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Monitoring of adalimumab and antibodies-to-adalimumab levels in patient serum by the homogeneous mobility shift assay

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ABSTRACT

This report describes the analytical validation and application of the homogeneous mobility shift assay (HMSA) method for the measurement of adalimumab and human antibodies-to-adalimumab (ATA) in serum samples from patients who have lost response to adalimumab treatment. Validation of the ATA- and the adalimumab-HMSA revealed a lower limit of detection to be 0.026 U/mL for ATA and 0.018 μg/mL for adalimumab in serum samples. Intra-assay and inter-assay precision determination yielded a coefficient of variation of less than 15%, and the accuracy of both assays was within 20%. Adalimumab drug tolerance in the ATA-HMSA was up to 20 μg/mL in the test serum. Serum samples from 100 drug-naïve healthy subjects were tested to set-up the cut point of 0.55 U/mL for ATA and 0.68 μg/mL for adalimumab. Analysis of 100 serum samples from patients who were losing response to adalimumab showed that 26% had an adalimumab level below the cut point, of these 68% were ATA positive. Overall, 44% of the patients (44/100) were positive for ATA. This study presents evidence that drug and anti-drug antibody levels are important determinants of patient response to therapy.

1. Introduction

Many chronic inflammatory diseases are mediated by up-regulation of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) [1]. The therapeutic use of anti-TNF-α antagonists such as infliximab, adalimumab, and certolizumab pegol has greatly improved the treatment of rheumatoid arthritis (RA), psoriasis (PS), and inflammatory bowel disease (IBD, Crohn's disease and/or ulcerative colitis) [2]. The anti-TNF-α therapeutics are effective in reducing disease activity, and offer significant benefits in quality of life and may have the potential to change the progression of the disease when given early [2,3]. However, over 30% of patients fail to respond to anti-TNF-α therapy, and approximately 60% of patients who responded initially lose the response over time, and require either drug dose-escalation or switch to an alternative agent in order to maintain response [4]. Anti-drug antibody formation may increase drug clearance in treated patients and/or neutralize the drug effect, thereby potentially contributing to the loss of response [5]. Anti-drug antibodies could also cause adverse events such as serum sickness and hypersensitivity reactions [6,7]. Moreover, recent data suggest that the standard dosing regimen for TNF-α-blocking drugs may be suboptimal in some IBD patients, and an individualized dosing regimen to achieve therapeutic drug levels may be needed in order to maximize the initial drug response and to maintain remission [8]. Therefore, accurate monitoring of serum drug and anti-drug antibody levels should be an important part of therapy for patients being treated with protein-based drugs. For this purpose, we have previously developed and validated a novel homogeneous mobility shift assay (HMSA) using size-exclusion high-performance liquid chromatography (SE-HPLC) to quantitatively measure both infliximab and antibody-to-infliximab (ATI) in human serum [9]. The HMSA method overcomes many limitations found in other methods, such as the bridging enzyme-linked immunosorbent assay (ELISA) method [10] and the radioimmunoassay (RIA) method [11]. The advantages of the HMSA method include high sensitivity, specificity, and accuracy and the ability to detect all isotypes of immunoglobulin and subtypes of IgG such as IgG_4. Using the HMSA method, it is possible to measure ATI in the presence of high levels of infliximab drug in patient serum [9], which is not possible with the ELISA method.

Adalimumab (Humira®) is a fully humanized monoclonal antibody against TNF-α, and is approved for the treatment of RA and Crohn's disease (CD) via subcutaneous injection. In the CLASSIC I and II clinical trials, adalimumab treatment resulted in a significantly higher rate of remission of CD [12,13]. Even though it is fully humanized, adalimumab does not eliminate the risk of

Abbreviations: ATA, antibodies-to-adalimumab; CD, Crohn's disease; HMSA, homogenous mobility shift assay; IBD, inflammatory bowel disease; PS, psoriasis; RA, rheumatoid arthritis; TNF-α, tumor necrosis factor-alpha; UC, ulcerative colitis.

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immunogenicity in both CD and RA patients [14,15]. Generation of antibodies-to-adalimumab (ATA) in the serum is associated with lower serum adalimumab concentrations and reduced response rate to treatment. In the present study, we evaluated the feasibility of using the HMSA method for the measurement of adalimumab and ATA levels in patients who have lost response to adalimumab therapy.

2. Materials and methods

2.1. Materials

Individual serum samples from healthy controls were obtained from blood bank donors (Golden West Biologics, Temecula, CA). Sera from patients with RA, PS, and IBD treated with adalimumab were drawn according to a protocol approved by an Institutional Review Board/Ethics Committee. Unless otherwise noted, all reagents and chemicals were obtained from Thermo Fisher Scientific (Waltham, MA) or Sigma Aldrich Corporation (St. Louis, MO).

