

ORIGINAL ARTICLE

Experimental Allergy and Immunology

IgG4 anti-infliximab in treated patients: Clinical impact and temporal evolution

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Abstract

Background: Infliximab (IFX) carries potential risk of immunogenicity with the production of anti-drug antibodies (ADA). ADA may belong to different isotypes and are usually measured by ELISA bridging assay. This test is not designed to detect IgG4 antibodies. The aim was to measure IgG4 anti-IFX antibodies in a cohort of IFX-treated patients and to evaluate their relationship with ADA and their clinical impact.

Methods: Anti-drug antibodies were detected using a bridging ELISA in the serum of 222 treated patients with different clinical outcomes to IFX. The same samples were analyzed for IgG4 anti-IFX antibodies using an experimental ImmunoCAP assay with reduced serum IgG4 background levels. A longitudinal evaluation was performed in a subgroup of 38 patients to define the temporal evolution of IgG4 anti-IFX.

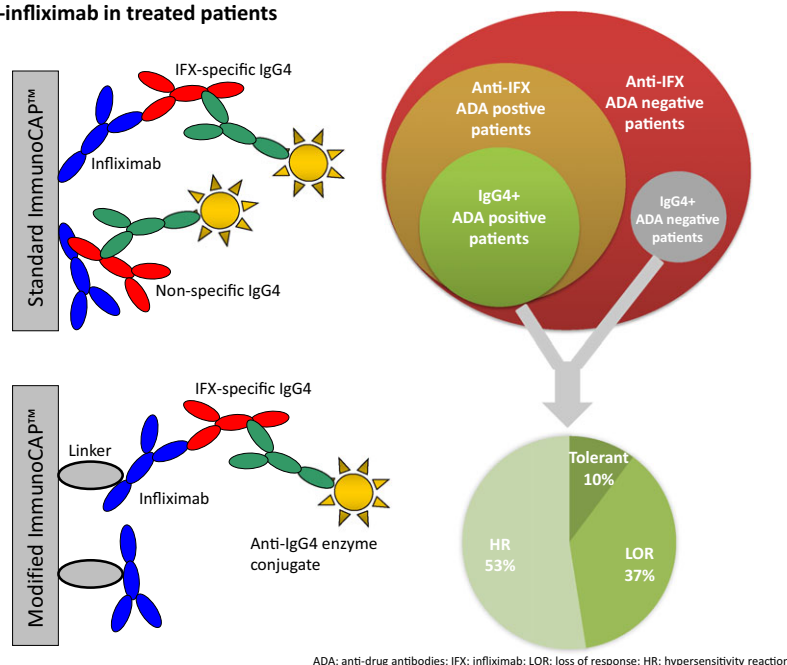
Results: IgG4 anti-IFX was found in 26.6% of patients. Eighty of 222 patients were ADA+ (36%) and the majority (57/80, 71.3%) had IgG4 anti-IFX. Two IgG4-positive but ADA-negative patients were identified. IgG4 anti-IFX levels correlated with the serum levels of ADA. IgG4 anti-IFX was more common in both reactive and nonresponder patients than in tolerant/responder patients. Patients who had experienced IgE-mediated reactions displayed significantly higher IgG4 anti-IFX than IgE-negative reactive patients. The majority of patients tested positive for IgG4 anti-IFX after the first seven infusions.

Conclusions: IgG4 anti-IFX is common in treated patients and a large part of ADA producing patients produce IgG4 antibodies. The IgG4 anti-IFX response does not prevent hypersensitivity reactions to IFX and correlates with the IgE anti-IFX response.

KEYWORDS

anti-drug antibodies, hypersensitivity reactions, IgG4, infliximab

IgG4 anti-infliximab in treated patients



GRAPHICAL ABSTRACT

IgG4 anti-IFX were measured using an experimental ImmunoCAP™ assay with reduced serum IgG4 background levels based on a modified IFX ImmunoCAP for research use only. IgG4 anti-IFX serum levels positively correlate with those of non-isotype-specific anti-drug antibodies (ADA) and ADA+ patients display higher levels of IgG4 anti-IFX than ADA– patients. IgG4 anti-IFX are not protective against clinical outcomes since are detectable more frequently in HR and LOR than in tolerant patients.

1 | INTRODUCTION

Infliximab (IFX) is a chimeric human-murine IgG1 monoclonal antibody that targets soluble and membrane-bound TNF- α and is largely used as a treatment for many immuno-mediated chronic diseases.^{1–3} Despite its beneficial effects, IFX treatment may be complicated by the development of unwanted immunogenicity that represents a major clinical concern.⁴ This is because the development of anti-drug antibodies (ADA) is associated with important clinical impact in terms of safety and efficacy. Although immunogenicity induced by IFX includes both humoral and cellular responses,⁵ the detection of ADA is currently the most feasible approach for assessing immunogenicity. Serological assays are required for the measurement of ADA in human clinical samples. Multiple formats are available aimed to discriminate between ADA-positive and ADA-negative samples (screening assay). Bridging ELISA is the most frequently used assay for the detection of ADA.⁶ This set-up uses the multivalency of most antibodies so that drug bound to the solid phase can be “bridged” by ADA to enzyme-tagged drug in the detection phase. For this reason, the bridging ELISA is not designed to detect functionally monovalent antibodies.

IgG4 is the less common subclass corresponding to about 4% of total IgG and is characterized by unusual structural and functional features. One of the most distinctive aspects is represented by the Fab arm exchange consisting of the random recombination of half-molecules of IgG4 with other half-molecules of IgG4. This phenomenon

leads to the formation of bispecific/monovalent antibodies.⁷ Several functional consequences of Fab arm exchange can be recognized.

