Development and validation of an optical biosensor for rapid monitoring of adalimumab in serum of patients with Crohn's disease

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Running title: rapid monitoring of adalimumab

Abbreviations: CV, coefficient of variation; FO-probe, fiber-optic probe; FO-SPR, fiber-optic surface plasmon resonance; ICC, intraclass coefficient; LLOQ, lower limit of quantification; MA-ADM, monoclonal antibodies towards adalimumab; TDM, Therapeutic drug monitoring; TNFα, tumor necrosis factor-alpha.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/dta.2250

Abstract

Therapeutic drug monitoring of adalimumab is recommended to improve therapeutic outcome in patients with Crohn's disease. Performing an ELISA requires a rather long time-to-result and the necessity of collecting multiple samples in order to decrease the cost per adalimumab determination. In this study, we aim to develop and validate a rapid assay suitable for measuring a single adalimumab serum sample using a fiber-optic surface plasmon resonance (FO-SPR) based sensor. Therefore, we have immobilized MA-ADM28B8 as capture antibody on a FO-probe and conjugated MA-ADM40D8 as detecting antibody to gold nanoparticles. A dose-response curve ranging from 2.5 – 40 ng/mL adalimumab was obtained in 1/400 diluted serum. Serum samples of patients with adalimumab concentrations between 1 and 16 µg/mL were measured whereas the negative control, a sample spiked with infliximab at a concentration of 16 µg/mL, showed no significant signal. Using a pre-functionalized FO-probe, the technology requires less than 45 min for measuring a single sample. Comparison of measurements between the biosensor and the ELISA revealed an excellent agreement with a Pearson r coefficient of 0.99 and an intraclass coefficient of 0.99. The reduced assay time and the possibility of measuring a single sample are major advantages compared to ELISA. The developed and validated optical adalimumab biosensor could be a valuable point-of-care diagnostic tool for adalimumab quantification in patients with Crohn's disease.

Keywords Therapeutic drug monitoring; Adalimumab; Crohn's disease; Surface plasmon resonance; Optical biosensor

INTRODUCTION

Adalimumab, a full human monoclonal antibody targeting tumor necrosis factor alpha (TNFα), has proved to be highly effective in inducing and maintaining clinical remission in patients with moderate-to-severe Crohn's disease and ulcerative colitis [1,2]. Therapeutic drug monitoring (TDM) guided dosing regimen based on adalimumab trough concentrations gains appreciation in clinical practice and is likely to offer the best route to maximize clinical response and remission of adalimumab treated patients. Recent studies have demonstrated a strong association between higher adalimumab concentrations and mucosal healing [3,4]. Besides, proactive TDM may be cost-saving in the long-term, especially when supra-therapeutic levels are identified and dose reduction can be performed [5]. It is therefore recommended to measure adalimumab concentrations to optimize therapy [6-8].

Several assays are currently available to support TDM of adalimumab. In a previous study, we have developed two types of ELISA to detect adalimumab concentrations in serum samples from patients with Crohn's disease and have validated these assays with other commercially available ELISAs [9]. However, ELISA requires a rather long time-to-result involving multiple steps like antigen coating, blocking of free binding sites, applying serum samples, washing unbound proteins, applying conjugated detecting antibody and applying chromogenic substrate. Moreover, the necessity of collecting multiple samples in order to decrease the cost per adalimumab determination also hampers its implementation. An optical biosensor such as the fiber-optic surface plasmon resonance (FO-SPR) based biosensor offers an option to solve this problem. We have previously demonstrated that an antibody pair selected for use in sandwich ELISA to determine infliximab concentrations can be successfully transferred to an optical biosensor resulting in excellent Pearson and intraclass correlation coefficient of 0.99 ad 0.98, respectively [10,11]. In this study, we aim to develop

and validate a rapid assay suitable for measuring a single adalimumab serum sample derived from adalimumab treated Crohn's disease patients.

