Spondyloarthritis: From disease phenotypes to novel treatments
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Adalimumab serum levels and antidrug antibodies towards adalimumab in peripheral spondyloarthritis: No association with clinical response to treatment or with disease relapse upon treatment discontinuation

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ABSTRACT

Introduction
To evaluate the clinical relevance of serum drug levels and anti-drug antibodies (ADAbs) in response to treatment as well as relapse upon treatment discontinuation in peripheral spondyloarthritis (pSpA) patients treated with adalimumab.

Methods
The study included 26 peripheral SpA patients treated with adalimumab for either 12 (n=12) or 24 (n=14) weeks in a randomized controlled trial. Patients achieving ASDAS inactive disease at the end of the treatment period were classified as responders. Clinical characteristics, serum trough adalimumab levels and ADAbs were assessed at the end of the treatment period and at follow-up (upon relapse or, in absence of relapse, at 16 weeks after discontinuation).

Results
Serum adalimumab levels measured 2 weeks after the last adalimumab administration ranged from <0.002 to 23.0 ug/mL, with a median of 11.5 ug/mL. These levels were neither associated with response to treatment or disease activity measurements at end of treatment, nor with the occurrence of relapse and time to relapse after discontinuation of treatment. Anti-adalimumab ADAbs were present in 23% of the patients at end of treatment and in 35% at follow-up after treatment discontinuation, indicating that ADAbs have been masked by the presence of the drug in some patients. However, ADAbs at end of treatment as well as at follow-up were not different between responders and non-responders and were not associated with relapse upon discontinuation of treatment.

Conclusions
There is no clear association between adalimumab serum levels or anti-adalimumab ADAbs and clinical response to treatment or with relapse upon treatment discontinuation in pSpA.
INTRODUCTION

Tumor necrosis factor (TNF) inhibition is a highly effective treatment for axial and peripheral spondyloarthritis (SpA). However, a significant proportion of patients fails to respond or does not tolerate the treatment because of side effects. Reasons for non-response or for intolerance are multiple, including potentially the development of anti-drug antibodies (ADAbs) directed towards the TNF blocker. It has been proposed that ADAbs may reduce therapeutic responses either by increasing the clearance of the TNF inhibitor or by direct neutralisation of the functional part of the drug. Accordingly, recent reviews suggest that monitoring of serum drug levels and ADAbs would be a promising tool for personalised cost-effective usage of biological therapies in immune-mediated inflammatory diseases (IMIDs).

Most studies on immunogenicity of TNF blockers have been performed in rheumatoid arthritis and Crohn’s disease. In SpA, the available studies on immunogenicity yielded conflicting results. For infliximab and adalimumab some groups reported that ADAbs towards these TNF inhibitors are associated with decreased clinical response and increased risk of hypersensitivity reactions, while others do not find this association and even conclude that serum anti-TNF drug levels are not associated with response to treatment. For etanercept these ADAbs have not been detected and it has been shown that the serum drug levels are similar in responders and non-responders. Recently, also with golimumab ADAbs do not appear to have a major role in treatment success or failure. Moreover, a recent meta-analysis on anti-TNF ADAbs in various IMIDs concluded that there was no relevant association of ADAbs with efficacy in SpA. This corresponds with the clinical experience that the treatment failure to TNF blockade is similar among the various TNF blockers, both the ones which do and do not cause ADAbs according to the before mentioned studies.

These conflicting results may be related to the diversity in methods and timing to evaluate the ADAbs as well as to the fact that the presence of detectable serum drug levels may mask the detection of ADAbs. The latter issue can be avoided by the use of novel assay methods and/or by assessing the ADAbs several weeks after stopping the TNF inhibitor. In this study, we assessed the potential clinical relevance of serum drug levels and ADAbs measured at the end of the treatment period as well as at a drug-free follow-up after a double-blind, placebo-controlled, randomized clinical trial (RCT) with adalimumab in peripheral SpA (pSpA). We correlated these serum levels both with clinical response at the end of treatment and with relapse upon discontinuation of the TNF inhibitor.

