

The Kinetics of Antidrug Antibodies, Drug Levels, and Clinical Outcomes in Infliximab-Exposed Patients with Immune-Mediated Disorders



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What is already known about this topic? Antidrug antibodies (ADAs) are associated with loss of response and hypersensitivity reactions (HRs) to infliximab. IgE ADAs are detectable in a proportion of ADA-positive patients with HRs.

What does this article add to our knowledge? ADA onset is an early event irrespective of morbidity. A second cycle of treatment after drug interruption increases the risk for ADA development. High ADA levels, early ADA onset, or drug disappearance are predictive of HR. Patients with strong and early ADA response more frequently develop anti-infliximab IgE.

How does this study impact current management guidelines? Drug levels and ADAs should be closely monitored during the first year of treatment or after the first infusion of the second cycle after interruption. The detection of IgE ADAs is advisable to be assessed when strong and early ADA response is detected.

BACKGROUND: Hypersensitivity reactions (HRs) and loss of response (LOR) to infliximab (IFX) are related to drug immunogenicity characterized by antidrug antibodies (ADAs).

OBJECTIVE: To analyze the timing of ADA appearance and its relationship with drug levels and clinical outcomes in IFX-treated patients with different diseases.

METHODS: Samples were longitudinally collected before each infusion from 91 IFX-treated patients and were assayed for ADA and drug levels by enzyme-linked immunosorbent assay and for IgE by ImmunoCAP system. Clinical data regarding efficacy and safety of therapy were also monitored.

RESULTS: The ADA onset occurred quite early, irrespective of the type of disease, during the first year and more frequently and earlier during the second cycle of therapy. Patients with HR were more frequently ADA-positive and with higher ADA titers compared with other patient groups. ADA onset tends to precede HRs and LOR; all HRs that occur after a period of drug interruption are preceded by ADA development. Before ADA detection, a progressive decline in IFX levels until a complete disappearance was observed. The ADA titer was maintained for years both in patients with ongoing therapy and in those who interrupted it. IgE ADAs are more frequently developed in patients with higher ADA levels and earlier ADA onset, but their rate of negativization is faster.

CONCLUSION: The present data suggest that most IFX-exposed patients develop ADAs within the first year of treatment irrespective of disease type. The clinical outcome to the treatment is preceded by ADA development, which in turn is associated with the reduction in drug serum levels. Both ADA evaluation and therapeutic drug monitoring may have a relevant impact on clinical practice, giving new insights to predict LOR and HRs. © 2018 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2018;6:2065-72)

Key words: Infliximab; Immunogenicity; Antidrug antibodies; IgE antidrug antibodies; Prognostic values

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INTRODUCTION

TNF- α blockers play a relevant role in the treatment of many chronic immune-mediated diseases. Infliximab (IFX) is a chimeric mAb composed of a constant region of human IgG1k linked to murine variable regions and has been successfully applied in the treatment of inflammatory bowel diseases (IBDs),

Abbreviations used

ADA- Antidrug antibodies

HR- Hypersensitivity reaction

IA- Inflammatory arthritis

IBD- Inflammatory bowel disease

IFX- Infliximab

LOR- Loss of response

MTX- Methotrexate

Vas- Vasculitis

inflammatory arthritis (IA: rheumatoid arthritis and seronegative arthritis), and vasculitis (Vas).¹⁻⁴ Like other biologicals, IFX is immunogenic and its administration may elicit a stable sensitization characterized by the development of antidrug antibodies (ADAs).⁵ The ADA onset has been described in about half the patients receiving repeated TNF- α monotherapy.⁶ ADAs lead to a lower drug concentration, essentially because of an increased drug clearance.⁷ It has been shown in previous studies that ADA levels inversely correlate with drug levels and therapeutic response.^{8,9} Previous reports also showed that ADAs decrease IFX response by as much as 80%.¹⁰ The development of ADAs seriously complicates IFX therapy because they affect not only the efficacy but also the safety of the treatment, leading to hypersensitivity reactions (HRs).¹¹⁻¹³ ADAs of IgE isotype (IgE ADAs) have been described in a proportion of ADA-positive patients who develop more severe HRs, and are associated with drug-specific skin test positivity.¹⁴

Some effects of ADAs on clinical outcomes are not fully understood, for instance, the occurrence of ADA-positive patients who are responsive to therapy or the ADA-negative patients who display loss of response (LOR) to the drug or, last, the meaning of transient ADA formation in a subgroup of responsive patients. Explanations for these discrepancies have been proposed as the sensitivity of different assays to detect ADAs, or the different timing of ADA detection during the course of therapy. This last problem has been recently studied by Ungar et al.¹⁵ who described the temporal evolution of ADAs in patients with IBD treated with IFX. This prospective study clearly indicates that such patients develop ADAs within the first year of treatment, whereas in the transient ADA-positive patients ADAs were detected throughout the duration of IFX therapy. In addition, the incidence of ADA onset is reduced by cotherapy with immunomodulators in scheduled-therapy patients even though it follows the same kinetics.¹⁶

On the basis of this report, the aim of our study was to analyze the timing of the onset of ADAs (ADA and IgE ADA) and their kinetics in a panel of patients with IBD, IA, and Vas treated with IFX for a long period of time. We also evaluated the relationship of ADAs to subsequent drug levels and HRs.

