Rheumatology International (2019) 39:841–849
https://doi.org/10.1007/s00296-019-04288-7

Rheumatology
INTERNATIONAL

OBSERVATIONAL RESEARCH

Clinical relevance of monitoring serum adalimumab levels in axial spondyloarthritis

José Miguel Senabre Gallego[1] · Jose Rosas[1] · Mariana Marco-Mingot[2] · José Alberto García-Gómez[3] · Gregorio Santos-Soler[1] · Esteban Salas-Heredia[1] · Ana Pons-Bas[1] · Xavier Barber-Vallés[4] · José Antonio Bernal-Vidal[1] · Catalina Cano-Pérez[1] · Mario García-Carrasco[5,6] · Emilio Flores-Pardo[7] · AIRE-MB Group

Received: 16 January 2019 / Accepted: 16 March 2019 / Published online: 21 March 2019

Abstract

Our aim was to assess the relationship between serum adalimumab levels, anti-drug antibodies (ADA) and disease activity in patients with axial spondylarthritis (SpA). We have carried out a single-centre cross-sectional study. adalimumab and ADA levels were analysed with ELISA and correlated with SpA activity using BASDAI and ASDAS scores. Adalimumab cut-off value was calculated to discriminate inactive disease/low disease activity (BASDAI < 4; ASDAS < 2.1) from moderate/high disease activity (BASDAI ≥ 4; ASDAS ≥ 2.1), using a receiver operating characteristic (ROC) curve. Up to January 2016, 51 consecutive patients were included. The median (range) age was 46.6 (18–68) and 47.1% were women. ADA prevalence was 27.5%, with none detected in the 21.6% receiving concomitant disease-modifying antirheumatic drugs (DMARDs) ($p = 0.021$). Adalimumab level was normal (> 3 mg/l) in 36 patients (70.6%), all without ADA. Fifteen patients (29.4%) had subtherapeutic adalimumab levels (< 3 mg/l), with ADA in 14 (93%). Median adalimumab (mg/l) was significantly higher in patients with inactive disease/low disease activity: BASDAI < 4 vs ≥ 4: 9.5 vs 2.6 ($p < 0.01$); ASDAS-CRP < 2.1 vs ≥ 2.1: 9.3 vs 0.3 ($p < 0.001$); ASDAS-ESR < 2.1 vs ≥ 2.1: 9.9 vs 3.0 ($p < 0.001$), and this finding was consistent with the result of the multivariate model. Patients with inactive disease/low disease activity presented significantly lower ADA levels. The adalimumab level cut-offs and area under the curve (AUC) obtained in the ROC curves were: ASDAS-CRP (< 2.1) 4.6 mg/l (AUC 81.2%; 95% CI 67.5–94.9; $p < 0.001$); ASDAS-ESR (< 2.1) 7.7 mg/l (AUC 82.4%; 95% CI 69.3–95.5; $p < 0.001$); BASDAI (< 4) 6.4 mg/l (AUC 73.5%; 95% CI 58.6–88.3; $p < 0.01$). In conclusion, presence of ADA in axial SpA patients treated with adalimumab was associated with lower serum drug levels. ADA levels were lower and adalimumab levels were higher in patients with inactive disease/low disease activity based on BASDAI and ASDAS indices. Concomitant treatment with MTX reduces de likelihood of finding ADA. Serum adalimumab levels above 4.6 mg/l are recommended to avoid compromising efficacy.

Keywords Spondylarthritis · Adalimumab · Antibody formation · Enzyme-linked immunosorbent assay · ROC curve · Treatment outcome

Introduction

Anti-tumour necrosis factor (anti-TNF) drugs have enabled great advances in the treatment of spondylarthritis (SpA) and, until the recent arrival on the scene of IL17 inhibitors, were the only effective biological agents in its axial manifestations, for which other disease-modifying antirheumatic drugs (DMARDs) are not recommended. There are currently five anti-TNF drugs approved for the treatment of SpA, including four monoclonal antibodies—infliximab, adalimumab, golimumab and certolizumab—and one soluble TNF receptor, etanercept. All of these drugs have demonstrated efficacy in clinical trials and around 60% of patients achieve an ASAS 20 response, vs 20% of patients assigned to placebo [1]. However, approximately 30–40% of patients do not respond to treatment initially or lose efficacy over time [2–4]. The mechanisms underlying these failures are not clearly understood, but the production of anti-drug

✉ José Miguel Senabre Gallego
josemisenabre@gmail.com

Extended author information available on the last page of the article

antibodies (ADA), causing the drug to be cleared from the blood and/or neutralising its effect, appears to play an important role [5]. These antibodies have also been associated with adverse events such as infusion reactions and injection site reactions [6]. Furthermore, given the high cost and adverse events that can be associated with biological therapies, in recent years strategies have been proposed to reduce drug doses in patients in prolonged clinical remission, thereby avoiding overtreatment [7].

Many studies have demonstrated the relationship between disease activity, serum levels of anti-TNF agents and their immunogenicity, including meta-analyses and systematic reviews [6, 8, 9], however, most of them were conducted in patients with rheumatoid arthritis (RA) or Crohn's disease. The available studies in SpA report controversial results. With regard to infliximab and adalimumab, some groups of researchers have reported that the existence of ADA was associated with reduced efficacy [5, 10–15], while others failed to find this relationship and concluded that drug levels were not associated with response to treatment [16]. In studies with etanercept, golimumab and certolizumab, the reported prevalence of ADA is very low [17–20].

The objective of this study was to assess the prevalence of ADA in patients with axial SpA receiving treatment with adalimumab, and to investigate their relationship with serum adalimumab levels and disease activity indices. A secondary objective was to establish a cut-off point for serum adalimumab levels at which patients have inactive disease/low disease activity.

Methods

Design and study population

This study was a cross-sectional study that included all patients with axial SpA being treated with adalimumab by the rheumatology section of our centre. Patients had to be over 18 years of age, be diagnosed with axial SpA according to the Assessment of Spondyloarthritis International Society (ASAS) 2009 criteria [21] and have received treatment with adalimumab, as per routine clinical practice, without interruptions, for at least 3 months.

