SEVERE INFUSION REACTIONS TO INFLIXIMAB: ETIOLOGY, IMMUNOGENICITY, AND RISK FACTORS IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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Casper Steenholdt, Morten Svenson, Klaus Bendtzen, Ole Østergaard Thomsen, Jørn Brynskov, et al.. SEVERE INFUSION REACTIONS TO INFLIXIMAB: ETIOLOGY, IMMUNOGENICITY, AND RISK FACTORS IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE. Alimentary Pharmacology and Therapeutics, 2011, 34 (1), pp.51. 10.1111/j.1365-2036.2011.04682.x . hal-00637801

HAL Id: hal-00637801
https://hal.science/hal-00637801

Submitted on 3 Nov 2011

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Alimentary Pharmacology & Therapeutics

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Journal:	*Alimentary Pharmacology & Therapeutics*
Manuscript ID:	APT-0297-2011.R1
Wiley - Manuscript type:	Original Scientific Paper
Date Submitted by the Author:	12-Apr-2011
Complete List of Authors:	Steenholdt, Casper; Herlev Hospital, Dept of Gastroenterology Svenson, Morten; Biomonitor A/S Bendtzen, Klaus; Biomonitor A/S; University Hospital of Copenhagen, Rigshospitalet, Institute for Inflammation Research Thomsen, Ole; Herlev Hospital, Dept of Gastroenterology Brynskov, Jørn; Herlev Hospital, Dept of Gastroenterology Ainsworth, Mark; Herlev Hospital, Dept of Gastroenterology
Keywords:	Crohn's disease < Disease-based, Inflammatory bowel disease < Disease-based, Ulcerative colitis < Disease-based, Biologics (IBD) < Topics, Clinical pharmacology < Topics, Immunology < Topics

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SEVERE INFUSION REACTIONS TO INFLIXIMAB: ETIOLOGY, IMMUNOGENICITY, AND RISK FACTORS IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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Running head: Severe infusion reactions to infliximab in IBD

Keywords: Inflammatory bowel disease, Crohn disease, ulcerative colitis, safety, acute severe infusion reactions, infliximab, TNF, adverse event, anti-drug antibody, IgE.

Word count (excluding title page, abstract, study highlights) references, figures, and tables):

3.175 words

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ABSTRACT (max 250 words)

Background: Infliximab (IFX) elicits acute severe infusion reactions in about 5% of patients with inflammatory bowel disease (IBD).

Aim: To investigate the role of anti-IFX antibodies (Ab) and other risk factors.

Methods: The study included all IBD patients treated with IFX at a Danish university hospital until 2010 either continuously (IFX every 4-12 weeks) or episodically (reinitiation after >12 weeks). Anti-IFX Ab were measured by radioimmunoassay.

Results: Twenty-five (8%) of 315 patients experienced acute severe infusion reactions. Univariate analysis showed that patients who reacted were younger at the time of diagnosis (19 vs 26 years, p=0.013) and at first IFX infusion (28 vs 35 years, p=0.012). Furthermore, they more often received episodic therapy (72 vs 31%, p<0.001), and logistic regression revealed this as the only significant predictor of reactions (OR 5 [2-13]; p<0.001). IFX reinitiation after 6 months intermission further increased the risk (OR 8 [3-20], p<0.001). Most reactions (n=14, 88%) occurred at 2nd infusion in the 2nd treatment series (p=0.006). Anti-IFX IgG Ab were highly positive in 19 of 20 patients (95%) shortly after the reactions (median 84 U/ml). Anti-IFX IgG Ab measured prior to the retreatment series were negative in 7 of 11 patients tested (64%). Anti-IFX IgE Ab were negative in all patients with reactions.

Conclusion: Acute severe infusion reactions were strongly associated with development of anti-IFX IgG Ab, but not with anti-IFX IgE Ab. The risk was particularly high at the 2nd infusion in retreatment series. Negative anti-IFX Ab before reinitiation did not rule out reactions.

