Factors Impacting Unbound Vancomycin Concentrations in Different Patient Populations

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The unbound drug hypothesis states that only unbound drug concentrations are active and available for clearance, and highly variable results regarding unbound vancomycin fractions have been reported in the literature. We have determined the unbound vancomycin fractions in four different patient groups by a liquid chromatography tandem mass spectrometry (LC-MS/MS) method and identified factors that modulate vancomycin binding. We have further developed and validated a prediction model to estimate unbound vancomycin concentrations. Vancomycin (unbound and total) concentrations were measured in 90 patients in four different hospital wards (hematology [n = 33 samples], intensive care unit [ICU] [n = 51], orthopedics [n = 44], and pediatrics [age range, 6 months to 14 years; n = 18]) by a validated LC-MS/MS method. Multiple linear mixed model analysis was performed to identify patient variables that were predictive of unbound vancomycin fractions and concentrations. The variables included in the model were patient age, ward, number of coadministered drugs with high protein binding, kidney function (estimated glomerular filtration rate [determined by Chronic Kidney Disease Epidemiology Collaboration formula]), alpha-1-acid glycoprotein, albumin, total bilirubin, IgA, IgM, urea, and total vancomycin concentrations. In the pediatric cohort, the median unbound vancomycin fraction was 81.3% (range, 61.9 to 95.9%), which was significantly higher (P < 0.01) than the unbound fraction found in the three adult patient cohorts (hematology, 60.6% [48.7 to 90.6%]; ICU, 61.7% [47.0 to 87.6%]; orthopedics, 56.4% [45.9 to 78.0%]). The strongest significant predictor of the unbound vancomycin concentration was the total drug concentration, completed by albumin in the pediatric cohort and albumin and IgA in the adult cohorts. Validation of our model was performed with data from 13 adult patients. A mean difference of 0.3 mg/liter (95% confidence interval [CI], −1.3 to 0.7 mg/liter; R² = 0.99 [95% CI, 0.95 to 0.99]) between measured and calculated unbound vancomycin concentrations demonstrated that the predictive performance of our model was favorable. Unbound vancomycin fractions vary significantly between pediatric and adult patients. We developed a formula to estimate the unbound fraction derived from total vancomycin, albumin, and IgA concentrations in adult patients.

Vancomycin, a glycopeptide antibiotic, is widely used to treat infections caused by methicillin-resistant Staphylococcus aureus and other β-lactam-resistant Gram-positive cocci (1, 2). The potential rise in the MICs of vancomycin makes it increasingly important to adjust its dose in order to ensure adequate concentrations in blood and other infected areas, as well as to avoid undue toxicity (3, 4). Generally, therapeutic drug monitoring (TDM) focuses on the total drug concentration in human plasma or serum, although it is hypothesized that only the “free” or “unbound” fraction of the total drug concentration is responsible for antimicrobial activity and potential toxicity and is available for clearance (5–8).

Vancomycin is generally considered a moderately (30 to 60%) protein-bound antibiotic, with albumin being an important binding protein (7). A protein binding proportion of 50% is generally used to calculate unbound vancomycin concentrations. However, protein binding of vancomycin shows considerable variability across studies (ranging from almost 0 to 90%), which could lead to different clinical responses even with the same total drug concentration (9–14). Unbound drug concentrations can vary among patients (hematology, intensive care, pediatric, etc.) and underlying disorders (burns, myeloma, obesity), possibly resulting in different responses to therapy or toxicity, as only unbound drug concentrations are considered pharmacologically active. Previous studies examining the correlation between unbound and total vancomycin concentrations did not distinguish between different patient populations (9, 12, 14). One study investigated only intensive care patients (10). Berthoin et al. measured unbound and total drug concentrations in three patient groups (i.e., hematology, intensive care, and orthopedic patients) (11). No differences in unbound drug concentrations among the three groups were demonstrated, but the numbers of patients studied in the different groups were rather low (11). Moreover, none of the different studies investigated unbound and total drug concentrations in pediat-
ric patients. Given the different behavior of drugs in this patient group, it is of special interest to measure the total and unbound drug concentrations in these populations.

