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Research Article

Golimumab Dried Blood Spot Analysis (GOUDA): a Prospective Trial Showing Excellent Correlation with Venepuncture Samples and More Detailed Pharmacokinetic Information

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Abstract. Development of a dried blood spot (DBS) method for golimumab will facilitate sample collection in a study setting and will give a more complete insight in the total drug exposure (area under the curve, AUC). We established a DBS method and assessed its robustness, user-friendliness and clinical usefulness in 10 patients with ulcerative colitis during golimumab induction and maintenance regimens. DBS was obtained through spotting of golimumab spiked in whole citrated blood to a filter paper. Several extraction conditions were evaluated and the selected extraction condition analytically validated. In a clinical setting, DBS and serum samples were taken simultaneously through intensive sampling regimens and a conversion factor was determined. Golimumab concentrations were measured using an in-house-developed ELISA and a CE-marked ELISA kit. User-friendliness was evaluated using a questionnaire. Mucosal healing was evaluated at week 14. A total of 79 matched pairs of serum and DBS sample golimumab concentrations revealed an overall conversion factor of 3.9. DBS golimumab concentrations after conversion correlated strongly with serum golimumab concentrations (ICC = 0.984). During induction, no linear correlation was found between golimumab trough concentration (TC) and AUC (R^2 = 0.29). Multiple peaks emerged during drug absorption. Patients who achieved mucosal healing appeared to have less fluctuating TC and a constant AUC over time. Nine out of 10 patients reported DBS sampling as user-friendly. The GOUDA study showed that DBS sampling is a robust and patient-friendly alternative to venous blood collection. DBS sampling may provide better insights into golimumab absorption and exposure. (ClinicalTrials.gov NCT02910375)

KEY WORDS: dried blood spots; golimumab; immunogenicity; pharmacokinetics; ulcerative colitis.

INTRODUCTION

Golimumab (Simponi®, Janssen Biologics, B.V.) is the most recently marketed anti-tumour necrosis factor (TNF) biologic originator available to treat patients with active, moderate-to-severe ulcerative colitis (UC). Preclinical work showed that golimumab has a higher affinity and a greater capacity to neutralize TNF compared to previously developed anti-TNF agents, such as infliximab and adalimumab (1). Subsequent demonstration of efficacy and safety in the PURSUIT-SC induction and PURSUIT-M maintenance trials have led to regulatory approval of subcutaneously administered golimumab for the treatment of UC (2,3).

In most European countries, patients receive the same induction treatment in daily clinical practice (200 mg at week 0 and 100 mg at week 2) followed by a body weight-based dose stratification during maintenance, i.e., 50 mg every four weeks for a patient with a body weight of less than 80 kg and 100 mg golimumab every four weeks for a patient with a body weight of at least 80 kg. However, as with the other anti-TNF agents, suboptimal response or loss of response to golimumab remains a major concern. Results from PURSUIT and observational clinical studies (4–6) reported Mayo clinical response rates of around 50% after golimumab induction therapy. An exposure-response relationship was observed, as patients with higher drug exposure were more likely to

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achieve improved outcomes (7,8). The exposure-response relationship indicates that therapeutic drug monitoring (TDM) of golimumab may be useful to establish a personalised therapy strategy for individual patients. In PURSUIT-M, week 6 non-responders had lower serum drug levels compared to responders at week 6. These early non-responders received 100 mg golimumab maintenance, and their drug exposure was increased to levels comparable with that of responders by week 14 (9). Since July 2018, the posology has changed and patients with an inadequate response to induction can have the dose increased to 100 mg at week 6 and every four weeks thereafter (10).

In order to adequately study absorption, distribution and clearance of the drug, rich sampling is required (i.e., at intermediate time points as well as at trough, before the self-administration of the drug) together with evaluation of patient- and disease-related characteristics. Using pharmacokinetic (PK) analyses, an ideal trough concentration (window) can be predicted which would allow for dose-to-target adaptations to increase the efficacy of golimumab treatment. We propose here an easy method *via* capillary puncture and dry blood spot (DBS) that avoids impracticalities associated with conventional venous sampling (e.g., extra hospital visits, processing of blood, and shipment of serum) and is less burdensome for patients. DBS sampling involves a finger prick (comparable to glycaemia measurement) to apply whole blood to a sampling paper, and these papers can be stored and transported at ambient temperature. Development of this self-sampling method would facilitate sample collection in a study setting and might improve TDM for self-administered drugs.

In the current study, DBS sampling was applied in a prospective cohort of ten patients with UC, either starting or maintaining golimumab treatment (GOlimUmab Dried blood spot Analysis, GOUDA). The reliability and user-friendliness of the DBS method were assessed, and the relation between golimumab concentration and drug exposure during both induction and maintenance regimens was explored.

