



# Dried blood spot testing for estimation of renal function and analysis of metformin and sitagliptin concentrations in diabetic patients: a cross-sectional study

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## Abstract

**Purpose** Dried blot spot (DBS) analysis of drugs or clinical parameters offers many advantages. We investigated the feasibility of using DBS for analysis of anti-diabetic drugs concomitantly with the estimated creatinine clearance ( $Cl_{crea}$ ).

**Methods** The cross-sectional study involved physicians in an enabling analysis with 70 diabetic patients and community pharmacists in a field investigation with 84 participants. All 154 DBS samples were analyzed for creatinine, metformin, and sitagliptin.

**Results** The diabetic patients revealed of a wide range of age (32–88 years), BMI values (19.8–54.7 kg/m<sup>2</sup>), and extent of polypharmacotherapy (1–21 drugs). A correlation factor to convert capillary blood creatinine from DBS into plasma concentrations was determined. Patients'  $Cl_{crea}$  ranged from 21.6–155.9 mL/min. The results indicated statistically significant correlations ( $p < 0.05$ ) between the use of two or three particular drug classes (diuretics, NSAIDs, renin-angiotensin system blockers) and a decreased renal function. DBS concentrations of metformin ranged between 0.23–4.99 µg/mL. The estimated elimination half-life ( $t_{1/2}$ ) of metformin was 11.9 h in patients with a  $Cl_{crea}$  higher than 60 mL/min and 18.5 h for diabetics with lower  $Cl_{crea}$ . Sitagliptin capillary blood concentrations ranged between 11.12–995.6 ng/mL. Calculated  $t_{1/2}$  of sitagliptin were 8.4 h and 13.0 h in patients with a  $Cl_{crea}$  above and below 60 mL/min, respectively.

**Conclusions** DBS allow for the analysis of concentrations of predominantly renally eliminated drugs and community pharmacists can provide a valuable contribution to DBS sampling.

**Keywords** Metformin · Sitagliptin · Creatinine · Clinical study · Dried blood spots · Capillary blood

## Introduction

Research and clinical application of compound analysis from dried blood spots (DBS) has significantly increased over the

past years [1, 2]. DBS, collected by fingerpicking and spotting capillary blood on filter paper, which is subsequently dried before analysis by liquid chromatography methods, offer many advantages such as the low blood volumes required, the usually high stability of the dried analytes, and the easy sampling process. Thereby, DBS sampling is attractive, e.g., therapeutic drug monitoring in children and adults. DBS home sampling offers a convenient and potentially cost saving alternative to conventional sampling [2, 3].

If used for therapeutic monitoring for predominantly renally eliminated drugs, the patient's plasma creatinine concentration allows the estimation of the glomerular filtration rate. The Cockcroft-Gault formula [4] has been suggested to perform best for renal function assessment in older adults [5]. However, to the best of our knowledge, there are no reports about a factor to convert capillary blood levels of creatinine from DBS into the serum/plasma concentrations.

For our investigations, we chose two model drugs that are frequently prescribed in diabetes. Metformin is a first line oral

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antidiabetic with beneficial effects on morbidity and mortality of type 2 diabetic patients [6]. Accumulation of metformin in blood in case of impaired renal function has been discussed to increase the risk of lactic acidosis [7]. Until recently, metformin use has been contraindicated in patients with an estimated glomerular filtration rate (eGFR) below 60 mL/min [8]. Metformin displays a significant distribution into erythrocytes [9, 10], and thus, it might be useful in monitoring capillary blood concentrations. The dipeptidyl peptidase-IV inhibitor sitagliptin is often combined with metformin. Like metformin it is predominantly cleared renally [11] and shows distribution into blood cells [12]. It is recommended to adapt the sitagliptin dose in patients with renal impairment [13].

Diabetes is a risk factor for kidney diseases [14], but also, various drugs can negatively impact renal function [15]. The combined therapy with diuretics, nonsteroidal anti-inflammatory drugs (NSAIDs), and inhibitors of the renin-angiotensin system (RAS) has been described as potentially nephrotoxic [16, 17]. Concomitant use of representatives of these highly prescribed three drug classes (“target drugs”) has been termed “triple whammy.” It might induce kidney injury based on glomerular hemodynamic alterations and result in a reduced glomerular filtration rate [18], particularly within the first 30 days of use [19].