METHODS

Patients and sampling

ADA and IFX levels as well as clinical outcomes were retrospectively investigated in more than 1000 serum samples from a cohort of 91 patients suffering from IBD ($n = 18$), Vas ($n = 28$), and IA ($n = 45$) collected longitudinally during 9 years (2007-2015) at the Clinical Immunology Center of Azienda Ospedaliero Universitaria of Careggi (Florence, Italy). The analysis was performed on consecutive samples taken at time 0 (before treatment

TABLE I. Patient characteristics

Patients	91
Female/male	45/46
Age (y), mean $\pm$ SD	52 $\pm$ 23
Disease	
IA	45 (49.5%)
IBD	18 (19.8%)
Vas	28 (30.7%)
Disease duration (y) (range)	9 (2-15)
Previous biological agents	9 (9.9%)
Concomitant therapy	
MTX	58 (63.7%)
Oral steroids	27 (29.7%)
Schedule therapy patients (first cycle of therapy)	76
Interrupted therapy patients (second cycle of therapy)	13

and at the subsequent 10 infusions. In 32 out of 91 patients, the samples were taken until the 40th infusion. To minimize drug interference, all samples were collected immediately before infusions.

Clinical responses were determined by disease-specific scores on the day of blood sampling. Specifically, for the patients with IBD, the Mayo score index and the Harvey-Bradshaw index score for ulcerative colitis and Crohn disease were used.^{17,18} For the patients with IA, the delta Disease Activity Score (Δ DAS28) according to the European League against Rheumatism response criteria was used.¹⁹ For the patients with spondyloarthritis, we used the Bath Ankylosing Spondylitis Disease Activity Score, and the response to treatment was defined according to the Assessment of SpondyloArthritis international Society consensus statement for the use of TNF- α inhibitors in spondyloarthritis.²⁰ For the patients with Vas, the clinical response was assessed using the Birmingham Vasculitis Activity Score version 3.²¹ During treatment, 41 patients out of the 91 developed ADAs. Regarding the clinical outcomes, 32 patients remained tolerant, 34 displayed LOR, and 25 experienced HRs, developed during the infusions or within 1 hour after the end of therapy. Clinical features of HRs are reported in Table E1 in this article's Online Repository at www.jaci-inpractice.org.

The demographic, clinical, and laboratory characteristics of the patients are presented in Table I. The study was approved by the local ethics committee (2012/0035982), and written informed consent was received from the participants before their inclusion in the study. A proportion of the patients were a part of previous studies.^{13,14}

ADA detection and IFX measurement

The ADA status of patients was evaluated by using a commercially available bridging format ELISA kit (Promonitor, Progenika, Spain), according to the manufacturer's instructions. An ELISA test (Promonitor, Progenika, Spain) was also applied to determine the serum levels of IFX.

IFX-specific IgE ADAs were measured via a streptavidin ImmunoCAP assay (kindly provided by Thermo Scientific-Phadia, Uppsala, Sweden) as previously reported.¹⁴ The threshold value for a positive result was 0.10 kUA/L.

Statistical analysis

Continuous variables were analyzed by a 2-tailed Student t test or the Mann-Whitney U test, as appropriate. Correlations were

analyzed by the Spearman rank correlation test. Categorical variables were analyzed by using the Fisher exact test. Kaplan-Meier curves were plotted to assess the temporal rate of events, and a log-rank test was conducted for a comparison between survival-free durations. A *P* value of less than .05 was considered statistically significant.

RESULTS

ADA developed in the first year of infusion in exposed patients with different diseases

We first observed preexisting ADAs before starting IFX therapy in 2 patients (2 of 91 [2.2%]). Thirty-nine out of the remaining 89 patients (43.8%) developed ADAs during the treatment (10 IBD, 16 IA, and 13 Vas).

We divided the 89 patients into 2 groups: the first group included patients longitudinally studied during their first cycle of therapy (*n* = 76), whereas in the second group we included patients who restarted a second cycle of therapy (*n* = 13) after drug interruption for at least 4 months. The percentage of patients who developed ADAs during the second cycle of treatment after interruption was higher than that observed in patients during the first cycle of therapy (69.2% vs 39.5%; *P* < .05).

