ARTICLE

Early Adalimumab and Anti-Adalimumab Antibody Levels for Prediction of Primary Nonresponse in Ankylosing Spondylitis Patients

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This study aimed at exploring the concentration-effect relationship of adalimumab and early adalimumab and anti-adalimumab antibody (AAA) levels in predicting primary nonresponse in a real-world pilot cohort of patients with ankylosing spondylitis. Thirty-one patients were included. The Ankylosing Spondylitis Disease Activity Score improved with increasing adalimumab trough level at week 12 and reached a major improvement with levels between 8 and 12 μg/mL. Moreover, weeks 4 and 2 adalimumab levels below 4.28 and 3.37 μg/mL were predictive of primary nonresponse (area under the curve (AUC) = 0.89, 0.88; $P = 0.0003$, $P = 0.034$, respectively). Week 4 AAA signal-to-noise levels were significantly higher among primary nonresponders, and the cutoff for primary nonresponse prediction was above 5.31 (AUC = 0.81; $P = 0.004$). Adalimumab trough levels in a range of 8–12 μg/mL are optimum to reach major improvement, and lower adalimumab with higher AAA levels at the early stage (week 4) predict primary nonresponse by supporting proactive monitoring to optimize adalimumab therapy.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ The concentration-effect relationship of adalimumab was previously established in several immune-mediated inflammatory diseases. However, this has not yet been validated in patients with ankylosing spondylitis (AS). Furthermore, the data regarding the role of proactive therapeutic drug monitoring of adalimumab at an early stage in patients with AS are limited.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ A concentration-effect curve of adalimumab in patients with AS and early adalimumab and anti-adalimumab antibody (AAA) levels in predicting primary nonresponse were explored.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ Adalimumab trough levels in a range of 8–12 μg/mL are optimal. Early adalimumab levels (at week 4, even at week 2) or AAA levels at week 4 can be used to predict primary nonresponse.

HOW MIGHT THIS CHANGE CLINICAL PHARMA-COLOGY OR TRANSLATIONAL SCIENCE?
☑ The results of this study suggest that adalimumab and AAA levels taken at an early stage may help physicians to prevent ineffective therapy, and measurement at steady-state may be a useful guide to reduce overtreatment and health care costs by supporting proactive monitoring to optimize adalimumab therapy.

Ankylosing spondylitis (AS) is an inflammatory arthritis of the axial skeleton. Patients with AS experience significant pain, stiffness, and lack of function that translates into important health care costs and increased mortality. Patients should be considered for antitumor necrosis factor α (TNF-α) therapy if they have active AS and have failed to respond to nonsteroidal anti-inflammatory drugs.[1,2]

Adalimumab, a humanized anti-TNF-α antibody, is effective in the treatment of AS and other autoimmune diseases. However, a substantial proportion (~ 30–40%) of patients

with AS show no clinical benefit and are considered primary nonresponders to adalimumab.[3,4] The mechanisms underlying primary nonresponse have not been clearly defined thus far. Low adalimumab concentration and the presence of anti-adalimumab antibodies (AAAs) may be important contributors. A concentration-effect curve was previously established in adalimumab-treated patients with rheumatoid arthritis (RA),[5] psoriatic arthritis (PsA),[6] psoriasis (PsO),[7,8] and inflammatory bowel disease (IBD).[9] However, this has not yet been validated in patients with

AS. Therefore, drug monitoring of adalimumab in patients with AS requires a better understanding of the association among the drug level, the AAA level, and the clinical response of adalimumab.

In the recently published guidelines used to inform appropriate utilization of therapeutic drug monitoring (TDM) with anti-TNF-α agents,[10] the American Gastroenterological Association advocates reactive TDM but makes no recommendation regarding the use of routine proactive TDM. Recent studies have shown the impact of low adalimumab levels after therapy induction on the clinical response in patients with IBD.[11] In addition, there is a variety of approved adalimumab dosing regimens in patients with IBD and AS (therapy induction with 160 mg loading dose and 80 mg subcutaneously at weeks 0 and 2 in patients with IBD, whereas 40 mg every other week is recommended in patients with AS). To our knowledge, the data regarding the role of proactive TDM of adalimumab at an early stage in patients with AS are limited.

The aims of the present study were, first, to determine a concentration-effect curve in patients with AS receiving scheduled adalimumab therapy, thus providing a therapeutic concentration range, and, second, to determine to which extent early adalimumab and AAA levels can predict primary nonresponse.

