

ORIGINAL ARTICLE

Cut-off levels and diagnostic accuracy of infliximab trough levels and anti-infliximab antibodies in Crohn's disease

CASPER STEENHOLDT¹, KLAUS BENDTZEN^{2,3}, JØRN BRYNSKOV¹,
OLE ØSTERGAARD THOMSEN¹ & MARK ANDREW AINSWORTH¹

¹Department of Medical Gastroenterology, University Hospital of Copenhagen, Herlev Hospital, Herlev, Denmark,

²Institute for Inflammation Research, University Hospital of Copenhagen, Rigshospitalet, Copenhagen, Denmark, and

³BioMonitor A/S, Copenhagen, Denmark

Abstract

Introduction. Reasons for infliximab failure in Crohn's disease and ulcerative colitis are debated. Serum levels of infliximab and anti-infliximab antibodies have been associated with loss of response. We aimed at determining cut-off levels for infliximab and anti-infliximab antibody concentrations associated with clinical response to infliximab maintenance therapy. **Methods.** Patients with inflammatory bowel disease ($n = 106$) were retrospectively classified as having maintained response or loss of response to infliximab maintenance therapy. Trough concentrations were measured by fluid-phase radioimmunoassays.

Results. Infliximab levels were significantly lower, and anti-infliximab antibody levels significantly higher, in Crohn's disease patients with loss of response (median infliximab 0 µg/ml, median anti-infliximab antibodies 35 U/ml) compared to patients with maintained response (median infliximab 2.8 µg/ml, median anti-infliximab antibodies 0 U/ml; $p < 0.0001$). Receiver operating characteristic (ROC) analysis identified optimal cut-off values: infliximab <0.5 µg/ml, which was associated with loss of response with sensitivity 86% [64–97] and specificity 85% [72–94]; and anti-infliximab antibodies ≥ 10 U/ml yielding a sensitivity of 81% [61–93] and specificity 90% [79–96]. Combined measurements of infliximab and anti-infliximab antibodies using these cut-off values had higher accuracy yielding a sensitivity of 81% [57–94] and specificity 94% [82–98]. Similar pattern was observed in a smaller cohort of patients with ulcerative colitis. **Conclusions.** Combined measurements of infliximab and anti-infliximab antibodies using cut-off levels provided high accuracy for discriminating between clinical response types to infliximab maintenance therapy. Cut-off levels are considered a prerequisite to further investigations of clinical usefulness of measurements of infliximab and anti-infliximab antibodies in patients failing infliximab therapy.

Key Words: Crohn's disease, inflammatory bowel disease, infliximab, TNF, ulcerative colitis

Introduction

Infliximab (Remicade), a chimeric mouse/human monoclonal antibody against tumor necrosis factor (TNF)-alpha, is highly effective in inducing and maintaining remission in patients with Crohn's disease (CD) and ulcerative colitis (UC) [1–4]. However, not all patients respond to infliximab therapy and a considerable fraction of initial responders lose effect over time [5–9]. These therapeutic failures, which have not yet been thoroughly addressed,

pose a challenge to clinicians; for example, there is no consensus regarding the optimal management of patients with therapeutic failure. This is largely due to lack of knowledge of the mechanisms leading to infliximab failure in the individual patients.

Reasons for infliximab failure may, for example, involve immunological mechanisms due to formation of anti-infliximab antibodies as well as pharmacokinetic and pharmacodynamic mechanisms [10]. The clinical relevance of anti-infliximab antibodies is disputed, even though anti-infliximab antibody

Correspondence: Casper Steenholdt, MD, Department of Medical Gastroenterology, University Hospital of Copenhagen, Herlev Hospital, Herlev Ringvej 75, DK-2730 Herlev, Denmark. Tel: +45 40529577. E-mail: steenholdt@brygge.dk

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formation has been associated with loss of treatment response, shorter response duration, and risk of infusion reactions in several independent studies of patients with CD [11–14]. Low serum infliximab concentrations have consistently been associated with failure of clinical response in both CD and UC [11,15–18].

