Linezolid in multidrug-resistant tuberculosis

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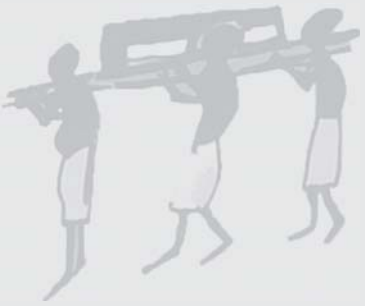

Chapter 4B

Clinical validation of the analysis of linezolid and clarithromycin in oral fluid of multidrug-resistant tuberculosis patients

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ABSTRACT

Linezolid plays an increasingly important role in the treatment of multidrug-resistant tuberculosis. However, patients should be carefully monitored due to time- and dose-dependent toxicity. Clarithromycin plays a more modest role. Therapeutic drug monitoring may contribute to assessing treatment regimens, helping to reduce toxicity whilst maintaining adequate drug exposure. Oral fluid sampling could provide a welcome alternative in cases where conventional plasma sampling is not possible or desirable. The aim of this study was to clinically validate the analysis of linezolid and clarithromycin and its metabolite hydroxyclarithromycin in oral fluid of patients with multidrug-resistant tuberculosis.

Serum and oral fluid samples were simultaneously obtained and analyzed using validated methods, after extensive cross-validation between the two matrices. Passing-Bablok regressions and Bland-Altman analysis showed that oral fluid analysis of linezolid and clarithromycin appeared suitable for therapeutic drug monitoring in MDR-TB patients. No correction factor is needed for the interpretation of linezolid oral fluid concentration with a linezolid serum / oral fluid ratio of 0.97 (95% CI, 0.92 – 1.02). However, the clarithromycin serum / oral fluid concentration ratio is 3.07 (95% CI, 2.45 – 3.69). Analysis of hydroxyclarithromycin in oral fluid was not possible in this study due to a non linear relationship between concentration in serum and oral fluid. In conclusion, the analysis of linezolid (no correction factor) and clarithromycin (correction factor *3) in oral fluid is applicable for therapeutic drug monitoring in multidrug-resistant tuberculosis as an alternative to conventional serum sampling. Easy sampling, using a non-invasive technique may facilitate therapeutic drug monitoring in specific patient categories.

INTRODUCTION

Tuberculosis is a mostly curable and preventable infectious disease caused by *Mycobacterium tuberculosis*. Approximately 3.7% of new tuberculosis patients and 20% of previously treated patients are infected with multidrug-resistant strains that are resistant to at least rifampicin and isoniazid (1). Treatment regimens of multidrug-resistant tuberculosis (MDR-TB) should consist of at least four anti-TB drugs for which the bacterium is susceptible (2).

The oxazolidinone linezolid is effective against *M. tuberculosis* and is increasingly used as a part of treatment regimens in patients with multidrug-resistant or extensively drug-resistant tuberculosis (3). However, patients should be carefully monitored due to time- and dose-dependent serious toxicity of linezolid, such as myelosuppression and polyneuropathy (4).

Clarithromycin has a less pronounced place in MDR-TB treatment regimens due to serum concentrations that usually do not reach minimal inhibitory concentrations (5). Nevertheless, sufficiently high local clarithromycin concentrations are reached in epithelial lining fluid and alveolar cells (6, 7). Furthermore, occasionally observed lower MICs [unpublished data], synergistic activity of clarithromycin against MDR-TB strains (8) and absence of severe adverse events (9) contribute to its place in anti-TB therapy.

Serum concentrations of linezolid have shown large inter-patient variability (10). Drug-drug interactions might further contribute to the observed variability in linezolid pharmacokinetics. For instance, clarithromycin has been observed to increase linezolid serum concentrations significantly (11). Therapeutic drug monitoring could potentially assist in identifying MDR-TB patients with too low or too high linezolid exposure. Conventional serum sampling is not always possible or desirable due to lack of venous access, complicated logistics, or due to the invasive character of the technique. Previously, we developed a dried blood spot analysis of linezolid (12) and clarithromycin (unpublished data) as an alternative to conventional serum sampling. A clinically validated method could be useful in patients that do not accept or tolerate an indwelling venous catheter, or who have difficult venous access. We therefore aimed to clinically validate the analysis of linezolid, clarithromycin, and hydroxyclarithromycin concentrations in oral fluid.