METHOD

Study design and patients

We conducted this observational cohort study consisting of 31 patients with AS (according to the modified 1984 New York Criteria) with prior documented radiologic evidence (X-ray) who received adalimumab therapy at the Department of Rheumatology, the First Affiliated Hospital of Soochow University (Suzhou, China). All patients were enrolled between December 2017 and August 2018, and who had active disease of at least 4.0 as indicated by the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). Patients were treated either with concomitant medication, including nonsteroidal anti-inflammatory drugs or sulfasalazine therapy, or with adalimumab monotherapy. None of the patients had previously received adalimumab. All patients received 40 mg of adalimumab subcutaneously every other week in outpatient clinics and were evaluated by a physician at baseline and 2, 4, 8, and 12 weeks. Blood samples were drawn for measurement of C-reactive protein at each evaluation visit before adalimumab administration, and then were frozen for determination of adalimumab and AAA levels. The study was approved by the Institutional Review Board of the First Affiliated Hospital of Soochow University, and all patients gave written informed consent.

Clinical response

Disease activity was assessed at baseline and after 2, 4, 8, and 12 weeks of treatment using BASDAI or Ankylosing Spondylitis Disease Activity Score (ASDAS) using C-reactive protein. Primary responders were defined as those who had either a decrease in ASDAS from baseline (ΔASDAS) ≥ 2.0 or a moderate disease activity achievement (ASDAS < 2.1) with ΔASDAS ≥ 1.1 by week 12.

Measurement of adalimumab concentrations

Plasma concentration was measured using a validated, indirect enzyme-linked immunosorbent assay method. Microtiter plates (Corning, Corning, NY) were coated with 1.6 µg/mL of rhTNF-α (PeproTech, Rocky Hill, NJ), and the drug was detected with a goat anti-human immunoglobulin G Fcγ-specific antibody conjugated to horseradish peroxidase (Sigma-Aldrich, St. Louis, MO). The limit of detection and lower limit of quantification of the assay were 0.43 and 0.63 µg/mL, respectively. The standard curve fitting with a 4-parameter curve ranged from 0.6320 µg/mL. A 10-fold and 100-fold dilution factor was validated at the 150 µg/mL level. The five quality controls (0.63, 1.50, 4.00, 15.00, and 20.00 µg/mL) were tested for intra-assay and interassay precision on six occasions. The coefficients of variation from the intra-assay were 8.9%, 1.3%, 1.31%, 7.89%, and 1.84%. The corresponding biases were 4.47%, −0.65%, −0.53%, −8.41%, and −1.82%, respectively. The interassay variabilities were 14.80%, 9.76%, 4.08%, 5.61%, and 3.80%, respectively. The corresponding biases were −7.08%, −4.34%, −1.41%, −2.12%, and −1.07%, respectively.

Measurement of AAA concentrations

AAA concentration was measured using an in-house high drug-tolerant assay modified according to the reports.[12–14] We developed a simple biotin-drug extraction and acid dissociation procedure to extract total AAAs to overcome interference of free drug and target antibody, as shown in **Figure S1**.

Serum samples were pretreated with acid dissociation (50 µL 300 mM acetic acid added to 100 µL of 10-fold diluted serum sample) to free total AAAs from all nonspecific or specific binding partners. One hundred microliters of biotinylated adalimumab (EZ-Link Sulfo-NHS-LC-Biotinylation Kit; Thermo Scientific, Rockford, IL) containing 25% 1 M Tris-HCl pH 8.8 and 50 µL of 2 mg/mL streptavidin-coated magnetic beads (Dynabeads MyOne Streptavidin T1; Invitrogen) was added successively to form biotin-adalimumab/AAA/bead complexes. After washing, AAAs were dissociated from complexes using acid (100 µL of 300 mM acetic acid) and then coated on a new microtiter plate (Corning). Plate-bound AAA was detected by adalimumab conjugated to horseradish peroxidase. HCA204 (human anti-adalimumab, clone AbD18655_hIgG1; Bio-Rad, Munich, Germany) was used as a positive control of AAAs in the present study. The method was validated using standard bioanalytical parameters and target acceptance criteria.[15–17] The preliminary validation was carried out with 51 normal human sera. The screening cutoffpoint factor was 1.122, and confirmatory cutoff point was established at 29.10% inhibition when spiked 10 mg/mL of adalimumab. Mass-based sensitivity was 32 ng/mL of positive control. Drug tolerance was up to 50 µg/mL of adalimumab for 500 ng/mL of positive control. Target tolerance was up to 500 ng/mL (4 µg/mL of positive control and 10 µg/mL of adalimumab). When individuals were spiked with 500 ng/mL of positive control, 80% of the individuals recovered 75–125% of the positive control. Of the 31 disease matrix samples from untreated