There is an obvious clinical need for development of a more rational therapeutic approach to patients with infliximab failure. Therefore, we and others have proposed treatment algorithms for CD patients with loss of response to infliximab maintenance therapy based on infliximab trough levels and anti-infliximab antibody levels at time of infliximab failure [10,19]. Despite a large number of publications assessing clinical implications of infliximab and anti-infliximab antibody levels, no previous studies have identified levels for clinically relevant infliximab and anti-infliximab antibody concentrations in patients with inflammatory bowel disease (IBD). It is unknown if cut-off levels will allow discrimination between different clinical response types to infliximab maintenance therapy. Furthermore, it is unknown if cut-off levels can be used in a binary way (high vs low), and prospectively as a guide in the treatment of patients with loss of response to infliximab maintenance therapy as suggested in the treatment algorithms [10,19].

In the present study, we determined cut-off values for clinically relevant concentrations of infliximab and anti-infliximab antibodies measured by radioimmunoassays (RIA). These cut-off values provided high diagnostic accuracy in terms of discrimination between different types of clinical response to infliximab maintenance therapy in patients with IBD, and may be useful in guiding treatment of individual patients with infliximab failure.

Materials and methods

Patients

All patients with IBD at our tertiary center in whom infliximab and/or anti-infliximab antibody trough levels had been determined during infliximab therapy in the period from January 2001 to June 2010 were included in the study [20,21]. Infliximab and/or anti-infliximab antibody concentrations were randomly measured in representative IBD patients as decided by the treating physician. The treating physician was blinded for the results of the analyses. Blood samples were available from 85 patients with CD and 21 patients with UC. Previously published data from 17 patients with CD were included in the study and

were evaluated in a secondary statistical analysis [22]. Trough level was defined as serum concentration immediately before an infliximab infusion. Retreatment was defined as minimum two separate infliximab treatment series at least 6 months apart. The study was approved by the regional ethics committee.

Based on patient records, each patient was retrospectively classified as having one of the following clinical response types to infliximab maintenance therapy: *Maintained response*, defined as a good clinical response to infliximab induction treatment (weeks 0, 2, 6) followed by a continued good clinical response to infliximab maintenance treatment; *Loss of response*, defined as an initial good clinical response to infliximab induction treatment followed by loss of clinical response to infliximab maintenance treatment leading to discontinuation of treatment. Infliximab maintenance treatment was defined as regular infliximab infusions every 4–12 weeks with at least one infusion 8 weeks after induction treatment. In maintained responders, measurement of infliximab and anti-infliximab antibody trough concentrations was done during maintenance treatment. In patients with loss of response, measurement was done when the treating physician determined to discontinue infliximab maintenance therapy due to lack of effect, and blood samples were sampled at trough levels at the time where the patient should have received the next scheduled infliximab infusion given the treatment had been continued. Study related classification of patients according to clinical response type was done without knowledge of the test results.

The decision of continuation or discontinuation of infliximab therapy was based solely on the treating senior gastroenterologist's overall evaluation of symptoms, findings of clinical examinations, biochemical analyses, and diagnostic procedures. The vast majority of patients were seen by the three authors (JB, OØT, MAA).

All patients were treated with hydrocortisone (100 mg intravenously), acetaminophen (1 g orally), and cetirizine (10 mg orally) prior to administration of infliximab to minimize risk of acute infusion reactions and prevent development of anti-infliximab antibodies [12].

Infliximab and anti-infliximab antibody measurements

All sera were tested at Biomonitor A/S (Symbion Science Park, Copenhagen, Denmark) under blinded conditions using fluid-phase RIAs for measurements of infliximab and anti-infliximab antibody levels as previously detailed [23–25]. Sera were stored at -80°C and usually tested within 1–2 weeks.

Functional infliximab concentrations were measured as the levels of infliximab providing the same TNF- α binding as those of the IgG-fraction of the serum samples tested. Briefly, 1% patient serum was incubated with ^{125}I -TNF- α (Perkin Elmer, Boston, MA, USA), and after separation of free and IgG-bound tracer using rabbit anti-human Fc- γ antibody (Dako, Glostrup, Denmark), the pellet activity was measured using a 1470 automatic gamma counter (Wallac, Alleroed, Denmark). Using infliximab as reference (Schering-Plough, Ballerup, Denmark), the TNF- α binding capacity was expressed as infliximab equivalents ($\mu\text{g/ml}$).

Anti-infliximab antibody levels were also tested by RIA. As infliximab is an IgG construct consisting only of kappa light chains, anti-human lambda light chain antibodies were used to distinguish radiolabeled infliximab in free form and complexed with any class of lambda-containing human immunoglobulin. Briefly, 1% patient serum was incubated with ^{125}I -infliximab, and immunoglobulin-bound tracer was precipitated with anti-human immunoglobulin lambda-chain antibody (Dako). After counting pellet activities, anti-infliximab antibody concentrations were expressed as arbitrary laboratory units (U) per ml, where <10 U/ml was considered negative (detection limit).