METHODS

Study patients

Twenty six patients from our RCT with adalimumab in peripheral arthritis in SpA patients not fulfilling the criteria for ankylosing spondylitis (AS) or psoriatic arthritis (PsA) were included in this study. The patients fulfilled the European Spondyloarthritis Study Group (ESSG) and/or the Amor criteria and had active disease at inclusion into the study. They were treated with either placebo (n=12) or adalimumab (n=14) for 12 weeks, followed by a 12 week open-label phase with adalimumab for all patients. After this period adalimumab was discontinued. Patients were
allowed to continue non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids (≤10 mg/day prednisone or equivalent), methotrexate and sulfasalazine on a stable dosage throughout the study. After discontinuation of the TNF inhibitor, patients were prospectively followed for 16 weeks and seen for a relapse visit upon worsening of symptoms or, in the absence of relapse, at the 16 weeks follow-up visit. The following disease activity parameters were measured: patient’s and physician’s global assessment of disease activity, 68/66 tender joint count (TJC) and swollen joint count (SJC), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Ankylosing Spondylitis Disease Activity Score (ASDAS), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Responders were defined as patients achieving ASDAS inactive disease at the end of treatment.28 Relapse was defined as increase of ≥1 swollen joint, or ≥2 points in patient’s or physician’s global assessment of disease activity or BASDAI.29 Fourteen patients (53.8%) reached ASDAS inactive disease at the end of the treatment period. Nineteen (73.1%) patients had a disease relapse within 16 weeks after discontinuation of adalimumab with a mean time to relapse of 10.0±3.2 weeks. The characteristics of the patients have been published previously.29 Written informed consent was obtained from each patient before study-related procedures were performed and the study was approved by the Medical Ethics Committee of the Academic Medical Center/University of Amsterdam.

Assessment of serum adalimumab levels

Serum samples were obtained two weeks after the last adalimumab administration (n=26) and at follow-up (upon relapse or 16 weeks after discontinuation in the absence of relapse, n=25). Trough serum adalimumab concentrations were measured by enzyme-linked immunosorbent assay (ELISA) developed by Wolbink and co-workers as previously described and as accredited by the RvA/CCKL (Dutch Accreditation Council/Dutch Accreditation Board for Medical Laboratories) according to the International Standardization Organization (ISO) guideline ISO17025.30 The detection limit of the assay is approximately 0.001 ug/mL; serum adalimumab levels <5.0 ug/mL were designated low, as previously described.15,30

Assessment of antibodies against adalimumab

The same serum samples were analyzed by a radio immunoassay (RIA) (Sanquin) to detect the presence of anti-adalimumab antibodies as previously described.30 After dilution of 1 μL of serum in phosphate-buffered saline/0.3% bovine serum albumin (pro analysi buffer), overnight incubation followed with 1 mg Sepharose-immobilized protein A (GE Health Care, Giles, England) in a final volume of 800 μl. Then, the samples were washed with phosphate-buffered saline 0.005% polysorbate. The anti-adalimumab binding was determined by overnight incubation with 20,000 disintegrations per minute (dpm [≈1 ng]) iodine 125-labeled F(ab)2 adalimumab diluted in Freeze buffer (Sanquin). Unbound label was removed by washing, and protein A-bound radioactivity was measured. Anti-adalimumab levels were expressed in arbitrary units (AU [1 AU=12 ng]) using a serum containing anti-adalimumab as standard. The mean cut-off value as derived from 100 healthy donors was set at 12 AU/mL.
Statistical analysis
Data are presented as median and interquartile range (IQR). Mann-Whitney U tests were used to compare differences in serum levels in case of unpaired samples and Wilcoxon signed rank tests in case of paired samples. Chi square tests were used for categorical variables. Logistic regression analyses were conducted to examine associations between the ASDAS response, relapse status and trough serum adalimumab and anti-adalimumab ADAbs levels. Correlations between the serum measurements and the clinical disease activity measurements were assessed using Spearman’s correlation tests. All statistical tests were 2-sided and *P*-values <0.05 were considered statistically significant.