patients that were screened and confirmed, two were positive for AAA. The signal-to-noise (S/N) ratio between the patient and normal matrix samples were similar (median 0.82 vs. 0.87; *P* = 0.85), indicating that the same cutoff point can be applied. Sample was defined as positive by S/N value > 1.122 and percent inhibition > 29.10%, and assay S/N was used for assessment of the AAA magnitude (negative sample was expressed as S/N = 1).

Statistical analysis

Continuous variables were expressed as median and interquartile range (IQR), and categorical variables were expressed as a percentage. Unpaired continuous variables were compared using the MannWhitney *U* test. To establish a concentration-effect curve at 12 weeks of treatment, all 31 patients were sorted from low to high adalimumab levels with correlating ΔASDAS and ΔBASDAI. These data were stratified into six groups of five patients (last group six patients), giving a mean trough level and a mean ΔASDAS and ΔBASDAI. Diagnostic performance was assessed with receiver operating characteristic (ROC) curve analysis. A clinically relevant threshold value was determined by the Youden index most accurate point. A two-tailed *P* value < 0.05 was considered statistically significant. All statistics and graphical figures were performed with GraphPad Prism 8 (La Jolla, CA).

RESULTS
Patient characteristics and clinical outcomes

Thirty-one patients with AS were included in the present study. All patients completed a 12-week follow-up and disease evaluation. Patient characteristics are shown in **Table 1**. Twelve (38.7%) patients experienced primary nonresponse.

Adalimumab and AAA levels

Of the 107 serum samples obtained from 31 patients at predose (*n* = 31), week 2 (*n* = 14), week 4 (*n* = 31), and week 12 (*n* = 31) analyzed in this study, 43 samples from 21 patients (67.7%) were defined as AAA-positive. There were high levels of pre-existing AAA in 2 patients, 16 patients developed stable AAA, and 5 patients developed transient AAA. Stable AAAs were defined by two consecutive positive AAAs at weeks 4 and 12, whereas transient AAAs were defined as the presence of only one positive AAA at weeks 4 and 12. At week 2, serum samples were drawn from 14 patients, and only 4 patients developed AAA.

Of the 137 serum samples obtained from 31 patients analyzed in this study, adalimumab was not detectable in any of the baseline samples. Of the serum samples available after administration, nine samples revealed a serum level below the lower limit of quantification. The adalimumab levels over time for patients with or without AAA are shown in **Figure 1**. Patients who were AAA-negative had significantly higher adalimumab levels than patients who were AAA-positive (week 4: median 7.53 μg/mL IQR 5.94–8.30 vs. 3.57 μg/mL IQR 2.33–6.42, respectively, *P* = 0.001; week 8: 11.35 μg/mL IQR 9.76–16.03 vs. 5.85 μg/mL IQR 2.69–10.07, *P* = 0.001; week 12: 16.57 μg/mL IQR 11.97–19.37 vs. 7.41 μg/mL IQR 3.07–12.22, *P* = 0.0005, **Figure 1a**). Patients who were AAA-positive can be divided into two parts, stable AAA and transient AAA. Median adalimumab trough levels at weeks 4, 8, and 12 was lower in patients who developed stable AAA as compared with those with AAA-negative or transient AAA (week 4: median 3.14 μg/mL IQR 1.46–5.21 vs. 7.53 μg/mL IQR 5.94–8.30 vs. 6.49 μg/mL IQR 5.77–7.76, respectively, *P* = 0.0006, *P* = 0.042; week 8: 4.64 μg/mL IQR 1.69–6.22 vs. 11.35 μg/mL IQR 9.76–16.03 vs. 10.89 μg/mL IQR 7.83–13.26, *P* = 0.0007, *P* = 0.059; week 12: 5.30 μg/mL IQR 1.52–9.24 vs. 16.57 μg/mL IQR 11.97–19.37 vs. 14.42 μg/mL IQR 10.93–16.02, *P* = 0.0003, *P* = 0.049); there was no statistical difference between patients with AAA-negative and transient AAA (**Figure 1b**).