2.2. Preparation of reagents

2.2.1. ATA calibration serum

ATA-positive sera were prepared by immunizing two rabbits with purified adalimumab (ProSci, Inc., San Diego, CA). Bleeds of anti-adalimumab positive sera from the rabbits were pooled and the relative amount of ATA was arbitrarily defined as 100 U/mL, equal to 1:100 dilutions. The pooled ATA calibration serum was aliquoted and stored at −70 °C.

2.2.2. Conjugation of adalimumab and TNF-α

The method for the conjugation of AlexaFluor-488 to adalimumab was same as described previously [9]. Briefly, commercially available adalimumab (Humira®, Abbott Laboratories, Abbott Park, IL) was buffer exchanged with phosphate buffered saline (PBS, pH 7.3) and labeled with AlexaFluor-488 (Life Technology, Carlsbad, CA) following the manufacturer's instructions. Only those conjugates containing 2–3 fluorescent dyes per antibody qualified for the ATA-HMSA. Conjugation of AlexaFluor-488 to TNF-α was performed as described previously [9].

2.3. HMSA for ATA and adalimumab

The procedure for the ATA-HMSA and the adalimumab-HMSA were similar to the ATI-HMSA as described previously [9], except that AlexaFluor-488 labeled adalimumab was used in the ATA-HMSA. In brief, serum samples were first acid dissociated with 0.5 M citric acid (pH 3.0) for 1 h at RT, and then neutralized with 10× PBS (pH 7.3) in the presence of adalimumab-AlexaFluor-488 in a 96-well plate format. The plate was incubated for 1 h at RT on an orbital shaker to complete the formation of the immune complexes. The equilibrated samples were filtered through a MultiScreen-Mesh Filter plate equipped with a Durapore membrane (0.22 μm; EMD Millipore, Billerica, MA) into a 96-well receiver plate (Nunc, Thermo Fisher Scientific, Waltham, MA). The recovered solutions were individually loaded into an HPLC system (Agilent Technologies 1200 series HPLC system, Santa Clara, CA) equipped with a BioSep SEC-3000 column (Phenomenex, Torrance, CA). The chromatography was run at the flow-rate of 1 mL/min with 1× PBS (pH 7.3) as the mobile phase for a total of 20 min, and was monitored with a fluorescence detector at excitation and emission wavelengths of 494 nm and 519 nm, respectively. ChemStation Software (Agilent Technologies, Santa Clara, CA) was used to set-up and collect data from the runs automatically and continuously.

To generate a standard curve, one aliquot of the stock ATA calibration serum was thawed and diluted to 2% in volume with rabbit serum (Sigma Aldrich, St. Louis, MO) in HPLC assay buffer (1× PBS, pH 7.3) to achieve final concentrations in the assay wells of 0.031, 0.063, 0.125, 0.250, 0.500, 1.000, 2.000, and 4.000 U/mL. Three quality control (QC) samples were prepared by diluting the calibration serum in assay buffer with 0.1% BSA to yield the high (1.600 U/mL), mid (0.600 U/mL), and low (0.200 U/mL) control concentrations. Similarly, adalimumab calibration standards were prepared by serially diluting purified adalimumab with assay buffer containing 0.1% BSA to achieve final concentrations of 0.013, 0.025, 0.050, 0.100, 0.200, 0.400, 0.800 and 1.600 μg/mL of adalimumab and final NHS concentration of 4% in the reaction mixture. Three adalimumab QC samples were prepared by diluting the adalimumab calibration standard with assay buffer and 0.1% BSA to yield the high (25 μg/mL), mid (10 μg/mL), and low (5 μg/mL) control concentrations.

2.4. ATA-HMSA and adalimumab-HMSA evaluation

The analytical validations including the performance characteristics for the ATA-HMSA and the adalimumab-HMSA (calibration standards, assay limits, assay precision [intra- and inter-assay], linearity of dilution, and substance interference) were performed based on the industrial recommendations [16]. A panel of serum samples from drug-naïve healthy donors (n = 100; Golden West Biologics. Temecula, CA) were analyzed to determine the assay cut points for the ATA-HMSA and adalimumab-HMSA. The samples were normally distributed and parametric statistics were applied to determine the cut point. The assay cut points were defined as the threshold above which samples were deemed to be positive, and was set to have an upper negative limit of approximately 99%, calculated by using the lowest mean value of individual samples interpolated from the standard curve + 3.0x the standard deviation (SD).

2.5. Data analysis

Data analysis was performed with the use of a proprietary automated program run on R software (R Development Core Team, Vienna, Austria) [9]. Briefly, the R program opened the ChemStation files, normalized the spectra, determined the area under each peak, and calculated the proportion of total peak areas shifted to the bound ATA/adalimumab-AlexaFluor-488 complexes over the total bound and free adalimumab-AlexaFluor-488 peak areas in the ATA-HMSA. An exponential association standard curve was generated from the standards and the measured ATA values were interpolated from the curve. To obtain the actual ATA and adalimumab concentrations in the serum, the interpolated results from the standard curves were multiplied by the dilution factor.

