Dried Blood Spot Analysis for Therapeutic Drug Monitoring of Clozapine

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ABSTRACT

Background: Schizophrenia is a psychiatric disorder that affects approximately 0.4%–1% of the population worldwide. Diagnosis of schizophrenia is based primarily on *Diagnostic and Statistical Manual of Mental Disorders,* Fifth Edition *(DSM-5)* criteria. Clozapine is an antipsychotic drug that is mainly used in the treatment of schizophrenia patients who are refractory or intolerant to at least 2 other antipsychotics. Due to the high variability in pharmacokinetics of clozapine, therapeutic drug monitoring (TDM) is highly recommended for clozapine therapy.

Objective: To develop and clinically validate a novel sampling method using dried blood spot (DBS) to support TDM of clozapine and norclozapine.

Methods: From June 2014 to September 2014, 15 schizophrenia patients (18–55 years) treated with clozapine were included. Plasma, DBS samples made from venous samples (VDBS), and finger prick DBS (DBS) samples were obtained before administration and 2, 4, 6, and 8 hours after clozapine intake. The study was repeated in 6 Russian patients for external validation. Passing-Bablok regression and Bland-Altman analysis were used to compare the DBS, VDBS, and plasma results for clozapine and norclozapine.

Results: The DBS validation results showed good linearity over the concentration time curve measured for clozapine and norclozapine. The accuracy and between- and within-day precision variation values were within accepted ranges. Different blood spot volumes and hematocrit values had no significant influence on the results. The DBS samples were stable at 20°C and 37°C for 2 weeks and at −20°C for 2 years. The mean clozapine and norclozapine DBS/plasma ratios were, respectively, 0.80 (95% CI, 0.76 to 0.85) and 1.063 (95% CI, 1.027 to 1.099) in Dutch patients. The mean clozapine DBS/DPS ratio in Russian patients was 0.70 (95% CI, 0.64 to 0.76).

Conclusion: DBS analysis is a reliable tool for blood sampling and performing TDM of clozapine and norclozapine in daily practice and substantially extends the opportunities for TDM of clozapine.

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Clozapine is an atypical antipsychotic drug used mainly in schizophrenia patients who are refractory or intolerant to at least 2 other antipsychotics, 1 of which is a second generation antipsychotic.[1,2] The drug has proven value in the treatment of therapy-resistant patients and causes less extrapyramidal side effect burden compared to typical antipsychotics.[3,4] Therapeutic drug monitoring (TDM) is highly recommended during clozapine therapy in order to improve efficacy and prevent dose-dependent toxic adverse events.[2,5–7] There seems to be a correlation between plasma clozapine concentration and the therapeutic effect of the drug, although there is still a notable difference in response between patients at any drug concentration.[8] Clozapine also displays large interindividual differences in the dose/plasma concentration ratio among patients.[5,9] Serum concentrations obtained when equal dosages of clozapine are given to different patients may vary by a factor of 6, caused by differences between patients in bioavailability and rate of metabolism.[10,11]

Clozapine is almost completely metabolized in the liver by cytochrome P450 (CYP) isoenzymes, mainly CYP1A2. Various genetic, environmental, and comorbidity factors, such as infections, affect plasma clozapine concentration by modulating CYP activity.[12–14] In addition, some individual factors such as age, gender, body weight, smoking habits, caffeine intake, and ethnicity contribute to the high interindividual variability in plasma clozapine concentrations.[11,15,16]

Regular TDM of clozapine is based on a venous sampling method. To extend the opportunities of TDM for patients in the ambulatory setting, dried blood spot (DBS) sampling may provide an alternative. DBS sampling has several advantages, including a less invasive and simplified sampling method and a smaller sampling volume. Also, to obtain DBS samples, no qualified personnel are required. The increased sample stability and the simple storage and transport requirements make the DBS method widely applicable.[17–19] A drawback for a widespread use of DBS as routine sampling method may be the influence of the hematocrit on the results.[20,21]

Clinical Points

- Dried blood spot (DBS) sampling of clozapine is a reliable, less invasive, and simpler tool for TDM of clozapine in daily practice.
- DBS sampling of clozapine substantially extends the opportunities of TDM of clozapine independent of the setting, inpatient or outpatient, and independent of the infrastructure level of the country.

The purpose of this study was to develop and clinically validate a method for DBS analysis of clozapine and norclozapine and to test this method in daily practice in schizophrenia patients treated with clozapine.

MATERIALS AND METHODS

Patients

From June 2014 to September 2014, 15 schizophrenia patients (aged 18–55 years) treated with clozapine were recruited from GGZ institution Noord-Holland Noord, The Netherlands. Patients could participate if they were on a stable dose of clozapine for at least 2 weeks and had Caucasian ethnicity. Information about comedication, caffeine intake, and smoking habits was recorded. Patients who started treatment or had dosage adjustments in their comedication known to influence clozapine pharmacokinetics (amiodarone, cimetidine, fluoroquinolones, fluvoxamine, furafylline, interferon, methoxsalen, mibefradil, insulin, methylcholanthrene, modafinil, nafcillin, β-naphthoflavone, or omeprazole) within 3 days before sampling were excluded from participation.