Statistical analyses

Descriptive statistics was stated as medians and interquartile ranges (IQR). Differences between response groups were evaluated by non-parametric methods using Mann-Whitney U test. Categorical variables were analyzed using Fisher's exact test.

Identification of optimal cut-off levels for infliximab and anti-infliximab antibodies were determined using receiver operating characteristics (ROC) analysis. The primary ROC-analysis was done using original data only. Data from 17 CD patients previously published in a different context were included in a secondary ROC-analysis [22]. Test characteristics were based on data from all patients. Identification of patients with loss of response and maintained response to infliximab maintenance therapy was considered equally important. Therefore, cut-off levels providing minimal difference between sensitivity and specificity were chosen (i.e. sensitivity = specificity) [26]. Confidence intervals (CI) of predictive values, and sensitivity and specificity of combined assessment of infliximab and anti-infliximab antibodies were determined using the efficient-score method [27].

Two sided p -values <0.05 were considered significant. Statistical analysis was done using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Crohn's disease

Patient characteristics. A total of 85 patients with CD were included. Fifty-nine (69%) were categorized as having maintained response to infliximab maintenance therapy (median number of infusions 8; IQR 6–17), and 26 (31%) were categorized as having lost response to infliximab maintenance therapy (median number of infusions 6; interquartile range (IQR) 5–11). Infliximab trough concentration was determined in 69 patients, and anti-infliximab antibody trough concentration was determined in 85 patients. Clinical characteristics of the two response groups appeared balanced (Table I).

Infliximab trough levels. Infliximab trough levels were significantly higher in CD patients with maintained response to infliximab maintenance therapy (median $2.8 \mu\text{g/ml}$, IQR 0.8–5.3, $n = 48$) compared to those who had lost response to infliximab maintenance therapy (median $0 \mu\text{g/ml}$, IQR 0–0, $n = 21$; $p < 0.0001$; Figure 1).

Infliximab cut-off levels. Applying ROC analysis on data from patients with CD, an infliximab trough concentration of $0.5 \mu\text{g/ml}$ provided an optimal cut-off value as previously defined. The same optimal cut-off value was found when including previously reported data in the ROC-analysis. Thus, infliximab trough levels $<0.5 \mu\text{g/ml}$ were associated with loss of response to infliximab maintenance therapy with sensitivity of 86% [95% CI 64–97] and specificity of 85% [72–94]. Seventy-three percent [52–88] of CD patients with infliximab trough levels $<0.5 \mu\text{g/ml}$ had lost response to infliximab maintenance treatment ($n = 19$), while 95% [83–99] of patients with infliximab trough levels $\geq 0.5 \mu\text{g/ml}$ had maintained response ($n = 41$). The ROC curve is shown in Figure 2, and sensitivity and specificity of selected cut-off values are listed in Table II.

The area under the ROC curve (AUC^{ROC}) represents the performance of the test. AUC^{ROC} was 0.93 [0.85–1.00] and significantly higher than 0.5 which characterizes an ineffective test ($p < 0.0001$). Accuracy was 87% (true positive and true negative tests compared to all tests). Test characteristics are summarized in Table III.

Anti-infliximab antibody levels. Anti-infliximab antibody trough levels were significantly higher in CD patients who had lost response to infliximab

Table I. Baseline characteristics.

	Crohn's disease		Ulcerative colitis	
	Maintained response	Loss of response	Maintained response	Loss of response
Number of patients	59	26	10	11
Male sex, <i>n</i> (%)	30 (51)	8 (31)	5 (50)	5 (45)
Age, year median (IQR)	39 (30–46)	36 (28–46)	30 (26–44)	41 (26–49)
Disease duration, year median (IQR)	10 (1–37)	8 (2–25)	6 (3–15)	3 (0,5–14)
Disease type, <i>n</i> (%)				
Luminal only	41 (69)	15 (58)	–	–
Fistulizing	18 (31)	11 (42)	–	–
Total infliximab infusions, <i>n</i>				
Median (IQR)	8 (6–17)	6 (5–11)	5 (4–8)	7 (4–10)
Previous anti-TNF treatment, <i>n</i> (%)				
Infliximab	10 (17)	11 (42)	1 (10)	4 (36)
Adalimumab	1 (2)	0 (0)	0 (0)	0 (0)
Concomitant medication, <i>n</i> (%)				
Azathioprine or mercaptopurin	40 (68)	12 (46)	10 (100)	4 (36)
Methotrexate	5 (8)	2 (8)	0	0
Cyclosporine	0 (0)	0 (0)	0	0
5-Aminosalicylates oral	4 (7)	3 (12)	9 (90)	8 (73)
Corticosteroids oral	0 (0)	0 (0)	0	1 (9)
None of abovementioned	11 (19)	9 (35)	0	0