In this study, we evaluated unbound vancomycin fractions in a larger cohort of different patient populations and identified factors associated with unbound vancomycin concentrations by using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for measurement.

**MATERIALS AND METHODS**

**Method validation.** (i) **Vancomycin determination.** Unbound vancomycin concentrations were determined with Centrffee Confirufal Filter Devices (molecular weight cutoff, 30,000; Millipore, Billerica, MA). Briefly, fresh lithium-heparin plasma samples (600 μl/sample) were incubated in a capped Centrffee Device for 30 min, after which the device was spun at 1,912 × g for 30 min at 37°C in a preconditioned Sigma 3-18K centrifuge (SciQuip, London, United Kingdom). The unbound vancomycin concentration is the concentration measured in the ultrafiltrate. Unbound and total vancomycin concentrations were determined by using the chromatographic conditions of a recently described method (within-run imprecision, 2.5 to 5.2%; total imprecision, 2.6 to 8.5%; limit of quantification, 0.3 mg/liter) (15). We further validated this method for the determination of unbound vancomycin concentrations. The unbound vancomycin fraction (percent) was calculated as follows: (ultrafiltrate concentration/total vancomycin concentration) × 100 (14).

(ii) **Analytical validation of LC-MS/MS method for determination of unbound vancomycin concentrations.** Method imprecision was evaluated by analysis of two randomly selected leftover patient samples (mean unbound and total vancomycin concentrations, 3.0 and 12.2 mg/liter and 4.3 and 18.6 mg/liter, respectively) in 10 consecutive runs on 10 different days. Accuracy was determined by measuring unbound vancomycin concentrations in two spiked ultrafiltrates (5 and 20 mg/liter) in 10 different runs. The percent deviation from the theoretically added vancomycin concentration was calculated. An accuracy of <15% was accepted (16).

Extraction recovery of the ultrafiltrate was evaluated by comparing the peak areas of vancomycin-spiked ultrafiltrate both before and after sample preparation (loss during extraction). The ultrafiltrate was spiked with three concentrations, i.e., 5, 20, and 40 mg/liter. The matrix effect (ME) was evaluated by comparing the peak areas of pure solvent (water) spiked with vancomycin at 5 and 20 mg/liter with the peak areas of ultrafiltrate of one blank plasma sample (healthy volunteer) and two different leftover patient plasma samples spiked with vancomycin at 5 and 20 mg/liter. The sample ME was calculated with the equation ME% = ((B/A) × 100, where B is the peak area of vancomycin obtained in the matrix and A is the peak area in solvent. Recovery of the ultrafiltration (UF) membrane (adhesion of vancomycin at the membrane) was assessed by measuring spiked ultrafiltrate in triplicate at three different vancomycin concentrations (5, 15, and 30 mg/liter) before and after filtration (second UF).

**Correlation study.** (i) **Patient population.** A retrospective study was conducted at the University Hospitals Leuven with leftover samples from patients who received vancomycin for suspected or proven Gram-positive infections and required TDM (routine total vancomycin) between April and June 2014. Unbound and total vancomycin concentrations of patients admitted to the hematology, intensive care, orthopedic, and pediatric (patients 6 months to 14 years old) wards were measured. Eight patients received continuous infusion, and 82 patients were treated by intermittent infusion of vancomycin.

(ii) **Data collection.** The baseline data collected from the laboratory information system were age, gender, diagnosis upon admission, and underlying condition. The treatment details consisted of the daily vancomycin dose and route of administration, drugs coadministered on the day of sampling, with a special focus on those with high plasma protein binding (PPB) (>70%), such as vitamin K antagonists, aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), phenytoin, and valproic acid (17). A score of 1 was attributed to each different drug. These summations were further included in the multivariate analyses.