The study procedures were reviewed and approved by the local ethics committee. Patients were included after providing written informed consent.

Sampling

The patients received clozapine as part of their regular treatment. The day before sampling, participants preserved half of their evening dose of clozapine for intake the next morning, to enable sampling during daytime. The venous blood samples from which a DBS was made (VDBS) and finger prick (capillary DBS) samples were obtained before administration of clozapine and 2, 4, 6, and 8 hours after drug intake. For the capillary DBS samples, participants were pricked in the finger with a lancet (BD Microtainer contact-activated lancet; 2.0 mm × 1.5 mm). The first drop of blood was wiped off and discarded. The next drops were dropped directly onto a Whatman FTA DMPK-C Card.[22] Dried blood spots from venous blood were prepared by pipetting 50 µL of venously drawn blood onto Whatman FTA DMPK-C Card (VDBS). The remaining venous drawn blood was centrifuged at 3,000 rpm for 5 minutes to obtain plasma. The plasma was stored at −20°C until analysis. The DBS and VDBS samples were left to dry at room temperature and were stored in sealed plastic bags with desiccant sachets at −20°C until analysis.

Table 1. Patient Characteristics in the Dutch and Russian Studies

Parameter	Dutch Patients	Russian Patients
Age, mean (SD), y	44 (11)	35 (14)
Male, n	12	3
Female, n	3	1
Body weight, mean (SD), kg	90 (13)	74 (11)
Height, mean (SD), m	1.81 (0.07)	1.71 (0.095)
Clozapine dose, mean (range), mg	287 (75–800)	150 (100–175)
Smoker, n	10	1
Nonsmoker, n	5	3
Comedication affecting plasma clozapine level, n		
Fluvoxamine	3	0
Omeprazole	3	0
Insulin	1	0
Sodium valproate	1	0

Russian External Validation Study

To examine whether it is feasible to perform TDM of clozapine with DBS samples taken abroad and analysis performed in The Netherlands, the study was repeated with 6 Russian patients from the Mental Health Research Institute, Tomsk, Russian Federation. The Russian study procedure was reviewed and approved by the local ethics committee. The inclusion criteria for the validation study were equivalent to the Dutch inclusion criteria, except for the Caucasian ethnicity. The venous blood and DBS samples were obtained before administration of clozapine and 1, 2, 3, 4, 6, 8, and 12 hours after drug intake. For obtaining plasma, DBS, and VDBS samples, the same procedures and equipment were used as in the Dutch study. To simplify the transport of the plasma samples to The Netherlands, dried plasma spot (DPS) samples were prepared by pipetting 50 µL of the prepared plasma onto Whatman FTAtm DMPK-C Cards. The DBS, VDBS, and DPS samples were left to dry at room temperature and stored in sealed plastic bags with desiccant at −20°C until transport to The Netherlands by express mail.

Analysis

To analyze the (V)DBS samples of clozapine and norclozapine, an 8-mm disc was punched out of each blood spot. A 400-µL extraction solution (20 µg/L [13C,2H3]-clozapine in a mixture of methanol and water [9:1, v/v]) was added to the punches, and the samples were vortexed for 1 minute. The samples were extracted from the punch by sonification (47 kHz) for 10 minutes. Afterward, the samples were vortexed for 1 minute, transferred into polypropylene vials, and subsequently centrifuged for 5 minutes at 11,000 rpm. Five µL of the clear upper layer was analyzed using a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method. Plasma samples were analyzed as routinely using a validated LC-MS/MS method.

DBS and DPS Method Validation

The DBS analytic method was validated based on the FDA guidance for bioanalytical method validation.[23] An 8-point calibration curve was prepared by mixing venous whole blood with clozapine or norclozapine stock solution

to obtain the desired concentrations. The DBS samples for the calibration curve were prepared by pipetting 50 µL of the spiked blood samples onto DBS paper. Linearity of the standard curve was assessed with $1/\chi^2$ weighting over a clozapine and norclozapine concentration range of 15 µg/L to 1,500 µg/L. Within-run and between-run accuracy and precision were evaluated for 4 quality control concentrations: 15 µg/L (lower limit of quantification [LLOQ]), 75 µg/L (low), 600 µg/L (medium), and 1,200 µg/L (high). Each validation level was analyzed in 5-fold on 3 consecutive days. The maximal tolerated bias was 20% for the LLOQ and 15% for the other validation levels. The recovery and the matrix effect of clozapine and norclozapine were determined as described by Li and Tse[17] and Vu et al.[20] Stability of the DBS samples was assessed by storing low and high concentrations at 20°C and 37°C for 14 days and at −20°C for 2 years. Stability was evaluated by comparing the analytic results with the nominal concentrations. Further, the impact of the hematocrit value and blood spot volumes on assay accuracy and precision was examined. DBS samples with hematocrit values of 20%, 25%, 30%, 35%, 40%, and 45% and blood spot volumes of 30, 50, 70, and 90 µL were assessed for this evaluation. For the method validation, the blood spot volume was standardized at 50 µL and the hematocrit value at 35%.

