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ORIGINAL ARTICLE

Factors impacting unbound vancomycin concentrations in neonates and young infants

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Abstract

Vancomycin pharmacokinetic (PK) and pharmacodynamic (PD) data in neonates are based on total concentrations. However, only unbound vancomycin is pharmacologically active. The objective was to determine vancomycin protein binding and the covariates impacting unbound vancomycin concentration in neonates and young infants. In neonates and young infants to whom vancomycin was administered intermittently for medical indications, total and unbound vancomycin plasma concentrations were determined using LC-MS/MS. Sampling occurred randomly during vancomycin exposure, covering a broad range of concentrations. Impact of covariates on unbound vancomycin concentration was determined using linear regression. Significant results of the univariate regressions were entered in a stepwise multiple regression. Passing-Bablok regression and Bland-Altman were used to assess the difference between measured and calculated unbound vancomycin concentration. Thirty-seven samples in 33 patients (median (interquartile range) gestational age 35 (29–39) weeks) were collected. Median total and unbound vancomycin concentrations were 14.2 (7.4–20.6) and 13.6 (7.2–22.5) mg/L, respectively. Median unbound fraction was 0.90 (0.77–0.98). Multiple regression revealed total vancomycin concentration ($\beta = 0.884$, $p < 0.001$) and albumin ($\beta = -0.323$, $p = 0.007$) as most important covariates of unbound vancomycin concentrations, with an R^2 adjusted of 0.953 ($p < 0.0001$). Mean absolute difference between calculated and measured unbound vancomycin was −0.008 (95% CI −0.92–0.91) mg/L. The unbound vancomycin fraction in neonates is higher compared to that in children and adults, and total vancomycin concentration and albumin were the most important covariates of unbound vancomycin concentration. Integration of protein binding in future PK/PD analyses is appropriate to optimize vancomycin dosing and to determine population-specific vancomycin PD targets for neonates.

Keywords Neonate · Vancomycin · Protein binding · Unbound concentration

Introduction

Vancomycin, a glycopeptide, is often administered to treat (suspected) serious gram-positive infections caused by Staphylococci, including methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative *Staphylococci* (CoNS) [1]. It is a hydrophilic compound, almost exclusively eliminated unchanged by glomerular filtration and to some extent by tubular secretion. Vancomycin disposition in neonates depends on weight, age, and renal function and is characterized

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by highly variable pharmacokinetics (PK) [2, 3]. An area under the 24-h concentration-time curve (AUC_{24}) divided by the minimal inhibitory concentration of the pathogen (AUC_{24}/MIC) ≥ 400 is considered as adequate vancomycin efficacy. However, this pharmacodynamic (PD) target is derived from adults with MRSA pneumonia. Suboptimal exposure in 47–81% of neonates [4–6] and the lack of appropriate dosing as well as population-specific PD targets illustrate the need for improved understanding of vancomycin PK/PD in neonates [7, 8].

One of the specific topics only very poorly explored in neonates is vancomycin protein binding. At present, available PK/PD data are based on total concentrations, while only unbound vancomycin leads to a pharmacological effect and is subsequently available for elimination. In plasma, vancomycin binds to human serum albumin and immunoglobulin A (IgA) [9]. Protein binding increases with higher IgA and albumin concentrations and decreases (nonlinearly) when vancomycin concentration increases except at very low IgA concentrations [9]. Recently, Oyaert et al. documented that the median unbound vancomycin fraction (0.81) in a pediatric cohort with median (range) age of 3 (1–14) years was significantly higher compared to three adult cohorts (0.61, 0.62, and 0.56 in respectively hematology, intensive care, and orthopedic adult patients) [10]. The most important predictors of unbound vancomycin concentration in the pediatric group were total vancomycin concentration and albumin, while in the adult patients, total vancomycin concentration, albumin, and IgA were revealed [10]. The aim of the current study was to explore vancomycin protein binding and its covariates in neonates and young infants.

Methods

Ethics, drug dosing, and clinical characteristics

Neonates and young infants, admitted to the Neonatal Intensive Care Unit of the University Hospitals Leuven, to whom vancomycin was administered for medical indications, were considered for inclusion after parental written informed consent. The study was registered at ClinicalTrials.gov (NCT02096536, EudraCT 2014-001124-29) and approved by the ethical board of the hospital. Vancomycin, co-administered with amikacin, is standard therapy for late-onset sepsis in our NICU. Each vancomycin dose (Vancomycine Actavis, 15 mg/kg) is intravenously administered over 60 min. The dosing interval depends on post-menstrual age (PMA) and plasma creatinine (mg/dL), with an interval of 24 h for PMA < 29 weeks, 12 h for PMA 29–35 weeks (24 h if creatinine > 0.60 mg/dL), and 8 h for PMA > 35 weeks (12 h if creatinine > 0.60 mg/dL) [11].

Clinical characteristics at birth (gestational age (GA), birth weight (BW)), as well as characteristics at the moment of vancomycin sampling (post-natal age (PNA), PMA, current weight (CW)), were extracted from the patient files. If available, plasma creatinine (mg/dL), albumin (g/L), total protein (g/L), and total and indirect bilirubin (mg/dL) within a time period of 24 h before or after vancomycin sampling were recorded. Results of blood and other cultures taken before the start of the vancomycin therapy were collected.

