Dried blood spots obtained by finger prick facilitate therapeutic drug monitoring of adalimumab and anti-adalimumab antibodies in patients with rheumatic inflammatory diseases

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ABSTRACT

Background Development of a self-sampling method for therapeutic drug monitoring (TDM) of biologicals will enhance TDM implementation in routine care and pharmacokinetic knowledge.

Objectives To compare adalimumab and anti-adalimumab antibody (ADA) level measurements in dried blood spots (DBS) obtained from finger prick with measurements in serum obtained via venepuncture, from patients with rheumatic inflammatory diseases.

Methods In this cross-sectional study, 161 consecutive patients were included. For clinical validation, DBS from finger prick and serum from venepuncture were collected simultaneously and adalimumab and ADA concentration were assessed by ELISA and antigen binding test (ABT), respectively. To convert DBS eluate results to values which can be compared to serum concentrations, five different methods were investigated, using a marker protein or a volumetric approach.

Results Adalimumab and ADA levels obtained from the finger prick/DBS method correlated well with serum results from the same patient (correlation coefficient >0.87). Interestingly, antibody concentrations (either adalimumab, ADA or total immunoglobulin G) in DBS from finger prick, but not albumin, were systematically lower compared to serum. Spike experiments demonstrated a quantitative recovery for all tested proteins in DBS, suggesting a slightly different protein composition of blood collected via finger prick vs venepuncture. We established a correction factor to relate finger prick/DBS values with serum values (approximately 1.2).

Conclusion We show here for the first time that adalimumab and ADA serum concentrations can be satisfactorily estimated by measuring levels in DBS eluates, collected by finger prick. This method offers great opportunity to simplify TDM of adalimumab.

INTRODUCTION

Adalimumab standard dosing can result in insufficient clinical response or overtreatment in a large proportion of patients. Several circumstances can affect clinical efficacy, ranging from undetectable drug levels due to immunogenicity, to serum trough levels substantially above the threshold necessary for complete target blockade. Therefore, it seems logical to use a personalised dosing scheme based on drug level and disease activity. Indeed, therapeutic drug monitoring (TDM) can help to identify causes for insufficient response, and concentration-effect relationships have been identified in multiple studies. Preliminary treatment algorithms and tapering strategies, making use of TDM, are currently available.

However, currently used methods for drug monitoring rely on blood collection by venepuncture. This requires qualified personnel and flexibility of the patient. Visiting an outpatient clinic for blood collection, and logistics of storing and shipping samples is cumbersome, in routine care as well as during clinical trials. Alternatively, development of a dried blood spot (DBS) obtained via an at home performed finger prick will enable self-sampling, with the results ready for immediate decision-making at consultation of the care giver when the next dose needs to be administered. Moreover, self-sampling is easy and minimally invasive. Only a small volume on a filter paper is required; it is convenient for storage and transportation can be performed by the regular mail service. Furthermore, development of a self-sampling method is also an important step forward in gaining more pharmacokinetic (PK) knowledge, needed for the implementation of TDM. In particular, it enables convenient data collection at multiple time-points, allowing e.g. the evaluation of drug concentrations at time points other than trough, for which there is currently a paucity of data.

Detection of antibodies in DBS has been described for screening of metabolic diseases, allergies, viral infections and vaccination efficacy. These studies did not address quantitative measurements of monoclonal antibody concentrations. One (small) study described preliminary results of their developed DBS method for detection of adalimumab and infliximab levels.

Here, we describe how DBS/finger prick can be used in a controlled environment in patients with rheumatic inflammatory diseases treated with adalimumab to obtain reliable estimates of serum concentrations of adalimumab and anti-adalimumab (ADA). To our knowledge this is the first extensive study in which a venepuncture and a finger prick are obtained simultaneously in patients, for clinical validation; and this is the first study to develop a DBS method for the measurement of ADA.
PATIENTS AND METHODS

Study design and patients
A cross-sectional study was performed in patients with Rheumatoid Arthritis (RA) (n = 96), Psoriatic Arthritis (PsA) (n = 31) and Ankylosing Spondylitis (AS) (n = 34) treated with adalimumab. The study was approved by the Medical Ethics Committee of the Slotervaart hospital and Reade Amsterdam, the Netherlands. All patients gave written informed consent according to the Helsinki declaration.

