Optimizing anti-TNF therapy in inflammatory bowel disease

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CHAPTER 4

Loss of infliximab into feces is associated with lack of response to therapy in patients with severe ulcerative colitis


Chapter 4

ABSTRACT
Background and aim
It is not clear why some patients with ulcerative colitis (UC) do not respond to treatment with antitumor necrosis factor (TNF) agents, such as infliximab. It could be that some patients have high level of inflammation, with large quantities of TNF to be neutralized by the drug. We investigated whether loss of anti-TNF agents through ulcerated intestinal mucosa reduces the efficacy of these drugs in patients with severe UC.

Methods
We collected fecal samples from 30 consecutive patients with moderate to severely active UC during the first 2 weeks of infliximab therapy at the University of Amsterdam hospital. Infliximab concentrations were measured in serum and supernatants of fecal samples using an enzymelinked immunosorbent assay (Sanquin Biologicals Laboratory, Amsterdam, The Netherlands). Clinical and endoscopic responses were assessed 2 and 8 weeks and 3 months after treatment began.

Results
Infliximab was detected in 129 of 195 fecal samples (66%); the highest concentrations were measured in the first days after the first infusion. Patients that were clinical nonresponders at week 2 had significantly higher fecal concentrations of infliximab after the first day of treatment than patients with clinical responses (median concentration, 5.01 mg/mL in nonresponders vs 0.54 mg/mL in responders; P=0.0047). We did not observe a correlation between fecal and serum concentrations of infliximab.

Conclusions
Infliximab is lost into stools of patients with UC. High fecal concentrations of infliximab in the first days after therapy begins are associated with primary nonresponse. Additional studies are needed to determine how therapeutic antibodies are lost through the intestinal mucosa and how this process affects treatment response.
Background

Monoclonal antibodies against tumour necrosis factor (TNF) have greatly improved the management of patients with inflammatory bowel disease (IBD). Infliximab is a chimeric anti-TNF antibody with proven efficacy in both Crohn’s disease (CD) and ulcerative colitis (UC). However, a substantial number of patients do not respond to anti-TNF treatment (primary non-responders) and many others develop a secondary loss of response over time. Both phenomena are still poorly understood.

Loss of response is often associated with undetectable serum drug concentrations and or the development of neutralizing anti-drug antibodies. In contrast, the reasons for primary non-response are largely unknown, although several potential mechanisms have been proposed. Certain subtypes of IBD may primarily be driven by pathophysiologic mechanisms that are less dependent on TNF. Furthermore, binding of anti-TNF antibodies to membrane-bound TNF has been associated with induction of T cell apoptosis and formation of wound healing macrophages. Both of these mechanisms of action depend on the presence of the membrane bound form of TNF and it has previously been suggested that the presence of membrane bound form of TNF may predict clinical response to treatment with anti-TNF.

An alternative mechanism for primary non-response is enhanced antibody clearance resulting in inadequate exposure to drug. Clearance of monoclonal antibodies is influenced by multiple factors including body mass index, gender, use of concomitant immunosuppressive agents, the serum albumin concentration, and inflammatory burden. Monoclonal antibodies typically undergo proteolysis within the reticuloendothelial system which is believed to be the primary route of clearance. In severe colitis, massive intestinal loss of proteins, electrolytes and other minerals occurs through the ulcerated epithelial surface. It is well established that patients with severe colitis often require higher than standard doses of anti-TNF antibodies to achieve clinical improvement. Based on these observations we hypothesized that fecal loss of antibodies could represent a yet unknown mechanism of increased drug clearance from a ‘leaky gut’, contributing to an insufficient therapeutic effect in patients with severe ulcerative colitis.
Methods

Anti-TNF naïve patients with moderate-to-severely active ulcerative colitis (endoscopic Mayo score 2/3), who were started on a regular dose of 5 mg/kg intravenous infliximab after failing corticosteroids and/or immunomodulators were studied prospectively. Patients collected at least 4 consecutive fecal samples during the first 2 weeks of treatment, at day 0, 1, 4, 7, 11, and/or 14. Clinical disease activity at baseline was documented with the Simple Clinical Colitis Activity Index (SCCAI), in addition to endoscopic assessment of the disease mucosa by endoscopic Mayo score. Furthermore, laboratory tests at baseline and week 2 included serum hemoglobin, albumin, C-reactive protein and fecal calprotectin (Bühlmann ELISA).