In the first group, the longitudinal analysis indicated that ADA onset occurs quite early within the first 10 infusions (mean $\pm$ SEM, 23.4 $\pm$ 2.4 weeks) in most of the patients (83.3%), ranging from the 6th to the 30th week of treatment (Figure 1, A [left panel]). The development of ADAs in patients during the second cycle of treatment was very early, being after the first infusion in all cases (Table II). Kaplan-Meier analysis indicated that the timing of ADA onset was similar in patients irrespective of their disease type (Figure 1, A [right panel]) and the type of treatment preceding IFX therapy (data not shown). Moreover, the proportion of patients with IA developing ADAs during the first cycle of therapy was slightly decreased, even if not significantly, in methotrexate (MTX)-cotreated (7 of 25 [28%]) in comparison with non-cotreated (7 of 15 [46.7%]; *P* = .23) patients (Figure 1, B). The timing of ADA onset was delayed in MTX-cotreated versus non-cotreated patients, although not significantly (25.4 $\pm$ 6.5 vs 14.6 $\pm$ 3.3 weeks; *P* = .27).

By monitoring the drug levels in ADA-positive patients, we found that IFX was detectable in all samples, but it declined until complete disappearance before ADA onset in all patients (Figure 1, C). The mean $\pm$ SEM for drug disappearance was 19.3 $\pm$ 1.8 weeks, whereas that for the first ADA detection was 23.4 $\pm$ 2.4 weeks (*P* < .05) (Figure 1, C).

The ADA onset precedes HRs in IFX-treated patients

When the cohort of 89 selected patients was categorized according to clinical outcomes to the treatment, a significantly higher frequency of ADA positivity was found in patients with HRs (21 of 23 [91.3%]) than in LOR (12 of 34 [35.3%]; *P* < .0001) or tolerant (6 of 32 [18.7%]; *P* < .0001) patients (Figure 2, A). Importantly, in the group of patients with HRs, the mean levels of ADAs were significantly higher than those showed by tolerant patients and, at some points, by LOR patients (Figure 2, B).

The analysis of ADA-positive patients showed that patients with HRs developed ADAs (mean $\pm$ SEM, 15.1 $\pm$ 3.5 weeks) before LOR (19.3 $\pm$ 3.4 weeks; *P* = .45) and tolerant (28.7 $\pm$ 3.2 weeks; *P* = .067) subjects, even not significantly.

When we compared the timing of ADA onset with that of HR development, during the first cycle of therapy, we found that

most patients developed ADAs before HRs (8 of 13 [61.5%]), the mean value being 23.2 $\pm$ 4.6 versus 36.1 $\pm$ 5.8 weeks, respectively (*P* < .01) (Figure 2, C). Also, in patients in the second cycle of treatment, ADA onset precedes HR development. In fact, in this group all patients with HR (*n* = 8) were ADA-positive, ADA became detectable in the meantime between the first and second infusions, and HR was experienced at the second infusion (Table II). Among patients who developed ADA and LOR (*n* = 12), about half of them (54.5%) developed ADAs before LOR, the mean values being 19.3 $\pm$ 3.4 versus 37.7 $\pm$ 18.1 weeks (*P* = .35).

We observed 1 ADA-positive patient with LOR during the second cycle of therapy (1 of 12 [8.3%]). He tested ADA-positive between the first and second infusions. He had never displayed any positive clinical response after restarting the treatment.

Kinetics of ADAs in patients receiving ongoing treatment or in out-of-therapy patients

We then monitored ADA levels from the first detection and up until the last dose of IFX was administered, either due to LOR or HR, and regardless not past the 10th infusion in the 30 ADA-positive patients of the first group (during the first cycle of therapy). We observed that they remained stable in most of them (24 of 30 [80%]), increased in a few patients (5 of 30 [16.7%]), and were transiently detectable in 1 (3.3%). In addition, to better evaluate the ADA kinetics, the longitudinal analysis was prolonged to more than 10 infusions in 6 ADA-positive patients (4 IA and 2 Vas) who continued therapy because no negative ADA-related clinical impact was observed; we assessed ADA every 5 infusions until the 40th infusion. We observed that in all 6 patients, ADA levels remained stable throughout the long-term continuation of therapy (Figure 3, A). We also evaluated ADA kinetics in a subgroup of ADA-positive patients (14 of 30) after drug interruption due to HR or LOR. We demonstrated a very slow decrease in ADA levels as shown in Figure 3, B, apparently irrespective of the type of novel regimen (see Table E2 in this article's Online Repository at www.jaci-inpractice.org).

In contrast, in 25 out of the 46 patients who tested ADA-negative at the 10th infusion and did not interrupt therapy, ADA remained undetectable in all subsequent samples (data not shown).

Kinetics of IgE ADAs

The IgE ADAs were detectable in 9 patients with HRs (4 IA and 5 Vas) and 5 of them developed IgE ADAs after the first infusion of the second cycle of therapy. All IgE-positive patients showed ADAs, and, notably, the amount of ADAs in IgE-positive patients was significantly higher than in IgE-negative patients (85.2 $\pm$ 17 vs 51.3 $\pm$ 4.6 AU/mL; *P* < .02). In addition, the ADA onset in IgE-positive patients was significantly (*P* < .02) earlier than in IgE-negative patients with HRs (Figure 4, A). The IgE ADA onset tightly followed that of ADAs, the mean $\pm$ SEM being 6.4 $\pm$ 2.3 and 10 $\pm$ 3.5 weeks (*P* was nonsignificant), respectively, even if IgE ADAs followed different kinetics from ADAs. In fact, IgE negativization occurred 30 to 60 days after the reaction, whereas ADA levels were detectable for more than 1 year.