Biochemical findings on the day of sampling included albumin, bilirubin (direct and total), and creatinine levels; estimated glomerular filtration rate (determined with the Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] formula); and total protein, urea, IgA, IgM, alpha-1-acid glycoprotein (AAG), and total plasma vancomycin concentrations. These biochemical data were obtained from the laboratory information system from samples obtained at the same time as the sample used for routine total vancomycin measurement. If one of the biochemical parameters was missing, an additional measurement was performed on leftover material (serum or lithium-heparin plasma) obtained at the same time as the sample used for vancomycin determination. All of the biochemical parameters described can be measured with serum as well as with lithium-heparin plasma (according to the respective method insert sheets from the manufacturers). This study was approved by the Ethics Committee of the University Hospitals Leuven. As the study was performed with leftover samples, it was not necessary to obtain the informed consent of each subject.

(iii) **Methods.** Leftover lithium-heparin plasma samples, sent to the clinical laboratory of the University Hospitals Leuven for clinically indicated total vancomycin measurements, from the different patient groups were collected from the laboratory sample storage system (2 to 8°C) at the end of the working day. Samples were centrifuged for 10 min at 1,920 × g (20°C). Only recent (same-day) samples were selected. Stability of vancomycin in plasma has been evidenced under these conditions and for even longer periods (15). An aliquot was frozen for measurement of total vancomycin by LC-MS/MS (−20°C), and another part was processed directly to obtain the unbound vancomycin fraction. Therefore, 600 μl of lithium-heparin plasma was centrifuged through a Centrffee UF device and stored at −20°C until analysis. Unbound and total vancomycin concentrations were measured by LC-MS/MS (15). Albumin (bromocresol), bilirubin (direct and total; diazonium, colorimetric), creatinine (enzymatic isotope dilution mass spectrometry traceable), total protein (biuret), and urea (kinetic urease-glutamate dehydrogenase) concentrations were determined with a Cobas 8000 c702 analyzer (Roche Diagnostics, Mannheim, Germany) in a clinical setting or with leftover lithium-heparin plasma or serum (leftover samples were collected and processed as described for vancomycin measurements). IgA, IgM, and AAG analyses were performed with an IMAGE nephelometer (Beckmann Coulter, Brea, CA).

(iv) **Statistical analysis.** Statistical analysis was performed with SPSS 22.0 for Windows (SPSS Inc., Chicago, IL). A P value of <0.05 was considered significant. Bland-Altman analysis, Passing-Bablok regression, and Spearman correlation coefficient determination were performed with Medcalc version 11.6.1.0. (Medcalc, Ostend, Belgium). Data were recorded as means ± standard deviations (SDs) or medians and interquartile ranges according to the data distribution. Univariate correlations were investigated by using scatterplots combined with Spearman’s rank correlation coefficients. Multivariate analysis was conducted by linear mixed modeling with random intercept. Spearman’s rank correlation coefficients were used instead of Pearson correlation coefficients because no assumptions of linearity were made in advance. However, because linear mixed models were used to create the prediction formula, Pearson correlation coefficients could also be used in this analysis. Differences between different patient groups were testing with a Mann-Whitney U test.

**Model validation.** The performance of the prediction model was assessed to determine the validity of the unbound vancomycin estimate (based on leftover samples). The study cohort for validation of the prediction tool consisted of another 13 hospitalized adult patients in three different adult patient wards with a clinically indicated vancomycin sample. Mean differences were calculated as the absolute difference between the calculated and measured unbound vancomycin concentrations divided by the measured concentration.
substantially alter the PPB of vancomycin. These results indicate that the freeze-thaw process does not (mean total vancomycin concentrations of 7.4, 16.5, and 22.8 mg/liter). The initial unbound vancomycin fractions (0.626, standard error [SE] 0.013, P < 0.001) and serum IgA (β = 0.806, SE = 0.032, P < 0.001, respectively). The R² values of the final model for the adult and pediatric cohorts were 0.952 and 0.866, respectively. Other variables found to be predictive of unbound vancomycin concentrations in the adult cohort included albumin (β = −0.162, SE = 0.019, P < 0.001) and serum IgA (β = −0.30, SE = 0.049, P < 0.001). On the contrary, in the pediatric cohort, only albumin was found to be predictive of unbound vancomycin concentrations (β = −0.212, SE = 0.053, P < 0.001). No significant differences between the adult cohorts were found, with the same variable retained in the formula describing unbound vancomycin concentrations in the individual adult patient groups. The results of the multiple linear mixed model analysis of the separate groups are summarized in Table 2. On the basis of these results, the unbound vancomycin concentrations in adult and pediatric patients can be predicted with the equation Unbound VAN = 0.63 × total VAN − 0.30 IgA − 0.16 HA + 5.57 for adult patients and the equation Unbound VAN = 0.81 × total VAN − 0.21 HA + 6.34 for pediatric patients, where the total vancomycin (VAN) concentration is in milligrams per liter, the IgA concentration is in grams per liter, and the human albumin (HA) concentration is expressed in grams per liter.