METHODS AND MATERIALS

Development of an adalimumab optical biosensor

The adalimumab optical biosensor was developed following the protocol described in Lu *et al*. [11] for the design of an optical biosensor for quantification of infliximab. For the bio-assay development, two anti-adalimumab monoclonal antibodies previously selected to quantify adalimumab in ELISA [9] were used. MA-ADM28B8 was taken as capture antibody and immobilized on a FO-probe at a concentration of 20 µg/mL in 10 mM sodium acetate buffer (pH 5.5) and MA-ADM40D8 was taken as a detection antibody and therefore conjugated to gold nanoparticles (20 nm, EM.GC20, BBI Solutions, Cardiff, UK) using a final concentration of 5 µg/mL in PBS with 0.5% of BSA buffer (pH 7.4). A schematic representation of the adalimumab optical biosensor is illustrated in Figure 1.

To establish a dose-response curve, a dilution series of adalimumab (0, 2.5, 5, 10, 20 and 40 ng/mL) was spiked in 1/400 diluted serum. The FO-probe was rinsed in regeneration buffer (50 mM NaCl, 1 M NaCl) in between two concentrations to remove the bound adalimumab and nanoparticles, facilitating the measurement of the entire range of adalimumab concentrations using one single FO-probe. The FO-SPR measurements were performed at least three times using probes and gold nanoparticles prepared at independent days. A volume of 150 µL of diluted sample was needed for a single measurement. Cross-reactivity of the developed adalimumab optical biosensor towards infliximab was assessed using a serum sample spiked with 16 µg/mL infliximab. An adalimumab neutralizing antibody, MA-ADM6A10, was selected to assess the impact of neutralizing antibody antibodies on adalimumab determination [12]. Briefly, 4 µg/mL adalimumab (final concentration) was

spiked into a 1/100 diluted healthy control serum in the absence and presence of two fold excess of MA-ADM6A10 (8 µg/mL), followed by 15 min incubation at 37°C and 300 rpm of shaking. After cooling on the ice for 5 min, the mixture was applied in a 1/400 dilution into the biosensor to determine the residual SPR shift.

Validation of the adalimumab optical biosensor

In order to validate the adalimumab optical biosensor, nine serum samples from patients with Crohn's disease, collected in the framework of the "adalimumab trough levels in Crohn's disease: a pilot pharmacokinetic study" (Eudract CT 2011-004632-54/CTC UZ Leuven S53297) were analyzed using both the adalimumab optical biosensor and the adalimumab ELISA [9]. For each patient sample, the FO-SPR measurement was performed at least three times using probes and gold nanoparticles prepared at independent days. A volume of 150 µL of diluted sample was taken for a single measurement. Samples with a concentration higher than 16 µg/mL were further diluted 1/800 allowing a quantification up to 32 µg/mL. The adalimumab concentrations were determined using a dose-response curve generated with adalimumab spiked to 1/400 diluted serum. Using a pre-functionalized FO-probe, i.e. probe coated with capture antibody, the biosensor required less than 45 min for measuring a single adalimumab serum sample. The lower limit of quantification (LLOQ) was determined using these nine patient samples and defined as the lowest adalimumab concentration that can be measured with a coefficient of variation (CV) of $\leq 25\%$ ($n = 3$) [13].

Statistics

To quantify the correlation and the agreement between adalimumab concentrations from both assays, the Pearson correlation coefficient and relative Bland-Altman plots were calculated using Graphpad Prism 7.0 (GraphPad Software, California, USA) and the intra-class correlation coefficient (ICC), using the "two-way mixed single measure test (absolute agreement)", was determined with SPSS Statistics v.22 (IBM, New York, USA).

RESULTS

Development of the adalimumab optical biosensor

To implement the adalimumab bioassay on the optical biosensor, MA-ADM28B8 was coupled to a FO-probe and MA-ADM40D8 was conjugated to gold nanoparticles. This sandwich based FO-SPR bioassay revealed a nonlinear adalimumab dose-response curve ranging from 2.5 – 40 ng/mL in 1/400 diluted serum, allowing detection of 1 to 16 µg/mL of adalimumab in serum (Figure 2). Negligible SPR shifts (0.03 ± 0.05 nm, n = 5) were obtained in 1/400 diluted serum in the absence of target. Infliximab, spiked in 1/400 diluted serum with a final concentration of 16 µg/mL showed no detectable signal (< 0.1 nm), indicating that infliximab does not interfere with the adalimumab determination. Addition of a two-fold excess of neutralizing antibody decreased the adalimumab concentrations to 10% (n = 4).

