CHAPTER 8

Dried blood spot sampling of nilotinib in patients with chronic myeloid leukemia: a comparison with venous blood sampling

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ABSTRACT

Objectives
To compare nilotinib concentrations obtained by venous blood sampling and dried blood spot (DBS) in patients with chronic myeloid leukemia (CML). It was investigated how to predict nilotinib plasma levels on the basis of DBS.

Methods
Forty duplicate DBS and venous blood samples were collected from 20 patients. Capillary blood was obtained by finger prick and spotted on DMPK-C Whatman sampling paper, simultaneously with venous blood sampling. Plasma concentrations were predicted from DBS concentrations using three methods: (1) individual and (2) mean hematocrit correction and (3) the bias between plasma and DBS concentrations. Results were compared using Deming regression and Bland-Altman analysis.

Key Findings
Nilotinib plasma concentrations ranged from 376 to 2663 µg/l. DBS concentrations ranged from 144 to 1518 µg/l. The slope was 0.56 (95% CI, 0.51 to 0.61) with an intercept of –41.68 µg/l (95% CI, –93.78 to 10.42). Mean differences between calculated and measured plasma concentrations were –14.3% (method 1), –14.0% (method 2), and –0.6% (method 3); differences were within 20% of the mean in 73%, 85% and 80% of the samples, respectively. The slopes were respectively 0.96 (95% CI, 0.86 to 1.06), 0.95 (95% CI, 0.86 to 1.03), and 1.00 (95% CI, 0.91 to 1.09).

Conclusions
Plasma concentrations of nilotinib could be predicted on the basis of DBS. DBS sampling to assess nilotinib concentrations in CML patients seems a suitable alternative for venous sampling.
BACKGROUND

Nilotinib is a second generation tyrosine kinase inhibitor (TKI) registered for the treatment of chronic myeloid leukemia (CML) [1]. Variability in the pharmacokinetics of nilotinib influences the blood concentration. Interpatient variability of nilotinib bioavailability has been reported to be high (62%)[2]. Drug-drug interactions [3] and food [4] contribute to this variability. In addition, patients’ drug intake behavior, for example skipping and forgetting doses as well as variation in the interval between intakes, also results in variable nilotinib concentrations [5]. Lower trough levels of nilotinib may result in treatment failure [2], whereas high trough levels of nilotinib may cause side effects [2, 6]. Therapeutic drug monitoring (TDM) may be useful to identify the cause of a lack of response to the treatment, which next to subtherapeutic drug levels may also be caused by resistance of leukemic cells to nilotinib due to mutations or other mechanisms [7]. In addition, patients with side effects and high nilotinib concentrations may have their dose reduced which can improve tolerability. The target trough plasma concentration (C_min) after administration of the initial 600 mg/day dose of nilotinib is suggested to be above 500 µg/l [2].

Blood samples for TDM are usually obtained by means of venepuncture by (para-)medical personnel. Compared to conventional venous blood sampling, dried blood spot (DBS) sampling is a convenient and simple sampling method with better patient comfort and lower costs [8]. With DBS sampling capillary blood is obtained by finger prick with an automatic lancet. This type of sampling is less invasive than venepuncture and requires a small volume of blood. DBS sampling can be performed by the patients themselves at any moment, which is convenient in the case of long distances to clinical practices [8, 9]. Moreover, it simplifies area under the curve (AUC) measurements as outpatients can be instructed to perform finger pricks over a time range at their homes. In addition, in countries where laboratory services are more centralized, DBS sampling may be a solution, because they can easily be sent by mail.

The impact of the hematocrit (Hct) level is a challenge for DBS sampling and can be divided into an analytical and a physiological aspect [8-10]. Blood with higher Hct will spread less easily on the DBS sampling paper resulting in the measurement of a higher concentration when a fixed diameter punch is taken compared to blood with lower Hct. The physiological aspect of
the Hct concerns the correlation between the nilotinib concentration measured in DBS and the plasma concentration [10].

