

Antidrug antibody detection for adalimumab depends on the type of assay used: an experimental approach to help clinicians interpret diagnostic data

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Abstract

Objective

To compare different methods of antidrug antibody (ADA) against adalimumab detection in ankylosing spondylitis (AS) patients and the impact of ADA on adalimumab drug levels and mean ASDAS-CRP.

Methods

We used the acid-dissociation-radioimmunoassay (ARIA), antidrug-binding-test (ABT) and a bridging Enzyme-linked Immunosorbent Assay (ELISA) to detect ADA at 4, 12 and 24 weeks of treatment. Patients were divided into groups; all assays negative (All-neg), only ARIA positive (ARIA-only-pos), ARIA and ABT positive, bridging ELISA negative (ARIA/ABT-double-pos) and all assays positive (All-pos).

Results

Eighty-three consecutive AS patient were included. At week 4, 18% compared to 11% and 0% of the patients tested positive for ADA in the ARIA, ABT and bridging ELISA, respectively. At week 12 and 24, cumulative 52% and 69% patients tested positive in the ARIA, compared to 27% and 30% patients in the ABT and 2% patients in the bridging ELISA. Adalimumab levels between All-neg and ARIA-only-pos were 9.1 (5.5-12.5) and 8.5 (5.7-12.3). Drug levels differed between ARIA/ABT-double-pos (2.7 (1.3-4.4)) and All-neg (9.1 (5.5-12.5)). All-pos patients had undetectable drug levels. Mean ASDAS-CRP at week 24 differs between All-neg (1.9 (\pm 1.2)), and All-pos (3.8 (\pm 1.9)) and ARIA/ABT-double-pos (2.0 (\pm 1.1)) and All-pos.

Conclusion

The majority of AS patients had detectable ADA against adalimumab in the ARIA. The ARIA detects more ADA compared to the less drug tolerant ABT and bridging ELISA. The clinical relevance depends on the impact on the bio-availability of the drug. A drug level measurement therefore helps to interpret ADA data regardless of type of assay used.

Key words

pharmacokinetics, immunogenicity, adalimumab, ankylosing spondylitis

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Introduction

Tumour necrosis factor inhibitors (TNFi) have changed the treatment for many inflammatory diseases, including ankylosing spondylitis (AS). In some patients TNFi are ineffective or lose effectiveness over the course of treatment (1). This phenomenon can partly be explained by immunogenicity. Immunogenicity is the formation of antibodies against a drug (anti-drug antibodies (ADA)) and reflects a natural reaction of the immune system to a foreign protein. Therapeutic monoclonal antibodies contain elements that are foreign to the patient, especially chimeric monoclonal antibodies, such as infliximab, induces an immune response, since the entire variable region is of mouse origin. Humanisation of the variable regions reduces the immunogenicity of the molecule. Adalimumab is an example of a fully human monoclonal antibody. But, even fully human monoclonal antibodies are potentially immunogenic with the ADA response usually directed against the antigen binding region of the molecule (2, 3).

For AS different percentages of ADA against adalimumab have been reported and range from 10% to 30% (4-7). However, direct comparison of immunogenicity data is hampered due to the different assays used with different susceptibilities for drug interference (8-10). Drug interference is a limitation of the assay to detect ADA in presence of the drug, due to the formation of ADA-drug complexes. The formation of ADA-drug complexes prevents that the Fab arms of the ADA bind the assay (11). This results in an under-estimation of the true ADA present in the serum (12). To overcome this limitation, drug tolerant assays have been developed, which are able to detect both free ADA and ADA in complex, by dissociating the ADA from the drug. These assays are for example the Meso Scale Discovery electrochemoluminescence (ECL) assay, an assay which gained popularity for pre-marketing ADA testing, the pH-shift-anti-idiotypic antigen binding test (PIA) and the acid-dissociation-radioimmunoassay (ARIA). Studies in for instance rheumatoid arthritis (RA) show different percentages of ADA de-

tection between the different assays (8, 11, 12).

