

ORIGINAL ARTICLE

Dried blood samples can support monitoring of infliximab concentrations in patients with inflammatory bowel disease: A clinical validation

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Aims: Therapeutic drug monitoring (TDM) can optimize the efficacy of infliximab (IFX) in patients with inflammatory bowel disease (IBD). Because of the delay between blood samples taken at trough and availability of results, dose adjustments can only be carried out at the next infusion, typically 8 weeks later. Dried blood samples (DBS) performed at home to measure IFX concentrations can reduce the time to adapt dose/dosing interval. Here, we aimed to validate the clinical application of DBS for IFX in IBD patients and to evaluate the feasibility of home sampling.

Methods: DBS results from 40 IBD patients on IFX treatment were compared to serum sample results at trough, peak, and 3–5 weeks after IFX infusion. Subsequently, patients performed DBS home sampling one week before the next IFX infusion. These were compared to serum concentrations as predicted by Bayesian analysis.

Results: IFX concentrations from finger prick and venous puncture correlate well. DBS IFX concentrations showed high correlation with serum IFX concentrations (Spearman correlation: ≥ 0.965), without bias. Passing-Bablok regression for IFX concentrations in DBS from home sampling also showed no bias (intercept: 1.02 mg L^{-1} (95% CI -1.77 – 2.04 mg L^{-1}), slope: 0.82 (95% CI 0.63 – 1.40)), with reasonable correlation (Spearman correlation: 0.671).

Conclusions: Timely adjustment of IFX dose/dosing interval can be facilitated by IFX concentration measurement in home-sampled DBS. DBS is a reliable method to measure IFX and can be used to predict IFX trough concentrations.

KEYWORDS

biologics, clinical pharmacology, gastroenterology, immunology, therapeutic drug monitoring

The authors confirm that the Principal Investigator for this paper is Prof. G.R. D'Haens and that he had direct clinical responsibility for patients.

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1 | INTRODUCTION

Therapeutic drug monitoring (TDM) can form an important tool to optimize efficacy of infliximab (IFX) treatment in patients with inflammatory bowel disease (IBD).¹⁻³ TDM for IFX in IBD patients is currently only applied in a reactive manner. Clinicians request a serum IFX concentration measurement when a patient does not respond to therapy (primary non-responders) or loses response while on treatment (secondary non-responders). Proactively maintaining trough concentrations above a target threshold, assures optimal treatment and can prevent deleterious anti-drug antibody formation.⁴⁻⁸ As venous blood samples are drawn when the patient presents at the clinic and samples are sent off for measurements, there is a delay between the collection of the serum sample and the availability of the results. As a consequence, dose adjustment usually can only be carried out at the following IFX infusion 8 weeks later, a delay that may significantly reduce the utility of TDM.⁷ Dried blood samples (DBS) with capillary blood obtained via a finger prick could greatly facilitate TDM, because patients can perform this finger prick anywhere and at any time prior to the next infusion. In this case, results of the measurements of DBS will be present when the patient visits the clinic and the dose can be adjusted immediately. At the same time, this alleviates the stress induced by venous blood collection.⁹ Therapeutic monoclonal antibodies and anti-drug antibodies can be accurately quantified in capillary blood samples.¹⁰ This is supported by further anti-tumor necrosis factor (TNF) measurements in capillary blood samples in patients with inflammatory disease treated with adalimumab.^{11,12} For these studies, Whatman® paper has been used to collect capillary blood. Because the handling of DBS on Whatman® paper is laborious, new methods for capillary blood sampling have entered the market. The Mitra™ microsampler, a novel device to collect capillary blood, also gives reliable results and, moreover, improves efficacy of sampling handling.¹³

The purpose of this study is to validate the clinical application of DBS for IFX in IBD patients using the Mitra™ microsampler. Capillary blood sampling consists of whole blood. To express the drug levels as weight per volume serum, volume whole blood needs to be converted to volume serum. Therefore, haematocrit (Hct) values are needed to convert DBS results into comparable serum IFX concentration. Here, different approaches of Hct estimations were evaluated. Additionally, in this study, the feasibility of using DBS to estimate serum IFX concentrations by patients at home was evaluated.

