

Clinical Implications of Variations in Anti-infliximab Antibody Levels in Patients with Inflammatory Bowel Disease

Casper Steenholdt, MD,* Magid Al-khalaf, MD,* Jørn Brynskov, MD, DMSc,* Klaus Bendtzen, MD, DMSc,[†] Ole Ø. Thomsen, MD, DMSc,* and Mark A. Ainsworth, MD, DMSc*

Background: The aim of the study was to investigate variations in anti-infliximab (IFX) antibody (Ab) levels and clinical implications thereof in patients with inflammatory bowel disease (IBD).

Methods: A retrospective, explorative, single-center study of patients with IBD who developed anti-IFX Ab and in whom anti-IFX Ab were reassessed.

Results: IFX was administered to 316 patients; anti-IFX Ab was determined in 180 patients and detected in 83 (46%). During ongoing IFX maintenance therapy, anti-IFX Ab disappeared at later reassessment in two-thirds of patients with clinical response after median 4 (3–5) infusions. In contrast, anti-IFX Ab persisted in all patients without clinical response. Anti-IFX Ab appeared pharmacologically active, as IFX levels were high when anti-IFX Ab disappeared (median 3.7 $\mu\text{g/mL}$, interquartile range [IQR] 2.8–5.5), while undetectable or low when anti-IFX Ab persisted (median 0 $\mu\text{g/mL}$, IQR 0–0). In 56 patients, anti-IFX Ab were assessed after IFX discontinuation. The proportion of patients with anti-IFX Ab gradually declined over time, with a few patients having anti-IFX Ab up to about 4 years after initial assessment. No variables were associated with anti-IFX Ab disappearance in multivariate analysis.

Conclusions: Discontinuation of IFX is advisable in patients with inadequate response and repeat positive anti-IFX Ab measurements. Anti-IFX Ab can persist for years after discontinuation, which could impact efficacy and safety at retreatment. Continued IFX treatment may, however, be considered in patients with clinical response and a single positive anti-IFX Ab measurement, as anti-IFX Ab disappears in two-thirds of these during continued treatment.

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Key Words: inflammatory bowel disease, Crohn's disease, ulcerative colitis, infliximab, TNF, antidrug antibody, anti-TNF antibodies, pharmacokinetics

B iologic drugs that specifically block the proinflammatory cytokine, tumor necrosis factor (TNF)-alpha, have proven highly effective in inducing and maintaining remis-

sion in moderate to severe luminal or fistulizing Crohn's disease (CD) or ulcerative colitis (UC).¹ Infliximab (IFX) is used as a TNF-blocking agent in inflammatory bowel disease (IBD).^{1–4} Despite its effectiveness, approximately one-third of IBD patients have no effect of IFX induction therapy (primary nonresponse), and about half the patients with response to IFX maintenance therapy lose the effect over time (secondary nonresponse).^{5,6}

IFX is a chimeric mouse–human IgG1 construct. Development of anti-IFX IgG antibodies (Ab) primarily directed against its murine F(ab)₂ component is frequent, and has been observed in 5%–18% of IBD patients on maintenance treatment and in up to 61% of patients receiving episodic treatment.^{7–9} Anti-IFX Ab formation is associated with loss of clinical response and infusion reactions, and may consequently lead to discontinuation of IFX therapy.^{6,9–13} Even though the risk of anti-IFX Ab formation is reduced by scheduled maintenance therapy and concomitant immunosuppression in episodically treated patients, anti-IFX Ab development appears to represent a substantial clinical problem in IBD. Accordingly, assessment of anti-IFX Ab has been proposed as an important element of

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From the *Department of Medical Gastroenterology, Herlev Hospital, Denmark, [†]Institute for Inflammation Research, Rigshospitalet, Denmark.

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Reprints: Casper Steenholdt, MD, Dept. of Medical Gastroenterology, Herlev Hospital, University of Copenhagen, Herlev Ringvej 75, DK, 2730 Herlev, Denmark (e-mail: steenholdt@brygge.dk).

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algorithms designed to optimize IFX treatment in IBD patients.^{2,6,10,14,15}

Existing trials have mainly focused on clinical implications of anti-IFX Ab formation, but little is currently known about the variation of anti-IFX Ab levels in individual patients. Data from a small subgroup of patients in the ACCENT1 therapeutic trial have shown that anti-IFX Ab appears to have a short half-life in patients who discontinue IFX.¹⁶ Of note, however, anecdotal experience, including our own (unpubl. data), suggest that anti-IFX Ab may also disappear in an as-yet undefined subgroup of IBD patients on IFX maintenance treatment.¹⁷ The aim of this study was to examine the therapeutic implications of variations in anti-IFX Ab levels during ongoing IFX therapy, as well as after IFX discontinuation in patients with IBD.