RESULTS
Clinical response to treatment and relapse after anti-TNF treatment discontinuation are independent of trough serum adalimumab levels
Trough serum adalimumab levels at the end of the treatment period (two weeks after the last injection) ranged from <0.002 to 23.0 ug/mL (median 11.5 ug/mL). Seven patients (26.9%) had serum adalimumab levels of <5.0 ug/mL. Levels were not different between patients treated with adalimumab for 12 or 24 weeks (*p*=0.292) (Figure 1A). There were no significant differences in trough serum adalimumab levels between responders (12.6 (IQR 7.3-16.2) ug/mL) and non-responders (9.3 (IQR 3.1-14.5) ug/mL) as defined by the achievement of ASDAS inactive disease (*p*=0.237) (Figure 1B). Serum adalimumab levels did also not correlate with end-of-study disease activity parameters such as patient’s global assessment (Figure 1C) and physician’s global assessment of disease activity, TJC, SJC, BASDAI, ASDAS, ESR (data not shown) and CRP (Figure 1D). Moreover, adalimumab levels at the end of treatment were not different between patients with and without subsequent relapse upon discontinuation of therapy (*p*=0.931) (Figure 2A) and were not correlated with time to relapse (*p*=0.984) (Figure 2B). Taken together, these data indicate that the amplitude and/or duration of clinical response to adalimumab in pSpA are not related to trough serum adalimumab levels.

Clinical response to treatment and relapse after anti-TNF treatment discontinuation are independent of the presence of anti-adalimumab ADAbs
At the end of the treatment period 6/26 patients (23.1%) tested positive for serum anti-adalimumab ADAbs: 4 were clearly positive with titres ranging from 89 to 2320 AU/mL and 2 were borderline positive both with a titre of 15 AU/mL. The presence of detectable anti-adalimumab ADAbs levels was similar between patients treated 12 (4/12 patients; 33.3%) or 24 weeks (2/14 patients; 14.3%) with adalimumab (*p*=0.250) (Figure 3A), and between responders (3/14 patients; 21.4%) and non-responders (3/12 patients; 25.0%) (*p*=0.829) (Figure 3B). The anti-adalimumab ADAb titres did not correlate with the various disease activity measurements (data not shown). Finally, the number of patients who tested positive for anti-adalimumab ADAb was not different between those with and without subsequent relapse upon discontinuation of therapy (*p*=0.518) (Figure 4A) nor was there a difference in time to relapse (*p*=0.488) (Figure 4B). As for the trough serum adalimumab levels,
Figure 1. Clinical response to treatment is independent of trough serum adalimumab levels. Trough serum adalimumab levels at end of treatment (two weeks after the last injection) were not different between patients treated with 12 or 24 weeks of adalimumab (A) or between responders and non-responders (B). Also there was no correlation between these drug levels and clinical disease activity parameters such as patient’s global assessment of disease activity measured on a 100 mm visual analogue scale (C) or C-reactive protein (CRP) (D).

Our data thus do not provide any evidence that anti-adalimumab ADAbs have a significant impact on the amplitude and/or duration of clinical response to adalimumab in pSpA.

Anti-adalimumab ADAbs can be masked by the presence of adalimumab but also ‘unmasked’ anti-adalimumab ADAbs do not correlate with clinical response to treatment.

As several factors can bias the measurement and/or interpretation of ADAbs, we conducted additional analyses to assess the potential clinical relevance of these antibodies. Firstly, there was a negative correlation between the anti-adalimumab ADAb titres and the serum trough adalimumab levels ($R = -0.709$, $p<0.001$). This may be explained by the fact that ADAbs contribute to the clearance of the drug or, alternatively, by the fact that serum adalimumab levels may interfere with the measurement of the ADAbs. To investigate the latter possibility, we obtained additional serum samples at follow-up in 25/26 patients at 10.1 (IQR 8.3-14.0) weeks after interruption of the adalimumab treatment. At this time point the median serum
Anti-drug antibodies (ADAbs) were not different between patients treated with 12 or 24 weeks of adalimumab (A) or between responders or non-responders (B).

