

Clinical relevance of monitoring serum levels of adalimumab in patients with rheumatoid arthritis in daily practice

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Abstract

Objective

We aimed to assess the usefulness of measuring serum levels of adalimumab (ADL) and anti-ADL antibodies in 57 patients with rheumatoid arthritis (RA) treated with ADL for at least 3 months in daily practice.

Methods

All patients received concomitant disease-modifying anti-rheumatic drug (DMARD). Receiver-operator characteristics (ROC) analysis was used to obtain the cut-off value of ADL for low disease activity (DAS28-ESR ≤ 3.2).

Results

Anti-ADL antibodies were detected in 4 (7%) patients with a mean (SD) DAS28 score of 4.6 (0.9). Patients with positive anti-ADL antibodies had significantly lower levels of ADL and higher DAS28 scores than those with negative antibodies.

Patients with DAS28 ≤ 3.2 as compared with patients with DAS28 > 3.2 showed significantly better SDAI score, higher serum concentrations of ADL and none of them showed anti-ADL antibodies. The cut-off of serum level of ADL for DAS28 < 3.2 was 4.3 mg/L. According to serum levels of ADL, patients were grouped into group 1 (low level) < 5.5 mg/L, group 2 (medium level) 5.5–11.3 mg/L and group 3 (high level) > 11.3 mg/L. Patients in the medium group were closed to clinical remission (median DAS28 2.7) and patients in the high group were on clinical remission (DAS28 2.1).

Conclusion

Serum levels of ADL should be maintained > 4.3 mg/L. In patients with ADL levels > 11.3 mg/L, a decrease of the dose of ADL or an increase in the interval between doses may be planned. The presence of anti-ADL antibodies was associated with a loss of clinical efficacy of ADL.

Key words

adalimumab, anti-TNF therapy, disease activity, rheumatoid arthritis, treatment outcome

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Introduction

The introduction of anti-tumour necrosis factor alpha (TNF- α) therapies has dramatically improved the management and outcome of patients with rheumatoid arthritis (RA) and spondyloarthritis. However, about 30% of patients with these diseases, receiving TNF inhibitors either do not respond to treatment or lose initial responsiveness (1, 2). The mechanisms underlying these failures are not entirely clear, but immunogenicity leading to production of anti-drug antibodies with removal of the drug from the circulation and/or direct neutralisation of drug activity seems to play a major role (2). In different series of patients with RA treated with biologics, anti-infliximab antibodies have been detected in 32.9% and 43% of patients (3, 4), anti-adalimumab (ADL) antibodies in 28% (5) and anti-etanercept antibodies in 7.9% (6). Therefore, accurate monitoring of serum drug and anti-drug antibody levels should be an important part of therapy for patients being treated with biological agents. However, data regarding the clinical relevance of measuring serum levels of anti-TNF drugs in RA patients to assess response to treatment and to allow for dose adjustment, as well as to provide a rationale for switching to another anti-TNF agent are scarce (7-11). Moreover, tailoring biological treatment to individual patients with RA starting adalimumab using drug levels and short-term outcome is cost-effective (12).

In patients with RA, clinical trials have shown that combination therapy with anti-TNF agents plus disease-modifying anti-rheumatic drugs (DMARDs), in particular methotrexate (MTX), is significantly superior to either MTX alone or anti-TNF therapy alone in improving signs and symptoms of disease (13-15). In case of ADL, MTX has been shown to have an effect in reducing immunogenicity in a dose-dependent manner (16) and could thereby result in higher ADL levels and enhanced therapy (8).

To provide further data on the usefulness of assessing ADL concentrations in RA patients treated with this anti-TNF- α drug in daily practice conditions, a cross-section study was conducted, the aims of which were: 1) to

assess the clinical relevance of serum levels of ADL to maintain RA patients on clinical remission or low disease activity, and 2) to determine the prevalence of anti-ADL antibodies in ADL-treated patients in association with DMARDs.

