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Clinical validation of an innovative dried whole-blood spot method to quantify simultaneously vancomycin and creatinine in adult patients

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Background: A drawback of vancomycin use is the need for therapeutic drug monitoring and renal function monitoring. Traditional blood sampling involves drawing blood through a venepuncture. An alternative method, dried blood spot (DBS) sampling allows for self-sampling at home.

Objectives: To clinically validate a DBS method for simultaneous monitoring of vancomycin and creatinine.

Methods: Hospitalized adults treated with intravenous vancomycin were included (trial registration NCT05257070). Blood sampling consisted of one venepuncture and one finger prick. Whole-blood DBS samples from patients were obtained by applying one drop of whole blood onto Whatman 903 filtrate paper. Bland–Altman analyses were used to assess the agreement and bias between the two measurements. Patients were asked to state their preferences for one of the two sampling methods.

Results: The study involved a final analysis of 39 patient samples for the clinical validation of vancomycin and 46 patient samples for the clinical validation of creatinine. The difference between plasma and DBS concentrations was ≤20% for 77% of the vancomycin samples, the mean bias was −0.1379% (95% limit of agreement −5.899–5.623). The difference between plasma and DBS concentrations was ≤20% for 89% of the creatinine samples, the mean bias was 2.656% (95% limit of agreement −26.16–31.47). Most patients (18 out of 31) preferred a finger prick over a venepuncture and 12 patients indicated no preference.

Conclusions: This is the first study that successfully clinically validated a DBS sampling method for simultaneous measurement of vancomycin and creatinine, allowing for direct use in (outpatient) practice.

Introduction

Outpatient parenteral antimicrobial therapy (OPAT) is a valuable approach for administering antimicrobial therapy via parenteral infusion without the need for hospitalization. One widely used antibiotic in OPAT is vancomycin, which is effective in treating complex Gram-positive infections such as infected thrombus, endocarditis, osteomyelitis and prosthetic bone and joint infection.[1] To ensure successful treatment and minimize nephrotoxicity, therapeutic drug monitoring (TDM) of vancomycin and monitoring of creatinine concentrations are crucial during vancomycin therapy.[1–3] Guidelines recommend monitoring plasma concentrations of vancomycin at least once weekly in haemodynamically stable patients.[4,5] The current conventional practice involves measurement of vancomycin in plasma obtained through the venepuncture method. This method of blood sampling can be painful, invasive, challenging and burdensome, particularly for OPAT patients. Limited access to a blood sampling facility due to either lack of transportation or geographic isolation further complicates TDM practice.[6] Despite these drawbacks, the use of vancomycin remains widespread in OPAT services.

In recent years, an alternative sampling method, dried blood spot (DBS) sampling, has gained interest for expanding healthcare services in TDM.[7] DBS involves collecting a small drop of

capillary blood onto filter paper, offering several advantages over venous blood sampling. The finger prick collection method used in DBS is less invasive and a common technique for blood sampling, similar to the heel prick screening method used for neonates.[7,8] Moreover, DBS allows for self-sampling at home, reducing the need for phlebotomy visits and enabling multiple time point sampling.[9] Furthermore, DBS requires lower blood volumes compared to venous blood sampling, making the process more patient friendly and sustainable.[7] The DBS method provides a promising alternate approach for TDM in patients receiving vancomycin treatment in the outpatient setting.

The Department of Hospital Pharmacy at Erasmus University Medical Centre successfully developed and validated a DBS method for the analytical quantification of vancomycin and creatinine in whole-blood DBS samples.[10] This technique used ultra-high performance liquid chromatography-tandem mass spectrometry) for whole-blood analyses and followed the FDA and EMA guidelines on bioanalytical method validation, as well as the guideline for development and validation of dried blood spot-based methods of the International Association for Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT).[11] Clinical validation is, however, necessary to demonstrate equivalence to venepuncture before this DBS sampling method may be interchanged for venepuncture sampling in a routine clinical setting.[11]

Patients and methods

Ethics

The study was conducted in accordance with the principles of the Declaration of Helsinki and the Medical Research Involving Human Subjects Act (WMO) to the standards of Good Clinical Practice. Approval was obtained from The Medical Ethics Review Committee of Erasmus MC (MEC-2021-0797). Written informed consent was obtained from every patient before inclusion in the study. This trial was registered under NCT05257070.

Study design

The ADVANCED study was performed at Erasmus University Medical Centre. Patients who received either continuous infusion (C-I) and intermittent infusion (I-I) of vancomycin were included, for intermittent infusion only through levels were obtained. Blood sampling involved simultaneously obtaining one venepuncture ($Plasma_{venous}$), which was drawn as part of standard clinical practice, and one DBS finger prick for research purpose (DBS_{finger}). Venepuncture sampling was performed by the attending nurse, DBS sampling was performed by a trained researcher. The time of the finger prick and the venepuncture were registered. Preferably, for vancomycin the time between the finger prick and the venepuncture was as short as possible. All patients were asked to complete a questionnaire to rate the pain of venepuncture versus finger prick sampling on a VAS score scale of 0–10 and to provide their preferences in sampling methods.

Patient population

Patients were included if they were >18 years of age and receiving intravenous vancomycin for treatment, and if vancomycin or creatinine was measured as part of their routine clinical care. The study aimed for a total inclusion of 40 paired DBS and venepuncture samples from a minimum of 25 different patients for each analyte.[11]

Dried blood spot samples

The DBS sample (DBS_{finger}) was collected from finger puncture using a single-use, automatic lancing device (BD Microtainer® Contact-Activated Lancet). Before punching, the finger site was pre-warmed and cleansed. The whole blood from the capillaries was spotted onto DBS filter paper (Whatman protein saver 903 card, Cardiff, UK). One drop of capillary blood was spotted onto the pre-marked circle of the DBS filter paper. A second DBS sample, DBS_{venous}, was prepared from the whole-blood venepuncture sample collection tube by pipetting 50 µL onto five pre-marked circles on the DBS filter paper. DBS card samples were dried in a flat position for at least 1 hour at room temperature prior to storage in a desiccator.

