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Analysis of capillary microsamples obtained from a skin-prick to measure vancomycin concentrations as a valid alternative to conventional sampling: a bridging study

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Highlights

- Bridging study conducted in critically ill patients
- Measurement of vancomycin in plasma sampled by finger prick collected as capillary microsample and by arterial cannula collected as conventional sample.
- No significant bias and strong correlation were found between both sampling methods.
- Capillary microsampling may serve as alternative to conventional sampling to support clinical studies.

Abstract

A bridging study is presented to investigate the applicability of measuring vancomycin concentrations obtained by finger-prick. A total of 25 paired plasma samples, collected from finger prick as capillary microsampling and arterial plasma samples collected from an indwelling cannula as conventional sampling, were obtained from critically ill patients receiving vancomycin. The maximum concentration (C_{max}) and the minimum concentration (C_{min}) measured were 66.2 mg/L and 29.7 mg/L for capillary microsampling and 78.9 mg/L, 25.6 mg/L for conventional sampling, respectively. The area under the concentration-time curve from 0 to 6 hours ($AUC_{0\text{-}6h}$) ranged between 94.8 and 269 mg/L.h for capillary microsampling and from 106 and 303 mg/L.h for conventional sampling. The comparative study conducted was assessed using three different statistical approaches: Bland-Altman and Passing-Bablok regression analyses and the USFDA criterion for the incurred sample reanalysis. The results of this analysis revealed no significant bias and a strong correlation between both sampling methods, with 95% of the calculated concentrations from the paired plasma samples laying within 20% of difference of the mean. This bridging study

verifies that capillary microsampling may serve as an alternative to conventional sampling techniques to support clinical applications for measuring vancomycin concentrations in plasma.

Key words: Vancomycin, bridging study, capillary microsampling, LCMS/MS

1. Introduction

Vancomycin is a glycopeptide antibiotic used to treat infections caused by Gram-positive organisms, such as methicillin-resistant *Staphylococcus Aureus* (MRSA) and penicillin-resistant *Corynebacterium jeikeium*, *Streptococcus pneumoniae* and *Clostridium difficile* [1,2]. The World Health Organisation has identified the optimal use of antibiotics as a core objective to combat antibiotic resistance [3]. Sub-optimal dosing of vancomycin can result in either supra-therapeutic concentrations, which is associated with nephrotoxicity [4-6], or sub-therapeutic concentrations, potentially increasing the risk of antibiotic resistance [7-9]. Monitoring vancomycin concentrations and pharmacokinetic studies are performed to optimise the dosing of vancomycin, particularly in patient groups known to commonly experience altered pathophysiology (such as critically ill patients) [10].

Conventional sampling techniques usually involve sampling relatively large volumes of blood (between 1 and 5 mL) obtained using arterial or venous cannula or direct venopuncture [11]. The frequency and volume of blood sampled can impact on the pathophysiology and/or the well-being of a patient. Using samples collected via microsampling techniques to describe the pharmacokinetics of a drug has gained interest as an alternative to conventional blood sampling [11,12]. Microsampling collects less than 50 µL of biological fluids by using minimally invasive procedures, such as a skin prick (usually taken from a finger or heel) [13].

To the best of our knowledge, we are the first to describe the application of a procedure to quantify vancomycin in plasma sampled by finger prick collected as capillary microsampling. The aim of this comparative study was to investigate the correlation between vancomycin

concentrations obtained in plasma samples collected by finger-prick as capillary microsampling, and samples collected by arterial cannula as conventional sampling.

2. Experimental

2.1 Reagents and materials

Vancomycin hydrochloride was purchased from Aspen Pharmacare (St Leonards, Australia), and the internal standard deuterated vancomycin TFA salt ([$^2H_{12}$] – vancomycin) was purchased from Alsachim (Illkirch, France). Acetonitrile was LC/MS grade, and formic acid was analytical grade; both chemicals were obtained from Fisher Chemicals (Fairlawn, New Jersey, USA). The ultrapure water was acquired using a Permutit system (resistivity at 25 ˚C < 18 ΩM). Drug-free plasma was purchased from and from Innovative Research (Novi, Michigan, USA). Heparinized capillary tubes (50 µL) were supplied by Radiometer Pacific Pty Ltd. (Mt Waverley, Australia). Heparinized vacuum tubes were supplied by Greiner Bio-One, Vacuette® liHep (Hieldelberg, Australia).

