Title: Measurement of teicoplanin concentration with LC-MS/MS method demonstrates the usefulness of therapeutic drug monitoring in hematologic patient populations

Running head: Teicoplanin drug monitoring using LC-MS/MS

Name of the authors:

Hyojin Chae, MD, PhD,*† Jeong Joong Lee, MSc,*‡ Kyoungho Cha, MSc,* Su Hyun Her, BSc,* Hyo-Young Kim, PhD,*‡ Eunhee Han, MD, PhD,*‡ Myungshin Kim, MD, PhD,*‡ Yonggoo Kim, MD, PhD,*‡ Sung-Yeon Cho, MD, PhD,*§ Dong-Gun Lee, MD, PhD,*§

From *Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea;

†Catholic Laboratory Development and Evaluation Center, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea;

‡Agilent Technologies, Seoul, Republic of Korea;

§Division of Infectious Disease, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea.
Corresponding Author:

Myungshin Kim, MD, PhD, Department of Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul, 06591, Republic of Korea.

E-mail: microkim@catholic.ac.kr
Phone: 82 2 2258 1645
Fax: 82 2 2258 1719

Disclosure: The authors report no conflicts of interest related to this work.

Abstract

**Background:** Teicoplanin is a glycopeptide antibiotic that has become increasingly popular with the spread of methicillin-resistant Staphylococcus aureus. The aim of the study was to develop and validate a UHPLC-MS/MS (ultra-high performance liquid chromatography tandem mass spectrometry) method for teicoplanin, and analyze trough teicoplanin concentrations achieved in patients with hematological diseases.

**Methods:** The UHPLC-MS/MS method for teicoplanin was developed, validated, and applied in a retrospective analysis of trough plasma teicoplanin concentrations from 305 patients receiving standard dose, and 17 patients receiving TDM-guided individualized dose.

**Results:** The linear range was 3.9–52.9 mg/L. The imprecision was less than 12%, the limits of detection and quantification were less than 0.13 and 0.72 mg/L, respectively. The sample carry-over and ion suppression were insignificant. In the standard dose group, the median teicoplanin concentrations were 7.5 mg/L (days 3-5) and 8.9 mg/L (on days 6-8) and the proportion of trough
levels achieving ≥10 mg/L, was 20% (days 3-5) and 38% (days 6-8), respectively. In the TDM-guided individualized dose group, median teicoplanin concentration was higher (16.9 mg/L), and the proportion of trough levels ≥10 mg/L was also higher (77%) when compared with the standard dose group.

**Conclusions:** Based on these results, the present UHPLC-MS/MS method can be considered suitable for routine TDM of teicoplanin. Also based on the insufficient trough teicoplanin concentrations achieved with standard dose regimen, and the higher trough teicoplanin concentrations achieved with TDM-guided individualized dose regimen, this study highlights the importance of TDM of teicoplanin, especially in high-risk patient groups.

**Key Words:** teicoplanin, therapeutic drug monitoring, liquid chromatography-tandem mass spectrometry

**INTRODUCTION**

The use of glycopeptides has rapidly increased in the last 10 years due to the exponential spread of methicillin-resistant Staphylococcus aureus (MRSA), and teicoplanin, a glycopeptide antibiotic derived from the actinomycete *Actinoplanes teichomyceticus*,\(^1,2\) has become increasingly popular as an antibiotic.\(^1\) Although the efficacy of teicoplanin is similar to that of vancomycin, it has distinct pharmacokinetic properties. Teicoplanin has a long half-life (88-182 hours vs. 4-6 hours for vancomycin) that allows once-daily dosing and can be given intravenously or intramuscularly. Also, teicoplanin is associated with a lower rate of adverse events including nephrotoxicity compared to vancomycin.\(^3\)
Five closely related glycopeptides characterized by different fatty acid chains of 10 and 11 carbon atoms (A2-1, A2-2, A2-3, A2-4, and A2-5) comprise the majority (90%–95%) of teicoplanin found in vivo. A2-2 is the most active compound and is the major teicoplanin component accounting for about 50% of the mixture. Measurement of teicoplanin can be performed using microbiological assay, immunoassay, and high-performance liquid chromatography (HPLC) methods. While the immunoassay is still considered to be the gold standard by many, only one commercial assay is available (Thermo Scientific fluorescence polarization immunoassay), which may not be available worldwide. Also immunological methods are more susceptible to interferences and cross-reactions, in general, and teicoplanin immunoassays have been reported to suffer from high imprecision especially at low concentrations around 10 mg/L, which is the target trough concentration for teicoplanin.