Firstly, the effective monovalency results in a low avidity molecule and prevents the formation of large immune complexes due to lack of cross-linking (even by other antibodies).⁸ This finding in association with a reduced binding of C1q-IgG4⁹ leads to a poor capacity to trigger complement cascade by IgG4 molecules. In addition, IgG4 is characterized by a low activity in binding Fc γ R and activating Fc γ R-mediated effector functions.¹⁰ Overall, these findings contribute to characterize IgG4 as “blocking antibodies.” Secondly, the monovalency does not allow the detection of IgG4 in ELISA bridging assays, as those designed to evaluate immunogenicity development during therapy with biological agents.⁷

IgG4 subclass presents another peculiar characteristic called Fc-Fc interaction that consists of its binding to other IgG molecules, especially IgG1 and IgG4, via their Fc parts. This tendency is increased by solid-phase immobilization of the IgG molecule, thus may cause high background signals or false-positive results in IgG-specific IgG4 immunoassays. This makes it a challenge to measure IgG4 against therapeutic IgG1 antibodies such as IFX in conventional solid-phase immunoassays.¹¹

The administration of biological therapy may lead to IgG4 anti-drug formation, as described for adalimumab, natalizumab, factor VIII, and IFN- β .^{12–16} Here, we study the development of IgG4 anti-IFX during IFX treatment and their clinical impact, in both a cross-sectional and a retrospective longitudinal analysis. IgG4 anti-IFX was measured

using an experimental ImmunoCAP™ assay with reduced serum IgG4 background levels based on standard ImmunoCAP Specific IgG4 assay reagents and a modified IFX ImmunoCAP for research use.

2 | METHODS

2.1 | Patients

Blood samples from 222 patients suffering from immune-mediated disease (95 inflammatory bowel disease [IBD], 30 rheumatoid arthritis [RA], 67 spondyloarthritis [SpA], 30 systemic vasculitis [Vas]) were collected. All patients were treated with IFX 3 or 5 mg/Kg (6.8% and 93.2%, respectively) at each infusion (range: 4-76; median number of infusions: 10). Regarding the clinical outcome to the treatment, 75 patients resulted tolerant, 89 displayed loss of response (LOR), and 58 experienced immediate hypersensitivity reactions (HRs). The reactions were graded as mild ($n = 25$) (erythema, urticaria/angioedema), moderate ($n = 18$) (dyspnoea, stridor, wheeze, nausea/vomiting, dizziness, chest or throat tightness, abdominal pain), and severe ($n = 15$) (cyanosis or $\text{SaO}_2 < 92\%$, hypotension [<90 mm Hg], confusion, loss of consciousness), according to Brown's classification. All patients had provided consent for serum collection and detection of anti-IFX antibodies.

In a subgroup of 38 patients (3 IBD, 13 RA, 6 SpA and 16 Vas), a longitudinal retrospective study was performed to analyze the temporal evolution of anti-IFX IgG4 antibodies. All patients used in this part of the study were never previously exposed to IFX. Pre-infusion sera were obtained in all patients before each infusion and analyzed for ADA, IgG4 anti-IFX, and IFX levels. In all longitudinally studied patients, sequential sera were available from at least the first 10 infusions (from T0 [first infusions] to T9 [10th infusion]). In addition, follow-up samples were analyzed for all 38 patients (mean follow-up time 142 ± 22 weeks and in total 166 samples analyzed). Regarding the clinical outcome to the treatment, 17 patients resulted tolerant, 17 displayed LOR, and 4 experienced immediate hypersensitivity reactions.

2.2 | Detection of ADA

All serum samples were evaluated for the presence of non-isotype-specific ADA using a commercially available bridging format ELISA kit (Promonitor®, Progenika Biopharma S.A., Spain), according to the manufacturer's instructions.

2.3 | Measurement of IFX-specific IgE and IgG4 antibodies

IgE and IgG4 antibodies to IFX were analyzed with experimental variants of the standard fluorescence enzyme immunoassays ImmunoCAP Specific IgE (measuring range: 0.10-100 kU_A/L) and ImmunoCAP Specific IgG4 (0.07-30 mg_A/L) using a Phadia™ 250 instrument (Phadia AB, Uppsala, Sweden).

Experimental tests for IFX-specific IgE analysis were developed by coupling IFX directly to the CNBr-activated ImmunoCAP matrix

(cellulose sponge) using the standard protocol of Phadia (standard IFX ImmunoCAP). The final assay has been described elsewhere.¹⁷

Experimental tests for IFX-specific IgG4 analysis were developed by coupling IFX to the ImmunoCAP solid phase via human serum albumin (HSA) as a linker by employing copper-free click chemistry (modified IFX ImmunoCAP).¹⁸ Briefly, *trans*-cyclooctene-modified HSA was first immobilized to the CNBr-activated solid phase and subsequently 6-methyltetrazine-modified IFX was conjugated to the HSA. The experimental assay for IgG4 anti-IFX was based on the modified IFX ImmunoCAP and commercially available assay reagents (Phadia AB). The standard procedure for ImmunoCAP assays has been described elsewhere.¹⁹ Specifically, patient samples were diluted 1:100 in sample diluent (Art No: 10-9361-01) prior incubation on the modified IFX ImmunoCAP. Bound IgG4 anti-IFX was detected by an IgG4-specific enzyme conjugate (10-9481-01), and finally, the generated fluorescence was automatically transformed to concentration by the instrument using IgG4 calibrators (10-9482-01). Due to known serum total IgG4 interference (high backgrounds) in direct sandwich-type IgG4 anti-IFX assays, it was important to establish the analytical cut-off (assay cut point). For this, thirty normal human serum samples were analyzed accordingly with the already published recommendations.²⁰

2.4 | Measuring of serum infliximab levels

An ELISA test (Promonitor, Progenika, Spain) was applied to determine the serum levels of IFX trough levels. Blood samples were obtained prior to application of IFX infusion.

