

Allergological *in vitro* and *in vivo* evaluation of patients with hypersensitivity reactions to infliximab

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Summary

Background The administration of biological agents is potentially affected by IgE-mediated infusion reactions.

Objective The aim of the study was to evaluate the utility of skin testing in patients who have experienced infliximab (IFX)-related reactions.

Methods Thirty patients with previous immediate hypersensitivity reaction to IFX, 20 disease-matched non exposed subjects, 15 IFX-treated disease-matched tolerant patients and 15 IFX non-responder patients were enrolled. Non-isotype-specific and IgE anti-drug antibodies (ADAs) were measured by a double-capture ELISA kit and ImmunoCAP assay, respectively. Prick and intra-dermal tests were carried out with the commercial IFX preparation serially diluted.

Results Skin testing, performed in 23 of 30 reactive patients, resulted positive in 7 of them (30.4%), whereas no positivity was found in other groups of patients. The majority of reactive patients displayed non-isotype-specific ADAs (23/30, 76.6%) and the presence of anti-IFX IgE antibodies was detected in 6 of them (26%). All 6 IgE-positive reactive patients showed skin testing positivity. One reactive ADAs-positive patient who resulted skin test positive, with no detectable serum IFX-specific IgE ADAs, was also found. Skin testing positivity was associated with severe and early reactions (within the 3rd dose). No unexpected adverse reactions to skin testing were recorded.

Conclusions and Clinical Relevance This study shows that about 30% of reactive patients display skin testing positivity. They usually develop severe reactions, mainly during the first administrations of IFX. The specificity and the safety of skin testing procedure for this biological agent are also confirmed.

Keywords adverse reactions, hypersensitivity, infliximab, skin testing

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Introduction

A new allergological field of clinical research is related to modern drug therapy with biological agents (BA). BA have the potential to induce an immune response leading to the production of specific anti-drug antibodies (ADAs) that can impact therapeutic efficacy as well as inducing hypersensitivity reactions (HRs) [1, 2]. The clinical manifestations of acute HRs, occurring during or within one hour after infusion, vary considerably, ranging from mild to severe. Important clinical consequences such as dropout therapy or even fatal cases may occur. This has significant implications for those

patients whose disease has progressed and therefore had limited treatment options. Drug-specific IgE antibodies associated with immediate adverse infusion reactions have been detected for different BA [3–7]. Accordingly, during the last few years positive skin testing has been reported in patients with immediate HRs caused by BA [8]. The availability of *in vitro* and *in vivo* diagnostic procedures able to detect ADAs is a key point in the management of BA-treated patients. The clinical utility of skin testing in routine practice has been unexplored up to now. This article is aimed to investigate the usefulness of skin testing in patients who have experienced infliximab (IFX)-related immediate HRs.

Methods

Patients and control subjects

Thirty patients (group I), who were referred to our Unit because of a previous immediate HR after IFX exposure, were enrolled in the study. The reaction was graded as mild (erythema, urticaria/angioedema), moderate (dyspnoea, stridor, wheeze, nausea/vomiting, dizziness, chest or throat tightness, abdominal pain) and severe [cyanosis or SaO₂ < 92%, hypotension (< 90 mmHg), confusion, loss of consciousness], according to Brown's classification [9]. Twenty non-exposed disease-matched patients (group II), 15 IFX-treated disease-matched tolerant patients (group III) and 15 IFX-treated patients who lost the response to the therapy (group IV) were also included as control groups. Informed consent was obtained from all enrolled subjects. Ethical approval for *in vivo* and *in vitro* diagnostic procedures for drug allergy was not necessary, since they are routinely performed. All clinical research has been conducted to GCP and according to local legislations. Table 1 summarizes the clinical characteristics of enrolled groups of subjects.

ADAs detection and isotyping

ADAs were measured by a double-capture ELISA kit (Immunodiagnostik AG, Bensheim, Germany) able to identify all anti-drug antibodies in an isotype independent manner (non- isotype-specific antibodies), according to the manufacturer's instructions. The screening cut-point was obtained from the evaluation of 56 untreated disease-matched patients (Optical density: 0.09) according

to the recommendation for the validation of immunoassay for detection of ADA [10]. This value, statistically determined, yields a 5% false positive rate.

IgE ADAs were measured with the use of the streptavidin ImmunoCAP assay (kindly provided by Thermo Scientific-Phadia, Uppsala, Sweden) as previously reported [11]. The threshold value for a positive result was 0.10 kUA/L.

