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**Author:** Wit, Djoek de  
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背景下

干血点（dbs）取样可能是一种更方便、更灵活的替代静脉取样方法来使用培唑帕滨。此研究旨在确定培唑帕滨dbs和血浆浓度之间的同意程度，以便于将dbs取样方法纳入临床实践。

患者和方法

在12名患者中，收集了配对的dbs和血浆样品。培唑帕滨血浆浓度通过dbs浓度计算得出，公式为：血浆浓度 = DBC/（1-血细胞比容）。通过Passing-Bablok和Bland-Altman分析来确定计算和测量血浆浓度之间的同意程度。我们预设了一个临床可接受的界限为25%的Bland-Altman分析。

结果

Passing-Bablok分析显示，计算和测量浓度之间存在一个小的恒定（截距估计值 -8.53（95% CI：-12.22至-4.41））和轻微的线性（斜率估计值 1.15（95% CI：1.04-1.24））偏差。这种偏差在临床意义上是无关的，因为Bland-Altman分析显示，计算到测量浓度的平均比值为0.94（95% CI：0.65-1.23）。临床可接受的界限均在95%的界限内。具体来说，92.6%的数据点在预定的可接受范围内。

结论

培唑帕滨血浆浓度可以从dbs浓度准确计算得出。尽管还需要对患者自己准备的dbs卡进行验证，但这些结果表明，dbs取样可以用于临床实践中监测培唑帕滨治疗的情况。
Introduction

Pazopanib hydrochloride (Votrient®) is an oral multi-targeted tyrosine kinase inhibitor (TKI) used for the treatment of metastatic renal cell carcinoma (mRCC) and metastatic soft tissue sarcoma (STS) [1,2]. For patients with mRCC, a correlation between pazopanib exposure and treatment outcome has been demonstrated [3]. Suttle et al. showed that a higher treatment response was seen for patients with a C\text{trough} level > 20.5 mg/L and a higher incidence of toxicity was seen in patients with a C\text{trough} level > 36 mg/L. This implies that the optimal therapeutic window for pazopanib in patients with mRCC lies between a C\text{trough} level of 20.5 - 36 mg/L. There is a large variability in pazopanib exposure between patients (40-60 CV% in AUC) leading to the risk of sub- or supra-therapeutic C\text{trough} levels and therefore to decreased therapeutic effects or more toxicity [3,4].

Theoretically, this might introduce problems when a patient has an exposure below the threshold for efficacy and needs an increased dose. However, the finding of a plateau is based on a limited number of patients and therefore not conclusive yet. In addition, results from two pazopanib TDM study suggest that exposure does increase with doses above 800 mg [4,8].

At present, pazopanib concentrations are monitored in plasma collected by venous sampling [9]. However, sampling by venipuncture has several disadvantages including its invasive character, the requirement for patients to travel to the clinic and the need for trained personnel. Compared to venous sampling, dried blood spot (dbs) sampling is a convenient, simple, flexible and more patient friendly alternative to collect blood in an at home setting. With clear instructions and adequate training, patients should be able to self-collect dbs samples. The added value and feasibility of dbs collection for TDM has been shown effective for several drugs including anti-epileptics, immunosuppressants and antiretroviral drugs [10-12].

Here, we describe the results of a study investigating the feasibility of dbs for TDM of pazopanib. The objective of this study is to determine the agreement between pazopanib dbs- and plasma concentrations in order to facilitate the future implementation of pazopanib dbs sampling into clinical practice.

Methods

Patients

The collection and analysis of dbs and plasma samples was part of a larger phase 1 study that investigated the feasibility of TDM for dose individualization of pazopanib [4]. Included patients were ≥ 18 years with progressive disease from an advanced solid tumor, a WHO performance status ≤ 2 that had no standard treatment options available. All patients had adequate haematologic, renal and liver function reserves. The study was approved by the institutional ethics committee (Leiden University Medical Center, The Netherlands) and all patients gave written informed consent before entering the study. Between July 2012 and June 2013, 13 patients were included of whom 12 also participated in the dbs part of the study.

Sampling

At day 14 of standard 800 mg pazopanib therapy, patients were admitted to the hospital for pharmacokinetic sampling. EDTA-blood samples were collected by venipuncture pre-dose and 1, 2, 3, 4, 6, 8, 10 and 24 hours after pazopanib intake. From these EDTA blood samples, 15 µL blood was collected into an EDTA capillary tube and spotted onto a Whatman FTA® dbs card. This procedure was repeated 2 times to fill the 3 pre-marked circles on the card. After spotting the dbs cards, venous blood samples were centrifuged at 3,000 rpm for 5 minutes; the supernatant plasma was stored at -20˚C until the day of analysis.