Collection material by venepuncture and finger prick
Venepuncture and finger prick were performed at the same visit by a trained laboratory assistant, at the hospital. Capillary blood from the finger prick was adsorbed on a piece of filter paper (Whatman 903, Whatman Germany) and air dried.

Elution of blood from DBS
Whole DBS was eluted from the filter by overnight incubation in phosphate-buffered saline (PBS) containing 0.05% Tween and 0.05% NaN₃ gently shaking at room temperature. Stability of DBS on filter cards kept at room temperature was tested and no effect in elution efficiency was seen for up to 3 months (data not shown). Eluates were kept at 4°C until further measurements were performed.

Haematocrit (Hct) measurements
Hct level of whole blood obtained from venepuncture was measured with the XN9000 from Sysmex (OLVG, Amsterdam) or with the ADVIA 2120 (Sanquin Diagnostics). The coefficient of variation (CV) for this measurement was 1.5% (Supplementary table S1).

DBS area measurements
The blood area of the DBS was quantified by scanning the filter paper, and measuring DBS area with the image processing program Image J. The average area of the front and the back was taken as representative for the average extent of the blood spot within the filter paper. The CV of the area measurements was 2% (Supplementary table S1).

DBS haemoglobin (Hb) level measurements
Hb levels were measured by Sanquin Diagnostics based on absorbance at 540 nm of the DBS eluates with a CV of 3.9% (Supplementary table S1).

Immunoglobulin (IgG) and albumin level measurements
IgG and albumin levels in DBS eluates and venepuncture serum (Vp-serum) were measured by nephelometry (Behring Nephelometer II) by Sanquin Diagnostics. CV of these assays is 5.4% and 5.96% (Supplementary table S1), respectively.

Adalimumab and ADA level measurements
Adalimumab and ADA levels were measured using an ELISA and antigen-binding test (ABT), respectively, both described previously. CV of both assays was 7% and 14% (Supplementary table S1), respectively. DBS eluates were tested starting with a 5 times dilution instead of the usual 50 times dilution in the anti-adalimumab ABT.

Conversion of DBS values into DBS-serum concentrations
Five different methods were investigated to convert values measured in DBS eluates (total IgG, albumin, adalimumab and ADA) into a value which can be compared with Vp-serum levels (the so-called DBS-serum concentrations). An overview is provided in Table 1. Calculations are given in Supplementary Methods.

Statistical analyses
Statistical analyses were executed using Graphpad Prism 6.04. Correlations were calculated as Spearman correlation coefficients. Kruskal-Wallis multiple comparisons were used to calculate differences in percentage deviation between the quartiles. One outlier in percentage deviation in IgG concentration was identified by Grubbs analysis. The threshold for significance was set at a P-value of less than 0.05.

Table 1: Description of 5 different methods to calculate serum fraction in order to convert DBS values into DBS-serum concentrations.

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Vp A</td>
<td>Serum fraction in DBS eluate is calculated with albumin marker protein concentrations in both serum and DBS eluate.</td>
</tr>
<tr>
<td>Vp H</td>
<td>Serum fraction in DBS eluate is calculated by DBS area in combination with the Vp Hct.</td>
</tr>
<tr>
<td>DBS A42</td>
<td>Serum fraction in DBS eluate is estimated based on albumin marker protein concentration in DBS eluate and a fixed albumin concentration of 42 g/L in serum</td>
</tr>
<tr>
<td>DBS H0.42</td>
<td>Serum fraction in DBS eluate is based on DBS area in combination with a fixed Hct factor of 0.42 L/L</td>
</tr>
<tr>
<td>DBS Hcomp</td>
<td>Serum fraction in DBS eluate is based on DBS area in combination with a Hct factor computed from Hb measurements</td>
</tr>
</tbody>
</table>

Vp = Venepuncture; DBS = dried blood spot; Hct = haematocrit;
RESULTS

Baseline characteristics and response rates of the 161 patients with RA, PsA or AS treated with adalimumab included in this study showed that this cohort is representative for patients treated in daily clinical practice (Table 2). Twenty-seven (16.8%) patients had detectable ADA.