2.5 | Drug tolerance assessment

Low and high ADA-positive control samples from IFX-treated patients, but without remaining free IFX, were pre-incubated at room temperature over night with increasing amount of spiked IFX (0, 0.013, 0.034, 0.32, 1.6, 8, 40, 200, 1000 µg/mL). The highest IFX concentration that still results in a signal above the assay cut point was defined as the drug tolerance level.

2.6 | Statistical analysis

Continuous variables were analyzed by a two-tailed Student *t* test. Correlations were analyzed by the Spearman's rank correlation test. Categorical variables were analyzed by Fisher's exact test. $P < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Clinical validation of the ImmunoCAP assay for IgG4 anti-IFX

The modified IFX ImmunoCAP was developed for IgG4 antibody measurements and optimized to reduce background binding of serum total IgG4 due to Fc-Fc interactions with the immobilized IFX

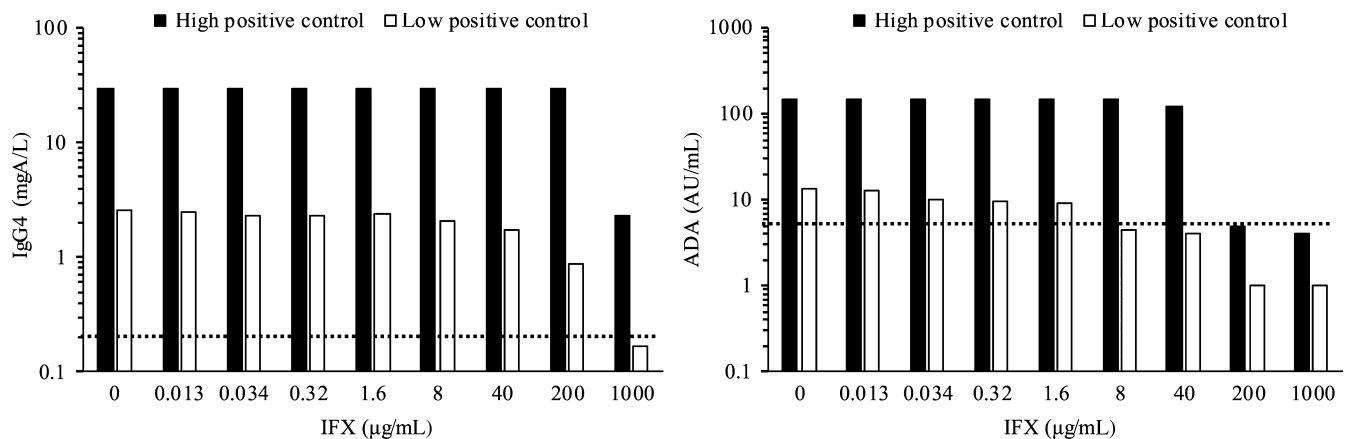


FIGURE 1 Drug tolerance evaluation of the modified ImmunoCAP IgG4 anti-IFX assay. Increasing doses (0–1000 µg/mL) of IFX have been added to two selected (high and low) positive sera in both the modified ImmunoCAP IgG4 anti-IFX assay and the non-isotype-specific ADA assay. Dotted lines represent cut-off values for both assays. ADA, anti-drug antibodies; IFX, infliximab

(which is a chimeric IgG1 monoclonal antibody). First, the reduction of IgG4 background binding to the modified IFX ImmunoCAP was confirmed by comparison with the standard IFX ImmunoCAP, a research ImmunoCAP originally developed for analysis of IgE antibodies using a standard coupling protocol for immobilization of IFX to the ImmunoCAP solid phase. When presumably negative serum samples from 30 healthy nontreated subjects were analyzed, the mean background level for the modified IFX ImmunoCAP was 0.08 mg_A/L (range: <0.07–0.40 mg_A/L) which was 100 times lower than the background for the standard IFX ImmunoCAP (mean: 8.98 mg_A/L, range: 0.43–18.5 mg_A/L). Thus, the modified IFX ImmunoCAP was used for all further analysis of IgG4 anti-IFX.

During the validation phase of the IgG4 anti-IFX assay, two parameters were assessed: the assay cut point and the drug tolerance. The assay cut point was calculated based on the results of the 30 negative samples as mean value + 1.645 standard deviations and resulted in a cut point for positivity corresponding to 0.203 mg_A/L.

Remaining free drug in the sample is one of the main interfering factors that may lead to false negative results in ADA assays having low free drug tolerance. To determine the drug tolerance of the ImmunoCAP IgG4 anti-IFX assay, both low and high positive control samples with no detectable free IFX were spiked with increasing concentrations of drug until the response in the assay fell below the assay cut point. The free drug tolerance displayed by the ImmunoCAP assay was very high with a tolerance of 200 and >1000 µg/mL IFX for the low and high positive control sample, respectively (Figure 1). As comparison, the corresponding free drug tolerance of the bridging ELISA for ADA was 1.6 and 40 µg/mL for the two control samples.

3.2 | IgG4 anti-IFX antibodies correlate with non-isotype-specific antibodies (ADA)

We investigated the frequency of IFX-specific ADA and IgG4 antibodies in IFX-treated patients (cross-sectional study), suffering from different immuno-mediated diseases. IgG4 anti-IFX antibodies were detected in 59 of 222 (26.6%) patients; 80 of 222 patients resulted

ADA+ (36%) and the majority of them (57/80, 71.3%) tested IgG4-positive (double positive) (Figure 2A). No differences were observed between patients with different type of disease or doses (3 or 5 mg/Kg) of IFX therapy (Figure 2B and data not shown).

