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ORIGINAL RESEARCH

Prediction of Unbound Vancomycin Levels in Intensive Care Unit and Nonintensive Care Unit Patients: Total Bilirubin May Play an Important Role

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Background: The mean unbound vancomycin fraction and whether the unbound vancomycin level could be predicted from the total vancomycin level are still controversial, especially for patients in different groups, such as intensive care unit (ICU) versus non-ICU patients. Other relevant potential patient characteristics that may predict unbound vancomycin levels have yet to be clearly determined.

Methods: We enrolled a relatively large study population and included widely comprehensive potential covariates to evaluate the unbound vancomycin fractions in a cohort of ICU (n=117 samples) and non-ICU patients (n=73 samples) by using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.

Results: The mean unbound vancomycin fraction was 45.80% ± 18.69% (median, 46.01%; range: 2.13–99.45%) in the samples from the total population. No significant differences in the unbound vancomycin fraction were found between the ICU patients and the non-ICU patients (P=0.359). A significant correlation was established between the unbound and total vancomycin levels. The unbound vancomycin level can be predicted with the following equations: unbound vancomycin level=0.395×total vancomycin level+0.019×total bilirubin level+0.468 (R^2=0.771) for the ICU patients and unbound vancomycin level=0.526×total vancomycin level-0.527 (R^2=0.749) for the non-ICU patients. Overall, the observed-versus-predicted plots were acceptable.

Conclusion: A significant correlation between the total and unbound vancomycin levels was found, and measurement of the unbound vancomycin level seems to have no added value over measurement of the total vancomycin level. The study developed parsimonious equations for predicting the unbound vancomycin level and provides a reference for clinicians to predict the unbound vancomycin level in adult populations.

Keywords: unbound vancomycin fraction, unbound vancomycin level, patient characteristics, predictors

Background

Vancomycin, an important antibacterial agent, has been recommended as a first-line treatment for gram-positive infections, especially methicillin-resistant *Staphylococcus aureus*.[1,2] It is well known that for vancomycin, a ratio of the area under the curve (AUC) concentration-time profile to the minimum inhibitory concentration (MIC) (AUC:MIC) of 400 or more can increase the likelihood of clinical cure and decrease the likelihood of toxicity.[3,4] In recent guidelines, serum trough concentrations of 15 to

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20 mg/L were stated to serve as a surrogate marker to reach this ratio at an MIC ≤ 1 mg/L for vancomycin.[5,6]

In the clinic, only the total vancomycin level is routinely measured.[4] Moreover, dosage adjustments depend on a total drug target, even though it is known that for most antibiotics, including vancomycin, only the unbound fraction of drug is critical for antimicrobial activity.[7–9] As a rule of thumb, a protein binding proportion of approximately 50% is assumed.[4,10,11] Some original studies concluded that the mean values of the unbound vancomycin fraction were 41.9% ± 14.1%[12] and 0.54 ± 0.08[13] and that the mean values of the bound vancomycin fraction were 41.5% ± 8.6%[14] and 54.6% ± 9.5%,[3] apparently justifying this rule of thumb. However, we found that the percentage protein binding of vancomycin reported in the literature ranges from nearly 0% to almost 100%,[15–21] with great controversy. This controversy might be due to the differences in different patient groups, such as patients with hypoalbuminemia, burns, myeloma, and obesity, where serum protein concentrations had high variability in these groups.[11,22–24] Notably, catabolism, systemic inflammation and multiple organ dysfunction may lead to plasma protein binding changes in ICU patients.[25,26] Some published literature indicates that plasma protein binding changes of drugs are common in intensive care unit (ICU) patients,[26,27] but this needs to be verified for the percentage protein binding of vancomycin.

In addition, some studies have concluded that the unbound vancomycin level could be predicted from the total vancomycin level.[11,14,19–21] Contradictory results have also been reported in which the unbound vancomycin level could not be predicted from the total vancomycin level.[18,25] Therefore, the mean value of the unbound fraction of vancomycin and whether the unbound vancomycin level could be predicted from the total vancomycin level remain controversial, especially for patients in different groups, such as ICU patients and non-ICU patients. In addition, other relevant potential patient characteristics, such as age, body mass index (BMI), creatinine level, cystatin C level, prealbumin level, total protein level, globulin level, albumin level, total bilirubin level, direct bilirubin level, and history of diabetes mellitus, heart failure, dialysis and hepatic dysfunction, that may account for alterations in the unbound vancomycin level have yet to be clearly determined.

Here, to address the aforementioned issues, we performed a study to evaluate the unbound vancomycin fractions in a large cohort of ICU and non-ICU patients by using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method and to examine the extent to which unbound and total vancomycin levels are correlated. In addition, this study also evaluated variables extracted from patient characteristics that may account for alterations in unbound vancomycin levels.

Patients and Methods

Patients

We conducted a retrospective, noninterventional study with archived samples from patients aged 18 years and older who received vancomycin for suspected or proven gram-positive infections and required routine total vancomycin therapeutic drug monitoring (TDM) between April 2018 and December 2019. Samples (n=71) from patients (n=57) were excluded from the following analysis because the archived plasma samples were insufficient. Samples (n=190) from patients (n=152) treated by intermittent infusion of vancomycin were analyzed. This study was approved by the Institute Medical Ethics Committee of the Affiliated Hospital of Qingdao University and was conducted in accordance with the ethical principles of the Declaration of Helsinki. As the study was performed with archived samples, the requirement for informed consent was waived by the Institute Medical Ethics Committee, but patient confidentiality was protected.