For the validation of the DPS method, plasma samples were prepared by mixing blank plasma with clozapine stock solution to obtain the desired concentrations. The DPS samples were prepared by pipetting 50 µL of plasma onto the DBS cards. Linearity of the calibration curve was assessed with $1/\chi^2$ weighting over a clozapine and norclozapine concentration range of 15 µg/L to 1,500 µg/L.

Statistics

In the method validation, bias was defined as the difference between the analytic result and the nominal concentration, expressed as a percentage. The DBS method was clinically validated by comparing the concentrations of clozapine and norclozapine in the (V)DBS samples with the concentrations measured in plasma/DPS using Passing-Bablok regression and Bland-Altman analysis using Excel 2003 expanded with the software tool Analyze-it version 2.30 (Analyze-it Software, Ltd). Conversion factors for DBS to plasma were calculated using the slope and intercept of the regression lines found with Passing-Bablok regression.

Table 2. Summarized Results of the Validation of DBS Analysis of Clozapine and Norclozapine

	Validation Level (n = 5)			
Validation Criterion	LLOQ	Low	Medium	High
Clozapine				
Nominal concentration (µg/L)	15	74.8	598	1,197
Reproducibility[a]				
Accuracy, bias (%)	0.6	2.8	4.8	7.7
Within-day precision, CV (%)	5.0	3.9	4.1	5.3
Between-day precision, CV (%)	0.0	2.5	3.3	4.1
Matrix effect (%)		105.2	96.1	101.6
Recovery (%)		98.3	102.0	96.1
Effect of hematocrit, range of bias (%)[b]		−16.3 to 15.0		−13.7 to 14.1
Effect of blood volume, range of bias (%)[c]		−2.6 to 5.5		0.6 to 6.4
Stability				
14 days at 20°C, bias (%)		−9.4		−5.9
14 days at 37°C, bias (%)		4.2		2.8
Stability				
2 years at −20°C, bias (%)		−11.1		−9.2
Norclozapine				
Nominal concentration (µg/L)	15.1	75.3	602	1,205
Reproducibility[a]				
Accuracy, bias (%)	−8.9	4.3	2.9	5.3
Within-day precision, CV (%)	4.5	5.7	3.2	5.4
Between-day precision, CV (%)	2.6	0.3	2.9	0.0
Matrix effect (%)		106.0	100.2	99.6
Recovery (%)		97.5	98.9	
Effect of hematocrit, range of bias (%)[b]		−14.7 to 12.4		−14.7 to 13.3
Effect of blood volume, range of bias (%)[c]		−0.4 to 4.2		−1.7 to 6.1
Stability				
14 days at 20°C, bias (%)		−2.0		−1.6
14 days at 37°C, bias (%)		1.7		2.3
Stability				
2 years at −20°C, bias (%)		2.8		−0.8

[a]Data are from 3 separate validation days.
[b]Comparison with sample of standardized hematocrit (35%).
[c]Comparison with sample of standardized blood spot volume (50 µL).
Abbreviations: CV = coefficient of variation, DBS = dried blood spot, LLOQ = lower limit of quantification.