Laboratory analysis

For this study, a total of three blood samples were collected and analysed for each patient: (i) a DBS sample obtained by a finger prick (DBS_{finger}), (ii) a DBS sample prepared from whole blood obtained by conventional venepuncture method (DBS_{venous}) and (iii) a plasma sample from whole blood obtained by conventional venepuncture, the standard clinical practice ($Plasma_{venous}$). The vancomycin and creatinine concentrations from the ($Plasma_{venous}$) samples were obtained from the electronic medical records from the patients.

The haematocrit of each DBS sample [$(Ht)DBS_{finger}$ and $(Ht)DBS_{venous}$] was measured pre-analysis to determine the effect of viscosity, according to a validated non-destructive method using near-infrared spectroscopy (NIR; Shimadzu, Den Bosch, The Netherlands) with a FlexIR NIR Fiber Optic Accessory probe (Pike Technologies, Madison, WI, USA).[12,13]

The venepuncture samples ($Plasma_{venous}$) were used to measure vancomycin and creatinine concentrations via enzymatic assay as part of standard clinical practice. The plasma creatinine concentration after venous sampling was analysed by an enzymatic assay on a Cobas8000 system (Roche Diagnostics, Basel, Switzerland). The bias and precision of this method are 6.1% and 2.4%, respectively, with a validated concentration range between 5 and 2700 µmol/L. The plasma vancomycin concentration after venous sampling was analysed with an immuno-assay on an Architect C4000 system (Abbott, IL, USA). The bias and precision of this method are 2% and 2%, respectively, with a validated concentration range between 1.1 and 100 mg/L.

DBS samples were assessed for spot quality by trained laboratory analyst. Spots were assessed for size, shape, overlap and colour consistency. In a case of multiple spots, the spot of the best quality was chosen and a sample of 6 mm diameter was punched using a manual disc puncher. In case of simultaneous inclusion of vancomycin and creatinine, both analytes were measured from one DBS spot. DBS analysis was performed using the LC-MS/MS method previously validated based on FDA/EMA guidelines and the IATDMCT guideline.[10,11] The DBS analysis method by Bahmany *et al*. was optimized to use whole blood of vancomycin patients as calibration standards and quality control (QC) samples instead of preparing calibration standards and QC samples by spiking stock solutions of vancomycin to vancomycin-free whole blood, due to the large variability in recovery of DBS versus plasma concentrations.[10] Independent whole-blood samples of patients with known vancomycin concentration were used for each calibration standard and QC level. Vancomycin concentrations in these samples were already measured with an immune-assay on an Architect C4000 system (Abbott, IL, USA) This method was partially validated in a concentration range of 3.8–76.60 mg/L for vancomycin, with an average bias of 4.4%, average repeatability of 12.8% and average reproducibility of 10.8%. Eight calibration standards and three QC levels (low, medium and high) were prepared in DBS for vancomycin and creatinine. Creatinine plasma concentrations were measured by an enzymatic assay (Cobas8000 system, Roche Diagnostics, Basel, Switzerland). Since it is impossible to obtain creatinine-free blood, independent, vancomycin-free, whole-blood samples of patients were used for each

calibration standard and QC level. Residues of, vancomycin-free, patient samples for creatinine were obtained from the laboratory of the clinical chemistry department. Residues of vancomycin patient samples were obtained from the laboratory of the hospital pharmacy. Before use, potential objection against the use of material for research purposes was checked for each patient in the laboratory information system of the Erasmus Medical Center. Finally, after mixing and homogenization, 50 µL was spotted on the DBS cards. DBS cards were stored in the desiccator and dried for at least 24 h at room temperature before use.

Study endpoints

The primary study endpoint was the agreement and bias between finger prick DBS concentrations (DBS_{finger}) and conventional plasma concentrations of vancomycin and creatinine ($Plasma_{venous}$). The Bland–Altman method was used to evaluate potential bias by testing the agreement between the two methods. For Bland–Altman analysis, the following acceptance limit from the EMA and IATDMCT guidelines was used: the difference of the two measurements should be within 20% of the mean of the concentrations for at least 67% of the samples.[11] Secondary study endpoints were (i) to evaluate if the difference between the measured vancomycin concentrations are correlated with the vancomycin dose, vancomycin dosing regimen (continuous or intermittent regimen), and the time between the two blood sampling methods, (ii) to evaluate if the plasma concentrations ($Plasma_{venous}$) are correlated with bias, (iii) to evaluate the effect of haematocrit on bias, (iv) to evaluate the effect of the DBS filter paper on vancomycin and creatinine concentration measurement by comparing the DBS finger samples (DBS_{finger}) and venepunctures ($Plasma_{venous}$), as well as DBS venous blood samples (DBS_{venous}) and venepunctures ($Plasma_{venous}$), (v) to evaluate the effect of the location of blood sampling (venous versus capillary) on vancomycin and creatinine concentration measurement by comparing DBS finger samples (DBS_{finger}) and DBS venous blood samples (DBS_{venous}), (vi) whether a correction factor for the DBS method can improve the agreement between DBS_{finger} and $Plasma_{venous}$ and (vii) the patients' experience with the blood sampling method (pain score on a VAS score scale and preference for sampling method).

Statistical analysis

Statistical analyses were performed with IBM SPSS statistics v.28.0 software for Windows (IBM, Armonk, NY, USA), and GraphPad Prism v.8 software (GraphPad Software, La Jolla, CA, USA). Correlations between non-parametric continuous variables were calculated using Spearman's correlation coefficient. The Mann–Whitney *U*-test was used to evaluate potential differences in non-parametric continuous variables between two groups.

Deming regression analyses were performed to assess any constant and/or proportional bias between plasma and DBS measurements. A proportional bias was obtained if the 95% confidence interval (CI) of the regression line slope did not contain 1. When the 95% CI of the regression line intercept did not contain 0, the data were considered to have a constant bias. Deming regression was used to calculate the difference in the effect of filter paper and location of blood sampling on drug concentrations.