As stated earlier, we observed preexisting ADAs in 2 patients and 1 of them also tested IgE-positive at baseline. This patient

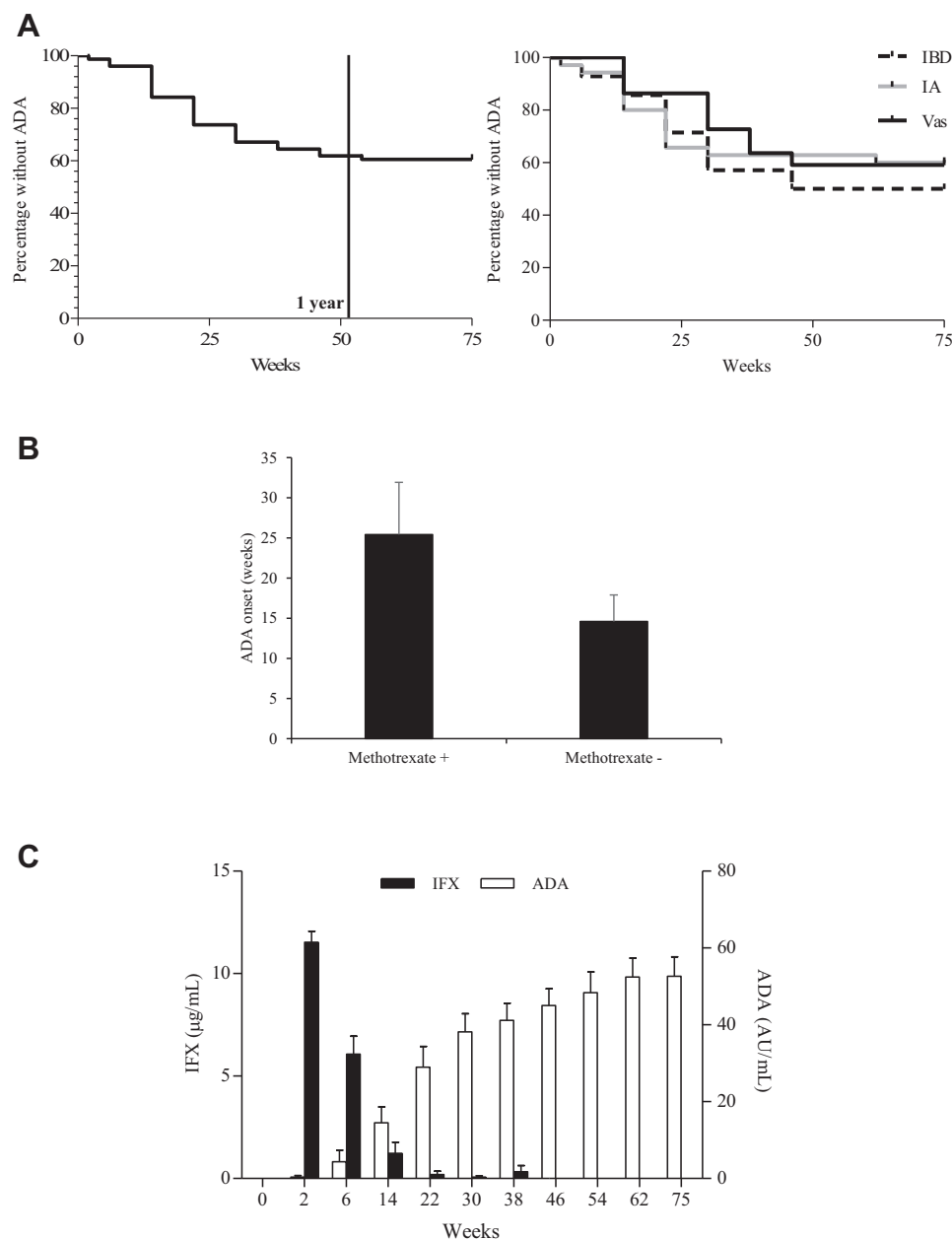


FIGURE 1. Incidence of ADA formation over time and chronological correlation between ADA and drug. **A**, Survival-free time of the development of ADAs during the first cycle of therapy in all exposed patients (left panel) or with different diseases (right panel). **B**, ADA onset in patients with IA receiving IFX monotherapy versus those receiving combination of IFX and MTX. Data are expressed as mean $\pm$ SEM ($P = .27$). **C**, Kinetics of ADAs and drug levels in patients who developed persistent ADAs at the same time points. Data are expressed as mean $\pm$ SEM.

showed a progressive increase in IgE ADA levels until week 110 when a severe HR occurred (Figure 3, *B* [left panel]). The second patient developed low levels of IgE ADAs after the first infusion and she experienced an HR after the second infusion (Figure 4, *B* [right panel]). Apparently, these patients did not display any difference from others who did not show ADAs before treatment, and were treated with corticosteroids plus MTX before starting IFX. The patients were not atopic or allergic to mouse and they had not previously received any other biologicals.