RESULTS

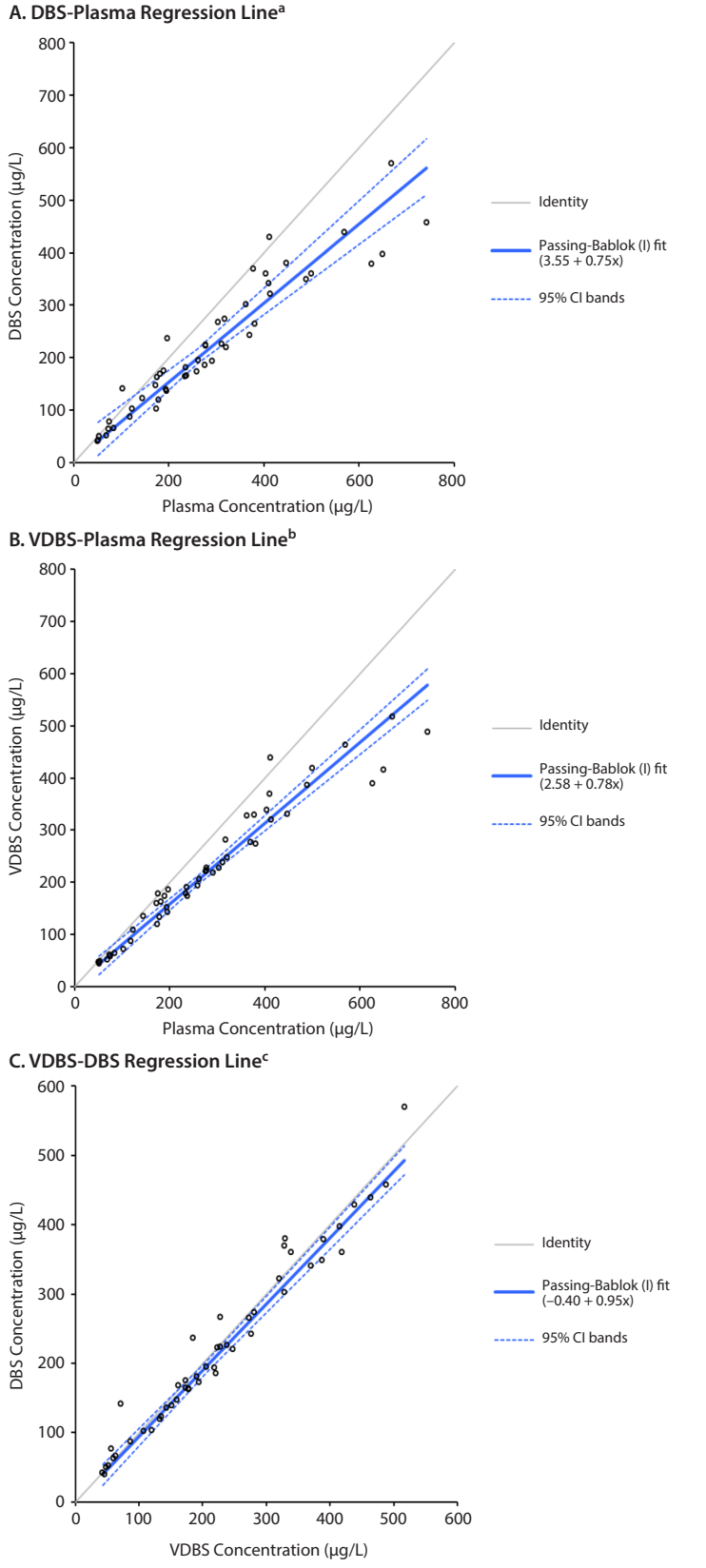
Patients

Fifteen Dutch patients (3 female/12 male, mean age of 44 years) participated in this study. The clozapine dosage of the patients varied between 75 mg and 800 mg once daily. Eight patients used comedication that could affect the clozapine concentration. Ten patients smoked, and all 15 patients drank caffeine-containing beverages (Table 1).

DBS Method Validation

The DBS assay method showed good linearity over the concentration range. The clozapine regression equation was $(0.00129 \pm 0.000696) + (0.00185 \pm 0.000019) \times$ response clozapine. The norclozapine regression equation was $(0.00273 \pm 0.000344) + (0.000835 \pm 0.000009) \times$ response norclozapine. The pooled correlation coefficient in both cases was 0.99. The mean measured concentration was between 100.6% and 107.7% of the nominal concentration for clozapine and between 91.1% and 105.3% for norclozapine. Within-run and between-run accuracy and precision coefficients of variation were, respectively, between 3.9% and 5.3% and 0.0% and 4.1% for clozapine and between 3.2% and 5.7% and 0.0% and 2.9% for norclozapine. They all fitted well within the accepted ranges. The recovery was between 96.1% and 102% for clozapine and between 97.5% and

Figure 1. Clinical Validation of (V)DBS Analysis by Passing-Bablok Regression Between Clozapine Concentrations in DBS, VDBS, and Plasma



[a]Slope = 0.75 (95% CI, 0.67 to 0.83); intercept: 3.55 (95% CI, −7.25 to 20.1).
[b]Slope = 0.78 (95% CI, 0.73 to 0.82); intercept: 2.58 (95% CI, −6.22 to 10.13).
[c]Slope = 0.95 (95% CI, 0.91 to 1.00); intercept: −0.40 (95% CI, −9.00 to 6.64).
Abbreviations: DBS = dried blood spot, VDBS = venous dried blood spot.

98.9% for norclozapine and fitted both within the required range of 85%–115%. The bias caused by the use of variable matrices was between 96.1% and 105.1% for clozapine and between 99.6% and 106.0% for norclozapine. No significant clozapine or norclozapine variation was observed after storage of the DBS samples at 20°C and 37°C for 14 days and at −20°C for 2 years. The bias in hematocrit values from 20% to 50% ranged from −16.3% to 15.0% and −13.7% to 14.1% for low and high clozapine concentrations, respectively, when compared with samples with a standardized hematocrit of 35%. The bias in hematocrit values from 20% to 50% for low and high norclozapine concentrations ranged from −14.7% to 12.4% and −14.7% to 13.3%, respectively. The bias caused by variation in blood spot volume between 30 µL and 90 µL ranged from −2.6% to 5.5% for low clozapine levels and from 0.6% to 6.4% for high clozapine levels. For norclozapine, the bias caused by variation in blood spot volume between 30 µL and 90 µL ranged from −0.4% to 4.2% for low levels and from −1.7% to 6.1% for high norclozapine levels (Table 2).

Comparisons of DBS, VDBS, and Plasma Analyses

One hundred forty-seven samples from 14 patients were included in the clinical validation of the DBS analysis. In Figure 1, the results of the Passing-Bablok regression between (V)DBS samples and plasma samples are shown. The slope of the regression line for the clozapine concentration between plasma and DBS samples was 0.75 (95% CI, 0.67 to 0.83); between VDBS samples and plasma, 0.78 (95% CI, 0.73 to 0.82); and between VDBS and DBS samples, 0.95 (95% CI, 0.91 to 1.00). The intercepts were 3.55 µg/L (95% CI, −7.25 to 20.1), 2.58 µg/L (95% CI, −6.22 to 10.13), and −0.40 µg/L (95% CI, −9.00 to 6.64), respectively. The slope of the regression line for the norclozapine concentration between plasma and DBS samples was 1.07 (95% CI, 0.99 to 1.17); between VDBS samples and plasma, 1.13 (95% CI, 1.05 to 1.20); and between VDBS and DBS samples, 0.97 (95% CI, 0.91 to 1.02). The intercepts were −2.30 µg/L (95% CI −13.57 to 6.66), −1.59 µg/L (95% CI, −9.20 to 8.16), and −2.09 µg/L (95% CI, −8.97 to 7.13).