The purpose of the present study was to investigate the feasibility of using DBS for concentration monitoring of anti-diabetic drugs in relation to the creatinine clearance ( $Cl_{crea}$ ) estimated according to Cockcroft and Gault (C&G  $Cl_{crea}$ ) as a measure of renal function [4]. We aimed at determination of “real-life” by recruiting a highly heterogeneous population of diabetic patients. The study was organized as joined project involving physicians specialized in diabetology and community pharmacists.

## Methods

### Study design and patients

The cross-sectional study was conducted in two phases. An enabling investigation was performed in cooperation with physicians; the field investigation involved community pharmacies. Patients with type 2 diabetes mellitus receiving a therapy with metformin and/or sitagliptin as fixed dose combination or monotherapy were recruited and enrolled in the study after written informed consent. Patients younger than 18 years and patients with an infectious disease were excluded.

In the enabling investigation, adult patients ( $n = 70$ ; 34 women, 36 men) were recruited in a medical practice of physicians specialized in diabetology near Würzburg (Bavaria, Germany). Technical assistants collected the capillary blood samples between February 2015 and May 2016 and sent the dried blood spots (DBS) to the Institut für Pharmazie for

analysis. Additionally, data determined in a clinical chemistry lab in the course of routine analysis of patients’ plasma were utilized. The study was approved by the ethics committee of the Medical Faculty of the University of Würzburg (reference number 287/14).

In the field study, 84 adult diabetic patients (39 women, 45 men) were recruited in 14 community pharmacies in middle and southern Germany. Pharmacists collected the capillary blood samples between August 2016 and November 2016 and sent the DBS for analysis. The study was approved by the Freiburg Ethics Committee International (FEKI) and registered by ISRCTN (ISRCTN14518136).

### Analytical methods

Samples of 40.0  $\mu$ L capillary blood were spotted on a precut filter paper disc and dried before packing in a plastic bag containing a desiccant. Concentrations of creatinine, metformin, and/or sitagliptin in DBS were determined using a validated liquid chromatography method as detailed previously [12]. The lower limits of quantification (LLOQ) were 0.2  $\mu$ g/mL (1.55  $\mu$ M) for metformin, 3 ng/mL (5.73 nM) for sitagliptin, and 0.15 mg/dL (13.26  $\mu$ M) for creatinine. The validated analytical ranges comprised 0.2–5  $\mu$ g/mL metformin, 3–500 ng/mL sitagliptin, and 0.15–1.5 mg/dL creatinine.

### Determination of a correlation factor of capillary blood to plasma creatinine

The creatinine concentrations of the 70 patients participating in the enabling study were analyzed in DBS and compared with patients’ plasma creatinine concentrations as determined with standard enzymatic methods in a clinical chemistry lab. A conversion factor was established by splitting the patient data set at a ratio of 65%/35% to be used for development and confirmation of a conversion factor  $F_0$ . The whole data set ( $n = 70$ ) yielded an optimized conversion factor  $F$ .

### Data analysis

For all patients, DBS concentrations of creatinine, metformin and/or, sitagliptin were determined. Depending on the time of withdrawal in relation to the last drug intake, the measured concentrations corresponded to mean steady state, peak, or trough concentrations. C&G  $Cl_{crea}$  [4] was calculated using the patients’ ideal body weight. For correlation analysis, the Pearson coefficient was determined. The elimination half-lives  $t_{1/2}$  for metformin and sitagliptin were calculated after dose normalization to 2000 mg metformin and 100 mg sitagliptin and assuming first-order kinetics. Plotting the normalized concentrations of all patients in relation to the time of the last intake of the medication, the elimination half-life of the population was calculated using the equation  $t_{1/2} = \ln 2/k_e$

with  $k_e$  (eliminations rate constant) being the negative slope of the linear regression line. Mean values were compared using a  $t$  test. Statistical significance was defined as  $p \leq 0.05$ .

## Results

The average age of all participants (73 female, 81 male) was  $67 \pm 11.8$  years (range, 32–88 years). The mean body mass index was  $30.2 \pm 5.5$  kg/m<sup>2</sup> (range 19.8–54.7 kg/m<sup>2</sup>). Patients concomitantly used 1–21 different drugs with a median drug number of 6 (Table 1). This information was derived from the patients' medication plans supplemented with interviews by participating physicians (enabling study) and pharmacists (field study). The groups of the enabling investigation ( $n = 70$ ) and the field study ( $n = 84$ ) did not differ statistically significantly regarding BMI or number of drugs used. Both groups differed slightly, but statistically significant regarding the age with  $64.6 \pm 12.1$  years in the enabling investigation and  $68.9 \pm 11.2$  years in the field study ( $t$  test,  $p = 0.02$ ).