Adalimumab level was 1.7 (IQR 0.2-4.4) μg/mL, which was significantly lower than at end of treatment (p<0.0001) (Figure 5A). Measurement of anti-adalimumab ADAbs at the same timepoint, allowing to exclude interference of circulating adalimumab levels, showed that the 4 patients with high ADAbs at end of treatment maintained high ADAbs levels after interruption of treatment, whereas in the 3 patients with low ADAbs titres at end of treatment the titres tended to increase over time (Figure 5B). Additionally, 3 patients without detectable ADAbs at end of treatment depicted titres between 15 and 57 AU/mL at follow-up, confirming that low to intermediate levels of ADAbs may be partially masked by circulating adalimumab. However, also these ‘unmasked’ anti-adalimumab ADAbs were not different between responders.
(33.3% positive for ‘unmasked’ anti-adalimumab) and non-responders (40.0% positive for ‘unmasked’ anti-adalimumab) \( (p=0.734) \), or between patients who did or did not experienced relapse after treatment discontinuation (respectively 42.1% and 16.7%, \( p=0.258 \)). The titres of the ‘unmasked’ ADAbs were neither correlated with the various disease activity measurements nor the time to relapse (data not shown).

Secondly, we investigated the effect of concomitant treatment with disease-modifying anti-rheumatic drugs (DMARDs) on ADAbs since this has been described to reduce the frequency of ADAb formation. \(^8,9,14,31\) Although numerically different, there was no statistical difference in anti-adalimumab ADAbs positivity between patients without DMARDs (5/15 patients, 33.3%) versus patients with DMARDs (1/11 patients, 9.1%) \( (p=0.147) \) (Figure 6A). Strikingly, this difference was not present anymore when analysing the ‘unmasked’ anti-adalimumab ADAbs: 5/14 patients (35.7%) positive in the without DMARDs group versus 4/11 patients (36.4%) in the DMARDs group \( (p=0.973) \) (Figure 6B).

**Adalimumab levels and anti-adalimumab ADAbs in patients with generalized drug reactions**

Two patients developed a generalized skin reaction upon adalimumab during the study. Both patients were able to continue adalimumab treatment after topical corticosteroids and oral

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**Figure 4.** Relapse after treatment discontinuation is independent of the presence of anti-adalimumab ADAbs. Anti-adalimumab anti-drug antibodies (ADAbs) at the end of treatment, two weeks after the last injection, were not different between patients who did or did not relapsed after discontinuation of adalimumab (A). There was no difference in time to relapse in patients who tested positive or negative for anti-adalimumab ADAbs (B).
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Figure 5. Anti-adalimumab ADAbs can be masked by the presence of adalimumab in the serum. Trough serum adalimumab levels (A) and anti-adalimumab anti-drug antibodies (ADAbs) titres (B) at the end of treatment (two weeks after the last injection) and at follow-up (upon relapse or, in absence of relapse, at 16 weeks after discontinuation). *p<0.05 assessed by Wilcoxon signed rank test.

Figure 6. The effect of DMARDs on anti-adalimumab ADAbs. At the end of treatment, two weeks after the last injection, anti-adalimumab anti-drug antibodies (ADAbs) were less often observed in patients who used concomitant disease-modifying anti-rheumatic drugs (DMARDs) compared to patients who did not use any DMARDs, although this was not statistically significant (A). However, this difference disappeared when assessing the ‘unmasked’ anti-adalimumab ADAbs at follow-up (B).

antihistaminic treatment. One of these patients had a low serum trough adalimumab level (<0.002 ug/mL) and tested positive for anti-adalimumab ADAbs with a titre of 2320 AU/mL. The second patient had a serum trough adalimumab level of 17.5 ug/mL and tested negative
for anti-adalimumab ADAbs both at the end of the study and at follow-up after interruption of treatment. Neither of the patients used concomitant DMARDs.

**DISCUSSION**

In this study we assessed the potential relevance of serum drug levels and ADAbs on various aspects of clinical response to adalimumab treatment in pSpA. The major findings were that a) trough serum adalimumab levels are heterogeneous but do not correlate with clinical response to treatment or relapse after anti-TNF treatment discontinuation, b) anti-adalimumab ADAbs are found in 1/4 patients but also did not correlate with clinical response to treatment or relapse after discontinuation of the TNF inhibitor, and c) low titer ADAbs can be masked by circulating adalimumab but also ‘unmasked’ ADAbs show no clear relationship with clinical efficacy.