Table 1 Demographic data and baseline characteristics

	Total patients (*n* = 31)	Primary responder (*n* = 19)	Primary nonresponder (*n* = 12)
Demographics			
Age, median (IQR), years	31 (28–37)	31 (26–35)	31 (28.25–38)
Male, *n* (%)	29 (93.5)	19 (100)	10 (83.3)
BMI, median (IQR)	23.0 (21.2–26.3)	21.8 (20.6–25.9)	23.7 (22.0–27.4)
Disease status			
Disease duration, median (IQR), years	7 (3–10)	7 (2–10)	9 (6.25–10.75)
CRP, median (IQR), mg/L	16.40 (9.57–46.70)	22.3 (13.8–75.9)	10.84 (5.57–26.85)
ESR, median (IQR), mm/hour	52 (33–101)	70 (42–108)	48 (29–76)
ASDAS-CRP, median (IQR)	4.06 (3.54–4.75)	4.12 (3.75–5.08)	3.87 (3.28–4.26)
BASDAI, median (IQR)	6.10 (5.20–7.50)	6.15 (5.20–8.00)	5.97 (5.13–6.88)
DMARD therapy			
NSAID use, *n* (%)	15 (48.4)	9 (47.4)	6 (50.0)
Sulfasalazine use, *n* (%)	3 (9.7)	2 (10.5)	1 (8.3)
Methotrexate use, *n* (%)	1 (3.2)	1 (5.3)	0 (0.0)

ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score using CRP; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BMI, body mass index; CRP, C-reactive protein; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; IQR, interquartile range; NSAID, nonsteroidal anti-inflammatory drug.

Clinical response and adalimumab

In **Figure 2a**, the relationship between adalimumab trough levels at week 12 and ΔASDAS is shown. All 31 patients were sorted from low to high adalimumab level, with each dot representing the mean concentration and correlating ASDAS improvement compared with baseline per five patients (the last dot is six patients), with SDs showing intervariability between patients. To reach clinically
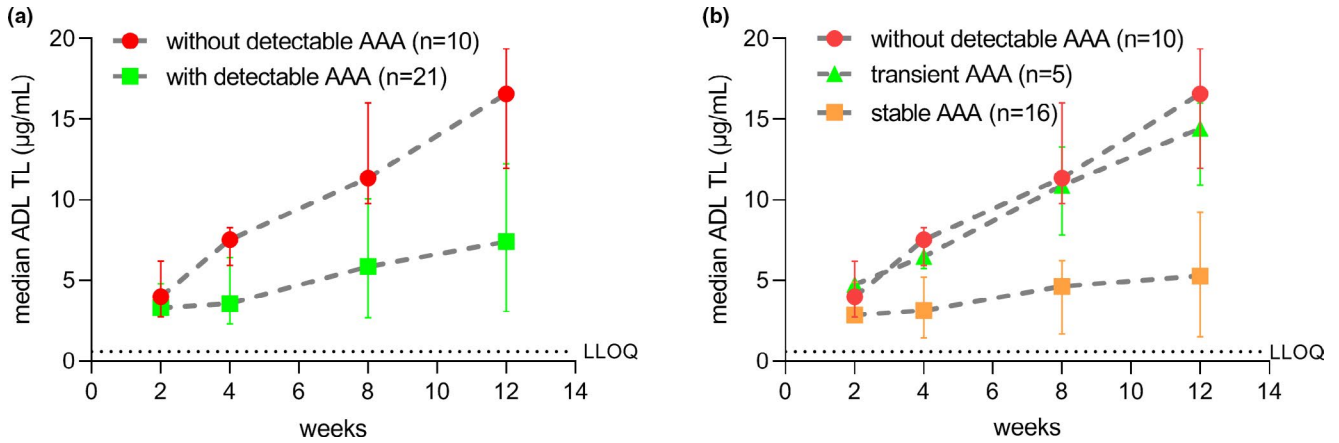