TABLE 1 Demographic, clinical, and biochemical characteristics on day of sampling of patients treated with vancomycin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ICU</th>
<th>Hematology</th>
<th>Orthopedics</th>
<th>Pediatrics</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>33</td>
<td>22</td>
<td>24</td>
<td>11</td>
<td>90</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>59 (17–80)</td>
<td>63 (17–81)</td>
<td>66 (32–84)</td>
<td>3 (1–14)</td>
<td>61 (1–84)</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>19/16</td>
<td>9/13</td>
<td>15/9</td>
<td>7/4</td>
<td>50/40</td>
</tr>
<tr>
<td>No. of samples</td>
<td>51</td>
<td>33</td>
<td>44</td>
<td>18</td>
<td>146</td>
</tr>
<tr>
<td>Albumin concn (g/liter)</td>
<td>28.4 (16.7–39.2)</td>
<td>29.4 (18.2–37.5)</td>
<td>35.0 (21.0–46.5)</td>
<td>28.9 (20.0–39.5)</td>
<td>31.4 (16.7–46.5)</td>
</tr>
<tr>
<td>IgA concn (g/liter)</td>
<td>3.5 (0.2–6.8)</td>
<td>1.2 (0.7–12.3)</td>
<td>1.9 (0.4–7.15)</td>
<td>0.4 (0.01–1.0)</td>
<td>1.7 (0.0–1.23)</td>
</tr>
<tr>
<td>M=concn (g/liter)</td>
<td>0.8 (0.1–2.8)</td>
<td>0.3 (0.1–5.4)</td>
<td>0.5 (0.3–1.7)</td>
<td>0.5 (0.1–1.2)</td>
<td>0.5 (0.1–5.4)</td>
</tr>
<tr>
<td>AAG concn (g/liter)</td>
<td>1.8 (0.4–3.9)</td>
<td>2.1 (0.8–3.0)</td>
<td>1.4 (0.4–2.5)</td>
<td>1.8 (1.0–3.9)</td>
<td>1.7 (0.4–3.9)</td>
</tr>
<tr>
<td>Total protein concn (g/liter)</td>
<td>55.0 (38.0–90.0)</td>
<td>60.5 (45.0–83.0)</td>
<td>61.2 (49–89)</td>
<td>52.0 (46.0–67.0)</td>
<td>58.4 (38.0–90.0)</td>
</tr>
<tr>
<td>Total bilirubin concn (mg/dl)</td>
<td>0.68 (0.1–35.9)</td>
<td>0.24 (0.01–3.69)</td>
<td>0.12 (0.008–1.19)</td>
<td>0.19 (0.11–0.90)</td>
<td>0.2 (0.0–3.56)</td>
</tr>
<tr>
<td>Urea concn (mg/dl)</td>
<td>80.0 (7.0–218)</td>
<td>27.0 (8.0–85.0)</td>
<td>34.0 (12–140)</td>
<td>9.5 (3.0–93.0)</td>
<td>33.0 (3.0–218.0)</td>
</tr>
<tr>
<td>Creatinine concn (mg/dl)</td>
<td>1.3 (0.1–10.0)</td>
<td>0.6 (0.2–3.3)</td>
<td>1.0 (0.6–1.9)</td>
<td>0.2 (0.1–0.9)</td>
<td>0.8 (0.1–1.0)</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>64.9 (6.0–&gt;90)</td>
<td>&gt;90 (14.1–&gt;90)</td>
<td>79 (22.6–&gt;90)</td>
<td>&gt;90 (&gt;90)</td>
<td>&gt;90 (6.4–&gt;90)</td>
</tr>
<tr>
<td>Total vancomycin concn (mg/liter)</td>
<td>17.8 (3.6–53.2)</td>
<td>11.8 (4.5–24.4)</td>
<td>13.0 (6.2–27.8)</td>
<td>10.7 (0.7–25.1)</td>
<td>13.5 (0.7–53.2)</td>
</tr>
<tr>
<td>Unbound vancomycin concn (mg/liter)</td>
<td>10.9 (2.3–34.2)</td>
<td>7.7 (2.2–20.8)</td>
<td>7.4 (3.2–16.1)</td>
<td>8.2 (0.6–22.6)</td>
<td>8.5 (0.6–34.2)</td>
</tr>
<tr>
<td>Unbound vancomycin fraction (%)</td>
<td>61.7 (47.0–87.6)</td>
<td>60.6 (48.7–90.6)</td>
<td>56.4 (45.9–78.0)</td>
<td>81.3 (61.9–95.9)</td>
<td>61.7 (45.9–95.9)</td>
</tr>
<tr>
<td>No. of coadministered drugs with &gt;70% PPB</td>
<td>7.0 (1.0–16.0)</td>
<td>8.0 (1.0–14)</td>
<td>6.0 (2.0–16.0)</td>
<td>4.0 (0.0–12.0)</td>
<td>6.0 (0.0–16.0)</td>
</tr>
</tbody>
</table>

