≤3) at the end of the induction program.

After treatment, the inflammatory infiltrate (Fig. 1) in SCAD patients dropped from the mean score 2.6 (±0.7) to
the mean score 1.1 (±0.3) (p=0.003) in SCAD B and from the mean score 2.7 (±0.09) to mean score 1.7 (±0.1) (p=0.04) in SCAD D.

In UC patients, the inflammatory infiltrate dropped from mean score 2.8 (±0.1) to the mean score 1.8 (±0.1) (p=0.003).

Even if the inflammatory score dropped in both forms of the disease, it was still higher than that of IBS patients (mean score 0.6±0.3). In particular, SCAD B patients did not show significant difference in inflammatory infiltrate in comparison with IBS patients (p=0.061), whilst SCAD D patients showed significantly higher inflammatory infiltrate than IBS patients (p=0.05). On the other hand SCAD B patients showed significantly lower inflammatory infiltrate than UC patients (p=0.023), whilst SCAD D patients showed no significant difference in inflammatory infiltrate in comparison with UC patients (p=0.3).

After treatment, the TNF-α expression in SCAD dropped from 42.7% (±7.58) to 15.7% (±2.6) in SCAD B patients (p=0.001), and from 40% (±5.9) to 28.6% (±5.3) in SCAD D patients (p=0.005) (Fig. 2).

In UC patients, the TNF-α expression dropped from 45.5% (±5.09) to 22.5% (±2.5) (p=0.001).

Even if the TNF-α expression dropped in both forms of the disease, it was still significantly higher than that of IBS patients (mean score 8%±4.95) (SCAD B vs IBS: p=0.0033; SCAD D vs IBS: p=0.001). Despite the fact that SCAD B patients showed lower TNF-α expression compared to UC, this difference was not statistically significant (p=0.08), whilst SCAD D patients showed TNF-α expression similar to that of UC after treatment (p=0.5).

Finally, the comparison of TNF-α expression in the two SCAD groups at the end of treatment showed that TNF-α was significantly overexpressed in SCAD D as compared to SCAD B (p=0.048).

**Discussion**

Segmental colitis associated with diverticulosis has been reported to occur in 1– 3.8% of patients with endoscopic evidence of diverticulosis [1, 29-33]. Although its pathogenesis is obscure, it has been suggested that an alteration of the local microenvironment due to the diverticular state could trigger an abnormal immune response [33]. With respect to IBD pathogenesis, Th1-lymphocytes have recently been found to be involved in the induction of cell-mediated immune responses through the production of selective cytokines, among which TNF-α appears to play a pivotal role [6].

In support of this, increased TNF-α concentrations have been reported in patients with CD and UC [6-8, 11, 12]. Moreover, TNF-α expression seems to be related to the severity of the endoscopic damage both in CD and UC [34, 35].

Recent data found TNF-α to be overexpressed also in SCAD [18, 19]. As in IBD, TNF-α expression in SCAD seems to be related to the severity of the endoscopic damage (21). These findings support the hypothesis that SCAD is a chronic disease in which the pathogenetic role of TNF-α seems to be similar to that played in IBD.

The results of this study provide further confirmation of this hypothesis. In fact the treatment of SCAD with steroids and a high-potency probiotic mixture seems to be able to obtain profound reduction of TNF-α expression, which could be related to the reduction of the diseases’ activity. These values persist higher than those expressed in IBS, confirming that SCAD is a chronic organic disease and not a functional disease.

Another important point is that TNF-α expression in SCAD seems to be related also to the clinical severity of the disease. We found that SCAD B patients, all of them in remission at the end of treatment, showed a higher reduction of TNF-α expression. On the contrary, none of the SCAD D patients were in remission but showed clinical response at the end of treatment: in these patients, significant reduction of TNF-α expression was assessed, with higher values than expressed in SCAD B patients at the end of treatment.
Finally, the comparison of TNF-α expression in the different groups at the end of treatment was significantly different. We do not know whether persisting TNF-α overexpression, with persistence of clinical, endoscopic and histological activity, may influence the long-term outcome of the disease. None of our SCAD D patients was in remission after 8 weeks of treatment, and all of them showed TNF-α values higher than SCAD B patients in remission at the end of treatment. A single case-report showed that infliximab successfully induced a long-term remission in a patient affected by a steroid-dependent SCAD, probably thanks to a profound TNF-α down-regulation after treatment [20]. We feel that our study further strengthens the indication of anti-TNF-α therapy in those very few cases of clinically severe SCAD patients who are also refractory to conventional therapies and with persisting TNF-α overexpression.

The limitation of our study is the small sample size of patients involved. Probably a larger sample size may provide more accurate results. Since the prevalence of this disease is quite low in clinical practice [1], a multicentre study may be of more interest. Moreover, it should be interesting to assess if there is a correlation between immunohistochemistry findings and serum markers, in particular to assess whether serum markers levels are correlated with higher TNF-α expression, and therefore could be an expression of severity of the disease. As in IBD [36], it is hypothesized that also SCAD serum markers levels (e.g. erythro-sedimentation rate or C-reactive protein) may be related to the severity of the disease. None to declare.

**Conflicts of interest**

None to declare.

**References**