3. Results and discussion

3.1. Evaluation of the ATA-HMSA and the adalimumab-HMSA

Because the maintenance protocol for patients treated with adalimumab requires biweekly dosing, and the estimated half-life of the drug in human blood is 15–20 days, the collection of a large quantity of ATA-positive sera from patients for use as calibration standards is a challenge. In theory, antisera from any mammalian species will bind to and form immune complexes with adalimumab, and show a similar SE-HPLC profile when compared to human immune complexes. Therefore, in order to produce a large quantity of ATA-positive sera for calibration needs, two rabbits were immunized with purified adalimumab and different bleeds of antisera were pooled to serve as calibration standards. The relative amount of ATA was arbitrarily defined as 100 U/mL, equal to 1:100 dilutions.

Table 1
Characteristics of the ATA-HMSA and the adalimumab-HMSA standard curves.

ATA-HMSA					Adalimumab-HMSA				
ATA Standard (U/mL)	Back calculated concentration (mean, U/mL)	Error (%)	CV (%)	*n*	Adalimumab Standard (μg/mL)	Back calculated concentration (mean, μg/mL)	Error (%)	CV (%)	*n*
4.000	3.820	4.51	0.47	29	1.600	0.973	39.17	0.31	29
2.000	2.099	4.94	4.87	29	0.800	0.898	12.21	4.89	29
1.000	0.970	3.03	7.76	29	0.400	0.405	1.35	10.36	29
0.500	0.496	0.71	9.31	29	0.200	0.192	3.94	13.41	29
0.250	0.263	5.16	10.45	29	0.100	0.104	4.12	16.09	29
0.125	0.132	5.58	15.8	29	0.050	0.055	9.39	18.92	29
0.063	0.062	1.66	19.73	29	0.025	0.026	2.46	24.93	29
0.031	0.017	44.84	29.03	29	0.012	0.006	51.84	34.58	29

When serial dilutions of the ATA calibration standards were incubated with adalimumab-AlexaFluor-488, dose-dependent immune complexes were formed with concomitant reduction of the free adalimumab-AlexaFluor-488. Analyses were then conducted by SE-HPLC, in which the shifted peak area of the immune complexes and free adalimumab-AlexaFluor-488 were calculated. The standard curve generated by plotting the proportion shifted area vs. ATA concentration is shown in Fig. 1A. The lowest concentration of ATA in the standard curve was 0.031 U/mL. The error for the back-calculated values of the 29 standard curve runs was within 10%, except for the lowest concentration (0.031 U/mL) (Table 1). The CV was <20% for concentrations above 0.031 U/mL, and the dynamic range of the assay was two orders of magnitude. The calculated limit of detection (LOD) was 0.026 U/mL from the 29 standard curve runs based on the method described previously [9]. The calculated lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) were 0.063 U/mL and 25.000 U/mL, respectively. Complete analytical validation of the ATA-HMSA was performed with the high (1.600 U/mL), mid (0.600 U/mL), and low (0.200 U/mL) QCs by multiple analysts using different instruments on different days. As shown in Table 2A, the ATA-HMSA intra-assay precision had a CV < 3% and an accuracy rate was <13% error. The inter-assay precision (run-to-run, analyst-to-analyst and instrument-to-instrument) had a CV of <9% and an accuracy of <18% error.

To evaluate the performance of the adalimumab-HMSA, purified adalimumab was used as the calibration standard and AlexaFluor-488 was used to label the TNF-α. The performance characteristics of the adalimumab-HMSA standard curve in the concentration range of 0.012–1.600 μg/mL (Fig. 1B) was similarly assessed over 29 experimental runs by multiple analysts using different instruments on different days (Table 1). The error for the back-calculated value of the 29 standard curve runs was within 15% except for the highest and lowest concentrations (Table 1). The CV was <25% except for the lowest concentration, and the dynamic range was two orders of magnitude. The calculated LOD, LLOQ, and ULOQ for the adalimumab-HMSA were 0.018 μg/mL, 0.040 μg/mL, and 1.100 μg/mL, respectively. As shown in Table 2B, the intra-assay precision and accuracy for the adalimumab-HMSA were <20% and <3%, respectively, whereas the inter-assay precision and accuracy for the adalimumab-HMSA were <12% and <22%, respectively.

To determine the linearity of dilution of both the ATA-HMSA and the adalimumab-HMSA, human serum samples containing a high titer of ATA or a high concentration of adalimumab were used. The samples were diluted serially 2-fold within the linear range of the standard curves and analyzed using the ATA-HMSA and the adalimumab-HMSA, respectively. The observed values of ATA or adalimumab were compared with the expected levels of ATA or adalimumab in the serum samples. The R^2 values for adalimumab and ATA were 0.9933 and 0.9977, respectively. Assays had slopes of 0.9747 for adalimumab and 0.876 for ATA, demonstrating accurate linear regression.

