Anti-TNF therapy in inflammatory bowel disease
Towards personalized medicine
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Monitoring of adalimumab concentrations at home in patients with inflammatory bowel disease by dried blood samples
ABSTRACT

Background
Adalimumab (ADL) is a subcutaneous administered anti-tumor necrosis factor (TNF) agent used in the treatment of inflammatory bowel disease (IBD) patients. Higher ADL trough concentrations are associated with improved clinical and endoscopic outcomes. Therapeutic drug monitoring of ADL might be facilitated by using dried blood samples (DBS) from capillary blood obtained at home. The aim was to compare serum ADL concentrations obtained via venepuncture to ADL DBS concentrations.

Methods
Crohn’s disease and ulcerative colitis patients receiving induction or maintenance ADL therapy were enrolled in this prospective cohort study. Blood was obtained via venepuncture and via DBS during a regular outpatient visit (time point 1). Just before the next ADL administration, patients performed DBS at home (time point 2). For this time point, serum ADL concentrations were estimated by Bayesian analysis.

Results
A total of 33 IBD patients were enrolled. During the outpatient visit, samples were obtained after a median [interquartile range (IQR)] of 6 [4-10] days after the last ADL dose. A high correlation was found between DBS and venepuncture results (Pearson correlation: ≥0.96), without any clinically relevant bias. For DBS performed by patients at home, initial comparison showed a moderate correlation between DBS results and predicted ADL serum concentrations (Pearson correlation: 0.51), although no bias was present. In addition, DBS eluate results compared to predicted ADL serum concentrations showed a mean absolute percentage error (i.e. accuracy) of 45%.

Conclusion
High correlations were found between ADL serum concentrations, obtained via conventional venepuncture, and DBS, which indicates that this home-based test can facilitate TDM-based ADL dose adjustments in daily practice.

Keywords: inflammatory bowel disease, adalimumab, therapeutic drug monitoring, dried blood sample
INTRODUCTION

Adalimumab (ADL) is a subcutaneous (SC) administered IgG monoclonal antibody directed against the pro-inflammatory cytokine tumor necrosis factor (TNF) and is used in the treatment of patients with inflammatory bowel disease (IBD). Interest in therapeutic drug monitoring (TDM) of anti-TNF agents has raised great interest to optimize treatment efficacy. Higher serum ADL trough concentrations, i.e. the lowest concentration before the next administration, are associated with improved clinical and endoscopic outcomes in patients with Crohn’s disease (CD) and ulcerative colitis (UC). To optimize treatment outcome in patients on ADL therapy, an ADL trough concentration between 4.9 and 12 mg/L is targeted.\textsuperscript{1–5}

Timing of TDM is still a matter of debate. Reactive TDM is applied in case of signs of secondary loss of response. When serum ADL trough concentrations are low, ADL maintenance treatment can be intensified from 40 mg SC every other week to 40 mg SC every week. In case of detectable anti-ADL antibodies (immunogenicity), addition of an immunomodulator (i.e. thiopurines or methotrexate) has shown to be beneficial.\textsuperscript{6,7} Proactive TDM during anti-TNF maintenance therapy may be useful to prevent flares in IBD patients caused by underdosing. Because serum ADL trough concentrations and anti-ADL antibodies are evaluated at a regular basis, pro-active dose adjustments (by changing the dose and/or dosing interval) can be made.

To facilitate TDM, capillary blood obtained via finger prick (i.e. dried blood samples (DBS)) can be used to measure anti-TNF serum concentrations.\textsuperscript{8,9} Patients can perform DBS themselves at home and send the samples by regular mail to the laboratory for subsequent evaluation of ADL trough concentrations, without coming to the hospital first. The primary aim of this study was to compare ADL serum concentrations and ADL concentrations measured in DBS from IBD patients in a clinical setting.