In addition to the dbs sampling cards prepared with venous blood, dbs sampling cards prepared by finger prick were collected pre-dose, and 3 and 8 hours after pazopanib intake. After disinfection of the skin with alcohol 70%, a lancet puncture was performed. The first drop of blood was discarded, thereafter 15 µL blood from the finger was collected using the above described capillary tube and spotted onto the bfs card. This procedure was repeated 2 times to fill the 3 pre-marked circles on the card.

After drying for at least 2 hours, dbs cards were stored at room temperature in a closed plastic bag containing 2 sachets of desiccant. Thereafter, finger prick dbs cards (n = 3), venous dbs cards (n = 9) and plasma samples (n = 9) were all sent to GlaxoSmithKline, USA for further bio-analytical analysis.

Analysis

For the analysis of dbs pazopanib concentrations, a 4 mm diameter disc was punched out from the 15 µL dried blood spot. Per subject only 1 blood spot out of 3 was analyzed. Pazopanib was extracted from this disc with the use of 50 µL formic acid and 400 µL methanol containing an isotopic labelled internal standard, [\text{[1H, 13C]}]-pazopanib. After through mixing and centrifugation, 200 µL of the extract was taken into an auto-sample...
two tested methods. We used Bland-Altman analysis to define the
accuracy of this method was between -10.3% and 5.5%. Samples were stable
on the DBS card for at least 75 days at ambient temperature. There was no
influence of haematocrit levels (0.2 to 0.65) on the performance of
this assay.

For analysis of pazopanib concentrations in plasma, 20 µL of plasma
was extracted by adding 500 µL of acetonitrile/10 mM ammonium acetate
(80/20 v/v) containing 10 ng/mL of [14C]-pazopanib as the internal
standard. This was followed by vortex mixing and centrifugation at
approximately 6200 g for 20 minutes. The supernatant was transferred
into clean tubes and injected onto a HPLC-MS/MS system for analysis.
This validated method was linear within the concentration range of
0.1 - 50 µg/mL pazopanib. The within- and between-run imprecisions
were ≤ 14.7% and ≤ 2.9% respectively and the accuracy of this method
was between -4.3% and 5.5%. Samples were stable in plasma for at least
530 days at -20 °C and 24 hours at ambient temperature.

Calculation of plasma concentration

Pazopanib plasma concentrations were calculated from DBS
concentrations using the previously described formula: plasma concentration
= DBS Concentration / (1-haematocrit) [13]. The blood:plasma ratio of pazo-
panib ranges from 0.59 to 0.93 which suggests only a minimal association
of pazopanib with blood cells [14]. In addition, only the unbound fraction
of a drug can partition into blood cells [15]. Since pazopanib has a high
protein binding of > 99.9%, the unbound fraction will be negligible [16].
Therefore, the fraction of pazopanib bound to red blood cells (haemat-
ocrit) was ignored in the above described formula. Plasma concentrations
were calculated using both patient specific measured haematocrit values
and fixed haematocrit values of 0.40 and 0.45 for males and females,
respectively. A paired Student’s t-test was used to test for a difference
in calculated plasma concentrations using measured and fixed
haematocrit values.

Statistics

Passing-Bablok regression and Bland-Altman analysis were used to deter-
mine the agreement between the two sampling methods [17,18]. Passing-
Bablok regression analysis tests for a constant bias and proportional bias
between two methods. If the 95%-ci for the intercept of the regression
line includes 0, no constant bias is observed. If the 95%-ci for the slope
of regression line includes 1, there is no proportional bias between the
two tested methods. We used Bland-Altman analysis to define the
clinical relevance of any found bias. As suggested by Bland and Altman,
a clinical and practical acceptance limit for the found ratio was deter-
mined [18]. A 25% range around the found ratio of the two methods was
determined to be clinically and practically relevant since pazopanib can
only be dose adjusted in steps of 25% of the total dose (200 mg tablets are
the lowest dose available). Hence, the difference should be > 25% to result
in a possibility to adjust the dose.

Analysis was performed with Microsoft office Excel (Microsoft Inc,
Redmond, WA) and add-in Analyse-it statistics software (Analyse-it

Results

Patients

Between July 2012 and June 2013, 12 patients were enrolled in this DBS
study. Characteristics of the patients included are summarized in Table 1.