DISCUSSION

The type of disease has been suggested as a potential patient-related factor influencing immunogenicity, but in this study both the incidence and the timing of ADA onset were similar in each group of patients irrespective of their morbidity. Our data confirm the early development (mainly within the first 6 months of treatment) of ADAs in IFX treatments, not only in patients with IBD and rheumatoid arthritis as already published,^{15,22} but also in patients suffering from Vas and other IA. We also demonstrate that patients who were ADA-negative after 12

TABLE II. Study of the patients who interrupted therapy with IFX and restarted a second cycle of treatment

Patient	Disease	Clinical outcome	ADA onset (wk)	Drug negativization (wk)	Clinical outcome onset (wk)
1	IA	HR	0-2	0-2	2
2	IA	HR	0-2	0-2	2
3	IBD	HR	0-2	0-2	2
4	Vas	HR	0-2	0-2	2
5	Vas	HR	0-2	0-2	2
6	IBD	HR	0-2	0-2	2
7	Vas	HR	0-2	0-2	2
8	IBD	HR	0-2	0-2	2
9	Vas	LOR	0-2	0-2	2
10	IA	LOR	—	—	2
11	Vas	Tolerant	—	—	—
12	Vas	Tolerant	—	—	—
13	IA	LOR	—	—	14

months of treatment are at low risk of developing ADAs later, regardless of disease. The ongoing therapy of ADA-negative patients did not elicit ADAs in any samples collected for many years, clearly suggesting that an active regulatory mechanism is persistently operating. Several mechanisms are likely responsible for avoiding ADA development. Salinas et al.²³ provided evidence that TNF- α blockers interfere with the germinal center-dependent B-cell maturation. Others have shown that TNF- α antagonists trigger transmembrane TNF- α -expressing cells by outside-to-inside (reverse) signaling, which leads to drug-mediated G0/G1 cell cycle arrest and apoptosis.^{5,12} It has been recently shown that TNF- α neutralization increases the development of memory CD4+ T cells producing IL-10, which display an overall regulatory profile and show enhanced suppressive function.^{24,25}

In a previous cross-sectional study, we found that about 30% of patients with HRs who tested ADA-positive (8 of 30 [26.6%]) were in their second cycle of therapy with IFX.¹³ In this study we confirm, in a longitudinal setting, that re-treatment after a period of drug interruption is a risk factor for a very early ADA onset. In fact, the present study demonstrates that the kinetics of ADA development during the first and second cycles of therapy are different, being much earlier at re-treatment than at the first course. These results agree with data obtained in patients with IBD, where the risk of reaction is higher at IFX reinitiation.²⁶⁻²⁹ It could be hypothesized that the decrease in the inhibitory mechanisms mentioned earlier, during the drug discontinuation in association with the maintenance of memory T effector cells, may favor and facilitate the ADA development observed at re-treatment.

In this study, both disease duration and the treatment before the IFX regimen seem to have a low impact on immunogenicity development. A clearly reduced immunogenicity in patients with rheumatoid and psoriatic arthritis who were also receiving MTX has been described.^{30,31} In our case series the cotherapy with MTX did not decrease the incidence of ADAs, but delayed their onset, although without reaching statistical significance, most likely because of the low number of MTX-treated cases included in our study.