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INTRODUCTION

Infliximab (IFX) (Remicade, Centocor) is a chimeric mouse-human antibody, which targets tumour necrosis factor (TNF) alpha. IFX has proven highly effective in inducing and maintaining remission in moderate to severe luminal and fistulizing Crohn's disease and ulcerative colitis.[1] Despite its effectiveness, several adverse drug reactions associated with IFX have been identified, such as opportunistic and non-opportunistic infections, skin reactions including malignancies, autoimmunity, and infusion reactions.[1;2] Infusion reactions to IFX are classified as acute or delayed. Acute infusion reactions appear in 10-40% of patients and are defined as reactions occurring during or within one hour after infusion.[2-4] Delayed infusion reactions occur 1-14 days after infusion in approximately 2% of patients and is commonly associated with myalgias, arthralgias, headache, fever, rash, and fatigue.[2;4] Acute reactions can be further categorized as mild, moderate, or severe; the latter occurring shortly after start of infusion necessitating immediate stop of the infusion due to ex. hypotension, chest tightness, respiratory distress with dyspnoea, bronchospasm, or laryngeal oedema, urticaria, or rash. Acute mild to moderate reactions are self-limiting and resolve spontaneously after temporary cessation of the infusion or reduction of the infusion rate. These reactions are usually associated with nausea, headache, fever, erythema, and itching.[2;4;5]

Acute severe infusion reactions to IFX are generally reported in about 5% of IBD patients and constitute a serious clinical problem because of the severity and the subsequent discontinuation of IFX therapy.[4] It is speculated that the reactions are caused by IFX immunogenicity.[4;5] However, little is known about the precise mechanisms including the role of anti-IFX antibodies (Ab) as well as risk factors. We aimed at investigating these factors and in particular the potential usefulness of measurements of IgG and IgE anti-IFX Ab to predict infusion reactions.

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METHODS

Patients

All IBD patients treated with IFX either continuously (i.e. IFX every 4-12 weeks) or episodically (i.e. reinitiation of IFX after more than 12 weeks without IFX therapy) from January 1999 until October 2010 were included in this single center study. Anti-IFX Ab, including cross reactivity with adalimumab (ADA), were measured before initiation of a new treatment series and/or after an acute severe infusion reaction. Blood samples were routinely obtained from all patients prior to IFX infusions and following an infusion reaction from May 2009 and onward as part of our local drug monitoring program. Before May 2009 samples were obtained sporadically as decided by the treating physician. Some blood samples were analyzed in conjunction with the reactions while others were stored in a biobank and analyzed as part of this study.

Acute severe infusion reactions were retrospectively identified using patient files. Reactions were defined as acute reactions occurring during IFX infusion which were judged severe by the treating physician, and resulting in immediate and permanent discontinuation of IFX and symptomatic treatment with antihistamines and hydrocortisone, and if necessary epinephrine.

All patients at our tertiary center were routinely treated with hydrocortisone (100 mg intravenously), cetrizine (10 mg orally), and acetaminophen (1 g orally) 30 minutes prior to administration of IFX to reduce the risk of acute infusion reactions and to prevent development of anti-IFX Ab.[6] The study was approved by the regional ethics committee.

Serum analyses

Anti-IFX Ab were measured in samples obtained 30 minutes prior to IFX infusion and/or in close connection with acute severe infusion reactions of which the majority of samples were obtained within one hour after the reactions (n=14) while a few were obtained up to nine days

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after the reactions (n=6). Anti-IFX Ab were exploratively assessed in samples from another four patients obtained longer time before or after the reactions (from two months prior to reactions up to seven years after reactions). Serum was collected after centrifugation of 10 ml blood, and stored at minus 80ºC until assay. Anti-IFX IgG and IgE Ab, and cross reactivity with ADA, were analyzed in the same sample material. All sera were tested under blinded conditions at Biomonitor A/S (Symbion Science Park, Copenhagen, Denmark).

Anti-IFX IgG Ab and cross reactivity with ADA

The fact that IFX is an IgG construct consisting only of kappa light-chains makes it possible to use anti-human lambda light-chain Ab to distinguish between free drug and drug in complex with any class of lambda-containing human immunoglobulin. The anti-IFX IgG assay was carried out as previously detailed.[7-9] Briefly, 1% serum was added 4,000-5000 cpm/100 microL of ^{125}I-IFX. After overnight incubation, free and immunoglobulin-bound (any isotype) tracer were separated by precipitation with rabbit anti-human lambda Ab (Dako, Glostrup, Denmark). Backgrounds were <4% and the inter- and intra-assay variations were below 20 and 10%, respectively. The data are given as U/ml based on a laboratory standard. Cross reactivity between anti-IFX Ab and ADA was tested by co-incubation of ^{125}I-IFX and sample as described above with and without IFX at 25 µg/ml and ADA at 50 µg/ml, respectively. The limit for positive ADA cross binding was set to 20% of the displaced ^{125}I-IFX obtained by the addition of IFX.