Bland-Altman analysis showed that the mean clozapine concentration ratio in DBS versus plasma was 0.803 (95% CI, 0.760 to 0.845). In 4 cases, the DBS/plasma ratio was larger than 1. The mean clozapine ratio between VDBS and plasma was 0.805 (95% CI, 0.779 to 0.832) and between VDBS and DBS samples was 0.999 (95% CI, 0.949 to 1.049). The 95% limits of agreement

are shown. Less than 5% of the values are out of these ranges (Figure 2).

The mean norclozapine concentration ratio found with Bland-Altman analysis in DBS versus plasma was 1.063 (95% CI, 1.027 to 1.099). The mean norclozapine ratio between VDBS and plasma was 1.121 (95% CI, 1.092 to 1.149), and between VDBS and DBS samples, 1.060 (95% CI, 1.038 to 1.082). The 95% limits of agreement are shown. Less than 5% of the values are out of these ranges (Figure 3).

Russian External Validation Study

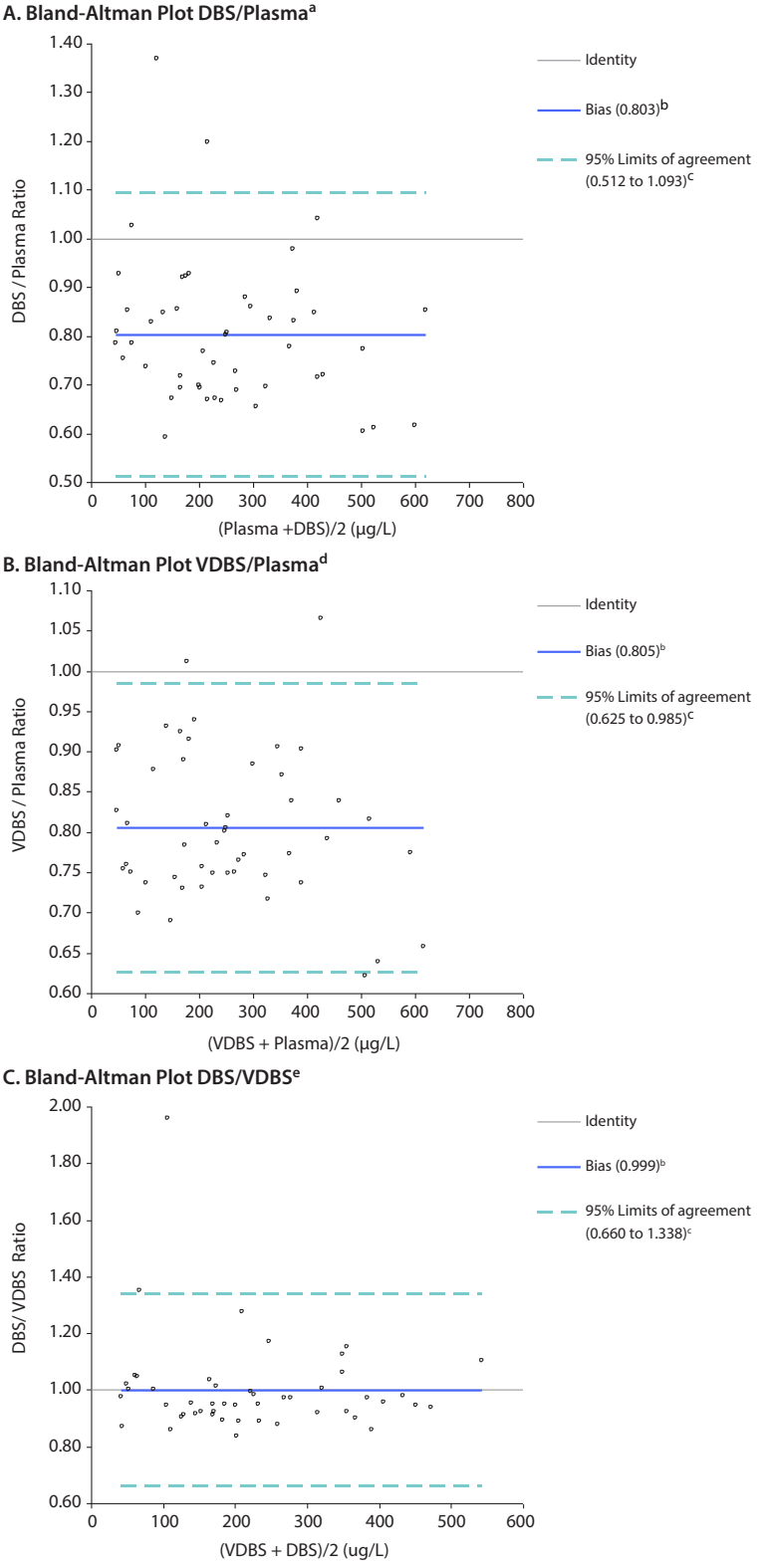
Six patients (3 female/3 male) participated in the control study. The 75 samples of 4 patients (1 female/3 male) were included in the control study in the end. The samples from 2 patients were excluded; 1 patient had not taken clozapine, and the spots from the other patient were too small for reliable measurement. The baseline characteristics of these 4 patients are presented in Table 1. In Figure 4, the results of the Passing-Bablok regression between DBS and DPS samples are shown. The slope of the regression line for the clozapine concentration between DBS and DPS samples was 0.75 (95% CI, 0.65 to 0.86); between VDBS samples and DPS samples, 0.75 (95% CI, 0.66 to 0.83); and between VDBS and DBS samples, 1.03 (95% CI, 0.95 to 1.08). The intercepts were −5.05 µg/L (95% CI, −12.76 to 0.76), −1.39 µg/L (95% CI, −10.61 to 2.47), and −2.71 µg/L (95% CI, −6.33 to 1.03).