The effect of the potential interfering substances present in the serum was also evaluated in both the ATA-HMSA and the adalimumab-HMSA as described previously (Supplementary Table 1) [9]. TNF-α and TNF-β do not interfere with the assay at their physiological levels of approximately 6.00 pg/mL. At concentrations of greater than 100 ng/mL, TNF-α begins to compete with the TNF-α-AlexaFluor-488 substrate for binding to adalimumab. However, this is 1.56×10^4 fold higher than the typical physiological levels seen in human sera. No significant interference was observed in the physiological levels of immunoglobulin, rheumatoid factor, hemolyzed serum, and lipemic serum. In addition, the presence of azathioprine up to 10 μM and methotrexate up to 2.0 mM did not affect the assays (data not shown). Testing of interfering agents also assists in demonstrating the specificity of the assay. In particular, none of the immunoglobulins spiked into normal human serum formed immunocomplexes with adalimumab-AlexaFluor-488 that could be detected by the HPLC assay. Further, polyclonal anti-adalimumab antibodies from the rabbit serum utilized for standard curve generation were purified by Protein G affinity chromatography. These purified, IgG anti-adalimumab antibodies showed specific, high affinity binding to adalimumab-AlexaFluor-488 (data not shown).

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2013.01.031.

Substantial concentrations of adalimumab may be present in the serum from patients, even if the blood is drawn at the trough

Table 2A
Assay precision of the ATA-HMSA.

	Intra-assay precision (*n* = 10)			Inter-assay precision								
				Run-to-run (*n* = 5)			Analyst-to-analyst (*n* = 3)			Instrument-to-instrument (*n* = 3)		
	High	Mid	Low	High	Mid	Low	High	Mid	Low	High	Mid	Low
Expected (U/mL)	1.600	0.600	0.200	1.600	0.600	0.200	1.600	0.600	0.200	1.600	0.600	0.200
Measured (mean, U/mL)	1.800	0.574	0.180	1.797	0.562	0.176	1.689	0.532	0.169	1.708	0.526	0.166
SD	0.039	0.009	0.016	0.125	0.024	0.012	0.142	0.040	0.011	0.123	0.031	0.010
CV (%)	2.170	1.600	1.830	6.94	4.33	6.96	8.420	7.450	6.530	7.190	5.950	5.970
Accuracy (%error)	12.530	4.410	10.250	12.33	6.41	12.12	5.590	11.250	15.560	6.750	12.370	17.180

Table 2B
Assay precision of the adalimumab-HMSA.

	Intra-assay precision (n = 10)			Inter-assay precision								
				Run-to-run (n = 5)			Analyst-to-analyst (n = 3)			Instrument-to-instrument (n = 3)		
	High	Mid	Low	High	Mid	Low	High	Mid	Low	High	Mid	Low
Expected (μg/mL)	1.000	0.400	0.200	1.000	0.400	0.200	1.000	0.400	0.200	1.000	0.400	0.200
Measured (mean, μg/mL)	0.974	0.392	0.189	1.016	0.446	0.215	1.032	0.487	0.220	0.917	0.442	0.202
SD	0.017	0.075	0.019	0.052	0.052	0.021	0.043	0.024	0.013	0.056	0.007	0.010
CV (%)	1.700	19.200	1.700	5.060	11.700	9.680	4.170	4.820	5.990	6.120	1.650	4.920
Accuracy (%error)	2.620	2.090	2.620	1.650	11.570	7.430	3.140	21.670	10.200	8.270	10.540	1.270