Anti-IFX IgE Ab

Ligand binding to IgE was detected using immunoadsorbed IgE, as previously detailed.[10] Briefly, a constant amount of anti-IgE monoclonal Ab coupled to paramagnetic beads was washed and added to 20% serum sample. After overnight incubation, the beads were washed two times and ^{125}I-IFX was added followed by co-incubation for 14-18 hours. Beads were sampled at

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intervals during the experiment and assessed with IgE anti-*Betula verrucosa*-positive serum and ^{125}I-*Betula verrucosa allergen no. 1* to include a positive control and to ensure the use of an optimal anti-IgE activity. The beads were carefully washed before detecting the amount of labeled ligand bound to IgE. Background binding was less than 0.5% of cpm added, and the lower limit of detection, set at 2x background level, was 0.14 kUa/l assessed from serial dilutions of sera testing positive for anti-Betv1 IgE in an ImmunoCAP-system (Phadia, Uppsala, Sweden).[10] Specificity was ascertained by lack of cross-binding to 20x surplus of a serum pool negative for specific IgE.

Statistical analyses

Statistical analyses were done in SPSS version 18 (IBM, Somer, NY, USA). Two sided p-values <0.05 were considered significant. Descriptive statistics were calculated as percentages for discrete data and medians with range or interquartile range (IQR) for continuous data. Baseline variables of patients with IBD were analyzed for association with development of acute severe infusion reactions to IFX using univariate analysis (χ^2 and Mann-Whitney test). Variables possibly associated with reaction ($p \leq 0.20$) were further evaluated in an unconditional multiple logistic regression analysis (enter method; Wald test used for assessing p-values). Potential risk variables were predefined as: Disease type, gender, age at diagnosis, age at first IFX infusion, disease duration at first IFX infusion, number of infusions, IFX dosing, IFX regimen, days between IFX series (not included in the multivariate model because not all patients were treated episodically), and concomitant immunosuppression during last IFX series (azathioprine, mercaptopurine, methotrexate, cyclosporine). Distribution of reactions during 1st and 2nd IFX series was compared using Log Rank test. Frequencies of reactions at different infusion numbers were assessed by Fisher's Exact test.

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RESULTS

Patients' characteristics

Twenty-five (8%) of 315 IBD patients (218 with Crohn's disease and 97 with ulcerative colitis) experienced an acute severe infusion reaction to IFX. Patients' characteristics are shown in **Table 1**. Clinical manifestations of reactions as noted in the patient files were: Acute severe malaise (100%), severe dyspnoea (60%), chest pain (44%), nausea (36%), universal erythema (32%), tachycardia (24%), chills and perspiration (24%), dizziness (16%), and hypotension (12%). Symptomatic treatment consisted of immediate cessation of IFX infusion, and intravenous infusion of antihistamine (n=22), hydrocortisone (n=6), and epinephrine (n=2). IFX was permanently discontinued in all patients due to the treating physician's overall evaluation of the reactions being severe.

Risk factors

Episodic therapy

Potential risk factors for development of acute severe infusion reactions were initially assessed by univariate analysis (**Table 1**). Patients who had experienced a reaction were significantly younger at time of diagnosis of IBD and at time of first IFX infusion as compared to patients without reaction. Furthermore, patients with reactions more often received episodic therapy than patients without reactions (**Figure 1**). The time period between 1st and 2nd IFX series was significantly longer among episodically treated patients with reaction compared to episodically treated patients without reaction (**Table 1**).

In order to create a multivariate model of potential predictors of infusion reactions, variables associated with reaction with $p \leq 0.2$ (disease, age at diagnosis, age at first IFX infusion, IFX regimen) were subjected to multiple logistic regression analysis. Episodic IFX therapy was

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identified as the only variable associated with development of acute severe infusion reactions; OR 4.9 [1.9-12.5], p<0.001 (**Figure 1**).

There is no official definition of episodic IFX therapy. Therefore, we investigated whether our findings depended on our definition of episodic therapy. Interestingly, the markedly increased risk observed during episodic therapy, was also evident in a sensitivity analysis where episodic therapy was defined as reinitiation of IFX after minimum 4 months without therapy; OR 5.5 [2.2-14.1], p<0.001 (18 reactions among 101 episodically treated patients, and 7 reactions among 214 continuously treated patients). Defining episodic therapy as minimum 6 months without IFX further increased risk of reaction during episodic therapy to OR 7.7 [3.0-19.5], p<0.001 (17 reactions among 75 episodically treated patients, and 8 reactions among 240 continuously treated patients).

