

# Antidrug antibodies against TNF-blocking agents: correlations between disease activity, hypersensitivity reactions, and different classes of immunoglobulins

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**Abstract:** Although anti-TNF drugs have changed the clinical course of rheumatoid arthritis (RA), survival rates and resistance-to-therapy data confirm that about 30% of RA patients fail to respond. The aim of this study was to evaluate the correlations between the development of antidrug antibodies, specific IgG<sub>4</sub> antibodies against TNF inhibitors, and resistance to therapy in RA patients. This retrospective study involved 129 patients with established RA naïve to biological agents (98 females and 32 males, mean age 56.7±12.3 years, disease duration 6.3±1.2 years, baseline Disease Activity Score [DAS]-28 3.2–5.6) who received treatment with anti-TNF agents after the failure of conventional disease-modifying antirheumatic drugs (32 received infliximab [IFX], 58 etanercept [ETN], and 39 adalimumab [ADA]). After 6 months of treatment, the patients were classified as being in remission (DAS28 <2.6), having low disease activity (LDA; DAS28 2.6–3.2), or not responding (NR: DAS28 >3.2). The patients were also tested for serum antidrug antibodies and IgG<sub>4</sub> antibodies against TNF inhibitors. After 24 weeks of treatment, 38% of the ETN-treated patients and 28% of those treated with ADA had injection-site reactions; the rate of systemic reactions in the IFX group was 25%. The differences among the three groups were not statistically significant ( $P=0.382$ ; ETN versus ADA  $P=0.319$ ). The percentages of patients with adverse events stratified by drug response were: LDA 8% and NR 18% in the ADA group; in remission 3%, LDA 22%, and NR 10% in the ETN group; and LDA 6% and NR 16% in the IFX group ( $P=0.051$ ). The percentages of patients with antidrug antibodies were: ADA 33.3%, ETN 11.5%, and IFX 10.3% ( $P=0.025$ ; ADA versus ETN  $P=0.015$ ). The percentages of patients with IgG<sub>4</sub> antibodies were: ADA 6%, ETN 13%, and IFX 26% ( $P=0.017$ ; ADA versus ETN  $P=0.437$ ). Associations between antidrug antibodies, specific IgG<sub>4</sub> antibodies, and adverse reactions were not significant for any of the three drugs. IgG<sub>4</sub> levels were higher in the ADA group than in the other two groups, and higher in the patients with worse DAS28 (NR) and in those experiencing adverse events. These data suggest a possible association between IgG<sub>4</sub> levels and worse DAS28 ( $r^2=5.8\%$ ,  $P=0.011$ ). The presence of specific IgG<sub>4</sub> antibodies against TNF blockers in patients with RA might affect the drugs' activity. Patients with injection-site reactions and IgG<sub>4</sub> against ETN may show a decreased response.

**Keywords:** antidrug antibodies, TNF-blocking agents, IgG<sub>4</sub> antibodies

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## Introduction

Over the last 20 years, biological therapies (especially TNF inhibitors) have revolutionized the management of chronic inflammatory diseases, including rheumatoid arthritis (RA). Disease management has been dominated by the three TNF inhibitors infliximab (IFX), adalimumab (ADA), and etanercept (ETN), but despite an acceptable response

rate of 60%–70%, a substantial proportion of patients fail to respond (primary failure) or experience significant side effects.<sup>1</sup> Some questions have also arisen concerning the safety of TNF inhibitors, because they can trigger immunization, induce rare type I and III hypersensitivity, and cause acute and delayed reactions.

There have been many reports of reactions in patients receiving intravenous IFX, a chimeric IgG<sub>1k</sub> anti-TNF agent,<sup>2</sup> and immunomediated side effects, such as cutaneous reactions, have been encountered during therapy with subcutaneous anti-TNF drugs. One recent paper described injection-site reactions in 29.3% of patients treated with ETN.<sup>3</sup> Adverse reactions to biological agents have been categorized into five types, including a complement-mediated reaction with immediate IgE or delayed IgG antibody formation.<sup>4</sup> The immunoglobulin IgG<sub>4</sub> is an IgG subtype that has been described by some authors (particularly Parish in the 1970s)<sup>5</sup> as potentially causing transient sensitization that leads to signs and symptoms comparable with those induced by IgE-mediated reactions; this was initially termed IgG short-term sensitizing by Parish, because upon passive transfer to normal skin, the sensitivity persists for only 2–4 hours. IgG<sub>4</sub> differs from IgE insofar as it is present in amounts that are large enough to be detected by agglutination or precipitation assays, and its sensitizing activity is not destroyed by heat or (in most cases) chemical reducing agents.<sup>4</sup>