IQR: Interquartile range.

maintenance therapy (median 35 U/ml, IQR 12–76, *n* = 26) compared to those with maintained response to infliximab maintenance therapy (median 0 U/ml, IQR 0–0, *n* = 59; $p < 0.0001$; Figure 3).

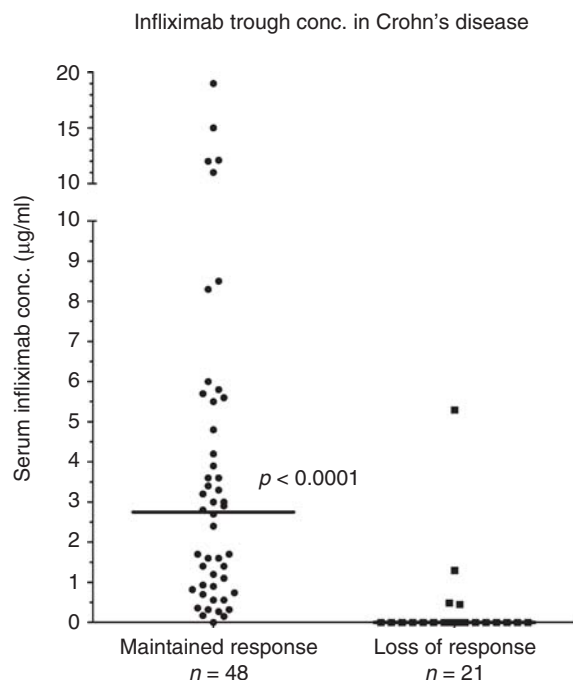


Figure 1. Infliximab trough serum concentrations in patients with Crohn's disease with maintained response and loss of response to infliximab maintenance treatment. Horizontal lines indicate median values.

Anti-infliximab antibody cut-off levels. Applying ROC analysis on data from CD patients, an anti-infliximab antibody trough level of 10 U/ml provided an optimal cut-off value as previously defined. This cut-off value corresponds to the detection limit of the assay. The same optimal cut-off value was found when including previously reported data in the ROC-analyses. Thus, the detection of anti-infliximab

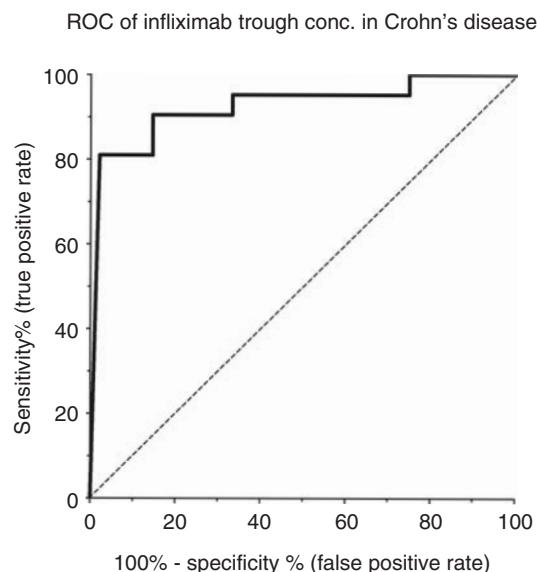


Figure 2. Receiver operating characteristic (ROC) curve of infliximab trough serum concentrations in patients with Crohn's disease to determine cut-off levels associated with clinical response type to infliximab maintenance treatment.

Table II. Selected cut-off values for infliximab trough concentrations associated with clinical response type to infliximab maintenance treatment in Crohn's disease. Optimal value in bold.