Number of infusions

A significant difference between distribution of reactions during 1st and 2nd IFX series was observed (p<0.0001) **(Figure 2)**. Eighteen reactions were observed during episodic IFX therapy: Sixteen occurred during the 2nd IFX series and 14 (88%) of these appeared at the 2nd infusion; thus, 19% of the 2nd infusions in 2nd IFX series resulted in reactions. The proportion of reactions at 2nd infusion was significantly higher than the proportion of reactions at other infusion time points during the 2nd IFX series (p=0.006). In contrast, there were no infusion time points associated with increased proportion of reactions among continuously treated patients (**Figure 2**).

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Anti-drug antibodies

Anti-IFX IgE Ab

Anti-IFX IgE Ab were measured in close connection with acute severe infusion reactions in 20 patients and all tests were negative despite the assay being able to detect even small amounts of IgE Ab against a birch pollen positive control. In another four patients, anti-IFX IgE Ab were exploratively measured in samples obtained longer time before or after the reactions (from two months prior to reactions up to 7 years after reactions with multiple assessments per patient). Anti-IFX IgE Ab were negative also in these patients. As false negative results can occur if anti-IFX IgE Ab are bound to IFX at the time of testing, we measured anti-IFX IgE Ab at multiple time points in 15 patients (median number of assessments 3, IQR 2-4; IQR 7 days before reaction to 35 days after reaction). Again, all tests were negative.

Anti-IFX IgG Ab

Anti-IFX IgG Ab were highly positive in 19 of 20 patients (95%) after the reactions (median 84 U/ml, IQR 30-100; **Figure 3**). Anti-IFX IgG Ab were undetectable in the four patients in whom explorative samples had been obtained longer time before or after the reactions as previously described. In the majority of patients with reactions during episodic therapy, anti-IFX IgG Ab measured prior to initiation of the new treatment series were negative (seven of eleven patients; **Figure 3**). Levels of anti-IFX IgG Ab increased after reaction in the four patients with Ab prior to reinitiation.

Levels of anti-IFX IgG Ab following the reactions were significantly higher than in a comparable group of patients with Crohn's disease with maintained good clinical response to IFX maintenance therapy (median 0 U/ml, IQR 0-0 U/ml, n=59; $p<0.0001$), as well as in patients with loss of response to IFX maintenance therapy (median 35 U/ml, IQR 12-76 U/ml, n=26; $p=0.045$) as previously described.[11]

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Nineteen patients were tested for cross reactivity of anti-IFX Ab with ADA. All were negative. A subset of 17 patients was later treated with ADA (median duration of treatment 177 days, IQR 56-770). All patients tolerated ADA without hypersensitivity reactions, except one. This patient experienced a late reaction 12 days after the 1st ADA injection and blood sample 19 days after reaction was highly positive for anti-ADA Ab (45 U/ml). Even though there was no demonstrable cross-reactivity between the previously developed anti-IFX Ab and ADA, the preexistence of low titers (below the detection limit) of cross reacting Ab below the detection limit cannot be ruled out.

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DISCUSSION

The clinical significance of immunogenicity and the potential usefulness of anti-IFX Ab measurements in acute severe infusion reactions in IBD patients receiving IFX therapy are disputed.[2;5;12] Several studies have reported a possible link between anti-IFX Ab and acute infusion reactions.[13-16] The only study having addressed immunogenicity in patients with acute severe infusion reactions exclusively, found all patients with reactions anti-IFX Ab positive.[6] Clinical resemblance with anaphylactic reactions suggests that some acute severe infusion reactions to IFX may be caused by a type 1 hypersensitivity reaction mediated by anti-IFX IgE Ab. In fact, an IgE mediated reaction to IFX has been reported in a child with Crohn's disease and in three patients with rheumatoid arthritis.[17;18] A single study has examined the putative role of IgE Ab in acute infusion reactions to IFX in IBD patients, showing that serum tryptase levels were normal after reactions of which only four were severe.[19] Tryptase is a mast cell enzyme and was used as a surrogate marker of IgE-mediated anaphylaxis; however, tryptase levels within the normal range do not exclude IgE mediated anaphylaxis, and other immunological mechanisms for reactions such as anti-IFX IgG Ab were not explored. [5;20] The fact that many milder reactions abate by slowing the infusion seems to support the hypothesis that at least some reactions are not IgE mediated.[2;21]

To systematically address the role of immunogenicity in acute severe infusion reactions in individual IBD patients, we measured anti-IFX IgG and IgE Ab after these reactions as well as prior to reinitiation of IFX after drug pauses. We found a clear association between reaction in the individual patients and development of high levels of circulating anti-IFX IgG Ab measured shortly after the reaction. In contrast, anti-IFX IgE Ab were not detected in any of these reactions. We conclude that the vast majority of acute severe infusion reactions to IFX are most likely not true anaphylactic reactions. Rather, they appear to be associated with anti-IFX IgG Ab. Our findings are supported by the previously published data from IBD patients as well as in line

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with newly published hypotheses.[5] Importantly, reactions could not be predicted by measuring anti-IFX Ab prior to reinitiation of IFX after a drug pause. It remains to be tested if Ab measurements following 1st infusion in a reinitiation series can help identify patients at risk of reaction at the 2nd infusion.