We found two IgG4-positive patients that were negative for ADA (2/142, 1.4%). ADA+ patients displayed significantly ($P < .0001$) higher serum levels of IgG4 anti-IFX than ADA– patients (Figure 2C, left panel) and a positive correlation (Spearman test, $r = .643$; $P < .0001$) between serum levels of anti-IFX IgG4 and non-isotype-specific ADA was observed (Figure 2C, right panel).

3.3 | IFX-specific IgG4 antibodies are not protective against either acute infusion reactions or loss of response

We then investigated the clinical impact of IgG4 formation in treated patients with different clinical outcome to the treatment. Specifically, we analyzed the presence of IgG4 anti-IFX antibodies in three different groups of patients (HRs, LOR, and tolerant patients), accordingly to the efficacy and safety of therapy. In our case series, we did not find an association between clinical outcome to IFX therapy and the type of underlying disease (Figure 3A). Both HR and LOR patients displayed a higher frequency of IgG4 positivity (Figure 3B left panel). In addition, higher serum levels of IgG4 antibodies were observed in HR than in tolerant patients, as shown in Figure 3B (right panel). When we analyzed the frequency of ADA in HR, LOR, and tolerant patients, we observed a significantly higher proportion of ADA-positive subjects among LOR ($P < .05$) and HR ($P < .0001$) than in tolerant patients, HRs patients showing the highest frequency of ADA (81%, Figure 3C, left panel). Among patients who had experienced an HR, the incidence of ADA positivity was significantly ($P < .005$) higher than the IgG4 positivity (53.4%). Both HR and LOR patients displayed higher ADA titer than tolerant subjects (Figure 3C, right panel).

Anti-drug antibodies -mediated reactions can be divided into IgE (IgE+, $n = 13$) and non-IgE-mediated (IgE–, $n = 45$) events. The large

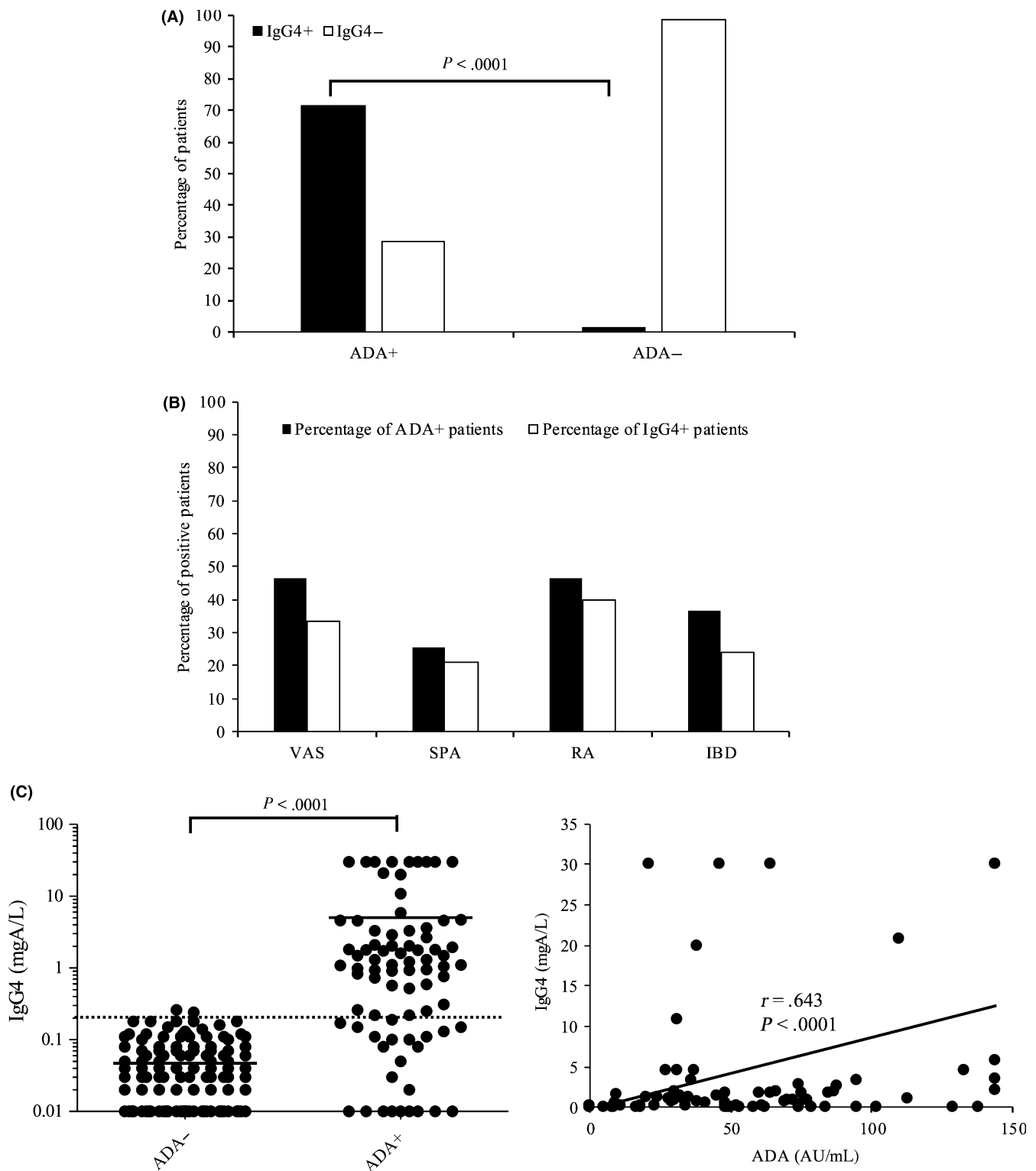


FIGURE 2 IgG4 anti-infliximab (IFX) antibodies in immune-mediated diseases and correlation with non-isotype-specific antibodies.