Variables and data collection

Information was collected on age, body mass index (BMI), date of diagnosis of SpA, laboratory data, including HLAB27, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), prior biological treatment for SpA, concomitant DMARDs, reasons for the interruption of adalimumab and duration of treatment with adalimumab. Treatment regimens with adalimumab correspond to routine clinical practice, at a dose of 40 mg every 14 days, at the discretion of the rheumatologist responsible for the patient [22].

Clinical assessments

Spondylarthritis disease activity was assessed using patient global assessment of disease activity (PGA) on a 100 mm visual analogue scale, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [23] and Ankylosing Spondylitis Disease Activity Score (ASDAS) [24]. The pre-established cut-off points for BASDAI were: <4 inactive disease activity and ≥ 4 active disease, and for ASDAS were: < 1.3 inactive disease, < 2.1 moderate activity, 2.1–3.5 high activity and > 3.5 very high activity. Patients were classified as responders if they had an ASDAS < 2.1 and BASDAI < 4. The Bath Ankylosing Spondylitis Functional Index (BASFI) was used to assess physical function.

Measurement of serum adalimumab and ADA levels

A 5-ml serum sample was taken from all patients just before the next adalimumab dose. It was stored at − 80 °C until analysis. The second version of the commercial enzyme-linked immunosorbent assay (ELISA) Promonitor® kit (Progenika Grifols SA, Spain), which has been clinically and analytically validated [25–27] was used. All serum samples were analysed under the standard conditions specified by the manufacturer. The serum samples underwent six dilutions for each curve (1.25–60 ng/ml for serum adalimumab levels and 3.13–200 AU/ml for ADA levels). All analytical work was conducted without knowledge of the clinical data. The technique's sensitivity is > 0.024 mg/l for serum adalimumab levels and > 3.5 AU/ml for ADA levels. Samples with drug levels below 3 mg/l were considered subtherapeutic. In those with negative levels and ADA results, an acid dissociation pre-treatment protocol recommended by the manufacturer was implemented, enabling the detection of ADA in the presence of antigen when possible drug-antibody complexes have broken down [28].

Statistical analysis

Statistical processing was performed using the SPSS 22.0 and R Core Team for Windows programs. The descriptive study of the subjects' quantitative variables was conducted using point estimation, processing the variables as means and standard deviations or medians and ranges depending on the distribution of each. Continuous variables with normal distribution were analysed using Student's *t* test and not-normally distributed variables with the Kruskal–Wallis method or the Mann–Whitney *U* test. Categorical variables were analysed using Chi-square and with the Fisher's exact test when necessary. Associations between continuous

variables were computed using Spearman Rank Correlation. The changes were considered significant when the probability associated with the comparison was < 0.05. A receiver operating characteristic (ROC) curve was calculated to obtain a cut-off value for trough adalimumab levels that would discriminate responder patients (BASDAI < 4 and ASDAS < 2.1). Linear multivariate models were created, using ASDAS-CRP, ASDAS-ESR and BASDAI as dependent variables. The normality of the variable was tested using the Shapiro–Wilk test. The Breusch–Pagan test was used for the homoscedasticity analysis. The possible correlation between the residues was analysed with the Durbin–Watson statistic.

Results

Baseline characteristics

Up to January 2016, 51 consecutive patients were included from a total of 60 screened patients. Two patients were excluded because they did not meet the diagnostic criteria, and another seven patients were excluded because clinical activity data were not available on the date when the levels were taken. The patients' baseline characteristics are summarised in Table 1. The median (range) age was 46.6 (18–68) and 47.1% were women. HLAB27 was positive in 42 patients (82.4%) and 25 patients (52.1%) had objective signs of sacroiliitis on X-ray. The median disease duration was 71 months (2–408) and the median duration of treatment with adalimumab was 9.8 months (1–69). Adalimumab was the first biological agent received in 36 patients (70.6%). Eleven patients (21.6%) were on concomitant treatment with DMARDs, mainly methotrexate (MTX) (15.7%) and sulfasalazine (5.9%). MTX doses varied from 10 to 25 mg per week, and median MTX dose was 20 mg. The median (range) BASDAI, ASDAS-CRP and ASDAS-ESR scores were 4.2 (0–9.6), 2.0 (0.1–4.3) and 2.3 (0.6–3.9), respectively.

Adalimumab and ADA levels and disease activity

The median adalimumab level was 7.9 mg/l (0.0–24.0). Therapeutic adalimumab levels (> 3 mg/l) were detected in 36 patients (70.6%), with ADA not being detected in any of these patients. Positive ADA levels (> 3.5 UA/ml) were detected in 14 patients (27.45%), all of whom had subtherapeutic serum adalimumab levels (< 3 mg/l), with a median adalimumab level of 0.024 mg/l (0.0–1.5). In one patient, neither the drug nor ADA was detected, despite performing acid dissociation. Since sample collection failure or detection method error is very unlikely, this finding was attributed to a lack of adherence to treatment.

Table 1 Demographic characteristics and activity scores by ADA presence

	All	ADA-negative	ADA-positive
n (%)	51 (100)	37 (72.6)	14 (27.4)
Age (years)	46.6 (18.5–68.5)	44.1 (18.5–68.5)	51.9 (25.9–67.9)*
Women [*n* (%)]	24 (47.1)	16 (43.2)	8 (57.1)
BMI	27.5 (18.8–36.1)	26.9 (18.8–33.6)	29.0 (23.1–36.1)*
Disease duration (months)	71.2 (2.3–408.4)	52.9 (2.3–338.2)	242.2 (10.9–408.4)**
HLAB27 + [*n* (%)]	42 (82.4)	31 (83.8)	11 (78.6)
X-ray sacroiliitis [*n* (%)]	25 (52.1)	8 (57.1)	17 (50.0)
BASFI	4.6 (0–8.7)	2.4 (0–8)	6.2 (2.4–8.7)***
BASDAI	4.2 (0–9.6)	2.6 (0–9.6)	5.9 (4–7.5)***
ASDAS-CRP	2.0 (0.1–4.3)	1.8 (0.1–4.3)	3.2 (2.0–4.3)***
ASDAS-ESR	2.3 (0.6–3.9)	1.7 (0.6–3.9)	2.8 (2.6–3.8)***
CRP (mg/l)	2.0 (0.1–30.0)	1.0 (0.1–6.0)	4.7 (2.0–30.0)***
ESR (mm/h)	5 (2–76)	4 (2–42)	13.5 (2–76)**
PGA	4.0 (0–10)	3 (0–10)	5 (3–8)**
Previous anti-TNF [*n* (%)]	15 (29.4)	11 (29.7)	4 (28.6)
Time on adalimumab (months)	9.8 (3.0–69.1)	9.4 (3.0–69.1)	11.9 (3.0–20.1)
Concomitant DMARD [*n* (%)]	11 (21.6)	11 (29.7)	0 (0)*