### Correlation between creatinine levels in capillary blood and plasma

The correlation between creatinine concentrations in capillary blood and plasma was determined in the enabling investigation with  $n = 70$  diabetic patients. Capillary blood concentrations in dried blood spots (DBS) and plasma values of the first 45 participants were used as a “training” data set and the ratio of patients' creatinine concentrations in capillary blood and plasma ( $c_{\text{Capillary Blood}}/c_{\text{Plasma}}$ ) was calculated. The mean ratios revealed moderate variability with a relative standard deviation of 11.80%. The resulting initial conversion factor  $F_0$  was  $0.918 \pm 0.108$  (mean  $\pm$  standard deviation).

To evaluate the precision of the conversion factor  $F_0$ , capillary blood concentrations of additional 25 patients were converted into plasma concentrations using  $F_0$ . There was no statistical significant difference between the means of calculated and measured plasma creatinine levels (paired  $t$  test). The maximal deviation between measured and calculated concentrations was 0.042 mg/dL, corresponding to 5.21%. The median of the differences was  $-0.0007$  mg/dL.

To improve the accuracy of  $c_{\text{Capillary Blood}}/c_{\text{Plasma}}$ , the ratio was calculated based on the data of all 70 study patients. The resulting conversion factor  $F$  of  $0.916 \pm 0.088$  displayed a slightly improved relative standard deviation of 9.58%. However, the interquartile range of the differences between calculated versus measured values was clearly smaller for  $F$  ( $-0.047$  to  $0.056$  mg/dL) compared to  $F_0$  ( $-0.098$  to  $0.085$  mg/dL) (Electronic supplement Fig. S1). Plotting measured against the calculated concentrations of creatinine in plasma showed a significant linear correlation ( $r = 0.944$ ;  $\alpha = 0.05$ ). The Bland-Altman plot revealed that the mean of the deviations of all data was 0.001 mg/dL (Fig. 1; line A) and that 95% of all results were distributed within the mean  $\pm 1.96$  standard deviations (0.165 mg/dL and  $-0.163$  mg/dL) of the differences (Fig. 1; lines B and C).

### Renal function estimation and the “triple whammy”

The plasma creatinine concentrations of all patients ( $n = 153$ ) were calculated based on the capillary blood concentration using the conversion factor  $F = 0.916$ . Patients' C&G  $Cl_{\text{crea}}$  ranged from 21.6–155.9 mL/min (median 67.46 mL/min; Table 2).

The serum creatinine concentrations of patients with a complete medication plan ( $n = 142$ ) were analyzed. Patients taking two or three target drugs (diuretics, NSAIDs, RAS

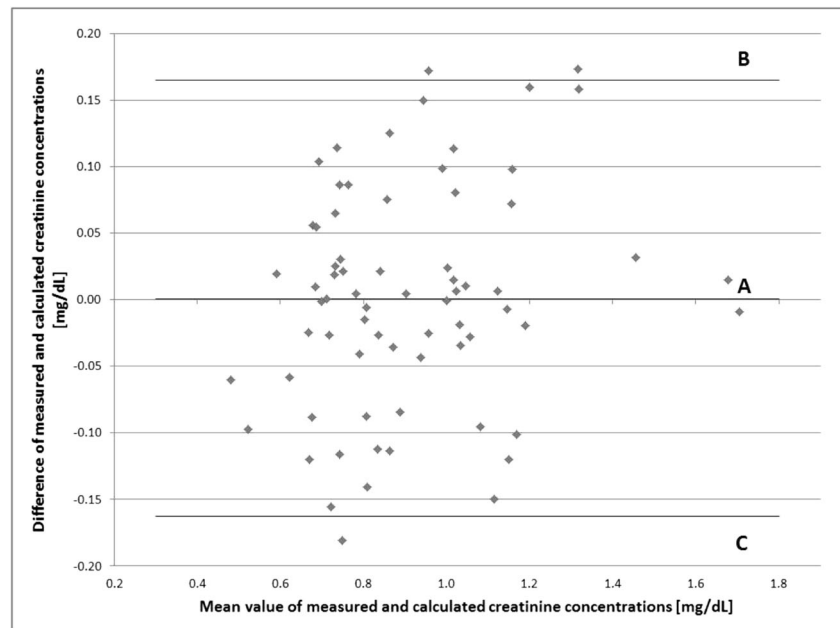
**Table 1** Descriptive patient characteristics. There were no statistically significant differences between the study groups regarding BMI and number of drugs used, but a slight, statistically significant difference in age ( $t$  test)