A, Frequency of IgG4 anti-IFX positivity in patients with ($n = 80$) and without ($n = 142$) non-isotype-specific anti-drug antibodies (ADA).

B, Incidence of ADA and IgG4 anti-IFX antibodies in patients suffering from vasculitis (VAS, $n = 30$), seronegative spondyloarthropathies (SpA, $n = 67$), rheumatoid arthritis (RA, $n = 30$), and inflammatory bowel disease (IBD, $n = 95$). C, Levels of serum IgG4 anti-IFX antibodies in ADA+ and ADA- IFX-treated patients (left panel); correlation between IgG4 and ADA antibodies (Spearman test) (right panel)

majority (12/13, 92.3% of IgE+ patients) resulted in the ADA+ group but we did not find significant higher titer of ADA in IgE+ patients (Figure 4A). We then evaluated the clinical impact of IgG4

development in both IgE+ and IgE- HRs patients. No difference in the frequency of IgG4 anti-IFX was observed (Figure 4B, left panel). Notably, HR patients who had experienced IgE-mediated reactions

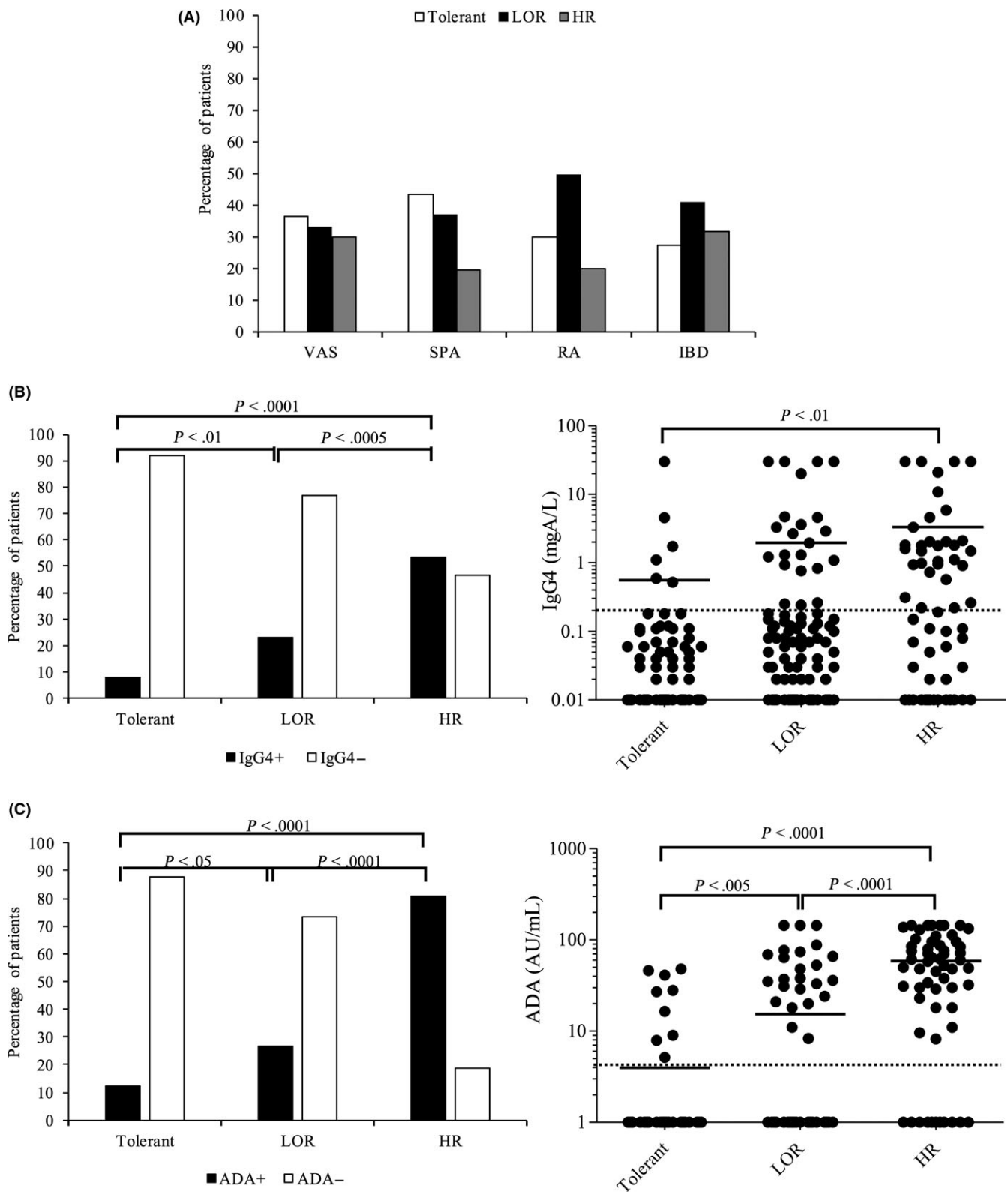


FIGURE 3 Infliximab (IFX)-specific IgG4 in patients with different clinical outcome to treatment. A, Incidence of different clinical outcomes in patients suffering from different immune-mediated disorders. Incidence (left panels) and serum levels (right panels) of IgG4 anti-IFX antibodies (B) and non-isotype-specific anti-drug antibodies (C) in patients treated with IFX with hypersensitivity reactions (HR, $n = 58$), loss of response to therapy (LOR, $n = 90$), or tolerant to the treatment ($n = 74$)

