Impact of vancomycin protein binding on target attainment in critically ill children: back to the drawing board?

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Objectives: The objectives of this observational study were to investigate plasma protein binding and to evaluate target attainment rates of vancomycin therapy in critically ill children.

Patients and methods: Paediatric ICU patients, in whom intravenous intermittent dosing (ID) or continuous dosing (CD) with vancomycin was indicated, were included. Covariates on unbound vancomycin fraction and concentration were tested using a linear mixed model analysis and attainment of currently used pharmacokinetic/pharmacodynamic (PK/PD) targets was evaluated. Clinicaltrials.gov: NCT02456974.

Results: One hundred and eighty-eight plasma samples were collected from 32 patients. The unbound vancomycin fraction (median = 71.1%; IQR = 65.4%–79.7%) was highly variable within and between patients and significantly correlated with total protein and albumin concentration, which were both decreased in our population. Total trough concentration (ID) and total concentration (CD) were within the aimed target concentrations in 8% of patients. The targets of AUC/MIC ≥400 and fAUC/MIC ≥200 were achieved in 54% and 83% of patients, respectively. Unbound vancomycin concentrations were adequately predicted using the following equation: unbound vancomycin concentration (mg/L) = 5.38 + [0.71 / total vancomycin concentration (mg/L)] / [0.085 / total protein concentration (g/L)]. This final model was externally validated using 51 samples from another six patients.

Conclusions: The protein binding of vancomycin in our paediatric population was lower than reported in non-critically ill adults and exhibited large variability. Higher target attainment rates were achieved when using PK/PD indices based on unbound concentrations, when compared with total concentrations. These results highlight the need for protein binding assessment in future vancomycin PK/PD research.

Introduction

The emergence of MRSA strains has led to an extensive use of vancomycin in the treatment of serious infections in critically ill children.1 Vancomycin is a glycopeptide antibiotic with a narrow therapeutic range.2 Achievement of pharmacokinetic (PK) and pharmacodynamic (PD) indices associated with maximum bactericidal killing are recommended to increase the probability of clinical cure and decrease the likelihood of toxicity.2 Studies in adults have shown that the advocated PK/PD index of favourable clinical outcome is an AUC over a 24 h period in steady-state divided by the MIC of the suspected pathogen (AUC/MIC) of ≥400.3 Despite its use in children, clinical studies are currently lacking to validate this target value.4–6 In routine clinical practice, trough concentrations are used as a ‘surrogate’ parameter to optimize vancomycin dosing regimens, because AUC/MIC calculations are labour- and cost-intensive.2,7 Both targets are based on total drug concentrations, whereas only the ‘unbound’ or ‘free’ drug exerts a pharmacological effect.8 A more direct fAUC/MIC target ≥200 has been advocated as the PK/PD target assuming a fixed unbound
vancomycin fraction of 50%\textsuperscript{,2,9} However, critically ill children exhibit marked variability in plasma protein concentrations (with albumin concentration ranging between 15 and 54 g/L), which may alter the protein binding.\textsuperscript{10,11} To date, no studies have investigated the implications of altered protein binding on target attainment rates.

This study had three aims: (i) to document plasma protein binding and factors that modulate the protein binding of vancomycin in critically ill children; (ii) to compare target attainment rates of three different currently used targets total (trough) concentration, AUC/MIC and $f_{AUC/MIC}$; and (iii) to develop a prediction model for the unbound vancomycin concentration.

**Methods**

Detailed methods are available as Supplementary data at JAC Online.

**Study design**

This two-centre, prospective, observational study enrolled children between 12 days and 15 years of age, admitted to the ICU, in whom intravenous vancomycin therapy was indicated, independently of the indication.

**Ethics**

The research was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Ghent University Hospital (EC2012/172). This study was registered at Clinicaltrials.gov (NCT02456974). Written informed consent was obtained from parents or a legally authorized representative and from the patient themselves if older than 12 years.

**Vancomycin treatment**

Patients received a weight-based vancomycin dosing regimen [either intermittent dosing (ID) or continuous dosing (CD)].