Since the immunogenicity of monoclonal antibodies is an important general safety and efficacy concern, understanding and interpreting different studies is essential.

The aim of this study was to compare the detection of ADA with a drug tolerant assay to less drug tolerant assays and combine this with drug concentration tests. This will help clinicians to interpret immunogenicity data in the context of the type of assay used. Furthermore, we studied the association between adalimumab drug levels, the proportion of detected ADA in the drug tolerant assay and clinical outcome. This is the first study to directly compare the detection of ADA against adalimumab in three different assay techniques, ARIA, Antibody binding test (ABT) and the bridging enzyme-linked immunosorbent assay (ELISA) in AS patients.

Patients and methods

Patients

We included AS patients (according to the 1984 modified New York Criteria) from our observational cohort study who received adalimumab 40 mg subcutaneously every other week at the Jan van Breemen Research Institute | Reade, Amsterdam. These patients were also described in previous papers (5, 13). All patients fulfilled the Assessment of Spondyloarthritis international Society (ASAS) criteria for TNFi, namely, failure to respond to ≥ 2 non-steroidal anti-inflammatory drugs (NSAIDs) in the maximal tolerable dosage or had contraindications for the use of NSAIDs before start of TNF inhibitors and a Bath ankylosing spondylitis disease activity index (BASDAI) > 4 (14). Some patients used concomitant disease-modifying anti-rheumatic drugs (DMARDs) and/or NSAIDs.

Previous studies show that the ARIA detects more ADA compared to the ABT and the bridging ELISA (8, 11). When the ABT is positive for ADA detection, the ARIA is also positive. Same holds for ADA detection in the bridging ELISA compared to the ABT. We divided the patients in four different groups

for clinical comparisons; all assays negative over all time points (All-neg), only ARIA positive over all time points (ARIA-only-pos), ARIA and ABT positive over all time points, bridging ELISA negative (ARIA/ABT-double-pos), and all assays positive over all time points (All-pos). The study was approved by the Medical Ethics Committee of Slotervaart hospital and Reade, Amsterdam, the Netherlands, according to the Declaration of Helsinki. All patients gave written informed consent.

Clinical response

Disease activity using the Ankylosing Spondylitis Disease Activity Score (AS-DAS) using C-reactive protein (CRP) was assessed at baseline, 4, 12 and 24 weeks of follow-up. The ASDAS-CRP includes three items of the BASDAI, namely back pain (10 cm VAS), duration of morning stiffness (10 cm VAS) and pain/swelling of peripheral joints (10 cm VAS), as well as patient global assessment of disease activity (10 cm VAS) and CRP level (mg/l). (15)

Measurement of adalimumab concentrations

Trough serum samples were taken at week 4, 12 and 24. Baseline samples were excluded from analyses, as previous studies demonstrated that no adalimumab and ADA are detected prior to starting adalimumab (5, 16). Adalimumab drug levels were measured by an ELISA, based on the principle that adalimumab is captured via its ability to bind TNF. Adalimumab binding was assessed by incubation with biotinylated F(ab')₂ fragments of rabbit IgG directed to the adalimumab idiotype. Details on this assay can be found in previous publications (12). The lower limit of quantitation of this assay is 0.01 mg/L.

Measurement of anti-adalimumab antibodies

ADA titres were measured using three different assay formats. Samples were included if they were measured in all three assays. All the assays below have been described in more detail in previous studies (8, 11, 12, 17).

ARIA: In summary, ADA in complex with adalimumab are dissociated by

acid. Upon neutralisation, biotinylated adalimumab F(ab')₂ is added in amounts that can compete with adalimumab present in the serum sample. Antibodies are subsequently captured by protein A sepharose and specific anti-adalimumab antibodies are detected by adding radio-labelled streptavidin. The cut-off is 30 AU/ml.