METHODS

Patients and samples

IBD patients, receiving ADL induction or maintenance therapy according to label, were prospectively enrolled during a scheduled routine visit to the outpatient clinic of the Amsterdam University Medical Centre, location Meibergdreef (Amsterdam, The Netherlands). During this visit, blood was obtained via venepuncture and in two-fold via DBS (time point 1, see Supplementary figure 1). The first DBS was performed by a trained healthcare professional and the second DBS by the patient after thorough instructions. Both DBS eluate results were compared to the ADL serum concentration obtained via venepuncture. To assess repeatability, DBS results from a trained healthcare professional were compared to DBS results obtained by the patient. Before their next ADL administration, patients were asked to perform a DBS at home (time point 2) and send the sample directly to Sanquin laboratories (Amsterdam, the Netherlands). The study was approved by the
Elution of capillary blood from DBS
The finger prick was performed using a contact-activated lancet (BD Microtainer 2.0 mm by 1.5 mm). Capillary blood was collected via a Mitra™ microsampling device with volumetric absorptive microsampler (VAMS) technology. The microsampling device consists of an absorbent polymeric tip designed to take up a fixed volume of blood by capillary action. The Mitra™ tips filled with blood were removed from the holder and eluted in elution buffer (PBS/0.05% Tween/0.05% NaN₃) vigorously shaking overnight (≥17 hours) on an orbital shaker. After removal, the eluate was kept at 4°C until measurements were performed. A fixed Hct value (0.42) was used to convert DBS eluate results to comparable serum ADL concentrations.

Adalimumab and anti-adalimumab antibody concentrations
ADL serum concentrations were measured using an enzyme-linked immunosorbent assay based on the principle that ADL is captured using its ability to bind TNF (Sanquin Diagnostic Services, Biologics Lab, Amsterdam, the Netherlands). ADL binding was assessed by incubation with biotinylated rabbit IgG directed to the ADL idiotype and subsequent detection with strep-poly horseradish peroxidase (HRP). The lower limit of quantification (LLOQ) of this assay is 0.01 mg/L. DBS eluate ADL concentrations were measured in the same dilution range as serum samples in the ADL serum concentration assay, resulting in a LLOQ of 0.6 mg/L after conversion to serum values. Accuracy of ADL measurements in serum is between 90.6 and 110% depending on the ADL concentration in serum and precision in % coefficient of variation (CV) is ≤5. Usage and elution from Mitra™ tips adds an extra variation component. For ADL level measurements this was extensively investigated and this results in an accuracy of 108.4% with a %CV of 10. Anti-ADL antibodies were measured in samples with serum ADL concentration <1 mg/L using an antigen binding test (ABT). Lower limit of detection (LOD) for serum anti-ADL antibodies is 12 AU/mL. DBS eluates were measured 20 times less diluted compared to serum samples in the ABT with a LOD of 12 AU/mL after conversion to serum values. Accuracy of anti-ADL measurements in serum is between 94 and 101% depending on the ADL concentration in serum and precision in %CV is ≤14.

Statistics
Correlations between DBS eluate results and ADL serum concentrations were calculated as Pearson or Spearman correlation coefficients, depending on normality. To assess the normality of all measured ADL concentrations, a Shapiro-Wilk test was used. Passing-Bablok linear regression was used to calculate the intercept and slope of the linear regression according to the following linear equation:
\[ Y = \beta_0 + \beta_1 X \]

Where \( \beta_0 \) represents the intercept and \( \beta_1 \) represents the slope of the linear regression line. The intercept represents the systematic bias between the two methods and the 95% confidence interval of the intercept should include 0. The slope represents the proportional bias between the two methods and the 95% confidence interval of the slope should include 1. For Bland-Altman plots, bias was defined as the difference between DBS eluate results and the serum ADL concentration, expressed as a percentage of the mean. Absolute agreement between the two methods was further assessed using the intraclass correlation coefficient (ICC; two-way mixed, absolute agreement, single measures). To evaluate repeatability of DBS, DBS eluate results performed by a trained healthcare professional were compared to DBS eluate results performed by the patient using Passing-Bablok regression, Bland-Altman plot and ICC. All statistical analyses were performed using R (version 3.5.2, Vienna, Austria).