Data Collection

Patient data were collected by a review of the medical records. The baseline data collected were age, sex, BMI and location (ICU versus non-ICU). The treatment details consisted of drugs with high plasma protein binding (PPB) (>70%) coadministered on the day of sampling, such as phenytoin, valproic acid, vitamin K antagonists, aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs).[19] Biochemical data collected from the laboratory information system included total protein, prealbumin, albumin, globulin, total bilirubin, direct bilirubin, creatinine and cystatin C levels. These reported laboratory data were obtained on the day of sample collection for routine total vancomycin measurement. If a laboratory value was not measured on the day of sample collection, the closest value within 3 days of sample collection was chosen when available.

Vancomycin Determination

Archived plasma samples sent to the clinical pharmacy laboratory of the Affiliated Hospital of Qingdao

University for total vancomycin measurements were collected from the laboratory sample storage system (2°C to 8°C) at the end of the measurement day. Samples were centrifuged at 2000×g (37°C) for 5 min. One part was frozen (−20°C) for measurement of total vancomycin by LC-MS/MS, and another part was processed directly to obtain the unbound vancomycin fraction.

The part of each sample used to obtain the unbound vancomycin fraction was centrifuged with a Centrifree Centrifugal Filter Device (molecular weight cutoff, 30,000; Millipore, Billerica, MA). Briefly, 600 μL of plasma was incubated in the capped Centrifree Centrifugal Filter Device and centrifuged at 2000×g (37°C) for 30 min. The unbound vancomycin samples were ultrafiltered and stored at −20°C until analysis.

Unbound and total vancomycin levels were measured by LC-MS/MS. Triazolam was chosen as the internal standard (IS). For total vancomycin, 25 μL of blank plasma sample was added to each 25 μL of thawed sample and mixed. Fifty microliters of the mixture was extracted with 150 μL of methanol (containing 0.1 mg/L IS) after appropriate dilution, and the supernatant was obtained after shaking completely. Fifty microliters of each extracted sample was diluted with 150 μL of 15% methanol before detection with LC-MS/MS. For unbound vancomycin, 10 μL of each thawed ultrafiltrate sample was appropriately diluted with 70 μL of blank ultrafiltrate (including 0.1 mg/L IS). The latter was achieved using a ChromCore^{TM} 120 C_{18} column (3 μm, 100 mm×2.1 mm; NanoChrom Technologies [Suzhou]). The upper and lower limits of quantification were 0.3125 mg/L and 20 mg/L, respectively. The inter- and intraday coefficients of variation as determined by high (10 mg/L), medium (1.25 mg/L) and low (0.67 mg/L) quality control samples were less than 12.6%.

Mass spectrometry analysis was performed on an API4000^{+} triple-quadrupole mass spectrometer equipped with a TurboIonSpray ionization source (AB Sciex Instruments, USA). Quantifications were performed in positive ionization mode with multiple reaction monitoring for the following transitions: m/z 725.6→144.3 for vancomycin and m/z 343.2→239.2 for IS. The detection parameters were optimized as follows: turbo gas temperature, 550°C; ion spray voltage, 5500 V; declustering potential, 42 for vancomycin and 100 for IS; and collision energy, 20 for vancomycin and 55 for IS. Gradient elution was applied with methanol (supplemented with 0.1% formic acid, mobile phase A) and 0.1% formic acid (v/v, mobile phase B) at a flow rate of 0.4 mL/min. The following gradient program was used for plasma separation: 0–0.3 min, 12% A; 0.3–0.8 min, 12→85% A; 0.8–2.2 min, 85% A. The column temperature was maintained at 40°C, and the injection volume was 5 μL.

The level of vancomycin was calculated by integration of the peak area ratio between vancomycin and triazolam based on standard calibration curves. The unbound vancomycin fraction (percent) was calculated according to the following equation: (ultrafiltrate level/total vancomycin level) × 100.[11,19]

Model Validation

The performance of the prediction model was assessed for model validation of the unbound vancomycin estimate. The study cohort for validation of the prediction tool consisted of an additional 13 hospitalized adult patients in the ICU ward and 6 hospitalized adult patients in the non-ICU ward with clinically indicated vancomycin samples (based on archived samples). The methodology used for modeling, including sample collection, data collection and unbound vancomycin measurement, was also used for model validation. Goodness of fit was assessed by regression with an observed-versus-predicted plot and Bland–Altman analysis, and model validation was performed based on bias and precision.[14] Mean bias was calculated according to the following equation as previously reported:[14] $(1/n)\times\Sigma\{$(predicted unbound vancomycin level - observed unbound vancomycin level)/predicted unbound vancomycin level$\}$, where n is the number of predicted unbound vancomycin levels. Mean precision was calculated according to the following equation as previously reported:[14] $(1/n)\times\Sigma\{(|$predicted unbound vancomycin level - observed unbound vancomycin level$|)$/predicted unbound vancomycin level$\}$, where n is the number of predicted unbound vancomycin levels.