MATERIALS AND METHODS

DBS Technique

Method Development

Different volumes (15–90 μL) of different concentrations (0.2–20 μg/mL) of golimumab, spiked in whole citrated blood from healthy donors (Valley Biomedical, Winchester, USA), were spotted to a piece of a filter paper (Whatman 903 Protein Saver Card, GE Healthcare, Diegem, Belgium) and air-dried overnight. A 6-mm-diameter disc from an area completely impregnated with DBS was punched out from the filter paper and eluted by adding 240 μL of elution buffer in an Eppendorf tube (1:25 pre-dilution). Several elution buffers, phosphate-buffered saline with combination of Tween 80 (0.1–1%), bovine serum albumin (0.1%) and ethylenediaminetetraacetic acid (0.1–1%) or SuperBlock®T20 buffer (Thermo Fisher Scientific, Rockford, USA), were tested to improve extraction recovery. The next steps included gently shaking (300 rpm, Eppendorf Thermomixer comfort) for 1 or 2 h at room temperature and centrifugation at 14,000 RCF (g) for 5 min. After extraction, DBS eluates were analysed on the MA-GOM171D8/MA-GOM159B8-HRP ELISA (7), starting with an additional 20-fold dilution (final dilution of 1:500). DBS eluates were undiluted stored at −20°C. The golimumab calibration curve and two serum control samples were prepared as described previously (7) and did not underwent spotting to a filter paper and subsequent extraction.

Method Validation

Analytical validation of the selected extraction condition followed the recommendations of the European Bioanalysis Forum (11).

Extraction Recovery. Extraction recovery was established by the analysis of 11 repeats of five different concentrations of golimumab (0.2–20 μg/mL), spiked in whole citrated blood, spotted onto the Protein Saver Card. An overall correction factor for extraction of spiked golimumab in whole citrated blood was determined.

Accuracy and Imprecision. Taking into account the overall correction factor for extraction of spiked golimumab in whole citrated blood, accuracy and imprecision of the method were assessed as described previously (7). The minimum acceptable criteria were an absolute mean percentage deviation ≤ 20% (for accuracy) and a coefficient of variation ≤ 15% (for imprecision).

Correlation Between Calculated Golimumab in DBS and Spotted Volume. Robustness of the method was assessed by spotting 10 different concentrations of golimumab (0.2–20 μg/mL) at the following different volumes: 15, 30, 45, 60 and 90 μL. The Jonckheere-Terpstra test was used to detect a trend in the observations with increasing volumes spiked. A two-sided p value of less than 0.05 was considered statistically significant.

Stability. The stability of the DBS method was tested by spotting different concentrations of golimumab (0.2–20 μg/mL) on different filter papers. The cards were kept for up to one month at room temperature and the eluates for up to three months at −20°C. Impact of the storage condition on golimumab recovery was assessed. Minimum acceptable criterion was an absolute mean percentage deviation ≤ 20%.

Impact of MA-GOM159B8 on Golimumab Concentration. Impact of anti-golimumab antibody (clone MA-GOM159B8) (7) on the detection of golimumab was investigated—both in serum and DBS extract—after incubation (for 15 min at 37°C) of golimumab (concentration of 1.3 μg/mL) with different molar ratios (up to 8-fold) of MA-GOM159B8. Complexes were spiked to serum and whole citrated blood, spotted onto the Protein Saver Card. The residual golimumab concentration in serum and DBS eluates was measured on the MA-GOM171D8/MA-GOM159B8-HRP ELISA (7).

Application in GOUDA Study

Study Design. The GOUDA study was a prospective trial of 10 patients initiating (n = 5) or under maintenance

($n = 5$) golimumab for moderate-to-severe ulcerative colitis at the University Hospitals Leuven (Leuven, Belgium). The primary objective of this trial was to compare golimumab concentrations obtained using venepuncture with golimumab concentrations obtained using finger pricks. Secondary objectives were the determination of a concentration-time profile (exposure) of golimumab in individual patients with UC and evaluation of user-friendliness of the DBS technology. This study was approved by the Ethical Committee of the University Hospitals Leuven (B322201630705/S59372). All patients provided informed consent.

Collecting Material by Venepuncture and Finger Prick. Blood samples were collected using intensified sampling regimens at pre-defined time points (displayed in Fig. 1). For patients initiating golimumab therapy (cohort A), the sampling schedule consisted of 13 venepunctures and 39 finger pricks during an 18-week period. For patients already under maintenance golimumab therapy (cohort B), the sampling schedule consisted of 8 venepunctures and 20 finger pricks during a 12-week period. Venepuncture was performed during standard outpatient clinic visits at the University Hospitals Leuven and during consultations at the general practice office. Serum was prepared by centrifugation in BD Vacutainer SST II Advance tube (BD Biosciences), containing a gel separator and clot activator. Finger pricks were performed by the patients themselves at home after proper education at the University Hospitals Leuven by a study nurse or pharmacist. Capillary blood from the finger prick was absorbed on the Whatman 903 Protein Saver Card and processed as described previously.

Conversion of DBS Values. At eight (cohort B) or thirteen (cohort A) time points (Fig. 1), venepuncture and finger prick were performed at the same time, which allowed converting golimumab concentrations measured in DBS eluates into values that can be compared with golimumab concentrations obtained *via* venepuncture. The golimumab concentrations measured in DBS eluates were divided by the golimumab concentrations measured in serum to establish a conversion factor for each patient in the GOUDA study. This conversion factor takes into account the overall correction factor for extraction and the capillary blood/serum ratio for golimumab. The Spearman's correlation and the intraclass correlation coefficient (ICC) were used to assess the correlation between golimumab in venepuncture serum and golimumab in DBS, after the latter was converted with an overall mean conversion factor.