DBS sampling of venous blood has been shown to be a feasible method of TKI blood testing in CML patients [11, 12]. To our knowledge, this is the first study of DBS sampling of nilotinib in daily clinical practice to evaluate the relationship between blood levels determined using DBS sampling and values conventionally obtained using venous blood sampling. The aim of this study was to compare the results of nilotinib plasma levels obtained by venous sampling and DBS, in CML patients. It was investigated how to predict nilotinib plasma levels on the basis of DBS.

**METHODS**

**Study design**
A validation study was conducted in the period February – September 2013 at VU University Medical Center (VUmc) in Amsterdam, the Netherlands. DBS and venous blood samples were collected simultaneously from patients. The study was approved by the ethics committee of VUmc and was conducted in accordance with the Declaration of Helsinki and its amendments and the Dutch Medical Research Involving Human Subjects Act (WMO). Written informed consent was obtained from each participating patient.

**Patients**
The study population consisted of adult patients (18 years or older) with CML on treatment with nilotinib. Data on date of birth, gender and nilotinib dose were documented. Patients were allowed to participate more than once. Patient data were processed anonymously.

**Sample collection**
Forty sets of DBS and venous blood samples were collected simultaneously. Five milliliters of lithium-heparin venous blood samples was obtained by venepuncture. The venous blood samples were centrifuged, and plasma was stored at −20 °C until analysis. DBS samples were taken within ten minutes after venepuncture by a trained nurse. DBS samples were obtained
by finger prick using an automatic lancet device. The first drop was discarded, and the next drops were collected to fill two 8-mm premarked circles on the sampling paper (Whatman™, FTA™ DMPK-C (GE Healthcare), WB129243, obtained from VWR International BV, Amsterdam, the Netherlands). The DBS samples were allowed to dry 3 h at room temperature and packed in sealable plastic minibags for transportation (at room temperature within the hospital building from the outpatient clinic to the laboratory). On arrival, the DBS samples were visually inspected on complete, homogenous and symmetric fullness of the 8-mm circle and presence of a dark-red color on both sides of the paper. The DBS samples were stored at 4 °C until analysis, which was performed within 6 months after entry. The long-term stability of nilotinib in DBS has been established to be at least 7 months. Nilotinib concentrations were analyzed in duplicate to determine the repeatability coefficient of variation.

**Bioanalytical validation**

The bioanalytical DBS method was validated according to FDA guidelines [13] and internal bioanalytical method validation guidelines [14-16]. To ensure the specificity and selectivity, cross-talk, carry-over and the contribution of matrix effect as described by Matuszewski et al [17, 18] on the method performance were determined.

**Chemicals and reagents**

Nilotinib and the internal standard nilotinib-d4 were purchased from ITK Diagnostics (Uithoorn, the Netherlands). HPLC-grade methanol was supplied by Biosolve (Valkenswaard, the Netherlands). The analytical grade solvents, formic acid (98%) and ammonium acetate were obtained from Merck (Darmstadt, Germany). Ultrapure water was obtained in-house via a Millipore Synergy UV system (Molsheim, France). Whatman DMPK-C sampling paper was obtained from VWR International (Amsterdam, the Netherlands). Drug-free blood was supplied by different healthy volunteers at VUmc (Amsterdam, the Netherlands).

**Standard solutions**

Nilotinib stock solution was prepared in methanol and DMSO (9 : 1 v/v) at free base concentration of 2 mg/ml. The calibration standard curves were prepared by spiking Li-heparin drug-free human blood with a Hct of 0.40. To cover the therapeutic windows of nilotinib, a six-point calibration curve of 51-4100 µg/l was made in 10.0 ml drug-free human blood. The
lower limit of quantification (LLOQ) was prepared at the concentration level of 17 µg/l. Forty microliters from the spiked blood was applied to the DMPK-C sampling paper and left to dry for 3 h at ambient temperature. Quality Control (QC) samples were prepared in a similar manner from a second independent weighting of nilotinib. QC samples were made at three concentration levels; low, medium and high at 75, 754 and 3017 µg/l, respectively. After dryness, the DBS samples were stored in a sealbag with a desiccant at refrigerator temperature. The internal standard contains nilotinib-d4 in methanol at 500 µg/l.