Skin testing

Skin testing at immediate reading (15 min) was carried out with the commercial IFX preparation on the volar forearm, after the serum analysis. The drug concentration for skin prick testing was from 0.01 mg/mL to 10 mg/dL and, if negative, intradermal testing (IDT) was performed by using 0.02 mL of 0.01 mg/mL dilution, and proceeding to 0.1 mg/mL, 1 mg/mL and 10 mg/mL. In both prick and IDT, a minimum weal area of 3 mm in diameter or an increase of area > 3 mm was considered as positive compared to the negative response obtained with the saline solution. Histamine was used as positive control. Skin testing was performed firstly with a mean delay of 48.6 ± 4.9 days after the IFX-induced HR and repeated 8 months later.

Statistical methods

Statistical analysis was performed using the Student's *t*-test and Fisher's exact test was used for statistical comparison between groups. Differences with *P*-values less than 0.05 were considered significant. Predictive values (PV) were estimated taking into account the prevalence of HRs to IFX recorded in our experience and in literature data [12].

Results

Clinical characteristics of the reported hypersensitivity reactions

We firstly analysed the clinical features of immediate HRs in our case series. Cutaneous and respiratory symptoms were the most frequent clinical features, in particular flushing, dyspnoea, throat constriction and itching, as reported in Fig. 1. Among the reactive patients, we observed the same incidence (33.3%) of mild, moderate and severe reactions. The majority (26/30; 86.6%) of the reactions were recorded before the 10th infusion, but never at the first dose of the drug, whereas 8 of them (26.6%) at the re-exposure after a period of interruption (Table 2). Time to onset of the reactions was known for all reactive patients and the mean value was 23.3 ± 4.2 min. Reaction within 1–15 min after the

Table 1. Patient characteristics

	Group I (ADR)	Group II (untreated)	Group III (tolerant)	Group IV (LOR)
Total number	30	20	15	15
M/F	14/16	9/11	5/10	6/9
Age (mean $\pm$ SE)	50.6 $\pm$ 5.4	46.5 $\pm$ 1.9	43.5 $\pm$ 2.1	44.7 $\pm$ 3.2
Disease				
RA	8	6	5	6
SPA	5	4	2	3
VAS	6	3	3	1
BID	11	7	5	5
Concomitant therapy				
MTX	5	6	5	2
Pred	7	2	2	1
MTX + Pred	3	3	/	7
5-ASA	8	5	4	1
5-ASA + Pred	3	4	1	4
Nil	4	/	3	/

RA, rheumatoid arthritis; SPA, spondiloarthritis; VAS, vasculitis; BID, bowel inflammatory disease; MTX, methotrexate; Pred, prednisone; 5-ASA, 5-aminosalicylic acid.

Table 2. Clinical informations and details of skin test results of reactive patients

Patient (disease)	Grade of reaction	Infusion with reaction	Non-isotype ADAs	IgE ADAs	Total IgE (kU/L)	Prick test	IDT
1 (BID)	Severe	2nd (re-exposure)	Pos	Pos	24.4	Neg	Pos
2 (BID)	Moderate	6th	Pos	Neg	22.4	Neg	Neg
3 (BID)	Mild	2nd (re-exposure)	Pos	Neg	79.1	Neg	Neg
4 (BID)	Moderate	7th	Pos	Neg	4.9	Neg	Neg
5 (BID)	Moderate	3rd	Pos	Neg	15.6	Neg	Neg
6 (BID)	Moderate	5th	Pos	Neg	68	NA	NA
7 (BID)	Severe	2nd (re-exposure)	Neg	Neg	9.5	NA	NA
8 (BID)	Mild	2nd	Pos	Neg	14	Neg	Neg
9 (BID)	Mild	14th	Neg	Neg	26.5	NA	NA
10 (BID)	Severe	3rd (re-exposure)	Pos	Neg	18.8	Pos	Pos
11 (BID)	Severe	4th (re-exposure)	Pos	Neg	51.5	Neg	Neg
12 (RA)	Severe	2nd (re-exposure)	Pos	Pos	58.8	Neg	Pos
13 (RA)	Moderate	7th	Pos	Neg	68.3	Neg	Neg
14 (RA)	Moderate	2nd	Pos	Neg	15.7	Neg	Neg
15 (RA)	Moderate	3rd	Pos	Neg	25.1	Neg	Neg
16 (RA)	Moderate	4th	Pos	Neg	< 2	Neg	Neg
17 (RA)	Mild	3rd	Pos	Pos	276	Neg	Pos
18 (RA)	Mild	6th	Neg	Neg	2.8	NA	NA
19 (RA)	Mild	4th	Neg	Neg	18.3	NA	NA
20 (SPA)	Mild	9th	Pos	Neg	48	Neg	Neg
21 (SPA)	Mild	3rd (re-exposure)	Pos	Pos	39.4	Neg	Pos
22 (SPA)	Mild	9th	Pos	Neg	21.1	Neg	Neg
23 (SPA)	Severe	2nd (re-exposure)	Pos	Pos	16.7	Neg	Pos
24 (SPA)	Moderate	4th	Pos	Neg	91.2	Neg	Neg
25 (VAS)	Severe	9th	Pos	Neg	529	Neg	Neg
26 (VAS)	Severe	2nd	Pos	Pos	12.3	Neg	Pos
27 (VAS)	Severe	11th	Neg	Neg	< 2	NA	NA
28 (VAS)	Moderate	12th	Neg	Neg	36.4	Neg	Neg
29 (VAS)	Mild	23th	Neg	Neg	4.1	Neg	Neg
30 (VAS)	Severe	9th	Pos	Neg	1431	NA	NA