2 | METHODS

2.1 | Patients and study design

In this prospective observational single-centre study, a total of 40 adult patients were enrolled. Patients had a diagnosis of Crohn's disease (CD) or ulcerative colitis (UC) and received IFX induction or maintenance therapy according to standard guidelines. Concentrations of IFX in DBS obtained by finger prick (i.e. capillary blood) were

What is already known about this subject

- Infliximab serum concentrations are associated with improved treatment outcomes in IBD patients.
- The use of capillary blood on Whatman® paper has been clinically validated for the measurement of therapeutic monoclonal antibody adalimumab.

What this study adds

- Infliximab can be accurately measured in capillary blood using Mitra™ tips in IBD patients.
- Dried blood sampling, with a finger prick, can be performed by the patient at home.

compared to concentrations of IFX in serum obtained by venepuncture. These samples were obtained at three different time points to measure the IFX serum concentrations, antibodies-to-IFX (ATI) and Hct. DBS via finger prick was simultaneously obtained at these time points with the help of a trained healthcare professional. At visit 1, samples were taken before and after the end of the IFX infusion, representing a trough concentration (time point 1) and a peak concentration (time point 2), respectively (Figure S1). At visit 2, 3–5 weeks after the infusion, samples were taken at the outpatient clinic representing a mid-infusion concentration (time point 3). One week before visiting the infusion unit for the next IFX infusion, patients independently performed a finger prick at home (time point 4) and sent the sample directly to Sanquin Diagnostic Services B.V. (Amsterdam, The Netherlands). Before performing the finger prick at home, patients were instructed by a trained healthcare professional how to use the device. Also, a leaflet was provided with detailed instructions on how to perform a finger prick at home which was guided by text and pictures. The study was approved by the local ethical committee and all patients gave written informed consent before enrolment.

2.2 | Infliximab and antibodies-to-infliximab measurements

All IFX concentrations were measured by an enzyme-linked immunosorbent assay (ELISA), as described in a previous study.¹⁴ 2 mg L⁻¹ monoclonal anti-TNF-7 (Sanquin) in phosphate buffered saline (PBS) was coated on Maxisorp ELISA plates overnight at room temperature. After washing with PBS/0.02% Tween (PT), recombinant TNF α (0.01 mg L⁻¹) (Active Bioscience, Hamburg, Germany) diluted in high performance ELISA buffer (HPE, Sanquin, Amsterdam, The Netherlands) was added and incubated for 1 hour at room temperature. Next, the plates were washed with PT and incubated for 1 hour with patient serum or DBS eluate, which was serially diluted in HPE. Subsequently, the plates were washed with PT and incubated for 1 hour with biotinylated IFX-specific rabbit anti-idiotypic antibody (0.25 mg L⁻¹ in HPE). After washing, streptavidin-poly-Horse Radish Peroxidase (Sanquin, Amsterdam, The Netherlands) (1/25 000, in

HPE) was added for 1 hour at 37°C. After washing, the ELISA was developed with 100 mg L⁻¹ tetramethylbenzidine in 0.11 M sodium acetate (pH 5.5) containing 0.003% (v/v) H₂O₂ and the reaction was stopped with 2 M H₂SO₄. Absorption was measured at 450 nm. Dilutions and ELISA were performed on an automated system (Tecan, Switzerland). Results were related to a titration curve of IFX on each plate.¹⁴ Lower limit of quantification (LLOQ) for serum IFX concentrations was 0.03 mg L⁻¹. DBS eluates were measured in the same dilution range as serum samples in the IFX concentration assay, resulting in a LLOQ of 0.6 mg L⁻¹ after conversion to serum values. ATI were measured in samples with serum IFX concentration <1 mg L⁻¹ using an antigen binding test (ABT). The ABT was performed in a slightly adjusted version compared to in a previous study.¹⁴ Serum (1 µl per test) or DBS eluate (20 µl per test) was pre-incubated with agarose-immobilized protein A (1 mg per test; GE healthcare, Chalfont St. Giles, UK) in Freeze buffer (Sanquin, Amsterdam, The Netherlands) in the presence of ~1 ng of ¹²⁵I-labelled pepsin-treated IFX. After overnight incubation, non-bound radiolabel was washed away and agarose-bound radioactivity was measured and related to a titration curve of a serum pool of patients containing high ATI. Lower limit of detection (LOD) for serum ATI was 12 AU mL⁻¹. DBS eluates were measured 20 times less diluted compared to serum samples in the ABT with a LOD of 12 AU mL⁻¹ after conversion to serum values. Both assays were developed by Sanquin Diagnostic Services, Biologics Lab, Amsterdam, The Netherlands.¹⁴