The present study aimed to develop and validate a robust liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay for quantitation of teicoplanin concentration in a routine clinical therapeutic drug monitoring (TDM) laboratory. We also performed a retrospective analysis of teicoplanin concentrations in patients with hematological diseases, a patient population representative of immunocompromised hosts with a high risk of developing life-threatening MRSA infections, to determine whether target trough concentrations were achieved with standard dosage regimen and to explore the effect of TDM-guided dosage adjustment of teicoplanin on attaining target concentrations.
MATERIALS AND METHODS

Materials

Teicoplanin was provided by Ildong Pharmaceutical (Seoul, Korea), and non-isotope-labeled vancomycin (C_{66}H_{75}Cl_{2}N_{9}O_{24}) was purchased from Sigma-Aldrich (St. Louis, MO, USA). LC-MS-grade acetonitrile and methanol were purchased from Honeywell Burdick & Jackson (Muskegon, MI, USA), and formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). De-ionized water was generated using a Millipore Milli-Q Gradient Water Purification System (Molsheim, France).

Calibration Standards and Quality Control Material

Two standard superstock solutions of 1 mg/mL teicoplanin were prepared by dissolving accurately weighed teicoplanin powder in de-ionized water. From one solution, working calibrators were made by dilution of the stock solution into drug-free plasma (DFP) to give calibrators with teicoplanin concentrations of 0, 3.13, 6.25, 12.5, 25, and 50 mg/L. The other stock solution was diluted with DFP to prepare three levels of quality control materials (3.13, 12.5, and 25 mg/L). A 1 mg/mL vancomycin internal standard superstock solution was also prepared by dissolving vancomycin in de-ionized water. A working concentration of 100 mg/L was used.

Sample Preparation

Plasma samples, calibrators, or quality control samples (100 µL) and 35 µL of internal standard (vancomycin) were protein precipitated by the addition of 500 µL of acetonitrile. Tubes were vortexed vigorously for 1 min and centrifuged at 3000 relative centrifugal force for 5 min. The clear supernatant (100 µL) was mixed with 200 µL of mobile phase A, which contained 0.1% (v/v) formic acid in de-ionized water, and was vortexed for 10 s, and 120 µL were transferred to HPLC sample vials.
Liquid Chromatography

Liquid chromatography was performed using an Agilent 1290 UHPLC system equipped with a binary solvent pump, autosampler, and column compartment (Agilent Technologies, Waldbronn, Germany). Sample was injected onto an Agilent Poroshell 120 EC-C18 2.7 µm (3.0 × 5.0 mm) column (Agilent Technologies). The mobile phase consisted of solution A (0.1% formic acid in deionized water) and solution B (0.1% formic acid in acetonitrile). The flow rate was 0.3 mL/min with the following gradient conditions: 0-0.5 min, 5% B (isocratic); 0.5-1.5 min, 5%-100% B (linear gradient); 1.5-2.5 min, 100% B (isocratic); 2.5-2.6 min, 100%-5% B (linear gradient); and 2.6-5 min, 5% B (isocratic). A needle wash solvent consisted of 40%, 40%, and 20% of acetonitrile, methanol, and propan-2-ol, respectively, and needle wash was used for 5 s between the sample injections to wash off the injection needle in the autosampler.

Mass Spectrometry

An Agilent 6460 triple-quadrupole mass spectrometer (Agilent Technologies) was operated in the positive electrospray ionization mode with the following parameters: dry gas temperature of 325°C, dry gas flow rate of 7 L/min, nebulizer pressure of 40 psi, sheath gas temperature of 350°C, sheath gas flow rate of 12 L/min, nozzle voltage of 500 V, and capillary voltage of 4000 V. An Agilent MassHunter workstation (Agilent Technologies) was used to control the equipment, data acquisition and analysis. Compounds were detected via multiple reaction monitoring mode employing the ion transitions of m/z 940.1 → 316.1 for teicoplanin A2-2/A2-3 and m/z 724.7 → 143.9 for the internal standard, vancomycin.
Patients