**Sample preparation**

The analytical samples were obtained by punching a 8-mm-diameter paper disc from the center of the DBS using a hole puncher. The paper discs were collected into test microcentrifuge tubes. To the tubes, 200 µl of methanolic internal standard solution (containing 500 µg/l nilotinib-d4) was added. The samples were shaken for fifteen minutes and centrifuged at 18 000g for five minutes. The clear supernatant was transferred to autosampler vials, and 7.5 µl of the aliquot was injected onto an ultraperformance liquid chromatography–mass spectrometric detection system (LC-MS/MS). An ion chromatogram obtained from an extracted blank DBS sample with an overlay of standard 4 and a sample injection of LLOQ is shown in Figure 1.

![Figure 1](image)

**Figure 1** A chromatogram of an overlay of standard 4 of dried blood spot (DBS) nilotinib (1025 µg/l), LOQ (17 µg/l) and a DBS blank sample.
Chromatography and Mass spectrometry

The analysis of extracted DBS samples was performed on a Waters Acquity UPLC separations module (Waters Ltd., Milford, MA, USA) equipped with a degasser, column heater, binary pump and sample manager. The Acquity UPLC system was controlled by MassLynx software (V4.1). The temperature of the sample manager and column manager was 10 and 50 °C, respectively. Chromatographic separation was performed on a Waters XBridge RP18 column (2.5 µm, 2.1 x 75 mm) using deionized water (A) and methanol (B), both containing 0.1% of formic acid and 2 mM ammonium acetate. A stepwise gradient was used with a flow rate of 0.400 ml/min during the analysis. A 7.5 µl injection of samples aliquots was loaded and eluted on to the column using the following gradient: 0–0.5 min; 5% B, 0.5–1.0 min; 65% B, 1.0–1.9 min; 65% B, 1.9–2.0 min; 5% B.

For the mass spectrometric analysis, an Acquity TQ detector with electrospray ionization (ESI) operating in positive mode at 800 V was used for detection. The source and desolvation temperature were set at 140 and 400 °C, respectively. The desolvation gas flow was set at 700 l/hr, the cone gas was set at 50 l/hr and the collision gas (Argon) was set at flow rate of 0.23 ml/min. Transitions from parent to product ions were monitored using multiple reactions monitoring (MRM) mode. The ion transitions were mostly selected on their predominant fragmentation as observed in the product ion mass spectra. A second product ion of nilotinib was monitored and used as a qualifier ion. The major MS/MS transitions utilized for analysis were m/z 530.33→259.26 (quantifier) and m/z 530.33→289.18 (qualifier). For the internal standard, one transition was used, m/z 534.33→293.18.

Linearity

Linearity was tested by analyzing three sets of calibration standard curves. Peak area ratio of nilotinib to the internal standard was used for the construction of calibration standard curves using 1/x weighted linear regression. Data acquisition was performed by MassLynx software and processed using QuanLynx. Calibration standard curves were found to be linear over the concentration range of 17–4100 µg/l. The linearity of the method expressed as correlation coefficient ($r^2$) was above 0.999. The statistical analyses were accomplished using Regres (Boss software, Tilburg, the Netherlands); no significant lack of fit was shown by ANOVA test.
Within and between run accuracy and imprecision

Within and between run accuracy and imprecision of the method were assessed by replicate (n = 6) analysis of the QC samples (low, medium, high, LLOQ). Accuracy and imprecision were considered acceptable when deviations of the mean values of back-calculated concentration from the nominal concentrations were within 15% for QC samples and 20% for LLOQ. The within and between imprecision expressed as % coefficient of variation (% CV) was between 1.7% and 11.5%. The within and between accuracy was ranged between 98.7% and 112.8%. Therefore, the method presented acceptable accuracy and imprecision.