Methods

Study population

For this cross-sectional study, data were obtained from a cohort of 57 consecutive RA patients attended in routine daily practice at the outpatient clinics of Rheumatology of three hospitals in Spain. Patients aged 18 years or older, diagnosed with RA according to 1987 revised criteria of the American College of Rheumatology (ACR) (17) and on current treatment with ADL, 40 mg s.c. every other week (Humira[®], Abbvie Laboratories, Madrid, Spain) for at least 3 months in association with one of the following DMARDs: MTX (oral or s.c., maximum weekly dose 25 mg), leflunomide (LFN, maximum daily dosing 20 mg) or hydroxychloroquine (OH-CLQ, maximum daily dosing 400 mg). The study was approved by the Ethics Committee of the participating centres and all patients gave written informed consent to participate in the study.

Data collection

In all patients information on age, sex, body mass index (BMI), date of diagnosis of RA, laboratory data including rheumatoid factor (RF), anti-cyclic citrullinated peptides (CCP) antibodies, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), previous and current treatment for RA (DMARDs and biological agents), reasons for withdrawal of anti-TNF drugs and duration of ADL treatment were recorded.

Clinical response

Disease activity was assessed using disease activity score in 28 joints (DAS28) (18) based on ERS (remission <2.6, low activity 2.6–3.1, moderate 3.2–5.1, severe >5.1) (18) and the simplified disease activity index (SDAI) (remission <3.3, low disease activity 3.3–11, moderate >11–26, high disease activity >26) (19).

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Competing interests: none declared.

Measurement of ADL concentrations and anti-ADL antibodies

In all patients, a 5 mL serum sample was obtained just before the next s.c. injection of ADL and was stored at -80°C until analysis. Serum concentrations of free ADL (trough level) and anti-ADL antibodies were measured using the second version of a commercialised enzyme linked immunosorbent assay (ELISA) (Promonitor®, Proteomika Derio, Vizcaya, Spain). Both versions of the commercialised immunoassay have shown adequate clinical and analytical validation criteria (20, 21) and an excellent correlation for the measurement of drug levels and anti-drug antibodies for infliximab, etanercept and ADL (22, 23). All sera were tested under standardised conditions specified by the manufacturer. Six dilutions of serum samples for each standard curve were made (1.25–60 ng/mL for serum levels of ADL and 3.13–200 AU/mL for anti-ADL antibodies). All the analytical development was carried out without knowledge of clinical data. The cut-off value were >0.024 mg/L for the serum levels of ADL and >3.5 AU/mL for positive anti-ADL antibodies. Samples with ADL trough level <3 mg/L were considered subtherapeutic (8, 11) and were analysed for the pres-

ence of anti-ADL antibodies with the standard assay and, when negative, using an acid dissociation pretreatment protocol recommended by the manufacturer in procedures of research and development, which allows for antibody detection in the presence of antigen in serum when possible drug-antibody complexes are disaggregated (24, 25).

Statistical analysis

Categorical variables are expressed as frequencies and percentages, and continuous variables with normal distribution of data as mean and standard deviation (SD). The chi-square (χ^2) test and the Student's *t*-test were used for the comparison of qualitative and quantitative variables, respectively. Statistical significance was set at $p < 0.05$. The relationship between serum concentrations of ADL and DAS28 score was analysed with the Pearson's product-moment correlation coefficient (*r*). Receiver operating characteristics (ROC) analysis was used to obtain a cut-off value for ADL trough levels between patients with low disease activity (DAS28 ≤ 3.2) versus those with moderate or high activity (DAS28 >3.2). The area under the ROC curve (AUC) was calculated using the trapezoidal rule. Confidence

intervals (CI) for the AUC, sensitivities and specificities were estimated using pROC package functions which compute the 95%CI with 2000 stratified bootstrap replicates (26).

