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Normalization of mucosal tumor necrosis factor-α: A new criterion for discontinuing infliximab therapy in ulcerative colitis

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ABSTRACT

Background: Biological agents such as anti-tumor necrosis factor (TNF) induce remission in ulcerative colitis. There is however no consensus regarding the discontinuation of this treatment.
Aim: The aim of this study is to assess whether clinical parameters and mucosal cytokine mRNAs in healed colonic mucosa can predict long-term remission in ulcerative colitis following discontinuation of infliximab (IFX) therapy.
Methods: The prospective Tromsø Inflammatory Bowel Disease (IBD) Study is based on an intensified induction treatment algorithm with IFX to achieve disease remission. Following clinical and endoscopic remission, IFX treatment was discontinued, and follow-up until relapse was performed. Patients who achieved clinical and endoscopic remission following an induction course of IFX were included. Expression levels of TNF alpha (*TNF*), interferon gamma (*IFNG*), interleukin (IL) 6 (*IL6*), *IL17A*, *IL23*, and transforming growth factor beta (*TGFB*) were quantified by real-time PCR in mucosal biopsies obtained at colonoscopy. Remission was defined as Ulcerative Colitis Disease Activity Index (UCDAI) below 3, and an endoscopic sub-score of 0–1. Relapse was defined as UCDAI score >3 and endoscopic sub-score >1. Mucosal cytokine transcript levels from 20 non-IBD patients with a normal colonoscopy served as control group.
Results: Of the 45 patients included, twenty patients (44%) had normalized levels of mucosal *TNF* expression at the time of mucosal healing, whereas 35 of 42 (83%) had normalized *IL17A* expression levels, and 31 of 36 (86%) had normalized *IFNG* expression levels. The median time to relapse was 8 months (range 4–12). Normalization of *TNF* gene expression predicted 20 months (1–39) relapse-free survival after withdrawal of IFX compared to 5 months (3–7) in the group with elevated *TNF* expression. Mucosal expression levels of *IL17A*, *IL23*, *IFNG*, *TGFB*, *IL6* did not predict long-term remission (>12 months)
Conclusion: Normalization of mucosal *TNF* predicts long-term remission after discontinuation of IFX.

1. Introduction

The treatment efficacy of inflammatory bowel disease (IBD) has been highly improved after the introduction of anti-tumor necrosis factor (TNF) such as infliximab in ulcerative colitis (UC) [1] and Crohn's disease (CD) [2], adalimumab in UC [3] and CD [4], golimumab in UC [5] and certolizumab in CD [6]. In cases where anti-TNF treatment does not induce a cure of the disease, most patients receive maintenance therapy for at least the first year after the induction period. Interestingly, after one year on anti-TNF maintenance treatment, 15–27% of patients on placebo still have a healed mucosa compared to 25–46% in the active group in UC [1,3,5,7], and from 0% to 18% in the placebo group compared to 10–44% in the active group in CD [6,8–10]. This implies that some patients are not in need of maintenance therapy during the first year after induction therapy. The main unresolved question is, who are they?

A few studies describe the risk of relapse after withdrawal of anti-TNF agents in CD and UC patients. In two prospective studies of CD, 60% [11], and 46% [12] were still in remission after 1 year with concomitant immunosuppressive (IS) treatment. In two retrospective IBD studies the remission rate after discontinuation of anti-TNF was 35% after 7 years in CD [13], 61% in CD and 75% in UC after 1 year [14]; and finally, in an prospective observational study 55% of CD patients were in remission after 1 year [15] (for

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review, see [16]). So far there is no general agreement on when to discontinue ongoing anti-TNF therapy in IBD. As far as we know, one guideline from 2015 recommends withdrawal of anti-TNF therapy – the English NICE guideline recommends that withdrawal should be considered for patients who have been in stable clinical remission for 12 months [17].

The prospective Tromsø IBD study is based on a treatment algorithm with an intensified induction course of biological therapy (anti-TNF) to achieve endoscopic remission, followed by discontinuation of anti-TNF treatment. The study aim is to define criteria for discontinuing biological therapy. The first report was published in 2013 showing that normalized mucosal gene expression levels of *TNF* and *IL17A* predicted a longer time to relapse after discontinuing adalimumab in CD. The median time to relapse for elevated and normal mucosal *TNF* expression following cessation of adalimumab was 20 and 68 weeks, respectively [18].

In this study we report the results of the Tromsø IBD study design as described below for UC patients treated to disease remission and, and the search for biomarkers for discontinuation of IFX.

Table 1
Demographic data in patients with inflammatory bowel disease treated with intensified induction therapy of infliximab until remission.