The inability of previous studies to show a clear connection between anti-IFX Ab development and acute severe infusion reactions may be due to inaccuracy of commonly used solid-phase enzyme immunoassays (EIA), as these assays are sensitive to false negative test results due to the inability to detect functionally monovalent immunoglobulins (IgG4), epitope masking, and to inconclusive anti-IFX Ab measurements due to interference with IFX.[11;22;23] We used a different technique based on fluid-phase RIA which has been clinically validated in both IBD and rheumatoid arthritis with high sensitivity and specificity.[7-9;11;22] This RIA is not influenced to the same degree by the potential artifacts encountered in solid-phase EIAs, it detects all isotypes of immunoglobulins and all IgG subclasses binding to IFX, and there is little or no interference with IFX. Time of IgE Ab measurement with respect to time of infusion reaction may be important for the ability of all types of assays to detect anti-IFX IgE Ab, because false negative results may occur if anti-IFX IgE Ab are bound to IFX at time of testing. To address this potential bias, we also measured anti-IFX IgE Ab before reactions and at multiple time points after reactions. Despite our assay being able to detect even low amounts of IgE Ab against a birch pollen antigen, all tests for anti-IFX IgE Ab were negative.

Acute severe infusion reactions to IFX were observed in 8% of patients in our IBD cohort with a substantially higher proportion of reactions during episodic treatment (17%) as compared to continuous treatment (3%). Previously reported prevalence has varied from about 2% in continuously treated patients up to 8.5% in episodically treated patients.[6;13;14;24;25] Some differences may be explained by the retrospective or prospective character of the data collection,

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by the mean number of infusions per patient, and by the definition of the reactions. We defined acute severe infusion reactions as reactions occurring during IFX infusion which were judged severe by the treating physician, and resulting in immediate and permanent IFX cessation. This is in accord with previous studies in which these reactions have been defined as acute reactions leading to IFX discontinuation[6;26], acute reactions judged life-threatening[13;24], or acute reactions leading to hospital admission[3]. The symptoms reported by our patients were similar to symptoms reported in other studies.[19;21] However, more restrictive definitions of reactions have been used, and the fact that only two of our patients were treated with epinephrine may indicate, that the severity of reactions in our cohort may be somewhat less than in other studies.[27]

We also investigated potential risk factors associated with acute severe infusion reactions. Episodic therapy defined as reinitiation of IFX after more than 12 weeks without therapy strongly increased risk of reaction with an OR of 5, and it was the only factor associated with increased risk in a multiple logistic regression analysis. In addition, we observed a longer period between 1st and 2nd IFX series among episodically treated patients with reactions. A strong association was also found in sensitivity analyses in which episodic therapy was defined as reinitiation of IFX after 4 months (OR 6), and risk further increased when episodic therapy was defined as reinitiation after 6 months (OR 8). Taken together, these data indicate that risk of reactions increases with time between IFX series. Risk of reaction was highest at 2nd infusion in a reinitiation series and particular high at 2nd infusion in the 2nd series. Previous studies have reported a protective effect of induction regimen and scheduled maintenance therapy[3;24], increased risk after drug pause[24;28;29], and a tendency of higher frequency of reactions at the 2nd IFX infusion[3;19;24-26]. Reporting of the importance of concomitant immunosuppression has been variable; some studies have found a protective effect[3;6;13;25] while others have not[26;30;31]. In general, the previous studies have examined relatively small cohorts of acute and/or delayed infusion reactions as a whole, and risk factors solely in patients with acute severe infusion

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reactions have not been assessed. Thus, our study is the first reporting in a larger cohort of acute severe infusion reactions only, and this is the first reporting of OR associated with increased risk at IFX reinitiation as well as a statistically increased risk at 2nd infusion in 2nd treatment series.