Golimumab Exposure and Treatment Outcome. The parameter $AUC_{(\text{time period expressed in weeks})}$ was calculated from the concentration-time profiles using non-compartmental analysis (PKNCA R package). Peak plasma concentration (*C*max) and time to reach *C*max (*t*max) were determined by visual inspection of the curve. The main treatment outcomes were week 14 mucosal healing (Mayo endoscopic sub-score ≤ 1) and drug failure (defined as need for golimumab dose increment from 50 to 100 mg every 4 weeks, introduction of other UC therapy or discontinuation of the drug due to lack of mucosal healing) during one-year follow-up study.

Patient Friendliness. At the end of the study, the participants were requested to fill out a questionnaire regarding the user-friendliness of the DBS methodology.

Laboratory Analysis. Anti-golimumab antibody concentrations were determined in venepuncture serum samples only by the "affinity-capture-elution" method, as described previously (7). The assay cut-off was set at 25 ng/mL taking into account a serum dilution of 1:18. Routine biochemical analyses (Department of Laboratory Medicine, University Hospitals Leuven) included determination of C-reactive protein (CRP) and serum albumin.

Comparison of the In-House-Developed Golimumab ELISA with a CE-Marked Golimumab ELISA Kit. For

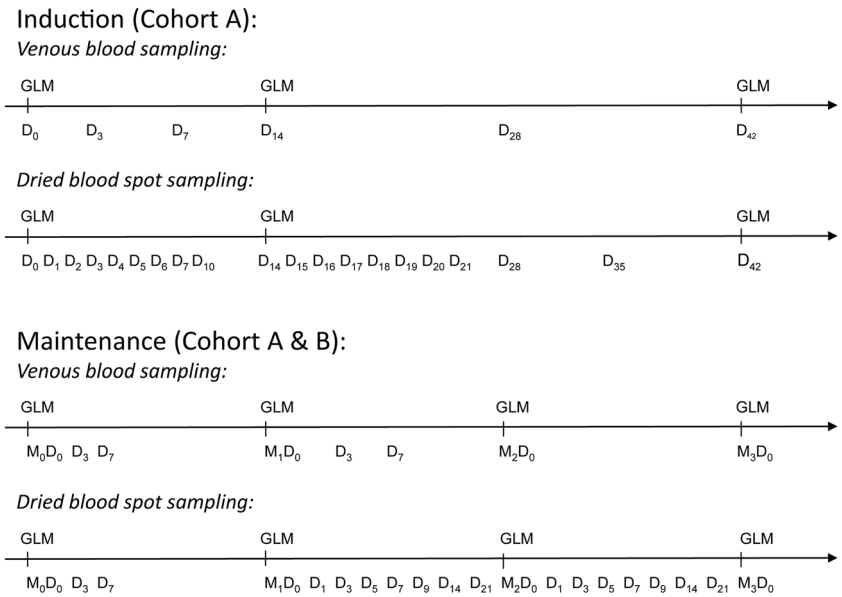

Fig. 1. Sampling times in the GOUDA study. GLM, golimumab; D, day; M, month

comparison purposes, all serum samples obtained through venepuncture in GOUDA, and analysed with the in-house-developed MA-GOM171D8/MA-GOM159B8-HRP ELISA (starting with a 2000-fold dilution), were re-analysed using a CE-marked golimumab ELISA kit (100-fold dilution) distributed by apDia (Turnhout, Belgium) as apDia Golimumab ELISA kit and as RIDASCREEN Golimumab Monitoring by R-Biopharm AG (Darmstadt, Germany). The kit uses TNF as coating and the in-house developed monoclonal antibody MA-GOM171D8 as conjugate. The Spearman's correlation and the relative Bland–Altman comparison between the CE-marked kit and the in-house-developed MA-GOM171D8/MA-GOM159B8-HRP ELISA were calculated using GraphPad Prism 7.0.

RESULTS

DBS Technique

Method Development

The different extraction conditions yielded extraction recoveries between 40 and 60%, with the highest values obtained using the SuperBlock®T20 buffer. The one-hour incubation was more time-efficient than the two-hour incubation without influencing the finally obtained golimumab concentrations. Consequently, all further extractions were performed by a one-hour incubation of the 6-mm-diameter disc in the SuperBlock®T20 buffer gently shaking (300 rpm, Eppendorf Thermomixer comfort) at room temperature followed by centrifugation at 14,000 RCF (g) for 5 min.

Method Validation

Extraction Recovery. Spotting of different concentrations (0.2–20 μg/mL) of golimumab, spiked in whole citrated blood, onto the Protein Saver Card yielded a mean (± SD) extraction recovery of 54.4% (± 9.0%) for golimumab determination in DBS eluates. Taking into account this average extraction recovery of 54.4%, we determined the overall correction factor for extraction of spiked golimumab in whole citrated blood to be 1.8.