Results

Patients

There were 57 patients: 12 men and 45 women, with a mean (SD) age of 63 (12) years and BMI of 28 (6) kg/m². The mean duration of RA was 13.9 (9.4) years. In 75% and 63% of the patients, positive RF and anti-CCP antibodies were detected, respectively. All patients had been treated with ADL at standard doses (40 mg s.c. every other week) for at least 3 months, with a mean (SD) of 2.7 (1.5) years. In 46 patients (80.7%), ADL was the first anti-TNF drug. Eleven patients (19.3%) had been previously treated with infliximab (*n*=9) and etanercept (*n*=2) without success. All patients were concomitantly treated with one DMARD: MTX (*n*=36, mean weekly dosing 15 mg), LFN (*n*=11, mean daily dosing 17 mg) and OH-CLQ (*n*=10, mean daily dosing 250 mg). The mean (SD) DAS28 score was 2.7 (1.1) and the mean SDAI 6.4 (6.2). Serum concentrations of ADL were performed in 74 cases (in 17 patients attended at

Table I. Characteristics of patients with RA treated with ADL according to the presence of anti-ADL antibodies and disease activity (DAS28).

Variables	Total patients (n=57)	Anti-ADL antibodies		<i>p</i> -value	DAS28 score		<i>p</i> -value
		Present (n=4)	Absent (n=53)		≤ 3.2 (n=37)	>3.2 (n=20)	
Measurements, n (%)	74	4 (5)	70 (95)	<0.001	52 (70)	22 (30)	0.004
Female patients, %	79	50	77	0.38	78	60	0.24
Age, years, mean (SD)	63 (12)	59 (9)	62 (13)	0.57	62 (12)	62 (13)	1
BMI, kg/m ² , mean (SD)	28 (6)	26 (7)	28 (5)	0.61	28 (6)	30 (7)	0.28
RF, positive, %	75	100	81	0.34	80	70	0.46
Anti-PCC antibodies, positive, %	65	100	78	0.29	66	60	0.71
Duration of RA, years, mean (SD)	13.9 (9.4)	12.5 (4.6)	12.8 (9.4)	0.91	13.5 (10.3)	14.9 (7.2)	0.55
Concomitant DMARDs, n (%)	57 (100)	4 (100)	53 (100)	-	37 (100)	20 (100)	-
MTX, %	72	75	74	0.97	70	55	0.29
LFN, %	11	0	18	1	19	20	0.96
OH-CLQ, %	17	25	8	0.63	11	25	0.59
DAS28, mean (SD)	2.7 (1.1)	4.6 (0.9)	2.7 (1)	0.02	2.2 (0.6)	4.3 (0.9)	<0.001
SDAI, mean (SD)	6.4 (6.2)	9.6 (6.3)	7.0 (6.6)	0.48	4.1 (2.9)	13.4 (8.1)	<0.001
Previous anti-TNF agent, n (%)	11 (19)	2 (50)	9 (17)	0.32	8 (22)	3 (15)	0.79
Infliximab, n (%)	9 (16)	1 (25)	8 (15)	0.79	7 (19)	2 (10)	0.76
Etanercept, n (%)	2 (3)	1 (25)	1 (2)	0.63	1 (3)	1 (5)	0.94
ADL first anti-TNF agent, n (%)	46 (81)	2 (50)	44 (83)	0.25	29 (78)	17 (85)	0.56
Time on ADL, years, mean (SD)	2.7 (1.5)	2.2 (2.0)	2.8 (1.6)	0.59	2.6 (1.6)	2.5 (1.5)	0.81
Serum ADL level, mg/L, mean (SD)	7.9 (4.6)	<0.024	8.0 (4.3)	<0.001	9.3 (4.1)	4.4 (4.0)	<0.001
Anti-ADL antibodies, n (%)	4 (7)	-	-	-	0	4 (20)	-

DMARDs: disease-modifying anti-rheumatic drugs; MTX: methotrexate; LFN: leflunomide; OH-CLQ: hydroxychloroquine.

Marina Baixa Hospital, more than one assessment of serum ADL levels was performed prospectively). The mean (SD) serum level of ADL of all measurements was 7.9 (4.6) mg/L.