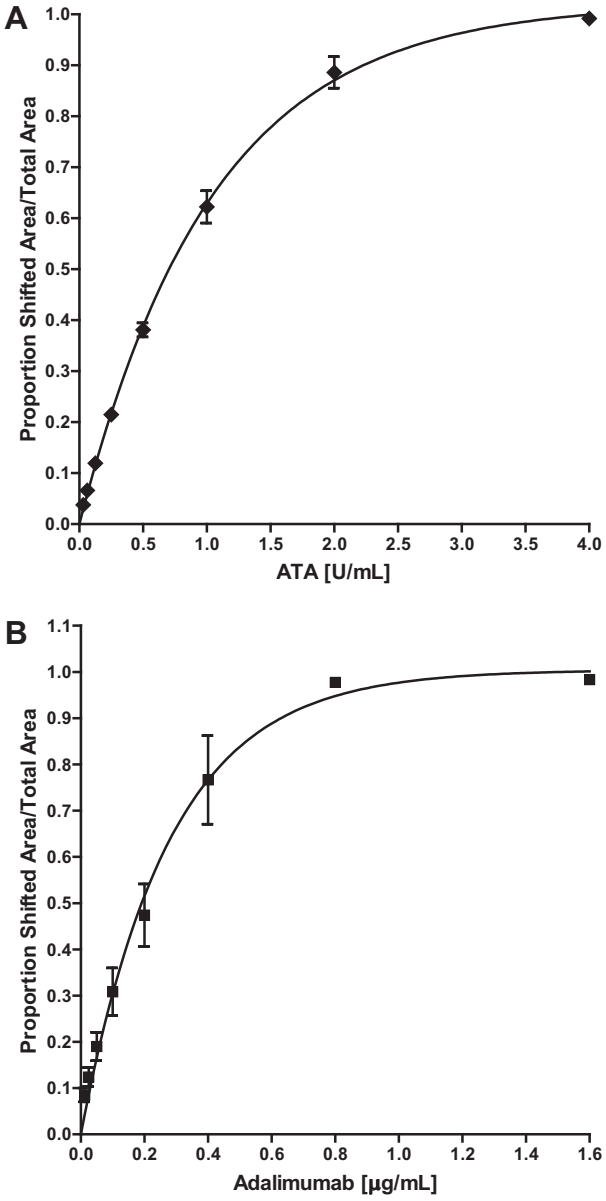

Fig. 1. The ATA-HMSA (A) and the adalimumab-HMSA (B) standard curves. Serial dilutions of the ATA calibration standards (A) or adalimumab calibration standards (B) were incubated with adalimumab-AlexaFluor-488 or TNF-α-AlexaFluor-488, respectively, which dose-dependently formed immune complexes. Immune complexes and remaining free adalimumab-AlexaFluor-488 or TNF-α-AlexaFluor-488 were resolved by SE-HPLC analysis. An exponential association standard curve was generated from the calibration standards. The means and standard deviations for three replicate values for each point on the curve were plotted.

time point due to the biweekly dosing regimen. As discussed previously, the presence of therapeutic antibody in the patient serum significantly affected the quantitative measurement of anti-drug antibodies in the bridging ELISA assay. To address this issue in the HMSA-based assays, ATA-positive human patient serum was tested in the presence of up to 40 μg/mL adalimumab to determine the concentration at which adalimumab would interfere with the ATA quantitation. When adalimumab was present in a sample, the total ATA was calculated using the equilibrium equation [9]. As shown in Fig. 2, the ATA-HMSA could detect ATA levels as low as 10 U/mL in serum samples containing 20 μg/mL of adalimumab with 68.5% recovery.

3.2. Cut point determinations for the ATA-HMSA and the adalimumab-HMSA

We screened 100 serum samples collected from adalimumab drug-naïve healthy subjects to establish the cut points for the ATA-HMSA and the adalimumab-HMSA. In the ATA-HMSA, the proportion of shifted area over the total area was near the LOD, and the mean value of the extrapolated ATA concentrations in serum from the standard curve (multiplied by the dilution factor of 50) was 0.329 ± 0.073 U/mL. The calculated cut point for ATA was 0.549 U/mL (mean + 3.0x SD). There was only one sample that contained ATA levels slightly higher than the cut point (0.630 U/mL), which resulted in a clinical specificity of 99% (Fig. 3). The same

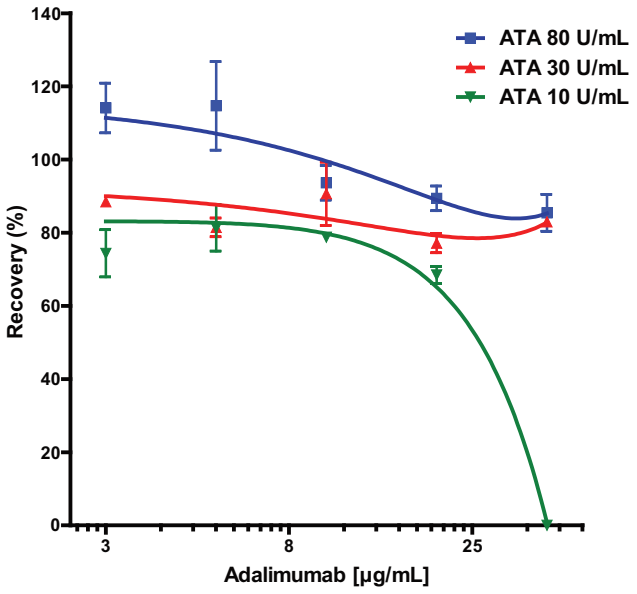

Fig. 2. ATA-HMSA drug tolerance. Interference by adalimumab in the ATA-HMSA was assessed by increasing doses of adalimumab (1.25–40 μg/mL) in each of the three different ATA concentrations (10, 30, and 80 U/mL). ATA-HMSA could detect an ATA level as low as 10 U/mL in the presence of up to 20 μg/mL of adalimumab.