	Enabling study ( $n = 70$ )	Field study ( $n = 84$ )	Statistical difference	Summary data ( $n = 154$ )
Age: mean $\pm$ SD (years)	$64.6 \pm 12.1$	$68.9 \pm 11.2$	$p = 0.02$	$67.0 \pm 11.8$
Age range (years)	32–86	35–88	–	32–88
Median age (years)	66	70	–	69
Sex	34 F/36 M	39 F/45 M	–	73 F/81 M
BMI: mean $\pm$ SD (kg/m <sup>2</sup> )	$30.0 \pm 6.0$	$30.3 \pm 5.1$	n.s.	$30.2 \pm 5.5$
BMI range (kg/m <sup>2</sup> )	19.8–54.7	20.1–42.2	–	19.8–54.7
Median BMI (kg/m <sup>2</sup> )	29	29.4	–	29.6
Number of drugs used: mean $\pm$ SD	$6.7 \pm 3.6$	$6.7 \pm 3.6$	n.s.	$6.4 \pm 3.2$
Range drug number	1–21	1–14	–	1–21
Median drug number	6	6	–	6

SD standard deviation, BMI body mass index, n.s. not statistically significant, F female, M male

– not determined

**Fig. 1** Bland-Altman plot to compare measured and calculated plasma concentrations of creatinine in the enabling study ( $n = 70$ ). Line A represents the mean value of the differences of the concentrations, B and C represent the mean  $\pm 1.96$  standard deviations of the differences between measured and calculated concentrations. More than 95% of the differences were located within this range



blockers) had a significantly lower  $Cl_{Crea}$  compared to patients without target drug prescription ( $t$  test,  $p < 0.05$ ; Fig. 2).

## Metformin

Metformin was prescribed to 139 patients, and DBS concentrations were quantified in all but two patients ( $n = 137$ ). Based on the prescription history, all patients were assumed to be in the steady state. Metformin concentrations ranged from 0.23  $\mu\text{g/mL}$  to 4.99  $\mu\text{g/mL}$  after daily intake of 500–2250 mg metformin with a median dose of 2000 mg (Table 2). The mean concentration ( $\pm$  SD) was  $2.22 \pm 1.16$   $\mu\text{g/mL}$ .

The interval to the last metformin intake ranged from 30 to 1545 min (0.5–25.75 h). Varying intervals allowed the estimation of the elimination kinetics of metformin after dose normalization to 2000 mg. Metformin then ranged between 0.35

and 7.26  $\mu\text{g/mL}$  with mean levels of  $2.69 \pm 1.34$   $\mu\text{g/mL}$ . The elimination half-life of metformin for patients with  $Cl_{Crea}$  above 60 mL/min was 12 h (714 min) and 18.5 h (1111 min) for the patients with the lower  $Cl_{Crea}$ .

Both the interval to the last metformin intake and the estimated  $Cl_{Crea}$  revealed a weak but significant correlation with the normalized concentration of metformin (Table 3; Pearson correlation;  $p < 0.05$ ; interval to last intake  $r = -0.319$ ,  $Cl_{Crea}$   $r = -0.368$ ). If patients with  $Cl_{Crea}$  lower and higher 60 mL/min were analyzed separately, the normalized concentration of metformin correlated stronger with the interval to the last metformin intake. The coefficient of correlation was  $r = -0.389$  ( $p < 0.05$ ) for patients with higher  $Cl_{Crea}$  ( $n = 78$ ) and  $r = -0.439$  ( $p < 0.05$ ) for patients with  $Cl_{Crea}$  lower than 60 mL/min ( $n = 57$ ). Metformin levels also significantly correlated with the patients' age ( $p < 0.05$ ,  $r = 0.259$ ).