MATERIALS AND METHODS

Study Design and Population

This was an observational retrospective study of IFX-treated IBD patients at a single Danish university hospital from January 1999 until October 2010. In this time period, IFX and anti-IFX Ab had been determined at the discretion of the treating physician. The study population comprised patients who had developed anti-IFX Ab and in whom anti-IFX Ab had been reassessed at least once at a later timepoint. The patients were identified by review of patient files of all patients having received IFX during the designated time period. These patients were then reassessed for anti-IFX Ab at time of this study (primo 2011) unless a repeat anti-IFX Ab measurement had already been collected routinely by the treating physician.

Definitions

Maintenance therapy was defined as regular IFX infusions every 4–12 weeks; episodic therapy as reinitiation of IFX after more than 12 weeks. Clinical response to IFX was classified according to the treating physicians evaluation as noted in the patient file as described previously and consisted of: 1) complete response, defined as clinical remission with no symptoms or findings indicating active disease; 2) no response, defined as no improvement of symptoms and findings of active disease; 3) partial response, defined as all other and intermediate response types and with a clear evidence of improvement of symptoms and findings of active disease.^{11,12} To reduce bias, evaluation of clinical response type and data analysis was done by separate investigators. Concomitant immunosuppression was defined as treatment with azathioprine, mercaptopurine, or methotrexate in standard doses during ongoing IFX therapy.

Serum Analyses

Samples

Blood samples were obtained as trough levels 30 minutes prior to IFX infusion; i.e., at least 4 weeks after the

previous IFX infusion. Three samples obtained at the time of first anti-IFX Ab detection during the IFX maintenance phase were not obtained as trough levels, and were consequently not analyzed for IFX levels. IFX and anti-IFX Ab concentrations were analyzed in parallel in the same sample material. Serum was collected after centrifugation of 10 mL blood and analyzed at Biomonitor A/S (Symbion Science Park Copenhagen, Denmark) under blinded conditions.¹⁸ Samples were sent at room temperature for immediate analysis; a few samples were stored at -80°C for later analysis.

IFX

Functional IFX concentrations were measured as the levels of IFX providing the same TNF- α binding as those of the IgG-fraction of the serum samples tested by radioimmunoassay (RIA).^{11–14,19–23} Briefly, 1% patient serum was incubated with ^{125}I -TNF- α (Perkin Elmer, Boston, MA), and after separation of free and IgG-bound tracer using rabbit anti-human Fc-gamma antibody (Dako, Denmark), the pellet activity was measured using a 1470 automatic gamma counter (Wallac, Denmark). Using IFX as reference (Schering-Plough, Denmark), the TNF- α binding capacity of a serum sample was expressed as the equivalent binding of IFX in $\mu\text{g/mL}$.

Anti-IFX IgG Ab

The anti-IFX IgG assay was carried out as previously detailed, using RIA and anti-human lambda light-chain Ab to distinguish between free drug and drug in complex with any class of lambda-containing human immunoglobulin.^{11–14,19–23} Briefly, 1% serum was added 4000–5000 cpm/100 μL of ^{125}I -IFX. After overnight incubation, free and immunoglobulin-bound (any isotype) tracer were separated by precipitation using a dose of rabbit antihuman lambda (Dako, Denmark) which had optimal precipitation of ^{125}I -IFX in seven randomly selected anti-IFX Ab-positive sera. The pellet activity was measured in a gamma-counter with backgrounds $<4\%$ and inter- and intraassay variations $<20\%$ and $<10\%$, respectively. The data are given as U/mL based on a laboratory standard set to 100 U/mL.

Statistical Analyses

Descriptive statistics were calculated as percentages for discrete variables, and median with interquartile range (IQR) or mean with standard error of the mean (SEM) for continuous variables, as appropriate. Fisher's Exact test was used for univariate analysis of discrete variables. Unpaired *t*-test or Mann-Whitney *U*-test, as appropriate, were used for univariate analysis of continuous variables. Multivariate logistic regression analysis was done by entering all parameters varying between groups on univariate analysis with a statistical significance of $P < 0.2$ into the model. The number of infusions until disappearance of anti-IFX Ab during maintenance treatment was estimated using Kaplan-Meier statistics. Variables associated with anti-IFX Ab disappearance after IFX discontinuation with $P < 0.2$ in univariate Cox proportional hazard regression analysis were included in a multivariate Cox model.

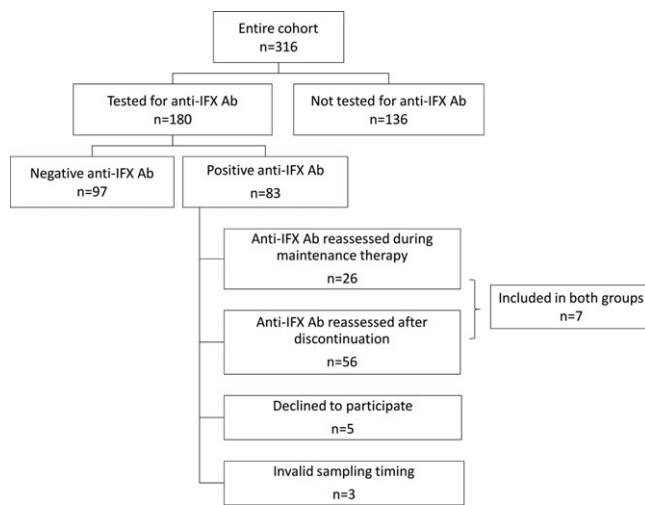


FIGURE 1. Study population.