Data are presented as the median value (range).

RESULTS

Method validation. The mean unbound vancomycin coefficients of variation (CVs) determined were 3.0 and 4.4% for the 5- and 20-mg/liter spiked ultrafiltrates, respectively. Accuracy ranged from 99.9 to 107.5% for the different concentrations. The average extraction recovery was 98.9% (CV, 1.4%) for unbound vancomycin. MEs ranged from 61.8 to 67.7% and from 49.0 to 71.4% for the 5- and 20-mg/liter spiked ultrafiltrates, respectively. When the response ratios (RRs; i.e., the area of vancomycin divided by the concentration in the adult (i.e., hematology, ICU, and orthopedic) and pediatric group was the total drug concentration (R, P < 0.01). No correlations with age (R = 0.13; P = 0.29), creatinine (R = 0.21; P = 0.01), IgM (R = 0.13; P = 0.11), AAG (R = −0.11; P = 0.20), or drugs with high protein binding (R = 0.01; P = 0.93) were seen in the univariate analysis. The same correlations were observed in the different individual patient populations, except for the pediatric cohort, in which no significant correlation with IgA was observed (R = −0.22, P = 0.39).

Multiple linear mixed model analysis revealed that the strongest significant predictor of unbound vancomycin concentrations was the total drug concentration, completed by albumin in the pediatric cohort and albumin and serum IgA in the adult cohorts. The strongest significant predictor of the unbound vancomycin concentration in the adult (i.e., hematology, ICU, and orthopedic) and pediatric group was the total drug concentration (β = 0.626, standard error [SE] = 0.013, P < 0.001 and β = 0.806, SE = 0.032, P < 0.001, respectively). The R² values of the final model for the adult and pediatric cohorts were 0.952 and 0.866, respectively. Other variables found to be predictive of unbound vancomycin concentrations in the adult cohort included albumin (β = −0.162, SE = 0.019, P < 0.001) and serum IgA (β = −0.30, SE = 0.049, P < 0.001). On the contrary, in the pediatric cohort, only albumin was found to be predictive of unbound vancomycin concentrations (β = −0.212, SE = 0.053, P < 0.001). No significant differences between the adult cohorts were found, with the same variable retained in the formula describing unbound vancomycin concentrations in the individual adult patient groups. The results of the multiple linear mixed model analysis of the separate groups are summarized in Table 2. On the basis of these results, the unbound vancomycin concentrations in adult and pediatric patients can be predicted with the equation Unbound VAN = 0.63 × total VAN − 0.30 IgA − 0.16 HA + 5.57 for adult patients and the equation Unbound VAN = 0.81 × total VAN − 0.21 HA + 6.34 for pediatric patients, where the total vancomycin (VAN) concentration is in milligrams per liter, the IgA concentration is in grams per liter, and the human albumin (HA) concentration is expressed in grams per liter.