All biological agents (whether of entirely human origin, chimeric, or “humanized”) can cause an immune response, leading to the formation of antidrug antibodies (ADABs), which are also known as human antichimeric antibodies or human antihuman antibodies, depending on the nature of the drug. The generation of ADABs is increasingly recognized as a mechanism explaining the failure of anti-TNF drugs in chronic inflammatory diseases. The lack of a clinical response in patients with ADABs may be due to the formation of an immune complex between TNF inhibitors and ADABs that suppresses the drug and restricts its therapeutic activity.<sup>1</sup>

The aim of this study was to evaluate the correlations between the development of ADABs and specific IgG<sub>4</sub> antibodies against TNF inhibitors, adverse local and general hypersensitivity events, and resistance to therapy in RA patients.

## Materials and methods

This retrospective study involved 129 patients with established RA naïve to biological agents (98 females and 32 males, mean age 56.7±12.3 years, disease duration 6.3±1.2 years, baseline Disease Activity Score [DAS]-28 scores 3.2–5.6)

who received treatment with anti-TNF agents after the failure of conventional disease-modifying antirheumatic drugs: 32 (24.8%) received IFX, 58 (44.9%) ETN, and 39 (30.3%) ADA. Table 1 shows their baseline characteristics.

After 6 months of treatment, the patients were classified as being in remission (DAS28 <2.6), having low disease activity (LDA; DAS28 2.6–3.2), or not responding (NR; DAS28 >3.2). During the 24 weeks of treatment, we also evaluated injection-site reactions in patients treated with ETN or ADA, and systemic reactions in those treated with IFX.

The patients were tested for serum ADABs with an anti-TNF $\alpha$ -blocker enzyme-linked immunosorbent assay kit (Immundiagnostik, Milan, Italy) and IgG<sub>4</sub> antibodies against TNF inhibitors (fluoroenzyme immunoassay kit for ImmunoCap® 250; Phadia, Uppsala, Sweden). ADAB (Immundiagnostik) titers higher than 0.44 OD for ETN, 0.18 OD for ADA, and 0.27 for IFX were considered positive. IgG<sub>4</sub> antibodies against TNF inhibitors (ImmunoCap) higher than 5.9 mg arbitrary unit/liter for ETA, 19.5 mg A/L for ADA, and 8.6 mg A/L for IFX were considered positive.

## Statistical analysis

Power calculation was performed on correlation coefficients considering Fisher's Z-transformation, with an  $\alpha$ -value of 0.05. For our simulation, we considered the following scenarios: a correlation coefficient ranging from 0.4 to 0.6 and a lower 95% confidence limit of the correlation coefficient (null correlation) ranging from 0.05 to 0.1. In the more conservative scenario ( $r_{sp}$  = 0.4 and null correlation of 0.1), we calculated that more than 90 subjects inclusive of up to 10% of missing values may be considered sufficient to achieve a nominal power over 90% (Figure S1).

**Table 1** Characteristics of patients at baseline

|                              | <b>Infliximab<br/>32 patients</b> | <b>Adalimumab<br/>39 patients</b> | <b>Etanercept<br/>58 patients</b> |
|------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Age (years)                  | 52.3±12.2                         | 53.6±11.8                         | 54.5±12.9                         |
| Disease duration             | 5.3±2.8                           | 5.5±3.1                           | 5.8±2.9                           |
| Rheumatoid factor positivity | 76%                               | 78%                               | 75%                               |
| APCA positivity              | 81%                               | 79%                               | 78%                               |
| ESR, mm                      | 54.3±8.4                          | 55.7±7.2                          | 53.5±6.7                          |
| CRP, mg/dL                   | 3.2±1.7                           | 3.4±1.5                           | 3.7±1.4                           |
| DAS28                        | 5.46±2.1                          | 5.43±1.9                          | 5.38±1.8                          |
| Corticosteroids dose, mg/day | 6.1±1.2                           | 6.4±1.1                           | 6.3±1.2                           |
| Methotrexate dose, mg/week   | 12.5±2.2                          | 12.1±2.1                          | 11.9±2.4                          |

**Abbreviations:** APCA, anti-citrullinated peptide/protein antibody; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DAS, Disease Activity Score.