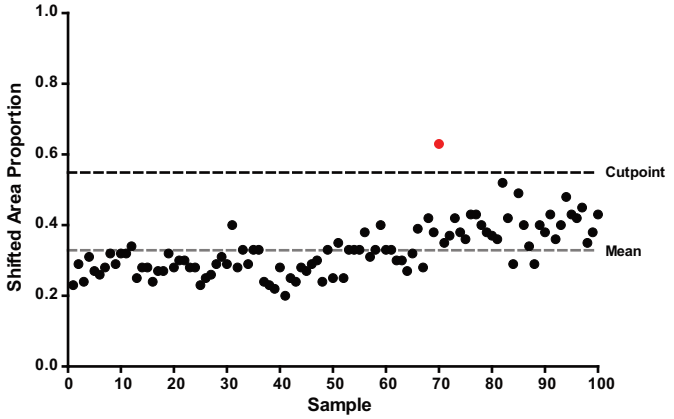

Fig. 3. Plot of the proportion shifted area of donors used to derive the cutpoint for ATA. 100 serum samples were collected from adalimumab drug-naïve healthy subjects to establish the cut points for the ATA-HMSA and the adalimumab-HMSA. The mean value of the extrapolated ATA concentrations in serum from the standard curve (multiplied by the dilution factor of 50) was 0.329 ± 0.073 U/mL. The calculated cut point for ATA was 0.549 U/mL (mean + 3.0*x* SD).

method was applied to calculate the cut point for the adalimumab-HMSA: 0.676 μg/mL, with a clinical specificity of 97%.

3.3. Measurement of adalimumab and ATA in serum samples from patients treated with adalimumab

Recent scientific publications have reported that the incidence of ATA in adalimumab-treated patients varies from 6% to 87% based on different assay methods [13,15]. To evaluate the performance of the adalimumab-HMSA and the ATA-HMSA in measuring adalimumab drug and ATA levels in patient serum, we collected serum samples from 100 patients treated with adalimumab. The basic characteristics of the patients are shown in Supplementary Table 2. All patients were treated with the adalimumab standard therapy for at least 3 months. All patients initially responded to therapy but then lost the response based on evaluation of the disease activity indices for each indication. All samples were diluted 25-fold for the adalimumab test and 50-fold for the ATA test. If the results of the test were above the ULOQ, the samples were retested with further dilutions in order to obtain accurate results. Of the 100 samples tested for adalimumab, 26 samples had drug levels below the cut point of 0.68 μg/mL, while 22 samples had the drug levels above 20 μg/mL. The distribution of the adalimumab levels in these 100 patients is shown in Fig. 4A. The mean average ATA levels in the studied samples were 4.644 ± 19.203 U/mL (mean ± SD, $n = 100$), significantly higher than the healthy controls (0.329 ± 0.073 U/mL, mean ± SD, $n = 100$, $P < 0.00001$). When the cut point of 0.55 U/mL was applied to the ATA-HMSA test, 44 samples were determined to be ATA positive (44%). There was an inverse relationship between adalimumab concentration and ATA positivity. The lower the adalimumab concentration in the patient serum, the more ATA positivity was detected. Sixty-eight percent of the serum samples were ATA positive when their adalimumab levels were below the cut point, while only 18.1% of the samples were ATA positive in patients with adalimumab levels >20 μg/mL (Fig. 4B). There was no statistically significant difference in ATA concentrations and ATA positivity between IBD, RA, and PS patient sera (Supplementary Fig. 1).

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2013.01.031.

Unfortunately, for patients who are losing response to adalimumab treatment, commercial assays are not yet available for the determination of adalimumab trough levels and anti-drug antibody formation. Significantly, the reported incidence of generating ATA in adalimumab-treated patients varies significantly based on

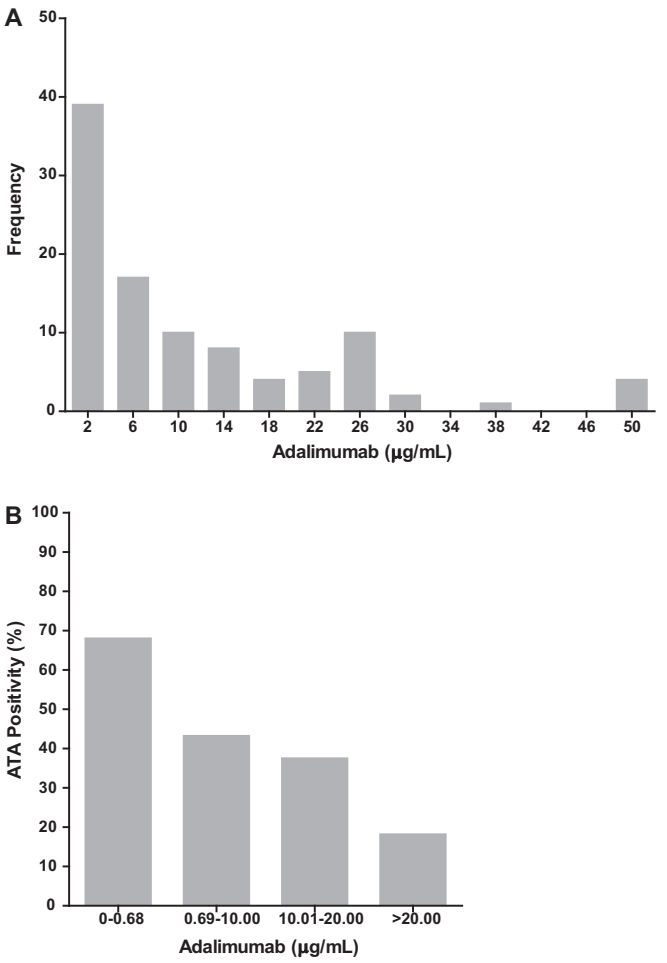

Fig. 4. Histogram showing distribution of adalimumab levels in patients who have lost response to treatment (A) and relationship between adalimumab and ATA levels in serum samples from patients treated with adalimumab (B). Higher frequency was observed in the lower adalimumab concentration range from patients who had lost response to drug treatment (A). The relationship between ATA positivity and adalimumab concentration was plotted (B).