All data shown are median (range), otherwise specified

ADA anti-drug antibody, *Anti-TNF* anti-tumour necrosis factor, *ASDAS* Ankylosing Spondylitis Disease Activity Score, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *BASFI* Bath Ankylosing Spondylitis Functional Index, *BMI* body mass index, *CRP* C-reactive protein, *DMARD* disease-modifying antirheumatic drug, *ESR* erythrocyte sedimentation rate, *PGA* patient global assessment of disease activity

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Significantly higher disease activity scores were observed among patients with ADA (e.g., BASDAI, ASDAS-CRP, ASDAS-ESR, PGA, ESR and CRP) (Table 1). In addition, these ADA positive patients were older and had a higher BASFI, BMI and longer disease duration. No differences were observed in regard to gender, HLAB27 presence, X-ray sacroiliitis, or previous use of anti-TNF agents. Concomitant use of DMARDs was associated with an absence of ADA, and it was notable that no patient with positive ADA was using DMARDs at the time of measurement ($p < 0.05$) (Table 1). Moreover, DMARDs use was associated with inactive disease/low disease activity, assessed with BASDAI, but not with ASDAS (Table 2). Likewise, patients classified as inactive disease/low activity (ASDAS-CRP < 2.1, ASDAS-ESR < 2.1 and BASDAI < 4) had higher median adalimumab levels (mg/l) compared to those with moderate/high activity: 9.3 vs 0.3, 9.9 vs 3.0 and 9.5 vs 2.6, respectively (Table 2; Fig. 1). In addition, these inactive disease/low disease activity patients had lower ADA levels, lower BASFI scores and lower BMI (Table 2). No differences were observed in age and the duration of treatment with adalimumab. The prevalence of ADA was lower in patients with inactive disease/low disease activity, assessed with ASDAS-CRP, ASDAS-ESR and BASDAI (Table 2). The inverse correlation between adalimumab and clinical activity according to the ASDAS-CRP, ASDAS-ESR and BASDAI indices was also observed in the multivariate model after adjusting for age, gender, BMI, concomitant use of DMARDs and previous use of anti-TNF agents.

The adalimumab level cut-offs and area under the curve (AUC) obtained in the ROC curves were 4.6 mg/l (AUC 81.2%; 95% CI 67.5–94.9), 7.7 mg/l (AUC 82.4%; 95% CI 69.3–95.5) and 6.4 mg/l (AUC 73.5%; 95% CI 58.6–88.3) for ASDAS-CRP, ASDAS-ESR and BASDAI, respectively (Table 3). Sensitivity and specificity of each cut-off point are detailed in Table 3.

Discussion

In this study, the prevalence of ADA in axial SpA patients on treatment with adalimumab is 27.5%. In previous studies, the reported ADA against adalimumab varies greatly from 13 to 58% [5, 10, 13, 14, 16, 29]. Significantly, our patients with ADA had lower serum adalimumab levels, and higher BASDAI, BASFI, ASDAS-CRP and ASDAS-ESR scores, and PGA, ESR and CRP levels. These results are similar to studies published by other authors [5, 10, 13, 14, 29]. de Vries et al. obtained 31% ADA using ELISA in 35 patients with ankylosing spondylitis (AS), and ADA were associated with a worse clinical response [5]. In the study by Arends et al., in 20 AS patients, an ADA rate of 30% was obtained using a radioimmunoassay, and ADA were correlated with lower serum adalimumab levels and increased clinical activity [10]. In the study by Paramarta et al., also using a radioimmunoassay, ADA were detected in 23% of 26 patients with peripheral SpA treated with adalimumab [16], but they found no relationship between ADA and clinical response or DMARDs use. One of the reasons for this result could be the limited number of patients. Using ELISA, Kneepkens et al. found ADA in 27% of 115 AS patients, and ADA were correlated with lower adalimumab levels and increased clinical activity [13]. The patients came from two cohorts in the Netherlands and Taiwan, and a higher ADA rate was found

Table 2 Demographic variables and drug level by disease activity assessed by ASDAS-CRP, ASDAS-ESR and BASDAI

	ASDAS-CRP < 2.1 ($n = 25$)	ASDAS-CRP ≥ 2.1 ($n = 21$)	ASDAS-ESR < 2.1 ($n = 19$)	ASDAS-ESR ≥ 2.1 ($n = 26$)	BASDAI < 4 ($n = 20$)	BASDAI ≥ 4 ($n = 30$)
Age (years)	41.9 (18.5–68.5)	49.7 (22.0–67.9)	40.8 (18.5–62.1)	50.7 (22.0–68.5)†	40.8 (18.5–62.1)	49.2 (22.0–67.9)
BASFI	2.1 (0.0–8.7)	6.2 (3.8–8.0)***	1.2 (0.0–4.5)	6.1 (3.1–8.7)†††	1.2 (0.0–6.0)	6.0 (2.4–8.7)§§§
BMI	26.3 (18.8–31.2)	28.7 (19.2–36.1)**	25.5 (18.8–30.4)	28.4 (19.2–36.1)††	26.0 (18.8–31.2)	28.2 (19.2–36.1)§
Time on adalimumab (months)	10.0 (1.4–65.9)	9.3 (1.5–69.1)	12.6 (1.4–66.5)	9.4 (1.5–44.1)	7.2 (1.0–65.9)	11.1 (1.5–69.1)
Adalimumab level (mg/l)	9.3 (0.0–24.0)	0.3 (0.0–14.0)***	9.9 (3.8–19.0)	3.0 (0.0–24.0)†††	9.5 (3.8–19.0)	2.6 (0.0–14.3)§§
ADA (mg/l)	3.5 (0.0–140.0)	35.0 (0.0–2000)***	3.5 (0.0–3.5)	3.5 (0.0–2000.0)††	3.5 (0.0–3.5)	3.5 (0.0–2000.0)§§
ADA [*n* (%)]	1 (4.0)	11 (52.4)***	0(0.0)	12 (46.2)††	0 (0.0)	14 (46.7)§§§
DMARD [*n* (%)]	8 (32.0)	3 (14.2)	6 (31.5)	4 (15.3)	7 (35.0)	3 (10.0)§

