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Reprint of "Anti-therapeutic antibodies and their clinical impact in patients treated with the TNF antagonist adalimumab"☆

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ABSTRACT

Patients treated with the TNF antagonist adalimumab develop anti-therapeutic antibodies (ATA), the prevalence of which varies depending on the assay used. Most assays are compromised due to the presence of adalimumab in the clinical samples. Our objective was to develop an antibody assay, applicable for clinical testing, which overcomes the limitation of therapeutic interference and to further determine the relationship between ATA development, adalimumab levels and disease activity in patients with rheumatoid arthritis (RA), psoriatic arthritis (PsA) or ankylosing spondylitis (AS). Use of an electrochemiluminescence platform permitted development of fit-for-purpose immunoassays. Serum samples from patients, taken prior to and at 12 and 24 weeks of treatment, were retrospectively analysed for levels of adalimumab and ATA. Overall, the antibody prevalence was 43.6% at 12 weeks and 41% at 24 weeks of treatment. Disruption of immune complexes by acid dissociation, a strategy often adopted for this purpose, only marginally increased the antibody prevalence to 48.7% and 46% at 12 and 24 weeks respectively. We found that antibody formation was associated with decreasing levels of circulating adalimumab, but no direct effect on disease activity was evident as assessed using DAS28 for RA patients and BASDAI for PsA and AS patients. However, a negative correlation of free adalimumab trough levels with disease activity scores was observed. Data showed that adalimumab levels can serve as an indicator of ATA development which can then be confirmed by ATA testing. Monitoring of both therapeutic and antibodies should be considered during adalimumab therapy to allow clinicians to personalise treatments for maximal therapeutic outcomes.

1. Introduction

Tumour necrosis factor alpha (TNF-α) antagonists, which include infliximab and adalimumab, are widely used for treatment of various chronic inflammatory or autoimmune diseases e.g. rheumatoid arthritis (RA), Crohn's disease, ankylosing spondylitis (AS) and psoriatic arthritis (PsA). However, some patients develop anti-therapeutic antibodies (ATAs) which alter the pharmacokinetics and in some instances neutralize the biological effects of these therapeutics, impacting on clinical outcome. Approximately 10–30% of patients fail to respond to anti-TNF-α therapy and up to 60% of patients who responded initially fail to respond to treatment over time and require either dose-escalation or a switch to an alternative therapeutic to maintain a clinical response [1,2]. The presence of ATA is thought to be responsible, at least in some patients, for the loss of clinical response.

Some studies have shown that adalimumab-treated patients develop ATA that are associated with lower serum adalimumab trough levels and loss of clinical response [3–6], depending on the magnitude of the

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Abbreviations: AS, ankylosing spondylitis; ATA, anti-therapeutic antibodies; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; DAS28, Disease Activity Score 28; DMARDs, disease-modifying antirheumatic drugs; ECL, electrochemiluminescence; LoD, limit of detection; nhs, normal human serum/sera; PC, positive control; PsA, psoriatic arthritis; RF, rheumatoid factors; RA, rheumatoid arthritis; SpA, spondyloarthritis

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immune response [7]. The reported incidence of anti-adalimumab antibodies varies considerably among studies, from less than 5% to over 80% of patients developing ATAs, sometimes transiently [5,8–10]. Such variation can be explained not only by differences in the population studied e.g., disease, therapeutic regimen, concomitant treatment with immunosuppressants and follow-up period but also by the heterogeneity in methodology employed for ATA assessment [8]. Antibodies to adalimumab have mainly been detected using radioimmunoassays [9] or pH-shift-anti-idiotype antigen binding test [7,10] but the limitation is that these require use of radioisotopes. Bloem et al. [11] compared antibody assays for evaluation of immunogenicity in adalimumab-treated RA patients and concluded that different assays correlated well quantitatively but differed in their discriminatory potential and ability to identify as positive those samples containing low amounts of ATA. This is not unexpected since accurate detection and quantitative measurement of ATA is fraught with technical problems associated not only with differential assay sensitivity for low and high affinity ATA but also with therapeutic and/or target interference. Mitigation of interference is crucial for a thorough assessment of ATA and is particularly relevant for monoclonal antibody therapeutics which persist in the circulation. Formation of immune complexes between circulating therapeutic and ATA compromises ATA detection and strategies for circumventing therapeutic interference are required. An acid dissociation step is often implemented in the immunoassay [10,12,13] but this has limitations and cannot be applied universally to eliminate therapeutic interference as acid can degrade the ATAs and/or therapeutic and, in some instances, provide false positive results due to increased target interference [14].