Fecal samples were diluted 1:5 in phosphate buffered saline containing 6% bovine serum albumin. Samples were then homogenized by vortexing for 60 minutes, centrifuged at 3000g for 5 minutes and 100 ul supernatant was collected and stored in freezer (-20°C). Infliximab concentrations were measured in serum at week 2 and fecal supernatants using an ELISA (Sanquin Biologicals Laboratory, Lower Limit of Quantitation 0.03ug/ml). Antibodies to infliximab were also measured at week 2 using an radioimmunoassay. Infliximab-specific IgG was measured by an antigen-binding test. The fecal calprotectin ELISA that was used (Bühlmann laboratories AG) had an upper detection limit of 1800ug/g, values above this limit were further noted as 1800ug/g.

Clinical response was assessed at week 0 and 2 and 3 months after initiation of treatment with infliximab by the treating physician who was unaware of either the serum or stool drug concentrations. Clinical response was defined as a total score of SCCAI ≤ 4 points or a drop in SCCAI of ≥ 50% from the baseline value. Endoscopic response was defined as improvement of the endoscopic Mayo score of at least one point from baseline to week 8.

Patients who needed additional/higher infliximab doses because of deteriorating symptoms or patients who required colectomy due to lack of response within the first 3 months were considered to be non-responders at 3 months.

This study was approved by the local ethical committee according to national Dutch legislation and written informed consent was obtained in all cases.
We used descriptive statistics and data were presented as non-normally distributed, with median value and interquartile ranges (IQR). Differences in infliximab serum and stool concentrations for responders and non-responders were analyzed with Fisher’s exact test was used for univariate analysis of predictors for response and for correlation analysis between serum and stool concentrations. Spearman rank was used. The area under the curve for the fecal infliximab concentrations versus time was calculated by using the trapezoidal rule. Specific power calculation was not performed because of lack of preliminary data. A P value <.05 was considered statistically significant. SPSS® software version 20.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All co-authors had access to the study data and had reviewed and approved the final manuscript.
Results

Thirty consecutive anti-TNF naïve patients with moderate-to-severely active colitis (18 pancolitis and 12 left-sided colitis) were included. No patients refused to participate. (TABLE 1)

<table>
<thead>
<tr>
<th>TABLE 1 Baseline Characteristics</th>
<th>N=30</th>
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<tbody>
<tr>
<td>Gender: male, n (%)</td>
<td>19/30 (63%)</td>
</tr>
<tr>
<td>Age, median (IQR)</td>
<td>40 (29-49)</td>
</tr>
<tr>
<td>Disease duration, median (IQR)</td>
<td>8 (0-15)</td>
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<tr>
<td>Left-sided colitis, n (%)</td>
<td>12/30 (40%)</td>
</tr>
<tr>
<td>Pancolitis, n (%)</td>
<td>18/30 (60%)</td>
</tr>
<tr>
<td>UC Endoscopic Mayo score 2 UC</td>
<td>4/30 (13%)</td>
</tr>
<tr>
<td>Endoscopic Mayo score 3</td>
<td>26/30 (87%)</td>
</tr>
<tr>
<td>Hospitalized, n (%)</td>
<td>10/30 (33%)</td>
</tr>
<tr>
<td>Corticosteroid refractory, n (%)</td>
<td>28/30 (93%)</td>
</tr>
<tr>
<td>Concomitant thiopurines</td>
<td>16/30 (53%)</td>
</tr>
<tr>
<td>UC Simple Clinical Colitis Activity Index, median (IQR)</td>
<td>10 (7-12)</td>
</tr>
<tr>
<td>Hb (g/dl), median (IQR)</td>
<td>11.5 (10.0-12.9)</td>
</tr>
<tr>
<td>CRP (mg/l), median (IQR)</td>
<td>13.6 (3.4-57.6)</td>
</tr>
<tr>
<td>Serum Albumin (g/l), median (IQR)</td>
<td>37 (30-41)</td>
</tr>
<tr>
<td>Fecal calprotectin (ug/g), median (IQR)</td>
<td>1800 (700-1800)</td>
</tr>
</tbody>
</table>

Legend: IQR; Interquartile range, Hb; Hemoglobin, CRP; C-Reactive Protein, UC: Ulcerative colitis.