Adalimumab measurements in DBS

Adalimumab levels were measured in DBS eluates and corresponding Vp-sera. Measured adalimumab levels in DBS eluates were converted into DBS-serum concentrations based on the five different methods described in Table 1 and Supplementary Methods. In short, the two reference methods use either albumin as an internal marker protein (Vp A) or a volumetric conversion in combination with whole blood Hct value (Vp H). Of the three diagnostic methods, two use either a fixed estimated albumin concentration of 42 g/L (DBS A42), or a fixed estimated Hct of 0.42 L/L in combination with the DBS area (DBS H0.42). These methods are expected to deliver results that may systematically deviate depending on the actual albumin or Hct values, respectively. The third diagnostic method uses a Hct computed from the measured DBS Hb value in combination with the DBS area (DBS Hcomp), and should not yield such a deviation, but may suffer from a larger coefficient of variation (CV) due to the additional Hb measurement.

Figure 1A shows the correlation of adalimumab concentrations in Vp-serum samples and corresponding DBS-serum concentrations calculated with the DBS H0.42 method. There was a good correlation between the two values (r = 0.9342). However, adalimumab DBS-serum concentrations were consistently lower compared to their corresponding Vp-serum concentrations; which was seen for all five different methods (Supplementary figure S2). By plotting the percentage of deviation in adalimumab DBS-serum vs Vp-serum concentration against the adalimumab Vp-serum concentration (Figure 2), a median deviation of ca. -16% was seen for all methods across the full range of adalimumab concentrations.

### Table 2: Baseline demographics and characteristics

<table>
<thead>
<tr>
<th></th>
<th>RA</th>
<th>PsA</th>
<th>AS</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>96 (59.6%)</td>
<td>31 (19.3%)</td>
<td>34 (21.1%)</td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years, mean ± SD</td>
<td>57.8 ± 15.3</td>
<td>51 ± 12.3</td>
<td>50.5 ± 11.5</td>
</tr>
<tr>
<td>Female, no (%)</td>
<td>78 (81.3)</td>
<td>13 (41.9)</td>
<td>12 (35.3)</td>
</tr>
<tr>
<td>BMI, mean ± SD</td>
<td>25.9 ± 5.5</td>
<td>27 ± 3.1</td>
<td>24.5 ± 3.1</td>
</tr>
<tr>
<td>Disease duration, years, median (IQR)</td>
<td>8 (5-14)</td>
<td>8 (4-13)</td>
<td>7 (4-14)</td>
</tr>
<tr>
<td>RF, no (%)</td>
<td>60 (62.5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ACPA, no (%)</td>
<td>48 (50)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HLA-B27, no (%)</td>
<td>-</td>
<td>-</td>
<td>29 (85.3)</td>
</tr>
<tr>
<td>DAS28, mean ± SD</td>
<td>2.3 ± 1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PASI, median (IQR)</td>
<td>-</td>
<td>0 (0.04)</td>
<td>-</td>
</tr>
<tr>
<td>BASDAI, mean ± SD</td>
<td>-</td>
<td>-</td>
<td>3.3 ± 2.2</td>
</tr>
<tr>
<td>Laboratory parameters (serum)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR mm/hr, median (IQR)</td>
<td>9 (5-21)</td>
<td>5 (2-10)</td>
<td>7 (2-11)</td>
</tr>
<tr>
<td>CRP mg/L, median (IQR)</td>
<td>1.8 (0.7-4.4)</td>
<td>1.1 (0.6-2.2)</td>
<td>1.9 (0.7-4.1)</td>
</tr>
<tr>
<td>Haematocrit (L/L), mean SD</td>
<td>0.41 ± 0.03</td>
<td>0.43 ± 0.03</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td>Haemoglobin (mmol/L), mean ± SD</td>
<td>8.4 ± 0.6</td>
<td>8.9 ± 0.7</td>
<td>8.9 ± 0.7</td>
</tr>
<tr>
<td>Albumin (g/L), mean ± SD</td>
<td>41 ± 3.3</td>
<td>43.5 ± 2.9</td>
<td>42.9 ± 3.2</td>
</tr>
<tr>
<td>Total IgG (g/L), mean ± SD</td>
<td>12.6 ± 3</td>
<td>12.4 ± 3.1</td>
<td>12.5 ± 2.9</td>
</tr>
<tr>
<td>Medication use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration adalimumab, years, median (IQR)</td>
<td>4 (2-8)</td>
<td>3 (1-7)</td>
<td>4 (2-7)</td>
</tr>
<tr>
<td>Adalimumab level, median, mg/L (IQR)</td>
<td>5.9 (3.9-7.6)</td>
<td>5.3 (4.2-9.10)</td>
<td>6.6 (3.9-9)</td>
</tr>
<tr>
<td>ADA+, AU/mL, no (%)</td>
<td>16 (16.7)</td>
<td>3 (9.7)</td>
<td>8 (23.5)</td>
</tr>
<tr>
<td>Methotrexate use, no (%)</td>
<td>76 (79.2)</td>
<td>25 (80.6)</td>
<td>-</td>
</tr>
<tr>
<td>NSAID use, no (%)</td>
<td>-</td>
<td>-</td>
<td>14 (41.2)</td>
</tr>
<tr>
<td>Clinical response **</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remission, MDA or inactive disease, no (%)</td>
<td>61 (63.5)</td>
<td>20 (64.5)</td>
<td>17 (50)</td>
</tr>
</tbody>
</table>