BID, bowel inflammatory disease; RA, rheumatoid arthritis; SPA, spondylarthritis; VAS, vasculitis; ADAs, anti-drug antibodies; IDT, intradermal test; NA, not available.

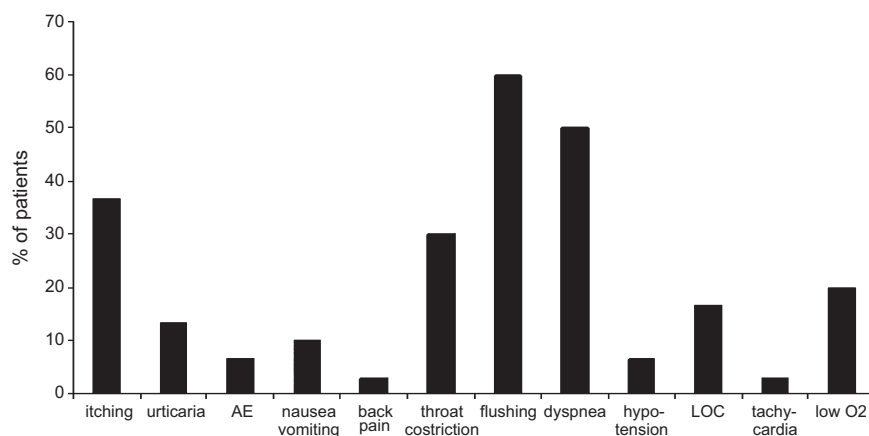


Fig 1. Clinical symptoms of recorded hypersensitivity reactions to infliximab.

beginning of the infusion was recorded in 17 patients, within 15–30 min in 3 and within 30–60 min in 10 of them. No reactions occurred after the end of the

infusion in our case series and concomitant therapy did not affect the incidence and severity of reactions (data not shown).

Sensitivity and specificity of skin testing

A preliminary study to establish an appropriate non-irritating concentration for prick and IDT was conducted. Twenty non-exposed subjects (group II), resulting negative at the detection of ADAs, were tested with sequentially increasing concentration of IFX. In contrast to what we had previously observed [4], irritant weals were noted in 3 subjects at IDT by using undiluted concentration (10 mg/mL) of the drug. Therefore, in the subsequent analysis of reactive patients we used only diluted solution of IFX (up to a maximum of 1 mg/mL). Twenty-three of 30 reactive patients (group I) accepted to be submitted to skin testing and positive results were found in 7 of them (30.4%) as compared with 1/30 (3.3%) patients with no HRs (group III and IV) ($P < 0.02$). The positive IDT was obtained with the IFX diluted at 1:1000 (0.01 mg/mL) in 1 case and 1:10 (1 mg/mL) in 6 cases (Table 2). Thus, the *in vivo* assay showed a sensitivity of 30.4% (7/23; CI 13.2–52.9%) and a specificity of 96.6% (29/30; CI 82.2–99.9%). The positive and negative predictive values could be estimated as being about 55% and 90% respectively.

Correlation between skin testing and serum IgE anti-IFX antibodies

To better define the role of skin testing for IFX in reactive patients, we compared the skin positivity with the presence of circulating specific IgE and non-IgE ADAs.