## Statistics revised

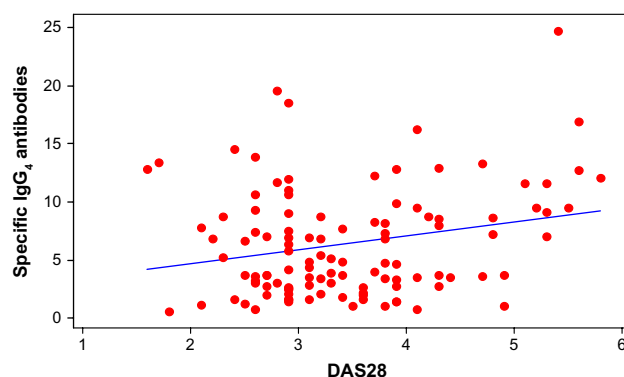
Continuous variables were expressed as means  $\pm$  standard deviation and categorical variables were described by percentages. The  $\chi^2$  test was used to compare categorical variables among groups, and the Pearson correlation coefficient was used to investigate for relations between quantitative variables. An  $\alpha$ -value of 0.05 was considered statistically significant, and all statistical tests were two-tailed. IgG<sub>4</sub> levels were considered positive if superior to the following cutoff values: ETN 5.9, ADA 19.25, and IFX 8.6. ADAb levels were considered positive if superior to the following cutoff values: ETN 0.446, ADA 0.187, and IFX 0.275. Analyses were performed using Minitab16 software.

## Results

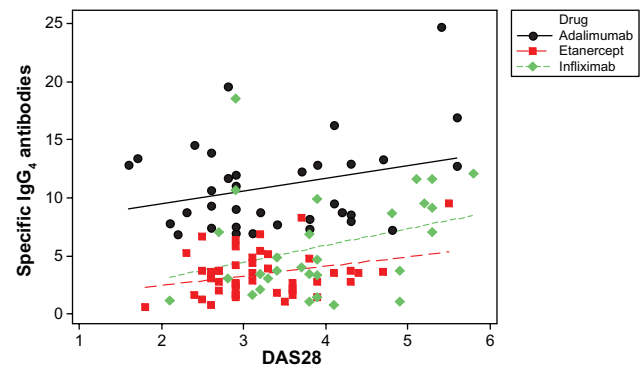
After 24 weeks of treatment, 38% of the ETN-treated patients and 28% of those treated with ADA had injection-site reactions; the rate of systemic reactions in the IFX group was 25%. The differences were not statistically significant ( $P=0.382$ ; ETN vs ADA  $P=0.319$ ).

The percentages of patients with adverse events stratified by drug response were: LDA 8% and NR 18% in the ADA group; remission 3%, LDA 22%, and NR 10% in the ETN group; and LDA 6% and NR 16% in the IFX group ( $P=0.051$ ). The percentages of patients with ADABs were: ADA 33.3%, ETN 11.5%, and IFX 10.3% ( $P=0.025$ ; ADA vs ETN  $P=0.015$ ). The percentages of patients with IgG<sub>4</sub> antibodies were: ADA 6%, ETN 13%, and IFX 26% ( $P=0.017$ ; ADA vs ETN  $P=0.437$ ). Associations between ADABs, specific IgG<sub>4</sub> antibodies and adverse reactions were not significant for any of the three drugs.

A scatter plot showed a positive correlation between IgG<sub>4</sub> levels and worse DAS28, (0.241,  $r^2=5.8\%$ ;  $P=0.011$ ; Figure 1). IgG<sub>4</sub> quantities were higher in the ADA group than in the other two groups (Figure 2), but the correlation



**Figure 1** Scatter plot of specific IgG<sub>4</sub> antibodies versus DAS28.  
**Abbreviation:** DAS, Disease Activity Score.



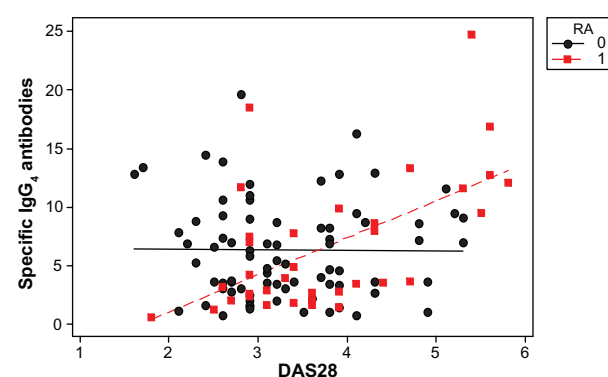
**Figure 2** IgG<sub>4</sub> specific antibodies against three different TNF blocking agents and DAS28.

**Abbreviation:** DAS, Disease Activity Score.

with DAS28 was only significant for the ETN group, (ADA 0.287,  $P=0.094$ ; ETN 0.299,  $P=0.039$ ; IFX 0.314,  $P=0.097$ ). The correlation between ETN and DAS28 was consistent with the higher percentage of ETN patients positive for IgG<sub>4</sub> (shown earlier) with respect to the ADA patients. There was a positive correlation between IgG<sub>4</sub> levels (regardless of the drug group) in patients experiencing adverse events (Figure 3, red line and dots; subjects having experienced adverse events correlating with DAS28 value  $=0.578$ ,  $r^2=33.5\%$ ;  $P=0.000$ ).