METHOD

From December 2011 to October 2012, patients were included from the Tuberculosis Center Beatrixoord (Haren, The Netherlands). Patients were ≥18 years old, were diagnosed with MDR-TB and provided written informed consent. The study protocol was approved by the local Medical Ethical Review Committee, as part of a previously published study. The prospective pharmacokinetic study is registered at www.clinicaltrials.org (NCT01521364).

All patients received oral dosages of linezolid 300 mg twice daily and clarithromycin 250 mg once daily. Full pharmacokinetic curves were obtained at steady state, after at least two weeks of administration of both drugs, using a practically feasible sampling schedule which resulted in adequate area under the time-concentration curves (AUC) in previous studies in our Center. Blood and oral fluid samples were collected simultaneously before and 1, 2, 3, 4, 8, and 12 hours after medication intake. Blood samples were drawn and after centrifuging serum samples were stored at -20°C until analysis. Oral fluid samples were collected using a small cotton roll on which the patients chewed for approximately two minutes (Salivette®, Sarstedt, Leicester, UK). Oral fluid samples were centrifuged and then stored at -20°C until analysis.

Linezolid and clarithromycin serum and oral fluid concentrations were analyzed using validated high performance liquid chromatography tandem mass-spectrometry methods (13, 14). Cross-validation between two matrices was performed by comparing calibration samples in pooled serum and non-stimulated, pooled oral fluid from six batches.

Pharmacokinetic parameters such as, most importantly, AUC were calculated using Kinfit software (MWPharm 3.60; Mediware, Groningen, the Netherlands) as described in a previous study using non-compartmental, trapezoidal calculations (10). Other pharmacokinetic parameters that were calculated using Kinfit software were C_{max}, C_{min}, apparent clearance (CL), elimination rate constant (k_{el}) and half-life ($t_{1/2}$). Of these parameters, CL, k_{el}, and $t_{1/2}$were determined using log-linear regression of the concentrations in the terminal period.

The method was clinically validated by comparing the linezolid, clarithromycin and hydroxyclarithromycin concentrations in serum samples with the concentration in oral fluid using Passing-Bablok regressions and Bland-Altman analysis (Analyze-it Software, Ltd.). The Pearson's correlation and Wilcoxon signed-rank test were applied to other comparisons.

RESULTS

Baseline patient demographics are presented in Table 1. Seven patients with MDR-TB, four male and three female, were included in the study. The isolates that were obtained from all patients displayed resistance for at least rifampicin and isoniazid. The patients had a median (interquartile range, IQR) age of 31 (25.5 – 33.5) years and weighed in at median (IQR) 71.5 (56.0 – 75.3) kg. Five patients were from Somalia, one from Turkey and one from the Netherlands. At the time of sampling, patients were on MDR-TB treatment for a mean (range) of 61.4 (33 – 149) days. One patient was HIV positive for which the patient was on cART with adequate virological and immunological response (before admission CD4 count: 380 cells/mm^3).

Table 1 Baseline demographics (n=7) and results from drug susceptibility testing

Parameter	Value
Age – year[†]	31.0 (25.5 – 33.5)
Male sex – no. (%)	4 (57%)
Bodyweight – kg[‡]	71.5 (56.0 – 75.3)
Height – m[‡]	1.73 (1.64 – 1.74)
Ethnicity – no. (%)	
African	5 (71%)
Caucasian	1 (14%)
Asian	1 (14%)
HIV positive – no. (%)	1 (14%)
Isolate resistant to drug based on DST – no./no. total	
Rifampicin	7/7
Isoniazid	7/7
Ethambutol	5/7
Pyrazinamide[ʃ]	4/6
Streptomycin	6/7
Capreomycin	2/7
Amikacin	0/7
Ciprofloxacin	1/7
Claritromycin[ʃ]	3/5
Clofazimin[ʃ]	0/4
Linezolid	0/7
Moxifloxacin	1/7
Protionamide[ʃ]	2/6
Rifabutin	6/7

[†] Data presented as mean (range), [‡] Data presented as median (interquartile range), [ʃ] Drug susceptibility testing (DST) was not available for all isolates of the included patients.