A retrospective analysis was conducted on teicoplanin-treated patients with hematologic disease at the Catholic Blood and Marrow Transplantation Center at Seoul St. Mary’s Hospital in Korea. For neutropenic patients, teicoplanin was not used as a routine antimicrobial therapy during chemotherapy or hematopoietic stem cell transplantation. Teicoplanin was used only when patient had 1) positive blood cultures for Gram-positive bacteria, 2) presentations of severe sepsis or shock, pending blood culture results 3) history of MRSA infection or colonization, 4) skin and soft tissue infection, or 5) a suspected catheter-related infection.\textsuperscript{11} And if the causative pathogen was MRSA and confirmed as susceptible to teicoplanin, infectious disease physicians adjusted the dose of teicoplanin while monitoring TDM and follow-up blood cultures. Patients aged 18 years or older, who were admitted from December 2015 and June 2017, were included if they had their plasma teicoplanin concentrations measured using the newly developed and validated LC-MS/MS method. Data collected from hospital records for each of the identified teicoplanin antimicrobial treatment episodes included gender, age, body weight, serum creatinine, underlying disease, administered dose, day of therapy, time of sample collection, and plasma teicoplanin concentration. The standard dosing regimen was three loading doses of 400 mg i.v. at 12-hour intervals followed by once-daily 400 mg i.v. maintenance dosing.\textsuperscript{12} Only trough concentrations were considered for inclusion in the analyses. And the adoption of an individualized dosage regimen based on teicoplanin TDM was at the discretion of infectious disease physicians. This study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board (IRB)/Ethics Committee of Seoul St. Mary’s Hospital.
Statistical Analyses

Continuous data are presented as medians (ranges) for non-normally distributed data, and categorical data are presented as numbers (%). Normality was assessed using the D’Agostino and Pearson normality test. Univariate analyses between the individual variables and the plasma trough teicoplanin concentrations were performed using the Kruskal-Wallis H-test with a post-hoc analysis for continuous variables and the Chi-square test for categorical variables. MedCalc version 12.1.4 (MedCalc Software, Mariakerke, Belgium) was used for all statistical analyses, and \( P < 0.05 \) was considered statistically significant.

RESULTS

Selectivity

Teicoplanin A2-2/A2-3 and vancomycin had elution times of 2.12 and 1.99 min, respectively (Fig. 1). In the selectivity test performed using six blank plasma samples from six different individuals, there was no endogenous interference at the mass transitions of teicoplanin A2-2/A2-3 and vancomycin at the same retention times, highlighting the selectivity of the assay.

Within- and Between-day Precision

Within- and between-day precision of the method was assessed by the analysis of three samples at various concentrations (3.5, 13.7, and 24.8 mg/L). These samples were analyzed 20 times each within a single run to determine intra-assay imprecision, and also analyzed in separate batches (n=20) over a period of 5 days to assess inter-assay imprecision. Both standard deviation and coefficient of
variation were calculated and used to determine the imprecision of the method. The intra-assay imprecisions were 9.2%, 2.8%, and 5.0% for mean concentrations of 3.5, 13.7, and 24.8 mg/L, respectively, and inter-assay imprecisions were 11.7%, 6.5%, and 4.6% for mean concentrations of 3.19, 13.97, and 25.23 mg/L, respectively.

**Linearity**

Linearity of the method was determined by analyzing a set of six levels of calibrators in duplicate according to the Clinical and Laboratory Standards Institute EP06 guideline. The linear regression equation obtained from estimated and actual measurements of teicoplanin A2-2/A2-3 was $y=1.0535x+0.251$, and linearity was observed in the concentration ranges of 3.855 to 52.940 mg/L. The coefficient of determination ($R^2$) was 0.09993, and the standard error of regression was 0.734 (Fig. 2).

**Limit of Detection and Quantification**

The lowest level calibrator was diluted two-fold, five-fold, and ten-fold with blank plasma, and each diluted sample was measured eight times. The limit of detection, defined as the lowest concentration at which the ion ratio exceeds the baseline signal-to-noise by a factor of 3, was 0.13 mg/L. The limit of quantification, defined as the lowest concentration at which the ion ratio exceeds the baseline signal-to-noise by a factor of 10 with a CV of less than 20% and accuracy within ±20% of the nominal concentration, was 0.72 mg/L (CV and relative error values were 16% and 14%, respectively).
Sample Carry-over

For carry-over evaluation, two levels of samples at high and low concentrations (H, high concentration: 50 mg/L; L, low concentration: 3 mg/L) were measured in the following sequence H1-H2-H3-L1-L2-L3, and carry-over (%) was calculated as (L1-L3)/(H3-H1)×100. The sample-to-sample carry-over was estimated as 0.8%, which was considered insignificant (<5%).

Ion Suppression

Ion suppression was evaluated by quantitative assessment of the absolute matrix effect. The mean absolute matrix effect (%) was defined by comparing the peak areas obtained from plasma sample with the peak areas of the same analyte present in the mobile phase. The mean absolute matrix effect was 91.3%, 86.1%, and 88.1% for 3.13, 6.25, and 12.5 mg/L of teicoplanin, respectively.