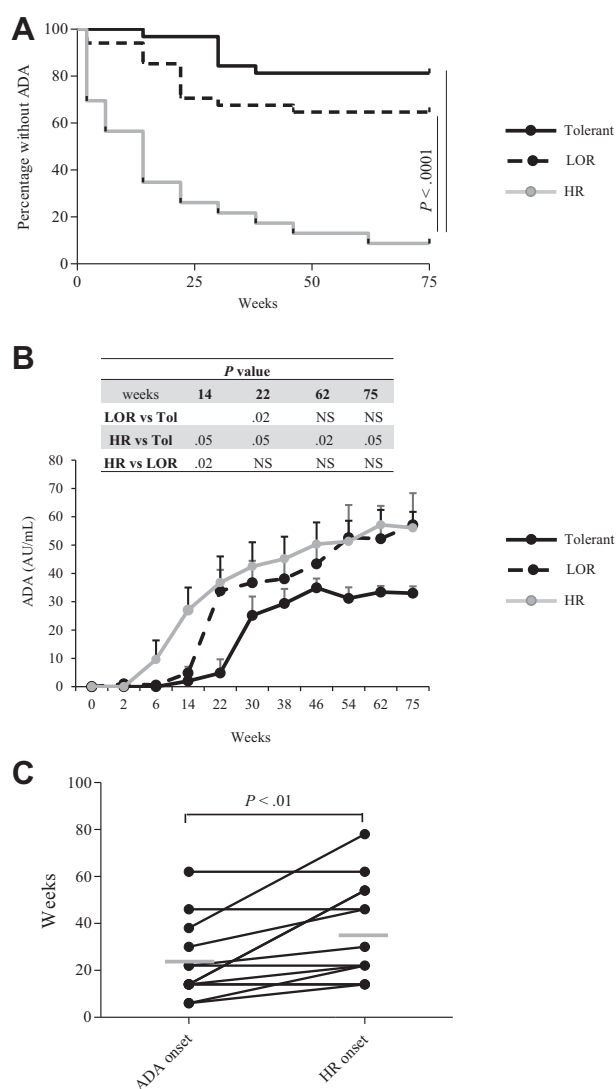


FIGURE 2. Chronological correlation between ADA and clinical outcome. **A**, Survival-free time of the development of ADAs in IFX-treated patients with different clinical outcomes. **B**, ADA measurement in tolerant patients with LOR and HR was studied from t0 to t10. Data are expressed as mean $\pm$ SEM. **C**, Time intervals between the onset of ADA and the onset of clinical outcome for a group of patients with HR. Each circle indicates each patient, and the gray line indicates the mean value. NS, Not significant.

Another important observation of the article concerns the relationship between the timing of ADA onset and of clinical outcome. Specifically, we found that in all ADA-positive patients who experienced an IFX-related infusion reaction, ADA development anticipates HRs. Similarly, about half the patients developed ADAs before LOR appearance. However, LOR preceded ADA detection in 45.5% of patients (data not shown). These results confirm the close relationship between HRs and ADAs, whereas mechanisms other than drug immunogenicity may be involved in LOR.³² In addition, the longitudinal analysis we performed in this study allowed us to show that IFX declines