It was evaluated whether a correction formula could optimize the agreement and minimize systematic differences between the standard and the DBS measurements. Because differences could have multiple causes and shapes, different correction formulae were evaluated. To evaluate whether haematocrit correction was required, correction factors with and without haematocrit correction were evaluated. Moreover, as the haematocrit effect might depend on the height of the concentration of the analyte, a correction formula including an interaction term between the haematocrit and the concentration of the analyte was evaluated. To determine whether correction for haematocrit was needed, uncorrected DBS haematocrit values measured with NIR [(Ht) DBS_{finger}] were used. Passing–Bablok regression and Bland–Altman analysis results were compared before and after correction for all correction formulae that were applied. For the Bland–Altman analysis, the proportion of samples within the 20% acceptance limit and the mean bias were evaluated. If results of these analyses were similar for multiple correction formulae, the simplest correction formula was chosen.

Results

In total, 39 patients with 40 vancomycin samples and 44 patients with 46 creatinine samples were included. The inclusion per analyte depended on whether it was measured as part of a patient's standard clinical care. One vancomycin patient sample was excluded from the analysis due to poor quality of the DBS sample (insufficient spot size).

Baseline characteristics

The baseline characteristics of the patients' samples included are presented in Table 1, stratified by analyte.

Table 1. Baseline characteristics

	Vancomycin ($n=39$)	Creatinine ($n=46$)
Gender		
Male (*n*, %)	22 (56%)	30 (65%)
Age [years, median (range)]	59 (20–84)	59 (21–84)
Bodyweight [kg, median (range)	89 (54–134)	82 (53.8–119)
Height [cm, median (range))]	176 (152–190)	178 (150–198)
Vancomycin dose [mg, median (range)]	2000 (500–4000)	NA
Vancomycin venous concentration [mg/L, median (range)]	19 (4.2–28.5)	NA
Creatinine venous concentration [µmol/L, median (range)]	NA	69 (28–510)
Vancomycin regimen (*n*)		
C-I	23	NA
I-I	16	NA

NA, not applicable.

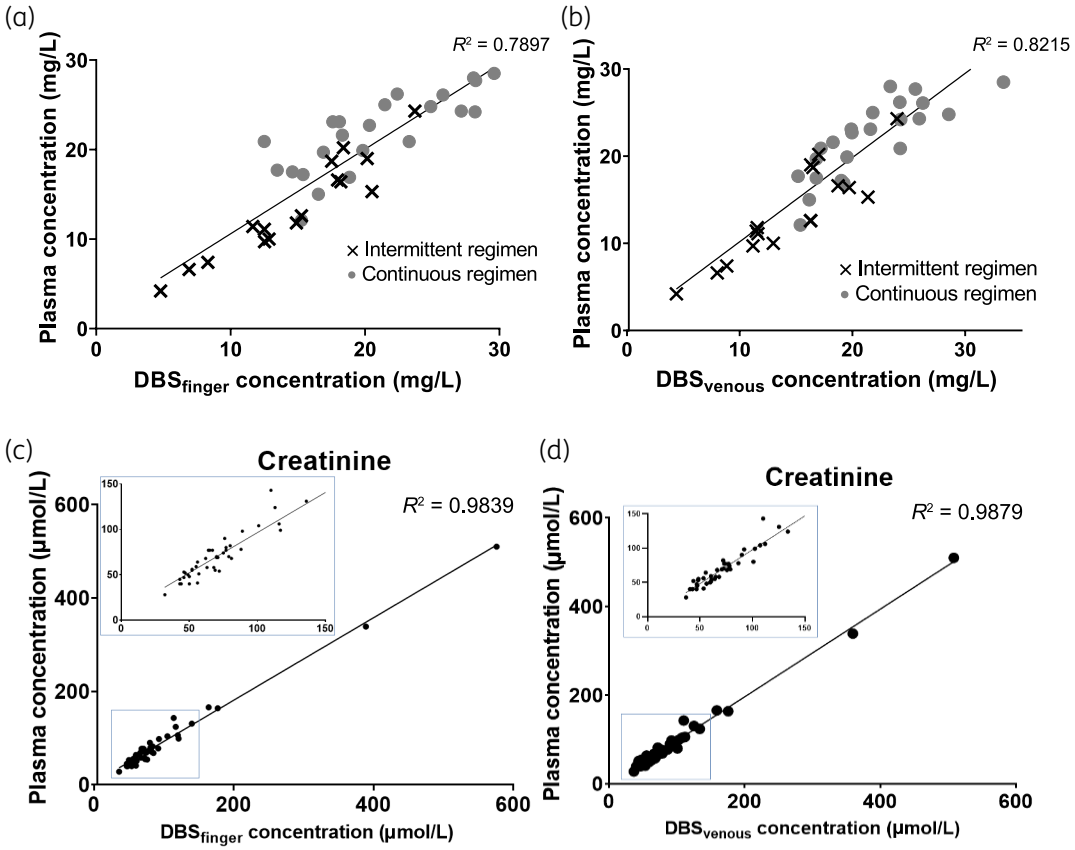

Figure 1. Plot of DBS$_{finger}$ and Plasma$_{venous}$ concentrations of (a) vancomycin and (c) creatinine, plot of DBS$_{venous}$ and Plasma$_{venous}$ concentrations of (b) vancomycin and (d) creatinine.

Table 2. Bland–Altman results of DBS$_{finger}$ and Plasma$_{venous}$ measurements

	Vancomycin (n = 39)	Creatinine (n = 46)
Δ Within ±20% of average	30 (77%)	41 (89%)
Mean bias	−0.1379	2.656
95% LOA	−5.899–5.623	−26.16–31.47

LOA, limit of agreement.

Agreement between DBS and plasma samples

The coefficient of determination (R^2) of DBS$_{finger}$ and Plasma$_{venous}$ was 0.7897 for vancomycin and 0.9839 for creatinine (see Figure 1). The R^2 of DBS$_{venous}$ and Plasma$_{venous}$ was 0.8215 for vancomycin and 0.9879 for creatinine (see Figure 1). Without a correction formula for vancomycin (n = 39), 77% of the measurements of DBS$_{finger}$ and Plasma$_{venous}$ met the 20% acceptance limit and for creatinine (n = 46), 89% of the measurements met the 20% acceptance limit (see Table 2). No systematic bias is observed from the Bland–Altman plots for vancomycin and creatinine. The Bland–Altman results are displayed in Table 2. Figure 2 shows the Bland–Altman plots of vancomycin and creatinine.