We firstly confirmed that the majority of reactive patients (group I) displayed ADAs-positivity (23/30; 76.6%) at the non-isotype-specific assay. Significant lower incidence of ADAs was recorded in group III (4/15; 26.6%, $P < 0.003$) but not in group IV (11/15; 73.3%). All patients belonging to group II resulted ADAs negative. Among ADAs-positive reactive patients, we showed the presence of IgE ADAs in 6 of 23 patients (26%), whereas three patients (3/30, 10%) with specific IgE antibodies were detected in ADAs-positive patients of group III and IV ($P = \text{NS}$) and none in untreated patients (0/20; $P < 0.05$). All 6 IgE-positive reactive patients showed skin testing positivity, but we also found 1 reactive ADAs-positive patient who resulted skin test positive with no detectable serum IFX-specific IgE (Table 2). Overall, by using a cut-off value of 0.10 kUA/L for the anti-IFX IgE, the *in vitro* test showed a sensitivity of 26% (6/23; CI: 10.2–48.4%) and a specificity of 90% (27/30; CI: 72.6–97.8%); the estimated positive and negative predictive values would be about 26% and 89% respectively.

We did not find any skin testing positivity 8 months later, according to the negative results of IgE ADAs (data not shown). Furthermore, 3 weeks after the second

skin testing procedure, we did not observe any IFX-specific IgE positivity (data not shown).

In skin positive patients reactions occurred early during the treatment with infliximab

We then analysed whether HRs had some peculiar clinical features in patients with skin testing positivity. We firstly observed a significant ($P < 0.02$) higher incidence of skin testing positivity in patients with severe reactions (5/7; 71.4%) than in those with mild-to-moderate events (2/16; 12.5%). Regarding the timing of the development of reaction, we showed that skin test positive patients displayed HRs significantly earlier (2.4 ± 0.2 infusions) during the course of treatment than both ATI-positive IgE-negative (5.3 ± 0.7 ; $P < 0.02$) and ATI-negative patients (6.6 ± 0.6 ; $P < 0.02$). Moreover, the majority of skin test positive patients (5/7; 71.4%), as opposed to skin test negative patients (2/16; $P < 0.02$), developed HRs at the re-exposure to the drug. In particular, among ATI-positive patients, all skin test positive subjects ($n = 7$) developed the reaction before or during the 3rd infusion compared to 5 of 16 skin test negative patients (31.2%, $P < 0.005$).

The safety of skin testing to infliximab

We then evaluated the safety of skin testing in patients with reported HRs. There were not unexpected adverse reactions to the skin testing procedure with the chosen dilutions of IFX in all patients, including IgE-positive subjects and, more importantly, in those who experienced severe anaphylactic reactions ($n = 10$). To analyse the impact of skin testing in the induction of a specific sensitisation, we repeated the measurement of serum antibodies in 20 ADAs negative patients belonging to group II ($n = 10$) and group III ($n = 10$) 4 and 8 weeks after the skin testing. We did not find any elicited humoral response towards IFX.

Discussion

This study attempts to evaluate the usefulness of skin testing as a diagnostic tool in the allergological work-up of patients who experienced HRs to IFX. It also proposes the methods and concentrations under which skin testing to IFX could be performed to detect patients with anti-IFX IgE antibodies. To date, very few studies, involving small case series, reported results of skin testing for biologicals confirmed by *in vitro* assay for serum specific IgE, measured by RAST or ImmunoCAP [4, 7]. Our results, according to previous data obtained with radiocontrast media and β -lactams [13–15], show that it is essential to perform IDT in patients with immediate reactions, as the prick test is usually negative. At present,

we suggest the use of IFX 1 mg/ml, since this concentration has not been shown to give false positive results in healthy donors and IFX-tolerant patients.

Although immediate IDT had a specificity of about 100%, the sensitivity is low. We evaluated patients within about 2 months of the reaction and we showed that about 30% of patients could be diagnosed by skin testing. Otherwise, our results show similar performance characteristics of *in vitro* IgE detection for BA. In our case series of IFX skin test positive patients, one of them resulted negative at the serum analysis with CAP system. The possible explanations may be the low serum concentration of IgE antibodies, all bound to the high affinity receptors on mast cells, as well as the interference of anti-IFX-specific IgG antibodies. In fact, one of the major topic of discussion concerns sensitivity of the *in vitro* IgE test assays, due to the low concentration of IgE compared to IgG antibodies in human serum. Furthermore, the majority of IFX-specific antibodies belong to the IgG isotype [16]. Although CAP system is highly specific and sensitive and IgE antibodies also have high avidity, excess IgG can deeply interfere with the detection of the relatively low amounts of IgE. The overall lack of sensitivity of IgE assays (*in vivo* and *in vitro*) could be also partially explained by other immunological models concerning non-IgE drug hypersensitivity. In particular, the existence of an IgG-mediated mechanism has been postulated in immediate HRs to BA [17, 18]. HRs may occur after immune complex formation between the drug and anti-IFX IgG antibodies, in fact various size of IFX/anti-IFX antibodies complexes, including very large ones, were demonstrated in patients who experienced severe infusion reactions [19].