Bland-Altman analysis showed a mean clozapine concentration ratio in DBS versus DPS samples of 0.70 (95% CI, 0.644 to 0.759). One DBS/DPS ratio was larger than 1. The mean ratio between VDBS and DPS was 0.718 (95% CI, 0.679 to 0.757) and between VDBS and DBS samples was 1.040 (95% CI, 0.990 to 1.091). The 95% limits of agreement are shown. Less than 5% of the values are out of these ranges (Figure 4).

DISCUSSION

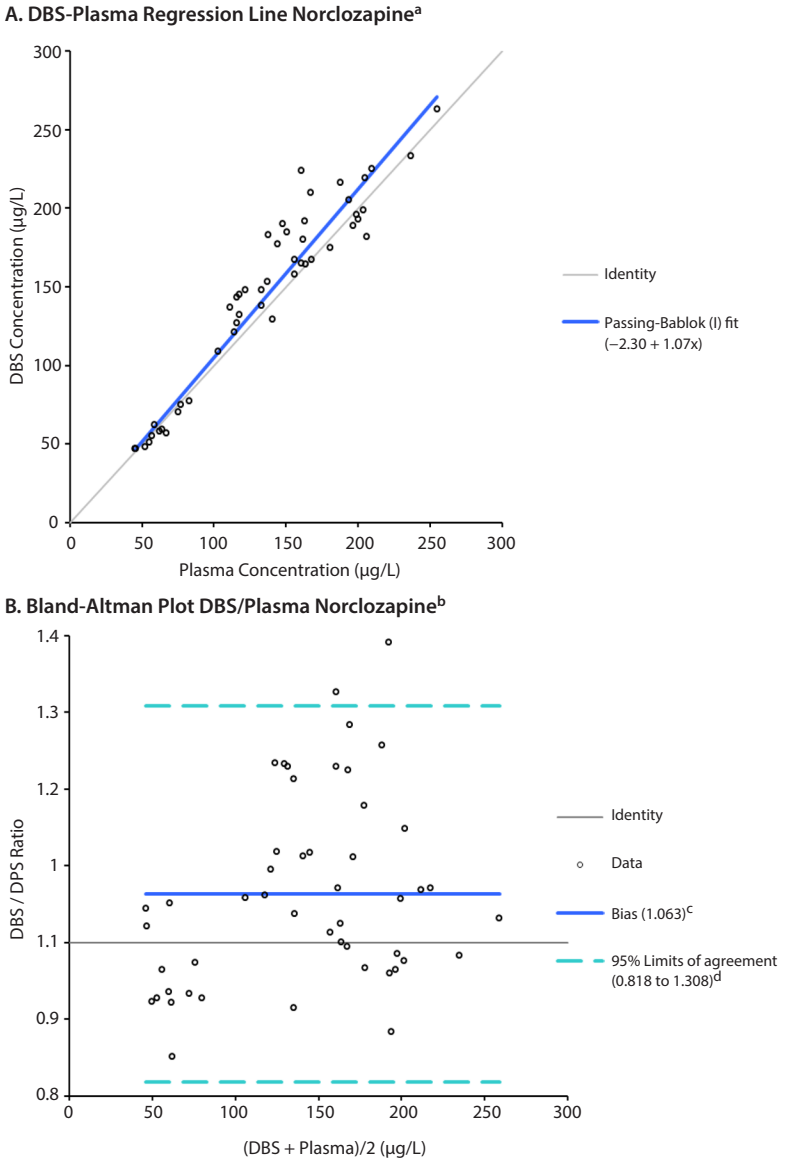
In this study, a novel method using DBS sampling for therapeutic drug monitoring of clozapine and norclozapine was developed, clinically validated, and tested in daily practice. An external validation of the method in Russian patients has also been conducted. The results indicate that DBS sampling of clozapine is a reasonable alternative to the conventional venous sampling. Further, we demonstrated that it is possible to perform TDM of clozapine with DBS samples taken abroad and analyzed in The Netherlands.