* Normally distributed continuous variables are represented by mean values ± standard deviation (SD) and non-normally distributed continuous variables are represented by median values (interquartile range, IQR); dichotomous variables are represented by numbers (percentages of total).

** Clinical response was defined according to disease specific criteria. Remission for RA was defined as a DAS28<2.6; MDA for PsA was defined by criteria drafted by Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA); and Inactive disease for AS was defined as a BASDAI<4.
To evaluate if adalimumab DBS-serum concentrations may systematically deviate from the ‘true’ Vp-serum concentrations as a function of Hct or albumin, minimum to maximum deviation of quartiles in adalimumab concentrations were plotted against these parameters (Supplementary figure S3). The Vp A method resulted in less deviation in adalimumab concentrations in the last vs first quartile of Vp-serum albumin concentrations, and vice versa for the DBS A42 method (Supplementary figure S3F and G). For the volumetric methods no correlation was seen of the Vp-concentrations with Hct (nor albumin or adalimumab, Figure 3A and B; Supplementary figure S3C-E, S3H-J and S3M-O), although a non-significant downward trend was observed for deviation in adalimumab concentration with increasing Hct (Figure 3B) for DBS H0.42. Indeed, the theoretical Hct effect on the discrepancy of adalimumab concentrations for the DBS H0.42 method with ‘true’ values is small in the range of Hct values between 0.38 and 0.45, which is the Hct value in almost 80% of the patients (Supplementary figure S1D). Therefore, the DBS H0.42 method is in fact a satisfactory option for this patient group. Besides the systematic lower antibody concentration in DBS eluates there was also a certain level of random variation. This random variation can almost completely be accounted for by the expected collective %CV of the combination of assays needed to calculate the deviation of DBS-serum over Vp-serum. For DBS H0.42 the estimated %CV is 10.10 and the %CV observed in this cohort was 13.41. The separate CVs of the different assays are given in Supplementary Table S1. If actual Hct values were estimated by measuring Hb, and these were taken into account when calculating adalimumab concentrations (DBS Hcomp), the CV increases from 13.4% to 15.0%. Since Hct was reasonably well computed using this method (Supplementary figure S1C), this might be the preferred method in case Hct values are expected to deviate more than for the current study population.

Since adalimumab is an IgG-antibody, we wondered if the aforementioned lower DBS-serum adalimumab concentration reflects a general difference in antibody concentration in Vp-serum compared to DBS-serum concentrations. Indeed, IgG DBS-serum concentrations were also lower compared to Vp-serum concentrations (Figure 4A), although the deviation was less pronounced compared to adalimumab (-11.43% for IgG; -16.26% for adalimumab). Notably, this decreased concentration of antibodies in DBS eluates was not an overall protein effect, since the albumin concentration was not significantly lowered in DBS-serum concentrations compared to serum (median deviation: 2.01; Figure 4B). To correct for this lower antibody concentration in the DBS-serum concentration, the calculated concentrations were multiplied with a constant factor 1.194 (1/(1-16.26%)). (Supplementary figure S4A).