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Multiple linear mixed model analysis was also performed with the bound vancomycin fraction, i.e., the total vancomycin concentration in milligrams per liter minus the unbound vancomycin concentration in milligrams per liter divided by the total vancomycin concentration in milligrams per liter, as the dependent variable. This analysis revealed that the bound vancomycin concentration can be predicted by the albumin concentration ($\beta = 1.826, SE = 0.271, P < 0.001$) in the pediatric cohort and albumin ($\beta = 1.027, SE = 0.076, P < 0.001$) and IgA ($\beta = 1.717, SE = 0.199, P < 0.001$) in the adult cohort. The total vancomycin concentration was not retained as an independent variable.

**Model validation.** Validation of the prediction model was evaluated by using data from 13 adult patients. The median total vancomycin concentration was 15.4 mg/liter (range, 11.6 to 31.8 mg/liter). The IgA and albumin concentrations ranged from 0.5 to 5.8 g/liter and from 18.8 to 38.8 g/liter, respectively. The mean unbound vancomycin concentration was 8.8 mg/liter (range, 5.1 to 17.3 mg/liter). The observed-versus-predicted plots for the patients included in the validation model are presented in Fig. 2. The $R^2$ was 0.99 (95% confidence interval [CI], 0.97 to 0.99), and the mean difference was 0.2% (95% CI, −14.2 to 13.8%) (Fig. 2).

**DISCUSSION**

Our study adds interesting information to the current understanding of factors associated with unbound vancomycin concentrations. Compared to other studies, ours examined relatively large patient populations and evaluated additional factors that are potentially associated with unbound vancomycin concentrations and consequently vancomycin PPB, like IgA and albumin concentrations or drugs with a PPB of >70% in a large cohort of patients from different wards (9, 10, 12, 14). Moreover, we were able to measure unbound vancomycin concentrations by means of an LC-MS/MS method validated for total and unbound drug concentrations, in contrast to other studies, in which an immunosay (fluorescence polarization immunoassay [FPIA] or particle-enhanced turbidimetric inhibition immunoassay [PETINIA]) or high-performance liquid chromatography (HPLC) with UV detection was used (9–14).

Although these methods offer sufficient sensitivity and selectivity for clinical purposes, they were developed and validated only in the author's institution. Further validation in other settings is required to confirm the generalizability of the findings.
for the determination of total drug concentrations in human plasma. Also, it is well known that some immunoassay methods are prone to interference by paraproteins or rheumatic factor (18) or can cross-react with crystalline degradation products, which comprise inactive metabolites of vancomycin found in plasma or serum samples from renally impaired and dialysis patients (19–21). As LC-MS/MS methods are more specific, it is generally accepted that they are less likely to suffer from these issues (22).

Differences in unbound vancomycin concentrations between immunoassays and HPLC were recently illustrated in a study by Crandon et al. in which unbound vancomycin concentrations were measured by an FPIA and an in-house-developed HPLC method (9). Although the clinical significance of this difference remains debatable, they found a significantly lower and more variable PPB with the FPIA than with HPLC (9). Another study that evaluated PPB by HPLC and a PETINIA assay found a good correlation between the two methods for the measurement of unbound and total vancomycin concentrations. Of note, comparison with calculated PPB values was not reported (11). Along with analytical issues (differences in the matrix between total and unbound drug concentrations), also preanalytical issues, like the conditions under which unbound vancomycin concentrations are obtained (temperature, pH, centrifugation speed, and time), can have a major impact on unbound vancomycin concentrations. It was recently reported that the unbound vancomycin fraction after UF at 4°C was, on average, 30.6% lower than after UF at 37°C. Moreover, UF at 37°C resulted in unbound vancomycin concentrations equivalent to those determined by equilibrium dialysis, which is considered the gold standard for the measurement of unbound drug concentrations (14). However, the latter method has the disadvantage that it needs several hours to reach an equilibrium (23). On the other hand, UF is a fast and easy-to-perform method but has the disadvantage of potential adsorption to the UF filter. Here, we confirmed previous reports that nonspecific adsorption of vancomycin to the UF filter is negligible and independent of the concentration.