Effect of vancomycin dose, dosing regimen and time difference between sampling methods

The total daily dose per kg was not significantly correlated with bias [(difference/mean (%)] in DBS$_{finger}$ and Plasma$_{venous}$ concentration for vancomycin (Spearman's correlation coefficient of 0.220, P = 0.178; Figure 3).

Both C-I (n = 23) and I-I (n = 16) vancomycin patients were included. The dosing regimen was significantly correlated with bias {[difference/mean (%)] in DBS$_{finger}$ and Plasma$_{venous}$ concentration}. The bias for vancomycin was higher for intermittent regimen compared to continuous regimen (median 10.99% and median −1.16% for intermittent and continuous regimen, respectively, P = 0.001 using the Mann–Whitney U-test). Deming regression showed no proportional or constant bias in the sub-analysis for C-I and I-I infusion groups.

As the difference in the concentration of vancomycin DBS$_{finger}$ and Plasma$_{venous}$ is dependent on the time difference, we evaluated the effect of timing on drug concentrations between the two different blood sampling methods. The median time between finger prick and venepuncture was 5 minutes (range −214–130 minutes). The time between the venepuncture and the finger prick was not significantly correlated with bias {[difference/mean (%)] in DBS$_{finger}$ and Plasma$_{venous}$ concentration} for vancomycin (Spearman's correlation coefficient of −0.261, P = 0.109; Figure 4). Here 75% of the vancomycin DBS samples

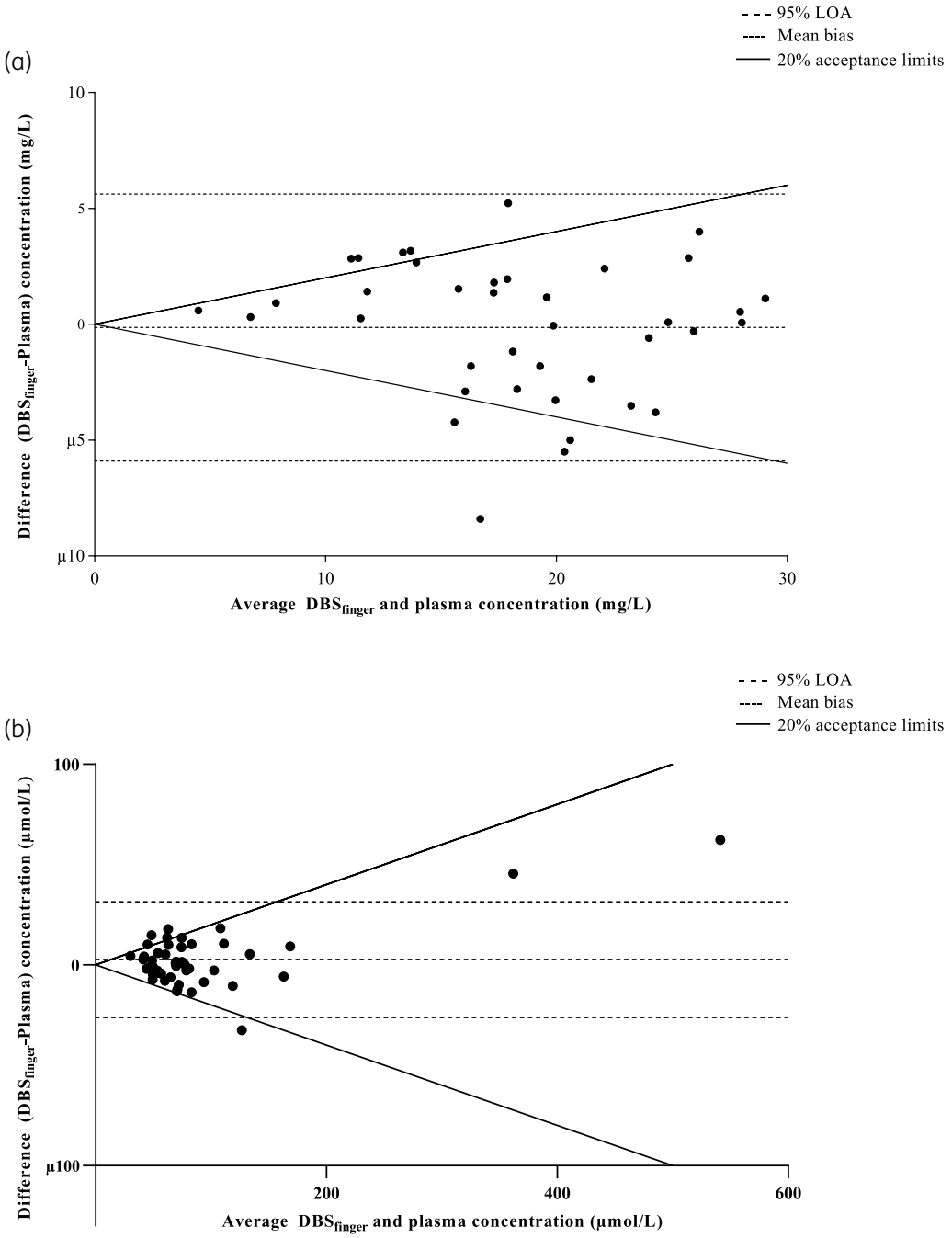

Figure 2. Bland–Altman plot of mean concentrations DBSfinger and Plasmavenous and difference in DBSfinger – Plasmavenous of (a) vancomycin and (b) creatinine. LOA, limit of agreement.

(12/16 samples) during intermittent infusion were sampled before venepuncture sampling, leading to a higher median bias during intermittent regimen (DBS concentrations are overestimated compared to the venepuncture concentrations).