## Discussion

Our results suggest that IgG<sub>4</sub> may play a role in the adverse reactions and therapeutic response to biological agents of patients with RA. The role of anti-ADA IgG<sub>4</sub> was first detected by means of an immunoassay in 271 consecutive RA patients during 3 years of treatment: anti-ADA antibodies were detectable in 32%, and specific IgG<sub>4</sub> antibodies in 29%. Although IgG<sub>4</sub> is often considered to be harmless due to its lack of an effector function, the neutralization of ADA by specific IgG<sub>4</sub> antibodies leads to a reduced clinical response.<sup>6</sup>



**Figure 3** Correlation between IgG<sub>4</sub> specific antibodies, adverse effects and DAS28.  
**Abbreviations:** DAS, Disease Activity Score; RA, rheumatoid arthritis.

In our 24-week study, we detected ADABs in 33.3% of our ADA patients and IgG<sub>4</sub> in only 6%.

Approximately 4% of the total IgG in the serum of Caucasian adults is IgG<sub>4</sub>,<sup>7</sup> which is considered an odd antibody insofar that it is the only IgG unable to activate complement and has a low affinity for Fcγ receptors.<sup>8,9</sup> Furthermore, it is able to exchange half-molecules in vivo, thus leading to bispecific antibodies that do not cross-link and the consequent formation of small immune complexes.<sup>10,11</sup> Because of its limited ability to trigger immunological effector functions and its tendency to form small immune complexes, it is thought that IgG<sub>4</sub> has less effect on the clearance of antigens and plays a limited role in inflammation.

Although the percentage of serum IgG<sub>4</sub> is generally low, antigen-specific IgG<sub>4</sub> has been described as the main isotype produced in some immune responses. As early as the 1970s, it was shown that chronic antigen exposure to grass pollen and bee venom led to the predominant formation of IgG<sub>4</sub>,<sup>12</sup> and increased allergen-specific IgG<sub>4</sub> is associated with a beneficial response to specific allergen immunotherapy in allergic patients.<sup>13,14</sup> Chronic treatment with biological agents, such as factor VIII, IFNβ, or therapeutic monoclonal antibodies can be considered long-term exposure to protein antigens. There have been frequent reports of the formation of IgG antibodies against biological agents, some of which have been found to produce IgG<sub>4</sub>. Prolonged exposure to IFNβ can also lead to the development of IgG<sub>4</sub> antibodies in patients with multiple sclerosis,<sup>15</sup> but although it has been shown that therapeutic monoclonal antibodies can give rise to the formation of IgG<sub>4</sub>, there is lack of long-term measurements.<sup>16,17</sup> The observation that the proportion of IgG<sub>4</sub> antibodies may diminish over time is somewhat unusual, and differs from what has been described in the various allergens.<sup>18</sup>

Our results showed more injection-site reactions in the ETN group than in the ADA group (38% vs 28%), and the presence of anti-ETN IgG<sub>4</sub> in 13% of the patients. Although it is not yet known what factors induce class switching to IgG<sub>4</sub>, it can be speculated that the immunological context of the patients and/or the intensity of treatment (dosing and frequency) may play a role. One of the reasons for the difference in IgG<sub>4</sub> responses between ADA-treated and allergic patients may be that the former received 40 mg every 2 weeks, whereas the patients receiving allergen-specific immunotherapy received 20 μg of allergen twice a week. The frequency of treatment could also explain the increase in anti-ETN IgG<sub>4</sub>.

Our findings indicate a correlation between adverse drug reactions and a poor response, and suggest that anti-ETN

IgG<sub>4</sub> may have a decreased response to ETN therapy. Other studies have shown that the formation of anti-ADA antibodies is related to lower functional drug levels and a diminished clinical response.<sup>19,20</sup> A large proportion of these antibodies are IgG<sub>4</sub> antibodies, which suggests IgG<sub>4</sub> is not simply an innocent bystander in this case.<sup>21</sup> Although IgG<sub>4</sub> antibodies cannot activate complement and have a low affinity for Fc receptors, they may still be able to compete with drug-induced TNFα binding to drugs, thus leading to a clinical nonresponse.