Bayesian analysis

After the scheduled routine visit to the outpatient clinic, patients were asked to perform a DBS at home and send directly to Sanquin. Because no venepuncture was performed at the time of home-sampling, serum ADL concentrations were predicted by maximum a posteriori Bayesian forecasting (NONMEM, Icon, Dublin, Ireland, software version 7.4). A population pharmacokinetic model, based on CD patients on ADL induction or maintenance therapy, was used.\(^{14}\) Data about anti-ADL antibody status and dosing regimen (every week/every other week) from each individual patient were entered in the model, as well as the measured ADL concentration obtained via venepuncture at time point 1. Evaluation of the model was qualified for usage by a visual predictive check (VPC), see Supplementary figure 2. The difference between predicted serum concentrations and measured DBS eluate results, was evaluated by calculating accuracy using mean absolute percentage error (MAPE):\(^{15,16}\)

\[
MAPE = \frac{1}{n} \sum \left| \frac{Obs - Ipred}{Obs} \right| \times 100
\]

where Obs denotes the observed DBS eluate ADL concentration and Ipred denotes the individual predicted ADL serum concentration. Bias was calculated using the mean error (ME):\(^{15}\)

\[
ME = \frac{1}{n} \sum (Obs - Pred)
\]

where Obs denotes the observed DBS eluate ADL concentration and Pred denotes the population predicted ADL concentration.
Evaluation sampling strategy

Serum ADL concentration at time point 2 was predicted for each individual patient based on one ADL concentration measurement after venepuncture at time point 1. Simulations were used to assess the predictive performance of the population pharmacokinetic model to predict ADL serum concentrations at time point 2 (home-sampling). For this purpose, the same population pharmacokinetic model from literature was used as the input model and typical values of pharmacokinetic parameters were used. A population of 1,000 patients was simulated, and for all patients an individual concentration-time curve was constructed. All patients received one dose of 40 mg ADL SC every other week, assuming steady-state, and no covariates were taken into account.

Two sampling strategies were evaluated, in which the first represents the current study design. For each individual, a simulated ADL concentration was observed at time point 1, ranging from day 1 to day 11 after the last ADL administration. Based on this concentration, a serum ADL concentration for each individual patient was predicted at day 14, assuming DBS was performed by the patient at trough concentration (time point 2, day 14). To evaluate the sampling strategy, predicted ADL concentrations at day 14, were compared to simulated ADL concentrations at day 14.

In the second sampling strategy, the ADL concentration was observed at time point 1 (day 1-11), followed by a second serum ADL concentration observation two days later (time point 2), which could range from day 3 to day 13. At trough concentration at day 14 (time point 3), ADL serum concentration was predicted and compared to the simulated ADL serum concentration of this time point to evaluate the sampling strategy.

Both sampling strategies were evaluated by calculating accuracy using mean absolute percentage error (MAPE): 15, 16

\[
MAPE = \frac{1}{n} \sum \left| \frac{Sim - Ipred}{Sim} \right| * 100
\]

where Sim denotes the prediction of the simulated ADL concentration and Ipred denotes the individual predicted ADL concentration. Bias was calculated using the mean error (ME): 15

\[
ME = \frac{1}{n} \sum (sim - pred)
\]

where Sim denotes the prediction of the simulated ADL concentration and Pred denotes the population predicted ADL concentration.
RESULTS

Patients and samples
Thirty-four patients were included in this prospective cohort study. Out of 34 patients, one patient withdrew informed consent and 33 patients (Crohn’s disease: 27, ulcerative colitis: 6) were evaluated. Thirty-one patients received ADL maintenance treatment. At baseline, median [interquartile range (IQR)] albumin and C-reactive protein (CRP) were 43 g/L [42 - 44 g/L] and 1.7 mg/L [1.0 - 3.4 mg/L], respectively. Patients had a median [IQR] haematocrit (Hct) of 0.43 L/L [0.40 - 0.45 L/L], see Table 1 for all patient characteristics.

Table 1: Patient characteristics at baseline

<table>
<thead>
<tr>
<th></th>
<th>N=33</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crohn’s disease (N, %)</td>
<td>27 (82%)</td>
</tr>
<tr>
<td>Ulcerative colitis (N, %)</td>
<td>6 (18%)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>16 (48%)</td>
</tr>
<tr>
<td>Induction (N, %)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>Maintenance (N, %)</td>
<td>31 (94%)</td>
</tr>
<tr>
<td>Every other week (N %)</td>
<td>17</td>
</tr>
<tr>
<td>Every week (N, %)</td>
<td>13</td>
</tr>
<tr>
<td>Every three weeks (N, %)</td>
<td>1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43 [31 - 56]</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74 [65 - 84]</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>14.3 [5.4 - 21.4]</td>
</tr>
<tr>
<td>Hematocrit (L/L)</td>
<td>0.43 [0.40-0.45]</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>43 [42-44]</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.7 [1.0-3.4]</td>
</tr>
<tr>
<td>Corticosteroids (N, %)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>Immunomodulators (N, %)</td>
<td>12 (35%)</td>
</tr>
<tr>
<td>Thiopurines (N, %)</td>
<td>5 (15%)</td>
</tr>
<tr>
<td>Methotrexate (N, %)</td>
<td>7 (21%)</td>
</tr>
</tbody>
</table>