2.2 Preparation of standard solutions and quality controls

The aqueous calibration stocks were prepared by dilution of formulated standard material with water to yield concentrations ranging from 1 to 100 mg/L. In processing the batch, the aqueous calibration solutions were combined with an equal volume of blank plasma to generate calibration standards. Aqueous calibration solutions were stored at -80°C in cryovial tubes.

The [$^2H_{12}$] – vancomycin (internal standard solution) was prepared in water at 100 mg/L. The solution was stored at -20°C.

The quality control samples were prepared from a stock solution that contained 10,000 mg/L of vancomycin in water. The quality control samples were prepared by diluting the stock solution with blank plasma to obtain vancomycin concentrations at 3, 10 and 80 mg/L. All quality control samples were processed alongside the clinical samples.

2.3 Analysis of samples

The liquid plasma samples were analyzed using a previously developed and validated method by Parker et al. [14], in accordance with the guidelines provided by the European Medicines Agency (EMA) [15] and the U.S. Food and Drug Administration (USFDA) [16]. Briefly, an aliquot of 10 µL of plasma (test sample, quality control sample, or blank plasma for calibrators and blanks) was combined with 10 µL of water (or aqueous calibration solution for preparation of calibration standards). [$^{2}H_{12}$] – vancomycin (5 µL) was added to all samples, with the exception of a drug-free plasma blank sample. Samples were vortex mixed for 5 seconds. Acetonitrile (40 µL) was then added to all samples in the batch, which was then vortex mixed. The samples were centrifuged at 12,000 x *g* for 5 minutes to precipitate proteins. Then, 0.5 µL of the resulting supernatant was injected onto a Shimadzu Nexera2 LC equipped with a Shimadzu 8030+ triple quadrupole mass spectrometer (MS) detector. The mobile phase A consisted of 30% acetonitrile, 10% acetone, 60% water and 0.1% formic acid (v/v), and the mobile phase B was 70% acetonitrile, 10% acetone, 20% water and 0.1% formic acid (v/v). The stationary phase was a SeQuant Zic-HILIC 2.1 x 20 mm (5.0 µm) analytical guard column (Merck, Darmstadt, Germany). Gradient-effected separations were between 100% and 0% of mobile phase B over 4.5 minutes at a flow rate of 0.3 mL/min, producing a back pressure of approximately 600 psi. The elution time was 1.6 minutes. The quantification of vancomycin and [$^{2}H_{12}$] – vancomycin was performed using

electro-spray ionization (ESI) source interface operated in positive-ion mode for the multiple reaction monitoring (MRM) analysis of *m/z* 725.1→144.1 and 746.1→144.2, and *m/z* 730.8 →144.1, respectively.

2.4 Bridging study

This study was approved by the Human Research & Ethics Committee of the Royal Brisbane and Women's Hospital for the project (HREC/15/ QRBW/249). Five patients receiving intravenous vancomycin (as prescribed by the treating physician) and admitted to the intensive care unit at the Royal Brisbane and Women's Hospital were eligible for the study.

Prior to sampling, written informed consent was obtained from either the patient or a close relative. Paired whole blood samples were simultaneously collected at five pre-defined time points: prior to administration of the vancomycin dose (time 0) and then at approximately one, two, four and six hours post-administration of the vancomycin dose. The sample collection process produced two sets of liquid plasma samples: one capillary microsample and one conventional plasma sample. For capillary microsamples, the patient's finger was cleaned with alcohol and puncture was performed using a lancet device. The finger was gently squeezed and held low below the heart of the patient until approximately 50 µL of whole blood was collected into a heparinized capillary tube. The whole blood was centrifuged at 2000 g for 10 minutes to obtain plasma. For conventional plasma samples, approximately 2 mL was obtained from an indwelling arterial cannula and was collected into a heparinized 5 mL vacuum tubes and centrifuged at 1500 *g* for 10 minutes to obtain plasma. After centrifugation, all plasma samples were transferred into screw-capped 2 mL polypropylene tubes and stored at -80°C until analysis.