Accuracy and Imprecision. The accuracy of the method was determined by analysis of five different concentrations of golimumab spiked in whole citrated blood, spotted onto the Protein Saver Card (final concentrations of 20, 10, 5, 2.5 and 1.3 μg/mL). The average measured value was recalculated with the overall correction factor for extraction of spiked golimumab in whole citrated blood of 1.8. Accuracy ranged from 83 to 88% and was found acceptable (Table I). Overall assay imprecision of golimumab-spiked DBS was < 15% for each concentration analysed and fulfilled the requirements as well.

Correlation Between Calculated Golimumab in DBS and Spotted Volume. Ten different concentrations of golimumab (0.2–20 μg/mL) were spotted at the following different volumes: 15, 30, 45, 60 and 90 μL. Figure 2 shows scatterplots of the recalculated golimumab concentration versus the volumes that were spotted. Analysis using the Jonckheere-Terpstra test for trend showed no increasing or decreasing trend in the observed golimumab concentrations with applied volume ($p = 0.732$). Consequently, the calculated golimumab concentration could be considered independent of the spotted volume when a minimum of 15 μL was applied.

Stability. The stability of the DBS method was tested by spotting different concentrations of golimumab (0.2–20 μg/mL) on different filter papers. The cards were kept for up to one month at room temperature and the eluates for up to three months at − 20°C. The requirement for golimumab recovery (see the "MATERIALS AND METHODS" section) was fulfilled under the given storage conditions (data not shown).

Impact of MA-GOM159B8 on Golimumab Concentration. Upon incubation with a two-fold molar excess of MA-GOM159B8, an inhibitory anti-golimumab antibody, golimumab could not be detected anymore, neither in serum nor in DBS eluate (Fig. 3).

GOUDA Study

Patient Characteristics

At study entry, mean (SD) age of the ten IBD patients (60% females) was 39(18) years. Body mass index (BMI), disease duration and smoking behaviour of the patients are shown in Table II. Nine out of 10 patients were not previously exposed to any therapeutic antibodies when initiating golimumab therapy. One patient was previously exposed to infliximab and adalimumab but experienced drug failure.

Golimumab Treatment

All five patients initiating therapy (cohort A) received golimumab 200 and 100 mg at weeks 0 and 2, respectively. Two patients used golimumab 50 mg every 4 weeks as maintenance treatment, whereas three patients (with body weight more than 80 kg) started on a 4-week maintenance treatment of 100 mg. Among the two patients who received golimumab 50 mg, one patient continued receiving 50 mg and one patient's dose increased to golimumab 100 mg after reaching week 14. Of the five patients maintaining golimumab treatment (cohort B), only one patient received 50 mg golimumab every 4 weeks. The four other patients receiving 100 mg golimumab every 4 weeks had either a body weight above 80 kg ($n = 1$) or were previously dose-optimised ($n = 3$).

Treatment Outcome

Two out of five patients in cohort A achieved mucosal healing at week 14. Golimumab was discontinued in three patients due to drug failure. Two stopped treatment during the GOUDA study, of whom one despite receiving a dose escalation, and a third patient during one-year follow-up of the GOUDA study. All five patients in cohort B had achieved

Table I. Accuracy of Spiked Golimumab DBS ($n = 3$)

Theoretically expected value (μg/mL)	Average measured value ± SD (μg/mL)	Corrected average measured value (μg/mL)	Accuracy (%)
20	9.2 ± 0.9	17	83
10	4.8 ± 0.6	8.6	86
5.0	2.4 ± 0.3	4.4	88
2.5	1.2 ± 0.3	2.2	88
1.3	0.7 ± 0.1	1.1	85

mucosal healing. After one-year study follow-up, none of the remaining six patients (one patient was lost to follow-up) experienced drug failure. The 100 mg golimumab dose was decreased in one patient (patient 4 in cohort A, see subsequent texts) because of adverse events.

Application of DBS Technique into the GOUDA Study

Conversion of DBS Values

A DBS eluate as well as a serum sample was available at 79 time points. Individual conversion factors ranged from 3.6 to 4.0, and a mean ± SD ($n = 79$) overall conversion factor of 3.9 ± 0.1 was determined. Figure 4 shows the correlation of golimumab concentrations measured in venepuncture serum and corresponding converted DBS golimumab concentrations.

The converted DBS golimumab concentrations are from now on reported.

Golimumab Exposure and Treatment Outcome

Golimumab exposure could be determined in the following three different ways: (1) sampling at trough, (2) sampling at trough and at some intermediate time points and (3) sampling at trough, peak and at multiple intermediate time points. Figure 5 shows the three different profiles generated from the respective sampling time points of one patient in cohort A of the GOUDA study.

Only when plotting the golimumab concentrations determined at peak and multiple intermediate time points (Fig. 5c), a clear multiple peak pattern emerged during drug absorption. Moreover, multiple sampling revealed that in most cases, but not for all patients at all intervals, the concentration just before the next injection can be considered the "trough (= the lowest)" concentration (Fig. 6a,b). For reasons of simplicity, however, we refer to trough as the concentration just before the next injection and not to the lowest concentration.

No clear association was found between AUC, golimumab trough concentrations (Fig. 6a,b) and achievement of mucosal healing.