ADA measurements in DBS
In addition to adalimumab level, we also investigated if ADA could be detected in DBS eluates. There was a decent correlation (r = 0.8741) between DBS-serum concentrations calculated with the DBS H0.42 method and corresponding sera for 16.8% (27 of 161) of serum samples with detectable ADA in serum samples (Figure 1B). A lower ADA level, similar to abovementioned result for adalimumab level, was seen in DBS eluates. After correction for this systematic deviation with the same factor as used for adalimumab level, two patients with low adalimumab levels (< 5 mg/L) and two patients with adalimumab levels in the therapeutic range (5-8 mg/L)1 were negative for ADA in serum samples and slightly positive for ADA (between 16 and 20.5 AU/ml) in the corresponding finger prick sample. The other way around, four patients with low adalimumab levels (< 5 mg/L) and three patients with adalimumab levels in the therapeutic range (5-8 mg/L) were negative for ADA in finger prick sample and slightly positive for ADA (between 12 and 28 AU/ml) in the corresponding serum sample.

Recovery of adalimumab, IgG and albumin in DBS
The systematic decrease in antibody concentration (adalimumab, ADA and IgG) in DBS eluates compared to Vp-serum can be an effect of loss of antibody protein during laboratory procedure to get eluates from DBS, an inability to detect the protein in the eluates, or a different composition of material obtained by finger prick compared to Vp. To exclude the first two options, we spotted whole Vp blood spiked with adalimumab on DBS cards, made eluates after 1 week storage, calculated the precise amount of plasma present in DBS eluates and compared the concentration in the DBS-plasma concentrations with the plasma concentration, which were not spotted. There was no significant loss in adalimumab, IgG or albumin with blood spotted on DBS cards (Supplementary figure 5 and data not shown).

DISCUSSION
Here we present a method that supports the usage of DBS obtained from finger prick as alternative for Vp-serum in the measurement of adalimumab and ADA concentrations in patients with rheumatic inflammatory diseases treated with adalimumab. This method would simplify the process of TDM, thereby increasing its possibility for implementation in routine care. However, advantages of a DBS method (e.g. less burden for patient and physician) should outweigh potential disadvantages compared to the conventional method (such as loss in precision and accuracy) and the amount of labour-intensity and costs should be within acceptable limits.15,21-23

To be able to compare the DBS values with serum concentrations we used five different methods to convert these values to DBS-serum concentrations. All methods showed a good correlation with Vp-serum results and the precision of all assays remained below 15%, in line with the European Medicine Agency (EMA) and Food and Drug Administration (FDA) guidelines of method validation.24,25 The precision of the final DBS assay to test adalimumab levels can be expected to be even lower, since no comparison with serum measurements (CV 7%) is needed.
One of the most important parameters that needs to be considered when optimising a DBS method, according to the White Papers of the European Bioanalysis Forum (EBF), is Hct. Hct influences recovery, spot size, and blood-to-plasma ratio. The effect of Hct on these parameters was investigated in this study. Recovery was not affected by Hct, since Hct levels did not significantly alter the deviation of DBS-serum over Vp-serum. Spot size and blood-to-plasma are clearly affected by Hct levels (Supplementary figure 1A), however these effects are completely (Vp H and DBS Hcomp) or partially (DBS H0.42) accounted for in the calculations to convert DBS eluates levels in DBS-serum concentrations. Theoretically, Hct values deviating from the average of 0.42 will result in a proportional deviation in DBS-serum concentrations calculated with the DBS H0.42 method from the ‘true’ value. However, since almost 80% of the patients in this study had a very limited Hct range from 0.38 to 0.45, the Hct effect was small (max 5%) compared to the random variation. This may explain why a significant influence of Hct on the calculated adalimumab concentration was not observed. Therefore, the DBS H0.42 method is a satisfactory alternative to Vp-serum adalimumab concentrations for this patient group, but the DBS Hcomp is most likely preferred in patient populations with known wider or aberrant Hct distributions. An accurate estimation of Hct levels can also be done using potassium levels in DBS eluates instead of the Hb levels used in this study.26

![Figure 1](image.png)

**Figure 1:** Correlation of adalimumab (ADL) (mg/L) (A) and anti-ADL (AU/mL) (B) measured in Vp serum versus the DBS-serum concentration calculated with the DBS H0.42 method. Spearman correlation coefficients were $r = 0.9342$ for ADL and $r = 0.8735$ for the anti-ADL positive Vp-serum samples. 4 DBS-serum concentrations were positive low positive (13.3 AU/ml – 17.1 AU/ml) whereas antibodies were not detected above the cut-off of 12 AU/ml in the corresponding sera.