Anti-ADL antibodies

Positive anti-ADL antibodies were detected in 4 patients (7%) (4 cases [5.4%] of the 74 measurements), three of them treated with MTX and another with OH-CLQ. The mean serum level of ADL was <0.024 mg/L and the mean anti-ADL antibody value of 1135 AU/mL (range 132–2000 AU/mL). When the groups of patients with and without positive anti-ADL antibodies were compared, patients with positive antibodies showed a significantly lower mean ADL concentration (<0.024 vs. 8.0 [4.3] mg/L, $p<0.001$) and a significantly higher mean DAS28 score (4.6 [0.9] vs. 2.7 [1], $p=0.02$) (Table I).

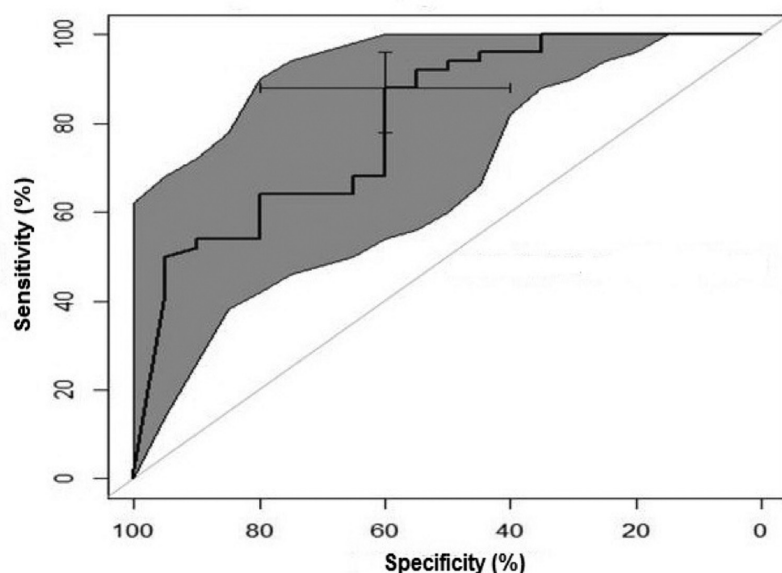
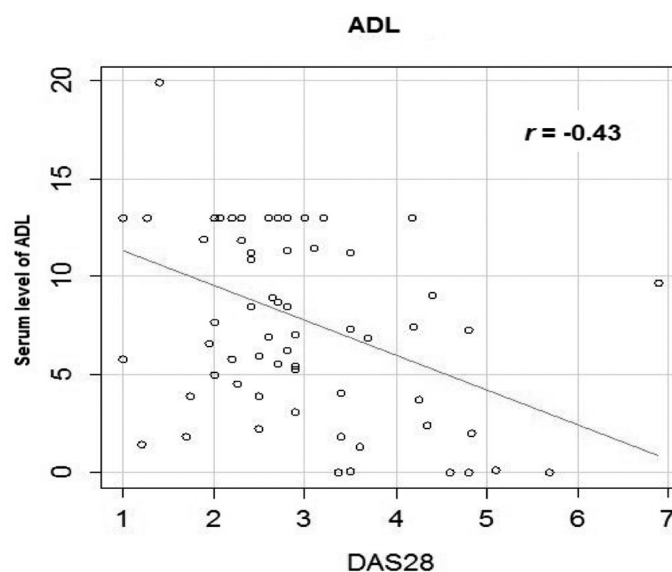
Correlation between ADL serum levels and DAS28

As shown in Figure 1, there was a negative correlation ($r=-0.43$) between serum ADL concentrations and DAS28 scores. Also, when patients were divided according to DAS28 score ≤ 3.2 or >3.2 , those with low disease activity showed higher serum ADL levels (9.3 [4.1] vs. 4.4 [4.0] mg/L, $p<0.001$) and lower SDAI scores (4.1 [2.9] vs. 13.4 [8.1], $p<0.001$). All four patients with positive anti-ADL antibodies had DAS28 scores >3.2 (20% vs. 0%, $p<0.001$).