For patients in cohort A, $AUC_{(0–18\ w)}$ was not linearly correlated with the golimumab trough concentrations just before the next injections at weeks 2, 6, 10, 14 and 18 ($R^2 = 0.29$). In these patients, a significant decrease in mean ± SD golimumab trough concentrations was observed from week 2 (11.0 ± 4.1 μg/mL) to week 10 (3.3 ± 1.9 μg/mL) ($p = 0.005$) but not in mean ± SD AUC within the respective dosing intervals ($AUC_{(0–2)}$ = 212.3 ± 82.4 μg*day/mL vs. $AUC_{(6–10)}$ = 174.7 ± 50.6 μg*day/mL) ($p = 0.085$). This decrease in golimumab trough concentrations was larger (79% compared to 54%) in patients without mucosal healing at 14 weeks of therapy (patients 1–3) than in patients with mucosal healing (patients 4 and 5) ($p = 0.028$). During maintenance phases (week 6–18 in cohort A versus the 12 weeks in cohort B), there was a trend for lower golimumab trough concentrations and AUC for patients in cohort A than for patients in cohort

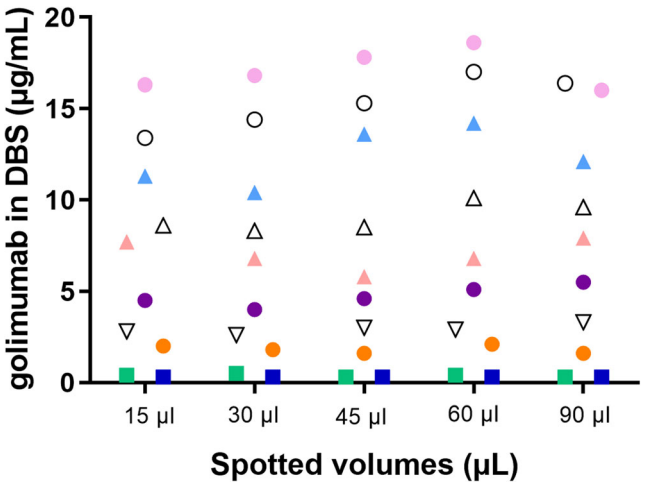

Fig. 2. Scatter plots of the recalculated golimumab concentration (μg/mL) versus the volumes that were spotted. Pink, open, purple and orange circles represent golimumab concentrations of 20, 15, 5 and 1 μg/mL, respectively; blue, open and pink triangles represent golimumab concentrations of 12.5, 10 and 7.5 μg/mL, respectively; open inverted triangles represent golimumab concentrations of 2.5 μg/mL; blue and green squares represent golimumab concentrations of 0.5 and 0.2 μg/mL, respectively

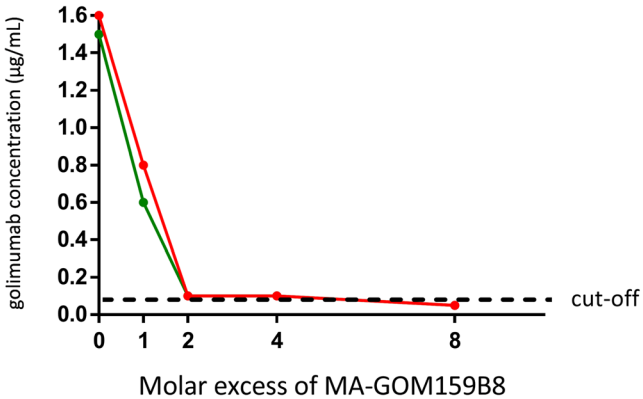

Fig. 3. Residual golimumab concentration (μg/mL) in serum (green line) and DBS eluate (red line), after incubation of golimumab with MA-GOM159B8 spiked in serum and whole citrated blood, spotted onto the Protein Saver Card

Table II. Characteristics of the UC Patients at the Start of the GOUDA Study

	GOUDA ($n = 10$)
Age (years), mean (SD)	39 (18)
Female/total (% female)	6/10 (60%)
Body mass index (kg/m^2), mean (SD)	25 (4)
Disease duration (years), median [IQR]	7 [3–22]
Biologic naive, *n* (%)	9/10 (90%)
Current smokers, *n* (%)	2/10 (20%)
Concomitant medication	
Percentage on mesalamine	7/10 (70%)
Percentage on corticosteroids	3/10 (30%)
Percentage on azathioprine	0/10 (0%)
Serum albumin (g/L), mean (SD)	43 (3)
C-reactive protein (mg/L), median [IQR]	2.5 [0.5–12.9]

B ($p = 0.064$ and $p = 0.053$). As mentioned previously, all patients in cohort B had achieved mucosal healing.

*C*max after the first dose of 200 mg (cohort A) ranged from 10.6 μg/mL (patient 4) to 31.2 μg/mL (patient 5) with a mean ± SD of 21.1 ± 7.4 μg/mL and was reached 3 to 6 days upon injection (mean *t*max of 5 days).

Patient Friendliness

Nine out of 10 patients reported DBS sampling an easy alternative and preferred this method to venous sampling (Supplementary Table S1).