ABT: The ABT uses protein A sepharose to capture ADA from serum, followed by detection of ADA by incubation with 125I labelled F(ab')₂ of Adalimumab. The limit of detection is 12 AU/ml and lower limit of quantification is 20 AU/mL.

Bridging ELISA: The assay was performed with the ADA adalimumab assay of Sanquin Reagents (M2950). Microtiter plates were incubated with 0.5 µg/ml adalimumab in PBS. After washing, the plates were incubated with patient serum which was serially diluted in High Performance Elisa Buffer (HPE, Sanquin Reagents). After washing, ADA are detected with biotinylated adalimumab. Outcome of the assay was either positive for ADA or negative for ADA against adalimumab. Only the samples with low or undetectable adalimumab levels (<0.5 mg/L) were tested. The other samples were assumed to be negative (11).

Statistical analysis

For statistical analyses SPSS v. 23.0 (SPSS, Chicago, IL) was used. Graphpad prism v. 6 was used for generating the figures. For differences in baseline demographic and clinical variables between sub-populations of patients, independent samples *t*-test, Kruskal Wallis test or one-way ANOVA test were used as deemed appropriate. Univariate linear regression was used to analyse the effect of ADA on clinical outcome and drug levels at week 24. For patients with missing drug level data, ADA and/or ASDAS week 24, last observation carried forward was used and a sensitivity analyses for missing data was performed. Clinical outcome data is corrected for the following confounders: methotrexate use at baseline, sulfasalazine use at baseline, gender and age at baseline. The threshold for statistical significance was set at $p < 0.05$.

Results

Patients

In this study we enrolled 86 consecutive patients. Three patients were excluded due to missing samples at all time points in one of the assays. No differences were found in baseline characteristics between the different groups (Table I). Seventy-three patients completed the 24 weeks of follow-up. During the course of treatment, 4 patients dropped out because of treatment failure, 3 patients due to side effects, one patient moved away, one patient developed multiple myeloma and one patient did miss a follow-up meeting for unknown reasons.

As shown in Figure 1, 15 patients (18%) tested positive for ADA in the ARIA compared to 9 (11%) in the ABT and 0 (0%) in the bridging ELISA at week 4. At weeks 12 and 24 respectively, the cumulative number of ADA-positive patients was 43 (52%) and 57 (69%) patients in the ARIA, compared to 22 (27%) and 25 (30%) patients in the ABT and 2 (2%) and 2 (2%) patients with the bridging ELISA.

Adalimumab drug levels

The median adalimumab levels at week 24 in the groups All-neg, ARIA-only-pos, ARIA/ABT-double-pos and All-pos are respectively: 9.1 mg/L (IQR 5.5–12.5), 8.5 mg/L (IQR 5.7–12.3), 2.7 mg/L (IQR 1.3–4.4) and 0.0 mg/L (0.0) (Fig. 2). No significant differences were found in median adalimumab levels between patients with no ADA detection (All neg) and patients tested positive in only the ARIA (ARIA-only-pos) ($p = 0.807$). However, when both ARIA and ABT tested positive (ARIA/ABT-double-pos) or positive in all three assays (All-pos), drug levels significantly differed from patients with no ADA detection (All-neg) ($p < 0.001$) and with only ADA detection in the ARIA (ARIA-only-pos) ($p < 0.001$). When patients tested positive in the ARIA, ABT and the bridging ELISA (All-pos) all patients showed undetectable drug levels.