Effect of plasma concentration

The vancomycin ($Plasma_{venous}$) concentration was significantly correlated with bias {[difference/mean (%)] in DBS_{finger} and $Plasma_{venous}$ concentration} for vancomycin (Spearman's correlation coefficient of −0.551, $P \leq 0.001$; Figure 5). In general, the bias increased with decreasing vancomycin plasma concentration, meaning that the DBS concentration was overestimated compared to plasma concentration. In the lower plasma concentration range more samples were included from intermittent infusions (see Figure 5a) and during intermittent infusion most of the DBS samples were obtained before the venepuncture sample (see Figure 4). By contrast, the creatinine concentration ($Plasma_{venous}$) was not

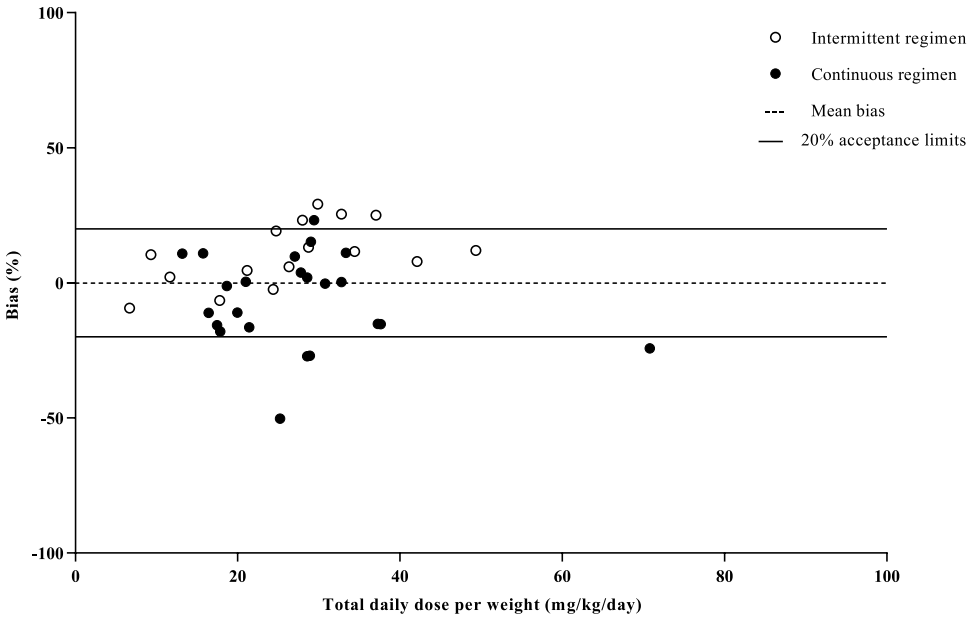

Figure 3. Vancomycin total daily dose per kg versus bias. Bias (%) = difference/average = $(DBS_{finger} - Plasma_{venous})/[(DBS_{finger} + Plasma_{venous})/2] \times 100$.

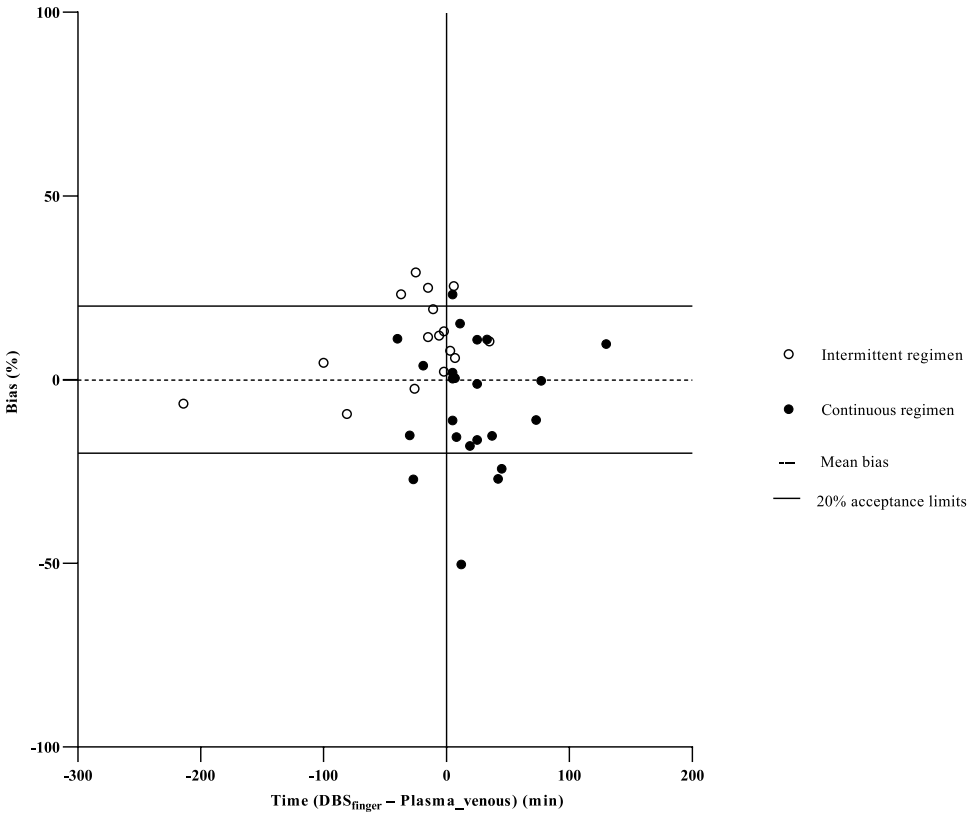

Figure 4. Time between finger prick (DBS_{finger}) and venepuncture ($Plasma_{venous}$) versus bias. Bias (%) = difference/average = $(DBS_{finger} - Plasma_{venous})/[(DBS_{finger} + Plasma_{venous})/2] \times 100$.