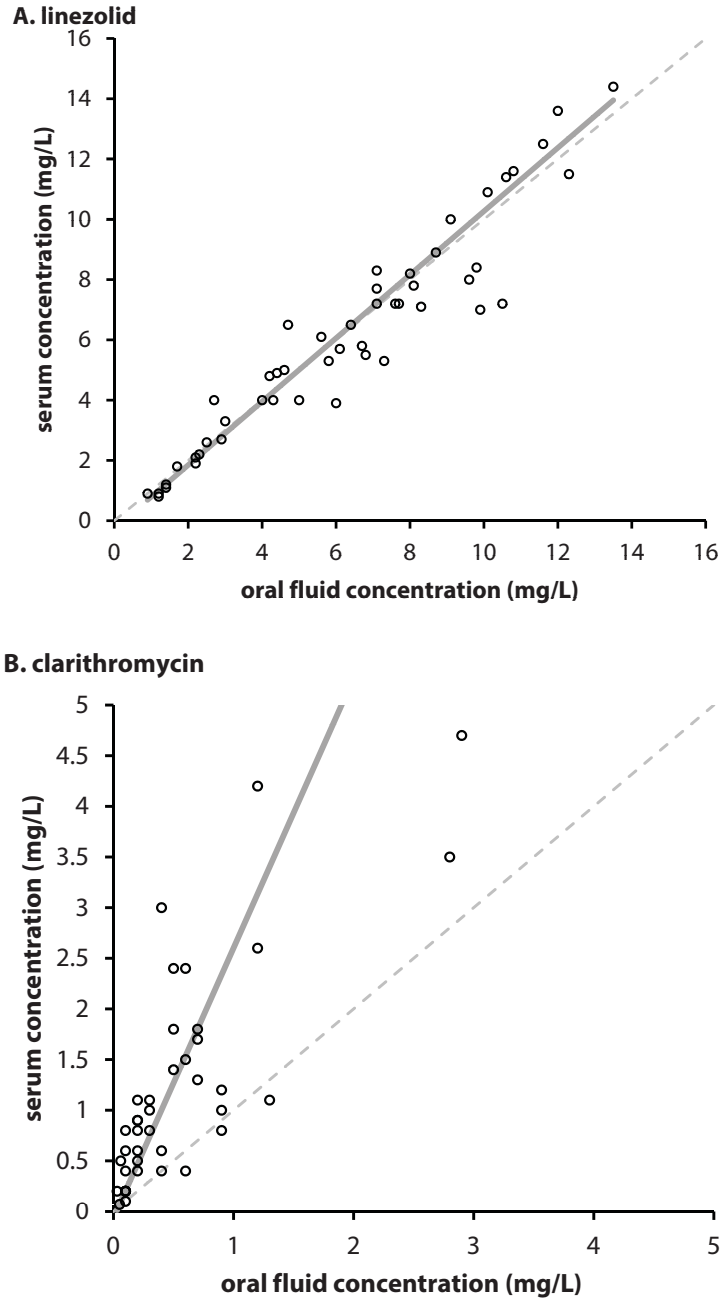

Figure 1 Scatter plot with Passing-Bablok fit of serum and oral fluid concentration in mg/L.
Identity lines are presented as dashed lines and regression lines as solid lines. A. linezolid (n=49): regression line of linezolid serum-oral fluid concentration has a slope of 1.05 (95% CI, 0.94 – 1.11) and intercept of -0.26 (95% CI, -0.52 – 0.05). B. clarithromycin (n=42): regression line of clarithromycin serum-oral fluid concentration has a slope of 2.67 (95% CI, 1.95 – 3.75) and intercept of -0.06 (95% CI, -0.18 – 0.21).

Comparison oral fluid and serum analysis method

Comparison of analysis in two matrices showed that linezolid, clarithromycin, and hydroxyclarithromycin had no significant differences of intercept and slope in serum and in oral fluid. The calibration curves in oral fluid were analyzed three times with all coefficients of variation (CVs) below 15%. All biases in concentration were <12% for linezolid and <8% for clarithromycin and its metabolite.