Categorical values are reported as count (percentage of total)
Continuous values are reported as median values [interquartile range]
CRP; C-reactive protein, N; number
**DBS ADL concentrations vs. serum ADL concentrations**

Samples at time point 1 (during a routine visit to the outpatient clinic) were obtained after a median [IQR] of 6 [4-10] days after the last ADL administration with a median [IQR] serum ADL concentration of 8.3 [5.6-12.6] mg/L. In total, 33 ADL serum concentrations were obtained via venepuncture at this time point (Figure 1). One patient sample was excluded because ADL concentrations were below LLOQ by using either DBS or venous blood. In both DBS and venous blood of this patient sample, anti-ADL antibodies were detected (>975 AU/mL).

![Figure 1: Measured adalimumab serum concentrations versus time after dose (days)](image)

**Figure 1: Measured adalimumab serum concentrations versus time after dose (days)**

![Figure 2: Passing-Bablok hospital visit healthcare professional + patient](image)

**Figure 2: Passing-Bablok hospital visit healthcare professional + patient**

Solid black circles represent measured adalimumab concentrations and the dashed black line indicates the line of identity. The solid grey line indicates the regression line and the grey shaded area represents the confidence interval of the regression line.
Healthcare professional
A high correlation was found between venepuncture and DBS results, performed by a trained healthcare professional (Pearson's correlation coefficient: 0.96). Passing-Bablok regression showed no systemic or proportional bias with the intercept of the regression line including zero (0.79 mg/L (95% CI -0.04 – 1.51 mg/L)) and the slope including 1 (0.91 (95% CI 0.79 – 1.01)) (Figure 2A). Bland-Altman plots showed a mean bias of -1.3% with lower and upper limits of agreement of -27 to 25% (Figure 3A). ICC was 0.96 (P<0.0001).

Patient
For DBS performed by a the patient, venepuncture and DBS showed high correlation (Pearson's correlation coefficient: 0.97). Passing-Bablok regression showed no systemic bias (intercept: 0.81 mg/L (95%CI 0.24 - 1.36 mg/L), but small proportional bias was shown (0.89 (95%CI 0.79 – 0.96) (Figure 2B). Bland-Altman showed a mean bias of 1.2% (limits of agreement: -23 – 26%) (Figure 3B). ICC was 0.96 (P<0.001) for DBS eluate results compared to venepuncture results.

Figure 3: Bland-Altman plots: (left) healthcare professional + (right) patients
Mean bias is indicated by the solid red line. Dashed lines represent the upper and lower limits of the bias

Healthcare professional vs. patient
To evaluate repeatability of DBS, DBS eluate results performed by a trained healthcare professional were compared to DBS eluate results from the patient. Passing-Bablok showed no systematic bias (intercept: -0.12 (95% CI -0.67 – 0.36 mg/L) or proportional bias (slope: 1.04 (95%CI 0.96 – 1.14). Bland-Altman showed a mean bias of 2.5% (lower and upper limits of agreement: -15 – 20%) and ICC was 0.96 (p<0.001), see Supplementary Figure 3.

Home-sampling
A total of 28 samples were performed by patients at home using DBS (time point 2), and samples were sent to Sanquin Laboratories (Amsterdam, the Netherlands). Samples were obtained after a median [IQR] of 13.5 [7.0 –14] days after the last ADL administration. An
initial comparison was made between DBS samples from time point 2 and obtained serum concentrations based on empirical Bayesian estimates. Passing-Bablok regression showed no structural or proportional bias (intercept: 0.04 mg/L (95%CI -7.57 - 2.22 mg/L), slope: 0.97 (95%CI 0.56 - 1.94), and Pearson’s correlation coefficient showed moderate correlation (0.51) . Bland-Altman plot showed mean bias: 2.4% (limits of agreement -102 - 107%), see Supplementary figure 4. MAPE (accuracy) and ME (bias), were 45% and 2.0%, respectively.