Statistical analyses were done with SPSS v. 18 (IBM, Somers, NY). All *P*-values were two-sided and *P* < 0.05 was considered significant.

Ethical Considerations

The study was approved by the regional Ethics Committee.

RESULTS

Patient Characteristics

IFX was administered to a total of 316 IBD patients among whom 219 had CD and 97 had UC diagnosed according to standardized diagnostic criteria (Fig. 1).^{24,25}

Anti-IFX Ab analysis was performed in 180 (57%) of these patients among whom 143 had CD and 37 UC. Sixty-six patients were tested one time, 63 twice, 36 three times, and 15 four or five times. Of the total of 180 patients tested, 83 (46%) had developed anti-IFX Ab in conjunction with IFX therapy (Fig. 1). Anti-IFX Ab were detected after median four IFX infusions (interquartile range [IQR] 3–7). Development of anti-IFX Ab was consistently associated with lack of concomitant immunosuppression and fewer IFX infusions in both univariate analysis and in multiple logistic regression analysis (Table 1).

Next, the patients were divided into two groups according to IFX therapy between initial and repeat anti-IFX Ab assessment: 1) patients in whom IFX maintenance therapy had been continued, and 2) patients in whom IFX therapy had been discontinued immediately after detection of anti-IFX Ab (samples obtained prior to last IFX infusion were included; Fig. 1). Five patients declined to participate, of whom all had discontinued IFX immediately after detection of anti-IFX Ab; three patients were excluded from the analysis due to invalid sample timing.

Anti-IFX Ab Levels During IFX Maintenance Therapy

IFX maintenance therapy was continued in 26 patients, 21 with CD and 5 with UC, after they had developed anti-IFX Ab. The initial median anti-IFX Ab level was 29 U/mL, IQR 16–59 and the corresponding IFX trough levels were either below the detection limit or very low: median 0.1 µg/mL, IQR 0–0.6 (Fig. 2). The reason for measuring anti-IFX Ab was incomplete response to IFX as judged by the treating physician. When anti-IFX Ab were reassessed at a later, scheduled infusion during the

TABLE 1. Characteristics of Patients Assessed for Anti-IFX Ab

Variables	Positive Anti-IFX Ab n=83	Negative Anti-IFX Ab n=97	Univariate Analysis <i>P</i>	OR [95% CI]	Multivariate Analysis <i>P</i>	OR [95% CI]
Crohn's disease – no. (%)	68 (82)	75 (77)	0.466	1.3 [0.6–2.8]	—	—
Male sex – no. (%)	38 (46)	45 (46)	1.000	1.0 [0.5–1.8]	—	—
Age at diagnosis – yr median (IQR)	23 (18–35)	25 (20–36)	0.430	1.0 [1.0–1.0]	—	—
Age at 1 st IFX infusion – yr median (IQR)	35 (24–46)	32 (26–41)	0.775	1.0 [1.0–1.0]	—	—
Disease duration at 1 st IFX series – yr median (IQR)	5 (2–13)	6 (2–9)	0.713	1.0 [1.0–1.1]	—	—
Total number of IFX infusions – no. median (IQR)	6 (4–9)	10 (4–17)	0.001	0.9 [0.0–1.0]	0.003	0.9 [0.9–1.0]
IFX dosing 5 mg/mL – no. (%)	82 (99)	96 (99)	1.000	0.9 [0.1–14.0]	—	—
IFX induction (week 0,2,6) ^b – no. (%)	39 (47)	56 (58)	0.178	0.6 [0.4–1.2]	0.079	0.6 [0.3–1.1]
Episodic IFX regimen – no. (%)	43 (52)	42 (43)	0.295	1.4 [0.8–2.5]	—	—
Concomitant immunosuppression ^a – no. (%)	42 (51)	69 (71)	0.006	0.4 [0.2–0.8]	0.011	0.4 [0.2–0.8]

Ab: antibodies; CI: confidence interval; IFX: infliximab; IQR: interquartile range; OR: odds ratio.

^aAzathioprine, mercaptopurine, or methotrexate.

^bIn case of episodic therapy, induction refers to the last IFX series.