Figure 1 Adalimumab trough level profile with different antiadalimumab antibody (AAA) types. (**a**) Median adalimumab concentration (IQR) per time point is shown for patients without detectable AAA (*n*=10) and with AAA (*n*=21). (**b**) Median adalimumab concentration (IQR) per time point is shown for patients without detectable AAA (*n*=10), with transient AAA (*n*=5) and with stable AAA (*n*=16). ADL, adalimumab; LLOQ, lower limit of quantification; TL, trough level.
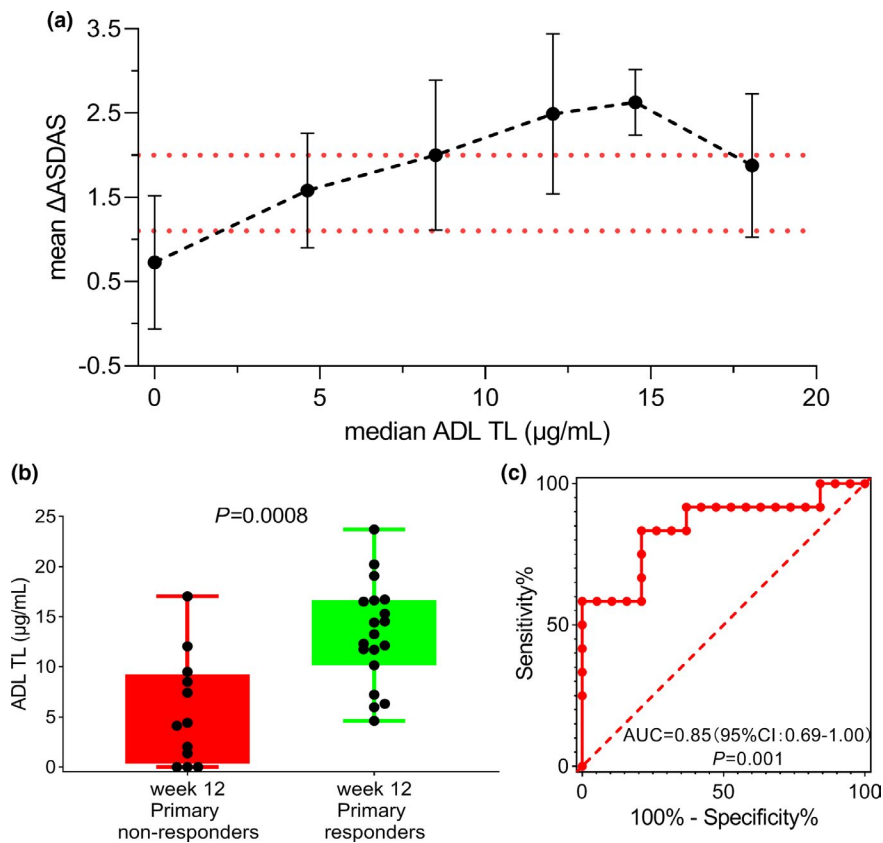

Figure 2 The relationship between adalimumab trough level at week 12 and clinical response. (**a**) Concentration-effect curve. Each point represents the mean of five data points of 31 trough level (the last dot represents six patients) measured at 12 weeks of treatment, stratified in ascending order with correlating ΔASDAS mean (SD). (**b**) Week 12 adalimumab levels were significantly lower among primary nonresponders than among primary responders (median adalimumab level 4.28 vs 13.26 µg/mL, IQR 0.34-9.24, 10.15-16.63 µg/mL among primary nonresponders vs primary responders, P=0.0008). (**c**) ROC curve analysis. Week 12 adalimumab levels < 9.82 µg/mL were significantly associated with primary nonresponse (AUC=0.85, P=0.001, sensitivity 83.3%, specificity 79.0%). ADL, adalimumab; CI, confidence interval; TL, trough level.

important improvement (ΔASDAS ≥ 1.1), concentrations of ~ 2.5 µg/mL seem to be already sufficient. Levels of ~ 8 µg/mL show major improvement (ΔASDAS ≥ 2.0). Serum levels up to 12 µg/mL show a positive association with ΔASDAS. However, it seems that concentrations above 12 µg/mL did not give further improvement of clinical efficacy. In general, adalimumab trough concentrations between 8 and 12 µg/mL seem optimal. No significant correlation between adalimumab levels at week 12 and ΔBASDAI was found.

Week 12 adalimumab levels were significantly associated with primary response at 12 weeks of treatment (median 4.28 vs. 13.26 µg/mL, IQR 0.34–9.24, 10.15–16.63 µg/mL among primary nonresponders vs. primary responders, P = 0.0008, **Figure 2b**). To establish a cutoff value, ROC curve analysis showed that week 12 adalimumab levels below 9.82 µg/mL were significantly associated with primary nonresponse (area under the curve (AUC) = 0.85, P = 0.001, sensitivity 83.3%, specificity 79.0%; **Figure 2c**).