Statistical Analysis

Statistical analysis was performed with SPSS statistical software (version 19.0; SPSS Inc., Chicago, Illinois, USA). Bland–Altman analysis and Spearman correlation analysis were performed with MedCalc statistical software (version 20; MedCalc, Ostend, Belgium). Collected data are expressed as the mean ± SD for continuous variables with a Gaussian distribution, while the median (min, max) was used for continuous variables without Gaussian distribution. Categorical variables are presented as

frequencies and percentages. Differences between different patient wards were assessed with the Mann–Whitney *U*-test.

Correlations between total and unbound vancomycin levels were investigated by using scatterplots combined with Spearman correlation analysis. To identify potential predictors from variables extracted from patient characteristics for the unbound vancomycin level, we used linear regression analyses. Patient baseline data, such as history of diabetes mellitus, heart failure, dialysis and hepatic dysfunction, were defined as patients with a history of diabetes mellitus = 1 and patients with no history of diabetes mellitus = 0. All tests were two-sided, and a P value of <0.05 was considered statistically significant.

Results

Patient Characteristics

A total of 190 samples from 152 patients (95 patients in the ICU ward and 57 patients in the non-ICU ward) aged 18 years and older who received vancomycin for suspected or proven gram-positive infections and required routine total vancomycin TDM were included. The mean age at the time of vancomycin treatment was 56.03 ± 16.68 years (median: 58 years; range: 18–90 years). When separated by ward, 95 patients were from the ICU ward, and 57 were from the non-ICU ward. In the ICU ward, there were 13 patients with a history of diabetes mellitus, 11 patients with a history of heart failure and 5 patients with a history of hepatic dysfunction. In the non-ICU ward, there were 14 patients with a history of diabetes mellitus, 13 patients with a history of heart failure, 12 patients with a history of dialysis and 5 patients with a history of hepatic dysfunction. The remaining demographic and patient characteristics collected are summarized in Table 1. The distributions of total bilirubin levels in the different patient populations are shown with box-and-whisker plots in Figure 1.

The Unbound Vancomycin Fractions and the Correlation Between Free Vancomycin Level and Total Vancomycin Level

The mean total vancomycin level was 16.57 ± 14.13 mg/L (median: 12.44 mg/L; range: 1.79–104.50 mg/L) among the samples from the total study population, 15.73 ± 13.80 mg/L (median: 12.55 mg/L; range: 1.79–104.50 mg/L) among the ICU ward samples and 17.90 ± 14.65 mg/L (median: 12.41 mg/L; range: 3.21–71.0 mg/L) among the non-ICU ward samples. The mean unbound vancomycin level was 7.78 ± 7.44 mg/L (median: 5.54 mg/L; range: 0.11–49.44 mg/L) among the total population samples, 7.10 ± 6.31 mg/L (median: 5.70 mg/L; range: 0.11–33.40 mg/L) among the ICU ward samples and 8.89 ± 8.90 mg/L (median: 5.12 mg/L; range: 0.50–49.44 mg/L) among the non-ICU ward samples. The mean unbound vancomycin fraction was 45.80% ± 18.69% (median, 46.01%; range: 2.13–99.45%) among the total population samples, 44.47% ± 17.15% (median, 45.19%; range: 2.13–92.07%) among the ICU ward samples and 47.94% ± 20.88% (median, 49.01%; range: 7.60–99.45%) among the non-ICU ward samples. As shown in Figure 2, no significant differences in the total vancomycin level (Figure 2A, P=0.475), unbound vancomycin level (Figure 2B, P=0.440) or unbound vancomycin fraction (Figure 2C, P=0.359) were found between the populations in the ICU ward and the non-ICU ward (Mann–Whitney *U*-test).

The correlation between the unbound vancomycin level and total vancomycin level among patients in the different wards is described in Figure 3. Among the total population samples, the unbound vancomycin level was strongly correlated with the total vancomycin level (Figure 3A; R=0.860, P<0.01). A significant correlation could also be established between unbound vancomycin level and total vancomycin level for the patients in the ICU ward (Figure 3B; R=0.871, P<0.01) and in the non-ICU ward (Figure 3C; R=0.866, P<0.01), respectively.

Regression Analysis

The variables included in the multiple linear mixed model were age, BMI, creatinine level, cystatin C level, prealbumin level, total protein level, globulin level, albumin level, total bilirubin level, direct bilirubin level, number of coadministered drugs with >70% PPB, history of diabetes mellitus, heart failure, dialysis, hepatic dysfunction and total vancomycin level. As shown in Table 2, the multiple linear mixed model analysis indicated that the total vancomycin level was the strongest predictor of the unbound vancomycin level among patients in the ICU ward, patients in the non-ICU ward and all patients (β=0.864, SE=0.023, P=0.000; β=0.866, SE=0.048, P=0.000; and β=0.863, SE=0.023, P=0.000, respectively). Other variables found to be predictive of the unbound vancomycin level were BMI (β=−0.102, SE=0.084, P=0.021) among all patients and total bilirubin (β=0.110, SE=0.009, P=0.035) among the ICU ward patients. The results indicated that the unbound vancomycin level can

Table 1 Patient Demographic and Baseline Characteristics on the Day of Sampling of Patients Treated with Vancomycin