Figure 2. Bland-Altman Plot of Clozapine Concentration Ratios in DBS and VDBS Samples Versus Plasma



[a]Geometric mean concentration ratio in DBS compared with plasma was 0.80 (95% CI, 0.760 to 0.845). [b]Mean ratio (%). [c]95% CI of mean ratio (mean ratio ± 1.96 × standard error of the ratio). [d]Geometric mean concentration ratio in VDBS compared with plasma was 0.81 (95% CI, 0.779 to 0.832). [e]Geometric mean concentration ratio in DBS compared to VDBS was 0.999 (95% CI, 0.949 to 1.049).
Abbreviations: DBS = dried blood spot, VDBS = venous dried blood spot.

Figure 3. Clinical Validation of (V)DBS Analysis by Passing-Bablok Regression and Bland-Altman Plot Between Norclozapine Concentrations in DBS, VDBS, and Plasma



[a]Slope = 1.07 (95% CI, 0.99 to 1.17); intercept: −2.30 (95% CI, −13.57 to 6.66).
[b]Geometric mean concentration ratio in DBS samples compared with plasma was 1.063 (95% CI, 1.027 to 1.099).
[c]Mean ratio (%).
[d]95% CI of mean ratio (mean ratio ± 1.96 × standard error of the ratio).
Abbreviations: DBS = dried blood spot, VDBS = venous dried blood spot.

Previous studies of the DBS method with other drugs revealed several technical factors that can influence the analytic results for DBS samples.[17–19,24] Two important factors are the blood spot volume and the hematocrit value. For the analysis of clozapine and norclozapine, variations in blood spot volume between 30 µL and 90 µL had minor influence on the analytic results. Biases were within 10% for both low and high clozapine and norclozapine concentrations. The hematocrit value had more impact on the DBS results. The biases measured in low clozapine concentration levels ranged from −16.3% to 15.0% and at very low hematocrit level fell out of the accepted range of 15%. Hematocrit levels found in psychiatric and schizophrenia patients in literature varied between 39.9% (± 4.2) and 42.3 (± 4) and fall within the range of hematocrit values validated by us.[25,26] It is very unlikely that the hematocrit value of one of the included patients was below 20%. The biases measured in high clozapine concentration and low and high norclozapine levels fell within the accepted range of 15%. The standardization of hematocrit at 35% during DBS validation seems acceptable.

Another limitation is the observed difference between clozapine concentrations in whole blood and plasma. In this study, the mean concentration of clozapine was lower in whole blood than in plasma. This effect is also described in the literature. Patteet et al described a validation of the DBS method for clozapine but tested the method in only 1 patient at 1 time point. Nevertheless, the clozapine concentration measured in the DBS sample of this patient was also lower than the corresponding plasma concentration.[27] Also, studies by Goossen et al[28] and Flanagan et al[29] describe 27% and 10%, respectively, higher clozapine concentrations in plasma when compared to whole blood concentrations. Goossen et al, however, did not measure norclozapine levels. Cheng et al present a mean clozapine concentration ratio between blood and plasma of 0.87 that is in agreement with the ratio of 0.80 measured in our study, but in this study also, no norclozapine levels were measured.[30]

The mean clozapine concentration ratio between VDBS and plasma of 0.81 almost equals the DBS/plasma ratio. It is likely that the difference between DBS and plasma levels is caused by the presence of red blood cells. This may dilute the concentration of clozapine, which is mostly distributed in plasma. The ability of clozapine to bind to plasma proteins and to or into red blood cells also differs, and so does, therefore, the distribution of clozapine between whole blood and plasma. This results in different clozapine concentrations between whole blood and plasma.[19,24,31]

Despite the higher mean clozapine concentration in plasma than in whole blood, there were 4 sample pairs in which the opposite effect was observed. Three of these pairs had been sampled in the absorptions phase ($t = 2$ hours) of clozapine. The other sample pair was taken at $t = 6$ hours, but the observed ratio between these samples was only slightly higher than 1, (ie, 1.027). The higher DBS clozapine levels in the

absorption phase are possibly caused by differences in clozapine concentration between venous blood and capillary blood taken from the finger. Capillary blood is a mixture of arterial and venous blood and, especially when the finger is warmed, mostly resembles arterial blood. In the absorption phase, before equilibrium is reached, this arterial component and the 2 different sampling spots, capillary and venous, may cause differences between DBS and plasma concentrations.[24]

To compare DBS and plasma clozapine concentrations for TDM, we calculated a conversion factor of 1.47 (1/0.68). In our calculation, samples taken on $t = 2$ were excluded.

