



Lack of penetration of amikacin into saliva of tuberculosis patients

To the Editor:

In the 2016 update of the World Health Organization treatment guideline for drug-resistant tuberculosis (TB), a shorter multidrug-resistant TB regimen was proposed because of its higher treatment outcomes [1]. However, therapeutic drug monitoring (TDM) is an excellent method to improve clinical outcomes as well and its practice is on the rise [2]. A well-known side-effect of group B injectable anti-TB drugs (e.g. amikacin) is ototoxicity [3]. TDM could also be a solution to minimise side-effects by lowering the drug exposure [4]. In the study by VAN ALTENA *et al.* [5], TDM was practised using the ratio of peak concentration (C_{max}) to minimal inhibitory concentration (MIC) and this resulted in a reduction in patients with hearing loss. Saliva is considered as an alternative matrix for TDM because it is easy, noninvasive and more patient friendly to sample [6]. Studies found a limited penetration of gentamycin and tobramycin into saliva [7], while detectable levels of amikacin in saliva of neonates were reported [8]. Given the low penetration of aminoglycosides into saliva and interest in C_{max} for TDM of amikacin, our objective was to study whether the salivary C_{max} of amikacin is measurable and useful in salivary TDM.

TB patients from the Tuberculosis Center Beatrixoord (Haren, The Netherlands) who were 18 years or older, who used amikacin as part of their TB treatment and in whom TDM using blood samples was routinely performed were eligible for inclusion. Written informed consent was obtained. This study was approved by the Ethical Review Committee of University Medical Centre Groningen (IRB 2016/069) and registered at Clinicaltrials.gov (NCT03080012).

Conventional TDM was part of routine treatment. Salivary samples were taken simultaneously with blood samples before, and 1, 2, 3, 4 and 8 h after administration.

After rinsing their mouth with water, the patients chewed on two cotton rolls (Orbis Dental, Münster, Germany) for 2 min. The cotton rolls were each placed in a 5-mL syringe connected to a membrane filter with pore size $\leq 0.22 \mu\text{m}$ (Millex-GP; Merck Milipore, Carrigtwohill, Ireland). Membrane filtration was used to decontaminate the saliva samples for laboratory safety reasons, as saliva of infectious TB patients contains *Mycobacterium tuberculosis* [9]. Saliva and serum samples were stored at -20°C until analysis. The recovery of the described saliva sampling method was determined in five-fold using solutions of amikacin in pooled saliva of $5 \text{ mg}\cdot\text{L}^{-1}$ and $20 \text{ mg}\cdot\text{L}^{-1}$.

Both saliva and blood samples were analysed with a calibrated particle-enhanced turbidimetric inhibition immunoassay (Architect; Abbott Diagnostics, Lake Forest, IL, USA) using the amikacin reagent kit 6L3520 (Multigent; Abbott Diagnostics). The lower limit of quantification (LLOQ) of the analytical method was $2.0 \text{ mg}\cdot\text{L}^{-1}$. Quality control samples of amikacin in pooled saliva were prepared at concentrations of $5 \text{ mg}\cdot\text{L}^{-1}$ and $20 \text{ mg}\cdot\text{L}^{-1}$.

In total, six TB patients (five males and one female) with a median (interquartile range) age of 47 (32–59) years, body weight of 65.4 (57.3–76.2) kg, creatinine clearance of 95 (69–106) $\text{mL}\cdot\text{min}^{-1}\cdot 1.73 \text{ m}^{-2}$ and amikacin dose of 7.19 (6.44–7.30) $\text{mg}\cdot\text{kg}^{-1}$ bodyweight were included in this study. All patients were treated with amikacin for more than 14 days before sampling. All *M. tuberculosis* isolates had MIC values of $1.0 \text{ mg}\cdot\text{L}^{-1}$. The recovery of the saliva sampling method was determined to be 42.9% with a coefficient of variation of 9.2%. The amikacin concentrations of the quality control samples were within an acceptable range of error (6.5–9.8%). The amikacin concentrations, including C_{max} , were not detectable in the saliva

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Salivary C_{max} TDM of amikacin was not feasible in TB treatment due to the very low penetration into saliva <http://ow.ly/d6v230h0bWG>

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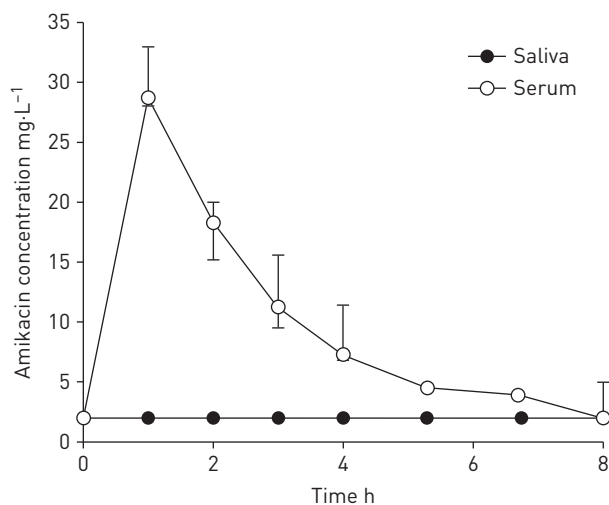


FIGURE 1 Median (interquartile range) amikacin concentration–time curves in serum and saliva of tuberculosis patients (n=6).

samples of the patients and did not exceed the LLOQ of 2.0 mg·L⁻¹, whereas amikacin could be quantified in all serum samples (figure 1).

Low penetration of amikacin into saliva could be explained by its physicochemical properties as it is a polycationic and highly polar compound. Amikacin cannot easily diffuse across membranes, such as in the salivary gland [6]. This effect also applies to other aminoglycosides and encourages the use of aerosolised administration [7].

A limitation of this study is the relatively high LLOQ of the immunoassay used. Other analytical methods with lower LLOQ values, such as liquid chromatography–tandem mass spectrometry, have been validated but are not available for TDM in high TB burden countries with limited resources [10]. In addition, the recovery of the sampling method was low due to adhesion of amikacin to the membrane filter or sampling material. Due to a high LLOQ and low recovery, we were able to measure salivary amikacin concentrations >5 mg·L⁻¹. As median serum C_{max} concentrations were 28.75 (28–33) mg·L⁻¹, we were able to quantify saliva/serum ratios up to 0.18. These low ratios are generally considered to be unsuitable for salivary TDM, unless a sensitive analytical method is used and other factors influencing variability in saliva penetration are absent.

In conclusion, the robust design using a full concentration–time curve enabled us to conclude that amikacin C_{max} concentrations were not measurable in saliva and the concept of simple salivary TDM of amikacin using immunoassay appeared not feasible.

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