	Sensitivity	Specificity
Infliximab <0.1 µg/ml	81 [58–95]	98 [89–100]
Infliximab <0.5 µg/ml	86 [64–97]	85 [72–94]
Infliximab <1.0 µg/ml	90 [70–99]	71 [56–83]
Infliximab <1.5 µg/ml	95 [76–100]	63 [47–76]
Infliximab <2.0 µg/ml	95 [76–100]	54 [39–69]

antibodies (i.e. ≥ 10 U/ml) was associated with loss of response to infliximab maintenance therapy with sensitivity of 81% [61–93] and specificity of 90% [79–96]. Seventy-eight percent [57–91] of CD patients with anti-infliximab antibody trough levels ≥ 10 U/ml had lost response to infliximab maintenance treatment ($n = 21$), while 91% [80–97] of patients with undetectable anti-infliximab antibodies had maintained response ($n = 53$). The ROC curve is shown in Figure 4, and sensitivity and specificity of selected cut-off values are listed in Table IV. AUC^{ROC} was 0.89 [0.79–0.98], and accuracy was 87%. Test characteristics are summarized in Table III.

Combined assessment of infliximab and anti-infliximab antibodies. Combined and simultaneous measurement of infliximab and anti-infliximab antibody trough concentrations was assessed in 48 CD patients with maintained response and in 21 CD patients who had lost response. Use of combined test for identifying patients who had lost response to infliximab maintenance therapy using the established cut-off values had a sensitivity of 81% [57–94], a specificity of 94% [82–98], and an accuracy of 90% (see Table III). Combined test was regarded positive if both tests were positive otherwise considered negative. Eighty-five percent [61–96] of CD patients with infliximab trough levels <0.5 µg/ml and detectable anti-infliximab antibodies (≥ 10 U/ml) had lost response to infliximab maintenance treatment ($n = 17$), while 92% [80–97] of patients with infliximab trough levels ≥ 0.5 µg/ml and/or undetectable anti-infliximab antibodies had maintained response ($n = 45$).

Ulcerative colitis

Patient characteristics. In order to explore whether our findings in CD would also apply to patients with UC, we next assessed infliximab and anti-infliximab trough levels in patients with UC. A total of 21 UC patients were included. Ten (48%) were categorized as having maintained response to infliximab maintenance therapy (median number of infusions 5; IQR 4–8), and 11 (52%) were categorized as having lost response to infliximab maintenance therapy (median number of infusions 7; IQR 4–10). Infliximab trough concentration was determined in 13 patients, and anti-infliximab antibody trough concentration was determined in 20 patients. Clinical characteristics of the two response groups appeared balanced (Table I).

Infliximab and anti-infliximab antibody trough levels

As in patients with CD, infliximab trough levels were significantly higher, and anti-infliximab antibody levels significantly lower, in patients with maintained response (median infliximab 3.8 µg/ml, IQR 1.1–8.5, median anti-infliximab antibodies 0 U/ml, IQR 0–0) compared to those who had lost response to infliximab maintenance therapy (median infliximab 0 µg/ml, IQR 0–0.9, median anti-infliximab antibodies 85 U/ml, IQR 10–100; $p = 0.0083$ and $p = 0.0007$, respectively; see Figures 5 and 6).

Infliximab and anti-infliximab antibody cut-off levels

Applying ROC analysis on data from patients with UC, an infliximab trough concentration of 0.8 µg/ml and detectable anti-infliximab antibodies (i.e. ≥ 10 U/ml) provided optimal cut-off values. Sensitivity and specificity of infliximab trough levels was 75% [35–97] and 100% [48–100], respectively, and AUC^{ROC} was 0.95 [0.83–1.07]. Sensitivity and specificity of anti-infliximab antibody levels was 80% [44–97] and 100% [69–100], respectively, and AUC^{ROC} was 0.90 [0.74–1.06].

Table III. Test characteristics for assessing clinical response to infliximab maintenance treatment in Crohn's disease.

	Sensitivity	Specificity	AUC^{ROC}	Accuracy
Infliximab <0.5 µg/ml	86% [64–97]	85% [72–94]	0.93 [0.85–1.00]	87%
Anti-infliximab antibodies ≥ 10 U/ml	81% [61–93]	90% [79–96]	0.89 [0.79–0.98]	87%
Combined infliximab <0.5 µg/ml and anti-infliximab antibodies ≥ 10 U/ml	81% [57–94]	94% [82–98]	NA	90%

AUC^{ROC} : Area under ROC curve.

NA: Not applicable.