All data shown are median (range), otherwise specified

ADA anti-drug antibody, *ASDAS* Ankylosing Spondylitis Disease Activity Score, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *BASFI* Bath Ankylosing Spondylitis Functional Index, *BMI* body mass index, *CRP* C-reactive protein, *DMARD* disease-modifying antirheumatic drug, *ESR* erythrocyte sedimentation rate

$^{**}p < 0.01$, $^{***}p < 0.001$ vs ASDAS-CRP < 2.1; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs ASDAS-ESR < 2.1; $^{\S}p < 0.05$, $^{\S\S}p < 0.01$, $^{\S\S\S}p < 0.001$ vs BASDAI < 4

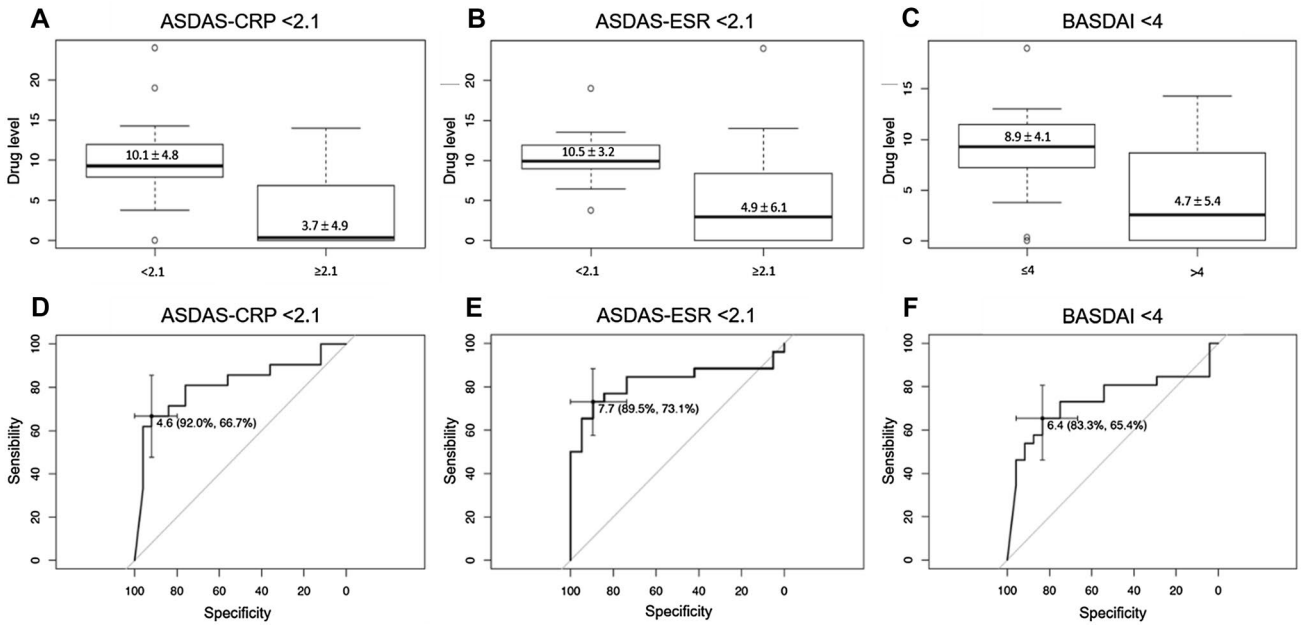

Fig. 1 Drug level by disease activity and ROC curves. *ASDAS* Ankylosing Spondylitis Disease Activity Score, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate. **a** Adalimumab level (mg/dl) by ASDAS-CRP < 2.1. **b** Adalimumab level (mg/dl) by ASDAS-ESR < 2.1. **c** Adalimumab level (mg/dl) by BASDAI < 4. **d** ROC curve of adalimumab level by ASDAS-CRP < 2.1. **e** ROC curve of adalimumab level by ASDAS-ESR < 2.1. **f** ROC curve of adalimumab level by BASDAI < 4

Table 3 Cut-off points and AUC of ROC curves for drug level vs activity

	Cut-off point (mg/l)	Sensitivity (%)	Specificity (%)	AUC (%)	95% CI
ASDAS-CRP < 2.1	4.6	92.0	66.7	81.2	67.5–94.9***
ASDAS-ESR < 2.1	7.7	89.5	73.1	82.4	69.3–95.5***
BASDAI < 4	6.4	83.3	65.4	73.5	58.6–88.3**

ASDAS Ankylosing Spondylitis Disease Activity Score, *AUC* area under the curve, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate

$^{**}p < 0.1$, $^{***}p < 0.001$

in the Taiwanese patients, at 40% vs 22% ($p = 0.06$), who had more severe disease and longer disease duration. The study by Cludts et al. is a prospective trial that used electrochemiluminescence in 21 AS patients, 58% of whom developed ADA. Lower adalimumab levels were found in these patients, but no differences were found in BASDAI scores at either 12 or 24 weeks. However, there was a negative correlation between adalimumab levels and BASDAI score ($r = -0.27$; $p = 0.032$) [14]. Finally, using ELISA, Bornstein et al. found ADA in 13% of 16 patients with axial SpA and correlated this with a worse clinical response [29].

Certain factors can influence serum adalimumab levels and the development of ADA, especially concomitant DMARDs use and BMI. In our study, 21.6% of patients received DMARDs, primarily MTX (15.7%), and none of these patients developed ADA. This correlation has previously been reported in RA studies [30–32], but seldom in axial SpA studies, since their concomitant use is less common. In the study by Plasencia et al. in 94 SpA patients treated with infliximab, development of ADA was more common in patients not receiving concomitant MTX (34% vs 11%; $p = 0.011$) [33]. Patients with non-radiographic SpA could had less clinical activity than AS patients. In our patients, we found a 52.1% prevalence of objective sacroiliitis signs on X-ray, with no differences regarding ADA presence (Table 1). For this reason, X-ray sacroiliitis does not seem to be a bias in our study.