Matrix interferences, recovery, cross-reactivity and carry-over

Matrix interferences were evaluated by qualifying and quantifying the influence of the matrix to LC-MS/MS analysis. To qualify the matrix effect, six analyte-free DBS samples spotted with six different batches of Li-Heparin blood were prepared. The extracts of these DBS samples were injected, while at the same time, a concentration of the highest standard separately was infused. A chromatogram is shown in Figure 1. No significant ion enhancement or suppression was observed. Quantifying of the matrix effect was evaluated by blank DBS postspiked with the analytes and the internal standards compared to neat solution samples (Slope comparison). A difference in slope <10% was acceptable. A small effect of 1.5% was quantified. Recovery was calculated by comparing the slopes of the DBS standards to pre-extracted spike standards. The recovery was 87.6%. Cross-reactivity was measured with the highest concentration of nilotinib in combination with nilotinib-d4. A negligible effect of less than 0.1% was found on nilotinib. Carry-over was determined by injecting the highest standard followed by a blank sample without internal standard. This process was carried out in triplo. Carry-over was found to be 0.11% for nilotinib.

Stability

The autosampler stability and long-term stability of DBS samples were determined in triplicate at two concentration levels (QC samples low and high). Auto-sampler (10 °C) stability was assessed after 24 h and long-term stability (refrigerator 2–8 °C) after 7 months. Freshly prepared DBS standards and QC samples were compared with extracts samples after 24 h and 7 months. A % CV less than 4% for both QCs of the autosampler stability was found. After 7
months, a slight decrease of -3.6% was observed for nilotinib for the long-term stability. The autosampler stability and long-term stability were within the acceptable limits (<15%).

**Influence of hematocrit**

To estimate the effect of Hct on the accuracy of the quantification of nilotinib, QC samples were prepared with nilotinib concentrations of 75 and 3018 µg/l at various Hct values of 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50. Nilotinib concentrations were analyzed in triplicate to determine bias. No significant impact of Hct on the accuracy was observed (0.5–15.3%).

**Determination of nilotinib in plasma**

To each 50 µl of plasma sample, 200 µl methanolic internal standard solution containing 450 µg/l nilotinib-d4 was added. The samples were shortly vortexed and stored at -80 °C for five minutes to achieve denaturation. Next, the samples were mixed again for five minutes and centrifuged at 18 000g for ten minutes. The clear supernatant was transferred into a vial, and a 5 µl aliquot was injected into the LC-MS/MS, in duplicate. The LLOQ of the assay was 11 µg/l. Assay imprecision and accuracy were 0.9–4.8% and 98.9–103.7%, respectively, with a concentration range of 11–5000 µg/l. The pretreatment and analysis were conducted in duplo. The method was validated according to FDA guidelines. All tested parameters met the specification limits. The nilotinib plasma method was based on the method as described by Haouala et al. [19].

**Calculation of nilotinib plasma concentrations on the basis of DBS**

DBS concentrations and plasma concentrations were compared with three methods. In methods 1 and 2, the blood-to-plasma concentration ratio was accounted for, and the analyzed DBS concentration was converted according to formula [8]:

\[
C_{\text{plasma}} = \frac{C_{\text{DBS}}}{(1 - \text{Hct}) + \text{Hct} \rho f_u}
\]

where \(C_{\text{plasma}}\) is the calculated plasma concentration, \(C_{\text{DBS}}\) is the concentration in DBS, \(f_u\) is the unbound fraction and \(\rho\) the erythrocyte-to-plasma concentration ratio. Plasma protein
binding is approximately 98% [20]; \( f_u \) is 0.02. The blood-to-plasma ratio of nilotinib is 0.71 [20]; \( \rho = \text{Hct} \times 0.71 \). Nilotinib plasma concentrations were calculated using the individual Hct values (method 1) as well as the mean Hct value of the study population of 0.41 (method 2). In method 3, plasma concentrations were predicted using the constant \( \left( b \right) \) and proportional \( \left( m \right) \) bias from the Deming fit between the analyzed plasma and DBS concentrations.