As shown in Figure 2, a cut-off value of serum ADL level of 4.3 mg/L was found for a low disease activity (DAS28 ≤ 3.2), with an AUC of 0.803 (95%CI 0.688–0.919) (sensitivity 88%, specificity 60%). Also, 88% of patients with an ADL serum level >4.3 mg/mL showed a mean DAS28 score ≤ 3.2 .

On the other hand, when serum ADL levels divided into tertiles (<5.5 , 5.5–11.3, >11.3 mg/L) were related to DAS28 scores, patients in group 1 (ADL <5.5 mg/L) showed higher mean (SD) values of DAS28 scores (3.34 [1.22]) as compared to both patients in group 2 (ADL 5.5–11.3 mg/L) (mean DAS28 score 3.04 [1.22]) and patients in group 3 (ADL >11.3 mg/L) (mean DAS28 score 2.23 [0.72]). Patients in group 3

Fig. 1. Correlation between serum ADL levels and DAS28 score (ADL: adalimumab).



Serum levels of ADL	DAS28 score	
	≤ 3.2	> 3.2
≤ 4.3 mg/L	6 (12%)	12 (60%)
> 4.3 mg/L	44 (88%)	8 (40%)

Fig. 2. ROC curve: cut-off of the serum level of ADL for DAS28 score of low disease activity (≤ 3.2); ADL: adalimumab.

showed DAS28 scores corresponding to clinical remission. As shown in Figure 3, higher values of ADL were associated with lower DAS28 scores.

Discussion

In this study carried out in patients with RA on treatment with ADL associated with DMARDs, the prevalence of anti-ADL antibodies and its relationship

with serum ADL concentrations and disease activity (DAS28) was assessed. The final goal of the study was to determine whether monitoring of ADL levels may affect clinical decision-making in routine daily practice.

The prevalence of anti-ADL antibodies in the present series of RA patients treated with ADL associated with a DMARD was 7%, which is lower than

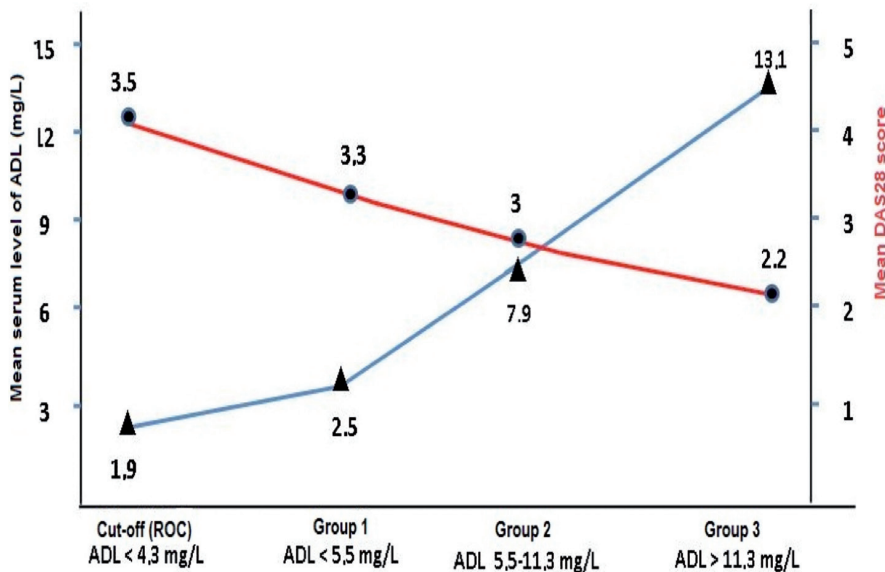


Fig. 3. Relationship between DAS28 score (▲) and serum levels of ADL (●) divided into tertiles (ADL: adalimumab).