Agreement between DBS concentrations prepared by finger prick
and with venous blood

Concentrations measured at the same time points in DBS samples prepared
by finger prick and prepared with venous blood were in good agreement
with each other (Figure 1A). Passing-Bablok regression showed that that
there was no constant (intercept estimate -0.71 (95%–ci; -3.41 to 2.23))
or proportional bias (slope estimate 1.05 (95%–ci; 0.93 to 1.17)) between
the two sampling methods for the preparation of DBS cards. In this study,
we collected 3 DBS cards prepared by finger prick per patient compared
to 9 DBS cards prepared with venous blood. Since both methods for DBS
card preparation were in agreement with each other and values therefor
interchangeable, we used the (more extensive) data from the venous DBS
cards for all further described analysis.

DBS vs. plasma concentrations

Pazopanib DBS concentrations (uncorrected concentrations in blood)
were on average 48.0% (sd 8.5%) lower than measured plasma concen-
trations (Figure 1B). Passing-Bablok regression analysis showed that
there was a constant (intercept estimate -4.68 (95%–ci; -6.48 to -2.47))
and proportional bias (slope estimate 0.63 (95%–ci; 0.57 to 0.68)).

Calculated vs. measured plasma concentrations –
Passing-Bablok analysis

Calculated plasma concentrations using patient specific haematocrit
values were on average 94.0% of measured plasma concentrations.
Variability was relatively large (sd 14.7%, range 61.6% - 134.9%). Using
a fixed haematocrit value, calculated plasma concentrations were on

average 95.0% of measured concentrations with comparable variability (SD 15.5%, range 65.0 - 144.0%). No significant differences between both approaches (measured and fixed haematocrit values) was observed (95%-CI of difference in calculated plasma concentrations; -0.19 to 0.57, $P = 0.315$).

Passing-Bablok analysis showed a small constant bias (intercept estimate -8.53 [95%-CI; -12.22 to -4.41]) and slightly proportional bias (slope estimate 1.15 [95%-CI; 1.04 to 1.24]) between calculated and measured plasma concentration when patient specific haematocrit values were used (Figure 2). Similar results were found when a fixed haematocrit value was used; intercept estimate -9.67 [95%-CI; -13.28 to -5.51] and slope estimate 1.17 (95%-CI; 1.07 to 1.26).
Calculated vs. measured plasma concentrations – Bland-Altman analysis

The difference in pazopanib concentrations between calculated and measured plasma concentrations using patient specific haematocrit values ranged from -19.2 to 13.2 µg/mL with a mean difference of -2.4 µg/mL (sd 6.8 µg/mL, Figure 3A). The mean ratio of calculated to measured plasma concentrations was 0.94 with the 95% limits of agreement of this ratio being 0.65 to 1.23 (Figure 3B). The clinical acceptance limits which were set at 25% around the found mean ratio, fell well within the 95% limits of agreement (0.71 to 1.18). More specifically, 92.6% (88 out of 95) of the data points were within the clinical acceptance limits.

Similar results were found when fixed haematocrit values were used. The difference ranged from -18.7 to 16.7 with a mean difference of -2.0 µg/mL (sd 7.1 µg/mL). The mean ratio of calculated to measured plasma concentrations was 0.95 with the 95% limits of agreement of this ratio being 0.65 to 1.25. The clinical acceptance limits (0.71 to 1.19) also fell well within the agreement limits. Using fixed haematocrit values, 12.6% (13 out of 103) of the data points exceeded the clinical acceptance limits.
Clinical relevance
A pazopanib trough concentration of 20.5 µg/mL is suggested as the threshold for efficacy in mRCC patients [3]. In Table 2, decision making based on measured and calculated plasma concentrations are compared. In 1 case (9.1%), there would have been a difference in decision making based on exposure. The measured plasma C\text{trough} was 24.7 µg/mL compared to a calculated C\text{trough} of 19.5 µg/mL from DBS. In all other cases, clinical decision making would have been the same based on either the measured or calculated plasma concentration. The same results were found when a fixed haematocrit value was used.

### Table 2

<table>
<thead>
<tr>
<th>Measured C\text{trough} level</th>
<th>Calculated C\text{trough} level</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20.5 µg/mL</td>
<td>2</td>
</tr>
<tr>
<td>≥ 20.5 µg/mL</td>
<td>0</td>
</tr>
<tr>
<td>≥ 20.5 µg/mL</td>
<td>1</td>
</tr>
</tbody>
</table>

*In bold: a difference in clinical decision making based on calculated plasma C\text{trough} levels using patient specific haematocrit and measured plasma C\text{trough} levels*

Based on the C\text{trough} levels, DBS cards were prepared by the research nurse with the use of a 15 µl capillary. It can be argued that sampling by the research nurse

Discussion
The present study shows that pazopanib plasma concentrations calculated with the use of DBS, are in good agreement with actually measured pazopanib plasma concentrations. This implicates that DBS sampling can be used as an alternative sampling strategy for the determination of plasma concentrations to monitor pazopanib therapy.