Anti-Golimumab Antibodies and Treatment Outcome

Since all serum samples showed detectable golimumab concentrations, presence of anti-golimumab antibodies was assessed using a drug-tolerant immunoassay, revealing antibodies in three out of ten patients. In patient 1 (Fig. 6a), a 56-year-old man receiving golimumab in combination with corticosteroids, anti-golimumab antibodies were detectable at weeks 14 (70 ng/mL equivalents with a golimumab concentration of 3.4 μg/mL) and 18 (92 ng/mL equivalents with a golimumab concentration of 2.2 μg/mL). As mucosal healing was not achieved after 14 weeks of therapy, the patient was switched to therapy with vedolizumab, an anti-α4β7 integrin. In patient 3 (Fig. 6a), a 20-year-old woman receiving golimumab in combination with corticosteroids and mesalamine, anti-golimumab antibodies were detectable at week 14 (44 ng/mL equivalents with a golimumab concentration of 1.6 μg/mL). Also, this patient was switched from therapy, as mucosal healing was not achieved even after a golimumab dose escalation at week 14. In patient 8 (Fig. 6b), a 59-year-old man receiving golimumab in monotherapy for 2.5 years, anti-golimumab antibodies were detectable at weeks 8 (42 ng/mL equivalents with a golimumab concentration of 2.1 μg/mL) and 12 (60 ng/mL equivalents with a golimumab concentration of 1.8 μg/mL) of the GOUDA study. Treatment outcome (achievement of mucosal healing) upon detection was not affected.

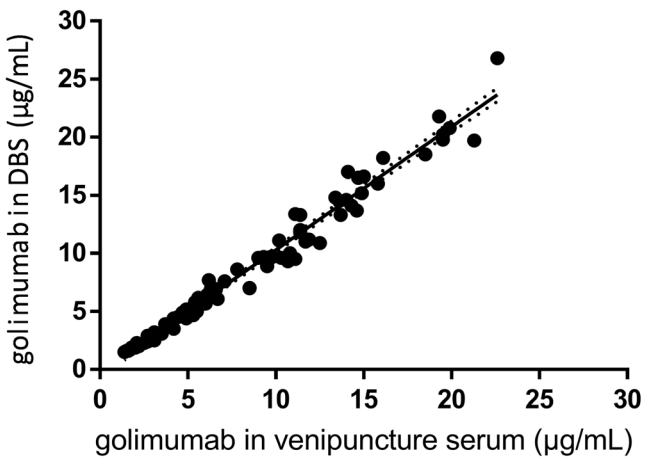

Fig. 4. Correlation of golimumab measured in serum obtained *via* venepuncture with the converted dried blood spot (DBS) golimumab concentration. Spearman's $r = 0.990$, $p < 0.0001$; interclass correlation coefficient = 0.984

Comparison of the In-House-Developed Golimumab ELISA with a CE-Marked Golimumab ELISA Kit

All serum samples from the GOUDA cohort were re-analysed using a CE-marked golimumab ELISA kit. Comparison of the golimumab serum concentrations between the in-house-developed MA-GOM171D8/MA-GOM159B8-HRP ELISA and a CE-marked golimumab ELISA kit revealed a Spearman's correlation of 0.962. The Bland-Altman plot showed an average bias of 16% with a 95% lower and upper limit of agreement of −20 and 53%, respectively (Fig. 7).

DISCUSSION

Compared to conventional therapy, the anti-TNF biologic golimumab allows a more profound control of the mucosal inflammation in patients with UC (12). However, loss of response remains an important problem in clinical practice. Recently, patients with a body weight of less than 80 kg who have an inadequate response can be dose increased to 100 mg at week 6 and every 4 weeks thereafter, according to the new Simponi® posology. Given the strong relationship between serum anti-TNF concentrations and long-term clinical outcome, therapeutic drug monitoring (TDM) based on drug concentrations is a promising strategy for treatment optimisation of golimumab in patients with UC. Up to now and because of practical reasons, TDM is mainly based on serum trough concentrations (TC) as indicators of drug exposure.

This study was initiated from the perspective that the current way of sampling is impractical and burdensome for patients. This hurdle hampers the evaluation of drug concentrations at time points other than trough, for which there is currently a paucity of data. Within the scope of a simplification of sample collection, the technique of the dried blood spots (DBS) seemed to fit well.

The DBS method in our study was developed and analytically validated by calculating the extraction recovery and the accuracy and imprecision of the recalculated recovery. Although the average extraction recovery (54.4%) did not equal 100%, the extent of recovery was consistent, precise and reproducible within a clinically relevant range of concentrations of drug, which is in line with the FDA