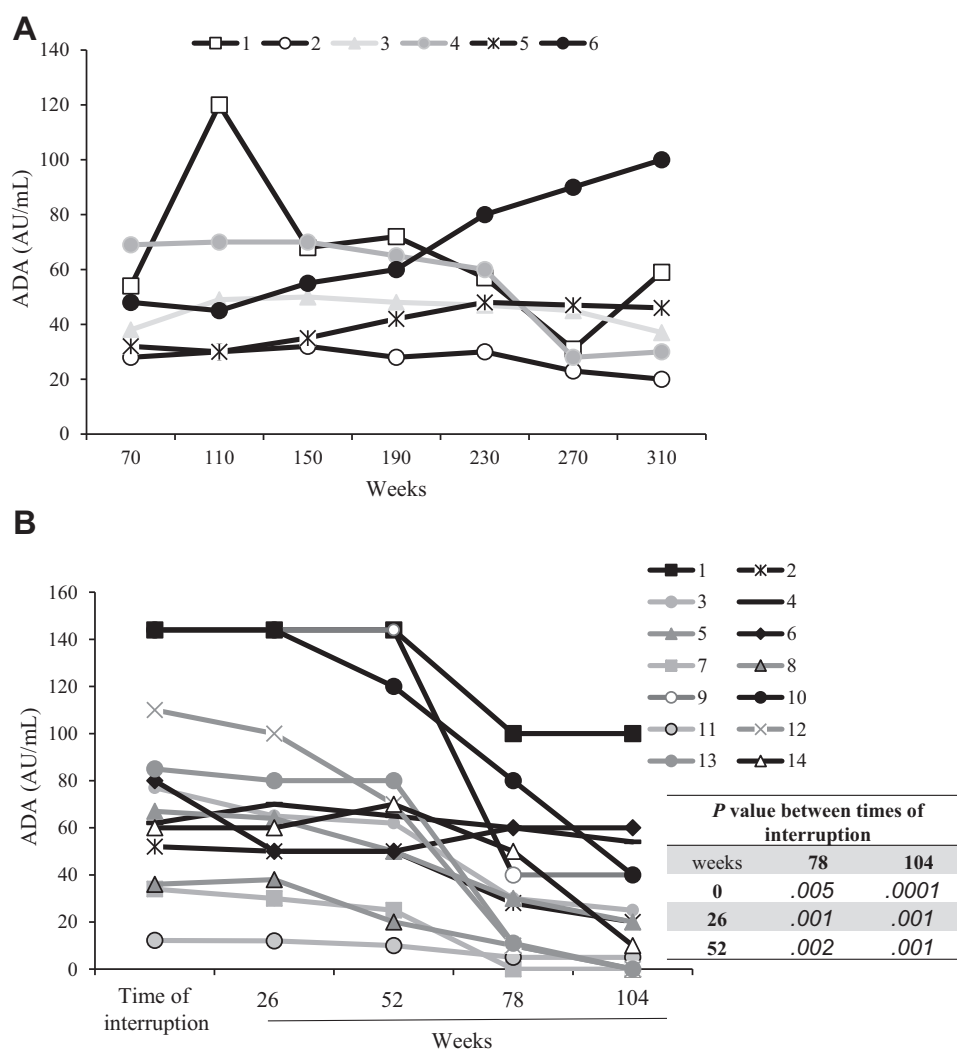


FIGURE 3. The kinetics of ADAs ranging from the 10th to the 40th IFX infusion. **A**, ADA levels in 6 ADA-positive patients who continued therapy without any clinical outcome. Each symbol and line identify each patient. **B**, ADA titer assessing of samples from 14 ADA-positive patients who interrupted therapy for LOR or HR. Each symbol and line indicate each patient.

until completely disappearing before ADA onset in most patients. The inverse relationship between ADAs and drug levels in a single sample has already been described, likely because of the fact that the quantification of ADAs is possible only when IFX levels are low.^{30,33} To our knowledge, this is the first longitudinal observation describing the temporal evolution of drug levels in relationship with ADA development, which suggests that the decrease in the drug levels may in fact favor its subsequent immunogenicity.

With regard to the clinical impact of ADA development, this study clearly shows that although patients may become ADA-positive during the treatment without any negative clinical consequences (they remain tolerant and good responders), their ADA levels are significantly lower than those of patients with immunogenicity-related clinical effects (LOR and HRs). Notably, we also analyzed ADA kinetics in these patients with long-term drug exposure who had not discontinued the treatment upon ADA onset. The analysis, which extended for more than 6 years, indicated that ADAs were consistently detectable

and were maintained at similar levels, with no negative clinical impact. The persistence of ADAs suggests that the bone marrow reservoir of ADA-producing plasma cells is supplied by new B cells/plasmablasts activated by memory T effector cells continuously stimulated by drug epitopes. However, ADA-positive patients who interrupted therapy maintained high ADA levels for more than 2 years and in some cases even longer, as also described by others.³⁴ Overall, these data suggest that once the immunological mechanism leading to ADA production begins, it may be difficult to stop.

Previous results have shown that IgE ADAs are detectable in a proportion of ADA-positive patients who have experienced HRs toward biologicals, including IFX.^{35,36} Several observations suggested that IgE-mediated HRs are more severe, occur earlier during the course of treatment, and occur more frequently during the second cycle of therapy.³⁷ In addition, a rapid decrease in IFX-specific IgE ADAs has been described in 3 patients.¹⁴ The analysis of IgE ADAs performed in this longitudinal study confirms that IgE-mediated reactions occur

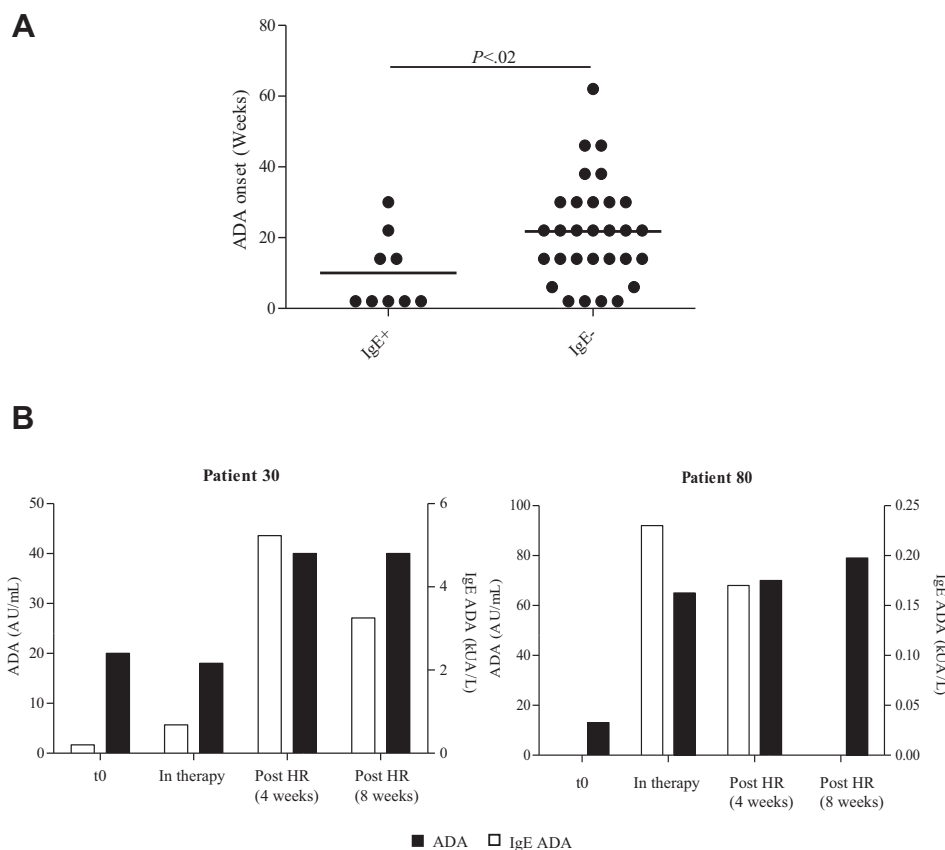


FIGURE 4. The kinetics of IgE ADAs. **A**, ADA onset in IgE-positive and IgE-negative patients. Each circle indicates each patient, the black line being the mean value. **B**, ADA and IgE ADA onset and kinetics in 2 patients showed ADAs before the treatment.