Early prediction of primary nonresponse

Primary nonresponders had significantly lower week 4 and week 2 adalimumab levels than primary responders (week 4: median 2.60 µg/mL IQR 0.30–3.55 vs. 7.07 µg/mL IQR 5.42–7.71, respectively, P < 0.0001; week 2: 2.73 µg/mL IQR 0.66–3.22 vs. 4.71 µg/mL IQR 3.14–4.95, respectively, P = 0.036; **Figure 3a,b**). Moreover, in ROC curve analysis, week 4 or week 2 adalimumab levels below 4.28 µg/mL or 3.37 µg/mL were significantly associated with primary nonresponse by week 12, respectively (week 4: AUC = 0.89, P = 0.0003, sensitivity 83.3%, specificity 94.7%; week 2: AUC = 0.88, P = 0.034, sensitivity 100%, specificity 70.0%; **Figure 3c,d**).

Similarly, week 4 AAA levels were significantly higher among primary nonresponders than among primary responders (median 7.52 IQR 3.93–10.78 vs. 1.00 IQR 1.00–3.77, respectively, P = 0.002; **Figure 3e**). Further ROC analysis showed that week 4 AAA S/N levels above 5.31 had a 66.7% sensitivity and 94.7% specificity for primary nonresponse (AUC = 0.81, P = 0.004; **Figure 3f**).

DISCUSSION

In this pilot cohort study, we identified the concentration-effect relationship of adalimumab in patients with AS, suggesting a therapeutic range of 8–12 µg/mL at steady-state. We also show that early adalimumab levels (at week 4, even at week 2) can be used to predict primary nonresponse at the treatment evaluation point (at week 12). Longitudinal data show that AAA appears as early as week 4 in 58% (18/31) of AAA-positive patients during week 12 treatment, which is associated with adalimumab level and primary response.

The therapeutic range for each disease plays an important role in optimizing treatment for individual patients. To our knowledge, therapeutic ranges of adalimumab trough level, corresponding to an optimal clinical effect, were reported in RA (5–8 µg/mL),[5] PsA (5–8 µg/mL),[6] PsO (3.5–7.0 µg/mL or 3.2–7.0 µg/mL),[7,8] and IBD (5–12 µg/mL).[9] Our findings are consistent with the above mentioned studies. However, the therapeutic range for adalimumab in

patients with AS has not been established in a previous study.[18] In an observational study, including two cohorts, adalimumab concentration was not related to clinical response by the BASDAI and ASDAS. The data regarding the study have been described previously.[19] The median adalimumab levels were significantly higher in Dutch patients than Taiwanese patients (12.6 vs. 6.1 µg/mL, P = 0.001), which may become a vital confounding factor in the pooled concentration-effect curve analysis. In patients with peripheral spondyloarthritis, there was no clear association between adalimumab serum levels and clinical response defined according to the ASDAS inactive disease achievement.[20] In contrast to previous findings, our results confirm the therapeutic range of adalimumab trough levels treated at 12 weeks in patients with AS, indicating that one-third of patients may be overtreated. Those patients treated at steady-state may be eligible for dose de-escalation and interval prolongation to reduce costs without loss of disease control.[21–23]

Our findings show that low adalimumab at week 4, even at week 2, was associated with poor clinical response at week 12 in patients with AS, offering a powerful opportunity to optimize therapy earlier in patients with low drug levels. To our knowledge, a similar study in patients with AS is lacking. In patients with PsO receiving the same treatment (adalimumab 40 mg every other week; n = 31),[24] adalimumab levels at 4 weeks were significantly higher in responders than in nonresponders, as validated in a subsequent real-world cohort (n = 47).[8] In patients with RA,[25] low adalimumab levels at week 12 were a significant predictor of nonresponse at 12 months. In patients with IBD receiving adalimumab induction therapy (loading doses of 160 mg and 80 mg at weeks 0 and 2, respectively), postinduction (week 4) adalimumab levels were associated with short-term mucosal healing and clinical response evaluated at weeks 12 and 52 in ulcerative colitis[26,27] and biological remission by week 12 in Crohn's disease.[28] Due to the lack of induction therapy in AS, RA, and PsA, clinical evaluations are conducted at 3–6 months after the start of therapy. Approximately one-third of patients will receive ineffective therapy during the treatment period.[3] In the era of treat-to-target based on the "hit hard, hit early" principle, an early marker of treatment could be helpful to identify nonresponsive patients who will benefit from dose escalation or other therapeutic antibodies.