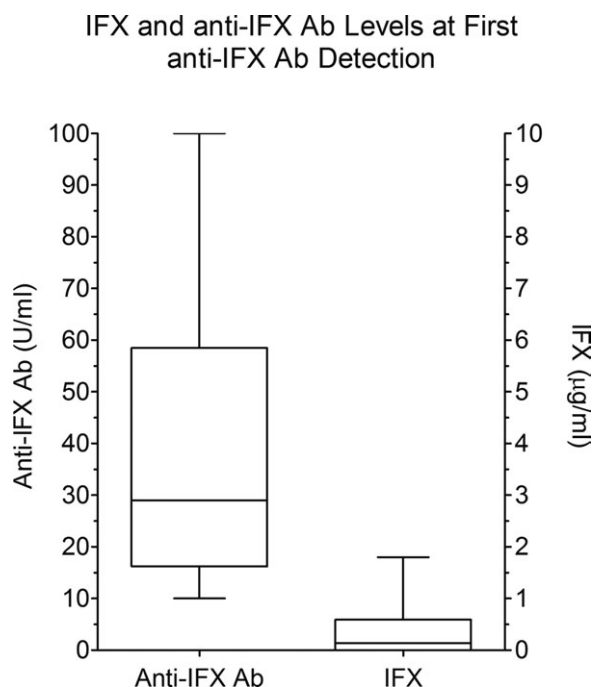


FIGURE 2. IFX and anti-IFX Ab concentration at time of first anti-IFX Ab detection during the IFX maintenance phase ($n = 26$). IFX was not measured in 10 patients because the samples had not been obtained as trough levels ($n = 3$), or because samples had been obtained at time of IFX reinitiation after a pause ($n = 7$). Box indicate median and interquartile range. Whiskers indicate range.

maintenance treatment, patients were then classified as having either clinical response (partial or complete as previously defined) or no response to the continued IFX treatment. The number of infusions between anti-IFX Ab assessments was higher among patients with response as compared with those without response: median five infusions, IQR 3–9 versus one infusion, IQR 1–4, $P = 0.019$. There was no difference between the proportion of patients having previously received an episodic IFX regimen: 11 of 17 (65%) with response and 2 of 9 (22%) without response, $P = 0.097$, and no difference between the proportion of patients with UC: 3 of 17 (18%) with response and 3 of 9 (33%) without response, $P = 0.628$.

As shown in Figure 3, anti-IFX Ab had disappeared completely at reassessment in 11 of 17 patients (65%) with clinical response to continued IFX maintenance therapy (nine with complete response of which anti-IFX Ab disappeared in seven; and eight with partial response of which anti-IFX Ab disappeared in four, $P = 0.335$). Disappearance of anti-IFX Ab occurred after median four (3–5) infusions, and the overall anti-IFX Ab concentration in this subgroup of patients decreased from median 22 U/mL, IQR 14–52 at first assessment to 0 U/mL, IQR 0–13 at reassessment, $P = 0.0009$. In contrast, anti-IFX Ab persisted in the subgroup of patients with no clinical response to continued IFX therapy (Fig. 3). Among these patients, the anti-IFX

Ab concentration increased from median 27 U/mL, IQR 17–76 at first assessment to 90 U/mL, IQR 62–100 at reassessment, $P = 0.0087$; IFX was discontinued shortly after anti-IFX Ab had been reassessed.

There was no difference in the initial anti-IFX Ab level between patients in whom anti-IFX Ab disappeared or persisted: median 31 U/mL, IQR 14–60, and median 27 U/mL, IQR 17–56, $P = 0.795$, respectively. There was no difference between the proportion of patients in whom anti-IFX disappeared or persisted in whom the IFX dose frequency was increased after detection of anti-IFX Ab (no patients were dose increased): 3 of 11 (27%) and 5 of 15 (33%), $P = 1.000$, respectively. There was no difference in the use of concomitant immunosuppression between patients in whom anti-IFX Ab disappeared or persisted: 7 of 11 (64%) and 8 of 15 (53%), $P = 0.707$, respectively. Finally, there was no difference between the proportion of patients in whom anti-IFX Ab disappeared or persisted who started or were dose increased on concomitant immunosuppression between anti-IFX Ab measurements: 1 of 11 (9%) and 2 of 15 (13%), $P = 1.000$, respectively. In the subgroup of 11 patients in whom anti-IFX Ab disappeared, two patients had two serial positive anti-IFX Ab tests obtained at different maintenance infusions; and six patients had a minimum of two negative anti-IFX Ab tests at later scheduled maintenance infusions. A single patient had two serial positive anti-IFX Ab tests at different maintenance infusions followed by two serial negative tests a

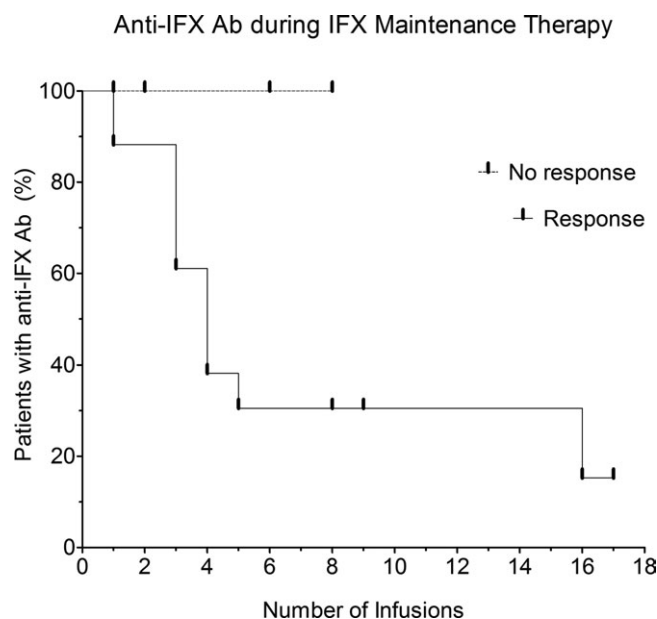


FIGURE 3. Number of infusions from detection of anti-IFX Ab during the IFX maintenance phase until disappearance of anti-IFX Ab after continued IFX maintenance therapy ($n = 17$ with clinical response to continued IFX treatment; $n = 9$ without response). A drop on the Y-axis indicates disappearance of anti-IFX Ab. Vertical bars are censored cases.