In our patients, the presence of ADA was associated with higher BMI, and the high clinical activity patients, as measured with both the ASDAS and BASDAI scores, had higher BMI (Tables 1 and 2). Other studies have analysed the relationship between BMI and poorer clinical response

in AS or SpA treated with anti-TNF agents. In a previous study in AS patients, BMI > 30 kg/m^2 was associated with lower adalimumab levels and greater clinical activity as measured by ASDAS and BASDAI, but not with the presence of ADA [34]. In the study by Ottaviani et al., of 155 AS patients treated with IFX, at 6 months the patients with the highest BMIs presented the worst BASDAI-50 response [35]. In the study by Simone et al., of 153 patients with AS treated with anti-TNF agents, the female gender and BMI were associated with a lower probability of clinical efficacy (OR 40; 95% CI 4.2–333.3) [36]. In the study by Gremese et al., of 170 patients with AS treated with IFX, adalimumab or etanercept, those with a high BMI achieved a worse clinical response, and the best predictive factor of lack of BASDAI-50 response at 12 months was a BMI > 30 kg/m^2 (OR 3.57; 95% CI 1.15–11.11) [37]. Some authors attribute these findings to adalimumab under-dosing in these patients [38], while another explanation could be an increase in the production of proinflammatory adipokines by fatty tissue [39].

It is relevant to the clinician to know the serum drug level at which adequate disease control is achieved. This question can be answered using ROC curves and obtaining the optimum serum drug level cut-off point to discriminate patients with low or moderate activity vs high activity. In our study, we obtained different cut-off points depending on the index used: 4.6 mg/l, 7.7 mg/l and 6.4 mg/l for ASDAS-CRP, ASDAS-ESR and BASDAI, respectively. To our knowledge, this is the first study to investigate this serum adalimumab level cut-off point in SpA patients. In RA patients, using ELISA, a serum adalimumab level cut-off point of > 4.3 mg/l was optimum to differentiate responders with a DAS28-ESR < 3.2 [31]. Other studies have investigated the serum adalimumab level that predicts persistent clinical remission after dose reduction in RA. Chen et al. obtain a serum adalimumab cut-off point of > 6.4 mg/l using DAS28-ESR [40], while Bouman et al. obtain > 7.8 mg/l using DAS28-CRP [41]. In Crohn's disease, other studies have investigated the serum adalimumab level cut-off point that best predicts clinical remission, with levels of > 5.85 mg/l reported by Mazor et al. [42] and > 5.0 mg/l by Nakase et al. [43] The ELISA technique was used in all of these studies. Another study, using the liquid-phase mobility shift assay technique, found a cut-off point of > 8.14 mg/l to distinguish patients with Crohn's disease in whom mucosal lesions had disappeared on the ileocolonoscopy [44]. Lastly, in patients with psoriasis, a serum adalimumab level > 7.84 mg/l, using ELISA, was the optimum to discriminate patients with PASI scores ≥ 75 [45].

This variability could exist for a number of reasons. First, the activity indices include different parameters. In the case of the BASDAI, they are completely subjective, while the ASDAS and DAS28 include objective analytical parameters. In addition, the threshold used to classify patients as responders determines a lower adalimumab level cut-off point on ROC curves. In our study, we chose the ASDAS threshold of < 2.1, classified as moderate activity, rather than < 1.3, classified as inactive disease, considering the latter to be too stringent. This choice raised the number of patients classified as responders, leading to a lower cut-off point on the ROC curves. Finally, it is likely that patients with SpA may need higher adalimumab levels to achieve an adequate clinical response, compared to patients with RA, since the majority of RA patients use DMARDs concomitantly.

One of the limitations of our study is its sample size. Although all patients who met the criteria were included consecutively, the estimated sample size was not reached. Nevertheless, a post-hoc analysis showed a power > 90% to find differences in adalimumab serum levels by disease activity. On the other hand, this was a cross-sectional study and causal relationships cannot be directly established. Other limitation of our study could be the presence of confounding factors as concomitant nonsteroidal anti-inflammatory drug (NSAID) intake or concurrence of other causes of pain, such as osteoarthritis, since both can influence the measure of SpA activity. Unfortunately, no data were collected about it.

We recognise the difficulty of measuring drug levels in routine clinical practice but knowing it may help us to detect non-responder patients, as well as to avoid dose reduction in patients with low drug levels. Additionally, it could help us to detect non-adherent patients, when neither the drug nor ADA was found, despite performing acid dissociation.

To conclude, in our study we found ADA in 27.5% of SpA patients treated with adalimumab, which is associated with lower serum adalimumab levels and greater clinical activity based on the BASDAI and ASDAS indices. Concomitant treatment with MTX reduces the likelihood of finding ADA. The adalimumab serum cut-off point to achieve optimum disease control according to the ASDAS-CRP index was 4.6 mg/l.

Acknowledgements Jordi Bozzo Ph.D. CMPP (Grifols, S.A.) is acknowledged for his editorial assistance in the preparation of the manuscript.

Author contributions JMS-G: conception, design, interpretation of data, writing original draft, final approval. JR: conception, data acquisition (patient recruitment), review and editing draft. MM-M: laboratory analysis. JAG-G: statistical analysis. GS-S: data acquisition (patient recruitment). ES-H: data acquisition (patient recruitment). AP-B: study coordination and data acquisition (sample collection). XB-V: statistical analysis. JAB-V: data acquisition (patient recruitment). CC-P: data acquisition (sample collection). MG-C: conception, review and editing draft. EF-P: conception, design, review and editing draft.

Funding The study was supported by a grant from "Asociación para la Investigación en Reumatología de la Marina Baixa" (AIRE-MB) and from the "Fundación Española de Reumatología" (FER 2012). The funds were used for the acquisition of the ELISA kits.

Compliance with ethical standards

Conflict of interest Dr. Senabre Gallego reports grants from Fundación Española de Reumatología, grants from Asociación para la Investigación en Reumatología de la Marina Baixa, assistance with translation of the manuscript from Grifols, S.A., during the conduct of the study; personal fees and non-financial support from Abbvie, non-financial support from BMS, personal fees from Celgene, personal fees from Janssen, non-financial support from Lilly, non-financial support from MSD, personal fees from Novartis, non-financial support from Pfizer, non-financial support from Roche, non-financial support from UCB, outside the submitted work. Dr. Rosas reports payment for the development of educational presentations including service on speakers' bureaus from Abbvie, MSD, Pfizer and UCB. Dr. Santos Soler reports travel/accommodations expenses covered from Pfizer, outside the submitted work. Mrs. Pons Bas reports travel/accommodations expenses covered from Pfizer, outside the submitted work. Dr. Bernal Vidal reports travel/accommodations expenses covered from Pfizer, outside the submitted work. Mrs. Cano Pérez reports travel/accommodations expenses covered from Pfizer, outside the submitted work.