Passing-Bablok regression (n=49) of linezolid concentration in serum and oral fluid showed a proportional bias of 1.05 (95% confidence interval [CI], 0.94 – 1.11) and a constant bias of -0.26 (95% CI, -0.52 – 0.05) (Figure 1A). For clarithromycin, the Passing-Bablok scatter plot (n=42) showed a proportional bias of 2.67 (95% CI, 1.95 – 3.75) and a constant bias of -0.06 (95% CI, -0.18 – 0.21) (Figure 1B). There were 7 missing clarithromycin and 8 missing hydroxyclarithromycin values due to concentrations below limit of quantitation of the applied method of 0.2 mg/L. A linear relationship was detected using Cusum linearity test ($p>0.1$) between oral fluid and serum concentrations of both linezolid and clarithromycin. However, the Cusum linearity test detected a non-linear relationship ($0.05<p<0.1$) between serum and oral fluid hydroxyclarithromycin concentration, with Passing-Bablok showing a constant bias of 0.02 (95% CI, -0.20 – 0.24) and proportional bias of 2.00 (95% CI, 1.14 – 3.00).

Bland-Altman assessment showed good agreement between analyses of linezolid and clarithromycin in serum and oral fluid; 4.1% (2/49) of observations falling outside 95% limits of agreement for linezolid, and 7.1% (3/42) for clarithromycin; see Figure 2. The observed bias for linezolid (n=49) was 0.97 with 95% confidence intervals below and above one (95% CI, 0.92 – 1.02) (Figure 2A). For clarithromycin, the observed bias was 3.07 (95% CI, 2.45 – 3.69) (Figure 2B). Pearson's test revealed that the analyses of linezolid and clarithromycin in serum and in oral fluid were correlated with $r = 0.95$ ($p<0.01$) and $r = 0.80$ ($p<0.01$) respectively.

Pharmacokinetic and pharmacodynamic evaluation

Pharmacokinetic parameters of linezolid and clarithromycin in serum and oral fluid are displayed in Table 2. Paired sample Wilcoxon Signed rank test showed no statistically significant difference between medians of all pharmacokinetic parameters in serum and oral fluid, except for linezolid k_{el} and $t_{1/2}$ ($p=0.018$). However, the clinical significance of this observed difference is little since the AUC is the most important pharmacokinetic parameter for therapeutic drug monitoring of linezolid and clarithromycin in multidrug-resistant tuberculosis patients.