different (non-commercial) assay methods [14,15,17,18]. The discrepancies observed in these reports are likely due to different assay technologies and the timing of blood drawn, as well as the clinical assessments of patient conditions. Most of the available assays for the assessment of anti-drug antibodies in serum are based on solid phase enzyme immunoassay methodology (ELISA) or derivatives thereof such as ECL. These assays differ significantly in clinical specificity and sensitivity with high risk of false-positives due to non-specific binding to immunoglobulin from serum substances other than the drug [19]. Furthermore, these assays cannot detect all the different forms of antibodies nor can they accurately measure the free anti-drug antibodies if an excess of drug is present in the serum [10]. RIA has also been developed to measure serum ATA and adalimumab concentrations and this assay has shown some advantages over ELISA [20]. However, the inherent RIA methodology is more cumbersome and commercially unavailable, and the use of radioactive materials is also a major drawback in many clinical labs. The results presented here show that there is higher incidence of ATA positivity (44%) in these secondary non-responding patients compare to all patients treated with adalimumab [15,17,21]. Twenty-six percent of the serum samples were found to be adalimumab drug negative (below cut point), which was unusual for patients under a biweekly dosing regimen. One of the reasons to explain this finding could be ATA generation since 68% of these patients were found to be ATA positive. ATA may

increase adalimumab clearance by forming immune complexes which are cleared by immunoglobulin Fcγ receptors. ATA positivity was significantly lower (18.1%) when the adalimumab concentration in the serum was above 20 μg/mL, and the loss of response to adalimumab treatment in this patient population may not necessarily be related to ATA formation. It is noteworthy that although the homogeneous mobility shift assay is capable of detecting low levels of ATA at very high concentrations of adalimumab, the significance of low levels of ATA in this patient population is currently unknown. It has been proposed that switching to another class of medication may be beneficial in this population subgroup of patients [22].

4. Conclusions

The ATA-HMSA and the adalimumab-HMSA are novel methods for measuring ATA and adalimumab levels in patient serum samples, and overcome many limitations encountered in solid-phase ELISA and RIA methods. The ATA-HMSA can be used to monitor anti-drug antibody formation even in the presence of high serum drug levels. Incorporation of routine measurement of anti-drug antibody and drug levels in patients treated with protein therapeutics is clinically useful and may help to optimize patient management. Prospective randomized clinical studies in different chronic inflammatory diseases are being conducted to confirm these current findings.

Conflict of Interest

Shui-Long Wang, Scott Hauenstein, Linda Ohrmund, Reshma Shringarpure, Jared Salbato, Rukmini Reddy, Kevin McCowen, Shawn Shah, Steven Lockton, Emil Chuang and Sharat Singh are employees of Prometheus Laboratories, Inc.

All authors contributed to this study's design, data collection, data analysis, and interpretation of data. All authors contributed to the writing of this manuscript, and in the decision to submit the article for publication. This study and analyses were funded by Prometheus Laboratories, Inc.