Other parameters that could affect DBS results are spot homogeneity and DBS stability. Since we eluted the whole DBS, the issue of spot homogeneity is excluded. Stability of DBS cards was tested for 3 months at room temperature and no loss of recovery was observed. This simplifies shipment of material and allows sending of DBS by regular mail if kept at room temperature during shipment.

The systematically lower antibody concentration in DBS eluates compared to Vp serum suggests that there is a discrepancy in protein concentrations in capillary compared to venous blood or that the capillary blood is diluted with fluids present between the capillary and the paper. The difference in molecular weight of IgG and albumin or the presence of albumin in dilution fluids (e.g. exudate) could explain why this lower concentration in DBS eluates was not observed for albumin. Although a definitive explanation for the lower levels of antibody in DBS when compared to serum is lacking, it can easily be corrected by multiplying the calculated DBS level with a constant factor of 1.194, as recommended by the EBF.21 This resulted in a good correlation between DBS and the Vp-serum values of adalimumab and ADA.

The current study was a cross-sectional analysis of patients with RA, PsA or AS treated with adalimumab. The number of patients with detectable ADA was limited (27 (16.8%)) in this study of patients. Possible explanations might be that non-responders due to ADA formation discontinued treatment prematurely, moreover, an ABT was used to measure ADA against adalimumab which is a drug-sensitive assay. Because of the low number of ADA positive patients (27 of 161 patients), we only clinically validated our assay based on the adalimumab level data. However, ADA levels in DBS and serum correlate well ($r = 0.8741$) and the patients with discrepancies between finger prick and Vp-serum had low ADA levels (max 28 AU/ml; threshold: 12 AU/ml).

Based on the similar behaviour of the three different antibodies in DBS tested in this study (adalimumab, ADA and IgG), we suspect that, although we focused in this study on adalimumab treated patients, TDM using DBS from finger prick can be used for patients treated with other biologicals as well. In the current study, finger prick is taken by the nurse in a controlled environment together with a Vp, to first investigate only the effect of DBS from finger prick versus Vp serum. In the study of Vande Casteele and colleagues, infliximab and adalimumab treated patients were included via an self-taken finger prick. These results together with our own results described in this paper support the feasibility of TDM studies of biologicals via self-sampling.

In conclusion, we have shown in this cross-sectional study including 161 patients with rheumatic inflammatory diseases treated with adalimumab whom are representative for patients treated in daily clinical practice, that adalimumab and ADA levels can be
properly measured in DBS samples obtained via finger prick. Precision and accuracy were within acceptable limits as described by FDA and EMA guidelines.\textsuperscript{24-25} Moreover, DBS can be stored at room temperature for 3 months which is convenient for shipment and only limited amount of blood is needed. In addition, DBS will reduce costs and time of physicians or nurses and patients, compared to serum withdrawal with Vp. Implementing this DBS method simplifies the TDM process and can provide more insight into PK of adalimumab, since frequent sampling within one dosing interval can easily be performed with an at home finger prick.

Figure 2: A-E Percentage deviation \( \frac{\text{DBS-Serum-Vp-serum}}{\text{Vp-serum}} \) in adalimumab (ADL) concentration (mg/L) calculated from the DBS eluates in the 5 different ways (DBS-serum concentrations) compared to the corresponding Vp serum concentrations. The dotted line represents the median deviation. The mean, % coefficient of variation (CV), estimated % CV and 95% confidence interval (CI) of the median are given in the imbedded box.

Figure 3: Percentage deviation in adalimumab (ADL) concentration (mg/L) calculated from the DBS eluates with the diagnostic method DBS H0.42 compared to the corresponding Vp serum concentration plotted against the quartiles of the ADL concentration (mg/L) (A) or the Hct values (L/L) (B). Box plots with minimum to maximum deviation are shown.

Figure 4: Percentage deviation in IgG concentration (g/L) calculated from the DBS eluates with the diagnostic method DBS H0.42 compared to the corresponding Vp serum concentration plotted against the Vp-serum IgG concentration (A). Percentage deviation in albumin concentration (g/L) calculated from the DBS eluates with the diagnostic method DBS H0.42 compared to the corresponding Vp-serum concentration plotted against the Vp-serum albumin concentration (B).
Supplementary Methods

Calculations of serum concentrations from DBS

To calculate the serum concentration of total IgG, adalimumab, anti-adalimumab or albumin (\(c_b\)), the concentration measured in the DBS eluate (\(c_e\)) was converted via one of the following methods.