Validation of the adalimumab optical biosensor using patient samples

The optical biosensor for adalimumab was validated towards the established adalimumab ELISA [9] using nine adalimumab serum samples (S1 – S9) from patients with Crohn's disease. The obtained mean adalimumab concentrations from both assays are summarized in Table 1. Based on the measurements of the nine samples, the lowest adalimumab concentration that resulted in a CV of ≤ 25% was 1.6 µg/mL, therefore the LLOQ of the adalimumab optical biosensor was set at 1.6 µg/mL. An overall inter-assay variation of 7-

25% was obtained for the samples above LLOQ. The two samples below the LLOQ were excluded from the comparison analysis. Comparison of the adalimumab concentrations obtained from the two assays revealed a Pearson r of 0.99 ($P < 0.0001$; Figure 3A) and an ICC of 0.99 ($P < 0.0001$; Figure 3B). Results are illustrated visually in Figure 3B using a Bland-Altman plot, demonstrating that the average difference is close to zero and that the two methods give superimposable results.

DISCUSSION

This paper reports on the development of an optical biosensor for measuring adalimumab in serum samples of patients with Crohn's disease. The results demonstrate the transferability of the antibody pair MA-ADM28B8/MA-ADM40D8, selected for ELISA, to an optical biosensor allowing specific detection of adalimumab concentrations between 1 and 16 µg/mL using a 1/400 dilution, requiring only 150 µL of diluted serum and delivering the result in less than 45 minutes. Comparing the adalimumab optical biosensor with a clinically validated adalimumab ELISA revealed excellent correlation and agreement with a Pearson r coefficient of 0.99 and ICC of 0.99.

The main advantages of the optical biosensor are the short time-to-result and the ability to analyze single samples on demand which will facilitate the implementation of therapeutic drug monitoring of adalimumab in daily clinical practice. The specificity of this biosensor for adalimumab will also allow to quantify adalimumab in patients previously exposed to infliximab, the first anti-TNFα agent approved for Crohn's disease, since no cross-reactivity with infliximab is determined. Previous studies have demonstrated that the antibody response against adalimumab is highly restricted and limited to the idiotype of the therapeutic antibody and more than 97% of anti-adalimumab antibodies developed in adalimumab-treated patients are neutralizing [14,15]. It is therefore of relevance to evaluate the impact of neutralizing

antibodies on adalimumab determination in the optical biosensor. As expected, neutralizing antibodies completely block the adalimumab detection, which is in line with what has been observed in the ELISA setup [9].

Although the current time-to-result is 45 min, which is already much shorter than that of ELISA taking at least 1.5h, research to reduce the assay time to less than 10 min is ongoing. Due to the matrix effect, serum samples require a 400-fold dilution, resulting in a lower limit of detection of 1 µg/mL and a lower limit of quantification of 1.6 µg/mL which is slightly higher compared to the reference adalimumab ELISA. However, during maintenance therapy, clinical response in patients with Crohn's disease is associated with adalimumab concentrations of 5.9 µg/mL or higher [16] and in this respect, the somewhat higher limit of quantification of the biosensor is not a major issue. Additionally, as shown in Table 1, in this assay an overall inter-assay variation of 7-25% was determined in serum samples above LLOQ, which is higher than that of the ELISA (7-14%), indicating the somewhat lower repeat-ability in the biosensor. Further optimization of the biosensor parameters and protocols will reduce the time to result, the inter-assay variation and lower the limit of quantification as already been shown for infliximab [11,17]. The performance and the availability of the biosensor was compared in detail to both the reference MA-coated adalimumab ELISA and a commercially available TNF-coated adalimumab ELISA[18] as shown in Table 2.

In summary, a rapid immunoassay for determination of adalimumab concentration using the adalimumab optical biosensor was established and revealed excellent correlation with ELISA. Considering its ongoing optimization with respect to analysis time and quantification limits, the possibility for full automation and miniaturization as well as the possibility of using multi-channel probes sensing, the optical adalimumab biosensor has a great potential to

be a new cost-effective point-of-care diagnostic tool for adalimumab measurements in a hospital setting or infusion center.