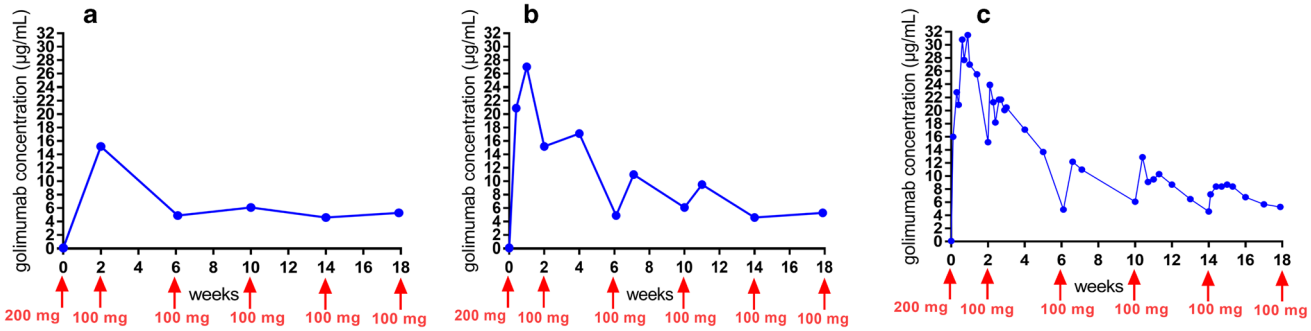

Fig. 5. Golimumab concentration-time profile of one patient in cohort A at trough sampling time points (**a**), at trough and intermediate sampling time points (**b**) and at trough, peak and multiple intermediate sampling time points of GOUDA (**c**)

requirements (11). Accuracy and imprecision of the recalculated recovery did fall within the predefined limits. The technique was furthermore insensitive to the spotted volume (15–90 µL) when a fixed diameter of 6 mm was punched out. The stability of the filter papers at room temperature (for at least one month) offered the opportunity for easier transportation and storage.

Therefore, in the next step, the method was applied in a "real-life" clinical setting: ten patients initiating or under maintenance golimumab therapy were asked to give blood by venepuncture (serum) and finger pricks (DBS) following intensive sampling regimens. Patients received education at the first visit on how to perform the finger prick and how to spot a sufficiently large droplet of blood onto the card. The majority of patients had a positive attitude towards the finger prick. Moreover, at the end of the study, this way of sampling was found to be easy and convenient, and most patients preferred to carry out a finger prick at home than to visit a doctor for a venepuncture.

When comparing the golimumab concentrations obtained by analysis of serum to the results obtained by analysis of DBS (performed at the same time), an excellent correlation was observed. Although a clear explanation for the systematically lower concentrations of golimumab in DBS eluates compared to those in venepuncture serum is lacking (a possible explanation might lie in a discrepancy in golimumab concentrations between capillary and venous blood), this was easily accounted for by using an overall correction factor of 3.9, which is based on the extraction recovery and the capillary blood/serum ratio for golimumab. Alternatively, other parameters can be used to convert a blood concentration into a serum/plasma concentration (13).

The haematocrit range (from 0.39 to 0.48) was for all men and women within the normal ranges; therefore, the effect of haematocrit on the calculated golimumab concentration was not studied and is presumed to be small in line with other studies on this matter (14). This is also supported by the fact that individual correction factors showed a small range (between 3.6 and 4.0), with typically low SDs within patients over time, and the fact that the overall correction factor of 3.9 could be successfully applied. However, as haematocrit has shown to influence spot size, recovery and blood-to-plasma ratio (15), an individual correction factor might need to be established first in case patients with aberrant haematocrit values are sampled. Recently, use of the Mitra® microsampler, as an alternative for capillary blood sampling by traditional DBS on filter paper, was investigated. The Mitra® microsampler consists of a porous material attached to a holder that should absorb a fixed volume of blood, irrespective of concentration of haematocrit. Overall, the Mitra® microsampler was less labour-intensive and yielded results quite similar to DBS performed using Whatman paper, but at a somewhat improved precision (13).

DBS sampling provided also better insights into golimumab exposure, as exposure was not captured well by the golimumab trough concentrations during induction. Moreover, through intensive sampling, a multiple peak pattern emerged during drug absorption, which would not

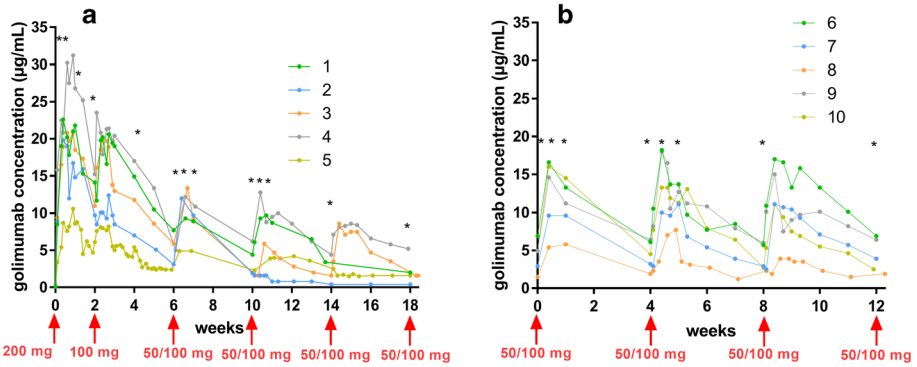

Fig. 6. **a** Golimumab concentration-time profiles of the five patients in cohort A. Patients 4 and 5 achieved mucosal healing. **b** Golimumab concentration-time profiles of the five patients in cohort B. All patients had achieved mucosal healing. *Time points where venepuncture and finger prick were performed simultaneously