Parameter	ICU	Non-ICU	ALL
No. patients	95	57	152
No. samples	117	73	190
No. patients with diabetes mellitus	13	14	27
No. patients with heart failure	11	13	24
No. patients with dialysis	0	12	12
No. patients with hepatic dysfunction	5	5	10
Age (year)	64 (18–88)	49 (18–90)	57 (18–90)
Male/female ratio	62/33	39/18	101/51
BMI (kg/m2)	24.22 (16.44–36.05)	23.81 (17.90–40.75)	24.21 (16.44–40.75)
Creatinine level (μmol/L)	68.10 (18.93–740.20)	83.40 (36.3–1104.9)	74.0 (18.93–1104.9)
Cystatin C level (mg/L)	1.12 (0.51–7.38)	1.21 (0.57–10.40)	1.14 (0.51–10.40)
Prealbumin level (g/L)	167.6 (48.0–383.8)	210.0 (42.0–418.5)	173.25 (42.0–418.5)
Total protein level (g/L)	57.82 (39.04–99.90)	59.34 (40.30–99.90)	59.02 (39.04–99.90)
Globulin level (g/L)	26.51 (13.15–40.10)	27.30 (14.50–62.10)	27.16 (13.15–62.10)
Albumin level (g/L)	32.31 (20.68–64.50)	35.10 (21.20–60.0)	33.8 (20.68–64.50)
Total bilirubin level (μmol/L)	10.91 (3.30–258.06)	9.22 (2.50–68.90)	10.0 (2.50–258.06)
Direct bilirubin level (μmol/L)	4.49 (0.74–228.26)	3.30 (0.11–38.57)	4.26 (0.11–228.26)
Type of infection			
Osteomyelitis	8 (6.8%)	5 (6.8%)	13 (6.8%)
Septicemia	33 (28.2%)	12 (16.4%)	45 (23.7%)
Intracranial infection	21 (17.9%)	12 (16.4%)	33 (17.4%)
Infective endocarditis	7 (6.0%)	14 (19.2%)	21 (11.1%)
Skin and soft tissue	21 (17.9%)	9 (12.3%)	30 (15.8%)
Other	27 (23.1%)	21 (28.8%)	48 (25.3%)
No. coadministered drugs with >70% PPB	0 (0–2)	0 (0–2)	0 (0–2)

Note: Descriptive data are presented as the median (min, max) or no. (%) of patients.
Abbreviations: ICU, intensive care unit; BMI, body mass index; PPB, plasma protein binding.

be predicted with the following equation: unbound vancomycin level=0.395×total vancomycin level+0.019×total bilirubin level+0.468 (R^2=0.771) for patients in the ICU ward, where the total vancomycin level is in milligrams per liter and the total bilirubin level is in micromoles per liter. The unbound vancomycin level can be predicted with the following equation: unbound vancomycin level=0.526×total vancomycin level-0.527 (R^2=0.749) for patients in the non-ICU ward, where the total vancomycin level is in milligrams per liter and the BMI is expressed in kilograms per square meter.

Model Validation

Validation of our prediction model for patients in the ICU ward was evaluated by using data from 13 patients aged 18 years or older in the ICU ward. The total vancomycin level was 15.50 ± 7.20 mg/L (median: 17.56 mg/L; range: 3.77–26.84 mg/L). The total bilirubin level was 26.31± 46.59 mg/L (median: 15.85 mg/L; range: 3.47–209.80 mg/L). The unbound vancomycin level was 7.46 ± 4.84 mg/L (median: 6.84 mg/L; range: 1.76–14.6 mg/L). The observed-versus-predicted plots and Bland–Altman analysis for the patients included in the validation model

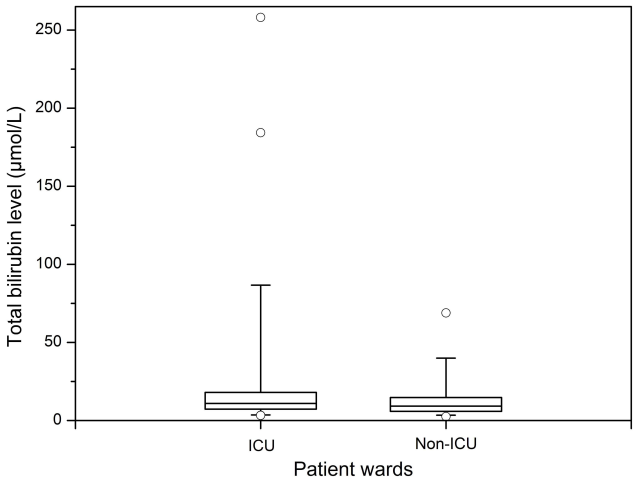

Figure 1 The distributions of total bilirubin level in the different patient populations are shown with box-and-whisker plots. The data are presented as boxes indicating the 2.5th, 25th, 50th (median), 75th, and 97.5th percentiles. Statistically nonsignificant difference (P>0.05, Mann–Whitney *U*-test) is not shown between patients in the ICU ward and non-ICU ward.

are presented in Figure 4. The R^2 was 0.907, the mean bias was −0.051, and the mean precision was 0.292, indicating that the observed-versus-predicted plot was acceptable.

Validation of our prediction model for patients in the non-ICU ward was evaluated by using data from 6 patients aged 18 years or older in the non-ICU ward. The total vancomycin level was 12.41 ± 5.84 mg/L (median: 10.18 mg/L; range: 7.29–20.85 mg/L). The unbound vancomycin level was 5.03 ± 2.11 mg/L (median: 4.78 mg/L; range: 1.97–7.88 mg/L). The observed-versus-predicted plots and Bland–Altman analysis for the patients included in the validation model are presented in Figure 5. Overall, the observed-versus-predicted plot was acceptable. The R^2 was 0.756, the mean bias was 0.114, and the mean precision was 0.241.