More and more research is done addressing the immunogenicity of TNF inhibitors in various IMIDs including SpA, since the development of ADAbs towards these TNF inhibitors are assumed to play a major role in loss of response to treatment. The mechanism behind this is thought to be either an increased clearance of the drug or neutralization of the active component of the compound. This hypothesis is supported by several studies with infliximab and adalimumab, which reported that treatment failure occurred more often in patients who test positive for ADAbs. However, there is also evidence which is not in line with this concept, since other studies with infliximab did not find a relation between ADAbs and response to treatment. Moreover, one of the studies which found serum trough infliximab levels to be significantly higher in responders compared to non-responders, showed that although statistical significance was reached, the difference between these groups was very low (8.2 ug/mL versus 6.3 ug/mL, respectively). Whether such a small difference is really clinically relevant is questionable. ADAbs towards etanercept have not been found nor is there an association between serum drug levels and clinical effect. Likewise, for golimumab there is no clear relation between ADAbs and clinical efficacy. Furthermore, a recent meta-analysis on various TNF-inhibitors and the clinical experience that there is no difference in efficacy or drug survival between the various TNF inhibitors also question the clinical relevance of anti-TNF ADAbs.

Several factors could be devised trying to explain these differences between the various studies. Firstly, not all TNF inhibitors are assumed to be equally immunogenic. E.g. the soluble dimeric fusion protein etanercept has a less immunogenic structure since only the fusion part of the molecule can contain immunogenic epitopes. Also it is administered more frequently than the other TNF inhibitors, thereby possibly creating more drug interference in ADAb detection. However, this does not explain why different studies in the same TNF inhibitor (e.g. infliximab) come to different conclusions. Secondly, there may be differences among the different SpA subtypes. However, even in studies with both the same disease as well as the same TNF inhibitor (e.g. infliximab in AS) contradicting conclusions are made. Hence, this could also not explain why we and others did not find a clinical association with anti-TNF drug levels or ADAbs in pSpA, while another study did conclude that these had clinical relevance. Thirdly, there is some variation in the size and duration of the studies but this did not influence the results whether ADAbs did or did not have clinical relevance. Fourthly, the use of DMARDs,
especially methotrexate, is described to decrease immunogenicity of TNF inhibitors through a mechanism which is not yet understood. However, this is not in line with the finding that the addition of methotrexate in the management of SpA does not have effect on the efficacy of the treatment or on drug survival of the TNF inhibitor. In our study we indeed found less ADAbs in patients using DMARDs, however this difference was not present anymore when analyzing the ‘unmasked ADAbs’. Fifthly, the detection of ADAbs is also influenced by the assay used. However, the method used in the current study is the same which has been used in other studies making it unlikely that this is the explanation for the differences in results. And finally, the timing of the samples also influences the measurement of ADAbs since the assays are sensitive for drug interference, even when measured before the next administration of the drug when the drug levels are the lowest. Indeed we showed that when anti-adalimumab ADAbs were measured at follow-up after discontinuation of the TNF inhibitor more patients tested positive compared to when measured at end of treatment. Previously it has been described that anti-TNF ADAb titres can decrease and increase over time and vice versa, causing a gradual increase in incidence over time when ADAbs status is presented cumulatively, but not when assessed at each time point independently. This shows that the timing of the measurement can influence the interpretation of the ADAbs status, making it very difficult to make strong conclusions about the relationship with clinical response, and to apply ADAbs measurement in clinical practice.

The limited number of patients is a limitation of our study. We can thus not exclude that clinical correlation with serum adalimumab levels and/or ADAbs would be found in larger patient cohorts. However, this would imply that this association is weak and thus anyway not relevant for treatment monitoring in individual patients. Also, one small study which investigated whether the serum trough infliximab levels modified therapeutic decisions in the management of AS did not find an improvement in the control of disease activity. Similar efficacy and drug survival of TNF inhibitors which induce and which do not induce ADAbs also question the relevance of testing the immunogenicity of these drugs. Although testing immunogenicity in clinical trials is standard practise and may yield interesting scientific insights, the real added value of the presence or absence of ADAbs in an individual patient in clinical practice remains thus to be demonstrated.

CONCLUSIONS

In conclusion, the link between either serum adalimumab levels or anti-adalimumab ADAbs with clinical response or relapse is not as strong as previously assumed. This argues against the use of these parameters in monitoring drug efficacy. And although the treatment with adalimumab and other TNF inhibitors is overall very successful in SpA, future research has to be done to unravel factors which can explain differences in clinical response to further improve disease management towards a personalized and more cost-effective approach.

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