2.3 | Elution of capillary blood from DBS

The finger prick was performed using a contact-activated lancet (BD Microtainer® 2.0 mm × 1.5 mm). Capillary blood was collected via a Mitra™ microsampling device with volumetric absorptive micro-sampler (VAMS) technology.¹⁵ The microsampling device consists of an absorbent polymeric tip designed to take up a fixed volume of blood by capillary action. This device overcomes some of the technical issues associated with blood volume variability and Hct that are reported when taking a punch from dried blood spots on Whatman® paper.¹⁶ 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.¹³

2.4 | Haematocrit (Hct) for conversion of DBS results

Hct values of each individual patient were used to convert DBS eluate results (whole blood) to serum concentrations. Because Hct cannot be measured directly in Mitra™ tips eluates, different methods for Hct estimation were evaluated. First, the use of a fixed Hct of 0.42 (method 'DBS H-fix') was evaluated. As Hct is typically rather stable, this method has been shown to be adequate for conversion of DBS eluate results from DBS on Whatman® paper in a cohort of adalimumab-treated patients with inflammatory diseases.¹² Second,

Hct values were calculated based on haemoglobin concentrations in the Mitra™ tips, a potentially more precise method because there is a linear relationship between haemoglobin and Hct (method 'DBS H-Hb'). This would account for varying Hct levels as seen in IBD patients. Haemoglobin concentrations were determined by absorbance at 415 nm. Two additional methods for conversion were evaluated which made use of Hct measured in whole blood: the use of Hct measured at time point 1 ('DBS H-T1') and the use of the most recent Hct ('DBS H-recent'). An overview of all methods is provided in Table S1. Hct was measured in whole blood with the XN9000 from Sysmex (Amsterdam UMC, The Netherlands) with linearity between 0 and 0.75 L L⁻¹.

2.5 | Bayesian pharmacokinetic analysis

One week before visiting the infusion unit, at time point 4, patients performed a finger prick at home without matching venepuncture. Because no venepuncture was performed at time point 4, Bayesian analysis (using the software program NONMEM®) was used to predict the corresponding venous serum IFX concentration for this time point. In this procedure, individual observed concentrations are combined with pharmacokinetic information obtained from a large population of patients. The latter consists of typical values for clearance and volume of distribution combined with their respective inter-patient variability. In the present study, Bayesian analysis was used to obtain individual pharmacokinetic parameter estimates based on the observed serum concentrations at time points 1, 2 and 3. These individual parameters were subsequently used to derive the concentration versus time profile and to predict the serum concentration at time point 4 (home sampling) for each individual patient. In this study population, the pharmacokinetic model was taken from literature describing pharmacokinetics of IFX in adult CD patients.¹⁷ In this model the concentration–time data were described using a two-compartment model with inter-patient variability on both clearance and central volume of distribution. Clearance increased when ATI were present and as serum albumin concentrations decreased. The use of concomitant immunomodulators was associated with a decrease in clearance. The population pharmacokinetic parameters are given in Table S2. In the present study, the predictive performance of the population pharmacokinetic model was evaluated with a visual predictive check (VPC) (Figure S2).^{18,19}

2.6 | Statistical analysis

All statistical analyses were performed using R (version 3.3.2, Vienna, Austria). To assess the normality of all measured IFX concentrations, a Shapiro–Wilk test was used. Correlations between DBS eluate results and IFX serum concentrations were calculated as Pearson or Spearman correlation coefficients, depending on normality. Passing-Bablok linear regression was used to calculate the intercept and slope of the linear regression according to the following linear equation²⁰:

$$Y = \beta_0 + \beta_1 X$$

where β_0 represents the intercept and β_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.

2.7 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY²¹ and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18.²²

3 | RESULTS

3.1 | Samples

A total of 40 IBD patients were enrolled. Patient demographics and other baseline characteristics are summarized in Table 1. One patient received IFX as induction treatment, and 39 patients received IFX as maintenance treatment. Median Hct was 0.42 L L⁻¹ (interquartile range [IQR], 0.38–0.44 L L). At time point 1 and time point 2, blood could not be successfully collected from one patient, resulting in 39 matching venepuncture/DBS samples from both time points 1 and 2. Twelve patients were lost to follow-up resulting in 28 samples from time point 3. In total, 106 IFX matching venepuncture/DBS samples were collected. Twenty-eight additional DBS samples, performed by the patient at home, were collected.