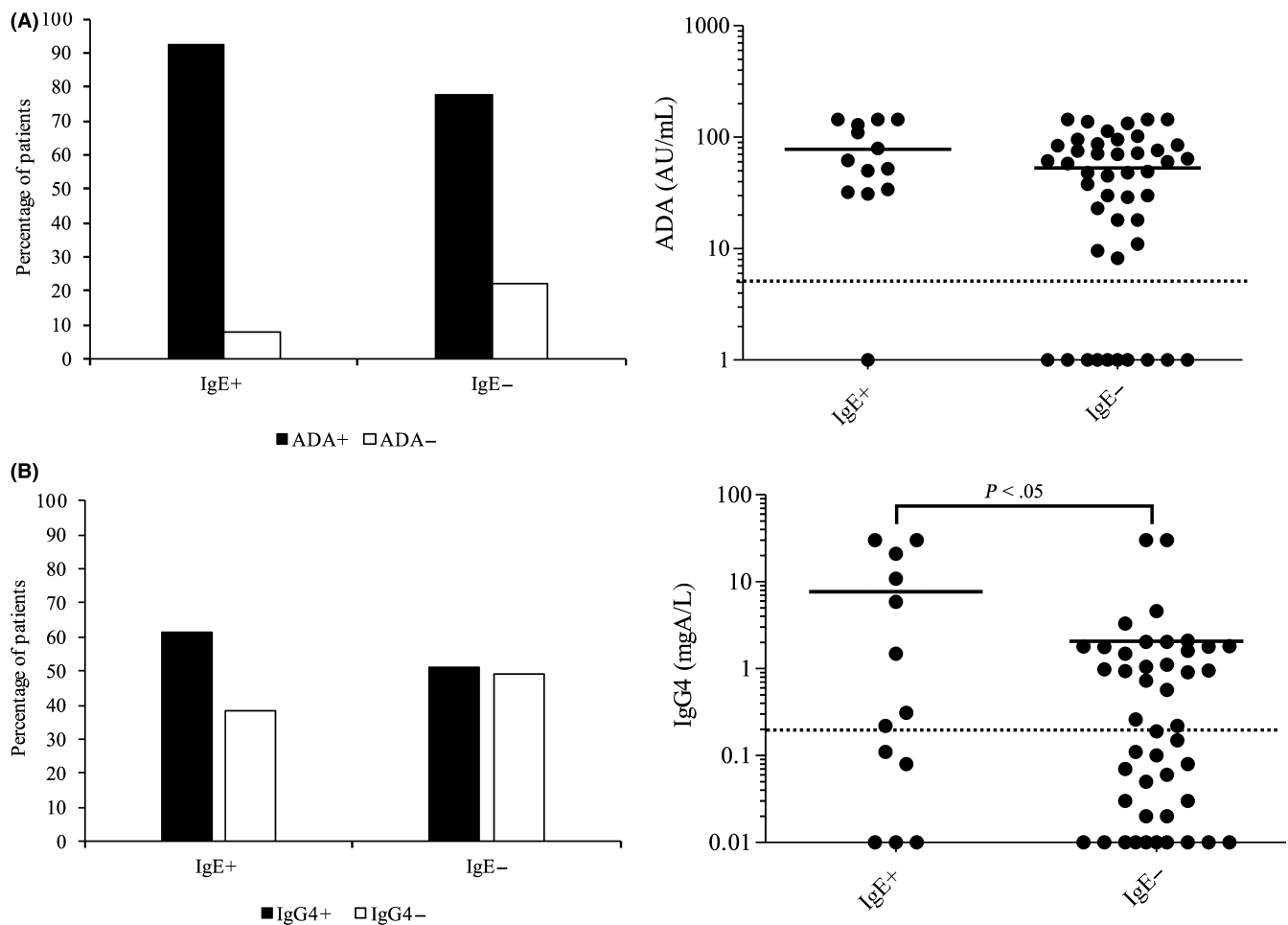


FIGURE 4 Anti-drug antibodies (ADA) and IgG4 development in patients with IgE-mediated hypersensitivity reactions. Evaluation of frequency (left panel) and serum levels (right panel) of ADA (A) and IgG4 anti-IFX antibodies (B) in reactive patients with ($n = 13$) or without ($n = 45$) IgE-mediated hypersensitivity reactions

were characterized by significantly ($P < .05$) higher serum levels of IgG4 than IgE-negative HR patients (Figure 4B, right panel). No differences in the frequency and the titer of IgG4 were found in patients with severe, moderate, or mild reactions (data not shown).

3.4 | Early development of IFX-specific IgG4 during the treatment

As humoral IgG4 development is usually regarded as a late immune response, occurring after repeated and prolonged exposure to the antigen, we investigated the timing of onset of IgG4 response after the treatment start, using a longitudinal analysis of a subgroup of 38 patients that were followed for 10–50 infusions (median value 10 infusions). Fourteen patients (14/38, 36.8%) developed IgG4 positivity, all of them within the first seventh infusion. Some of them tested positive starting from the third infusion. None of them showed pre-existing IFX-specific IgG4. All 14 IgG4+ patients also tested positive for ADA; notably in 3 cases, IgG4 antibodies were detectable before ADA positivity (Figure 5A). All 24 patients that tested IgG4 negative at 7th infusion remain negative also after prolonged treatment (range 10–52; median value: 10 infusions). The majority of patients that

developed IgG4 antibodies (9/14; 64.3%) interrupted the course of treatment due to immunogenicity-related events ($n = 5$ LOR and $n = 4$ HRs). IFX treatment was maintained in five patients despite serological positivity due to the absence of any clinical impact. In two of the five patients, IgG4 anti-IFX levels remain stable over time. In the other three patients, the IgG4 anti-IFX decreased to levels near or below the assay cut point (Figure 5B).