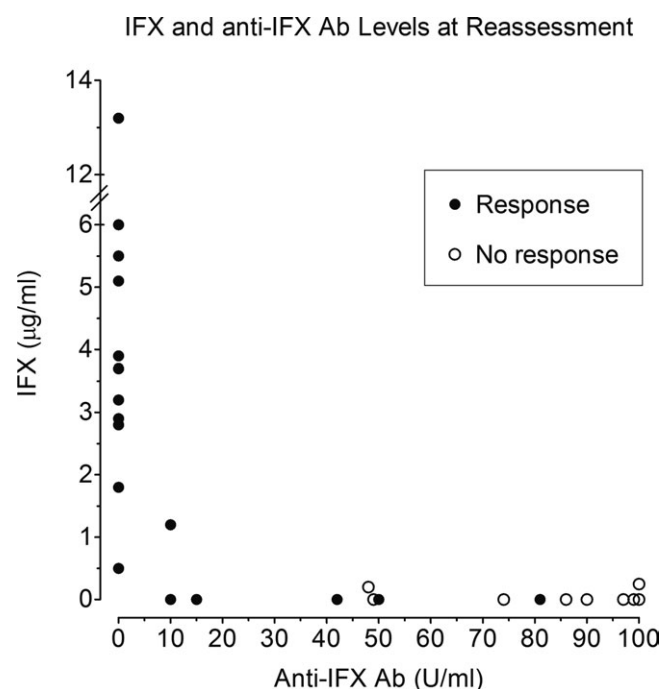


FIGURE 4. IFX and anti-IFX Ab trough concentration at time of anti-IFX Ab reassessment during the IFX maintenance phase in patients with ($n = 17$) and without ($n = 9$) response to continued IFX therapy.

later infusions. In the subgroup of 15 patients in whom anti-IFX Ab persisted at repeat measurements, three patients had three serial positive anti-IFX Ab tests obtained at different scheduled maintenance infusions.

Figure 4 shows the interplay between anti-IFX Ab levels, IFX trough levels, and clinical response type at the time of anti-IFX Ab reassessment. Of note, anti-IFX Ab appeared to also be functionally active at this timepoint, because anti-IFX Ab persistence was associated with undetectable or very low IFX trough levels: median 0 $\mu\text{g/mL}$, IQR 0–0; while Ab disappearance was associated with relatively high IFX trough levels: median 3.7 $\mu\text{g/mL}$, IQR 2.8–5.5, $P < 0.0001$.

Finally, biochemical markers of disease activity supported the clinical classification of IFX response types: C-reactive protein (CRP) was significantly higher among patients with no response to continued IFX treatment as compared with patients with response: 31 versus 3 mg/mL, $P < 0.003$; albumin was significantly lower among patients with no response: 613 versus 659 μM , $P = 0.008$; and there was a nonsignificant trend toward a decreased hemoglobin concentration among patients with no response: 7.9 versus 8.6 mM, $P = 0.055$ (Fig. 5).

Anti-IFX Ab Levels After IFX Discontinuation

Fifty-six patients, 46 with CD and 10 with UC, had detectable anti-IFX Ab at the time of IFX discontinuation

or shortly thereafter: median 59 U/mL, IQR 26–100. Anti-IFX Ab were initially assessed median 12 days, IQR 0–43 after discontinuation of IFX, and patients had received median five infusions, IQR 3–7 prior to discontinuation.