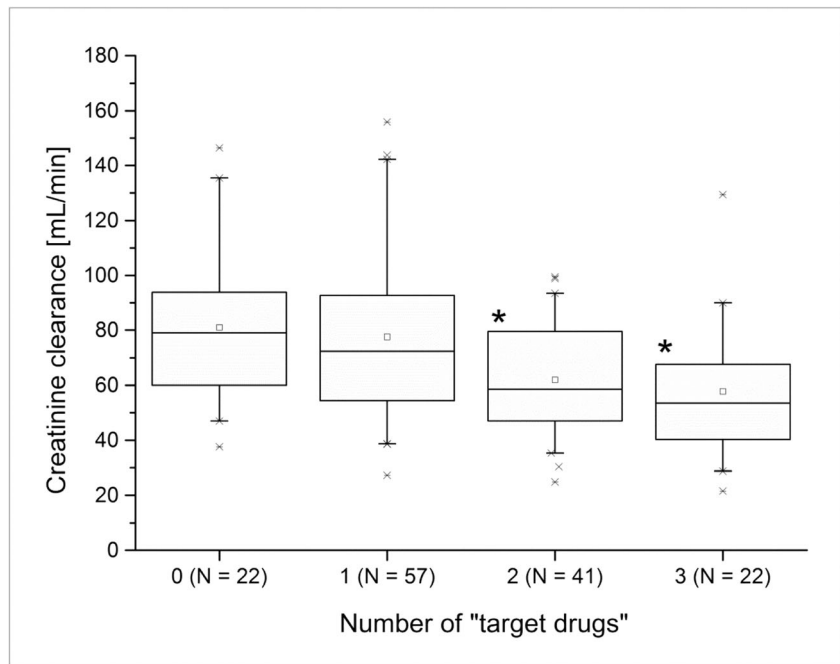
**Table 2** Estimated creatinine clearance (C&G  $Cl_{Crea}$ ) and measured concentrations of metformin and sitagliptin from dried blood spots (DBS). C&G  $Cl_{Crea}$  was calculated using the determined conversion factor of 0.916. Variations in population size is due to different numbers of patients taking metformin and/or sitagliptin and missing values ( $Cl_{Crea}$ :  $n = 1$ ; metformin:  $n = 2$ , sitagliptin:  $n = 2$ ) due to analytical problems

	$Cl_{Crea}$ (mL/min)	Metformin ( $\mu\text{g/mL}$ )	Sitagliptin (ng/mL)
Population	$n = 153$	$n = 137$	$n = 67$
Mean value $\pm$ SD	$71.22 \pm 27.0$	$2.22 \pm 1.16$	$432.2 \pm 268.8$
Range	21.6–156.0	0.23–4.99	11.1–995.6
Median value	67.46	2.01	388.9
Interval drug intake–DBS sampling (min)	n.a.	30–1545	35–1545
Dose range (mg)	n.a.	500–2550	25–100
Mean dose (mg)	n.a.	$1688.5 \pm 464.8$	$95.8 \pm 15.6$
Median dose (mg)	n.a.	2000	100
Estimated $t_{1/2}$ (h)*	> 60 mL/min	11.9 ( $n = 78$ )	8.4 ( $n = 40$ )
	< 60 mL/min	18.5 ( $n = 57$ )	13.0 ( $n = 24$ )

n.a. not applicable, SD standard deviation

\*After dose normalization to 2000 mg metformin and 100 mg sitagliptin

**Fig. 2** Combined therapy with diuretics, nonsteroidal anti-inflammatory drugs, inhibitors of the renin-angiotensin system (“target drugs”) and estimated creatinine clearance of the diabetic patients. Taking two or three target drugs correlated with a significant lower renal function compared to taking none of the drugs (\* $p < 0.05$ )



### Sitagliptin

Sitagliptin was prescribed to 69 patients and concentrations were quantified in all but two patients ( $n = 67$ ) whose samples revealed concentrations below the LLOQ of 3 ng/mL or above of the validated range, respectively. The concentrations ranged from 11.12–995.6 ng/mL after daily intake of 25–100 mg metformin (median dose 100 mg). The mean concentration ( $\pm$  SD) was  $432.2 \pm 268.8$  ng/mL. The high variability of capillary blood concentrations was also seen after dose normalization to 100 mg sitagliptin per day, ranging between 11.12 and 1226 ng/mL with mean levels of  $458.4 \pm 289.1$  ng/mL.