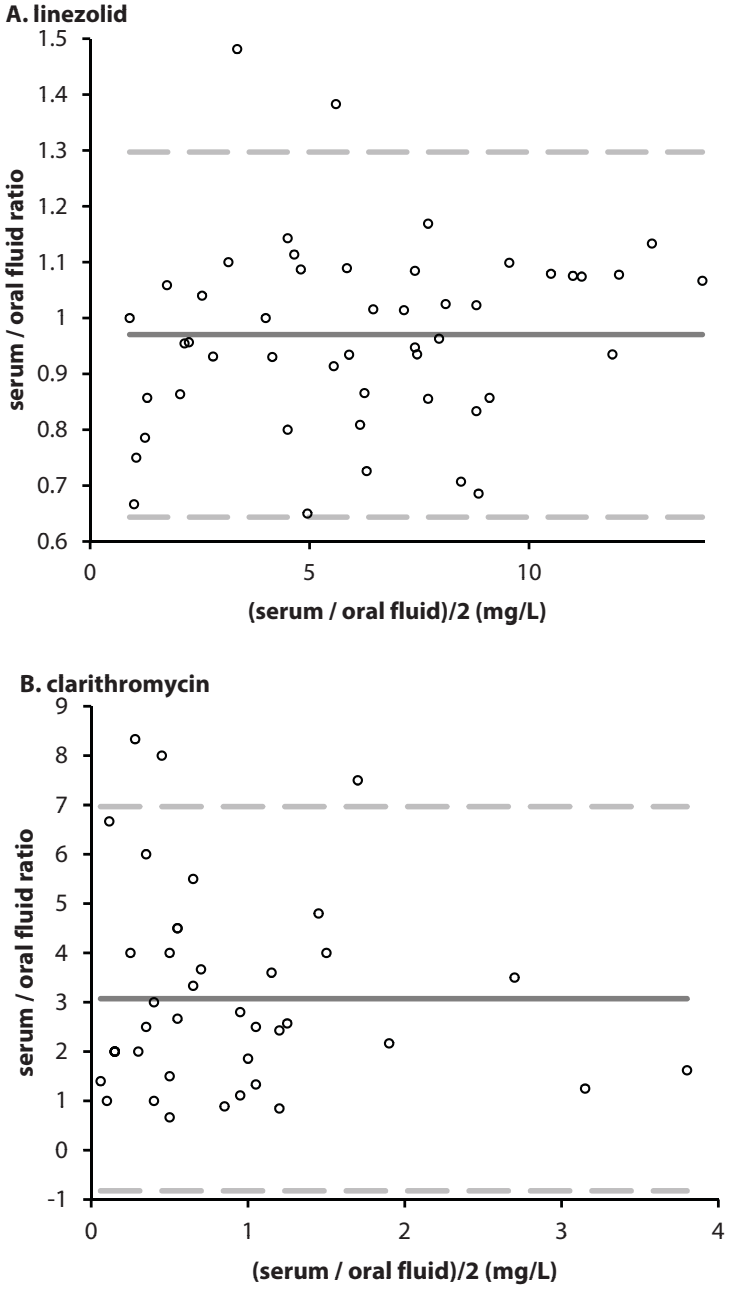

Figure 2 Bland-Altman plot of serum/oral fluid concentration ratio compared to average serum and oral fluid concentration.

The line representing the bias is presented as a solid line and the 95% limits of agreement as dashed lines. A. linezolid (n=49): bias is 0.97 (95% CI, 0.92 – 1.02), the lower and upper 95% limits of agreement are respectively 0.64 (95% CI, 0.56 – 0.73) and 1.30 (95% CI, 1.22 – 1.38). B. clarithromycin (n=42): bias is 3.07 (95% CI, 2.45 – 3.69), the lower and upper 95% limits of agreement are respectively -0.82 (95% CI, -1.89 – 0.24) and 6.97 (95% CI, 5.90 – 8.03).

Pharmacokinetic and pharmacodynamic parameters of linezolid from all patients are displayed in Table 3. Isolates from all patients were susceptible for linezolid with a MIC of median 0.25 mg/L. Patients had a linezolid AUC_{0-12h} of median (IQR) 63.9 (47.8 – 83.8) mg*h/L in serum and 62.1 (50.5 – 59.2) mg*h/L in oral fluid. All patients had an AUC_{0-24}/MIC ratio of >100 in both serum and oral fluid. For patient 2, AUC_{0-24h}/MIC ratio reached approximately 1000. Median (IQR) linezolid AUC_{0-24h}/MIC ratio in serum was 277 (260 – 517) mg*h/L and in oral fluid 288 (262 – 594) mg*h/L. Paired sample Wilcoxon Signed rank test showed no statistically significant difference between AUC_{0-12h} or AUC_{0-24h}/MIC ratio in serum or oral fluid (p=0.296).

Table 2 Pharmacokinetic parameters of linezolid and clarithromycin in serum and in oral fluid (n=7)

	Serum	Oral fluid	p-value†
Linezolid			
AUC_{0-12u} (mg*h/L)	63.9 [47.8 – 83.8]	62.1 [50.5 – 89.2]	0.296
C_{max} (mg/L)	10.9 [6.8 – 11.5]	10.1 [8.2 – 10.7]	1.0
C_{min} (mg/L)	2.2 [1.5 – 4.2]	2.3 [1.7 – 4.2]	0.084
CL (L/h)	3.5 [2.4 – 5.9]	3.6 [2.2 – 5.0]	0.063
k_{el} (/h)	0.14 [0.10 – 0.17]	0.13 [0.08 – 0.16]	0.018¥
$t_{1/2}$ (h)	4.9 [4.2 – 7.9]	5.2 [4.5 – 9.8]	0.018¥
Clarithromycin			
AUC_{0-12u} (mg*h/L)	8.2 [6.2 – 12.2]	10.7 [9.4 – 12.1]	0.091
C_{max} (mg/L)	1.7 [1.3 – 2.7]	2.8 [2.0 – 3.4]	0.063
C_{min} (mg/L)	0.01 [0.01 – 0.04]	0.03 [0.03 – 0.06]	1.0
CL (L/h)	28.5 [19.3 – 39.1]	62.2 [52.8 – 81.0]	0.237
k_{el} (/h)	0.21 [0.19 – 0.23]	0.64 [0.49 – 1.06]	0.667
$t_{1/2}$ (h)	3.3 [3.1 – 3.6]	10.2 [6.4 – 13.5]	1.0

Data are presented as median [interquartile range]. † p-values comparing pharmacokinetic parameters between serum and oral fluid. ¥ Statistical significant difference between median of the parameter in serum and in oral fluid.