Extract Stability

Extract stability was evaluated according to existing guidelines\textsuperscript{14} by measuring stored extracts of three levels of QC (n=8 at each level) maintained at autosampler temperature 2-8°C for 72 hours. The final extract stability was demonstrated for at least 72 hours (see Supplement Table 1, http://links.lww.com/TDM/A238 ) with a mean accuracy within ±15% of the nominal concentration and CV less than 15%.

Patient Characteristics

A total of 350 patients who were treated with standard dosage regimen were included in the analysis, with a median age of 53 years (range: 18-85 years), median weight of 62 kg (range: 37-122 kg), and 177 (51%) were male. The demographic and clinical characteristics of the patients are shown in Table 1.
Plasma Teicoplanin Concentrations in Patients Under Standard Dosage Regimen

A total of 585 trough plasma teicoplanin measurements were measured from 350 patients. In treatments with more than one trough measurements performed on days 3-8, the first trough level was used. Considerable variation in trough concentrations was observed despite the administration of same dose. The distributions of the plasma teicoplanin concentrations according to the day of therapy are shown in Fig 3. There was a significant difference between the plasma teicoplanin concentrations according to the day of therapy (Kruskal-Wallis test, \( P < 0.005 \)). And post-hoc analysis confirmed a significant difference between the teicoplanin concentrations measured on days 3-5 and on days 6-10 (\( P < 0.05 \)). The median plasma teicoplanin concentrations on days 3-5 were 7.5 mg/L (range: 1.1-22.2 mg/L) and on days 6-8 were 8.9 mg/L (range: 2.3-46.2 mg/L). The proportion of trough levels \( \geq 10 \) mg/L on days 3-5 and on days 6-8 was 20% and 38%, respectively (\( P < 0.05 \) by Chi-square test).

Plasma Teicoplanin Concentrations in Patients Under Individualized TDM-guided Dosage Regimen

Apart from the 350 patients who followed a standard dosage regimen, 17 patients with hematologic diseases were treated with an individualized escalating dosage regimen of teicoplanin, guided by frequent teicoplanin TDM measurement and a close clinical surveillance (Table 1). In this patient group, the median plasma teicoplanin concentration was 16.9 mg/L (range: 3.8-36.0 mg/L), and the proportion of trough levels \( \geq 10 \) mg/L was 77%.
DISCUSSION

Teicoplanin is effective and safe in the treatment of staphylococcal infections, including endocarditis, osteomyelitis, and septic arthritis.\(^\text{15}\) It is generally accepted that a serum trough concentration (\(C_{\text{min}}\)) \(> 10 \text{ mg/L}\) is appropriate for MRSA infections.\(^\text{16}\) However, recent studies and meta-analyses have indicated that \(>20 \text{ mg/L}\) \(C_{\text{min}}\) may be needed for effective treatment of septic arthritis, endocarditis, and possibly other deep-seated staphylococcal infections.\(^\text{12}\) Therefore, TDM of teicoplanin is advocated to ensure efficacy, particularly in patients who have high clearance, such as children, burn patients, and patients with neutropenia, and in the management of serious infections to ensure adequate dosing.\(^\text{17}\) Moreover, the correlation between trough levels of teicoplanin and clinical outcome is supported by several studies and highlights the importance of TDM to ensure therapeutic concentrations of teicoplanin.\(^\text{1,16,18}\)

With the emergence of teicoplanin as an alternative glycopeptide antibiotic to vancomycin, and the recognized importance of trough teicoplanin concentration monitoring, it was our goal to develop a robust and high-throughput LC-MS/MS assay for the determination of teicoplanin. Although a deuterated teicoplanin would be ideal as an internal standard, this was found to be cost-prohibitive,\(^\text{8}\) and other potential internal standards including ristocetin\(^\text{8}\) and polymixin B\(^\text{19}\) could not be used because of their differences in polarity and structure. The former led to larger deviations in the retention time between the internal standard and teicoplanin and the latter led to excessive imprecision. Thus, we adopted vancomycin,\(^\text{7}\) which showed similar behavior to teicoplanin during chromatography and ionization, as the internal standard. Although vancomycin can also be used as a therapeutic antimicrobial agent, since teicoplanin and vancomycin have similar mechanisms of action, it is unlikely that patients are administered teicoplanin in conjunction with vancomycin. Even in those patients for whom vancomycin is switched to teicoplanin, since teicoplanin TDM is
recommended at a steady state, this will leave enough time for vancomycin to be excreted completely from the body.  