TABLE 1 Baseline patient characteristics (n = 40)

	n (%)
Male	18 (45%)
Crohn's disease	29 (73%)
Ulcerative colitis	11 (27%)
Induction	1 (2.5%)
Maintenance	39 (97.5%)
Infliximab dose, mg	400 [338–413]
Infliximab dosing interval, days	56 [48–56]
Patient body weight, kg	76 [66–80]
Age, years	41 [32–50]
Disease duration, years	13 [6–18]
Albumin, g L ⁻¹	43 [41–45]
C-reactive protein, mg L ⁻¹	1.3 [0.4–4.4]
Haematocrit (L L ⁻¹)	0.42 [0.38–0.44]

Data are expressed as n (%) or median [interquartile range]

3.2 | Infliximab measurements in DBS

The IFX serum concentrations collected at time point 1 (trough), time point 2 (peak), and time point 3 (mid-infusion concentration) are depicted in Figure S2. Median (IQR) IFX serum concentrations were 3.7 mg L⁻¹ (2.4–6.4 mg L⁻¹), 103 mg L⁻¹ (85–135 mg L⁻¹) and 15 mg L⁻¹ (11–21 mg L⁻¹), respectively. Results for two alternative conversion methods of Hct, 'DBS H-T1' and 'DBS H-recent', are summarized in Table S3.

In two DBS samples, both collected at time point 1 (at trough concentration), the measured concentrations were below the LLOQ of 0.6 mg L⁻¹ for IFX in DBS samples and the results were discarded. Both methods to determine the serum volume, 'DBS H-fix' and 'DBS H-Hb', resulted in serum concentrations that showed high correlation between DBS and venepuncture results with Spearman correlation ≥ 0.965 (Figure 1). For results using 'DBS H-fix' serum volume estimation, Passing-Bablok regression showed no systematic or proportional bias because the 95% confidence interval of the calculated intercept (0.08 mg L⁻¹ [95% CI -0.22–0.47 mg L⁻¹]) and slope (1.01 [95% CI 0.95–1.07]) enclosed 0 and 1, respectively. For results using the 'DBS H-Hb' serum estimation, Passing-Bablok regression showed slightly wider confidence intervals, with a calculated intercept of -0.02 mg L⁻¹ (95% CI -0.47–0.50 mg L⁻¹) and slope of 0.96 (95% CI 0.90–1.09), but without systematic or proportional bias.

3.3 | Feasibility of sampling at home

At time point 4, patients performed a finger prick at home and the DBS sample was sent by the patient to an analytical laboratory for subsequent analysis. IFX concentrations in DBS eluate from home sampling were compared to the serum IFX concentrations predicted by Bayesian analysis. The predictive performance of the population pharmacokinetic model was assessed using a visual predictive check and showed good agreement between simulated and observed serum IFX concentrations obtained after venepuncture (Figure S2). Median (IQR) IFX concentration in DBS eluate was 5.7 mg L⁻¹ (4.6–7.5 mg L⁻¹) with a median (IQR) time after dose of 48 days (39–71 days). Spearman correlation coefficients for this time point were 0.697 and 0.671, using 'DBS H-fix' and 'DBS H-Hb', respectively (Figure 2). For 'DBS H-fix' Passing-Bablok regression showed small systematic and proportional bias with a 95% confidence interval of the intercept of the regression line not enclosing 0 (intercept: 1.77 mg L⁻¹ [95% CI 0.29–2.63 mg L⁻¹]), and with 95% confidence interval of the slope of the regression line not enclosing 1 (slope: 0.63 [95% CI 0.55–0.91]) (Figure 2A). Using 'DBS H-Hb' (Figure 2B), Passing-Bablok regression showed no bias with 95% confidence interval of the regression line enclosing 0 and 1, for calculated intercept and slope respectively, but with wider confidence intervals (intercept: 1.02 mg L⁻¹ [95% CI -1.07–2.02 mg L⁻¹], slope: 0.80 [95% CI 0.63–1.14]).