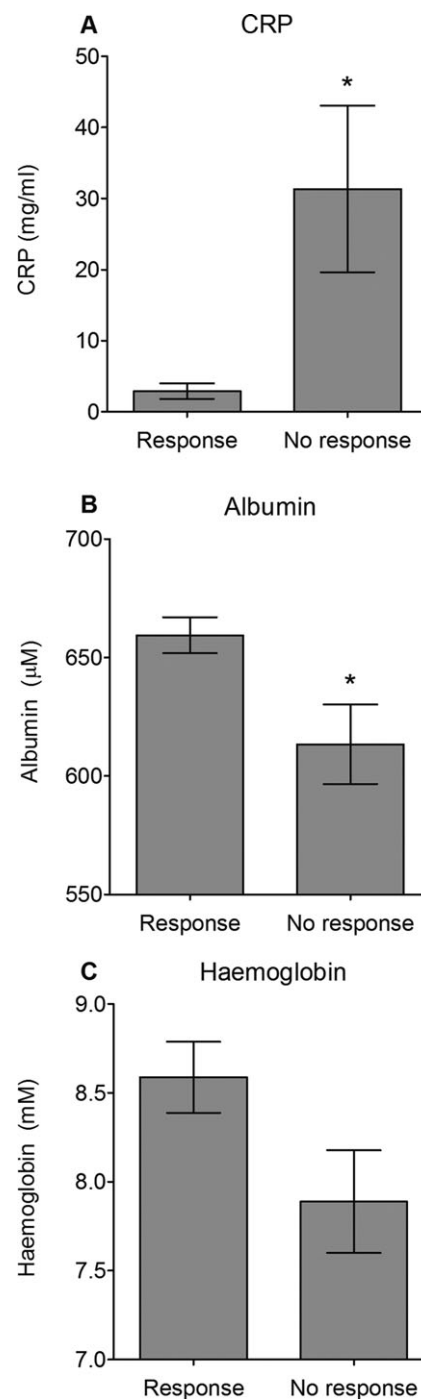


FIGURE 5. Biochemical markers of disease activity at time of anti-IFX Ab reassessment during the IFX maintenance phase in patients with ($n = 17$) or without ($n = 9$) clinical response to continued IFX therapy; (A) CRP, (B) albumin, (C) hemoglobin. Columns indicate mean $\pm$ SEM. * $P < 0.01$.

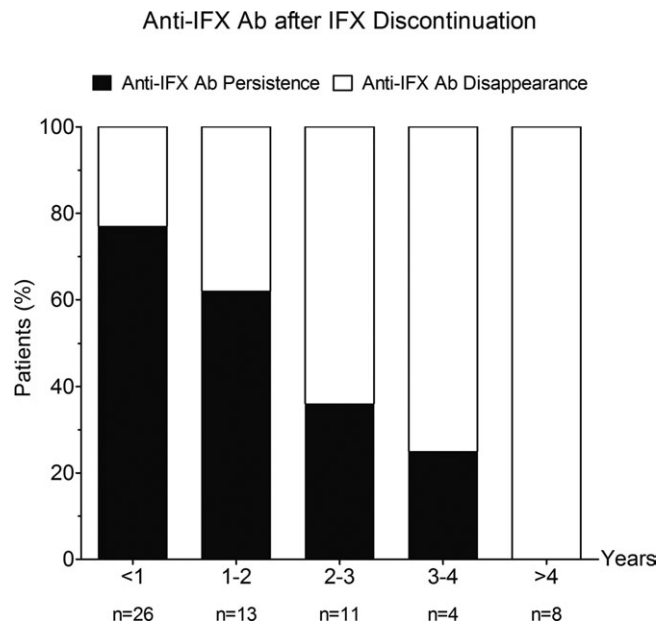


FIGURE 6. Time until disappearance of anti-IFX Ab in patients who had discontinued IFX shortly after detection of anti-IFX Ab.

Reasons for discontinuation and hence for measuring anti-IFX Ab were loss of response to IFX maintenance therapy ($n = 21$), acute severe infusion reactions ($n = 17$), delayed infusion reactions ($n = 11$), or clinical remission ($n = 7$). Anti-IFX Ab were reassessed at a later timepoint in each patient; 50 patients were reassessed one time, six patients

two times. The proportion of patients with detectable anti-IFX Ab gradually declined over time (Fig. 6). A fraction of patients still had anti-IFX Ab after 3 years, and anti-IFX Ab could be detected up to nearly 4 years (1334 days) in one patient.

Characteristics of patients with and without disappearance of anti-IFX Ab after IFX discontinuation are displayed in Table 2. We further performed univariate Cox regression analysis that revealed no variables significantly associated with anti-IFX Ab disappearance except administration of another TNF-inhibitor (adalimumab [ADA]) between the anti-IFX Ab measurements, which reduced the hazard of anti-IFX Ab disappearance (Table 2). This, however, was not confirmed in the multivariate Cox regression analysis.

DISCUSSION

The clinical impact of anti-IFX Ab on efficacy and safety in IBD is well recognized, and even though the predictive value of anti-IFX Ab measurements to identify patients at risk of infusion reactions has been questioned, anti-IFX Ab status is increasingly recommended as a key element in algorithms designed for optimizing IFX therapy in IBD and other immunoinflammatory diseases.^{6,8,13–15,26,27} Formation of anti-IFX Ab may occur shortly after IFX initiation as well as after prolonged drug exposure, but the clinical significance of variations in anti-IFX Ab levels in individual patients has not previously been investigated.^{8,12,19}

TABLE 2. Variables Associated with Anti-IFX Ab Disappearance After IFX Discontinuation

