Dose optimisation of first-line tuberculosis drugs using therapeutic drug monitoring in saliva: feasible for rifampicin, not for isoniazid

To the Editor:

The persisting worldwide burden of tuberculosis (TB) is worrisome. In 2018, an estimated 10 million individuals developed TB and 1.45 million infected individuals died [1]. The increase in drug resistance is an important point of concern. Resistance can be acquired by inappropriate drug management, noncompliance and insufficient drug exposure [2, 3]. The last is frequently described for the first-line TB drugs rifampicin and isoniazid due to large interindividual pharmacokinetic variability [3]. Therapeutic drug monitoring (TDM) can be used to verify drug exposure and adjust individual drug dosages if needed [4]. The efficacy of rifampicin and isoniazid is associated with the ratio of the steady-state area under the concentration–time curve from 0 to 24 h (AUC_{0-24}) to minimal inhibitory concentration with a target value of >271 for rifampicin and >567 for isoniazid [5, 6]. Traditional TDM uses plasma or serum samples, whereas other matrices such as dried blood spot and saliva have been recommended as alternatives suitable for programmatic use [4, 7]. Collecting saliva samples is noninvasive and simple with the perspective of home-based self-sampling [8]. Salivary concentrations of rifampicin and isoniazid have been studied before, but highly variable saliva/serum concentration ratios across studies were observed [8]. Moreover, none of these studies assessed the feasibility of TDM using saliva samples.

Therefore, the aim of this prospective study was to evaluate the feasibility of saliva instead of serum samples for TDM of rifampicin and isoniazid in patients with TB.

Adult patients with TB admitted at the Tuberculosis Center Beatrixoord (Haren, the Netherlands) who were treated with rifampicin and/or isoniazid and had routine TDM for rifampicin or isoniazid were eligible for inclusion. All patients provided informed consent. This study was approved by the ethical review board of the University Medical Center Groningen (IRB 2016/069) and registered at Clinicaltrials.gov (NCT03080012).

All samples were taken after >14 days of treatment (steady state) and stored at −80°C pending analysis. Saliva and serum samples were collected simultaneously according to the routine TDM schedule which usually included samples drawn before, and 0.5, 1, 2, 3, 4 and 6 h after drug intake. Two different methods of saliva collection were used. The Salivette (Sarstedt, Nümbrecht, Germany) was used for sputum culture-negative patients. Membrane filtration was applied to the samples of sputum culture-positive patients to minimise infection hazard [9, 10]. The recovery of both sampling methods was determined for rifampicin and isoniazid at concentrations of 1 and 7 $mg{\cdot}L^{-1}$ as described [11]. Rifampicin recovery at 1 $mg{\cdot}L^{-1}$ was 64% (coefficient of variation (CV) 9%) using the Salivette and 67% (5%) using membrane filtration, while at 7 $mg{\cdot}L^{-1}$ recovery was 102% (2%) and 99% (8%), respectively. For isoniazid, recovery (CV) at 1 $mg{\cdot}L^{-1}$ was 77% (8%) using the Salivette and 68% (4%) using membrane filtration, whereas at 7 $mg{\cdot}L^{-1}$ recovery was 91% (1%) and 88% (3%), respectively. After analysis, the salivary drug concentrations were corrected for the recovery of the applied sampling method. The pH of each saliva sample was determined by two independent researchers using pH indicator strips (range 4.0–7.0 and 2.0–9.0; Merck KGaA, Darmstadt, Germany).

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Therapeutic drug monitoring using saliva samples is feasible for rifampicin, despite low penetration, but is not feasible for isoniazid, which showed inexplicable highly variable saliva/serum concentration ratios https://bit.ly/2yAS2Jc

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Saliva and serum samples were analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods [12, 13]. The method for rifampicin was recently updated and validated using the more suitable internal standard [$^2H_8$]-rifampicin. Cross-validation in saliva was successfully performed for both drugs. Bias and precision of spiked pooled saliva met the pre-set criteria of <20% for lower limit of quantification (rifampicin 0.1 mg·L^{-1}, isoniazid 0.2 mg·L^{-1}) as well as <15% for low (rifampicin 0.5 mg·L^{-1}, isoniazid 0.4 mg·L^{-1}), medium (rifampicin 5.0 mg·L^{-1}, isoniazid 4.0 mg·L^{-1}) and high (rifampicin 8.0 mg·L^{-1}, isoniazid 6.4 mg·L^{-1}) concentrations.

Saliva/serum ratios were calculated using the paired drug concentrations for each time point as well as the noncompartmental AUC_{0-24} (MWPharm version 3.82; Mediware, Groningen, the Netherlands) in both matrices. The saliva/serum concentration ratios were evaluated using Passing–Bablok regression and Bland–Altman plots (Analyze-it 4.81; Analyze-it Software Ltd, Leeds, UK). C_{max} was defined as highest observed drug concentration and t_{max} as time of C_{max}. Intraindividual variation was assessed as CV (%) of the saliva/serum ratios within one pharmacokinetic curve, while interindividual variation was calculated as CV of the mean saliva/serum ratios of all curves.

Characteristics of the study population, pharmacokinetic parameters (C_{max}, t_{max}, AUC_{0-24}) in both matrices, and saliva/serum ratios are shown in table 1.