Table 3 Pharmacokinetic and pharmacodynamic parameters of linezolid

		AUC_{0-12h} (mg*h/L)		AUC_{0-24h}/MIC ratio	
Patient	MIC (mg/L)	Serum	Oral fluid	Serum	Oral fluid
1	0.25	34.6	42.1	277	337
2	0.25	120.1	126.4	961	1011
3	0.5	61.0	62.1	244	248
4	0.5	63.9	58.8	256	235
5	0.25	33.0	34.5	264	276
6	0.5	76.6	72.0	306	288
7	0.25	90.9	106.3	727	850
Total¥		63.9 [47.8 – 83.8]	62.1 [50.5 – 89.2]±	277 [260 – 517]	288 [262 – 594]±

¥ Median [interquartile range], ± no statistically significant difference between serum and oral fluid (p=0.296).

Patients had a clarithromycin AUC_{0-12h} of median (IQR) 8.2 (6.2 – 12.2) mg*h/L in serum and 3.5 (3.1 – 4.0) mg*h/L in oral fluid. One patient was inadvertently administered 500 mg, instead of 250 mg clarithromycin at the day of sampling. The samples obtained from this patient were included in the evaluation. The clarithromycin AUC_{0-12h} after administration of 500 mg clarithromycin was 29.1 mg*h/L in serum and 15.7 mg*h/L in oral fluid, well above the AUCs of the patients receiving 250mg clarithromycin. After applying the correction factor of 3.07 as determined using the Bland-Altman assessment, patients had an adjusted clarithromycin AUC_{0-12h} of median (IQR) 10.7 (9.4 – 12.1) mg*h/L in oral fluid. Paired sample Wilcoxon Signed rank test showed no statistically significant difference, but did show a trend towards difference between clarithromycin AUC_{0-12h} in serum and in oral fluid after applying a correction factor of 3.07 (p=0.091).

DISCUSSION

The clinical validation performed in this study showed that oral fluid analysis of linezolid and clarithromycin are suitable for TDM in MDR-TB patients. No correction factor is needed for the interpretation of linezolid oral fluid concentration. However, clarithromycin oral fluid concentration should be corrected by multiplying by three to enable comparison to clarithromycin serum levels. After applying a correction factor of three in case of clarithromycin or no correction factor in case of linezolid, the pharmacokinetic parameter AUC_{0-12h} as calculated from oral fluid samples are applicable for TDM and could assist in identifying patients with too high or too low exposure.

Unfortunately, analysis of hydroxyclarithromycin in oral fluid is not possible due to a non-linear relation with concentrations in serum. Nevertheless, the analysis of hydroxyclarithromycin shows good linearity over a range of 0.2 – 10 mg/L in both serum and oral fluid. A possible explanation for the observed non-linear relation between analysis of hydroxyclarithromycin in serum and in oral fluid might be the low hydroxyclarithromycin concentrations that were observed. In this concentration range, around the limit of quantitation, CVs are relatively high although within acceptable limits of <20%. This could explain a non-linear relationship between analysis of hydroxyclarithromycin in serum and oral fluid in this low concentration range. Possibly, analysis of a larger cohort or higher clarithromycin doses with corresponding higher hydroxyclarithromycin concentrations would reveal a linear relationship. Furthermore, this could confirm that there is no significant difference between clarithromycin exposure in serum and oral fluid

(after correction), despite a trend towards statistical significance that was observed in our cohort.