Variables	Anti-IFX Ab Disappearance <i>n</i> =29	Anti-IFX Ab Persistence <i>n</i> =27	Univariate Cox Analysis <i>P</i>	HR [95% CI]	Multivariate Cox Analysis <i>P</i>	HR [95% CI]
Crohn's disease – no. (%)	26 (90)	20 (74)	0.448	1.6 [0.5-5.2]	—	—
Male sex – no. (%)	15 (52)	11 (41)	0.772	1.1 [0.5-2.4]	—	—
Age at diagnosis – yr median (IQR)	20 (18-28)	35 (21-47)	0.460	1.0 [0.9-1.0]	—	—
Age at 1 st IFX infusion – yr median (IQR)	33 (23-41)	36 (26-48)	0.102	1.0 [0.9-1.0]	0.988	1.0 [1.0-1.0]
Disease duration at 1 st IFX series – yr median (IQR)	6 (3-18)	4 (1-7)	0.097	1.0 [0.9-1.0]	0.678	1.0 [0.9-1.1]
IFX infusions – no. median (IQR)	5 (4-7)	4 (3-7)	0.636	1.0 [0.9-1.1]	—	—
IFX dosing 5 mg/mL – no. (%)	28 (97)	27 (100)	0.275	0.3[0.0-2.0]	—	—
IFX induction at last IFX series – no. (%)	12 (41)	13 (48)	0.128	0.5 [0.2-1.2]	0.281	0.6 [0.3-1.4]
Episodic IFX regimen – no. (%)	16 (55)	12 (44)	0.312	1.5 [0.7-3.4]	—	—
Concomitant immunosuppression ^a – no. (%)	19 (66)	7 (26)	0.082	2.0 [0.9-4.4]	0.337	1.6 [0.6-4.2]
Anti-IFX Ab conc. (U/ml) – median (IQR)	52 (22-85)	80 (35-100)	0.419	1.0 [1.0-1.0]	—	—
TNF-blocker btw anti-IFX Ab measurements ^b – no. (%)	18 (62)	18 (67)	0.037	0.4 [0.2-1.0]	0.208	0.5 [0.2-1.4]
Transfusions btw anti-IFX Ab measurements – no. (%)	2 (7)	0 (0)	0.499	1.7 [0.4-7.5]	—	—

Ab: antibodies; ADA: adalimumab; Btw: between; CI: confidence interval; HR: hazard ratio; IFX: infliximab; IQR: interquartile range.

^aAzathioprine, mercaptopurine, or methotrexate.

^bAdalimumab.

Here we report that anti-IFX Ab actually disappear in a significant fraction of patients with presumed clinical response to continued IFX maintenance treatment, and that anti-IFX Ab persist in some patients for years after discontinuation of IFX.

The association between anti-IFX Ab and loss of response to IFX maintenance therapy has only recently been acknowledged, and is still disputed by some clinicians.^{6,8,12,14,15} Accordingly, certain patients in our IBD cohort continued IFX treatment based on an overall clinical judgment despite incomplete response and development of anti-IFX Ab. For the purposes of the present study the clinical course of the disease as well as anti-IFX Ab levels were reassessed at later timepoints during the maintenance therapy. Anti-IFX Ab appeared to be functionally active both at the initial detection and at reassessment because the corresponding IFX trough levels were low, and in the vast majority of cases even below the detection limit of the assay. Thus, all trough levels were above a clinically validated cutoff level for anti-IFX Ab, which has previously been shown to associate with loss of treatment response with high sensitivity and specificity.¹²

The majority of patients with initial anti-IFX Ab appeared to benefit from continued IFX therapy. Surprisingly, anti-IFX Ab had disappeared in two-thirds of these responders when reassessed before subsequent infusions. Disappearance was independent of initial anti-IFX Ab titers, IFX dose escalation, and use of concomitant immunosuppression. Thus, we here describe a novel subgroup of patients in whom anti-IFX Ab disappears during continued IFX treatment. The reason(s) for this is unknown. It may be caused by immunological tolerance but could also be related to anti-IFX Ab absorption due to persistent “antigen” (i.e., IFX) administration. Based on data from this study, it cannot be firmly concluded that the favorable clinical response observed is caused by continued IFX administration, and this needs to be investigated further.

In the remaining one-third of patients with a clinical response to continued IFX dosing, anti-IFX Ab persisted also at repeat measurements, and because the corresponding IFX trough levels were undetectable or low we suggest that IFX should be discontinued here, as a clinical response is unlikely to be caused by IFX, and due to increased risk of infusions reactions.^{13,26} We speculate that this subgroup of patients may have low disease activity due to previous induction of remission by IFX or by natural fluctuations of disease activity. A minority of patients with initial anti-IFX Ab had no clinical effect of continued IFX therapy. In all these patients, anti-IFX Ab persisted at the time of reassessment and, as a consequence, IFX was discontinued shortly thereafter. We believe this subgroup represents a subpopulation of patients with loss of response to IFX maintenance therapy because of drug immunogenicity.^{11–13,15} We con-

clude that clinical response to ongoing IFX therapy needs to be taken into account when evaluating the importance of a single positive anti-IFX Ab assessment and that measurement is preferably repeated. In case of persisting anti-IFX Ab and low IFX trough levels at repeat measurements, IFX should be discontinued because patients are unlikely to benefit from further treatment and have increased risk of infusion reactions.^{13,22,28}

Kinetic studies have demonstrated that anti-IFX Ab markedly increase clearance of IFX.^{17,29} In agreement with this, several studies have described a correlation between anti-IFX Ab, low IFX trough levels, and loss of response.^{7,11,12,16,19,30–32} A minority of studies, however, have failed to detect these correlations.^{8,33–35} The reasons for this discrepancy are currently unknown, but differences between study populations, laboratory techniques, and timing of sampling may all contribute, as previously suggested.⁷ Our novel observation that anti-IFX Ab disappear in a large fraction of patients during ongoing IFX treatment may also add to the explanation.