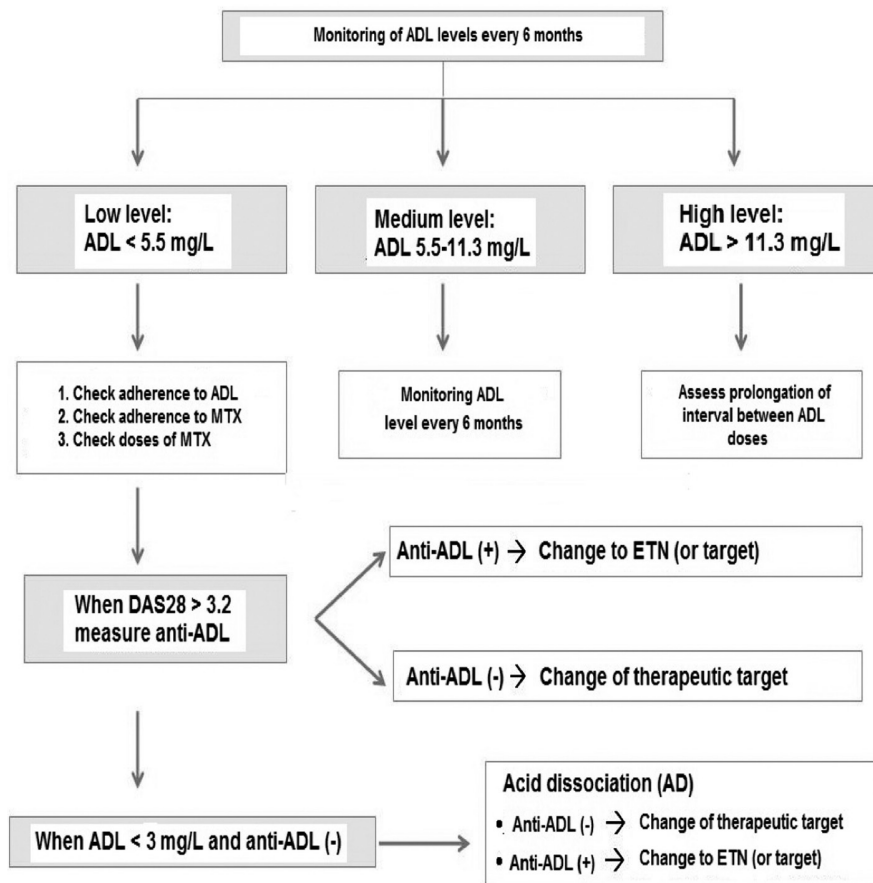


Fig. 4. Treatment algorithm based on monitoring of serum ADL levels and the presence of positive or negative anti-ADL antibodies (ADL: adalimumab; MTX: methotrexate; ETN: etanercept).

rates reported in other studies (7, 27). In our study, like others, the development anti-ADL antibodies was associated with almost negligible serum ADL

concentrations. Moreover, all patients received DMARDs (MTX in 63% of cases). The efficacy of ADL combined with MTX is higher than ADL admin-

istered as monotherapy (13-15). The mechanism of action of MTX is unclear, although lowers the frequency and amount of antibodies formed, whereby the efficacy of biologicals is improved. Also, MTX decreases inflammation and may act as an immunomodulator with early suppression of expansion of B and T cells (28). Different studies have shown that MTX reduced immunogenicity in RA patients treated with ADL, and that this effect is dose-dependent (8, 16). The PREMIER study has shown that in patients with early aggressive RA, combination therapy was superior to both MTX and adalimumab monotherapy in all outcomes measured (13). Although different studies have shown that the percentage of patients with anti-ADL antibodies is higher among those treated with ADL alone as compared with patients treated with ADL and MTX (5, 7, 8), there are no conclusive data in relation to other DMARDs. In the study of De Stefano *et al.* (29), anti-TNF- α drugs can be used in combination not only with MTX, but also with LFN, with the same probability of achieving significant clinical improvement in RA patients.

However, when the effectiveness and safety of a therapeutic regimen associating subcutaneous anti-TNF- α , etanercept (ETN) and ADL, with LFN or MTX in a 2-year open-label study performed in clinical practice, at 18 months, improvement was present in 33.3% of the patients in the LFN group and in 81.5% of the patients in the MTX group ($p=0.001$) (30). In our study, of the four patients with anti-ADL antibodies, three patients received MTX and one patient received OH-CLQ.