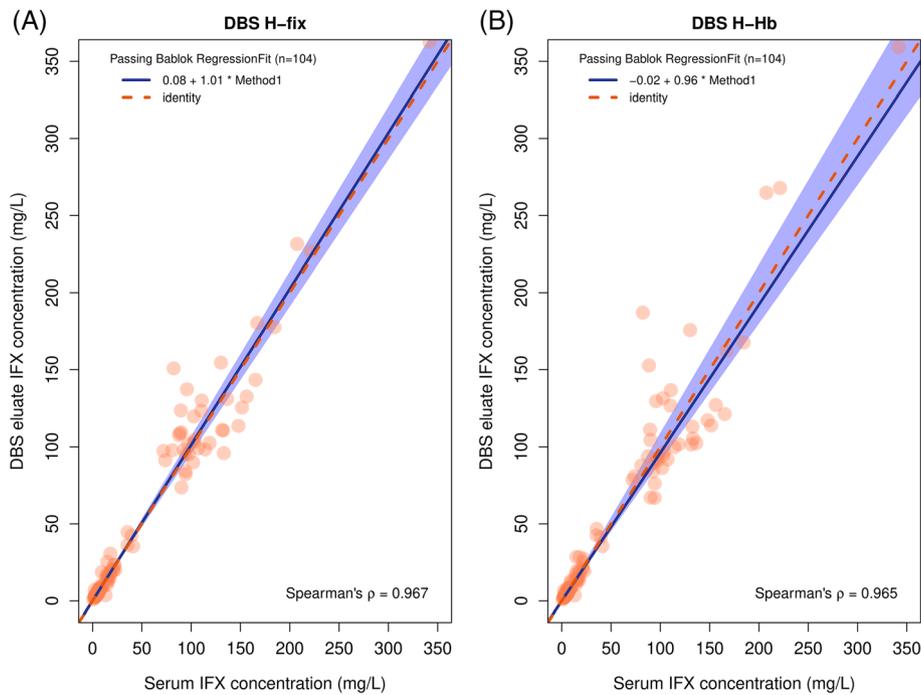


FIGURE 1 Passing-Bablok regression. (A) DBS H-fix: Intercept: 0.08 mg L^{-1} (95% CI -0.22 – 0.47 mg L^{-1}), slope: 1.01 (95% CI 0.95 – 1.07). (B) DBS H-Hb: Intercept: -0.02 mg L^{-1} (95% CI -0.47 – 0.50 mg L^{-1}), slope: 0.96 (95% CI 0.90 – 1.09). Red dots represent measured samples. The solid blue line represents the regression line, the dashed red line represents the line of identity, and 95% confidence intervals are represented by the blue shaded area. DBS, dried blood sample; IFX, infliximab

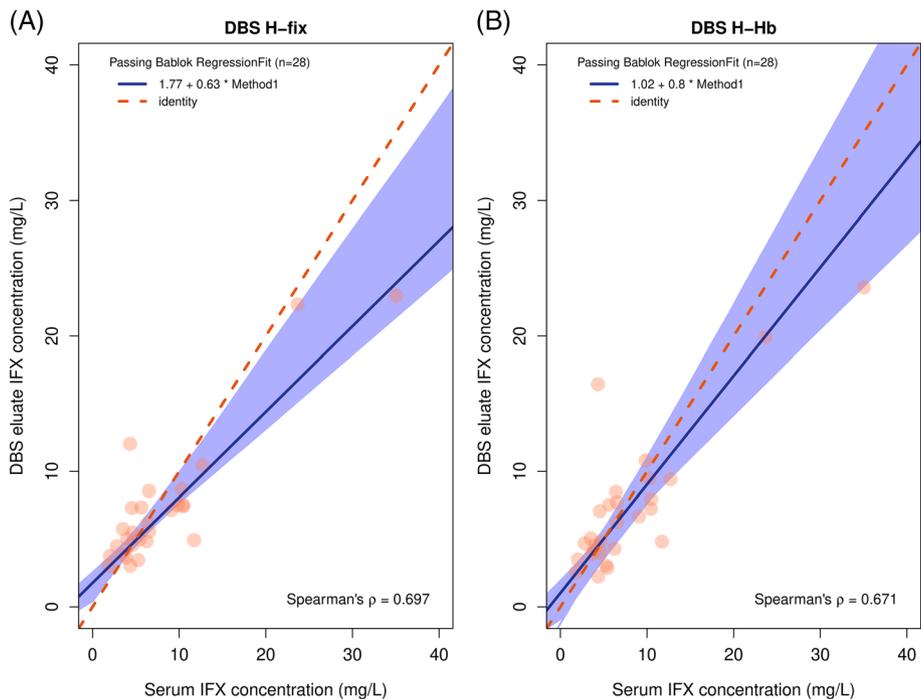


FIGURE 2 Passing-Bablok regression home-sampling. (a) DBS H-fix: Intercept: 1.77 mg L^{-1} (95% CI 0.29 – 2.63 mg L^{-1}), slope: 0.63 (95% CI 0.55 – 0.91). (B): DBS H-Hb: Intercept: 1.02 mg L^{-1} (95% CI -1.07 – 2.02 mg L^{-1}), slope: 0.80 (95% CI 0.63 – 1.14). Red dots represent measured samples. The solid blue line represents the regression line, the dashed red line represents the line of identity, and 95% confidence intervals are represented by the blue shaded area. DBS, dried blood sample; IFX, infliximab