2.5 Data analysis

The concentration of each clinical sample was obtained using the data from a matrix-matched calibration curve that had been prepared within the batch. The mathematical basis of the quantification was applied as a linear regression with peak-area ratio (drug/internal standard response) against concentration (x), with a $1/x^2$ weighting. The capillary microsampling and conventional sampling concentrations were compared using Bland-Altman and Passing-Bablok regression analyses, performed using XSTAT for Microsoft Excel® (XSTAT Software, version 2018.6, New York, United States).

3. Results and discussion

A validated LCMS-MS method was successfully applied to measure vancomycin in capillary microsamples and conventional liquid plasma samples [14]. Twenty-five paired plasma samples collected using both capillary microsampling and conventional sampling techniques were obtained from five critically ill patients receiving vancomycin. Five capillary microsamples were lost during sample collection either due to an inability to collect a peripheral sample from the patient or loss during centrifugation.

The maximum concentrations (C_{max}), the minimum concentrations (C_{min}) and the areas under the concentration-time curve from 0 to 6 hours ($AUC_{0\text{-}6h}$) for five patients receiving vancomycin and for both capillary microsampling and conventional sampling are illustrated in Table 1. No significant differences were observed between the results of both sampling techniques. An example of a plasma concentration – time profile from one patient is presented in Figure 1.

The degree of error between the results obtained from capillary microsampling compared with those obtained by conventional sampling was assessed using Passing-Bablok analysis [17], in which the plasma concentrations calculated from capillary microsamples were plotted against measured conventional plasma concentrations (Figure 2). The assessment of the width of the 95% of the confidence interval (CI) provided a description of the variability of the results from the mean, indicating good overall agreement as values one and zero are included in the 95% CI of the slope (0.85 to 1.19) and the intercept (-6.43 to 3.41), respectively. Therefore, no proportional or constant errors were found in the comparison of the sampling methods.

A Bland-Altman comparison analysis [18,19] was also used to calculate the agreement between measured vancomycin concentrations collected from finger-prick using capillary microsampling and from samples obtained from an indwelling cannula using conventional sampling. The agreement was calculated using the bias described as the mean of the difference between measurements, the precision described as the standard deviation (SD) of the differences between measurements and the upper and lower limits of agreement (set at 95% confidence interval, CI) based on the SD and set to ±1.96 from the bias (Figure 3). The mean difference is less than 1 μg/mL (-0.52 [95% CI: -2.94 to 1.91]), lower limit of agreement (95% CI: -10.7) and upper limit of agreement (95% CI: 9.64) with a value of zero included in the 95% CI. These results reveal no significant bias and a strong correlation between the calculated capillary microsampling vancomycin concentrations and the conventional plasma vancomycin concentrations.

The USFDA guidelines recommend that a minimum of 20 paired samples are used in conducting a bridging study to evaluate the agreement between microsampling and

conventional sampling [16]. The criterion recommended by the FDA guidelines is the incurred samples reanalysis, where a minimum of 67% of the samples the percentage difference between the results obtained by the two methods for comparison should be within 20% of the mean. This criterion was satisfied, as 20 paired samples were collected in this bridging study and 95% of the paired samples were within 20% of the mean value of vancomycin plasma concentrations obtained from capillary microsampling and conventional sampling.

The data obtained in this bridging study demonstrates a good correlation between samples obtained by finger prick using capillary microsampling and samples obtained from an indwelling arterial cannula using conventional sampling for the measurement of vancomycin concentrations.

4. Limitations

It is important to recognize that some limitations remain as there is some complexity of obtaining multiple capillary microsamples from a finger prick to conduct pharmacokinetic studies [20,21], especially for critically ill patients. Some of the samples from our patients were taken by repeated puncture of the same finger, and in some cases, it was necessary to gently squeeze the puncture side until enough blood was collected in the capillary tube.

Despite these limitations, the correlation between plasma concentrations obtained by capillary microsampling and conventional sampling remained high, suggesting that for this particular drug, capillary microsamples can be collected by repeated punctures on the same finger and/or by gently squeezing the finger to obtain the whole blood sample.