Due to the fact that our study is a case-control project, the predictive values can only be estimated on the basis of prevalence recorded in our previous study and review of cohort studies [12]. The prevalence of reactions in our experience is about 12% and it is the median of a wide prevalence range of 1% to 27%. Given this prevalence, positive and negative predictive values would be about 26% and 90% respectively.

Our previous observation in IFX-reactive patients showed a rapid decrease of IFX-specific IgE antibodies [4]. Accordingly, none of the patients in this study showed skin test positivity to IFX and specific serum IgE when studied 8 months after the first analysis. Even if we do not have data on the negativization rate of skin testing for biologicals, an obvious factor is that patients can become skin test negative over time, similar to patients with allergic sensitization to β -lactam antibiotics [20]. These data suggest that the allergological work-up of reactive patients should be performed quite early, including both skin testing and CAP assay. Even though it would have been interesting to re-expose these patients to the biological drug after the

negativization of the skin tests, we preferred treating the IFX-reactive patients with an alternative TNF- α blocker. In our experience with a different chimeric monoclonal antibody (rituximab), we observed in a reactive patient that re-treatment after negativization of serum specific IgE led to the development of a new and more severe reaction associated with higher levels of serum IgE antibodies and skin testing positivity [5].

One limit of our study is the absence of a prospective design to really define the role of skin testing in the identification of patients at risk of severe reactions. Preliminary data obtained showed that patients with ADA positivity but skin testing negativity are able to tolerate ongoing treatment with IFX (unpublished personal data).

Cutaneous reactions (flushing, itching) along with respiratory symptoms (dyspnoea, throat constriction) were the most common features noted in our patient population (both IgE positive and IgE negative), but we observed higher frequency of positive skin tests in patients with severe reactions compared to those with mild or moderate events. Regarding *in vitro* tests, we observed variable levels of anti-IFX IgE ADAs not correlating with the severity of reaction. It is well known that low levels of drug and food-specific IgE antibodies are also able to induce HRs, especially in presence of low levels of total IgE. In fact, the specific/total IgE ratio might reflect the probability that IFX-specific IgE on the mast cells are adjacent and available for cross-linking. Accordingly, we can suggest that the low concentration of total IgE levels observed in our IFX-IgE positive patients may favour the mast cells/basophils activation. These effector cells are activated when a threshold number of Fc ϵ RI, which have been bound by specific IgE, is cross-linked by allergen molecules.

Concerning the safety, there were no unexpected adverse reactions to the skin testing procedure with IFX also in patients who experienced previous severe infusion HRs. Furthermore, the concentration of IFX used in our protocol did not elicit an IgE antibody response to the BA as confirmed by the negative results of serum samples tested up to 8 weeks after skin testing.

Our data confirm that not all HRs to IFX are IgE-driven, thus leading to the low sensitivity and positive predictive value of skin testing. Although these *in vivo* procedures are not fully standardized for BA, the easy clinical application and particularly their high specificity may offer a new tool for the diagnosis and prevention, at least, of IgE-mediated HRs. We recommend that further studies are performed to obtain a standardization of skin testing procedures for biologicals in the clinical practice, as well as to better define the diagnostic performance of skin testing in a wider range of cases. We also showed that IgE-mediated reactions usually occur at the beginning of the treatment, especially within the first 3 doses, thus suggesting that the

immune response against IFX which leads to the development of IgE ADAs is a quite early phenomenon. Even though the mechanism leading to the IgE response during the early phase of IFX treatment is still unknown, the use of skin testing could be proposed along with ADA detection, during the first four administrations of the drug, as a preventive strategy to avoid reactions, especially after a period of interruption [21]. Furthermore, if an alternative therapy is not available, in the case of skin testing positivity in a reactive patient, a desensitization procedure could be considered.

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Conflict of interests

All authors declare no conflict of interests.

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