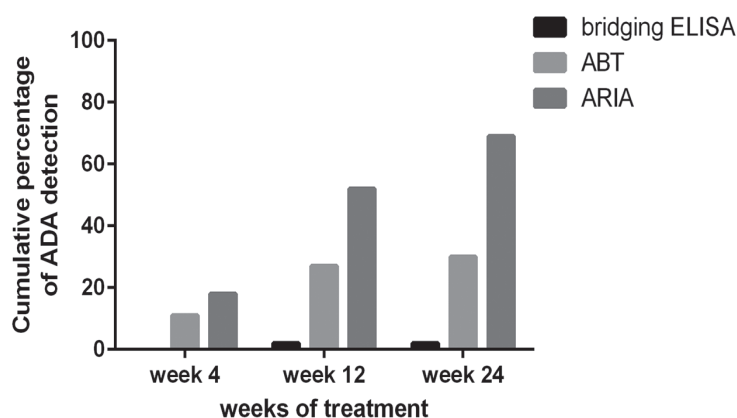
Clinical outcome

Mean (\pm SD) ASDAS-CRP at week 24 are 1.9 (\pm 1.2), 2.2 (\pm 0.9), 2.0 (\pm 1.1) and 3.8 (\pm 1.9) for All-neg, ARIA-only-pos, ARIA/ABT-double-pos and

Table I. Baseline characteristics.

	Total n=83	All-neg n=26	ARIA-only-pos n=29	ARIA/ABT-double-pos n=26	All-pos n=2
Demographics					
Age, mean±SD years	43.5 ± 10.4	42.6 ± 9.6	42.7 ± 10.1	44.6 ± 11.6	52.01 ± 11.6
Male, n (%)	50 (60.2)	16 (61.5)	17 (58.6)	16 (61.5)	1 (50.0)
Disease duration, median (IQR) years	7.0 (2.0-14.0)	7.5 (1.5-12.5)	7.0 (1.0-15.0)	4.5 (2.0-12.5)	16.0*
HLA-B27 positive, n (%)	64 (77.1)	20 (76.9)	23 (79.3)	20 (76.9)	1 (50.0)
Disease status					
CRP, Median (IQR)mg/L	5.0 (2.8-12.3)	5.5 (2.8-12.3)	5.0 (3.0-17.0)	3.0 (2.0-6.5)	31.0*
BSE, Median (IQR)mm/h	14.5 (7.0-31.0)	11.5 (4.8-26.0)	19.0 (10.0-34.0)	10.0 (6.0-34.0)	35.5*
ASDAS-CRP, mean±SD	3.2 ± 1.0	3.0 ± 1.2	3.4 ± 0.9	3.0 ± 0.9	3.8 ± 2.1
DMARD therapy					
Prior biologicals, n (%)	22 (26.5)	9 (34.6)	4 (13.8)	9 (34.6)	0 (100)
Methotrexate, n (%)	7 (8.4)	5 (19.2)	2 (6.9)	0 (100)	0 (100)
Sulfasalazine, n (%)	9 (10.8)	5 (19.2)	3 (10.3)	1 (3.85)	0 (100)
NSAIDs, n (%)	56 (67.5)	14 (53.8)	21 (72.4)	20 (76.9)	1 (50)

HLA-B27: human leukocyte antigen B27. CRP: C-reactive protein ESR: erythrocyte sedimentation rate; ASDAS-CRP: Ankylosing Spondylitis Disease Activity Score using CRP. NSAID: non-steroidal anti-inflammatory drug. ARIA: acid-dissociation-radioimmunoassay. ABT: antibody binding test. ELISA: enzyme linked immune sorbent assay; All-neg: all assays negative over all time points; ARIA-only-pos: only ARIA positive over all time points; ARIA/ABT-double-pos: ARIA and ABT positive over all time points, bridging ELISA negative; All-pos: and all assays positive over all time points). * not included in statistical tests due to low numbers of patients.



ARIA: acid-dissociation-radioimmunoassay, ABT: antibody binding test, ELISA: enzyme linked immune sorbent assay

Fig. 1. Cumulative percentages of ADA detection per assay.

All-pos respectively. Differences are found in detection of ADA at week 24 and mean ASDAS-CRP between All-neg and All-pos ($p=0.024$) and ARIA/ABT-double-pos and All-pos $p=0.025$, corrected for confounding; $p=0.025$ and $p=0.026$ respectively (Fig. 3).