Ethical approval The study was approved by our local Ethics Committee for Clinical Research of Hospital de San Juan, Alicante. All study procedures were performed according to the ethical principles of the Declaration of Helsinki and Good Clinical Practice.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. van der Heijde D, Sieper J, Maksymowych WP, Dougados M, Burgos-Vargas R, Landewé R, Rudwaleit M, Braun J (2011) 2010 Update of the international ASAS recommendations for the use of anti-TNF agents in patients with axial spondyloarthritis. Ann Rheum Dis 70(6):905–908. https://doi.org/10.1136/ard.2011.151563
2. van der Heijde D, Schiff MH, Sieper J, Kivitz AJ, Wong RL, Kupper H, Dijkmans BA, Mease PJ, Davis JC (2009) Adalimumab effectiveness for the treatment of ankylosing spondylitis is maintained for up to 2 years: long-term results from the ATLAS trial. Ann Rheum Dis 68(6):922–929. https://doi.org/10.1136/ard.2007.087270
3. van der Heijde D, Dijkmans B, Geusens P, Sieper J, DeWoody K, Williamson P, Braun J (2005) Efficacy and safety of infliximab in patients with ankylosing spondylitis: results of a randomized, placebo-controlled trial (ASSERT). Arthritis Rheumatol 52(2):582–591. https://doi.org/10.1002/art.20852
4. Senabre-Gallego JM, Santos-Ramirez C, Santos-Soler G, Salas-Heredia E, Sanchez-Barrioluengo M, Barber X, Rosas J (2013) Long-term safety and efficacy of etanercept in the treatment of ankylosing spondylitis. Patient Prefer Adherence 7:961–972
5. de Vries MK, Brouwer E, van der Horst-Bruinsma IE, Spoorenberg A, van Denderen JC, Jamnitski A, Nurmohamed MT, Dijkmans BA, Aarden LA, Wolbink GJ (2009) Decreased clinical response to adalimumab in ankylosing spondylitis is associated with antibody formation. Ann Rheum Dis 68(11):1787–1788. https://doi.org/10.1136/ard.2009.109702
6. Martín-López M, Carmona L, Balsa A, Calvo-Alén J, Sanmartí R, Tornero J, Rosas J (2018) Serum drug levels of biologic agents in the management of rheumatoid arthritis and spondyloarthritis: a systematic review. Rheumatol Int 38(6):975–983. https://doi.org/10.1007/s00296-018-4022-4
7. Navarro-Compán V, Plasencia-Rodríguez C, de Miguel E, Balsa A, Martín-Mola E, Seoane-Mato D, Cañete JD (2016) Anti-TNF discontinuation and tapering strategies in patients with axial spondyloarthritis: a systematic literature review. Rheumatology 55(7):1188–1194. https://doi.org/10.1093/rheumatology/kew033
8. Garcês S, Demengeot J, Benito-Garcia E (2013) The immunogenicity of anti-TNF therapy in immune-mediated inflammatory diseases: a systematic review of the literature with a meta-analysis. Ann Rheum Dis 72(12):1947–1955. https://doi.org/10.1136/annrheumdis-2012-202220
9. Maneiro JR, Salgado E, Gomez-Reino JJ (2013) Immunogenicity of monoclonal antibodies against tumor necrosis factor used in chronic immune-mediated Inflammatory conditions: systematic review and meta-analysis. JAMA Intern Med 173(15):1416–1428. https://doi.org/10.1001/jamainternmed.2013.7430
10. Arends S, Lebbink HR, Spoorenberg A, Bungener LB, Roozendaal C, van der Veer E, Houtman PM, Griep EN, Limburg PC, Kallenberg CG, Wolbink GJ, Brouwer E (2010) The formation of autoantibodies and antibodies to TNF-α blocking agents in relation to clinical response in patients with ankylosing spondylitis. Clin Exp Rheumatol 28(5):661–668
11. de Vries MK, Wolbink GJ, Stapel SO, de Groot ER, Dijkmans BA, Aarden LA, van der Horst-Bruinsma IE (2007) Inefficacy of infliximab in ankylosing spondylitis is correlated with antibody formation. Ann Rheum Dis 66(1):133–134. https://doi.org/10.1136/ard.2006.057745
12. Krzysiek R, Breban M, Ravaud P, Prejean MV, Wijdenes J, Roy C, Henry YD, Barbey C, Trappe G, Dougados M, Emilie D (2009) Circulating concentration of infliximab and response to treatment in ankylosing spondylitis: results from a randomized control study. Arthritis Rheumatol 61(5):569–576. https://doi.org/10.1002/art.24275
13. Kneepkens EL, Wei JC, Nurmohamed MT, Yeo KJ, Chen CY, van der Horst-Bruinsma IE, van der Kleij D, Rispens T, Wolbink G, Krieckaert CL (2015) Immunogenicity, adalimumab levels and clinical response in ankylosing spondylitis patients during 24 weeks of follow-up. Ann Rheum Dis 74(2):396–401. https://doi.org/10.1136/annrheumdis-2013-204185
14. Cludts I, Spinelli FR, Morello F, Hockley J, Valesini G, Wadhwa M (2017) Anti-therapeutic antibodies and their clinical impact in patients treated with the TNF antagonist adalimumab. Cytokine 96:16–23. https://doi.org/10.1016/j.cyto.2017.02.015
15. Bornstein G, Lidar M, Langevitz P, Fardman A, Ben-Zvi I, Grossman C (2018) The prevalence and clinical effect of immunogenicity of TNF-alpha blockers in patients with axial spondyloarthritis. Clin Exp Rheumatol 36(2):228–232
16. Paramarta JE, Baeten DL (2014) 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. Arthritis Res Ther 16(4):R160. https://doi.org/10.1186/ar4675
17. de Vries MK, van der Horst-Bruinsma IE, Nurmohamed MT, Aarden LA, Stapel SO, Peters MJ, van Denderen JC, Dijkmans BA, Wolbink GJ (2009) Immunogenicity does not influence treatment with etanercept in patients with ankylosing spondylitis. Ann Rheum Dis 68(4):531–535. https://doi.org/10.1136/ard.2008.089979
18. Inman RD, Davis JC, Heijde D, Diekman L, Sieper J, Kim SI, Mack M, Han J, Visvanathan S, Xu Z, Hsu B, Beutler A, Braun J (2008) Efficacy and safety of golimumab in patients with ankylosing spondylitis: results of a randomized, double-blind, placebo-controlled, phase III trial. Arthritis Rheumatol 58(11):3402–3412. https://doi.org/10.1002/art.23969
19. Gehin JE, Goll GL, Warren DJ, Syversen SW, Sexton J, Strand EK, Kvien TK, Bolstad N, Lie E (2018) FRI0645 Anti-drug antibodies to certolizumab pegol are associated with low drug levels