Consistent with previous studies,[19,29–31] AAA development was associated with a reduced adalimumab level and subsequent treatment nonresponse. The reported incidence of AAA varies widely from 554% among studies due to the use of different assays.[32,33] For example, in the commonly used radioimmunoassay, the sample is considered positive when the AAA level exceeded 12 AU/mL and the adalimumab level was below 5 µg/mL. Thus, the reported incidence was underestimated due to drug interference in several clinical studies (median adalimumab trough level ranged from 5–10 µg/mL).[19,29,31] In a biosimilar study that aimed to demonstrate equivalence of SB5 and adalimumab,[34] all healthy subjects were AAA-positive in the US-adalimumab group due to highly sensitive and drug-tolerant assay using Meso Scale Discovery system. Bridging enzyme-linked
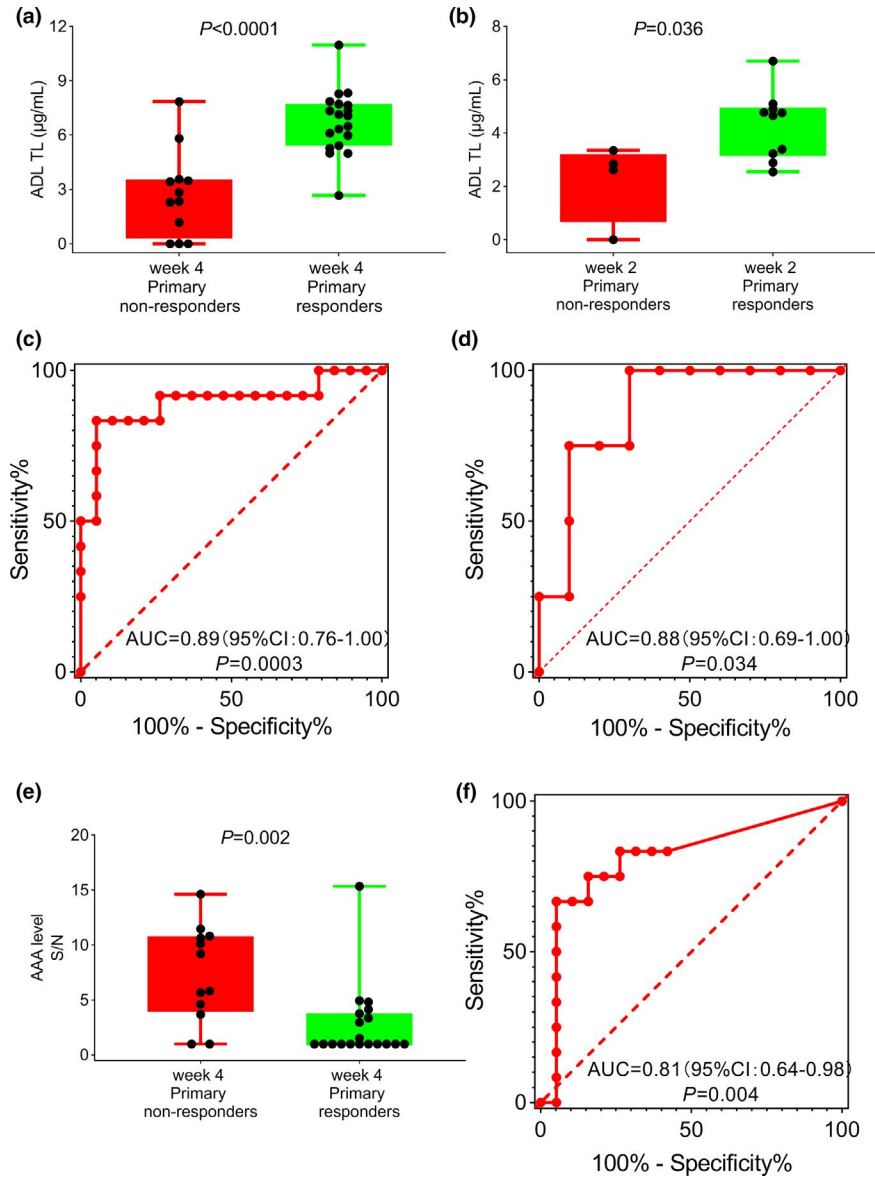
Figure 3 Early adalimumab and anti-adalimumab antibody (AAA) serum levels as predictors of primary nonresponse. Adalimumab trough levels were significantly lower among primary nonresponders than among primary responders at both week 4 (median adalimumab level 2.60 µg/mL interquartile range (IQR) 0.30–3.55 vs. 7.07 µg/mL IQR 5.42–7.71, respectively, *P* < 0.0001) (**a**) and week 2 (2.73 µg/mL IQR 0.66–3.22 vs. 4.71 µg/mL IQR 3.14–4.95, respectively, *P* = 0.036) (**b**). Receiver operating characteristic curve analysis. (**c**) Week 4 adalimumab levels < 4.28 µg/mL were significantly associated with primary nonresponse by week 12 (area under the curve (AUC) = 0.89, *P* = 0.0003, sensitivity 83.3%, specificity 94.7%), and (**d**) week 2 adalimumab levels < 3.37 µg/mL were significantly associated with primary nonresponse by week 12 (AUC = 0.88, *P* = 0.034, sensitivity 100%, specificity 70.0%). (**e**) AAA levels were significantly higher among primary nonresponders at week 4 (median AAA S/N level 7.52 IQR 3.93–10.78 vs. 1.00 IQR 1.00–3.77, respectively, *P* = 0.002). (**f**) Week 4 AAA S/N levels above 5.31 had a 66.7% sensitivity and 94.7% specificity for primary nonresponse (AUC = 0.81, *P* = 0.004). ADL, adalimumab; CI, confidence interval; TL, trough level.