Female/male (number)	16/29
Age	36 (15–70)
Duration of disease (years)	5 (0–17)
Baseline CRP	5.5 (0–97)
UCDAI before IFX (mean)	10.1 (6–12)
UCDAI after IFX (mean)	1.6 (0–2) ($P < 0.0005$[b])
Endoscopic pre-score (mean)	2.7 (1–3)
Endoscopic post-score (mean)	0.7 (0–1) ($P < 0.0005$[b])
Infusions of IFX (mean)	3.8 (2–9)
Pre-calprotectin (μg/g)	2100 (210–2500)
Post-calprotectin (μg/g)	65 (20–270) ($P < 0.0005$[b])
Medication at start	
5ASA[a]	43/45 (93%)
Steroids	41/45 (85%)
Immunosuppressive[a]	38/45 (84%)

Values are median (range) if not other indicated.
[a] Medication throughout induction period and after discontinuation of infliximab.
[b] Pre- and post comparisons; Wilcoxon signed rank.

2. Methods

2.1. Patients

The present study is based on a treatment algorithm with an intensified induction therapy to induce remission by biological therapy (anti-TNF), followed by withdrawal of biological therapy. The recruited subjects were patients with moderate to severe UC, treatment-dependent or resistant to steroid. Infliximab (IFX). 5 mg/kg (Remicade; Centocor Inc., Horsham, Pa., USA) was given as repeated infusions at 0, 2 and 6 week and then every 4 weeks until endoscopic remission. The UC diagnosis was based on established clinical, endoscopic and histological criteria [19]. Patients in disease remission (see below) following 2 or more infusions with IFX were included. Response to therapy was evaluated by clinical examination and colonoscopy 2–6 weeks after the last IFX infusion. Remission was defined as a reduction of the UCDAI score to less than 3 in addition to a reduction of the endoscopic sub-score to 0 or 1 [20]. Patients with severe co-morbidity were excluded. Following clinical and endoscopic remission, IFX therapy was discontinued and patients were followed-up with regular clinical evaluations. Relapse was defined using endoscopic and clinical disease activity indices (UCDAI-score >3 and endoscopic score >1) [21]. The patients were followed-up in a period of up to 104 months. Demographic data and co-medication are listed in Table 1.

Biopsies from subjects with a normal colonoscopy and normal colonic histological examination served as controls. All participants were informed and gave written consent. The Regional Committee of Medical Ethics of North Norway and the Norwegian Social Science Data Services approved the study and the storage of biological material (ID: P REK NORD 14/2004).

2.2. Tissue samples

Colonic mucosal biopsies at remission (2 biopsies from each patient) were sampled from the bowel region previously showing the most severe inflammation. Biopsy specimens for RNA extraction were immediately immersed in RNA *later* (Ambion Inc, Austin, USA) and stored at 4 °C overnight.

2.3. Cytokine gene expression

Real-time PCR procedures have previously been described in detail [22,23] RNA was extracted from biopsies by the Trizol method (Invitrogen, Paisley, UK). Total RNA concentration was measured at 260 nm with *U-1500 UV/Vis* spectrophotometer (Hitachi Instruments Inc, San Jose, CA, USA).

Reverse transcription of total RNA was performed by iScript (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Levels of mRNA for *TNF, IL-17, IL-23, IFN-γ, TGF-β, IL-6 a*nd beta-actin (*ACTB*) (house-keeping gene) were determined in duplicates by real-time quantitative RT-PCR using TaqMan chemistry (Applied Biosystems, Foster City, CA, USA) and a standardized threshold value. Except for the *IFNG* assay, all assays were in-house; primer sequences are listed in Table 2.

Stability of *ACTB* as housekeeping gene in the present context has been ascertained earlier [23,24]. The CT values were analyzed according to the delta-delta-C_T method [25].

2.4. Statistics

UCDAI scores defined outcome and relapse. Post-treatment remission was strictly defined as UCDAI less than 3 with endoscopic sub score of 0 or 1. Cytokine transcript levels were analyzed as "normalization/not normalization" as defined by the 97.5th percentile of the normal controls. Time to relapse was analyzed using Kaplan–Meier survival analysis, and different potential predictors were tested. Factors showing a Log-Rank $P < 0.10$ were then analyzed in a multiple regression by Cox proportional hazard analysis. The resulting model was then reduced until all remaining predictors were significant. However, due to the small sample size, the number of covariates we were able to include in the model was limited. *P*-values below 0.05 were considered significant. All statistical analyses were performed in IBM SPSS Statistics 22.

3. Results

3.1. Study population

Forty-five patients in endoscopic and clinical remission after IFX induction treatment were included. After discontinuation of IFX therapy patients were followed up with regular clinical evaluations. IBD co-medication at start of the IFX induction treatment is shown in Table 1, where the 5ASA and immunosuppressive agents (azathioprine or methotrexate) were continued during the induction period and after discontinuation of IFX. The steroids were stopped in all patients during the initial induction period of IFX. Other demographic and clinical data are presented in Table 1.