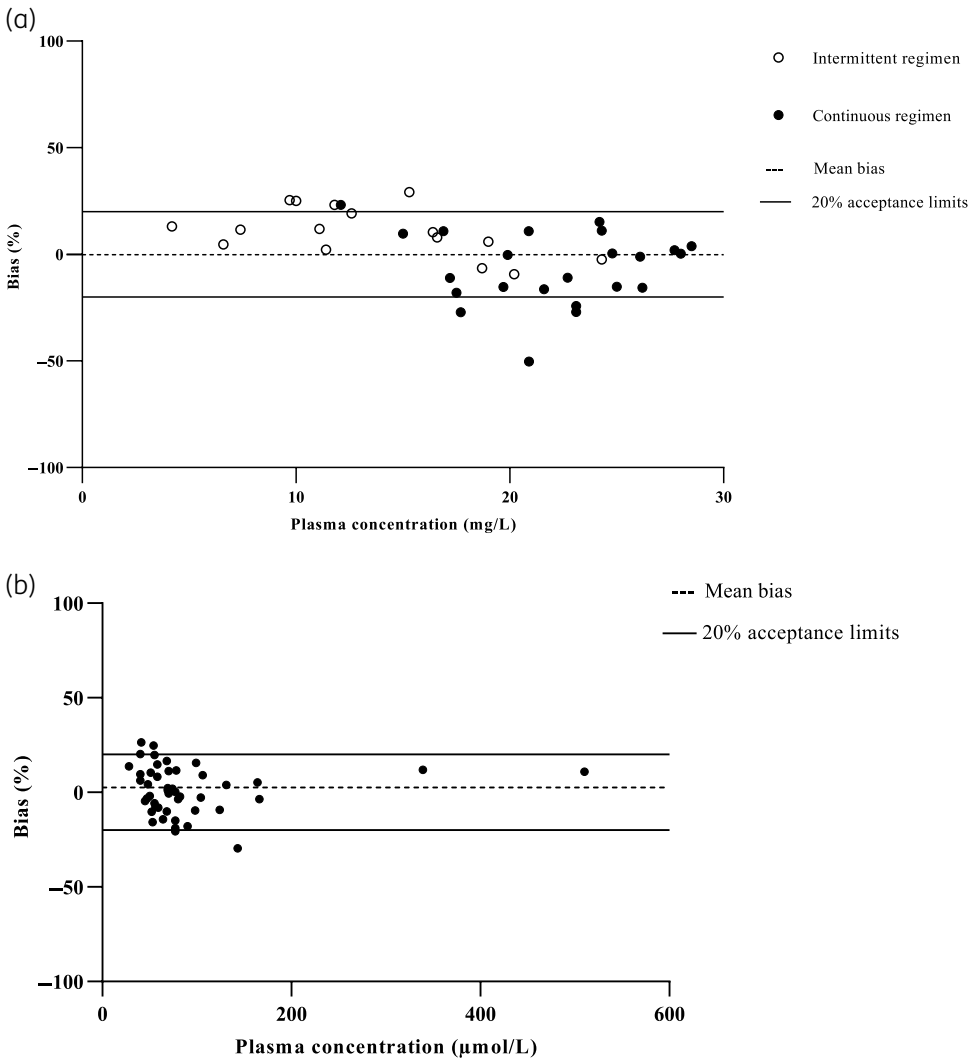

Figure 5. $Plasma_{venous}$ concentrations versus bias for (a) vancomycin and (b) creatinine. Bias (%) = difference/average = $(DBS_{finger} - Plasma_{venous})/[(DBS_{finger} + Plasma_{venous})/2] \times 100$.

significantly correlated with bias for creatinine (Spearman's correlation coefficient of −0.219, $P = 0.144$; Figure 5).

Haematocrit effect

The haematocrit was not significantly correlated with bias {[difference/mean (%)] in DBS_{venous} and $Plasma_{venous}$ concentration} for vancomycin and significantly correlated with bias for creatinine (Spearman's correlation coefficients of −0.180 and 0.386, respectively; $P = 0.273$ and $P = 0.008$, respectively; Figure 6).

DBS filter paper and sampling location effects

No proportional or systemic bias was observed for vancomycin (DBS_{finger} and $Plasma_{venous}$). No effect of the filter paper and sample location was observed for vancomycin (Table 3). For creatinine, a proportional and systemic bias was observed between DBS_{finger} and $Plasma_{venous}$ and regarding the sample location (Table 3).

Correction formulae

Several correction formulae were investigated to improve the agreement between DBS_{finger} and $Plasma_{venous}$ concentrations. No improvement was seen in the agreement between the two sampling methods for vancomycin and creatinine if a correction formula was applied (Table 4). Small improvements were seen regarding the mean bias, however, this was not clinically significant. Consequently, the simplest formula was adopted (no correction formula). Table 5 shows the results of the Passing–Bablok analyses before and after application of a correction formula.

The patients' experience with DBS

The questionnaire on patient's experience with the DBS method was completed by 31 participants. The median pain score of the venepuncture (3.0; range 0.0–8.0) was significantly higher compared to the median pain score of finger prick

(2.0; range 0.0–6.0; Wilcoxon signed ranks test, $P=0.009$). Most patients preferred a finger prick over a venepuncture ($n=18$; 58%), one patient preferred a venepuncture over a finger prick ($n=1$, 3%) and 12 patients stated no preference (39%).