\[
C_{\text{plasma}} = \frac{C_{\text{DBS}} + b}{m}
\]

**Safety parameters**

As the occurrence of all-grade elevations in total bilirubin was found to be higher in patients with higher nilotinib exposure and to a lesser extent also elevations of other liver function parameters [6], total bilirubin, ASAT and ALAT levels were determined as safety parameters according to routine analysis.

**Statistical analysis**

Nilotinib concentrations as measured by DBS and venous blood sampling methods were compared according to the EP9-A2 guideline [21]. This is a statistically robust protocol designed for method comparison studies. The collected data were processed using Microsoft Office Excel 2010 and analyzed using SPSS version 20 for Windows (IBM Corp, Armonk, NY, USA) and Analyse-it (Analyse-it Software Ltd, Leeds, UK). Outliers were determined with the outliers test on within-method duplicates and the test for between-method outliers, according to the EP9-A2 guideline [21]. For the analysis of the primary outcome, the comparison of the nilotinib concentration in DBS and the plasma concentration, general Deming regression was used. The slope was computed, with a standard error (SE) and a 95% confidence interval (95% CI). Agreement between the two methods was presented using a Bland-Altman difference plot. The mean bias and the 95% limits of agreement were calculated. Acceptance criteria for the agreement between calculated and measured plasma concentrations were based on the guidelines for bioanalytical method validation of the FDA and EMA [13, 22]. The difference in concentration between the two methods should be within 20% of the mean difference for at least two third of the samples.
RESULTS

Study sample
Twenty patients participated, of whom most (85%) were treated with 300 mg nilotinib twice daily (Table 1). The dose was unchanged during the study period. Forty sets of DBS and venous blood samples were collected. Mean Hct was 0.41 ± 0.05. There was no statistically significant difference in nilotinib concentration between the blood samples from males (n=25) and females (n=15).

Ten out of eighty DBS (12.5%, from six patients) were not filled completely. For these samples, a 5.5-mm paper disc was punched and calculated on a 5.5 mm punched DBS of the calibration standard curve. The mean Hct of the patients with these samples was 0.39 ± 0.06. Outliers were not detected.

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Demographics (n=20)</th>
<th>Mean age, years (SD)</th>
<th>56 (14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n (%) male</td>
<td>13 (65)</td>
<td></td>
</tr>
<tr>
<td>Nilotinib dose, n (%) (n=20)</td>
<td>150 mg, bid</td>
<td>1 (5)</td>
</tr>
<tr>
<td></td>
<td>300 mg, bid</td>
<td>17 (85)</td>
</tr>
<tr>
<td></td>
<td>400 mg, qd</td>
<td>1 (5)</td>
</tr>
<tr>
<td></td>
<td>400 mg, bid</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Safety parameters (n=40)</td>
<td>Median total bilirubin, µmol/l (range)</td>
<td>13 (5-31)</td>
</tr>
<tr>
<td></td>
<td>Median ASAT, µmol/l (range)</td>
<td>23 (16-37)</td>
</tr>
<tr>
<td></td>
<td>Median ALAT, µmol/l (range)</td>
<td>24 (16-52)</td>
</tr>
</tbody>
</table>

ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; qd, quaque die (once a day); bid, bis in die (twice a day); SD, standard deviation.