A small constant, and slightly proportional bias was shown between calculated and measured pazopanib plasma concentrations. However, these biases were clinically not relevant as the vast majority of data points were within the predefined clinical acceptance limits. In addition, the difference between calculated and measured plasma concentrations would have resulted in different clinical decision making in only one out of 11 cases. It should be noted that in this case the difference between calculated and measured concentrations was small and concentrations were close the defined target of 20.5 µg/mL. Overall, these results show that DBS sampling can be used as an alternative – more patient friendly – sampling strategy to monitor pazopanib therapy in clinical practice.

Previously, Kralj et al investigated DBS sampling and analysis for the TKIs imatinib, nilotinib and dasatinib [19]. They used the same formula and also found good agreement between calculated and measured plasma concentrations. In the current study, we used both patient specific as well as fixed haematocrit values for the estimation of pazopanib plasma concentrations. The percentage of data points within the clinical acceptance limits when fixed haematocrit values were used, was slightly lower in comparison to when patient specific haematocrit values were used. However, no significant difference between calculated plasma concentrations using patient specific or fixed haematocrit levels could be shown. In addition, there was no difference in clinical decision making based on C\text{trough} levels when patient specific or fixed haematocrit values were used. This indicates that fixed haematocrit values can be interchangeably used instead of measured haematocrit values for the calculation of pazopanib plasma concentrations when patient haematocrit levels are within the normal range.

The binding of pazopanib to red blood cells is thought to be limited and we did not take this into account for the calculation of pazopanib plasma concentrations. This may potentially cause bias in the calculation of plasma concentrations from DBS concentrations. However, calculated plasma concentrations from DBS were on average 6% lower than the measured concentrations. This demonstrates that the possibility of pazopanib partitioning into red blood cells is minimal since the calculated concentration would then have been higher otherwise. In addition, plasma concentrations could be readily predicted from DBS concentrations which also indicates that the uptake of pazopanib into red blood cells is small. This is also in agreement with the fact that pazopanib has a high plasma protein binding (> 99.9%) and the assumption that only the free unbound amount of a drug can participate into red blood cells.

In this study, DBS cards contained relatively high concentrations of pazopanib since samples were taken as part of rich \text{pk}-\text{curves} shortly after pazopanib intake to calculate pazopanib AUCs. As a consequence, there is only a limited number of DBS samples within the lower concentration range. Although splitting the Passing-Bablok regression into C\text{trough} DBS samples and all other samples or into different concentration ranges did not change the results, it can be doubted whether the small proportional bias is caused by the fact that there are not enough samples in the lower concentration range or whether there is truly a proportional bias. In addition, this limits the amount of data on which agreement is based within the lower concentration range. Both bio-analytical assays for the determination of pazopanib in DBS and plasma were validated according to international guidelines, excluding an analytical cause.

DBS cards were prepared by the research nurse with the use of a 15 µl capillary. It can be argued that sampling by the research nurse
with a capillary does not truly reflect an at home sampling setting where patients spot themselves, which is a limitation of this study. On the other hand, previous studies with antiretroviral and immunosuppressive drugs have shown that 87.5 to 98% of the 

\[ \text{dbs} \]

samples obtained by patients were suitable for analysis [20,21]. Although these cards were prepared by blood drop and not capillary, it suggests that preparation of a 

\[ \text{dbs} \]

card by patients after a clear instruction is highly feasible. The perfect agreement between 

\[ \text{dbs} \]

cards prepared by finger prick and those prepared with whole blood shows that there is no engorgement of blood when a 

\[ \text{dbs} \]

is prepared by finger prick.

**Conclusion**

This study shows a good agreement between pazopanib levels measured in plasma and concentrations calculated from the corresponding 

\[ \text{dbs} \]

card. Although validation of clinical utility with 

\[ \text{dbs} \]

cards prepared by patients themselves is necessary, the results from this study show the feasibility of the use of 

\[ \text{dbs} \]

cards. With the ease and convenience of sample collection, 

\[ \text{dbs} \]

could be very useful for TDM of patients treated with pazopanib and potentially other TKIs in the future.
References