The interval to the last sitagliptin intake ranged from 35 to 1545 min (0.6–25.75 h). The elimination half-life of sitagliptin for patients with  $Cl_{Crea}$  above 60 mL/min was 8.4 h (505 min) and 13 h (779 min) for the patients with the lower  $Cl_{Crea}$ .

Sitagliptin concentrations showed a significant weak correlation with the  $Cl_{Crea}$  (Table 3; Pearson correlation;  $p < 0.05$ ;  $r = -0.294$ ) and a stronger correlation with the interval to the last intake (Pearson correlation;  $p < 0.05$ ;  $r = -0.522$ ;  $n = 64$

**Table 3** Statistically significant ( $p < 0.05$ ) correlations (Pearson correlation) among the patient measures. Other parameters (BMI, glucose concentration,  $HbA_{1c}$ ) revealed no significant correlations

	Metformin	Sitagliptin
$Cl_{Crea}$	-0.368	-0.294
Interval intake-sampling	-0.319	-0.522
Age	0.259	/
Hematocrit	/	0.396

patients). Sitagliptin capillary blood concentrations and the interval to the last intake correlated stronger for patients with higher  $Cl_{Crea}$  ( $n = 40$ ;  $r = -0.570$ ,  $p < 0.05$ ) compared to patients with  $Cl_{Crea}$  lower than 60 mL/min ( $n = 24$ ;  $r = -0.479$ ,  $p < 0.05$ ). Sitagliptin levels also significantly correlated with the patients’ hematocrit ( $p < 0.05$ ,  $r = 0.396$ ; hematocrit concentrations were available for  $n = 35$  patients).

### Discussion

In the present cross-sectional study, we investigated the feasibility of using dried blood spots (DBS) for concentration monitoring of predominantly renally eliminated anti-diabetic drugs in relation to the estimated creatinine clearance (C&G  $Cl_{Crea}$ ).

For calculating plasma creatinine levels based on DBS samples, a conversion factor was established based on the strategy described for the development of the MDRD formula [20]. Therefore, the patient data set was split at a ratio of 65%/35% to be used for development and verification of an initial factor ( $F_0$   $0.918 \pm 0.108$ ) which was optimized using the whole data set, yielding the improved conversion factor ( $F$   $0.916 \pm 0.088$ ).

There are controversial reports about the deviation of creatinine concentrations determined in plasma and venous or capillary blood samples. Higher mean creatinine concentrations in capillary dried blood spots compared to plasma samples have been communicated [21, 22]. In contrast, others found lower creatinine levels in capillary blood [23] or dried venous blood samples [24] in relation to plasma concentrations which is consistent with the results of the present study.



The patients' renal function was calculated using the Cockcroft-Gault formula and the patients' ideal body weight, which has been shown to yield best results [5]. The estimated C&G  $Cl_{crea}$  revealed a decrease by 1.26 mL/min per year in the diabetic patients in the current study. This is consistent with a yearly decline of 1.18 mL/min reported for a cohort of 230 subjects with different comorbidities such as hypertension, diabetes mellitus, or impaired fasting glucose [25]. Most of the diabetes patients in the present study also suffered from hypertension as comorbidity.

A reduced glomerular filtration rate due to glomerular hemodynamic alterations has been reported for patients concomitantly taking non-steroidal anti-inflammatory drugs (NSAIDs), diuretics, and inhibitors of the renin-angiotensin system (RAS) ("target drugs"). This association has been termed "triple whammy effect" [16, 18]. Although a polypharmacotherapy can also be the consequence of an impaired renal function, the mechanism of kidney injury caused by a combination of particular drug classes has been analyzed in detail [18]. The number of patients taking no/1/2/3 target drugs in our study (22/57/41/22) was lower than in other clinical investigations (e.g. 135/87/60/19; [16]). However, the correlation of lower  $Cl_{crea}$  and number of target drugs was still statistically significant. Though this does not prove a causality the results are consistent with the described "triple whammy effect".