Discussion

The aim of this study was to find out whether there are differences in ADA detection between no (bridging ELISA) or less drug tolerant assay (ABT) and a drug tolerant assay (ARIA). We show indeed that the assays differ in their ADA detection: at 24 weeks of treatment, a large portion of our AS patients (69%) tested positive in the ARIA for ADA detection, compared to 30% and

2% of ADA detection in respectively the ABT and the bridging ELISA.

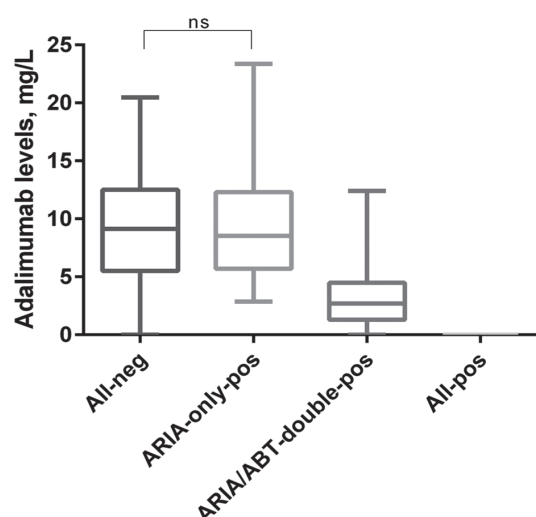
The clinical impact of immunogenicity depends on the altered pharmacokinetics of the drug. The normal half-life of IgG1 antibody is around 3 weeks. Immune complexes, depending on their size, have a faster clearance (18). However, the main effect of the formation of ADA is due to the neutralisation of the drug. This gives an inverse relationship between free drug and antidrug antibodies. In other words, the detection of ADA depends on the relative amount of ADA produced and the amount of free drug. The clinical relevance of measuring only ADA is limited since the amount of free active drug determines the clinical outcome. Thus, to analyse

the impact of ADA on clinical outcome, it is essential to measure concentrations of active drug, which is drug that is not blocked by ADA and can still fulfill its function.

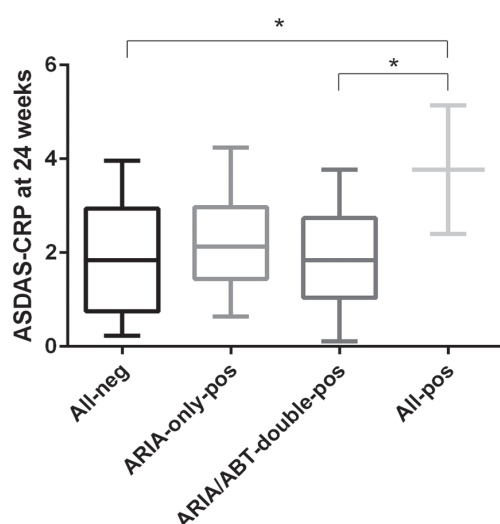
The latter is confirmed by our results as the ASDAS-CRP was comparable between almost all the groups. However, when the bridging ELISA detects ADA, which means there is no free drug available, ASDAS-CRP was significantly higher compared to the patients with no ADA detection.

Nevertheless, it has to be taken into account that there is as yet no established concentration-effect relationship for AS, which is inconsistent with findings in RA and psoriatic arthritis (PsA) (13, 19, 20). If we look at our findings, a clinical effect is achieved with low drug levels and it could be hypothesised that a lower dose is sufficient to reach a clinical effect in AS patients compared to RA and PsA.