DBS concentration versus plasma concentration
Nilotinib plasma concentrations ranged from 376 to 2663 µg/l, and the median repeatability coefficient of variation was 3.9% (interquartile range 2.8–5.0%). Concentrations measured in DBS ranged from 144 to 1518 µg/l, with a median repeatability coefficient of variation of 4.8% (interquartile range 2.4–11.1%). Figure 2a shows the relationship between the analyzed plasma and DBS concentrations. The slope value was 0.56 with a SE of 0.03 (95% CI, 0.51 to 0.61). The intercept was −41.68 with a SE of 25.74 (95% CI, −93.78 to 10.42).
Calculated plasma concentration on the basis of DBS versus plasma concentration

Plasma concentrations calculated using method 1 (with individual Hct correction) ranged from 232 to 2745 µg/l. Comparing these concentrations with the measured plasma concentrations, the slope value was 0.96 with a SE of 0.05 (95% CI, 0.86 to 1.06) (Figure 2b). The intercept was −83.12 with a SE of 44.35 (95% CI, −172.90 to 6.66). For method 2 (with mean Hct correction), calculated plasma concentrations ranged from 243 to 2562 µg/l. The slope value was 0.95 (SE 0.04; 95% CI, 0.86 to 1.03) with an intercept of −70.26 (SE 43.52; 95% CI, −158.36 to 17.84) (Figure 2c).

In method 3, plasma concentrations were predicted using the constant \( b = -41.68 \) and proportional bias \( m = 0.56 \) from the Deming fit between the analyzed plasma and DBS concentrations. Calculated plasma concentrations ranged from 331 to 2780 µg/l. The slope value was 1.00 (SE 0.04; 95% CI, 0.91 to 1.09) with an intercept of 0.34 (SE 46.27; 95% CI, −93.34 to 94.01) (Figure 2d).

Figure 3 shows the Bland-Altman difference plots of the calculated and measured plasma concentrations. The difference in nilotinib concentrations between calculated and measured plasma concentrations using method 1 (with individual Hct correction) ranged from −56% to 24%, with a mean difference of −14.3% (SE 2.8%; 95% CI, −19.9% to −8.8%). The 95% limits of agreement were −50.1% and 21.4% (Figure 3b). The difference between the calculated and measured plasma concentration using method 2 (with mean Hct correction) was similar (mean bias of −14.0%; SE 2.6%; 95% CI, −19.3% to −8.7%; 95% limits of agreement −48.0% and 20.1%) (Figure 3c). With method 3, the difference in nilotinib concentrations between the two methods ranged from −32.9% to 35.4%, with a mean difference of −0.6% (SE 2.3%; 95% CI, −5.3% to 4.1%). The 95% limits of agreement were −30.9% and 29.6% (Figure 3d). The differences between the calculated plasma concentrations and the measured plasma concentrations were within the predefined acceptance criteria for agreement. With methods 1, 2 and 3, the difference was within 20% of the mean for 73%, 85% and 80% of the samples, respectively.
Figure 2 Relationship between nilotinib plasma concentration and (a) nilotinib concentration measured in dried blood spot, (b) calculated plasma concentration using individual hematocrit, (c) calculated plasma concentration using mean hematocrit of 0.41, and (d) calculated plasma concentration using the constant and proportional bias between the analyzed plasma and dried blood spot concentrations.
Figure 3 Difference between nilotinib plasma concentration and (a) nilotinib concentration measured in dried blood spot, (b) calculated plasma concentration using individual hematocrit, (c) calculated plasma concentration using mean hematocrit of 0.41, and (d) calculated plasma concentration using the constant and proportional bias between the analyzed plasma and dried blood spot concentrations.
DISCUSSION

The present study aimed to compare nilotinib concentrations using DBS samples of capillary blood obtained by finger prick and plasma concentrations in venous blood samples in patients with CML. Nilotinib concentrations measured in DBS were lower than the corresponding plasma concentrations measured in venous blood samples. The present study shows that plasma concentrations of nilotinib can be predicted on the basis of DBS. Differences between the calculated and measured plasma concentrations were within the acceptance criteria for agreement.