Although LC-MS/MS is capable of separating the teicoplanin components, to optimize the suitability of the method for routine clinical application, only the largest fraction, A2-2/A2-3, was used for calibration and quantification as recommended in other methods.  

This is possible because the sub-composition of teicoplanin is regulated by governmental bodies, and therefore always similar.  

Possibly due to the molecular size of teicoplanin and its ionization properties, there are only a few LC-MS/MS methods developed and validated for teicoplanin measurement using multiple reaction monitoring method (see Supplement Table 2).  

Our method has a run time of 5 min and hence amenable for routine use in the clinical TDM laboratory. Also, this method is robust in terms of measuring teicoplanin in plasma (cumulative results of calibrators during a 6-month period of teicoplanin TDM monitoring are provided, see Supplement Table 3) with wide linear range spanning the therapeutically relevant range of teicoplanin concentrations.

While the long half-life of teicoplanin allows once-daily dosing, such a pharmacokinetic characteristic has the disadvantage of a slow onset to reach a steady-state concentration, and therefore an initial loading dose should be administered to attain therapeutically relevant concentrations early in the treatment period.  

The standard dosing regimen includes a loading dose of 400 mg administered three times at 12-hour intervals, followed by a once-daily dose of 400 mg thereafter.  

In this retrospective, single-center cohort study of 350 patients with hematologic diseases under standard dosing regimen, a significant proportion of patients failed to achieve a trough concentration ≥10 mg/L at early days of therapy. This is in line with previous observations that the conventional loading dose regimen is insufficient to achieve optimal trough concentrations in patients.  

Therefore, a higher loading dosage regimen might be warranted to reach steady-
state teicoplanin concentrations more rapidly, in certain clinical setting, including infections in patients with hematological diseases.\textsuperscript{10,27} In this regard, measuring teicoplanin by LC-MS/MS substantially improves the reliability in TDM of teicoplanin, whose trough concentrations have to be quickly determined to ensure adequate teicoplanin dosing. The clinical implications of TDM of teicoplanin is clearly demonstrated by the fact that of the patients in which individualized TDM-guided escalating dosage regimen was employed, the majority of the patients achieved a trough concentration $\geq 10$ mg/L, compared with the standard dosing regimen of which the majority failed to attain.

**CONCLUSION**

In conclusion, the present method is the third LC-MS/MS method employing multiple reaction monitoring published to date, and the method has been validated to be robust for high-throughput and efficient TDM of teicoplanin in a routine clinical laboratory. Our retrospective analysis of trough teicoplanin concentrations using the newly developed method also emphasizes the importance of performing TDM of teicoplanin and using individualized dosing regimen in attaining target plasma trough concentrations in certain clinical settings.

**ACKNOWLEDGMENTS**

The author wishes to acknowledge the financial support of the Catholic Medical Center Research Foundation made in the program year of 2015.
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**Figure Legends**

**FIGURE 1.** Representative ion chromatograms of (A) a blank plasma sample, (B) a sample spiked at the LLOQ, and (C) a patient sample with a teicoplanin concentration of 13.7 mg/L.

**FIGURE 2.** The linearity of the standard curve was observed at teicoplanin concentrations that ranged 3.855 to 52.940 mg/L.

**FIGURE 3.** The longitudinal plasma teicoplanin concentration distributions according to the sampling day. The lower and upper limits of the boxes represent the 25th and 75th percentiles, respectively. The horizontal lines within the box represent the median value, and the lower and upper whiskers represent the 10th and 90th percentiles, respectively. *P < 0.005* by Kruskal-Wallis H-test. Significant differences between pairs (*P < 0.05* by post-hoc analysis) are indicated with different lower-case characters (a, b).
Table 1. Patient characteristics

<table>
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<td>51 (32-71)</td>
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<tr>
<td>Gender</td>
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<td>177 (51%)</td>
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HLH, hemophagocytic lymphohistiocytosis; ITP, idiopathic thrombocytopenia purpura; NS, not significant (P > 0.05)
Peak-area ratio of teicoplanin to internal standard

\[ y = 124.88x + 31.893 \]

\[ R^2 = 0.9967 \]
![Box plot showing teicoplanin concentration (mg/L) over different days of therapy.](image)

- *P < 0.005

**Teicoplanin concentration (mg/L)**

**No. of days of therapy**

D3, D4, D5, D6, D7, D8