Conflicts of Interest

SV received grant support from Centocor, MSD, Abbvie and UCB Pharma, lecture fees from Abbot, Abbvie, MSD, Ferring Pharmaceuticals and UCB Pharma and consultancy fees from Pfizer, Ferring Pharmaceuticals, Shire Pharmaceuticals Group, MSD and AstraZeneca Pharmaceuticals. AG has served as a speaker for MSD, Janssen Biologicals, Pfizer and Abbvie, as consultant for UCB and has received Investigator Initiated Research Grants from Pfizer. KU Leuven licensed infliximab and adalimumab ELISA to apDia. SB, JL, FD, DS and JL declare not to have a conflict of interest.

Acknowledgements

This study was financially supported in part by grant G.0617.12 from FWO-Flanders and grants IOF/KP/12/002 and C22/15/025 from the KU Leuven. SB acknowledges the China Scholarship Council (CSC) for the scholarship.

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Table 1. Mean results of the measurements of nine adalimumab serum samples from patients with Crohn's disease using both the optical biosensor and the sandwich ELISA. Errors represent standard error of the mean.

Clinical samples	Optical biosensor (µg/mL, n = 3)	ELISA (µg/mL, n = 3)
S1	< 0.3	< 0.3
S2	1.1 ± 0.4	1.2 ± 0.1
S3	6.5 ± 0.8	5.6 ± 0.4
S4	10 ± 3	9.4 ± 0.6
S5	12 ± 3	13 ± 2
S6	19 ± 1	18 ± 2
S7	3.0 ± 0.7	3.1 ± 0.2
S8	1.4 ± 0.2	2.0 ± 0.2
S9	5.3 ± 0.7	5.3 ± 0.4

Table 2. Comparison of the performance and availability of the adalimumab biosensor, the in-house MA-coated ELISA and a commercially available TNF-coated adalimumab ELISA kit.

Items	**Optical biosensor**	**MA-coated ELISA** (in house developed[9])	**TNF-coated ELISA** (Ridascreen® ELISA kit, R-biopharm[18])
principle	MA-ADM28B8/ MA-ADM40D8-nanoparticle	MA-ADM28B8/ MA-ADM40D8-HRP conjugate	TNF/MA-ADM40D8-HRP conjugate
assay time	10-45 min[a]	1.5 day	2 h
amount of samples to be analyzed	1-4[b]	70 + controls	80 + controls
serum volume	10 µL	10 µL	10 µL
operation	automated	by hand	automated
availability	expected to be commercially available summer 2017, RUO	not commercially available	commercially available (R-biopharm)

Note: [a] The total sample analysis time has potential to be reduced to 10 min as demonstrated by Lu *et al.* [17] for infliximab detection. [b] A device with 4 probes allowing measurement of 4 samples in parallel is under development.