In conclusion, acute severe infusion reactions to IFX appear not to be true IgE-mediated anaphylactic reactions but rather associated with development of anti-IFX IgG Ab. Risk of reaction is relatively high during episodic or reinitiation therapy, especially at the 2nd infusion in a new series, but negative anti-IFX Ab before reinitiation does not exclude reactions.

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Acknowledgements

The authors would like to thank Tobias Klausen for statistical assistance (Dept of Haematology, Herlev University Hospital, Denmark), and Charlotte Kühnel, Yvonne Krogager, Lene Neergaard, Lise Olsen, Anne Hallander, Anni Petersen, Vibeke Hansen, Hanne Fuglsang, and Birgit Kristensen for technical assistance (Dept of Medical Gastroenterology, Herlev University Hospital, Denmark).

Support for this study was provided by Aase and Ejnar Danielsen's Foundation, Beckett Foundation, Danish Biotechnology Program, Danish Colitis-Crohn Society, Danish Medical Association Research Foundation, Frode V. Nyegaard and wife's Foundation, Health Science Research Foundation of Region of Copenhagen, Herlev Hospital Research Council, Lundbeck Foundation, and P. Carl Petersens Foundation.

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Table 1: Patient characteristics

Variables	Reaction n=25	No reaction n=290	P
Disease – no. (%)			
Crohn's disease / Fistulizing	21 / 6 (84) / (29)	197 / 84 (68) / (43)	0.095
Ulcerative colitis	4 (16)	93 (32)	
Male sex – no. (%)	10 (40)	146 (50)	0.321
Age at diagnosis – yr median (IQR)	19 (15-29)	26 (20-37)	**0.013**
Age at 1st IFX infusion – yr median (IQR)	28 (19-35)	35 (26-45)	**0.012**
Disease duration at 1st IFX series – yr median (IQR)	5 (2-8)	5 (2-11)	0.620
IFX infusions – no. median (IQR)	5 (5-9)	5 (3-10)	0.543
IFX dosing – no. (%)			
5 mg/kg	24 (96)	288 (99)	0.220
10 mg/kg	1 (4)	2 (1)	
IFX regimen – no. (%)			
Continuous	7 (28)	200 (69)	**<0.001**
Episodic	18 (72)	90 (31)	
Concomitant immunosuppression* at last IFX series – no. (%)	14 (56)	188 (65)	0.377
Variables assessed only in episodically treated patients:			
Patients having received 2nd IFX series – no. (%)	18 (72)	90 (31)	**<0.001**
Time period 1st to 2nd IFX series – days median (IQR)	861 (286-1728)	211 (146-458)	**<0.001**
Patients having received 3rd IFX series – no. (%)	2 (8)	24 (8)	1.000
Time period 2nd to 3rd IFX series – days median (IQR)	1608 (375-2841)	220 (155-767)	0.124
Patients having received 4th IFX series – no. (%)	1 (4)	5 (2)	0.424
Time period 3rd to 4th IFX series – days median (IQR)	329 (NA)	526 (203-1092)	0.770

* Azathioprine, mercaptopurine, or methotrexate.

IFX: Infliximab

IQR: Interquartile range

NA: Not applicable

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FIGURE LEGENDS

Figure 1

Risk of acute severe infusion reactions to infliximab (IFX) during continuous and episodic therapy.

Figure 2

Distribution of acute severe infusion reactions to infliximab (IFX) during 1st and 2nd IFX series.

Figure 3

Anti-infliximab (IFX) IgG antibodies (Ab) in individual patients before reinitiation of IFX and after acute severe infusion reactions. Samples from the same patients are connected by dotted lines.