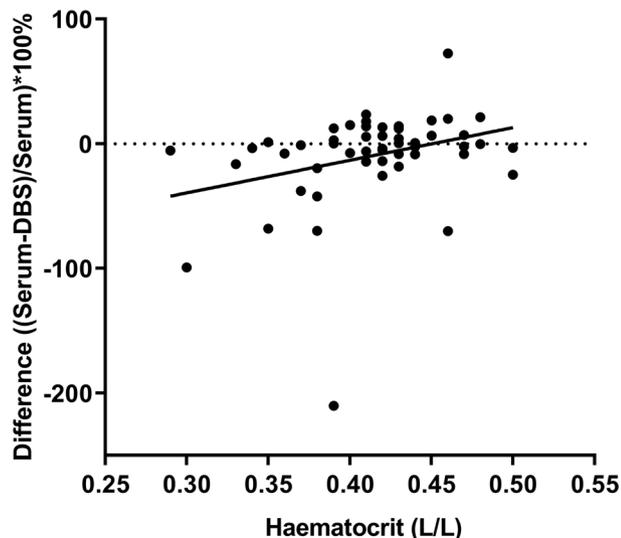


FIGURE 3 Difference between serum and DBS eluate results (%) versus haematocrit. The solid line represents the trend line ($P = 0.01$) DBS results are based on 'DBS H-fix', using a fixed haematocrit value of 0.42

3.4 | Influence of Haematocrit

To evaluate the influence of Hct on DBS outcomes, the difference between IFX concentration measured in serum and in DBS was plotted against all Hct values measured. A weak, but significant correlation between the difference in serum and DBS results and Hct values was shown ($P = 0.01$) (Figure 3).

3.5 | Antibodies-to-infliximab measurements in DBS

ATI were measured in all DBS samples with low ($<1 \text{ mg L}^{-1}$) IFX concentrations ($n = 3$). Only one patient sample showed a high level of ATI in DBS (1705 AU ml^{-1}). The corresponding serum sample of this patient had IFX serum trough concentrations below LLOQ and also a high level of ATI ($>880 \text{ AU ml}^{-1}$).

4 | DISCUSSION

This study shows that DBS via finger prick is a reliable method to measure IFX concentrations in IBD patients. Results for measured IFX serum concentrations using DBS are highly comparable to measured IFX serum concentrations after conventional venepuncture. Most importantly, we are the first to show that sampling at home leads to accurate IFX concentration results which will optimize the use of TDM in IBD patients treated with the anti-TNF monoclonal IFX.

The use of capillary blood for measuring drug concentrations finds application in various fields. So far, the use of capillary blood has been described mainly for bioanalysis of small molecules.²³ Therapeutic and toxic drug concentrations can be measured in

capillary blood for antibiotics, anti-epileptics, anti-HIV agents, immune-suppressants and in the cardiovascular field, among others.²⁴ The use of capillary blood in (young) children also is of great interest, because blood collection from children is always challenged by ethical issues. Besides the ease of blood collection, using capillary blood can be cost-saving as it reduce the need for visits to the (outpatient) clinic.²⁵