frequently at re-treatment and that IgE ADAs quickly decrease after drug interruption. One explanation may be that IgE ADAs are undetectable because of interference in the assay by non-IgE ADAs. We have previously shown that drug skin testing closely correlates with IgE ADA positivity, but we do not know whether such *in vivo* assay could override the interference issues of *in vitro* IgE detection.^{13,14} In addition, this study shows (1) a different kinetics of IgE isotype in comparison with ADAs with a faster disappearance and (2) that IgE ADAs are more frequently detectable in patients with a stronger and earlier ADA response.

CONCLUSIONS

The present data suggest that most IFX-exposed patients develop ADAs within the first year of treatment irrespective of disease type. The clinical outcome to the treatment is preceded by ADA development, which in turn is anticipated by the reduction in drug serum levels. Both ADA evaluation and therapeutic drug monitoring may have a relevant impact on clinical practice, giving new insights to predict LOR and HRs.

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TABLE E1. Clinical features of patients with HR

Patient	Disease	Age (y)/sex	Grade of reaction	Cycle of therapy	Nonisotype ADAs	IgE ADAs	Skin testing	MTX cotherapy
8	IA	53/M	Severe	2*	Pos	Pos	Pos	Yes
17	IA	55/F	Severe	2*	Pos	Pos	Pos	No
21	Vas	73/F	Severe	9	Pos	Pos	NA	No
31	BID	53/F	Severe	2*	Pos	Pos	Pos	No
78	Vas	56/M	Severe	9	Pos	Neg	Neg	No
1	BID	35/M	Moderate	3	Pos	Neg	Neg	No
16	IA	44/M	Moderate	4	Pos	Neg	Neg	No
23	Vas	76/F	Moderate	12	Neg	Neg	Neg	No
27	BID	56/F	Moderate	5	Pos	Neg	NA	No
29	IA	40/M	Moderate	6	Neg	Neg	Neg	No
42	Vas	42/M	Moderate	2*	Pos	Pos	Pos	No
43	Vas	33/F	Moderate	2*	Pos	Pos	Pos	No
55	BID	32/F	Moderate	7	Pos	Neg	Neg	No
57	Vas	41/F	Moderate	2*	Pos	Neg	Neg	No
63	IA	60/F	Moderate	4	Pos	Neg	Neg	Yes
70	BID	44/F	Moderate	4*	Pos	Neg	Neg	No
26	IA	51/M	Mild	4	Neg	Neg	Neg	Yes
12	Vas	70/M	Mild	4	Pos	Pos	Neg	No
25	BID	57/F	Mild	6	Pos	Neg	Neg	No
34	IA	46/F	Mild	4	Pos	Neg	Neg	Yes
44	BID	45/F	Mild	8	Pos	Neg	Neg	No
50	BID	44/F	Mild	2*	Pos	Neg	Neg	No
74	IA	58/M	Mild	9	Pos	Neg	Neg	No
Patients with preexisting ADAs								
80	Vas	48/F	Severe	2*	Pos	Pos	Pos	No
30	RA	57/F	Mild	3	Pos	Pos	Pos	Yes

NA, Not available; *Neg*, negative; *Pos*, positive; RA, rheumatoid arthritis.

*Second course of treatment.

TABLE E2. ADA levels in patients who interrupted the first cycle of therapy for LOR or HR

Patient	Disease	ADA levels (AU/mL) (weeks after IFX interruption)					Therapy after IFX interruption
		0	26	52	78	104	
1	IA	144	144	144	100	100	Etanercept
2	Vas	52	50	50	28	20	Tocilizumab
3	IA	77	65	62	30	25	Etanercept
4	IA	62	70	65	60	54	Adalimumab
5	IA	67	64	50	30	20	Etanercept
6	IBD	80	50	50	60	60	Adalimumab
7	IA	34	30	25	0	0	Etanercept
8	IA	36	38	20	10	0	Adalimumab
9	Vas	144	144	144	40	40	Etanercept
10	IBD	144	144	120	80	40	Adalimumab
11	IBD	12	12	10	5	5	Adalimumab
12	Vas	110	100	70	10	0	Etanercept
13	IA	85	80	80	11	0	Abatacept
14	Vas	60	60	70	50	10	Etanercept