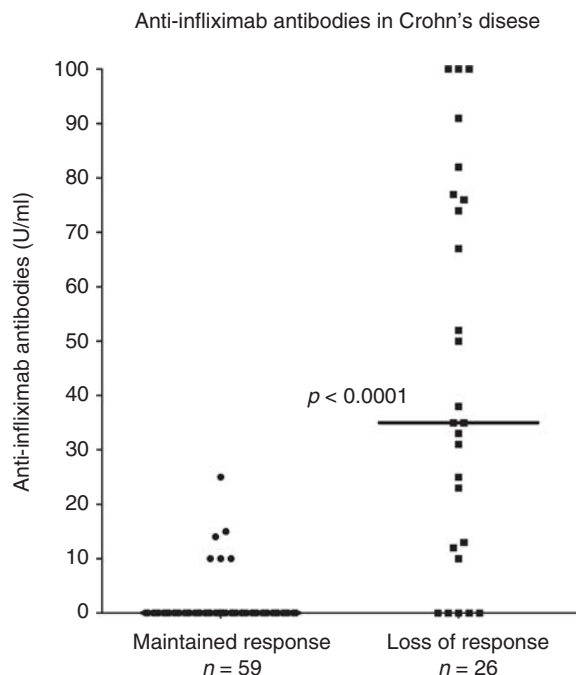


Figure 3. Anti-infliximab antibody concentrations in serum of patients with Crohn's disease with maintained response and loss of response to infliximab maintenance treatment. Horizontal lines indicate median values.

Predisposing factors for development of anti-infliximab antibody and low infliximab levels

The number of patients developing anti-infliximab antibodies was significantly higher among IBD

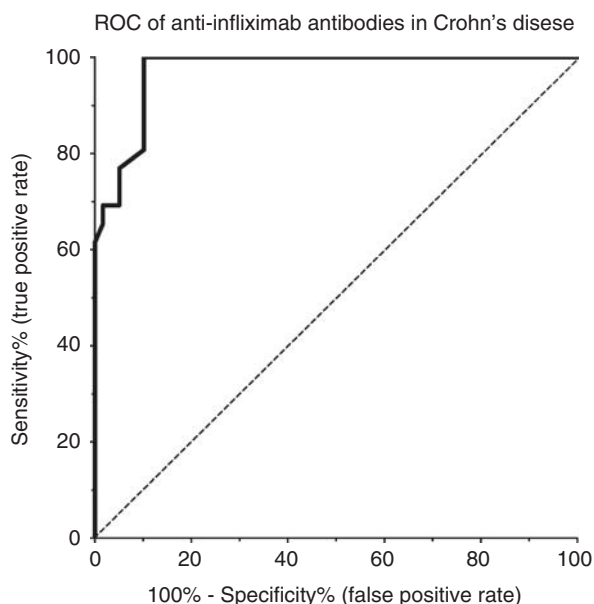


Figure 4. Receiver operating characteristic (ROC) curve of anti-infliximab antibody trough serum concentrations in patients with Crohn's disease to determine cut-off levels associated with clinical response type to infliximab maintenance treatment.

Table IV. Selected cut-off values for anti-infliximab antibody trough concentrations associated with clinical response type to infliximab maintenance treatment in Crohn's disease. Optimal value in bold.

	Sensitivity	Specificity
Anti-infliximab antibodies ≥ 10 U/ml	81 [61–93]	90 [79–96]
Anti-infliximab antibodies ≥ 11 U/ml	77 [56–91]	95 [86–99]
Anti-infliximab antibodies ≥ 12 U/ml	73 [52–88]	95 [86–99]
Anti-infliximab antibodies ≥ 13 U/ml	69 [48–86]	95 [86–99]
Anti-infliximab antibodies ≥ 14 U/ml	69 [48–86]	97 [88–100]

patients who did not receive concomitant immunosuppressives (16 of 32 patients) compared to those treated with azathioprine, 6-mercaptopurine, or methotrexate (19 of 73 patients); odds ratio 2.8 [1.2–6.8] ($p = 0.02$). Furthermore, development of anti-infliximab antibodies was significantly higher among retreated CD patients (11 of 21 patients) as compared to patients having received only one treatment series (16 of 64 patients); odds ratio 3.3 [1.2–9.2] ($p = 0.03$).