Discussion

Despite its history of extensive use, the unbound vancomycin fraction has not been adequately studied with high variability when evaluated in clinical patient data. Since only the unbound drug is thought to be pharmacologically active, a better understanding of the unbound vancomycin fraction is clearly of great importance and may assist in guiding dosing recommendations.[3,21] In this study, we evaluated unbound vancomycin fraction in a relatively large cohort of different patient populations, including 117 samples from 95 ICU patients and 73 samples from 57 non-ICU patients. Previous studies have already found a large variation in the unbound vancomycin fraction, ranging from nearly 0% to almost 100%.[15–21] Our present study showed that the unbound vancomycin fraction ranged from 2.13% to 99.45% in the overall patient population, which is consistent with the abovementioned previous studies.

Because PPB changes of drugs are common among ICU patients,[26,27] we compared the unbound vancomycin fraction between ICU patients and non-ICU patients but found no significant differences (P=0.359). Consistent with our findings, some studies also examined the effect of ICU location on the unbound vancomycin fraction and found no significant differences.[18,19] Another previous study showed that ICU patients had a protein binding value that was 8.4% lower than that of non-ICU patients.[3] Notably, however, only 55 patients were included in that study, including 14 ICU patients.

Whether the unbound vancomycin level can be predicted from the total vancomycin level remains controversial. We performed a study with a relatively large cohort to evaluate the extent to which unbound and total vancomycin levels are correlated. We found that a significant correlation could be established between the unbound vancomycin level and the total vancomycin level among all patients (R=0.860, P<0.01), among ICU patients (R=0.871, P<0.01) and among non-ICU patients (R=0.866, P<0.01), which more or less agrees with the viewpoints of previous studies.[11,14,19–21] Contrasting results concluded that the unbound vancomycin level is independent of the total vancomycin level.[18] Of note, the study did not include a large patient population, with 65 samples from 15 patients, and the patients received vancomycin by continuous infusion, whereas our patients received vancomycin by intermittent infusion, potentially explaining this contradiction.

Linear regression was selected to model the data because stepwise regression could be used to attempt to relate the unbound vancomycin level to one or more of the available clinical patient data points and establish a parsimonious equation for predicting the unbound vancomycin level. We found that total bilirubin was a predictor of unbound vancomycin levels in ICU ward patients, a novel finding that has not been reported in previous models to our knowledge. As described in Figure 1, the level of total bilirubin ranges widely among ICU patients and may function as a predictor of unbound vancomycin level because it can expel vancomycin from albumin and bind to it,[28] elevating unbound vancomycin levels. Our results therefore indicate that total bilirubin