Sampling strategies
For evaluation of the sample schemes, MAPE for accuracy and ME for bias were calculated. MAPE and ME for sample scheme 1 (representing the current study design) were 23% and -0.76 mg/L, respectively. For the sampling strategy with an additional measured ADL serum concentration, MAPE and ME were 12% and -0.35 mg/L, respectively.

DISCUSSION
This prospective cohort study showed that DBS-based ADL concentration measurements can be used to measure ADL serum concentrations in IBD patients. Higher ADL trough concentrations, are associated with improved therapy outcomes during ADL maintenance treatment in IBD. Implementation of TDM in the routine management of IBD patients receiving ADL is hampered by time between blood sample withdrawal and availability of results. Clinical decisions, that are based on ADL trough concentrations and anti-ADL antibody status can be delayed for up to 2 weeks. In addition, TDM for ADL is designated to be performed at trough and is not directly interchangeable with concentrations at non-trough. In 1963, the use of capillary blood was described as a novel method to screen for metabolic disorders in neonates. The scope of applications for DBS has expanded over time and now includes the measurement of monoclonal antibodies. By obtaining capillary blood via a finger prick for DBS, patients can send in a DBS from home which can be performed just before the next ADL administration. When a patient visits the outpatient clinic afterwards, ADL trough concentration and anti-ADL antibody results are known which can guide treatment decisions.

Next to evaluation of DBS used in a clinical setting, we evaluated the use of DBS by the patient at home. To assess DBS eluate results from home-sampling, serum ADL concentrations were predicted for this time point using Bayesian analysis. A pharmacokinetic model from literature was used based on CD patients using adalimumab on maintenance therapy, which is similar to the current study population. No proportional or systemic bias was seen, but results showed high variability between DBS eluate and predicted ADL concentrations (accuracy: 45%). First, this higher variability in results can be attributed to the use of Bayesian analysis. Although Bayesian analysis is a reliable tool to predict drug concentrations, it also contributes to some extend to the variability seen. In addition, this variability might be
caused by the sampling strategy used, as only one serum ADL concentration was used as input to predict ADL concentration at trough to compare with the DBS result. Simulation of the current sampling strategy showed reasonable accuracy (23%), which can be reduced to 12% by means of a second serum ADL concentration measurement. Hence, for optimal prediction by Bayesian analysis of an ADL serum concentration at trough (i.e. to compare to an ADL DBS eluate result), two obtained serum ADL samples could reduce variability. Lastly, this variability might be the result of performance of DBS by the patient at home without supervision. Collection of capillary blood should be performed carefully to prevent under or over filling of the Mitra™ tip.

Even though this study has a relative small sample size, this is the first study that shows that DBS is a reliable tool to measure ADL concentrations in IBD patients by making use of a Mitra™ micro-sampling device. High agreement was seen between DBS results and venepuncture results, which supports the use of DBS in clinical practice. DBS measurements will likely reduce the time to ADL dose adjustments in IBD patients who lose their response, resulting in optimized treatment outcomes.
REFERENCES


**SUPPLEMENTARY**

Supplementary Figure 1: Study design

Supplementary Figure 2: Visual Predictive Check

Individual observations of ADL are represented by the solid circles. The red solid line depicts the median of the observed ADL concentrations and the blue solid lines depict the observed 5th and 95th percentiles. The red shaded area represents the 95% confidence interval of the median of the simulated data and the blue shaded areas represent the 95% confidence intervals of the 5th and 95th percentiles of the simulated data.
Supplementary Figure 3: Passing-Bablok (A) and Bland-Altman (B) analysis for repeatability

3A: Solid black circles represent measured adalimumab concentrations and the dashed black line indicates the line of identity. The solid grey line indicates the regression line and the grey shaded area represents the confidence interval of the regression line.

3B: Mean bias is indicated by the solid red line. Dashed lines represent the upper and lower limits of agreement of the bias.
Supplementary Figure 4: Passing-Bablok (A) and Bland-Altman (B) analysis for home-sampling

4A: Solid black circles represent measured adalimumab concentrations and the dashed black line indicates the line of identity. The solid grey line indicates the regression line and the grey shaded area represents the confidence interval of the regression line.

4B: Mean bias is indicated by the solid red line. Dashed lines represent the upper and lower limits of agreement of the bias.