In CD, there was no association between lack of concomitant immunosuppression and low infliximab trough levels ($<0.5 \mu\text{g/ml}$): 12 of 23 without concomitant immunosuppressive treatment had low infliximab levels, while 14 of 46 receiving concomitant immunosuppressives had low infliximab levels ($p = 0.11$). Also, there was no association between retreatment of CD patients and low infliximab trough

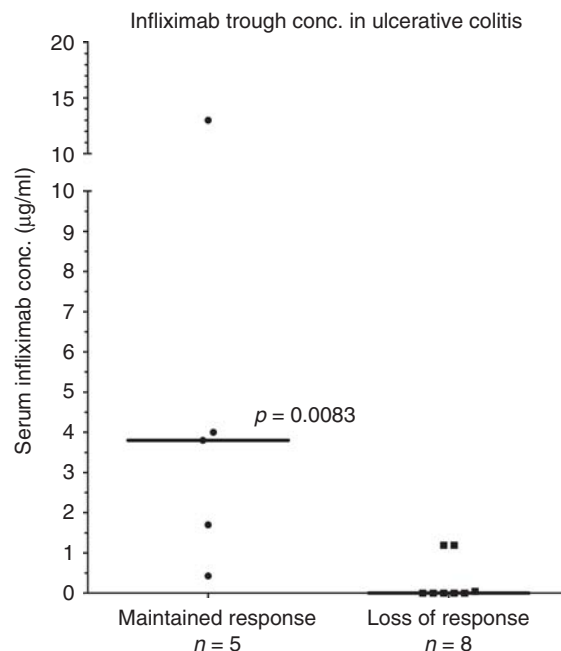


Figure 5. Infliximab trough serum concentrations in patients with ulcerative colitis with maintained response and loss of response to infliximab maintenance treatment. Horizontal lines indicate median values.

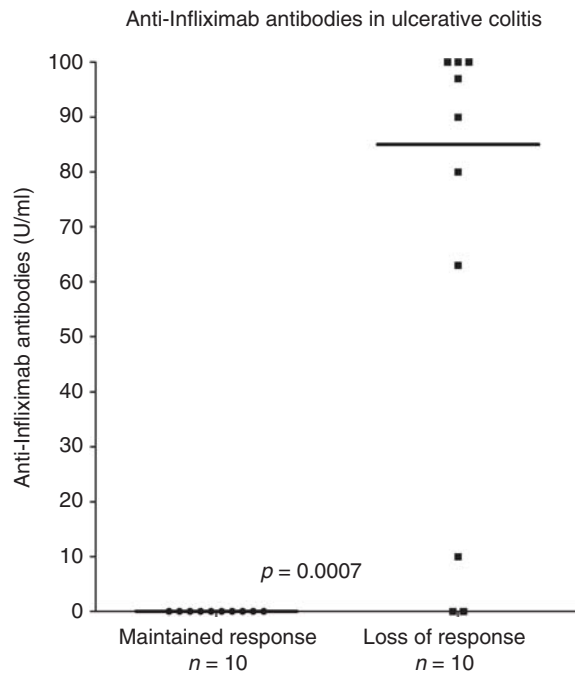


Figure 6. Anti-infliximab antibody trough serum concentrations in patients with ulcerative colitis with maintained response and loss of response to infliximab maintenance treatment. Horizontal lines indicate median values.

levels: 9 of 17 retreated patients had low infliximab, while 17 of 52 continuously treated patients had low infliximab levels ($p = 0.16$).

Discussion

The present study has identified cut-off levels for clinically relevant concentrations of infliximab and anti-infliximab antibodies in patients with CD. Cut-off levels could be used in a binary way, and it allowed discrimination of different clinical response types to infliximab maintenance therapy with high accuracy. Combined measurements of infliximab and anti-infliximab antibodies had the highest overall accuracy as compared to individual assessments of infliximab and anti-infliximab antibody concentrations. Even though the immunogenicity in CD and UC may not necessarily be the same, we found comparable cut-off values in a small cohort of UC patients, indicating that our results from CD may in fact also apply to UC. Larger and prospective studies are needed to confirm and extend these findings.

In agreement with previously published studies, we found that patients with CD who have lost effect of infliximab maintenance therapy have significantly lower serum trough levels of infliximab and significantly higher serum trough levels of anti-infliximab antibodies as compared to patients

with adequate clinical response to infliximab maintenance therapy [11,15–18,22]. However, previously published data on the clinical importance of anti-infliximab antibodies in CD have been ambiguous. Thus, expression of anti-infliximab antibodies has been associated with loss of response in some studies but not in others [5,11–15,28,29]. A study of UC patients showed only a correlation between infliximab and clinical response [17]. This is in contrast to our findings, which in a small cohort of UC patients demonstrated significantly lower levels of infliximab and significantly higher levels of anti-infliximab antibodies in UC patients having lost effect of infliximab maintenance therapy as compared to those with maintained response. In our cohort of IBD patients, lack of concomitant immunosuppressive therapy and retreatment with infliximab were separately associated with development of anti-infliximab antibodies, which is in accord with previous findings [11,12,15,28–31].