4 | DISCUSSION

Treatment with IFX may be associated with immunogenicity that results in the development of ADA and important clinical outcomes such as loss of response and adverse reactions. ADA may belong to several isotypes and it has been shown that anti-IFX antibodies mainly belong to the IgG1 subclass.²¹ In this study, including patients suffering from different immune-mediated diseases, ADA developed in 36% of treated patients and ADA positivity was closely related to the onset of HRs and partially to the loss of response to the treatment. ADA were analyzed using an ELISA bridging format assay that, even if it is a non-isotype-specific test, is not designed to detect

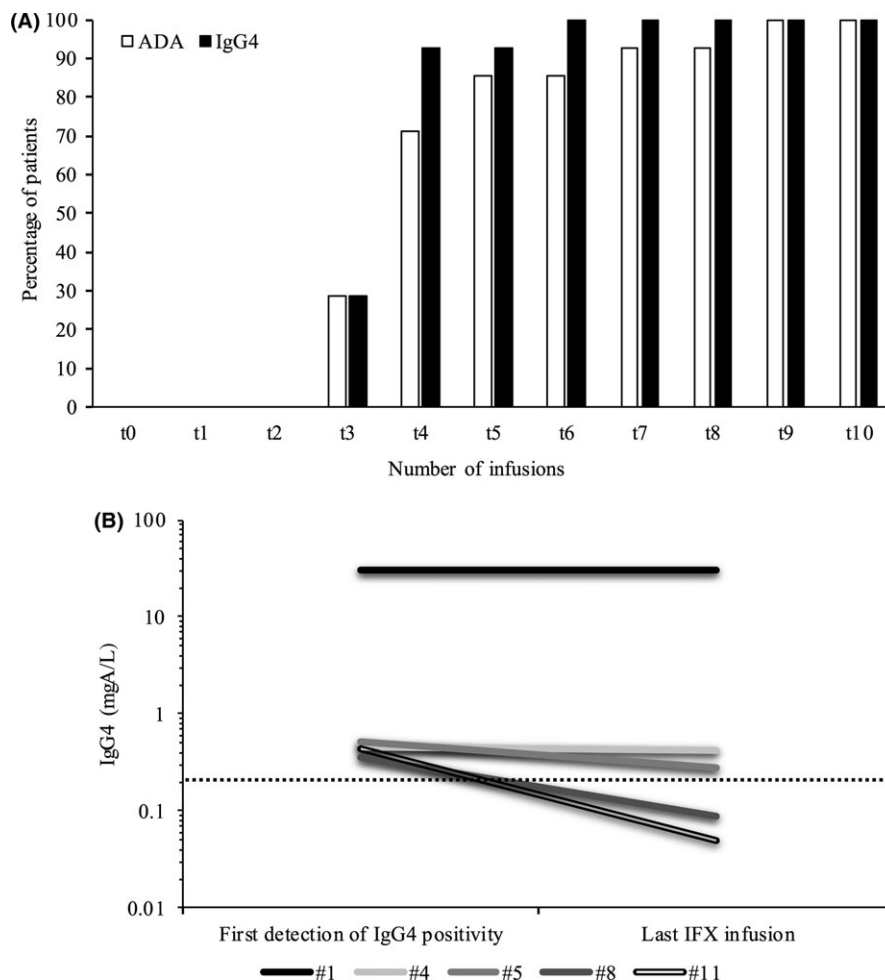


FIGURE 5 Early development of drug-specific IgG4 during infliximab (IFX) treatment. A, Monitoring of both anti-drug antibodies (ADA) and IgG4 antibodies during the first 10 IFX infusions in a longitudinal analysis of 38 patients. B, Evolution of serum levels of anti-IFX IgG4 antibodies in five patients who did not stop the IFX treatment after IgG4 development. Patient #1 was monitored for 40 infusions, patient #4 for 33 infusions, patient #5 for 38 infusions, patient #8 for 52 infusions, and patient #11 for 50 infusions

IgG4 anti-IFX antibodies. IgG4 may undergo Fab arm exchange and if this event occurs, the bridging ELISA is not able to detect it (potential false negative result). We then performed the analysis of serum samples using ImmunoCAP Specific IgG4 and a modified IFX ImmunoCAP where the drug was coupled to the ImmunoCAP solid phase (cellulose sponge) via HSA as linker. The aim was to reduce the serum total IgG4 background binding to the test via Fc-Fc interaction with IFX that has been previously described.¹¹ The assay validation showed that the serum background was reduced 100 times with the modified IFX ImmunoCAP compared to using experimental ImmunoCAP with conventional coupling of IFX to ImmunoCAP, resulting in a sensitive IgG4 anti-IFX assay with a cut point of 0.203 mgA/L and high free drug tolerance (200 µg/mL IFX for the low positive control sample). The high drug tolerance of the IgG4 anti-IFX assay can be derived to the high amount of immobilized IFX in the test and the short diffusion distances within the 3-dimensional sponge matrix of the ImmunoCAP solid phase, factors that favors antibody binding due to the law of mass action.²² Bridging ELISAs for ADA are known for their limited free drug tolerance, and other immunogenicity assays with improved drug tolerance like the antigen binding test have been presented.²³ Furthermore, a recently published ADA assay for IFX with improved free drug tolerance is the affinity capture elution assay by Van Stappen et al.²⁴