**Results**

Thirty-two patients were included resulting in a total of 188 plasma samples. All samples were analysed for total and unbound vancomycin concentration. Six samples were excluded from the analysis because there was an implausible result (unbound concentration being higher than total concentration). Demographic and clinical data, sampling characteristics and disease severity are summarized in Table S1 (available as Supplementary data at JAC Online). The median unbound fraction was 71.1% (IQR 65.4%–79.7%), ranging from 49.4% to 98.1%. The median difference between the lowest and highest value of the unbound fraction within patients was 14.3% (IQR = 8.7%–18.9%), ranging from 3.1% to 41.8%. The median difference results are based on values from 31 patients, because 1 patient had only one sample. Saturation of plasma protein binding did not occur within the range of clinically achieved concentrations. Pathogens were isolated in nine patients and included eight staphylococcal infections, one infection with *Streptococcus pyogenes* and one infection with *Enterococcus faecalis*.

**Mixed model analysis on unbound vancomycin fraction**

Total protein ($P < 0.001$) and albumin concentration ($P < 0.001$) were found to be significant covariates on the unbound fraction.

**PK/PD target attainment evaluation (Figures 1 and 2)**

We evaluated 32 trough samples for patients who received ID and 6 samples for patients who received CD. The median total trough (ID) and total concentrations (CD) were 6.7 mg/L (IQR 4.7–8.7 mg/L) and 14.5 mg/L (IQR 10.2–18.7 mg/L), respectively. Only three trough samples (ID) achieved the target range (one after first dose and two after steady-state dose) and all of the measured total concentrations (CD) were below the target range. For 24 patients, the (f)AUC was accurately calculated (8 were excluded as the AUC could not be precisely calculated). The median AUC/MIC and $f_{AUC/MIC}$ were 425 (IQR 293–497) and 294 (IQR 222–357), respectively.

**Mixed model analysis on unbound vancomycin concentration**

The model with total vancomycin ($P < 0.001$) and total protein concentration ($P = 0.001$) [Akaike’s Information Criterion (AIC): 790] performed slightly better than the model with total vancomycin ($P < 0.001$) and albumin concentration ($P = 0.008$) (AIC: 792); including a third covariate did not lead to a better fit of the data. The unbound vancomycin concentration in our patient population could be predicted by the following equation:

$$C_{\text{unbound}} = 5.38 + (0.71 \times C_{\text{total}}) - (0.085 \times C_{\text{tp}})$$

In this equation, $C_{\text{unbound}}$ represents the unbound vancomycin concentration (mg/L), $C_{\text{total}}$ the total vancomycin concentration (mg/L) and $C_{\text{tp}}$ the total protein concentration (g/L).

![Figure 1. Correlation between total trough concentrations and (f)AUC/MIC for patients who received ID ($n = 21$). The broken line indicates the target AUC/MIC of 400, the continuous line indicates the target $f_{AUC/MIC}$ of 200, filled circles represent the calculated AUC/MIC and open circles represent the calculated $f_{AUC/MIC}$. Spearman’s rank correlation coefficient: AUC/MIC, $R = 0.85$ ($P < 0.01$); and $f_{AUC/MIC}$, $R = 0.82$ ($P < 0.01$). Twelve (57%) patients reached the AUC/MIC of 400 (above the broken line) and 17 (81%) patients reached the $f_{AUC/MIC}$ of 200 (above the continuous line).](https://academic.oup.com/jac/article-abstract/72/3/801/2691394)
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Figure 2. Target attainment based on AUC/MIC and fAUC/MIC for patients who received ID (n = 21) and CD (n = 3). The vertical broken line indicates the target AUC/MIC of 400 and the horizontal continuous line indicates the target fAUC/MIC of 200. Spearman’s rank correlation coefficient: R = 0.88 (P < 0.01). Thirteen (54%) patients reached the AUC/MIC of 400 (first quadrant), 20 (83%) patients reached the fAUC/MIC of 200 (first and second quadrants) and 4 (17%) patients did not reach the fAUC/MIC (third quadrant).

Model validation

The final model for the unbound vancomycin concentration was validated using another six patients (51 samples) (details are available as Supplementary data).