The application of electrochemiluminescence (ECL) technology for ATA testing has gained prominence in recent years [14,15], based on its increased sensitivity, large dynamic range and greater therapeutic tolerance. We therefore evaluated the utility of ECL-based assays and compared ATA results obtained with or without inclusion of acid treatment, in samples from adalimumab-treated patients in three disease groups - RA, AS and PsA. ATA specificity was confirmed by competitive inhibition and the neutralizing potential of the ATAs assessed in a reporter gene assay [16]. As the half-life of adalimumab is 15–19 days [17], the levels of residual therapeutic were also determined in an ECL assay. Finally, we tried to ascertain if the presence of ATA has any effect on trough levels of adalimumab and on disease progression.

2. Materials and methods

2.1. Patients

Sera from 3 distinct patient cohorts, RA (n = 18), PsA (n = 9) and AS (n = 12), were obtained from the Rheumatology outpatients clinic at the Sapienza University of Rome (Italy). Demographic data are shown in Table 1. Patients were treated with 40 mg adalimumab every other week as monotherapy or in combination with DMARDs therapy. For all patients, sequential samples were collected prior to the next adalimumab injection and retrospectively assessed (baseline - T0, after 12 weeks of therapy - T3 and after 24 weeks of therapy - T6). Therapeutic-naïve patient sera (n = 10) with high titers of rheumatoid factors (RF) and pooled or individual normal healthy sera (nhs, n = 17) were also included in the study. Ethical approval and informed consent was obtained in accordance with the guidelines in the Helsinki Declaration.

Disease activity was assessed at baseline and after 12 and 24 weeks of therapy by Disease Activity Score 28 (DAS28) for RA patients and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) for PsA and AS patients. For clinical assessment, PsA and AS patients were grouped together as SpA since all PsA patients showed axial involvement. For RA patients, low disease activity is DAS28 > 2.6 and < 3.2 and remission is DAS28 < 2.6. For SpA patients, the minimal clinically meaningful BASDAI reduction is 2 and remission is BASDAI < 4.

2.2. Reagents

Commercially available adalimumab (AbbVie Inc, Illinois, USA), recombinant TNF-α (Xiamen Amoytop Biotech Co., China) and the 3rd WHO International Standard (IS) for TNF-α (coded 12/154) were used. An affinity purified hyperimmune sheep polyclonal, specific for the $(Fab')_2$ portion of adalimumab, generated at NIBSC served as positive control (PC) in the antibody assays.

2.3. Protein labelling

Adalimumab and TNF-α were labelled with EZ-link Sulfo-NHS-LC-Biotin (Thermo Scientific, MA, USA) and with ruthenium-NHS-ester (sulfo-tag NHS Ester, MesoScale Discovery (MSD), Gaithersburg, USA) as per manufacturers' instructions.

2.4. Detection of anti-therapeutic antibodies (ATA)

Dilution series of controls and test sera were incubated with biotinylated adalimumab and ruthenium-conjugated adalimumab (both at 0.5 µg/ml in PBS-0.5% BSA) overnight at room temperature (RT) in polypropylene plates, the mixtures (25 µl per well) transferred to pre-blocked MSD streptavidin-coated plates and incubated for a further 2 h. The plates were washed twice with PBS-0.05% Tween and after addition of read buffer T, the plates were read using a SectorImager 2400 (MSD).

For acid dissociation, test sera were diluted 1:10 with acetic acid 300 mM in polypropylene plates, mixed at RT for 30 min, neutralized with Tris 1 M pH 9.5 and incubated for 2 h at RT with biotinylated adalimumab and ruthenium-conjugated adalimumab, both at 1 µg/ml. The mixtures (50 µl per well) were transferred to pre-blocked MSD

Table 1
Demographic data of study patients.

Disease cohort	RA	SpA
Number of patients	18	21
Rheumatoid factor positive	13	N/A
Female	15	11
Male	3	10
Age, yrs. (mean ± standard deviation)	50.3 ± 10.7	47.5 ± 11.9
Disease duration, yrs. (mean ± standard deviation)	10.5 ± 8.9	8.3 ± 6.9
Concomitant therapy n (%)	18 (100)	10 (47.6)
Glucocorticoids n (%)	16 (88.9)	2 (9.5)
Methotrexate n (%)	13 (72.2)	3 (14.3)
Leflunomide n (%)	4 (22.2)	0
Sulphasalazine n (%)	1 (5.6)	8 (63.4)