The k_{el} and $t_{1/2}$ of linezolid showed a statistical significant difference of medians in oral fluid compared to serum. The parameters k_{el} and $t_{1/2}$ are calculated from concentrations obtained in the terminal period, i.e. in the last 3 – 4 samples in a relatively small cohort. The relevance should be confirmed in a larger cohort or using curves with more samples in the terminal period. In clinical practice, not the paramater k_{el} or $t_{1/2}$, but the pharmacokinetic/pharmacodynamic parameter, AUC/MIC ratio of linezolid, is used for therapeutic drug monitoring (10–11).

To date, there has been no comparison in the literature of the analysis of clarithromycin and linezolid between serum and oral fluid in patients with MDR-TB. However, there are several studies describing pharmacokinetics of clarithromycin and hydroxyclarithromycin in saliva (15, 16), but none describing pharmacokinetics of linezolid in oral fluid. Clarithromycin administered to 12 healthy volunteers in a dose of 500 mg twice daily, resulted in an AUC_{0-12h} of 18.0±5.0 mg*h/L (15). We observed a similar AUC_{0-12h} of 15.7 mg*h/L in the one patient that was administered 500 mg clarithromycin. Saliva-serum ratios of around two were reported, lower than the ratio of three observed in our study. However, no data were presented comparing results from the analysis of clarithromycin in serum and oral fluid, since the study aimed to describe kinetics, not to clinically validate the analysis in oral fluid (15). Another study, described pharmacokinetics of clarithromycin in saliva and serum after a single dose of 500 mg clarithromycin (16). However, the aim was to describe the penetration of clarithromycin into saliva, not to clinically validate the analysis of clarithromycin in saliva. The Summary of Product Characteristics (SmPC) of linezolid describes a linezolid oral fluid – plasma concentration ratio of 1.2, comparable with the bias of 0.97 that was observed in the Bland-Altman assessment (17). Our current study describes the method of analysis of clarithromycin and linezolid, cross-validation between serum and oral fluid, and most importantly clinical validation in MDR-TB patients. No statistically significant differences were found between AUC_{0-12h} or AUC_{0-24h}/MIC ratio of serum and oral fluid.

TDM could potentially assist in identifying MDR-TB patients with too low or too high linezolid exposure. Analysis of anti-TB drugs in oral fluid may be advantageous in patients without readily accessible venous access, hindering blood sampling. Furthermore, the non-invasive sampling could be suitable in children with MDR-TB, in whom indwelling intravenous catheters are no option, and in whom no study data on pharmacokinetics

are available to guide therapy. Oral fluid sampling in pediatric patients is preferred over conventional serum sampling by a majority of children and their parents (18). Oral fluid sampling might even reduce costs due to the higher level of trained personnel needed for blood sampling and since less time is needed (18). Oral fluid sampling might even take place at home. No children were included in this study. A clinical validation of oral fluid sampling in pediatric MDR-TB patients is urgently needed. Furthermore, the applicability of saliva and/or other collection devices than the Salivette® (Sarstedt, Leicester, UK) for pharmacokinetic analysis and therapeutic drug monitoring in MDR-TB patients should be clinically validated.

In conclusion, the clinically validated analysis of clarithromycin and linezolid in oral fluid could provide a helpful alternative if conventional blood sampling is not possible or desirable. Using a correction factor of 3.07 for clarithromycin oral fluid concentrations and no correction factor for linezolid makes the oral fluid sampling readily applicable in clinical practice and allows for easy interpretation.