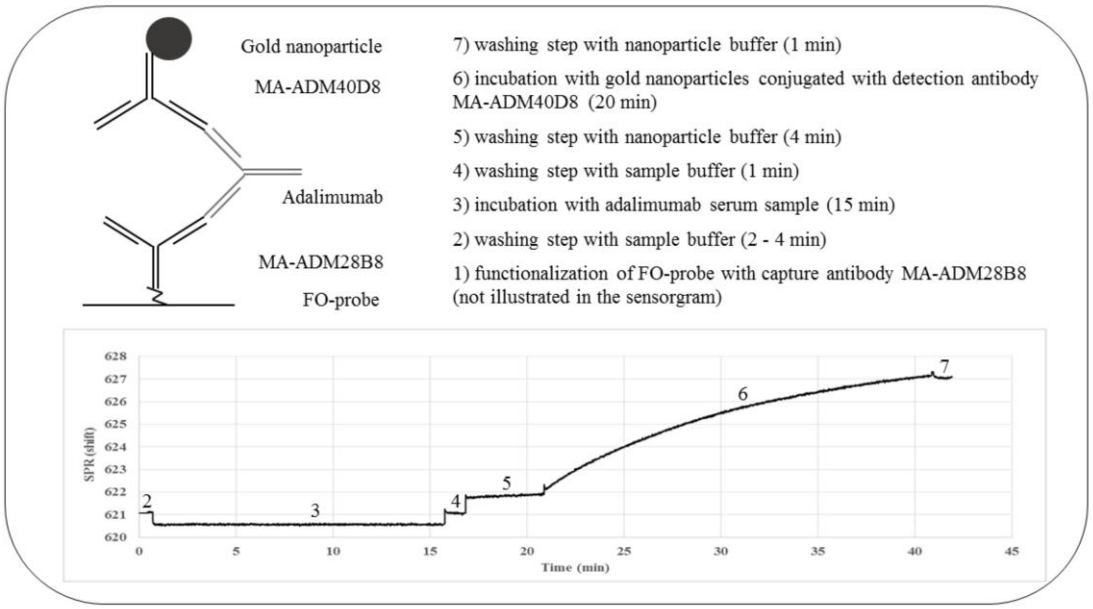

Figure 1. The adalimumab optical biosensor diagram. Upper: a schematic representation of the adalimumab biosensor. Down: a typical optical biosensor sensorgram using a pre-functionalized fiber (with capture antibody coated and unbound surface blocked) representing two major steps including specific binding to adalimumab in a single sample (step 3) and signal amplification using gold nanoparticles conjugated with detection antibody (step 6).

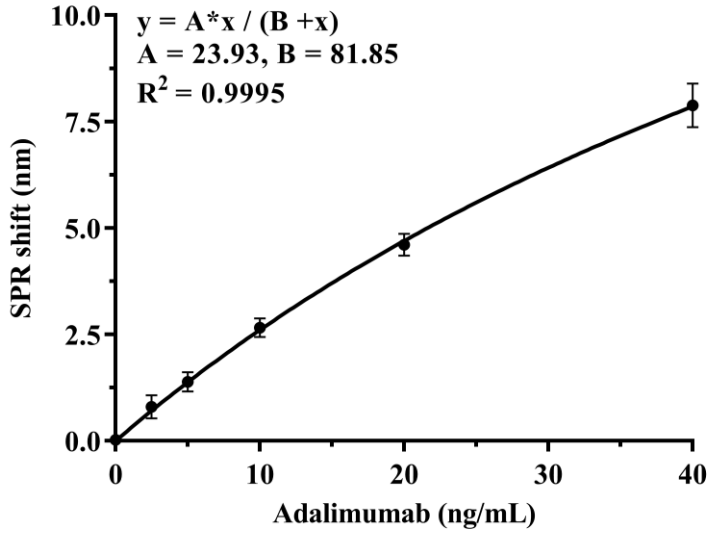

Figure 2. Nonlinear dose-response curve obtained from a dilution series of adalimumab in 1/400 diluted serum. Fitting parameters are indicated on the left of the graph. Error bars represent standard deviations (n = 4).

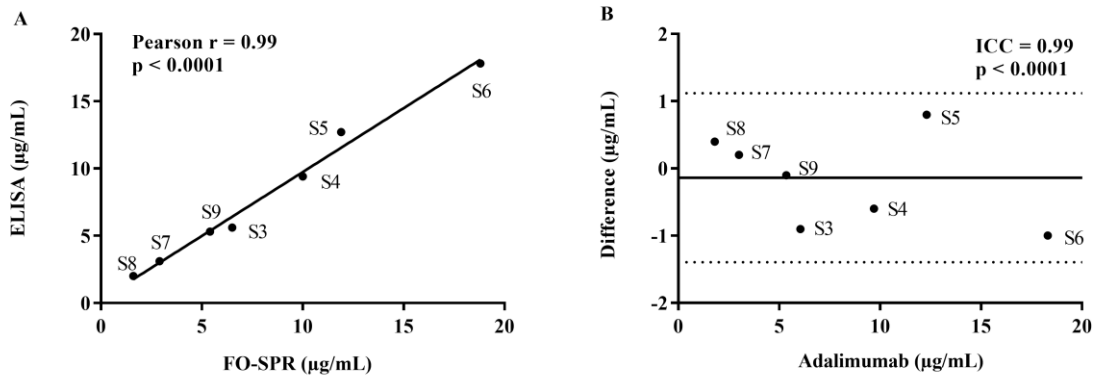

Figure 3. Correlation (A) and Bland-Altman comparison (B) of adalimumab levels as measured by the optical biosensor and the ELISA. The absolute difference between the two measurements (µg/mL) is plotted on the Y-axis and the average of the two measurements (µg/mL) on the X-axis (B). Dark line represents the average of the difference and dotted lines represent the 95% limits of agreement. Each point represents a single patient sample.