When comparing nilotinib concentrations assessed with DBS and venous sampling, no direct conversion of one value into another is generally possible [10]. The blood-to-plasma concentration ratio, which is primarily determined by Hct, should be taken into account [8, 10]. In the present study, nilotinib plasma concentrations were predicted using the individual Hct values as well as the mean Hct value of the study population of 0.41. Both conversion methods showed acceptable slope values of 0.96 and 0.95, respectively, explaining the relationship between calculated plasma concentrations and the corresponding measured plasma concentrations. As both methods provided similar results, it can be concluded that calculation of plasma levels on the basis of DBS does not require a correction for the individual Hct. This offers logistical advantages and increases the usefulness of this sampling method. An alternative, which also implies logistical advantages, is to measure an endogenous compound in DBS to predict the approximate individual Hct. Capiau et al. [23] found that the potassium concentration in DBS can also be used to assess Hct.

Nilotinib plasma concentrations were also predicted using the constant and proportional bias between the analyzed plasma concentrations and analyzed DBS concentrations. With a perfect slope value of 1.0 explaining the relationship between calculated plasma concentrations on the basis of DBS and the corresponding plasma concentrations, this method appeared most accurate to predict plasma concentrations. This method has previously been shown useful for calculation of vemurafenib plasma levels assessed by means of DBS [24].
Several other studies on the development and validation of assessment of plasma levels of TKIs on the basis of DBS sampling have been performed [11, 12, 24, 25]. In these studies, there was also no effect of Hct on the accuracy of the assessment of plasma level. One study investigated the possibility of assessment of plasma levels of three TKIs in CML treatment by means of DBS [11]. However, only two patients using nilotinib were included in the study. This study used the individual Hct to translate the DBS concentrations to plasma concentrations and found a higher slope value of 1.1. It should be noted that blood was obtained by means of venepuncture and spotted onto the sampling paper, while in the present study capillary blood was used. The concentration in capillary blood may deviate from the venous concentration because the material in DBS consists of blood cells, plasma and interstitial fluid.

A strength of the present study is the use of capillary blood obtained by finger prick to fill the premarked circles on the DBS sampling paper. The use of capillary obtained blood corresponds to the clinical practice of DBS sampling. Furthermore, we used the EP9-A2 guideline [21] for the comparison of the two sampling methods, according to which we collected forty sets of DBS and venous blood samples simultaneously which were analyzed in duplicate.

This study also has some limitations. Several DBS did not meet the requirement of ‘completeness’. We decided not to exclude these samples from the data set. After adjustment for surface of the nilotinib concentrations of these DBS samples, the difference between the calculated plasma concentration and the corresponding plasma concentration was within the 95% limits of agreement. In addition, it should be recognized that another type of sampling paper requires renewed validation of the method. In this study, DMPK-C sampling paper of Whatman was used. Due to variability in properties of the paper, extrapolation to other types of sampling paper than the type used in the present study cannot be recommended. Because of the twice daily dosing schedule of nilotinib at a fasted condition, we were not able to collect blood samples at trough condition, nor to provide information on whether there would have been a difference in clinical decision making based on the two sampling methods.

Nilotinib bioavailability is subject to high variability [2] as the result of drug-drug interactions [3], food [4] and drug taking behavior [5]. Plasma levels too low may result in the risk of low or delayed responses and a dose too high may result in side effects [6], with QT interval
prolongation considered the most feared risk. TDM can be used to identify unacceptably low or high nilotinib plasma concentrations. The results of TDM can be used to adapt the dose or to optimize the intake conditions. In addition, TDM may identify possible medication nonadherence. The use of DBS for the assessment of nilotinib plasma levels facilitates the assessment of nilotinib plasma levels.

**Conclusion**

Nilotinib plasma concentrations could be calculated on the basis of the DBS. DBS sampling of capillary blood obtained by finger prick to assess the nilotinib concentration in CML patients seems to be a suitable alternative for conventional venous blood sampling and may be useful for TDM of CML patients in clinical practice.
REFERENCES