Marker protein methods

Albumin was used as an internal reference protein. For the reference method \(V_p A\), the measured albumin concentration in serum (\(alb_s\)) was used:

\[
\text{formula 1)} \quad c_s = c_e \cdot \frac{alb_s}{alb_e}
\]

with \(alb_e\) the albumin concentration measured in the DBS eluate, whereas for the diagnostic method \(DBS A42\), a constant value of 42 was used:

\[
\text{formula 2)} \quad c_s^{\text{calc}} = c_e \cdot \frac{42}{alb_e}
\]

This will lead to a theoretical deviation from the ‘true’ value (\(c_s\)), which is given by

\[
\text{formula 3)} \quad \frac{c_s^{\text{calc}}}{c_s} = \frac{42}{alb_e}
\]

Volumetric methods

Alternatively, concentrations were based on estimating the original volume of the blood sample on the filter paper in order to calculate the dilution factor of the blood sample into the eluate, in combination with estimating the serum fraction of the blood sample using one of several ways to estimate the cell fraction (hct).

The blood concentration of total IgG, adalimumab, anti-adalimumab or albumin (\(c_b\)) is given by

\[
\text{formula 4)} \quad c_b = c_e \cdot d
\]

with \(d\) the dilution factor of the DBS sample after elution, i.e., the ratio’s of the original volume of the blood sample (\(V_b\)) and the volume of the eluate (\(V_e\)):

\[
\text{formula 5)} \quad d = \frac{V_e}{V_b}
\]

 Whereas \(V_e\) is known, \(V_b\) needs to be estimated. For this, we measure the area of the blood spot \(A(hct)\) and multiply this with \(\nu(hct)\), the blood volume per unit area:

\[
\text{formula 6)} \quad V_b = A(hct) \cdot \nu(hct)
\]

Thus, \(V_b\) can be calculated if \(A(hct)\) is measured and \(\nu(hct)\) is known.

We observed that the blood volume per unit area depended on the cell fraction, or hematocrit (hct), to some extent. In other words, a given volume of blood resulted in somewhat different areas depending on hct, therefore, both \(A(hct)\) and \(\nu(hct)\) are functions of hct. We postulate that \(\nu(hct)\) can be approximated as

\[
\text{formula 7)} \quad \nu(hct) = \nu_0 + \nu_1 \cdot hct
\]

where \(\nu_0\) and \(\nu_1\) are parameters that were obtained from fitting this function to blood spot area data converted to \(\nu(hct)\) values for blood samples with a precisely chosen range of hct values applied onto filter paper (see Supplementary figure 1A). In this way, the expression for the blood concentration becomes

\[
\text{formula 8)} \quad c_s = c_e \cdot \frac{V_e}{A(hct) \cdot (\nu_0 + \nu_1 \cdot hct)}
\]

To convert blood concentration into serum concentration we use

\[
\text{formula 9)} \quad c_s = \frac{c_b}{1 - hct}
\]

For the reference method \(V_p H\), this leads to the expression

\[
\text{formula 10)} \quad c_s = c_e \cdot \frac{1}{1 - hct} \cdot \frac{V_e}{A(hct) \cdot (\nu_0 + \nu_1 \cdot hct)}
\]

For the diagnostic method \(DBS H0.42\), a constant value for hct of 0.42 is assumed:

\[
\text{formula 11)} \quad c_s^{\text{calc}} = c_e \cdot \frac{1}{1 - 0.42} \cdot \frac{V_e}{A(hct) \cdot (\nu_0 + \nu_1 \cdot 0.42)}
\]

For this method, the theoretical deviation from the ‘true’ value is given by

\[
\text{formula 12a)} \quad \frac{c_s^{\text{calc}}}{c_s} = \frac{1}{1 - 0.42} \cdot \frac{1}{\nu_0 + \nu_1 \cdot 0.42} \cdot (1 \cdot hct) \cdot (\nu_0 + \nu_1 \cdot hct)
\]
In other words, deviation for this method is dependent on hct (which is assumed to be constant but in reality is not), which affects both the serum fraction, as well as flow of blood through the filter paper. The latter contribution is smaller than, and partially compensates the former theoretically (Supplementary figure 1D).