The mean concentration of norclozapine was, in contrast to clozapine, approximately 6% higher in whole blood than in plasma. This effect is probably caused by the difference in protein binding between clozapine and norclozapine. Norclozapine binds less to plasma proteins than clozapine, making more norclozapine available for partition into red blood cells.[29]

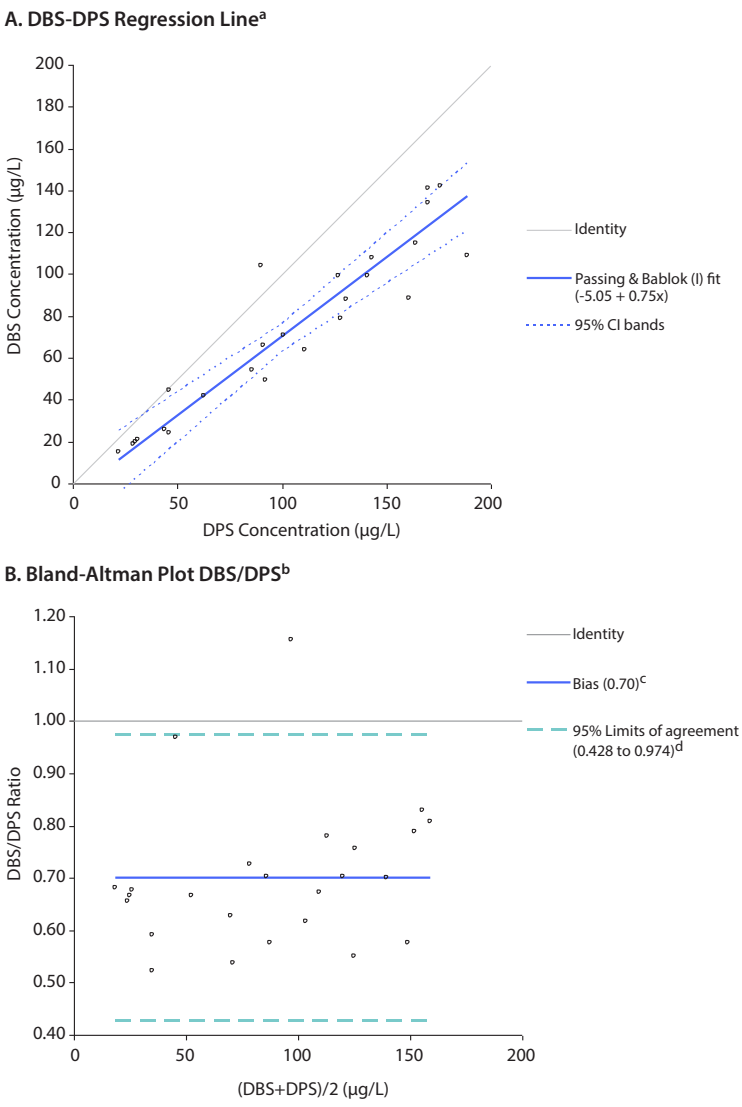
To compare DBS and plasma norclozapine concentrations for TDM, no conversion factor is needed. The 95% of the slope of the regression line found with Passing-Bablok regression encloses 1 (ie, 95% CI, 0.95 to 1.14), and the 95% CI of the intercept encloses zero (ie, 95% CI, −11.64 to 11.10). In our calculation, samples taken on $t = 2$ were excluded.

The results of the Russian external validation study correspond with the outcomes of the Dutch study. The mean clozapine concentration ratio between blood and plasma found in the Russian samples was, however, lower than in the Dutch study, ie, 0.70 (95% CI, 0.64 to 0.76). The difference between the Russian and Dutch mean DBS/plasma clozapine ratio is significant and presumably caused by the small number of patients included in the Russian control study. Also, the use of DPS instead of liquid plasma may have had an effect.

The mean clozapine concentration ratio between VDBS and DPS was 0.72, which reconfirms that the difference between DBS and plasma levels (DPS) is caused by the use of different matrices.

Several studies indicate that DBS sampling is an easier and more patient-friendly method for TDM.[17,32] Clozapine DBS samples can be taken by patients at home or by their caregivers. After drying, the samples can be mailed to a laboratory. For schizophrenia patients, this saves traveling time to a clinic, and sampling can take place at convenient times. This is especially relevant for countries where laboratory facilities are sparsely available.

Figure 4. Clinical Validation of DBS Analysis by Passing-Bablok Regression and Bland-Altman Plot in the Russian Validation Study



[a]Slope = 0.75 (95% CI, 0.65 to 0.86). Intercept: −5.05 (95% CI, −12.76 to 0.76).
[b]Geometric mean concentration ratio in DBS samples compared with DPS was 0.701 (95% CI, 0.644 to 0.759).
[c]Mean ratio (%).
[d]95% CI of mean ratio (mean ratio ± 1.96 × standard error of the ratio).
Abbreviations: DBS = dried blood spot, DPS = dried plasma spot.

In addition, the stability of DBS samples at room temperature and the minimal biohazard risks during shipping make TDM of clozapine with DBS also feasible for countries that are devoid of the equipment to measure clozapine levels effectively and for low or middle income countries with less developed or less reliable infrastructure.[17,32,33]

In conclusion, in this study in 14 Dutch and 4 Russian patients with schizophrenia, a DBS analysis method for clozapine TDM was successfully developed and clinically validated. An external validation was carried out to demonstrate its appropriateness. Clozapine and norclozapine concentrations in both DBS and VDBS samples showed good correlation with the corresponding plasma concentrations. There is preliminary evidence that samples should not be drawn during the absorption phase of clozapine. DBS is a reliable tool for TDM of clozapine

in daily practice that substantially extends the opportunities of TDM of clozapine independent of the setting, inpatient or outpatient, and independent of the infrastructure level of the country. In short: it makes clozapine TDM available worldwide.

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