Assessment and monitoring serum levels of anti-TNF- α agents may be useful to optimise treatment in different clinical scenarios, including patients in clinical remission, patients with disease activity despite treatment with ADL and patients with low serum levels of the drug and without anti-drug antibodies. A reduction of the dose or a prolongation of the time interval between doses may be adequate in patients who are on clinical remission (8), which in turn would reduce costs without affecting treatment efficacy. Monitoring of drug

levels and anti-drug antibodies would justify therapeutic intensification and/or drug switching after primary or secondary failure of anti-TNF therapy (1, 31). In patients with low serum drug levels and negative anti-drug antibodies even after an acid dissociation procedure, a change of therapeutic target seems the most reasonable approach (31).

In our study, like others (5, 8), there was a negative correlation between serum levels of ADL and DAS28 score, with a cut-off value of 4.3 mg/L (88% of patients with DAS28 score ≤ 3.2 showed serum ADL levels above this cut-point). This value is close to the cut-off concentration of 5 mg/L reported in the study of Pouw *et al.* (8). The small difference may be attributed to the use of their newly validated and automated ADL ELISA concentration method, which is different than the ELISA kit used in our study as well as the characteristics of the population included in the study. Also, our value of sensitivity is comparable, whereas the AUC (0.803) and the specificity are better than those reported by Pouw *et al.* (8).

The distribution of patients into three groups according to serum levels of ADL together with data of DAS28 may be useful for decision-making in daily practice allowing developing a treatment algorithm. As shown in Figure 4, patients in group 1 (serum ADL < 5.5 mg/L) on clinical remission or with low disease activity, a reduction of the doses of ADL or an increase in the interval between doses would probably result in clinical reactivation of symptoms. Therefore, monitoring serum ADL levels may prevent the appearance of relapses. In case of DAS28 > 3.2 , measurement of anti-ADL antibodies would support the decision to change to another anti-TNF agent or therapeutic target. In all cases, however, adherence to ADL and DMARDs (and even to increase the dose of DMARD) should be assessed. Patients in group 2, with serum ADL concentrations between 5.5 and 11.3 mg/L and a median DAS28 score of 2.7 are probably in a clinically safe range with negative anti-drug antibodies (32), so that monitoring drug levels and DAS28 at regular intervals could be recommended as well as to check

adherence to DMARD treatment at adequate doses. Finally, in group 3 patients with high serum levels of ADL (> 11.3 mg/L) and on clinical remission (median DAS28 score 2.1), it may be able to successfully increase the dose interval without losing clinical efficacy. The study of Pouw *et al.* (8) showed no additional improvement of disease activity in patients with ADL concentrations exceeding 8 mg/L. This value coincides with the mean level in Group 2 (7.9 mg/L), which in turn is identical to 7.4 mg/L reported by these authors in the population co-treated with DMARDs. Recently, algorithms to approach patients with RA receiving TNF inhibitors introducing immunogenicity assessment have been reported (1, 33), with decision trees based on measurements of serum drug levels (and anti-drug antibodies). These algorithms including that presented in our study represent preliminary tools to aid decision making among clinicians, and how these assessments can be integrated in the care of patients in routine clinical practice, leading to personalised and more cost-effective strategies to RA treatment.

Our cross-sectional study has some limitations including the small study population and large differences in the duration of RA, which may affect the results of DAS28. Also, serum ADL levels were measured prospectively in a subset of patients but these preliminary results are the basis of an ongoing prospective project of periodic monitoring of serum drug levels at our institution in routine clinical practice.

Conclusion

In summary, the prevalence of anti-ADL antibodies in RA patients treated with ADL associated with DMARDs was 7%. Serum ADL concentrations were inversely correlated with DAS28 score, so that higher levels of ADL were associated to lower levels of disease activity. Serum ADL levels should be monitored periodically to maintain levels > 4.3 mg/L. Patients with ADL levels > 11.3 mg/L may be candidates to increase the interval between doses. Measurement of serum anti-drug antibodies may be restricted to patients with low or undetectable serum ADL concentrations.

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