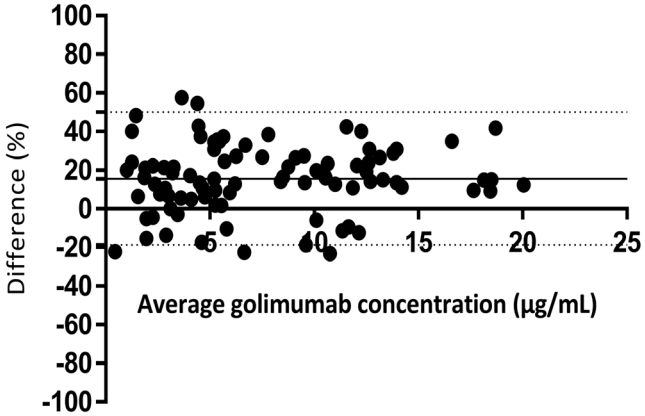

Fig. 7. The Bland–Altman comparison of golimumab concentrations determined with the MA-GOM171D8/MA-GOM159B8-HRP ELISA and the CE-marked golimumab apDia ELISA kit. The relative difference (%) in golimumab concentrations between both assays is plotted against the average measured golimumab concentration (µg/mL). Average bias (grey line) and the 95% lower and upper limits of agreement (dotted lines) are represented

have been detected otherwise. Although the mechanism responsible for this phenomenon is currently unknown, a possible explanation may lay in first-pass catabolism of the drug at the subcutaneous administration site or in the draining lymphatics. In this regard, non-linear disposition pharmacokinetics has been frequently observed with biotherapeutics undergoing target-mediated drug disposition before reaching the systemic circulation (16). Furthermore, a pen delivery device was used to administer golimumab into the thigh in nine out of ten patients. One patient used a syringe to administer golimumab into the abdomen. The multiple peak pattern seemed to occur independently of delivery device (as it occurred in all patients) and independently of the dosage or the number of injections per patient/occasion used. Whether or not this multiple peak pattern influences treatment outcome needs further investigation.

Because of the limited sample size in our study, we were not able to establish a pharmacokinetic/pharmacodynamic correlation—although we did observe a trend—between golimumab exposure and achievement of mucosal healing. However, we and others previously confirmed the existence of such a correlation for golimumab (7,17). Moreover, some patients may have a good response with lower golimumab concentrations than others. In this regard, Ducourau *et al.* showed that an individual disease activity at baseline is an essential factor in determining the target concentration for adalimumab to achieve a clinical outcome in rheumatoid arthritis patients: the target concentration will be higher in patients with very active disease at baseline and lower in patients with moderate disease (18). The fact that patients who achieved mucosal healing in our cohort did not necessarily have the highest, but rather the "most stable", exposure over time seems to be in favour of this hypothesis. Furthermore, we did observe an inter-individual difference in apparent drug absorption after the first administration of golimumab 200 mg. The latter may indicate that a part of the observed variability in golimumab exposure results from differences in absorption instead of only clearance of the antibody. Extensive population-based PK studies are needed to further identify and quantify sources of variability in golimumab absorption and exposure.

An important cause of non-response to adalimumab or infliximab treatment is subclinical drug concentrations due to antibody formation (19). Here, we show that anti-golimumab antibodies were detected in three out of ten patients using a drug-tolerant immunoassay; one patient maintained mucosal healing and two patients had to be switched to another biological agent. The clinical relevance of low-concentration anti-drug antibody detectable in a drug-tolerant but not in a drug-sensitive assay, remains, however, to be proven (7).

The results described in this paper support the feasibility of TDM of golimumab *via* self-sampling. This may facilitate TDM on a larger scale and allows to prospectively study clinically relevant golimumab concentration cut-offs. When applying TDM, peak and trough golimumab concentrations cannot be used interchangeably, because of the observed differences in peak-to-trough ratios. The availability of a CE-marked ELISA kit greatly facilitates utilisation, automation and standardisation of golimumab testing. However, although the performance between the in-house developed golimumab ELISA and the CE-marked golimumab apDia ELISA kit was comparable, absolute golimumab concentrations showed systematic differences. Therefore, only results obtained with the same assay can be compared or should be recalculated (e.g., with a mean difference of 16%, a golimumab concentration of 5.0 µg/mL determined with the in-house-developed golimumab ELISA corresponds to a golimumab concentration of 4.2 µg/mL determined using the CE-marked golimumab apDia ELISA kit). Because of insufficient sample volume, DBS eluates could only be measured using the in-house-developed golimumab ELISA. Finally, adding DBS to data collection protocols for population-based studies could provide TDM data from infants, young children, pregnant women or the elderly, for whom venepuncture is even more cumbersome.

CONCLUSION

The GOUDA study showed that DBS sampling is a reliable and patient-friendly alternative to venous blood collection. DBS sampling simplifies the TDM process and can provide more insight into the PK of golimumab, as frequent sampling within one dosing interval can be easily performed with a finger prick taken at home. Our prospective study with intense collecting samples at peak, intermediate and trough provided better insights into golimumab absorption and exposure.

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