The high number of AS patients developing ADA shows a natural response of the immune system to a foreign protein, which is not a surprise as this assay, the ARIA, can detect all ADA and is not hampered by ADA being bound to the drug. This is consistent with results shown in previous reports using the drug tolerant ARIA and PIA assay in adalimumab treated RA patients (8, 16). In the most recent study, 66% of RA patients showed detectable ADA



ns: non-significant; all other values were significantly different from each other: p-value < 0,05
 All-neg: all assays negative; ARIA-only-pos: only ARIA positive; ARIA/ABT-double-pos: ARIA and ABT positive, bridging ELISA negative and All-pos: all assays positive.
 ARIA: acid-dissociation-radioimmunoassay, ABT: antibody binding test, ELISA: enzyme linked immune sorbent assay



* p-value < 0,05
 All-neg: all assays negative; ARIA-only-pos: only ARIA positive; ARIA/ABT-double-pos: ARIA and ABT positive, bridging ELISA negative and All-pos: all assays positive.
 ARIA: acid-dissociation-radioimmunoassay, ABT: antibody binding test, ELISA: enzyme linked immune sorbent assay
 ASDAS-CRP: Ankylosing Spondylitis Disease Activity Score using C-reactive protein

Fig. 2. Differences in adalimumab drug levels and detection of antidrug antibodies in different assays at 24 weeks of treatment.

Fig. 3. Differences in ASDAS-CRP and detection of antidrug antibodies in different assays at 24 weeks of treatment.

in the ARIA at 52 weeks of treatment, similar to the 69% of AS patients in our study. Of the RA patients, methotrexate use was significantly less frequent in patients testing positive for ADA (16). Although, the use of methotrexate in AS is limited because of its lack of efficacy, both patients who developed ADA detected in all three assays did not use methotrexate.

The observed differences in ADA detection using different assays is explained by the various susceptibilities for drug interference of these assays. (10) Drug tolerant assays, such as the ARIA, are designed to measure not

only the free ADA but also – to a certain extent – the ADA in complex, using methods to separate the ADA from the drug. In this way free ADA can be detected in the presence of the therapeutic monoclonal antibody (8, 16). Overall, as expected, studies show that the ARIA indeed has a higher drug tolerance compared to the ABT and bridging ELISA, and therefore detects the most ADA.(8) The bridging ELISA is hampered mostly by drug interference and detects only ADA when drug levels are very low or undetectable (21). The clinical relevance of relying only on the presence of ADA detected with

a drug tolerant assay, such as the ARIA, is therefore limited, since there is, in the majority of patients, still free drug present in serum. This in contrast to the ADA detected by a drug sensitive assay (ABT and bridging ELISA), when detection of ADA means that there is no or little free drug present in patients' serum. In this study we show that indeed patients with no ADA detection had significant higher drug levels compared to patients with ADA detection in all three assays who had undetectable drug levels. The drug levels of patients who were only positive for ADA detection in the ARIA had adequate drug levels and were not significantly different from the patients with drug levels with no ADA detection. The importance of interpreting immunogenicity data within the confines of the assay methodology used is emphasised by the development phase of biosimilars. The higher percentages of ADA found in biosimilar studies compared to the original studies is due to use of a drug tolerant assay compared to drug sensitive assays, used in the original studies (22-24).

There are some limitations to this retrospective study. Since adalimumab is an at home administered drug, all patients received instructions to have blood samples taken before their next adalimumab injections. Thus, the time between blood sampling and next adalimumab injection might show some variability between patients, adding to the variation in drug levels. Second, it should be mentioned that the number of patients was low in the All-pos group, but still none of these patients had detectable drug.

In conclusion, the majority of the AS patients showed detectable ADA against adalimumab in the drug tolerant ARIA. Immunogenicity data differ depending on the assay used; the more drug tolerant, the more ADA is detected. The clinical relevance depends on the impact on the bio-availability of free drugs. A drug level measurement therefore helps a clinician interpret ADA data regardless of the source of the ADA measurement. We would recommend to always measure drug levels. When these are low/undetectable, an ADA test could provide additional information.

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