5. Conclusions

We successfully applied a validated method to measure vancomycin concentrations as microsamples collected using a finger-prick in a clinical environment. We were able to correlate measured vancomycin concentrations in samples collected using finger-prick by capillary microsampling to plasma samples collected using an indwelling arterial cannula by conventional sampling. The results support the use of capillary microsampling to support clinical studies, particularly in patients for whom conventional sampling methods may impose a burden.

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Figure 1. Plasma vancomycin concentrations (mg/L) versus time (min) for a critically ill patient with samples collected using a finger-prick as a capillary microsample (solid line) and from an arterial cannula using conventional sampling (dotted line).

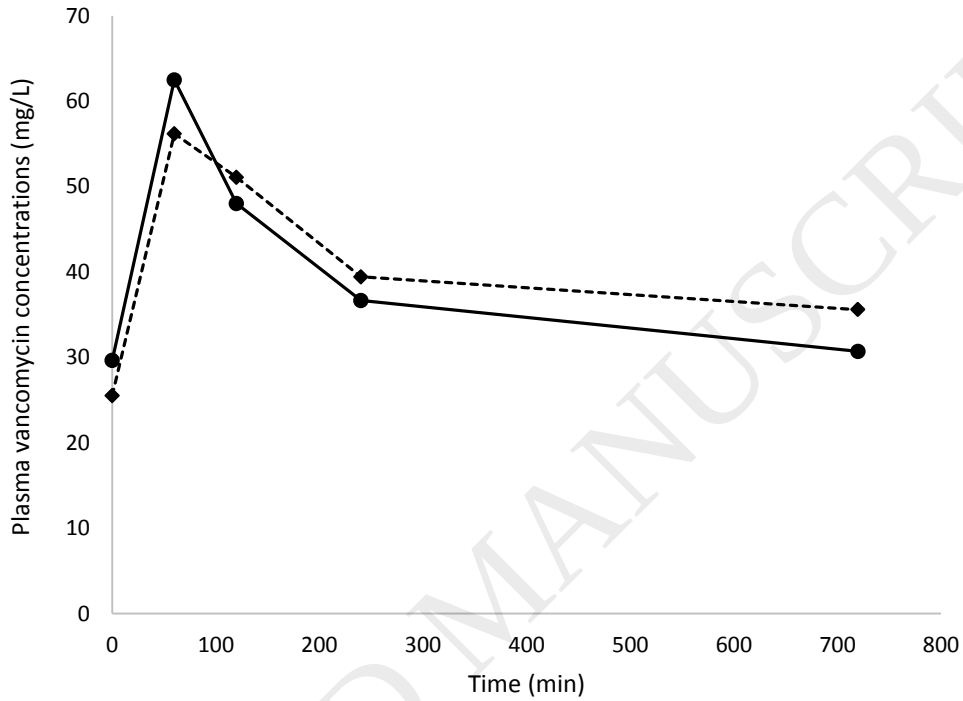

Figure 2. Passing-Bablok regression analysis plotting vancomycin concentrations obtained from a finger-prick using a capillary microsample (CMS) versus those obtained from an arterial cannula using conventional sampling. The solid line is the slope and intercept of the regression line. The dash line represents the 95% confidence interval. The dotted line is the identity line.