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Figure 1

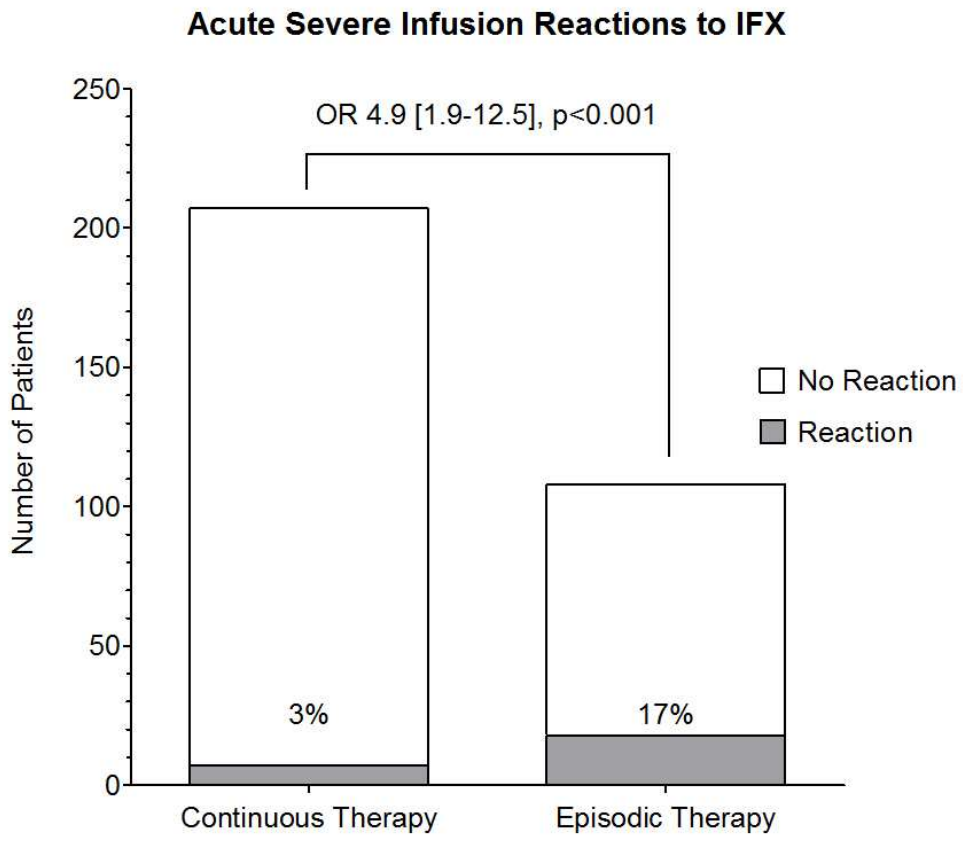

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Figure 2

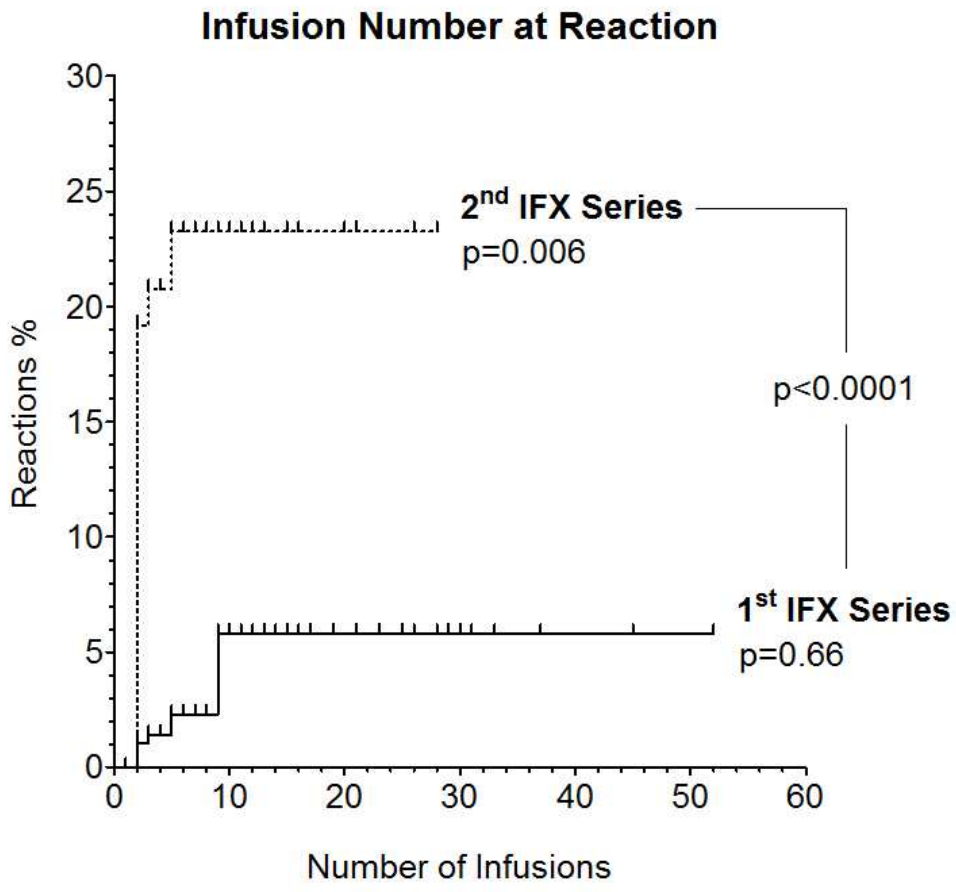

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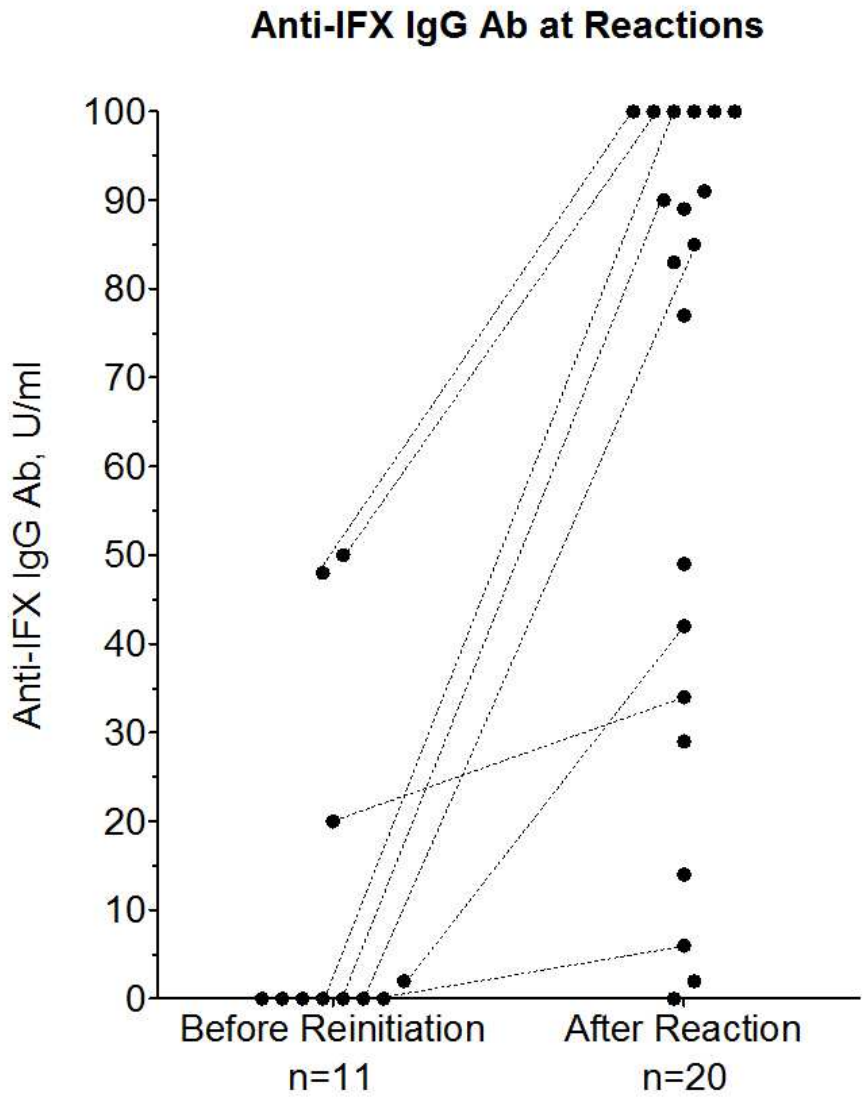
Figure 3


Anti-IFX IgG Ab at Reactions
Anti-IFX IgG Ab, U/ml
100
90
80
70
60
50
40
30
20
10
0
Before Reinitiation
n=11
After Reaction
n=20

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DECLARATION OF INTERESTS

Sources of financial support the last two years must be acknowledged.

1. Authors' declaration of personal interests:

(i) Klaus Bendtzen has served as a speaker for Pfizer, Wyeth, Roche, Bristol-Meyers Squibb, and Biomonitor A/S.

Ole Østergaard Thomsen has served as a speaker and consultant for Schering-Plough, UCB, and Zealand Pharma.

(ii) Morten Svenson is an employee at Biomonitor A/S.

(iii) Klaus Bendtzen owns stocks in Biomonitor A/S.

Casper Steenholdt, Jørn Brynskov, and Mark Ainsworth have no personal interests to declare.

2. Declaration of funding interests:

This study was funded by independent research grants from: Aase and Ejnar Danielsen's Foundation, Beckett Foundation, Danish Biotechnology Program, Danish Colitis-Crohn Society, Danish Medical Association Research Foundation, Frode V. Nyegaard and wife's Foundation, Health Science Research Foundation of Region of Copenhagen, Herlev Hospital Research Council, Lundbeck Foundation, and P. Carl Petersens Foundation.

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