and reduced clinical response at 3 months in patients with inflammatory joint diseases. Data from the NOR-DMARD study. Ann Rheum Dis 77(Suppl 2):844. https://doi.org/10.1136/annrheumdis-2018-eular.5257
20. Martínez-Feito A, Plasencia-Rodriguez C, Navarro-Compán V, Jurado T, Kneepkens EL, Wolbink GJ, Martín S, Ruiz Del Agua A, Navarro R, Mezcua A, Jochems A, Peiteado D, Bonilla MG, Balsa A, Pascual-Salcedo D (2018) Optimal concentration range of golimumab in patients with axial spondyloarthritis. Clin Exp Rheumatol 36(1):110–114
21. Rudwaleit M, van der Heijde D, Landewé R, Listing J, Akkoc N, Brandt J, Braun J, Chou CT, Collantes-Estevez E, Dougados M, Huang F, Gu J, Khan MA, Kirazli Y, Maksymowych WP, Mielants H, Sørensen IJ, Ozgocmen S, Roussou E, Valle-Oñate R, Weber U, Wei J, Sieper J (2009) The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part II): validation and final selection. Ann Rheum Dis 68(6):777–783. https://doi.org/10.1136/ard.2009.108233
22. European Medicines Agency (2008) Humira® EPAR—Product Information. https://www.ema.europa.eu/documents/product-information/humira-epar-product-information_en.pdf, Accessed Dec 2017
23. Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A (1994) A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol 21(12):2286–2291
24. van der Heijde D, Lie E, Kvien TK, Sieper J, Van den Bosch F, Listing J, Braun J, Landewé R, (ASAS) AoSiS (2009) ASDAS, a highly discriminatory ASAS-endorsed disease activity score in patients with ankylosing spondylitis. Ann Rheum Dis 68 (12):1811–1818. https://doi.org/10.1136/ard.2008.100826
25. Llinares-Tello F, Rosas J, Senabre-Gallego J, Santos-Soler G, Santos-Ramirez C, Salas-Heredia E, Molina-Garcia J (2012) Analytical and clinical evaluation of a new immunoassay for therapeutic drug monitoring of infliximab and adalimumab. Clin Chem Lab Med 50(10):1845–1847. https://doi.org/10.1515/cclm-2012-0050
26. Llinares-Tello F, Rosas J, De La Torre I, Valor L, Senabre JM, Barber X, Hernandez D, Carreno L, Santos-Soler G, Salas E, Santos-Ramirez C, Sanchez-Barrioluengo M, Molina-Garcia J (2013) Comparative study of both versions of an immunoassay commercialized for therapeutic drug monitoring of adalimumab. Ann Rheum Dis 72:A234
27. Ruiz-Argüello B, del Agua AR, Torres N, Monasterio A, Martínez A, Nagore D (2013) Comparison study of two commercially available methods for the determination of infliximab, adalimumab, etanercept and anti-drug antibody levels. Clin Chem Lab Med 51(12):e287–e289. https://doi.org/10.1515/cclm-2013-0461
28. Llinares-Tello F, Rosas-Gómez de Salazar J, Senabre-Gallego JM, Santos-Soler G, Santos-Ramírez C, Salas-Heredia E, Barber-Vallés X, Molina-García J (2014) Practical application of acid dissociation in monitoring patients treated with adalimumab. Rheumatol Int 34(12):1701–1708. https://doi.org/10.1007/s00296-014-3032-0
29. Bornstein G, Lidar M, Langevitz P, Fardman A, Ben-Zvi I, Grossman C (2018) The prevalence and clinical effect of immunogenicity of TNF-α blockers in patients with axial spondyloarthritis. Clin Exp Rheumatol 36:228–232
30. Bartelds GM, Krieckaert CL, Nurmohamed MT, van Schouwenburg PA, Lems WF, Twisk JW, Dijkmans BA, Aarden L, Wolbink GJ (2011) Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. JAMA 305(14):1460–1468. https://doi.org/10.1001/jama.2011.406
31. Rosas J, Llinares-Tello F, de la Torre I, Santos-Ramírez C, Senabre-Gallego JM, Valor L, Barber-Vallés X, Hernández-Flórez D, Santos-Soler G, Salas-Heredia E, Carreño L (2014) Clinical relevance of monitoring serum levels of adalimumab in patients with rheumatoid arthritis in daily practice. Clin Exp Rheumatol 32(6):942–948
32. Krieckaert CL, Nurmohamed MT, Wolbink GJ (2012) Methotrexate reduces immunogenicity in adalimumab treated rheumatoid arthritis patients in a dose dependent manner. Ann Rheum Dis 71(11):1914–1915. https://doi.org/10.1136/annrheumdis-2012-201544
33. Plasencia C, Pascual-Salcedo D, Nuño L, Bonilla G, Villalba A, Peiteado D, Díez J, Nagore D, del Agua AR, Moral R, Martin-Mola E, Balsa A (2012) Influence of immunogenicity on the efficacy of longterm treatment of spondyloarthritis with infliximab. Ann Rheum Dis 71(12):1955–1960. https://doi.org/10.1136/annrheumdis-2011-200828
34. Rosas J, Llinares-Tello F, Senabre-Gallego JM, Barber-Vallés X, Santos-Soler G, Salas-Heredia E, Pons Bas A, Cano Pérez C, García-Carrasco M, Group atA-M (2017) Obesity decreases clinical efficacy and levels of adalimumab in patients with ankylosing spondylitis. Clin Exp Rheumatol 35(1):145–148
35. Ottaviani S, Allanore Y, Tubach F, Forien M, Gardette A, Pasquet B, Palazzo E, Meunier M, Hayem G, Job-Deslandre C, Kahan A, Meyer O, Dieudé P (2012) Body mass index influences the response to infliximab in ankylosing spondylitis. Arthritis Res Ther 14(3):R115. https://doi.org/10.1186/ar3841
36. Simone D, Canestri S, Nowik M, Messuti L, Miceli M, Gremese E, Di Mario C, Tolusso B, Ferraccioli G (2014) Genetic and clinical predictors of response to TNF blocker in an Italian axial-SPA cohort. Clin Exp Rheumatol 32:802
37. Gremese E, Bernardi S, Bonazza S, Nowik M, Peluso G, Massara A, Tolusso B, Messuti L, Miceli MC, Zoli A, Trotta F, Govoni M, Ferraccioli G (2014) Body weight, gender and response to TNF-α blockers in axial spondyloarthritis. Rheumatology 53(5):875–881. https://doi.org/10.1093/rheumatology/ket433
38. Micheroli R, Hebeisen M, Wildi LM, Exer P, Tamborrini G, Bernhard J, Möller B, Zufferey P, Nissen MJ, Scherer A, Ciurea A (2017) Impact of obesity on the response to tumor necrosis factor inhibitors in axial spondyloarthritis. Arthritis Res Ther 19(1):164. https://doi.org/10.1186/s13075-017-1372-3
39. Gómez R, Conde J, Scotece M, Gómez-Reino JJ, Lago F, Gualillo O (2011) What's new in our understanding of the role of adipokines in rheumatic diseases? Nat Rev Rheumatol 7(9):528–536. https://doi.org/10.1038/nrrheum.2011.107
40. Chen DY, Chen YM, Hsieh TY, Hung WT, Hsieh CW, Chen HH, Tang KT, Lan JL (2016) Drug trough levels predict therapeutic responses to dose reduction of adalimumab for rheumatoid arthritis patients during 24 weeks of follow-up. Rheumatology 55(1):143–148. https://doi.org/10.1093/rheumatology/kev298
41. Bouman C, van Herwaarden N, van den Hoogen F, van der Maas A, van den Bemt B, den Broeder AA (2017) Prediction of successful dose reduction or discontinuation of adalimumab, etanercept, or infliximab in rheumatoid arthritis patients using serum drug levels and antidrug antibody measurement. Expert Opin Drug Metab Toxicol 13(6):597–604. https://doi.org/10.1080/17425255.2017.1320390
42. Mazor Y, Almog R, Kopylov U, Ben Hur D, Blatt A, Dahan A, Waterman M, Ben-Horin S, Chowers Y (2014) Adalimumab drug and antibody levels as predictors of clinical and laboratory response in patients with Crohn's disease. Aliment Pharmacol Ther 40(6):620–628. https://doi.org/10.1111/apt.12869
43. Nakase H, Motoya S, Matsumoto T, Watanabe K, Hisamatsu T, Yoshimura N, Ishida T, Kato S, Nakagawa T, Esaki M, Nagahori M, Matsui T, Naito Y, Kanai T, Suzuki Y, Nojima M, Watanabe M, Hibi T (2017) Significance of measurement of serum trough level and anti-drug antibody of adalimumab as personalised pharmacokinetics in patients with Crohn's disease: a subanalysis of the