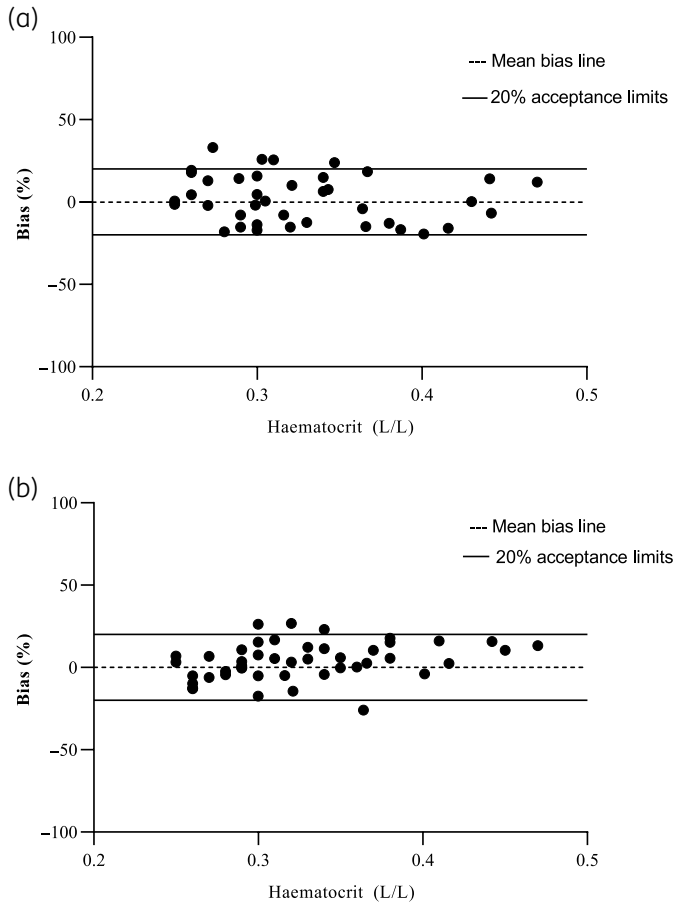

Figure 6. Haematocrit of DBS_{venous} samples versus bias for (a) vancomycin and (b) creatinine. Bias (%) = difference/average = $(DBS_{venous} - Plasma_{venous})/[(DBS_{venous} + Plasma_{venous})/2] \times 100$.

Discussion

This is the first study that successfully clinically validated a DBS sampling method through direct finger prick for simultaneous measurement of vancomycin and creatinine concentrations. Previous studies have prepared vancomycin DBS cards by spotting whole blood or plasma on the DBS cards.[14,15] However, the IATDMCT guidelines state that the sampling method used during clinical validation should be the same as the sampling method that will be used in daily practice.[11] The spotting of venous blood on a DBS card, used for the validation in other studies, is thus not appropriate for a clinical validation study for vancomycin. The study by Scribel *et al.* did compare DBS samples through finger prick in patients with venepuncture samples.[16] However, in their study vancomycin plasma could not be accurately predicted from DBS measurements in clinical specimens (plasma to DBS drug concentration ratios were highly variable, ranging from 1.148 to 5.022). By contrast, creatinine plasma concentrations were satisfactorily predicted from DBS measurements. They hypothesized that this may be due to a variable partition between plasma and blood cells, or even due to interactions to the paper substrate and may be affected by drying time.[16] However, we believe that the use of calibration standards and QC samples prepared by spiking working solutions of vancomycin to venous blood may be the reason for the unsatisfactory results. We believe that, due to the properties of vancomycin, only patient-derived calibration standards and QC samples lead to satisfactorily results. This may be due to the protein binding of vancomycin, which results in less homogenous distribution into the red blood cells. In addition, we also hypothesize that working solutions have different flow properties, affecting the calibration standards and QC DBS samples. In our study, we used whole blood of patients with known vancomycin concentrations as calibration standards and QC samples.

Our DBS method fulfilled the requirements for clinical validation, as described in the IATDMCT guidelines.[11] As the method easily fulfils the official requirements for clinical validation, the method could be used for vancomycin and creatinine monitoring in clinical practice. However, one might consider a slightly elevated variability in DBS measurements compared to venous blood sampling. We propose several explanations for this difference between plasma and DBS concentration. In this trial, patients were included with varying disease severity, whereas the target population for at-home sampling consists of outpatient patients who are, in general, stable. The inclusion of hospitalized patients limited

Table 3. Results from the Deming regression analyses

No correction factor	Slope	95% CI	Intercept	95% CI
Vancomycin ($n=39$)				
$Plasma_{venous} \sim DBS_{finger}$	0.9326	0.8200–1.045	1.104	−1.032–3.239
$Plasma_{venous} \sim DBS_{venous}$	0.9321	0.7821–1.082	1.357	−1.119–3.833
$DBS_{venous} \sim DBS_{finger}$	1.002	0.8526–1.151	−0.2783	−2.795–2.238
Creatinine ($n=46$)				
$Plasma_{venous} \sim DBS_{finger}$	1.124	**1.058–1.190**	−8.448	**−14.28–−2.617**
$Plasma_{venous} \sim DBS_{venous}$	1.005	0.9428–1.067	1.874	−3.104–6.852
$DBS_{venous} \sim DBS_{finger}$	1.118	**1.010–1.227**	−10.51	**−19.23–−1.788**

Bold numbers: constant or proportional bias.

Table 4. Bland–Altman results of different correction formulae

	Vancomycin ($n=39$)	Creatinine ($n=46$)
No correction factor		
Δ Within ±20% of average	30 (77%)	41 (89%)
Mean bias	−0.1379	2.656
95% LOA	−5.899–5.623	− 26.16–31.47
Correction formula 1	$DBS_{finger}/0.9761$	$DBS_{finger}/1.067$
Δ Within ±20% of average	29 (74%)	38 (83%)
Mean bias	0.3097	−3.113
95% LOA	−5.490–6.110	−25.26–19.03
Correction formula 2	$DBS_{finger}/0.9326$	$DBS_{finger}/1.124$
Δ Within ±20% of average	28 (72%)	35 (76%)
Mean bias	1.183	−7.480
95% LOA	−4.732–7.099	−27.46–12.50
Correction formula 3	$(DBS_{finger} - 1.104)/0.9326$	$(DBS_{finger}+8.448)/1.124$
Δ Within ±20% of average	30 (77%)	40 (87%)
Mean bias	−0.0004540	0.03640
95% LOA	−5.916–5.915	−19.94–20.01
Correction formula 4	$-2.605+(0.931\times DBS_{finger}+(12.135\times(Ht)DBS_{finger}$	$11.993+(0.885\times DBS_{finger})+(-12.405\times(Ht)DBS_{finger}$
Δ Within ±20% of average	29 (74%)	40 (87%)
Mean bias	−0.004233	−0.00370
95% LOA	−5.600–5.592	−19.91–19.90
Correction formula 5	$-30.403+(2.345\times DBS_{finger})+(102.348\times[(Ht)DBS_{finger})$ $+(-4.580\times DBS_{finger}\times(Ht)DBS_{finger}$	$3.313+(0.979\times DBS_{finger}+(10.072\times(Ht)DBS_{finger}$ $+(-0.235\times DBS_{finger}\times(Ht)DBS_{finger}$
Δ Within ±20% of average	28 (72%)	40 (87%)
Mean bias	−0.002821	−0.05766
95% LOA	−5.018–5.012	−19.85–19.74

Correction formulae: 1, correction for the linear regression slope (with the regression line forced through 0); 2, correction for the Deming regression slope; 3, correction for the Deming regression slope and intercept; 4, correction for haematocrit using simple linear regression with the plasma concentration as dependent variable and the DBS concentrations and haematocrit as independent variables; 5, correction for haematocrit using linear regression with the plasma concentration as dependent variable and the DBS concentrations and haematocrit as independent variables and with an interaction term between DBS concentrations and haematocrit.