It should be noted that the groups of patients with and without response to continued IFX treatment are not directly comparable due to the retrospective nature of this exploratory study, resulting in lack of randomization and lack of strict study protocol determining treatment approach and timing of anti-IFX Ab reassessments. The difference in follow-up time between patients with and without response is a potential confounder to both response, i.e., a longer follow-up increases response in placebo groups in randomized controlled trials, as well as to antibody titers, i.e., disappearance of anti-IFX Ab occurred after a median of four infusions. Another limitation is the absence of response criteria based on a formal disease activity index, such as the CDAI, which is not routinely used in our clinic. However, clinical classification and data analysis were done by two independent investigators, and the biochemical data strongly support the classification used. From a practical point of view, the clinician's evaluation of response type and decision regarding continuation or change in the therapeutic regimen probably reflects the clinical setting at most institutions, and a similar approach has been used in other studies.^{7,11,12,15,36}

We cannot rule out that disappearance of anti-IFX Ab during continued IFX treatment is caused by previous false-positive anti-IFX Ab detections. However, our observations are supported by the fact that several patients had serially negative and/or positive anti-IFX Ab measurements. Furthermore, disappearance of anti-ADA Ab has recently been observed in six patients with rheumatoid arthritis who were dose increased.³⁷ In addition, we used a clinically validated and commercially available technique based on fluid-phase RIA, which has been used in both IBD and rheumatoid arthritis with high sensitivity and

specificity, and with low inter- and intraassay variation.^{12,19–21} This RIA is not influenced to the same degree by artifacts encountered in solid-phase enzyme-linked immunosorbent assays (ELISAs), as it detects all isotypes of immunoglobulins and all IgG subclasses binding to IFX, and with low drug sensitivity; i.e., IFX levels seen in trough serum samples do not interfere with detection of anti-IFX Ab.^{14,20,38,39} Thus, it is unlikely that the results originate from methodological inaccuracy. The fact that disappearance of anti-IFX Ab was observed in a relatively large subgroup and not only in a few patients, as well as the clear correlation between IFX concentration and presence or absence of anti-IFX Ab, adds weight to our results.

The biologic half-life of human IgG at normal blood levels varies from 7 to 21 days depending on subclass.³⁹ We expected a similar half-life of anti-IFX Ab in patients who discontinued IFX, as they are no longer exposed to the antigen driving T- and B-cells for production of specific anti-IFX Ab. However, a substantial fraction of patients had circulating anti-IFX Ab several years after IFX cessation. None of the variables assessed were associated with anti-IFX Ab disappearance, and anti-IFX Ab concentration at baseline did not seem to influence the rate of disappearance. Taken together, these data indicate that the immunogenicity of IFX resembles commonly used vaccines consisting of other protein antigens and leading to prolonged persistence of anti-IFX Ab, at least in some individuals.⁴⁰ This novel observation may have clinical implications, because of increased risk of infusion reactions and loss of response if anti-IFX Ab is present at IFX reinitiation after a drug pause.^{12,13}

This study did not address the kinetics of anti-IFX Ab. Hence, anti-IFX Ab levels were not measured at pre-specified and regular time intervals in individual patients. Furthermore, anti-IFX Ab measurements obtained immediately prior to the last IFX infusion as well as some time after IFX discontinuation were allowed as baseline samples. Thus, our data do not allow conclusions or generalizations concerning the kinetics of anti-IFX Ab.

Anti-IFX Ab measurements have been used in the clinical management of IBD patients at our institution for several years. Anti-IFX Ab have been assessed in around half of our patient cohort, and about half of these have developed anti-IFX Ab. This relatively high incidence of anti-IFX Ab was not explained by frequent use of episodic therapy, which is known to increase risk of anti-IFX Ab formation.^{13,16,33} Instead, we observed an association between lack of concomitant immunosuppression and anti-IFX Ab irrespective of treatment regimen, contrasting previous studies in which this has only been evident among episodically treated patients.^{16,33,41} Patients who had detectable anti-IFX Ab received fewer IFX infusions as compared with patients without anti-IFX Ab, suggesting that

IFX was usually discontinued when anti-IFX Ab were detected and/or that continued IFX administration is more effective in removing anti-IFX Ab from the circulation.

In conclusion, anti-IFX Ab disappear in two-thirds of patients who respond to ongoing IFX treatment for reasons that are yet unknown. Therefore, clinical response needs to be taken into account when evaluating the importance of detected anti-IFX Ab. Persistence of anti-IFX Ab must be considered if reinitiation of IFX therapy is contemplated even years after discontinuation.

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