We detected anti-ADA antibodies more frequently than anti-ETN antibodies (33% vs 11.5%), whereas the rate of detection of IgG<sub>4</sub> anti-ETN was 13%, which may predict a lower response to ETN therapy in patients with injection-site reactions. One limitation of this study is that it did not measure the serum levels of the three drugs. Our study confirms that patients with injection-site reactions and anti-ETN IgG<sub>4</sub> may not respond to ETN therapy.

## Acknowledgment

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## Author contributions

MB and FA designed the study and drafted the paper. MM, MI, and FM performed the laboratory analysis. FLG and MB recruited the patients. PSP commented on and critically edited the paper. All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

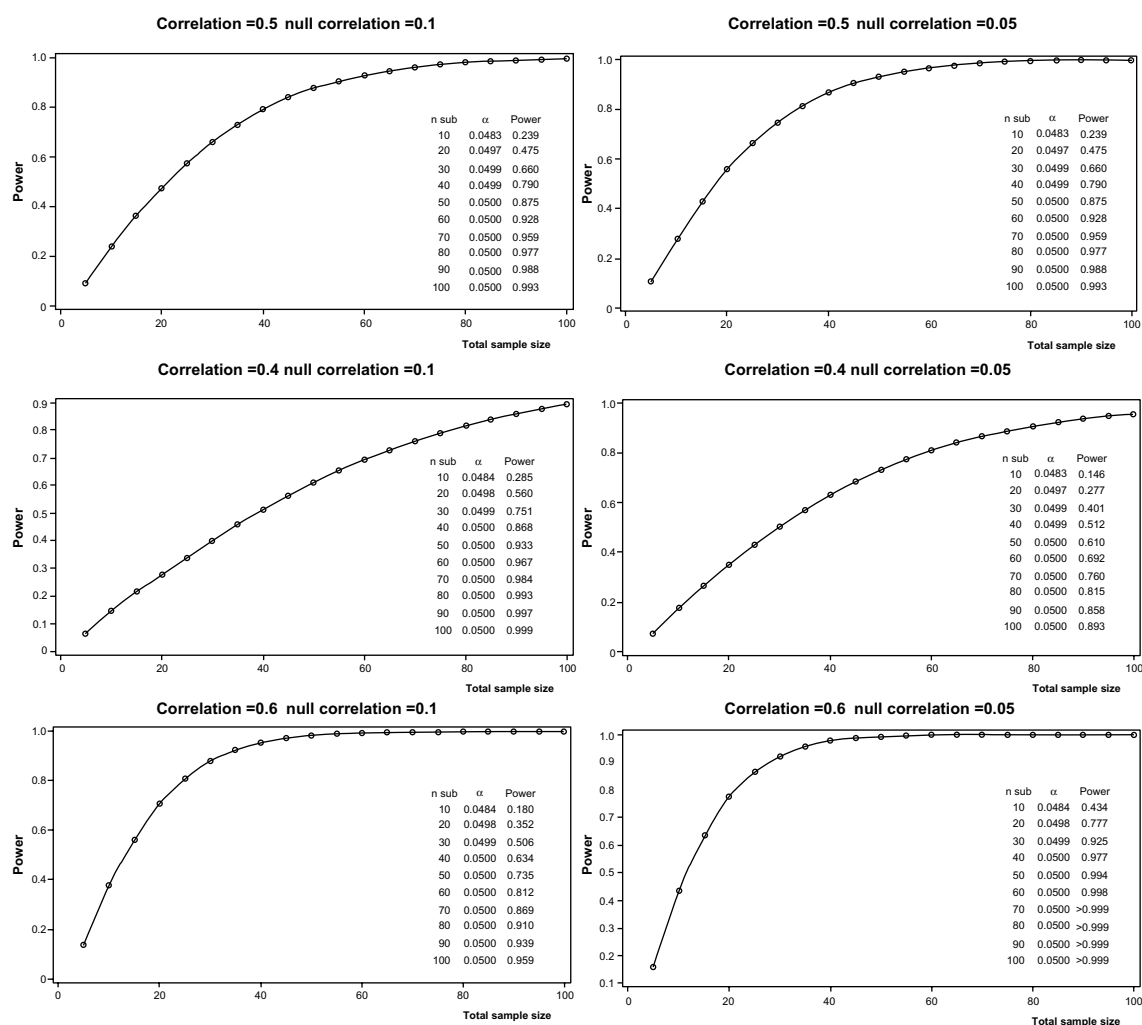
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## Supplementary material



**Figure S1** Power calculations.

**Notes:** Power calculation reporting power (1-beta) vs sample size (number of pairs) under the assumption of a correlation coefficient ranging from 0.4 to 0.6 (moderate weak to moderate strong) with a null correlation of 0.1 and 0.05 for the left and the right panels respectively.

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