All patients received infliximab 5 mg/kg at week 0 and 2, except for 2 patients who failed to demonstrate any clinical and biochemical improvement at day 5 and therefore received a second infusion of 10 mg/kg at that time. These patients were considered to be non-responders. The demographic characteristics of the patients are shown in table 1. One third of patients were hospitalized for severe disease and had failed intravenous treatment with corticosteroids.

Infliximab was not detected in any of the fecal samples collected before the start of treatment. During the first 2 weeks of treatment, infliximab was detectable in feces of 25/30 patients (83%) and in 129/195 (66%) of all fecal samples. (FIGURE 1) The highest concentrations were measured in the first days following the first infliximab infusion (fecal concentration day 1 (median, IQR): 1.70, 0.01-9.75 ug/ml). At day 1, Infliximab was detected in fecal samples in 22 out of 30 patients. At week 2 serum infliximab concentrations (median, IQR) were 17.15 (13.25-25.01) ug/ml.
Loss of infliximab into feces in UC

Figure 1. Infliximab concentrations in fecal supernatant 14 days after the first infliximab infusion.

Clinical response was observed in 18/30 patients (60%) by week 2 and in 14/30 patients (47%) by month 3. Twenty-three patients underwent a second endoscopy at week 8 including 2 patients receiving an extra IFX dose. Of the 21 patients on regular treatment, 12/21 (57%) had an endoscopic response. (TABLE 2)

<table>
<thead>
<tr>
<th>TABLE 2 Effects of infliximab induction at week 2, week 8 and month 3</th>
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<tbody>
<tr>
<td>Week 2 clinical response, n (%)</td>
</tr>
<tr>
<td>Week 8 endoscopic response, n (%)</td>
</tr>
<tr>
<td>Month 3 clinical response, n (%)</td>
</tr>
</tbody>
</table>

Week 2 non-responders had significantly higher fecal infliximab concentrations on the first day after the infliximab infusion than week 2 responders (median 5.01 (1.91-20.14) vs. 0.54 (0.0-4.40) ug/ml, P= .0047). (FIGURE 2). Endoscopic non-responders at week 8 (9/21) had higher fecal infliximab concentrations compared to patients with endoscopic improvement (median 4.66 (1.49-16.29) vs 1.16 (0.0-4.82) ug/ml respectively, P=.0588). Patients without response by month 3 (16/30) also ended to have higher fecal infliximab concentrations (median 2.51 (1.23-11.58) vs. 0.54 (0.0-8.07) ug/ml) at day 1 compared to responders (P=.1081).
The areas under the curve of fecal loss of infliximab during the first 2 weeks were 16.8 for non-responders at week 2 versus 4.6 ug/ml/day for responders (12 vs. 18; P=.0048), 16.5 versus 4.8 ug/ml/day for endoscopic (non-)response at week 8 (9 vs. 12; P=.09) and 12.8 versus 4.8 ug/ml/day (16 vs. 14; P=0.15) at month 3. While multiple biochemical parameters including C-reactive protein, hemoglobin and fecal calprotectin were analyzed, fecal infliximab concentration at day 1 was the only predicting factor for endoscopic response to infliximab (TABLE 3).

For clinical non-responders at week 2, serum infliximab concentrations (median, IQR) were 16.55 (8.55-24.02) ug/ml compared to responders 17.15 (13.75-26.25) ug/ml (P=.81).