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References

[1] A.G. Suryaprasad, T. Prindiville, The biology of TNF blockade, Autoimmun. Rev. 2 (2003) 346–357.
[2] D. Tracey, L. Klareskog, E.H. Sasso, J.G. Salfeld, P.P. Tak, Tumor necrosis factor antagonist mechanisms of action: a comprehensive review, Pharmacol. Ther. 117 (2008) 244–279.
[3] F. Magro, F. Portela, Management of inflammatory bowel disease with infliximab and other anti-tumor necrosis factor alpha therapies, BioDrugs 24 (Suppl. 1) (2010) 3–14.
[4] J.P. Gisbert, J. Panes, Loss of response and requirement of infliximab dose intensification in Crohn's disease: a review, Am. J. Gastroenterol. 104 (2009) 760–767.
[5] P. Miheller, L.S. Kiss, K. Lorinczy, P.L. Lakatos, Anti-TNF trough levels and detection of antibodies to anti-TNF in inflammatory bowel disease: are they ready for everyday clinical use? Expert Opin. Biol Ther. 12 (2012) 179–192.
[6] F.R. Brennan, L.D. Morton, S. Spindeldreher, A. Kiessling, R. Allenspach, A. Hey, P.Y. Muller, W. Frings, J. Sims, Safety and immunotoxicity assessment of immunomodulatory monoclonal antibodies, MAbs 2 (2010) 233–255.
[7] A.N. Emi, J.F. de Carvalho, S.C. Artur Almeida, E. Bonfa, Immunogenicity of anti-TNF-alpha agents in autoimmune diseases, Clin. Rev. Allergy Immunol. 38 (2010) 82–89.
[8] J.F. Colombel, B.G. Feagan, W.J. Sandborn, A.G. Van, A.M. Robinson, Therapeutic drug monitoring of biologics for inflammatory bowel disease, Inflamm. Bowel Dis. 18 (2012) 349–358.
[9] S.L. Wang, L. Ohrmund, S. Hauenstein, J. Salbato, R. Reddy, P. Monk, S. Lockton, N. Ling, S. Singh, Development and validation of a homogeneous mobility shift assay for the measurement of infliximab and antibodies-to-infliximab levels in patient serum, J. Immunol. Methods 382 (2012) 177–188.
[10] F. Baert, M. Noman, S. Vermeire, A.G. Van, G.D'. Haens, A. Carbonez, P. Rutgeerts, Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease, N. Engl. J. Med. 348 (2003) 601–608.
[11] L. Aarden, S.R. Ruuls, G. Wolbink, Immunogenicity of anti-tumor necrosis factor antibodies-toward improved methods of anti-antibody measurement, Curr. Opin. Immunol. 20 (2008) 431–435.
[12] S.B. Hanauer, W.J. Sandborn, P. Rutgeerts, R.N. Fedorak, M. Lukas, D. MacIntosh, R. Panaccione, D. Wolf, P. Pollack, Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial, Gastroenterology 130 (2006) 323–333.
[13] W.J. Sandborn, S.B. Hanauer, P. Rutgeerts, R.N. Fedorak, M. Lukas, D.G. MacIntosh, R. Panaccione, D. Wolf, J.D. Kent, B. Bittle, J. Li, P.F. Pollack, Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial, Gut 56 (2007) 1232–1239.
[14] K. Karmiris, G. Paintaud, M. Noman, C. Magdelaine-Beuzelin, M. Ferrante, D. Degenne, K. Claes, T. Coopman, S.N. Van, A.G. Van, S. Vermeire, P. Rutgeerts, Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease, Gastroenterology 137 (2009) 1628–1640.
[15] G.M. Bartelds, C.L. Krieckaert, M.T. Nurmohamed, P.A. van Schouwenburg, W.F. Lems, J.W. Twisk, B.A. Dijkmans, L. Aarden, G.J. Wolbink, Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up, JAMA 305 (2011) 1460–1468.
[16] G. Shankar, V. Devanarayan, L. Amaravadi, Y.C. Barrett, R. Bowsher, D. Finco-Kent, M. Fiscella, B. Gorovits, S. Kirschner, M. Moxness, T. Parish, V. Quarmby, H. Smith, W. Smith, L.A. Zuckerman, E. Koren, Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products, J. Pharm. Biomed. Anal. 48 (2008) 1267–1281.
[17] R.L. West, Z. Zelinkova, G.J. Wolbink, E.J. Kuipers, P.C. Stokkers, C.J. van der Woude, Immunogenicity negatively influences the outcome of adalimumab treatment in Crohn's disease, Aliment. Pharmacol. Ther. 28 (2008) 1122–1126.
[18] T.R. Radstake, M. Svenson, A.M. Eijsbouts, F.H. van den Hoogen, C. Enevold, P.L. van Riel, K. Bendtzen, Formation of antibodies against infliximab and adalimumab strongly correlates with functional drug levels and clinical responses in rheumatoid arthritis, Ann. Rheum. Dis. 68 (2009) 1739–1745.
[19] N. Vande Casteele, D.J. Buurman, M.G. Sturkenboom, J.H. Kleibeuker, S. Vermeire, T. Rispens, D. van der Kleij, A. Gils, G.G. Dijkstra, Detection of infliximab levels and anti-infliximab antibodies: a comparison of three different assays, Aliment. Pharmacol Ther. 36 (2012) 765–771.
[20] K. Bendtzen, P. Geborek, M. Svenson, L. Larsson, M.C. Kapetanovic, T. Saxne, Individualized monitoring of drug bioavailability and immunogenicity in rheumatoid arthritis patients treated with the tumor necrosis factor alpha inhibitor infliximab, Arthritis Rheum. 54 (2006) 3782–3789.
[21] G.M. Bartelds, C.A. Wijbrandts, M.T. Nurmohamed, S. Stapel, W.F. Lems, L. Aarden, B.A. Dijkmans, P.P. Tak, G.J. Wolbink, Clinical response to adalimumab: relationship to anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis, Ann. Rheum. Dis. 66 (2007) 921–926.
[22] W. Afif, E.V. Loftus Jr., W.A. Faubion, S.V. Kane, D.H. Bruining, K.A. Hanson, W.J. Sandborn, Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease, Am. J. Gastroenterol. 105 (2010) 1133–1139.