DIAMOND trial. Aliment Pharmacol Ther 46(9):873–882. https://doi.org/10.1111/apt.14318
44. Zittan E, Kabakchiev B, Milgrom R, Nguyen GC, Croitoru K, Steinhart AH, Silverberg MS (2016) Higher adalimumab drug levels are associated with mucosal healing in patients with Crohn's disease. J Crohns Colitis 10(5):510–515. https://doi.org/10.1093/ecco-jcc/jjw014
45. Takahashi H, Tsuji H, Ishida-Yamamoto A, Iizuka H (2013) Plasma trough levels of adalimumab and infliximab in terms of clinical efficacy during the treatment of psoriasis. J Dermatol 40(1):39–42. https://doi.org/10.1111/j.1346-8138.2012.01679.x

Affiliations

José Miguel Senabre Gallego[1] · Jose Rosas[1] · Mariana Marco-Mingot[2] · José Alberto García-Gómez[3] · Gregorio Santos-Soler[1] · Esteban Salas-Heredia[1] · Ana Pons-Bas[1] · Xavier Barber-Vallés[4] · José Antonio Bernal-Vidal[1] · Catalina Cano-Pérez[1] · Mario García-Carrasco[5,6] · Emilio Flores-Pardo[7] · AIRE-MB Group

Jose Rosas
j.rosas.gs@gmail.com

Mariana Marco-Mingot
marco_marmin@gva.es

José Alberto García-Gómez
codicealberto@gmail.com

Gregorio Santos-Soler
santosgre@gmail.com

Esteban Salas-Heredia
esalasheredia@gmail.com

Ana Pons-Bas
cuartes@hotmail.com

Xavier Barber-Vallés
xbarber@goumh.umh.es

José Antonio Bernal-Vidal
josantonio.bernal@gmail.com

Catalina Cano-Pérez
linetacape@hotmail.com

Mario García-Carrasco
mgc30591@yahoo.com

Emilio Flores-Pardo
eflores@umh.es

1 Sección de Reumatología, Hospital Marina Baixa, Av. Alcalde En Jaume Botella Mayor 7, 03570 Villajoyosa, Alicante, Spain

2 Laboratory Department, Hospital Marina Baixa, Villajoyosa, Alicante, Spain

3 Infectious Disease Department, Hospital General Universitario de Elche, Elche, Alicante, Spain

4 Center of Operations Research, Universitas Miguel Hernández, Elche, Alicante, Spain

5 Systemic Autoimmune Disease Research Unit, HGR-36-CIBIOR IMSS, Puebla, Mexico

6 Department of Rheumatology and Immunology, Benemérita Universidad Autónoma de Puebla, Puebla, Mexico

7 Clinical Medicine Department, Universitas Miguel Hernández, Elche, Alicante, Spain