This current study is a prospective observational study of IBD patients treated with IFX. We show here that the Mitra™ tip is a reliable tool to measure IFX concentrations in IBD patients. To the best of our knowledge, we are also the first to show that the Mitra™ tip can be used by the IBD patient at home to measure IFX concentrations. Admittedly, correlation between DBS eluate results and IFX serum concentrations was higher in DBS acquired in the hospital compared to samples performed by the patient at home (Spearman correlation: ≥ 0.965 vs. ≥ 0.671 , respectively). Home sampling is challenged by the lack of experience of the patient to properly collect capillary blood and careful instructions are needed to prevent under- or over-filling of the Mitra™ tip. Secondly, results from DBS performed by the patient at home were compared to predicted serum concentrations by Bayesian analysis. This resulted in variation as introduced by the model as well and this could explain part of the bias and wider confidence intervals seen. Patients in the IBD population generally show high rates of anaemia (10–70%) during their disease course, which results in a low Hct (<0.40).²⁶ We validated four different methods of handling Hct values, to convert IFX DBS eluate results to values comparable to IFX serum concentrations. For method 'DBS H-fix', a fixed Hct value of 0.42 was chosen as the population average. Although men and women have different standard Hct values, no distinction was made for conversion because this is impractical to implement in standard procedures when implementing DBS in clinical practice. For patients with lower Hct, higher IFX concentrations were measured in DBS compared to serum, because patients with an Hct value below 0.42 have a larger serum fraction in their DBS than calculated with the 'DBS H-fix' method. Using haemoglobin in DBS to calculate Hct ('DBS H-Hb') results gives an accurate estimate of the serum IFX concentration and is therefore a more accurate approach when a patient has decreased Hct values, although the percentage of such patients was limited in our study population.

The use of DBS has been suggested before as a tool to facilitate TDM of IFX in IBD patients.¹¹ DBS can be performed by the patient at any time (between hospital visits) and everywhere without the need to come to the medical centre. Using DBS, IFX and other results can be available for the clinician when the patient visits the clinic and dose adjustments can be made directly before the patients' next IFX infusion. Exact timing of the mid-infusion sampling can be entered in a Bayesian dashboard to interpret the results.²⁷ This way, using DBS will greatly facilitate both proactive and reactive TDM of IFX.

Our study has several limitations. First, our validation of home sampling is limited by the number of samples collected ($n = 28$).

Twelve patients were lost to follow-up before time point 3, mainly because these patients refused an additional hospital visit. The latter emphasizes the need for DBS for TDM and pharmacokinetic studies as it minimizes the patients' burden and the frequency of hospital visits. Second, as already mentioned, DBS samples performed by the patient at home were compared to predicted serum concentrations by Bayesian analysis which could explain part of the bias and wider confidence intervals seen. In addition, only one patient showed a high level of ATI, and DBS could therefore not be clinically validated for the measurement of ATI compared to venepuncture.

In conclusion, IFX concentrations can be accurately measured using DBS, independent of the Hct values of the IBD patient. This shows that home sampling of capillary blood results in reliable IFX serum concentrations, which will greatly facilitate optimal treatment for IBD patients in a timely manner.

COMPETING INTERESTS

G.R.A.M.D. has served as advisor for Abbvie, Ablynx, Amakem, AM Pharma, Avax-ia, Biogen, Bristol Meiers Squibb, Boehringer Ingelheim, Celgene, Celltrion, Cosmo, Covidien, Ferring, DrFALK Pharma, Engene, Galapagos, Gilead, Glaxo Smith Kline, Hospira, Immunic, Johnson and Johnson, Lycera, Medimetrics, Millenium/Takeda, Mitsubishi Pharma, Merck Sharp Dome, Mundipharma, Novonordisk, Pfizer, Prometheus laboratories/Nestle, Protagonist, Receptos, Robarts Clinical Trials, Salix, Sandoz, Setpoint, Shire, Teva, Tigenix, Tillotts, Topivert, Versant and Vifor, and received speaker fees from Abbvie, Ferring, Johnson and Johnson, Merck Sharp & Dohme, Mundi-pharma, Norgine, Pfizer, Shire, Millenium/Takeda, Tillotts and Vifor. T.R. has received honoraria for lectures from Pfizer, Abbvie and Regeneron, and a research grant from Genmab. R.A.A.M. has received unrestricted research grants from Bayer, Shire and Merck Sharpe & Dome and consultancy fees from Bayer and Shire. S.E.B., T.S., A.d.V. and K.B. have no competing interests to declare.

CONTRIBUTORS

S.E.B. was responsible for the study design, data collection, data analysis, data interpretation, drafting the manuscript. G.R.A.M.D. took part in study design feedback and revising the manuscript. T.S. was responsible for data collection and data analysis, and A.d.V. for data collection, study design feedback and revising the manuscript. T.R. was responsible for study design feedback, technical feedback, data interpretation and revising the manuscript. K.B. took part in data collection, data analysis, data interpretation and revising the manuscript, and R.A.A.M. in revising the manuscript, data interpretation and manuscript supervision. All authors approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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