Penetration of rifampicin into saliva was low and slightly delayed. This resulted in undetectable salivary concentrations when collected before drug intake and 0.5 h or 1 h after drug intake. Saliva and serum concentrations (>1 h after drug administration) correlated well with a regression line of saliva concentration=0.074+0.112×serum concentration (95% CI of intercept −0.0311–0.161, 95% CI slope 0.087–0.138; r=0.803). Bland–Altman analysis led to a mean±SD (95% CI) saliva/serum concentration ratio of 0.13±0.04 (0.12–0.14). The AUC_{0-24} saliva/serum ratio was slightly higher, but comparable (table 1). An AUC_{0-24} conversion factor was calculated as serum/saliva AUC_{0-24} ratio and resulted in a median (interquartile range) of 6.5 (6.2–7.9). Inter- and intraindividual variation were both ∼20%.

Isoniazid saliva/serum ratios were much higher than for rifampicin and can be explained by the difference in protein binding (10% *versus* 90%). Passing–Bablok regression resulted in a regression line of saliva concentration=−0.055+0.812×serum concentration (95% CI intercept −0.556–0.460, 95% CI slope 0.185–1.244; r=0.889). The Bland–Altman analysis showed a mean±SD (95% CI) saliva/serum concentration ratio of 0.80±0.46 (0.65–0.95). Intraindividual variation was 22.3%, while interindividual variation was relatively

TABLE 1 Patient characteristics, noncompartmental pharmacokinetic parameters in serum and saliva, salivary pH and saliva/serum ratios

	Rifampicin	Isoniazid
Subjects	11	8
Study population		
Male	9 (82)	6 (75)
Age years	34 (25–54)	54 (49–58)
Bodyweight kg	69 (58–71)	68 (57–72)
Creatinine concentration μmol·L^{-1}	62 (51–72)	65 (49–75)
Dose mg·kg^{-1}	10.2 (8.5–12.3)	5.4 (4.2–6.5)
Serum pharmacokinetics		
C_{max} mg·L^{-1}	8.70 (5.99–12.12)	3.50 (1.65–4.75)
t_{max} h	2 (2–3)	2 (1–2)
AUC_{0-24} mg·h·L^{-1}	38.01 (34.44–76.50)	17.83 (7.80–20.74)
Saliva pharmacokinetics		
C_{max} mg·L^{-1}	1.21 (1.08–1.35)	1.57 (0.93–2.75)
t_{max} h	3 (2–4)	1 (1–2)
AUC_{0-24} mg·h·L^{-1}	5.88 (5.08–7.94)	7.62 (7.28–11.73)
Salivary pH	6.1 (5.5–7.0)	6.1 (5.8–6.8)
Saliva/serum ratio		
Paired concentration ratio	0.126 (0.109–0.154)	0.763 (0.413–1.158)
Interindividual variation %CV	21.5	48.3
Intraindividual variation mean (range) of %CV	17.2 (7.4–24.0)	22.3 (9.2–36.5)
AUC_{0-24} ratio	0.154 (0.127–0.162)	0.824 (0.492–1.200)

Data are presented as n, n (%) or median (interquartile range), unless otherwise stated. C_{max}: highest observed drug concentration; t_{max}: time of C_{max}; AUC_{0-24}: steady-state area under the concentration–time curve from 0 to 24 h; CV: coefficient of variation.

large (48.3%), which could suggest that isoniazid penetration into saliva is influenced by other factors. Salivary pH was not related to the saliva/serum ratio of isoniazid and rifampicin.

A limitation of this study is the lack of data on salivary flow and protein binding. Both could introduce variation in the saliva/serum ratios [8]. However, we aimed to evaluate the feasibility of salivary TDM and consider it unfeasible if protein binding and salivary flow have to be determined in each patient. Moreover, no influence of salivary pH on saliva/serum ratios was detected, whereas salivary pH is related to salivary flow [8].

Despite this limitation, we propose that rifampicin AUC_{0-24} in serum can be estimated satisfactorily using the AUC_{0-24} in saliva applying a conversion factor of 6.5 and used for AUC_{0-24}-guided dose optimisation in TB patients. The sampling burden can be reduced by collecting samples only at 2, 3, 4 and 6 h after drug intake, since the other salivary rifampicin concentrations (0, 0.5, 1 h) were undetectable. Simple high-performance liquid chromatography–ultraviolet methods [14] are available in TB-endemic areas, but not usually LC-MS/MS. Additional testing is recommended to determine whether these analytical techniques are also able to assess low rifampicin concentrations in saliva.

The results for isoniazid are less encouraging. Based on the findings in this study, we would not recommend TDM of isoniazid in saliva. The major cause of the large variation of isoniazid saliva/serum ratios remains unclear, as is the case with moxifloxacin [10]. A future study could focus on the identification of acetylator phenotype using saliva samples. Unfortunately, our sample size was too small to distinguish three groups with different drug clearance rates and we did not perform NAT2 genotyping.

In general, we conclude that TDM for isoniazid using saliva samples will not be an equivalent alternative to traditional TDM, as already shown for moxifloxacin [10] and amikacin [15], but it can be useful in home screening of rifampicin drug exposure in patients with TB, as has been established for linezolid [10] and levofloxacin [11].

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