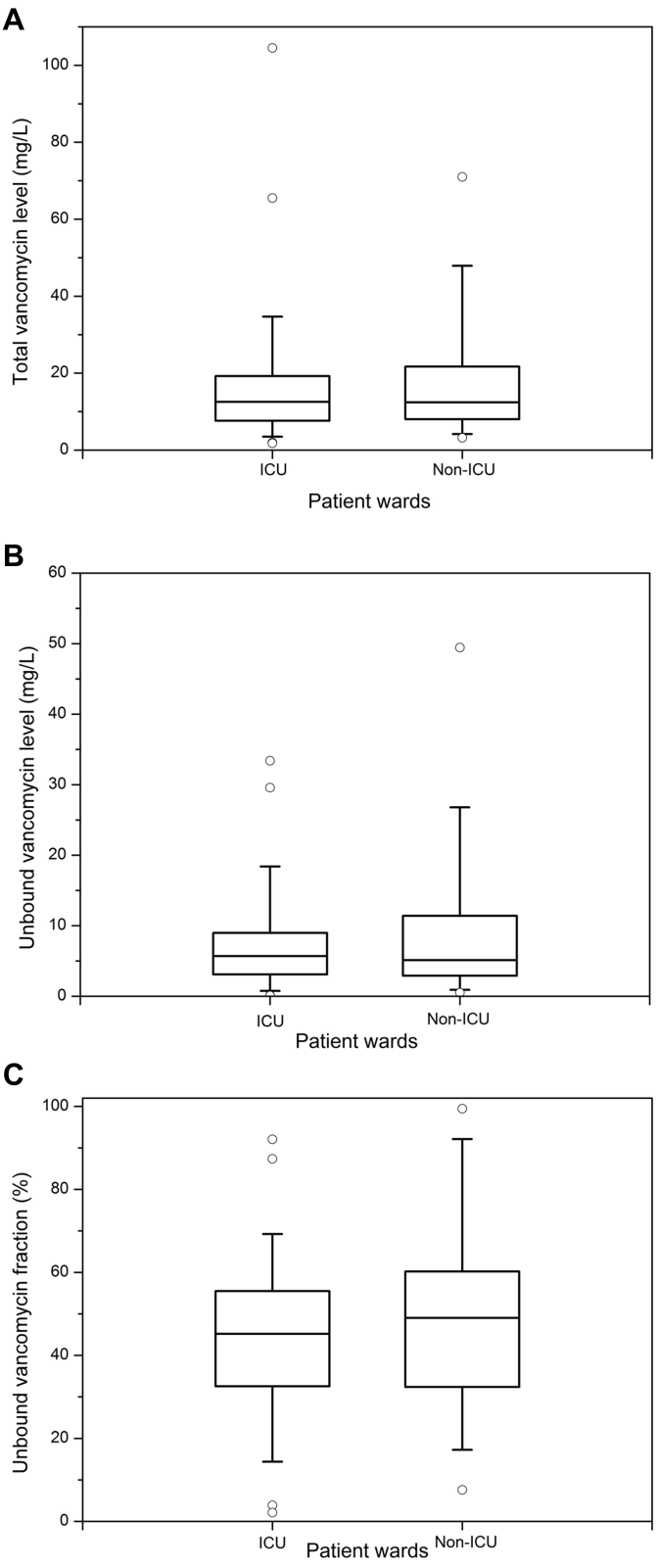

Figure 2 The distributions of total vancomycin level (**A**), unbound vancomycin level (**B**) and unbound vancomycin fractions (**C**) in the different patient populations are shown with box-and-whisker plots. The data are presented as boxes indicating the 2.5th, 25th, 50th (median), 75th, and 97.5th percentiles. Statistically nonsignificant differences (P>0.05, Mann–Whitney *U*-test) are not shown between patients in the ICU ward and non-ICU ward.

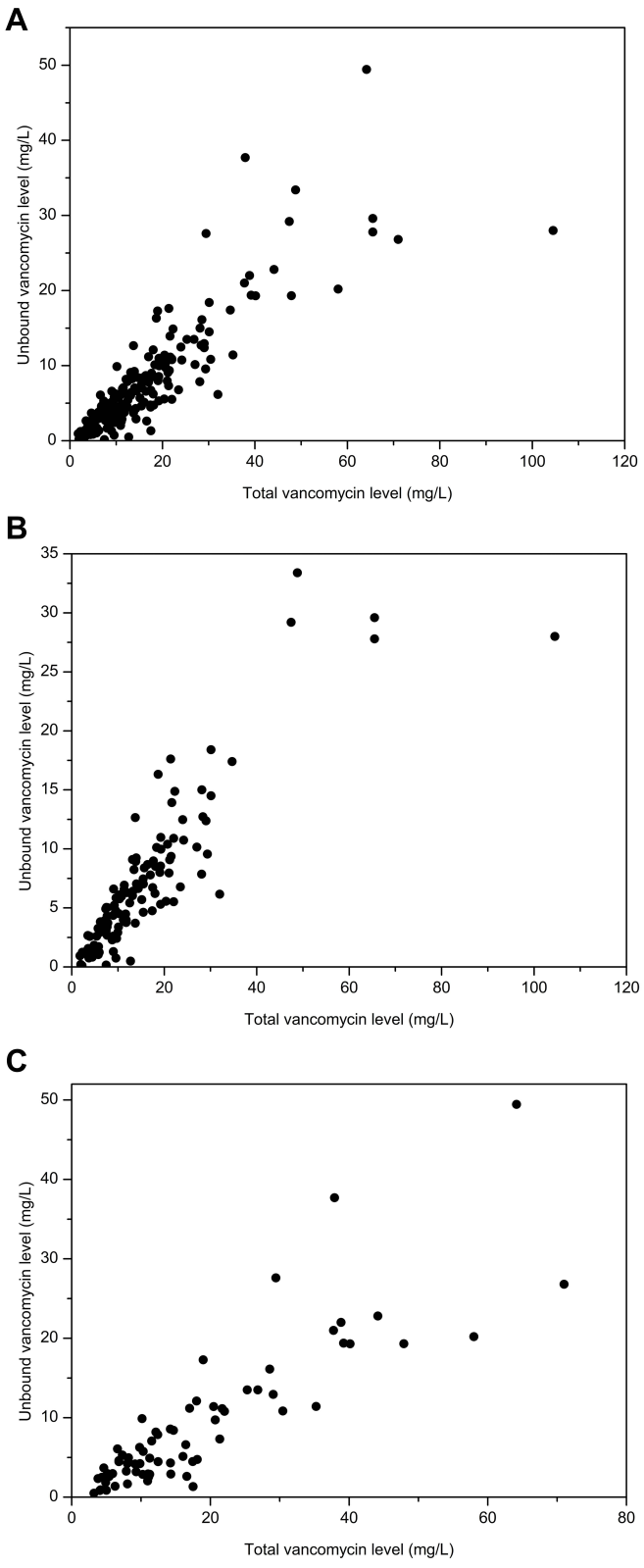

Figure 3 The correlation between the unbound vancomycin level and total vancomycin level among the total (**A**), ICU ward (**B**) and non-ICU ward (**C**) patient populations.