REFERENCES

1. **World Health Organization (WHO).** 2012. Global tuberculosis report 2012. World Health Organization (WHO), Geneva, Switzerland.
2. **World Health Organisation (WHO) (ed.),** 2011. Guidelines for the programmatic management of drug-resistant tuberculosis. World Health Organization, Geneva, Switzerland.
3. **Lee, M., J. Lee, M. W. Carroll, H. Choi, S. Min, T. Song, L. E. Via, L. C. Goldfeder, E. Kang, B. Jin, H. Park, H. Kwak, H. Kim, H. Jeon, I. Jeong, J. S. Joh, R. Y. Chen, K. N. Olivier, P. A. Shaw, D. Follmann, S. D. Song, J. Lee, D. Lee, C. T. Kim, V. Dartois, S. Park, S. Cho, and C. E. Barry.** 2012. Linezolid for Treatment of Chronic Extensively Drug-Resistant Tuberculosis. N. Engl. J. Med. **367:**1508-1518.
4. **Xu, H. B., R. H. Jiang, L. Li, and H. P. Xiao.** 2012. Linezolid in the treatment of MDR-TB: a retrospective clinical study. Int. J. Tuberc. Lung Dis. **16:**358-363.
5. **Truffot-Pernot, C., N. Lounis, J. H. Grosset, and B. Ji.** 1995. Clarithromycin is inactive against Mycobacterium tuberculosis. Antimicrob. Agents Chemother. **39:**2827-2828.
6. **Conte, J. E.,Jr, J. A. Golden, S. Duncan, E. McKenna, and E. Zurlinden.** 1995. Intrapulmonary pharmacokinetics of clarithromycin and of erythromycin. Antimicrob. Agents Chemother. **39:**334-338.
7. **Rodvold, K. A., M. H. Gotfried, L. H. Danziger, and R. J. Servi.** 1997. Intrapulmonary steady-state concentrations of clarithromycin and azithromycin in healthy adult volunteers. Antimicrob. Agents Chemother. **41:**1399-1402.

8. **Cavalieri, S. J., J. R. Biehle, and W. E. Sanders Jr.** 1995. Synergistic activities of clarithromycin and antituberculous drugs against multidrug-resistant Mycobacterium tuberculosis. Antimicrob. Agents Chemother. **39:**1542-1545.

9. **Chu, S. Y., L. T. Sennello, S. T. Bunnell, L. L. Varga, D. S. Wilson, and R. C. Sonders.** 1992. Pharmacokinetics of clarithromycin, a new macrolide, after single ascending oral doses. Antimicrob. Agents Chemother. **36:**2447-2453.

10. **Alffenaar, J. W., R. van Altena, I. M. Harmelink, P. Filguera, E. Molenaar, A. M. Wessels, D. van Soolingen, J. G. Kosterink, D. R. Uges, and T. S. van der Werf.** 2010. Comparison of the pharmacokinetics of two dosage regimens of linezolid in multidrug-resistant and extensively drug-resistant tuberculosis patients. Clin. Pharmacokinet. **49:**559-565.

11. **Bolhuis, M. S., R. van Altena, D. R. Uges, T. S. van der Werf, J. G. Kosterink, and J. W. Alffenaar.** 2010. Clarithromycin significantly increases linezolid serum concentrations. Antimicrob. Agents Chemother. **54:**5418-5419.

12. **Vu, D. H., M. S. Bolhuis, R. A. Koster, B. Greijdanus, W. C. de Lange, R. van Altena, J. R. Brouwers, D. R. Uges, and J. W. Alffenaar.** 2012. Dried blood spot analysis for therapeutic drug monitoring of linezolid in patients with multidrug-resistant tuberculosis. Antimicrob. Agents Chemother. **56:**5758-5763.

13. **Harmelink, I. M., J. W. Alffenaar, A. M. Wessels, B. Greijdanus, and D. R. Uges.** 2008. A rapid and simple liquid chromatography-tandem mass spectrometry method for the determination of linezolid in human serum. EJHP Science. **14:**3-7.

14. **de Velde, F., J. W. Alffenaar, A. M. Wessels, B. Greijdanus, and D. R. Uges.** 2009. Simultaneous determination of clarithromycin, rifampicin and their main metabolites in human plasma by liquid chromatography-tandem mass spectrometry. J. Chromatogr. B. Analyt Technol. Biomed. Life. Sci. **877:**1771-1777.

15. **Burkhardt, O., K. Borner, H. Stass, G. Beyer, M. Allewelt, C. E. Nord, and H. Lode.** 2002. Single- and multiple-dose pharmacokinetics of oral moxifloxacin and clarithromycin, and concentrations in serum, saliva and faeces. Scand. J. Infect. Dis. **34:**898-903.

16. **Wust, J., and U. Hardegger.** 1993. Penetration of clarithromycin into human saliva. Chemotherapy. **39:**293-296.

17. **Pfizer.** 2005. Zyvoxid. Product Information.

18. **Gorodischer, R., P. Burtin, P. Hwang, M. Levine, and G. Koren.** 1994. Saliva versus blood sampling for therapeutic drug monitoring in children: patient and parental preferences and an economic analysis. Ther. Drug Monit. **16:**437-443.