The cause of the variable and to some extent contradictory findings regarding clinical relevance of infliximab and anti-infliximab antibody levels is unknown but may be related to methodological differences including the shortcomings of commonly used enzyme-linked immunosorbent assays (EIAs) for detection of infliximab and anti-infliximab antibodies [10,32]. Thus, it is debated whether EIAs actually measure bioactive infliximab concentrations, and whether EIAs detect anti-infliximab antibodies that interfere with infliximab bioactivity [33–35]. False-positive and false-negative results contribute to additional discrepancy in many EIA formats. False positive findings are, for example, caused by non-specific binding of low affinity heterophilic antibodies, rheumatoid factors, or complement factors to the Fc-parts of immunoglobulins including infliximab. This is particularly problematic in bridging-type EIAs. False negative findings are, for example, caused by matrix effects in solid-phase assays resulting in epitope masking, and the inability to detect functionally monovalent immunoglobulins, for example, IgG4 in bridging-type EIAs. Inconclusive anti-infliximab antibody measurements due to interference with infliximab contribute to the problem and further decrease the clinical usefulness of often used EIAs [10,34,36].

Consequently, we have used clinically validated fluid-phase RIAs which mimics the actual conditions in the patients and are not influenced to the same degree by the potential artifacts encountered in solid-phase EIAs [10,22,24,25,36–38]. Thus, the RIA for detection of infliximab measures the functional bioactive infliximab concentration; that is, the fraction of infliximab which is not neutralized by anti-infliximab antibodies and is therefore capable

of neutralizing TNF- α – the TNF-binding capacity. Furthermore, the RIA for detection of anti-infliximab antibodies measures all isotypes of immunoglobulins binding to infliximab [24,25].

Limitations of our study include a retrospective design and classification of clinical response type based on the overall clinical evaluation without strict and well defined objective criteria for this. Patients were not routinely assessed by a clinical activity index score and/or endoscopic evaluation. Data from a small number of CD patients have previously been published in a different context [22]; however, optimal cut-off values were established using original data only. Cut-off values were the same when including previously published data, which indicates that these values are robust and reproducible. RIA requires specialized equipment due to usage of radioactive substances and is therefore difficult to use as an in house laboratory technique. However, the RIAs used are commercially available and therefore generally available for detection of infliximab and anti-infliximab antibodies [23]. It should be noted that the established cut-off values applies to RIA and may not necessarily be generalized to other assays. This is, however, the case for all types of assays including different subtypes of EIAs.

It is widely acknowledged that development of anti-infliximab antibodies does not explain all cases of loss of response to infliximab maintenance treatment [15,35]. Our results are in accordance with this: 20% (5 of 26) of CD patients who had lost response had undetectable levels of anti-infliximab antibodies. Half of these patients had infliximab trough levels below the established cut-off level. In these cases, we hypothesize that antibodies may have escaped notice or that loss of response was due to altered pharmacokinetics, and that the condition might be treated with increased infliximab dosage. By contrast, the other half had infliximab trough levels above the established cut-off level. In these cases, we hypothesize that loss of response was caused by a pharmacodynamic problem, for example, due to TNF-independent disease. For these patients, we speculate that TNF-inhibitors are ineffective and should be discontinued. We have previously suggested a treatment algorithm based on these considerations [10]. A recently published retrospective study has added weight to our hypothesis by indicating higher response rates when IBD patients with loss of response and positive anti-infliximab antibodies were changed to another TNF inhibitor as compared to dose escalation, as well as when patients with undetectable infliximab levels were dose escalated as compared to change of anti-TNF drug [19]. Although intuitively appealing, our algorithm needs of course to be tested prospectively.

In conclusion, this study is the first to provide cut-off values for clinically relevant concentrations of infliximab and anti-infliximab antibodies in patients with IBD. Using these cut-off values, combined measurements of infliximab and anti-infliximab antibodies using commercially available RIA techniques could differentiate patients who had lost effect of infliximab maintenance therapy and patients with maintained response to infliximab maintenance therapy with high accuracy. Cut-off levels are considered a prerequisite to further investigations of the clinical usefulness of measurements of infliximab and anti-infliximab antibodies in patients failing infliximab therapy.

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