Table 2 Variables Used in Constructing a Model Estimating Unbound Vancomycin Level by Multivariable Linear Regression

Variable	β	SE	P value
Total patients			
Total vancomycin level (mg/L)	0.863	0.023	0.000
BMI (kg/m^2)	−0.102	0.084	0.021
ICU patients			
Total vancomycin level (mg/L)	0.864	0.023	0.000
Total bilirubin (μmol/L)	0.110	0.009	0.035
Non-ICU patients			
Total vancomycin level (mg/L)	0.866	0.048	0.000

Abbreviations: BMI, body mass index; ICU, intensive care unit; β, β coefficient; SE, standard error.

could be a potential predictor of unbound vancomycin level in the clinic. Similar to previous observations,[18,25] significant correlations between the unbound vancomycin level and total protein level and albumin in our study population could not be demonstrated. This is puzzling since the PPB characteristics of vancomycin were also believed to be correlated predominantly with albumin,[15,19,29] total protein[14] and IgA[11,15,19,30] levels in previous studies. Although we included a relatively large study population, we were still unable to correlate the unbound vancomycin level and the albumin level. In future studies, we will include a larger population, which may finally uncover a correlation between these two compounds. Regarding the impact of the IgA level, as the study was retrospective and noninterventional, the IgA level was not measured in our study population, thus excluding comparisons with previous studies.

Compared with previous studies, we included a larger study population and more comprehensive potential covariates, such as coadministration of drugs with PPB>70% and baseline data, including not only routine biochemical data but also history of diabetes mellitus, heart failure, dialysis and hepatic dysfunction. A linear regression model was applied to attempt to relate the unbound vancomycin level to one or more of these additional parameters but without success. Coadministration of drug combinations with PPB>70%, including vitamin K antagonists, aspirin and NSAIDs, on the day of sampling in our study was known, especially to expel vancomycin from albumin.[19,31] Although these drugs are expected to be able to expel vancomycin from albumin and elevate unbound vancomycin levels, no significant correlation between the unbound vancomycin level and the number of coadministered drugs with a PPB>70% was found in either our study or a previous study.[19] Among patients on dialysis, Tan et al[32] studied 6 patients with renal failure and concluded that this population had a higher unbound vancomycin fraction and that the mechanism may be attributed to a reduced albumin binding affinity and competition with accumulated endogenous substrates due to reduced renal clearance.[33] The opposite result was observed by Butterfield et al[14] who found

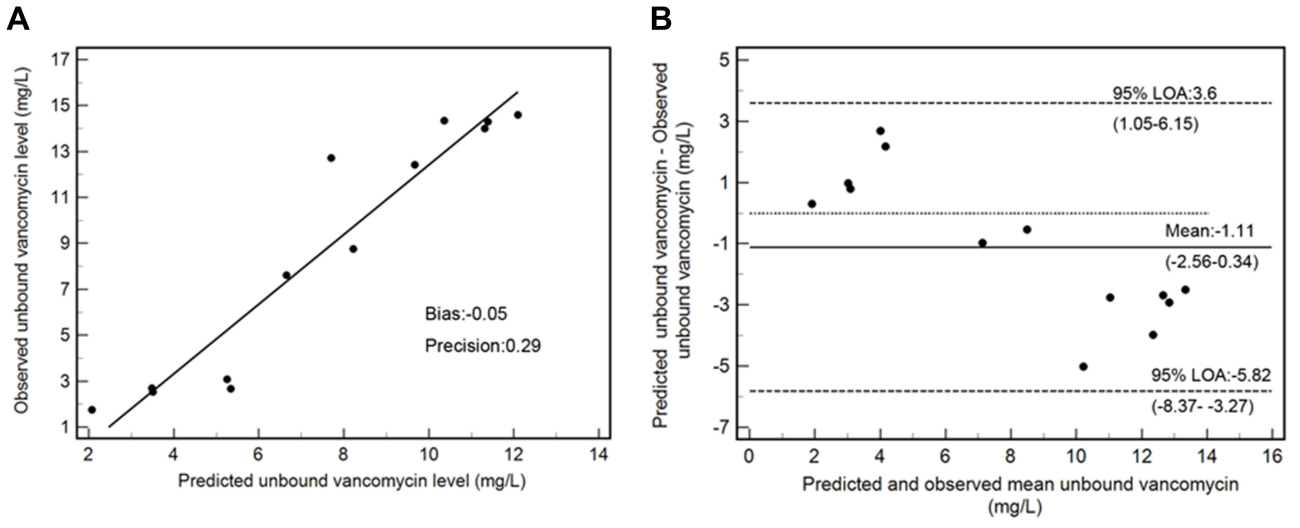

Figure 4 Validation of the prediction model for patients in the ICU ward. (**A**) Correlation between the observed unbound vancomycin level and predicted unbound vancomycin level. (**B**) Bland–Altman analysis of the observed unbound vancomycin level plotted against the predicted unbound vancomycin level. Horizontal dashed lines are drawn at the mean difference (milligrams per liter) and at the limits of agreement (LOAs).