No direct correlation between fecal levels and serum levels was observed. (r=-0.15, P=.44). (Supplementary FIGURE 3) Patients with detectable infliximab levels in feces had a significantly more severe colitis, indicated by higher clinical scores and fecal calprotectin concentrations at baseline compared to patients in which infliximab could not be detected the first day after infusion. (Supplementary TABLE 4) In addition, patients with relatively lower (than our median) baseline serum albumin levels were found to have significantly higher fecal IFX concentrations at day 1 and lower serum IFX concentrations at week 2. (Supplementary TABLE 5) Moreover, patients with a fecal calprotectin of >1000ug/g at week 2 had significantly higher median (IQR) fecal IFX AUC compared to patients with lower calprotectin levels at week 2: 16.49 (7.97-43.33) ug/ml/day vs. 0.15 (0.0-5.08) ug/ml/day (P<0.01). Finally, week 2 fecal Calprotectin levels showed a correlation with fecal AUC: r:0.56 (P<0.01).
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Patients with detectable infliximab levels in feces had a significantly more severe colitis, indicated by higher clinical scores and fecal calprotectin concentrations at baseline compared to patients in which infliximab could not be detected the first day after infusion. (Supplementary TABLE 4)

In addition, patients with relatively lower (than our median) baseline serum albumin levels were found to have significantly higher fecal IFX concentrations at day 1 and lower serum IFX concentrations at week 2. (Supplementary TABLE 5)

Moreover, patients with a fecal calprotectin of >1000ug/g at week 2 had significantly higher median (IQR) fecal IFX AUC compared to patients with lower calprotectin levels at week 2: 16.49 (7.97 - 43.33) ug/ml/day vs. 0.15 (0.0 - 5.08) ug/ml/day (P<0.01). Finally, week 2 fecal Calprotectin levels showed a correlation with fecal AUC: r:0.56 (P<0.01).

TABLE 3 Early predictors for endoscopic response (N=21)

<table>
<thead>
<tr>
<th>Predictor, median (IQR)</th>
<th>Responders(n=12)</th>
<th>Non-responders(n=9)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease duration (years)</td>
<td>10 (0-15)</td>
<td>4 (2-12)</td>
<td>.67</td>
</tr>
<tr>
<td>Concomitant Thiopurines</td>
<td>4/12 (33%)</td>
<td>5/9 (56%)</td>
<td>.40</td>
</tr>
<tr>
<td>Baseline Colitis Activity Index</td>
<td>10 (6-12)</td>
<td>10 (10-13)</td>
<td>.35</td>
</tr>
<tr>
<td>Baseline Hb (g/dl)</td>
<td>11.0 (10.0-13.1)</td>
<td>11.6 (10.8-13.2)</td>
<td>.35</td>
</tr>
<tr>
<td>Baseline CRP (mg/l)</td>
<td>13.5 (6.4-45.5)</td>
<td>11.0 (2.4-75.0)</td>
<td>.72</td>
</tr>
<tr>
<td>Baseline Albumin (g/l)</td>
<td>37 (32-41)</td>
<td>38 (32-40)</td>
<td>.89</td>
</tr>
<tr>
<td>Fecal calprotectin (ug/g)</td>
<td>1800 (625-1800)</td>
<td>1800 (981-1800)</td>
<td>ns</td>
</tr>
<tr>
<td>Fecal IFX day 1 (ug/ml)</td>
<td>1.16 (0.0-4.82)</td>
<td>4.66 (1.49-16.29)</td>
<td>.0588</td>
</tr>
</tbody>
</table>

Legends: IQR; Interquartile range, Hb; Hemoglobin, CRP; C-Reactive Protein, IFX; infliximab
Discussion

We report that intestinal loss of therapeutic anti-TNF antibodies is associated with treatment failure in patients with moderate-to-severely active ulcerative colitis. This is the first observation showing that a therapeutic antibody can be found at detectable levels in feces. Our data is likely a conservative estimate of the quantity of antibody lost in the gut, since therapeutic proteins are degraded by proteases that are abundantly present in the intestinal lumen of IBD patients and proteases were not neutralized in our assay.27 The assay that we used in this study detects intact antibody and Fab2 fragments, but may not detect Fab fragments or other/further breakdown products of infliximab.21 Fecal samples were deep frozen and homogenized and analyzed several weeks later. Therefore, degradation of part of the antibody cannot be excluded.