immunosorbent assays may not be adequately robust for detecting the IgG4 subclass, which may also underestimate the levels of AAA. In the immune response against adalimumab in patients with RA, a considerable part of the AAA is IgG4.[35] A drug-resistant assay that incorporated a combination of adalimumab/AAA complex precipitation and the acid dissociation procedure was reported previously[13] and then used to determine the AAA in patients with Crohn's disease.[28] A total of 21.4% of the available samples were

identified as presence of AAA (> 0.77 µg/mL-eq), lower than the rate in the present study (67.7%) due to the sensitivity of the assay and different patients with different therapeutic regimens. Early adalimumab levels were lower in patients with AS without induction phase than those in patients with IBD, which may provoke AAA formation.[28,36] In the treatment of patients with AS, methotrexate and thiopurines are not used, which might be an explanation for the higher incidence of AAA formation.[37,38] In agreement with published

data,[39] we also observed the early onset of immunogenicity response using a longitudinal analysis, which could indicate the time of immunogenicity assessment and be helpful to making therapeutic decisions earlier.

We realize that our study is considered a pilot and exploratory study and has several limitations. First, a small sample size was the most relevant when interpreting the results, although we did our best to collect serum and medical records, and large-scale, multicenter prospective studies are required to validate and confirm our findings. In addition, randomized control trials comparing proactive-TDM-guided treatment with routine care should be conducted to determine whether the TDM-guided individualized therapy is beneficial. The treatment algorithm in the TDM-guided group should be well designed and consider lots of factors, such as accessibility of drugs, healthcare resources, and wishes of patients. Finally, the measurement of AAA levels should be unified when the value will be applied in clinic setting. We used the S/N value to indicate the magnitude of AAA in the present paper, perhaps the AAA levels expressed as μg/mL-calibrator may be overcome by the interference of interday or interanalyst assay.

In conclusion, in the present prospective study, our findings confirm the existing concentration-effect relationship of adalimumab in patients with AS and provide evidence that lower early adalimumab levels and higher early AAA levels predict primary nonresponse. These results indicate that adalimumab and AAA levels taken at an early stage may help physicians to prevent ineffective therapy, and measurement at steady-state may be a useful guide to reduce overtreatment and health care costs by supporting proactive monitoring to optimize adalimumab therapy.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Figure S1. BEAD assay diagram. Excess biotin-drug was added to acidified serum sample for biotin-ADL/AAA complexes formation, which were captured by SA-Bead. The beads were washed and then acidified. After that, supernatant containing AAA was immobilized onto another plate and detected using specific HRP-ADL followed.

Conflict of Interest. The authors declared no competing interests for this work.

Author Contributions. X.L.D., R.F.Z., and L.Y.M. wrote the manuscript. X.L.D. and L.Y.M. designed the research. X.L.D., R.F.Z., L.X., and J.W. performed the research. X.L.D., M.H.G., and L.Y.M. analyzed the data.

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