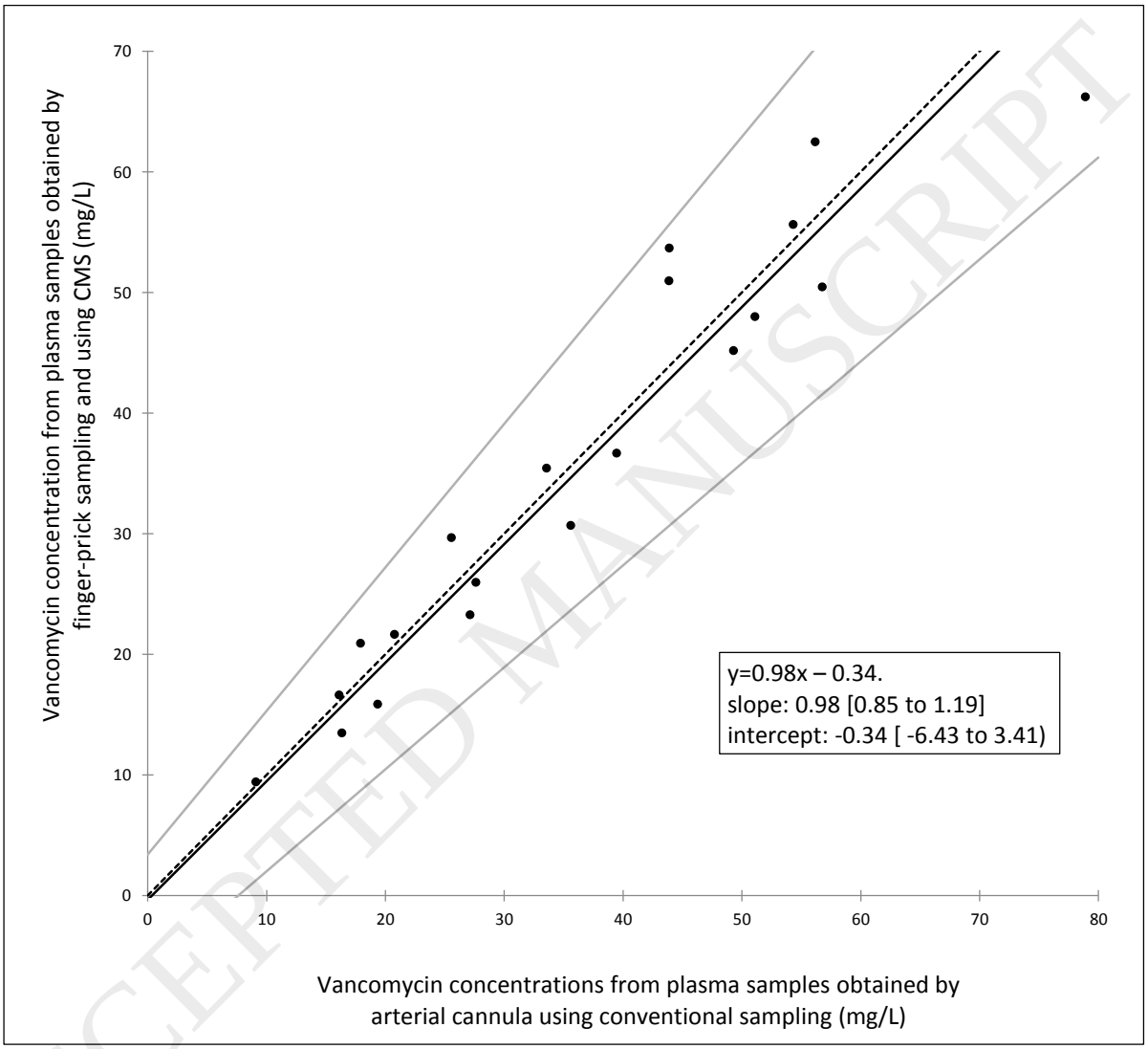

Figure 3. Bland-Altman analysis plotting the differences between liquid plasma vancomycin concentrations obtained from a finger-prick using a capillary microsample (CMS) from those obtained from an arterial cannula using conventional sampling, (*y*-axis) against the average of both sampling techniques (*x*-axis). Mean difference and the upper limit of agreement (ULA) and lower limit of agreement (LLA) set to ±1.96 SD with its 95% confidence interval (CI).