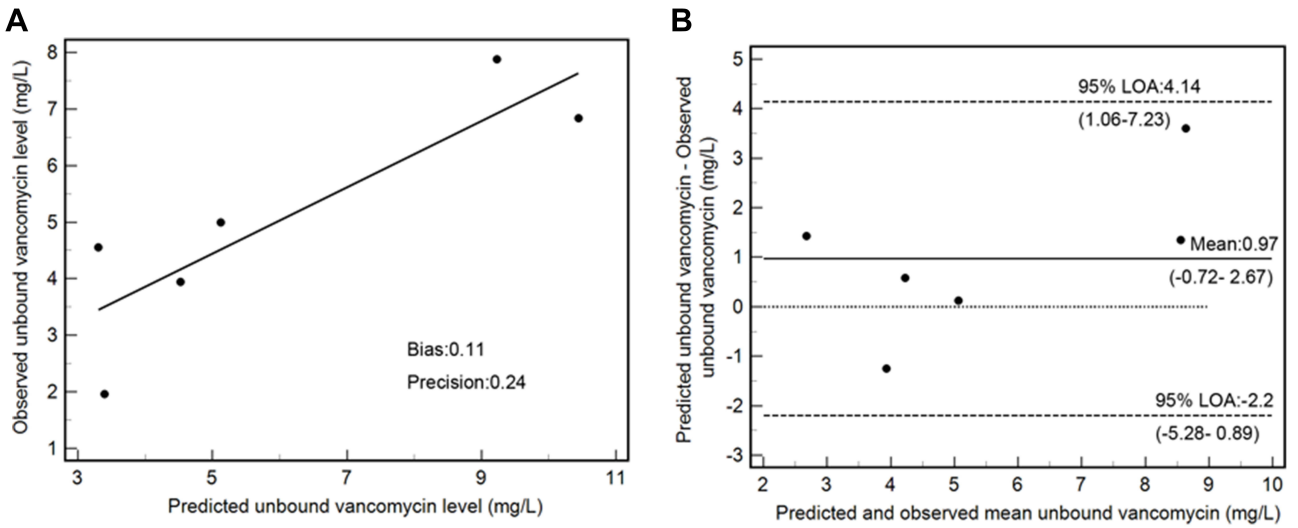

Figure 5 Validation of the prediction model for patients in the non-ICU ward. (**A**) Correlation between the observed unbound vancomycin level and predicted unbound vancomycin level. (**B**) Bland–Altman analysis of the observed unbound vancomycin level plotted against the predicted unbound vancomycin level. Horizontal dashed lines are drawn at the mean difference (milligrams per liter) and at the limits of agreement (LOAs).

a reduced unbound vancomycin level in patients on dialysis. Our study concluded that dialysis had no effect on unbound vancomycin levels, which is not congruent with the above studies. The different conclusions may be explained by the fact that various proteins affect protein binding, and it is possible that proteins unaffected by reduced renal clearance played a role in the unbound vancomycin level in our study population.[14] It should also be pointed out that vancomycin (with a molecular weight of 1450 Da) is not considered "dialyzable" with older (pre-1990s) hemodialyzers.[5] As hemodialysis technology has produced far more permeable membranes, vancomycin is currently thought to be cleared substantially by contemporary high-permeability hemodialyzers.[5,34,35] Moreover, different methods of administration, such as the intravenous administration of vancomycin after the dialysis treatment used in our study population or administration of vancomycin during the last hour of dialysis treatment, may also influence unbound vancomycin levels or fraction. These reasons may explain the different conclusions. To further shed light on this disputed conclusion, an additional investigation in a larger dialysis population should be performed to evaluate whether dialysis can influence the unbound vancomycin fraction and which accumulated endogenous substrates are able to expel vancomycin from proteins.

Several notable limitations also exist and should be mentioned. First, this study employed a retrospective and noninterventional design with a limited sample size (eg, only 12 patients on dialysis were in the non-ICU ward group in this study) and the possibility of including other unknown factors. Second, the findings are limited to our center because this study was based on patients at a single center rather than at multiple centers. Future studies should include a larger study population at multiple centers, which may provide a more reliable model for clinicians to estimate the unbound vancomycin level. Third, due to a deficiency of the IgA test obtained on the day of vancomycin sample collection for routine total vancomycin measurement, we were not able to assess the correlation between the unbound vancomycin level and the IgA level, which has been analyzed in many other studies.[11,15,19,30] However, despite these limitations, this study still provides a reference for clinicians to improve the understanding of the predictors of the unbound vancomycin level. Our study refines the current understanding of the factors that modify the unbound vancomycin level and improves the clinician's ability to predict the unbound vancomycin level in an adult patient.

Conclusions

As we observed a significant correlation between total and unbound vancomycin levels in the ICU patient population and non-ICU patient population, measurement of the unbound vancomycin level seems to have no added value over measurement of the total vancomycin level. In addition, we also found that total bilirubin was a predictor of the unbound vancomycin level in ICU ward patients. Furthermore, the favorable predictive performance of the

prospective model validation suggests the final parsimonious mathematical equations that can be used as a practical alternative to predict the unbound vancomycin level in a given adult being treated with vancomycin.

Abbreviations

ICU, intensive care unit; AUC, area under the curve; MIC, minimum inhibitory concentration; BMI, body mass index; LC-MS/MS, liquid chromatography-tandem mass spectrometry; TDM, therapeutic drug monitoring; PPB, plasma protein binding; NSAIDs, nonsteroidal anti-inflammatory drugs; IS, internal standard.

Data Sharing Statement

All data generated or analyzed during this study are included in the article.

Ethical Considerations and Publications

This study was approved by the Institute Medical Ethics Committee of the Affiliated Hospital of Qingdao University on 3 January 2018. This study was conducted in accordance with the Declaration of Helsinki. As the study was performed with archived samples, the requirement for informed consent was waived by the Institute Medical Ethics Committee, but patient confidentiality was protected. Written informed consent for publication was obtained from all coauthors participated to the research.

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Author Contributions

All authors made significant contributions to the research reported, whether in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the research work.

Disclosure

The authors report no competing interests in this work.

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