We found a median unbound vancomycin fraction of 61.7% (range, 45.9 to 95.9%) when using UF at 37°C. These unbound vancomycin fractions are lower than those reported in the study by Stove et al., who reported a value of 74.4% (SD, 6.6%) (14). These differences could possibly be attributable to the different methods that were used to measure unbound and total vancomycin concentrations (immunoassay [FPIA] versus LC-MS/MS).

Second, vancomycin was probably measured in other patient populations that could receive other drugs or endogenous molecules that could contribute to different vancomycin protein binding.

The PPB characteristics of vancomycin have been studied in detail and found to be related predominantly to albumin and IgA contents (24, 25). This is in line with our observations, as multiple linear mixed model analysis revealed a statistically significant correlation between unbound vancomycin concentrations and albumin and IgA in the total and separate adult data sets.

Our study clinically supports the results of other studies that have shown a negative correlation between serum IgA and unbound vancomycin concentrations (14, 24–26). This is of particular interest in IgA myeloma patients or in populations with low IgA concentrations (like selective IgA-deficient patients, immunocompromised patients, etc.), as the high or low IgA concentrations will decrease or increase the unbound vancomycin concentration and hence will reduce or increase the length of time for which unbound vancomycin is above the MIC, respectively.

We used a validated LC-MS/MS method to measure total and unbound vancomycin concentrations. In our previous paper, we compared four different immunoassays with our LC-MS/MS method (15). With some assays, acceptable agreement was obtained, making our formula theoretically valid for total vancomycin measurements with these assays. Commercially available immunoassays are, however, not validated for the measurement of unbound vancomycin concentrations in ultrafiltrate matrix. For instance, Crandon et al. identified important differences in unbound vancomycin fractions between immunoassays and an inhouse HPLC method (9).

Previous studies of vancomycin PPB included only adult patients, whereas we were able to measure unbound vancomycin
concentrations in a pediatric group. Although this group was rather small (11 patients), a significant difference from the unbound vancomycin concentrations in adult patients was demonstrated. This could be explained in part by the fact that pediatric patients have lower serum IgA concentrations (27). Future studies with pediatric patients and neonates should confirm and further elucidate the difference in pediatric patient groups.

We found a significant and strong relationship between the total and unbound vancomycin concentrations. This is similar to the results of other studies, which demonstrated that the unbound vancomycin concentration is highly predictable by the total drug concentration (12, 14). Compared to those studies, we evaluated vancomycin concentration is highly predictable by the total drug concentration. This is similar to vancomycin binding sites to expel vancomycin from the protein (29). Frequent administration of drugs in our study included NSAIDs (PPB, >90%), aspirin (PPB, >99%), analgesics (PPB, >95%), and vitamin K antagonists (PPB, >90%), drugs known especially for the ability to expel drugs from albumin (17, 30). Future studies with vancomycin and albumin and known concentrations of the displacing agents should clarify if the coadministration of highly protein binding drugs has an effect on unbound vancomycin concentrations.

In conclusion, we have refined the current understanding of unbound vancomycin by measuring unbound and total vancomycin concentrations in different patient populations by means of a validated LC-MS/MS method. As we observed a significant correlation between total and unbound vancomycin concentrations in the four patients populations, measurement of unbound vancomycin concentrations seems to have no added value over measurements of total vancomycin concentrations. This implies that dose changes based on unbound vancomycin concentrations would probably have been marginally different from dose changes based on routinely measured total vancomycin concentrations, except for patients with severe hypo- or hyperalbuminemia, IgA myeloma, or IgA deficiency, etc. We further developed and validated a formula based on IgA and albumin for adult patients to calculate unbound vancomycin concentrations. Although our formula was developed by using LC-MS/MS, we expect the formula to be equally valid for immunoassays with good agreement with the method we have developed for total vancomycin measurement. Last, we observed a significantly larger unbound fraction in the pediatric cohort. The clinical implications of this finding need to be examined further.

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