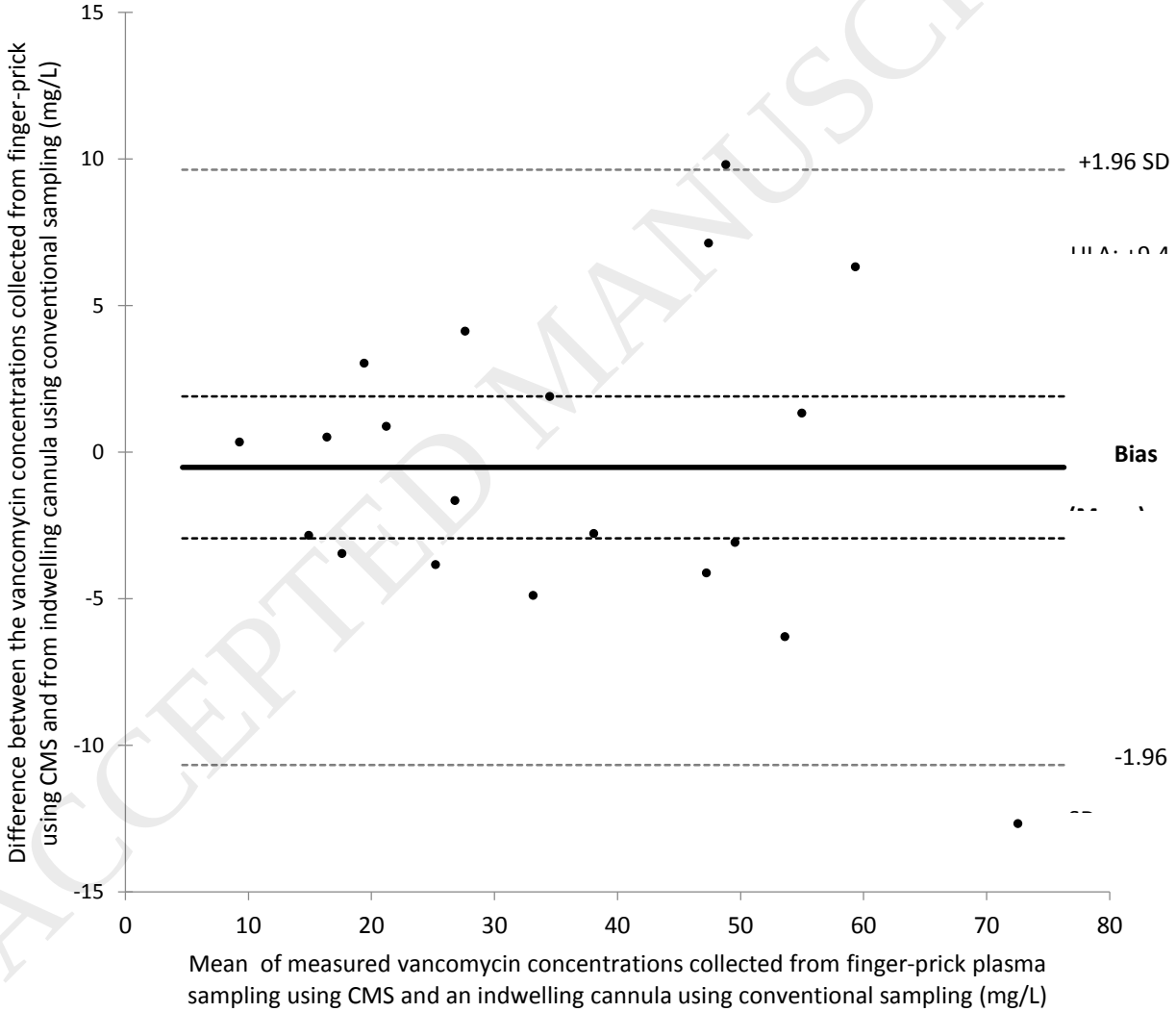

Table 1. C_{max}, C_{min} and AUC from 0 to 6 hours for five critically ill patients receiving vancomycin

Subject	Capillary microsampling			Conventional sampling		
	C_{max} (mg/L)	C_{min} (mg/L)	$AUC_{0\text{-}6h}$ (mg/L.h)	C_{max} (mg/L)	C_{min} (mg/L)	$AUC_{0\text{-}6h}$ (mg/L.h)
1	62.5	29.7	253	56.2	25.6	260
2	26.0	15.9	94.8	27.6	19.4	106
3	66.2	13.5	269	78.9	16.3	303
4	53.7	9.4	266	43.9	9.1	233
5	55.6	ND	ND	54.3	ND	ND

ND: no data available, only one sample was obtained for this patient