Discussion

The present study revealed that protein binding of vancomycin in critically ill children is comparable to what has been described in critically ill adults, but is substantially lower in comparison with healthy volunteers and non-critically ill adults (50%–55%).\textsuperscript{12-15} The lower vancomycin binding may be explained by differences in protein concentrations as we found that protein binding depended on total protein concentrations (of which albumin is the most important fraction). Both total protein (range = 21.1–74.2 g/L) and albumin concentrations (range = 14.2–45.1 g/L) were decreased in our study population and exhibited marked variability.\textsuperscript{10,11,16} Oyaert et al.\textsuperscript{12} observed a similar trend of reduced protein binding in children, when compared with adults. Besides the observation of reduced protein binding, a considerably high intra- and intervariability in protein binding was found.

As protein binding was altered and highly variable, our study aimed to compare target attainment rates using different proposed PK/PD targets, i.e. total (trough) concentration and fAUC/MIC. The most commonly used PK/PD parameter, i.e. total (trough) concentration, was within the aimed target ranges in only 8% of cases. Since trough concentrations of 10–15 mg/L are believed to be a good surrogate to achieve an AUC/MIC ≥400, it would be reasonable to assume that the latter target would not be achieved in the majority of patients.\textsuperscript{2} However, our study revealed that an AUC/MIC ≥400 was achieved in 54% of patients. Total trough concentrations correlated well with the calculated AUC/MIC (R = 0.85) and a total trough concentration of ~7 mg/L corresponded to an AUC/MIC of 400. This is in agreement with two previous studies in children, which predicted that achievement of an AUC/MIC of 400 (assuming MIC = 1 mg/L) corresponded with lower trough concentrations (8–10 mg/L) due to altered PK.\textsuperscript{17,18} More importantly, our target attainment analysis showed that in the same group of patients, the fAUC/MIC ≥200 target was reached in an even larger proportion (83%) of our study population. The fAUC/MIC target reflects directly the exposure to the unbound pharmacologically active concentration. Consequently, attempts to achieve the AUC/MIC target of 400, for the 29% of cases who already achieved the fAUC/MIC target, may not result in additional clinical benefit but may lead to unnecessary drug exposure and potential toxic effects (Figure 2; quadrant 2).

Despite the fAUC/MIC target value of 200 being a more arbitrary goal, based on the most conservative and fixed protein binding of 50%, to our knowledge there is no superior alternative target available.\textsuperscript{9} In each case, these findings question the magnitude of underdosing as suggested in previous studies, which only used total trough concentration and AUC/MIC for target attainment analysis.\textsuperscript{17,19,20} In clinical practice, monitoring vancomycin exposure by fAUC (with MIC if available), and thereby taking into account the protein binding, might be a more justified target to prevent underdosing or overexposure. Furthermore, given the high variability in protein binding in our study population, it seems not advisable to assume a fixed unbound fraction to calculate this fAUC/MIC ratio. In this heterogeneous population with different types of infection, we were able to accurately predict the unbound vancomycin concentration in plasma based on total vancomycin and total protein concentration.

Our study has a number of limitations to consider. First, we did not determine actual vancomycin MIC values to accurately calculate the (f)AUC/MIC, mainly because in 72% of cases therapy was started empirically. Instead, we used a commonly used MIC breakpoint of 1 mg/L to compare target attainment with previous study results.\textsuperscript{17,18} Second, a small number of subjects on CD were included, so no definite conclusions could be drawn with regard to target attainment. Third, tissue concentrations were not measured and higher targets may be needed to ensure adequate penetration in tissues.

In conclusion, our study demonstrates that the unbound vancomycin fraction in our population is higher than generally assumed, exhibiting high intra- and intervariability. The number of patients achieving target vancomycin concentrations varied widely depending on the type of PK/PD target used. These results argue against currently used PK/PD indices for making quantitative exposure–response assessments in critically ill children. Further clinical and bacteriological outcome studies should be performed in specific populations to define appropriate PK/PD indices based on unbound concentrations. We provide a validated prediction tool for unbound vancomycin concentrations and offer an easy alternative for measuring unbound concentrations in clinical practice.

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Transparency declarations
None to declare.

Supplementary data
Supplementary methods and results data and Table S1 are available at JAC Online (http://jac.oxfordjournals.org/).

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