Table 5. Results Passing–Bablok analyses $Plasma_{venous} \sim DBS_{finger}$

	Slope	95% CI	Intercept	95% CI
Vancomycin ($n=39$)				
Correction formula 1	0.945	0.810–1.126	1.768	−2.082–4.479
Correction formula 2	0.989	0.825–1.166	1.850	−1.598–5.028
Correction formula 3	0.989	0.833–1.160	0.666	−2.772–3.768
Correction formula 4	0.887	0.747–1.007	1.895	−0.671–5.044
Correction formula 5	0.901	0.767–1.008	1.796	−0.799–4.850
Creatinine ($n=46$)				
Correction formula 1	0.929	0.795–1.045	1.099	−6.671–8.353
Correction formula 2	**0.881**	**0.781–0.992**	1.099	−6.684–7.676
Correction formula 3	**0.881**	**0.757–0.991**	8.615	0.960–15.823
Correction formula 4	**0.878**	**0.769–0.982**	8.374	**1.727–15.332**
Correction formula 5	**0.900**	**0.790–0.993**	7.233	0.964–14.203

Bold numbers: constant or proportional bias.

sometimes the quality of the dried blood spots as patients were not able to warm their hands adequately or were expressing motoric restlessness. In case of a limited blood flow from the finger prick, it might require pressuring the finger, which might enhance haemolysis and contamination with more interstitial fluid. Differences between DBS and plasma can also be explained

from blood spot quality. Differences can be attributed to too small spot sizes, irregularities, spot overlap or multiple spots in one area. Some suboptimal spots were also seen in our samples and dilution due to pressuring the finger is not visible from the DBS spot. It is therefore recommended to evaluate spot quality before analyses. This also highlights the importance of adequate patient training before a patient can start DBS sampling. Furthermore, the time difference between the two sampling methods can also contribute to more variability. A slight discrepancy was found between the DBS_{finger} and DBS_{venous} concentrations. For vancomycin, this discrepancy was larger than for creatinine. The DBS_{venous} was prepared from the $Plasma_{venous}$sample, eliminating any effect of time difference between DBS_{venous} and $Plasma_{venous}$, whereas the correlation between DBS_{finger} and $Plasma_{venous}$, although not significantly, was affected by the time difference between the two sampling methods.

We investigated whether the vancomycin dose, dosing regimen, time between the two blood sampling methods, plasma concentrations and haematocrit were correlated with bias. We found that the dosing regimen and the vancomycin plasma concentration were significantly correlated with bias. During intermittent infusions the DBS samples were generally sampled before the venepuncture samples, leading to a higher positive bias in these samples. In the lower plasma concentration range, more intermittent infusions were included, leading indirectly to a significant correlation with bias. We also observed that some patients with a large time difference exhibited a lower bias. We believe that this is due to the renal elimination rate of vancomycin in these specific patients. Patients with a faster renal clearance will be more impacted from even a small time difference and vice versa. In patients with a slower clearance rate, the vancomycin level will vary less through time. One patient exhibited a bias ∼50%. We have identified this as an outlier with the Tukey method. For the assessment of the haematocrit effect, the DBS_{venous} samples were used to eliminate any influence of spot quality and time differences. For creatinine, a significant effect of haematocrit was seen, however this was not considered clinically relevant. Several correction formulae were investigated, however, this did not result in an improvement in the agreement for both compounds. The robustness of the DBS measurement was investigated by evaluating the effect of the location of blood sampling and the effect of filter paper. Both appeared to have no effect on the measurements of vancomycin. It did have an effect on the measurement of creatinine, but the application of a correction formula did not result in a higher agreement.

A limitation of this study is that venepuncture samples measured by enzymatic assay were compared with DBS samples measured with LC-MS/MS. This can contribute to a lower agreement between the two sampling methods, as venepuncture samples measured by LC-MS/MS would be preferable. However, we wanted to mimic clinical practice, as vancomycin and creatinine are normally measured with enzymatic assay. Furthermore, we clinically validated plasma concentrations versus whole-blood concentrations, the latter not being a regular matrix on which vancomycin TDM references are based and is not described in the guidelines. Another limitation is that for vancomycin 39 patient samples were included instead of the required 40 patient samples by the guidelines. However, we expect that this has not affected the results. Last, even though the time between venepuncture and DBS sampling was kept to a minimum, this was not always possible in clinical practice.

This is the first study that successfully clinically validated a DBS sampling method by finger prick from patients for simultaneous measurement of vancomycin and creatinine, allowing for direct use in practice and proceeding a new era to implement patient-friendly DBS sampling at home. We performed a solid clinical validation according to the current international guidelines, using typical patients in a real-life clinical setting. In this way, we have been able to simulate implementation in real practice. Most of the patients (58%) preferred a finger prick over a venepuncture. We hypothesize that the benefit of a finger prick will increase if it is implemented in the OPAT setting, eliminating the need for a patient to travel to a phlebotomy facility. The use of vancomycin DBS in OPAT setting is currently being studied in a multicentre randomized controlled trial (ADVANCED OPAT, NCT06283433).

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Transparency declarations

None to declare.

Author contributions

M.H.: conceptualization, methodology, formal analysis, investigation, writing—original draft, writing—review and editing, S.B.: conceptualization, formal analysis, writing—review and editing, H.A.W.v.O.: conceptualization, writing—review & editing, J.v.O.: conceptualization, writing—review and editing, B.C.P.K.: conceptualization, writing—review and editing, B.C.M.d.W.: conceptualization, writing—review and editing, supervision.

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