To the best of our knowledge, capillary blood concentrations for metformin have not been reported. However, it is well-known that metformin significantly distributes into erythrocytes and therapeutic values for plasma and erythrocyte concentrations have been published [9, 10, 13]. In the present study, analysis of metformin in DBS revealed mean capillary blood concentrations of  $2.22 \pm 1.16$   $\mu\text{g/mL}$  (median 2.01  $\mu\text{g/mL}$ ) which approximate published values if concentrations suggested for plasma ( $0.5 \pm 0.4$   $\mu\text{g/mL}$ ) and erythrocytes ( $0.8 \pm 0.4$   $\mu\text{g/mL}$ ) were combined [9]. Metformin concentrations determined under "real-life" use of the drug were reported as  $2.7 \pm 7.3$   $\mu\text{g/mL}$  in plasma and  $2.0 \pm 4.4$   $\mu\text{g/mL}$  in erythrocytes, and values up to 5  $\mu\text{g/mL}$  in plasma were assessed as "slightly to moderately elevated" [10]. This would cover the range of DBS concentrations (0.23 and 4.99  $\mu\text{g/mL}$ ) in the present study, allowing for the conclusion that most of the patients were within the therapeutic range.

The negative correlation between  $Cl_{crea}$  and plasma metformin concentrations was known [26, 27] and also observed in our study. Although pharmacokinetic parameters depend on the eGFR (corresponding to the  $Cl_{crea}$ ) according to a continuous function, we chose dichotomous categories with a threshold of 60 mL/min. This value has been meeting the National Kidney Foundation's definition of a chronic kidney disease and until recently metformin use has been contraindicated in patients with an eGFR below this threshold [8]. Based on the estimated  $Cl_{crea}$  and the elimination calculated using

population pharmacokinetic methods, a prolonged metformin half-life of 18.5 h was determined in patients with  $Cl_{crea}$  below 60 mL/min. In contrast, patients with higher  $Cl_{crea}$  revealed a  $t_{1/2}$  of about 12 h. These elimination half-lives are considerably longer than the reported 5–6 h [6, 28], but consistent with the published  $t_{1/2}$  of 11–14 h [29]. In the present study the elimination was determined based on capillary blood, not on plasma samples. Due to the distribution of metformin into erythrocytes serving as an additional compartment, a longer  $t_{1/2}$  in capillary blood as compared to plasma should be expected.

We are not aware of any published full or capillary blood concentrations of sitagliptin. Peak plasma concentrations of sitagliptin in the steady state levels were reported to be 383 ng/mL (941 nM) after 100 mg [30] and 167–174 ng/mL after 50 mg doses [29]. Sitagliptin also distributes into erythrocytes [12], and thus, mean DBS concentrations of  $432.2 \pm 268.8$  ng/mL detected in the present study are consistent with the documented range. In contrast to published data, sitagliptin concentrations in the present study displayed a pronounced variability; they ranged between 11.1 and 995.6 ng/mL.

Compared to metformin, sitagliptin values showed a weaker negative correlation with  $Cl_{crea}$ . Yet, the calculated  $t_{1/2}$  of sitagliptin was clearly longer in patients with  $Cl_{crea}$  below 60 mL/min (13 h) compared to patients with higher  $Cl_{crea}$  (8.4 h). This is consistent with reported plasma half-lives between 10 and 14 h [11, 29, 30]. Apparently, the distribution of sitagliptin into erythrocytes does not significantly influence its  $t_{1/2}$ .

A limitation of this study is that only a single DBS sample was collected per patient, and thus, the individual pharmacokinetics could not be determined. However, after demonstrating the general feasibility of the approach, this can be realized in future studies. Another limitation was that we did not compare the capillary blood concentrations of metformin and sitagliptin with simultaneously obtained venous blood levels of the same patients. Lastly, we had to trust the patients' medication plans being complete and up-to-date for prescribed and OTC medications.

To summarize, for the first time, we report DBS concentrations of metformin and sitagliptin in relation to the patients'  $Cl_{crea}$ . The diabetic patient population was highly heterogeneous including patients of a wide range of age, BMIs, and varying extent of polypharmacotherapy, so that the results can be regarded as "real-life" data. The fact that the determined drug concentration ranges are consistent with data published for plasma and the detail that we uncovered are indications for the "triple whammy" effect in the study population suggest that DBS can be used for concentration monitoring of predominantly renally eliminated drugs.

In the field study part, DBS samples were collected in community pharmacies. There are sparse reports about such an approach [31]. The present study confirms that community pharmacists can provide a valuable contribution to DBS sampling.

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## Compliance with ethical standards

Patients were recruited and enrolled in the study after written informed consent. The study was approved by the ethics committee of the Medical Faculty of the University of Würzburg (reference number 287/14) and the Freiburg Ethics Committee International (FEKI) and registered by ISRCTN (ISRCTN14518136).

**Conflict of interests** The authors declare that they have no conflict of interest.

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