For the diagnostic method DBS Hcomp, a value for hct is estimated by measuring Hb in the DBS eluate. Since the cell fraction in blood is predominantly made up by red blood cells (RBC), and RBC count correlates linearly with Hb (Supplementary figure 1B), the following relationship holds:

(formula 12b) \[ \text{hct} = y \cdot \text{Hb}_e \]

Furthermore, \( \text{Hb}_e \) can be obtained as

(formula 13) \[ \text{Hb}_e = \frac{\text{Hb}_b \cdot d}{A(\text{hct}) \cdot (V_e + V_i \cdot \text{hct})} \]

with the latter approximation based on equations 5-7.

We observed a small loss of hemoglobin in the DBS eluates of the adalimumab treated patients, since the \( \text{Hb}_b \) based on the \( \text{Hb}_e \) was on average 11% lower compared to \( \text{Hb}_b \) measured in whole blood. To compensate for this loss, the \( \text{Hb}_e \) values were multiplied with 1.13 as correction factor.

The expression for hct becomes

(formula 14) \[ \text{hct} = \frac{y \cdot \text{Hb}_b \cdot V_e}{A(\text{hct}) \cdot (V_e + V_i \cdot \text{hct})} \]

Rearranging and solving for hct results in

(formula 15) \[ \text{hct} = \frac{-V_e + \sqrt{V_e^2 + 4 \cdot \frac{y \cdot \text{Hb}_b \cdot V_e}{A(\text{hct}) \cdot V_i}}}{2 \cdot V_i} \]

which can be inserted into equation 10 to calculate \( C_e \). In this way, serum concentrations are calculated based on a volumetric conversion of DBS concentrations that take into account actual hct values for estimating serum fraction and correcting for hct-dependent flow of blood through the filter paper; the actual hct value being based on measurement of Hb. The correlation between the actual hct and the hct value based on measurement of Hb for the adalimumab treated patients in this study is shown in Supplementary figure 1C.

Supplementary table

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<th>% CV</th>
<th>ADL</th>
<th>Anti-ADL</th>
<th>IgG</th>
<th>Albumin</th>
<th>Hct</th>
<th>Area</th>
<th>Hb</th>
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<td>2%</td>
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Supplementary material

Supp. Figure S1: Blood volume (µl) per area (mm²) plotted against the Hct values of the spotted blood. Average with standard deviation (SD) of 5 different volumes between 100 and 200 µl is shown (A). Hb in blood is plotted against Hct values of spotted blood. Hb in blood is calculated from Hb measured in eluates multiplied by the dilution factor of spotted blood in elution buffer (B). Computed Hct based on Hb with the DBS Hcomp formula given in Supplementary methods plotted against Hct values of spotted blood (C). Theoretical percentage deviation of plasma proteins calculated with the DBS H0.42 method compared to concentration in Vp-serum plotted against hypothetical Hct values (D). Formula is given in Supplementary methods.

Supp. Figure S2: Correlation of adalimumab (ADL) concentration (mg/L) measured in Vp serum and calculated from DBS eluates with the 5 different methods described in Patients and Methods. Spearman correlation coefficients (r) were r = 0.9506 for Vp A, r = 0.9534 for DBS A42, r = 0.9349 for Vp H, r = 0.9342 for DBS H0.42 and r = 0.9168 for DBS Hcomp.
Supp. Figure S3: Percentage deviation in adalimumab (ADL) concentration (mg/L) in the DBS serum concentrations calculated in the 5 different ways compared to Vp serum concentrations plotted against the quartiles of the ADL concentration (A-E), albumin concentration (g/L) (F-J) or haematocrit (Hct) values (L/L) (K-O). Box plots with minimum to maximum deviation are shown.
Dried blood spots obtained by finger prick to monitor adalimumab treatment

Supp. Figure S4: Correlation of adalimumab (ADL) (mg/L) (A) and anti-ADL (AU/mL) (B) measured in Vp serum and calculated from DBS eluates with the DBS H0.42 method after correction with conversion factor.

Supp. Figure S5: Vp blood was spiked with adalimumab (ADL) and spotted on DBS cards; eluates were made and ADL, IgG and albumin concentration were measured. The plasma concentration was calculated based on the spotted amount of blood in combination with the measured hct value. DBS-plasma concentration is presented as percentage recovery compared to Vp-plasma concentration.
REFERENCE LIST