Given that the peak fecal infliximab concentration was observed approximately 2 days after the first infusion, it appears that the greatest loss of infliximab occurs at the time when serum drug concentrations are the highest and when the mucosa is most severely inflamed and, hence, more ‘leaky’. Patients with significant fecal loss of infliximab had more severe disease at baseline. Patients with low serum albumin concentrations at baseline had higher fecal IFX concentrations at day 1 and lower serum IFX concentrations at week 2. This underscores the probable role of protein loss in lowering serum IFX concentrations and the idea that severe disease and extensively ulcerated surface is the cause of intestinal loss, similar to observations made with immunoglobulins.18 Because the current cohort mainly consisted of patients with extensive colonic disease (with large ulcerated surfaces), no significant association was found between extent of disease and fecal loss.

This cohort only included patients with ulcerative colitis. However, in an additional pilot study we analyzed fecal samples of 4 CD patients (2 patients with colonic disease and 2 patients with isolated small bowel disease). Three of them also had measurable fecal infliximab levels, suggesting that antibody loss may not be limited to the ulcerated colon alone.

Anti-infliximab antibodies were not detectable in serum samples at week 2. The ability to evaluate antibodies to infliximab is hampered by the fact that the antibody assay that was used cannot detect anti-infliximab antibodies in the presence of high circulating infliximab concentrations. Antibodies to infliximab were also not detected in...
feces. Furthermore, no significant difference was observed in patients with or without concomitant thiopurines with regard to serum or fecal levels of infliximab.

In this cohort, we observed a rather high rate of primary non-response to infliximab in comparison with previously reported studies. However, this is most likely due to the fact that this particular cohort mainly consisted of patients with severely active colitis (endoscopic Mayo score 3). Moreover, our early and strict definition of response may have contributed to this high ‘failure’ rate.

Although fecal loss of infliximab appears to contribute to primary non-response in ulcerative colitis, it is probably not the only factor influencing the pharmacokinetics of these therapeutic antibodies. The lack of correlation between early fecal infliximab concentrations and day 14 serum infliximab concentrations could be explained by other factors influencing the serum concentration of the drug. Although it has been suggested that serum drug concentrations are highly relevant for clinical effect, recent data indicate that mucosal concentrations may be even more important, at least in severe types of colitis. Mucosal anti-TNF concentrations and the ratio of anti-TNF-to-TNF in tissue were reported to be lower in severely inflamed parts of the colon, which suggested that mucosal inflammation is the most likely cause of antibody loss. Fecal loss of infliximab could hence represent a separate elimination route, affecting the mucosal compartment separately of the blood compartment. Its contribution in relation to proteolysis within the reticuloendothelial system remains to be determined. Recently, molecular imaging of intestinal membrane-bound TNF positive immune cells with fluorescent antibodies, at the site of action (mucosa), has been proposed as a potential modality to predict therapeutic response to biological treatment.

Evidently, fecal loss of monoclonal antibodies may represent a reflection of disease activity rather than only a causal factor of primary non-response. As such, it could even be considered as a biomarker for disease activity, identifying patients at high risk for colectomy. The importance of early detection of non-response is exemplified by the two patients who were given an ‘accelerated’ 10 mg/kg (double dose) infusion and consequently avoided colectomy. Both patients that had received IFX 10mg/kg at day 5, had high serum CRP (306 and 128 mg/l) and low serum albumin (25 and 29 g/l) and were hospitalized for severe symptoms. After initial slight improvement of CRP and Albumin the first days after a regular dose of IFX (5 mg/kg), those parameters deteriorated again at day 5 in both patients: CRP (217 and 111.8 mg/l), Albumin (21 and 27 g/l). Because the clinical condition of the patients deteriorated, it was decided to administer a second dose of IFX at day 5.
This intervention led to rapid improvement of the clinical condition of both patients and patients were discharged. Week 2 CRP were 23.2 and 3.9 mg/l and Albumin 32 and 35 g/l. Endoscopy at week 8 showed mucosal healing (endoscopic Mayo score ≤1) in both patients. Up to date both patients have not had a colectomy (follow-up 34 and 18 months respectively). In line with this observation, a recent study showed that an intensified infliximab induction strategy for acute severe colitis reduced colectomy rates significantly.\textsuperscript{32}

In conclusion, intestinal loss of infliximab in moderate-to-severely active ulcerative colitis is associated with a diminished response to this treatment. Patients with severe disease may therefore benefit from more intensive dosing regimens. This strategy warrants a prospective clinical trial.
Loss of infliximab into feces in UC

REFERENCES