In our study, only two ADA+ patients tested positive for IgG4 against IFX. On the other hand, IgG4 antibodies were more frequent in ADA+ patients. The majority of ADA+ patients were IgG4+ and a correlation between serum levels of ADA and IgG4 anti-IFX was observed. Overall, these findings suggest that IgG4 antibodies are part of the heterogeneous humoral response against IFX. IgG4 antibodies were previously analyzed in patients treated with different biological agents such as adalimumab, natalizumab, and erythropoietin.^{12–16} Specifically, in adalimumab-treated patients, IgG4 was detectable in similar frequency as in our IFX-treated patients, and the authors reported that IgG4 anti-adalimumab antibodies may be present early on during the treatment (after 16 weeks).¹² Similar results have also been obtained in natalizumab-treated patients in which IgG4 antibodies have developed after 3 months of therapy.¹⁵ Accordingly, in our longitudinally analyzed cases, we observed an early development of IgG4 response toward IFX occurring within the first seven infusions (corresponding to about 9 months). While concentrations of IgG4 antibodies induced by allergen-specific immunotherapy continuously rise during treatment, as well as a correlation between IgG4 levels and the number of annual stings in beekeepers exists,^{25,26} we did not observe any increase in IgG4 levels during prolonged drug exposure. This data, together with the observation that patients who do not develop IgG4 anti-IFX early on remain negative despite very long IFX treatment,

suggest that repeated antigenic (drug) stimulation is not a key factor for IgG4 induction toward IFX. Notably, in some patients, IgG4 antibodies may be detected before ADA. This fact, as well as the observation of two IgG4+ but ADA− patients in our case series, may be due to a higher free drug tolerance of the ImmunoCAP assay in comparison with the bridging ELISA for ADA, presumably due to the high antibody-binding capacity of the large ImmunoCAP solid phase and the intrinsic sensitivity of drug interference of a bridging assay set-up.

One of the main significant observations in our study is the analysis of the correlation between IgG4 anti-IFX positivity and the clinical outcome to IFX treatment, taking into account that IgG4 is usually regarded as a blocking antibody with very poor effector functions. Overall, our findings suggest that IgG4 does not inhibit or prevent immediate HR, as reactive patients have a stronger humoral IgG4 response against IFX than other patients. On the other hand, an IgG4 involvement in the induction of HR is not plausible, as there is no evidence that IgG4 antibodies can mediate mast cells or basophil activation.²⁷ Accordingly, a significant proportion of patients who have experienced an immediate HR does not exhibit IgG4 positivity. Furthermore, when reactive patients were grouped according to their expression of IFX-specific IgE, it was indicated that IgG4 antibodies had no functions as inhibitors of IgE-mediated reactions. This result may be in contrast with some studies that have shown a correlation between the appearance of allergen-specific IgG4 antibodies and the development of tolerance during allergen-specific immunotherapy or desensitization for inhalants and foods.^{28,29} On the other hand, our results agree with the description that IgG4 antibodies against rodents in laboratory animal workers do not protect against allergic sensitization.³⁰ Regarding patients with loss of response to IFX, they tested IgG4 anti-IFX positive more frequently than treatment-responders, thus showing no protective role for IgG4 against loss of response. On the other hand, an involvement of IgG4 antibodies in the onset of LOR cannot be ruled out, taking into account that IgG4 antibodies are usually characterized by high antigenic affinity and associated neutralizing capability. Due to the heterogeneity of anti-drug immune responses in terms of antibody isotypes, specificity, and affinity, the clinical consequences of immunogenicity of biological agents are dependent upon the characteristics and composition of ADA. Probably, the ratio between IgG4 and IgG1 (IgG4/IgG1) and IgE (IgG4/IgE) could be a more informative parameter in the evaluation of clinical impact of IFX immunogenicity.³¹

Even if the molecular mechanisms involved in IgG4 induction have not completely clarified until now, IL-10 seems to be one of the most frequently involved switching factors for IgG4. In particular, studies suggest an important role for T regulatory cells in the development of an IgG4 humoral response via multiple factors including IL-10.³¹ In fact, IL-10 interferes with the class switching and in addition, it seems to be involved in the differentiation of IgG4-switched B cells to IgG4-secreting plasma cells.³² Interestingly, it is important to underline that IFX treatment is able to induce IL-10-producing regulatory memory T cells in treated patients.⁵ In addition, in vitro studies indicate that TNF- α blockers, including IFX, preferentially favor the onset of regulatory monocytes able to produce IL-10 or

participate in the reverse signaling on mTNF- α -bearing cells inducing apoptosis, cell cycle block, and IL-10 production.^{33,34} In other words, IFX administration leads to an IL-10-rich microenvironment through several mechanisms, including the development of regulatory T cells that may favor IFX-specific IgG4 production. Besides IL-10, Th2 cytokines such as IL-4 and IL-13, cytokines that are responsible for the switch to IgE, are also important switch factors for IgG4 onset.^{35,36} A sequential Th2-driven switch can transform an IgG4-producing B cell into an IgE-producing B cell. This finding may be the explanation of our results displaying higher serum levels of IgG4 in IgE+ than in IgE− reactive patients. The presence of IFX-specific IgG4 antibodies in IgE+ patients is probably the consequence of a given IL-4 plus IL-10-driven immune response against IFX. In line with our results, a positive correlation has been recently described between serum levels of IgE and IgG4 in patients with IgG4-related disease.³⁷

In conclusion, the ImmunoCAP technology has allowed us to demonstrate the production of IgG4 anti-drug antibodies during IFX treatment in a proportion of patients in both a cross-sectional and a longitudinal analysis. The presence of IgG4 response to IFX, which is associated with the production of ADA and correlates with IgE anti-IFX response, is not protective against immunogenicity-related events such as HRs and LOR.

CONFLICTS OF INTEREST

Dr. Moverare, Dr. Eriksson, and Dr. Venemalm report personal fees from Phadia AB/Thermo Fisher Scientific, during the conduct of the study. In addition, Dr. Moverare, Dr. Eriksson, and Dr. Venemalm have a patent ANALYSIS OF ANTIBODIES (WO 2015/091438) pending.

AUTHOR CONTRIBUTIONS

AV, FN, and EM conceived the study, designed the experiments, interpreted the results, and drafted the manuscript. AM participated in study design and interpretation. FN, AC, and SP performed experiments. CE and LV developed, and partly validated, the new modified infliximab ImmunoCAP test for specific IgG4 measurement. RM participated in the development of the modified infliximab test, the planning and evaluation of the study, and writing of the manuscript.

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