Blood sampling

Blood samples (0.60 mL, Pediatric Lithium Heparin Microtainer tube) to determine total and unbound vancomycin concentrations were collected via arterial line if available for medical reasons, or by venous puncture only in case of a planned blood sampling for medical reason. The number of samples collected in each neonate depends on the weight of the baby since a total sampling volume of maximum 1 mL/kg in each patient was respected as predefined by the protocol. Samples were collected during vancomycin exposure, but time of sampling was not further specified to facilitate exploration of protein binding in a broad range of vancomycin concentrations.

Drug assay

Blood samples were centrifuged (Centrifuge 5451R, Eppendorf, 1.800×*g*, 10 min) as soon as possible after collection and subsequently stored at − 80 °C until analysis. After thawing, 40 μL of plasma was used for total vancomycin analysis, the remainder (260 μL) was immediately filtrated. Ultrafiltration (Centrifree, Millipore, 2000 g, 30 min, 37 °C) was used to separate unbound (i.e., free from albumin as well as IgA) from bound vancomycin as previously described [12, 13]. Total and unbound vancomycin concentrations were determined using a previously developed and validated LC-MS/MS method (within-run imprecision ranging from 2.5 to 5.2%; total imprecision ranging from 2.6 to 8.5% and limit of quantification 0.3 mg/L). For further analytical and chromatographic details, we refer to the respective reports [10, 14]. An additional validation documented that at least two plasma freeze-thaw cycles have no impact on unbound fraction. Total and unbound vancomycin were measured in three routine left-over plasma samples. The remaining plasma was frozen. In every thaw cycle, sufficient amount was taken to measure total vancomycin and ultrafiltrate for unbound vancomycin determination. All obtained values were well within 20% deviation from the first measurement (average 102%, range 95–111% for total vancomycin (100% = same as first measurement), 103% (93–116%) for unbound vancomycin, 101% (96–113%) for the unbound fraction). The unbound vancomycin fraction was calculated as vancomycin concentration in ultrafiltrate/total vancomycin plasma concentration [15].

Biochemical assays

Albumin (bromocresol), bilirubin (diazonium, colorimetric), creatinine (enzymatic isotope dilution mass spectrometry (IDMS) traceable), and total protein (biuret) concentrations were determined with a Cobas 8000 c702 analyzer (Roche Diagnostics, Mannheim, Germany) in our hospital laboratory.

Statistical analysis

Statistical analysis was performed using Medcalc® version 18 (Medcalc Software, Ostend, Belgium). Clinical characteristics were reported by median and interquartile range (IQR), or incidence. Vancomycin plasma concentrations (mg/L) were also reported by median and interquartile range. The impact of continuous covariates on the unbound vancomycin concentration was determined using linear regression analysis. Significant results of the univariate linear regressions were subsequently entered in a stepwise multiple regression analysis. Spearman correlation was performed to identify significantly correlated covariates. Passing-Bablok regression and Bland-Altman analysis were used to assess the difference between measured and calculated (i.e., based on the multiple regression equation) unbound vancomycin concentration. A p value of < 0.05 was considered as significant.

Results

Thirty-seven samples (13 arterial, 24 venous) in 33 neonates (male/female 19/14) were collected. Demographic, clinical, and biochemical characteristics of the patients are provided in Table 1. Median (IQR) total and unbound vancomycin plasma concentrations were 14.2 (7.4–20.6) and 13.6 (7.2–22.5) mg/L, respectively. Median unbound vancomycin fraction was 0.90 (0.77–0.98). The samples were collected 72 (25–110) h after initiation of vancomycin therapy (median dose 14.60 (14.10–15) mg/kg). Nine of 37 samples were collected in neonates with a blood culture-proven infection (*Staphylococcus epidermidis* ($n = 4$), *Staphylococcus aureus* ($n = 1$, *S. aureus*), *Staphylococcus capitis* ($n = 2$), *Enterococcus faecium* ($n = 1$), *Klebsiella oxytoca* ($n = 1$)). Other samples were related to subcutaneous ($n = 5$) or respiratory ($n = 3$) infections. Table 2 presents the univariate linear regressions. CW was significantly correlated with BW ($r = 0.843$, $p < 0.0001$), GA ($r = 0.796$, $p < 0.0001$), and PMA ($r = 0.875$, $p < 0.0001$). Of these covariates, CW was included in the multiple regression since vancomycin is usually administered in the late neonatal period. In a multiple regression analysis including total vancomycin concentration, albumin, and CW, two independent covariates of the unbound vancomycin concentration were retained, i.e., total vancomycin concentration ($\beta = 0.884$, standard error 0.042, $p < 0.001$) and albumin ($\beta = -0.323$, standard error 0.110, $p = 0.007$), with an R^2 adjusted of 0.953 ($p < 0.0001$). Linear regression graphs of both covariates are provided in Fig. 1a, b. Repetition of the multiple regression with PMA or GA instead of CW provided identical results. Unbound vancomycin concentration can be calculated using the following equation: unbound vancomycin concentration (mg/L) = 0.884 × total vancomycin concentration (mg/L) − 0.323 albumin concentration (g/L) + 10.609. Bland-Altman analysis revealed a mean absolute difference between calculated and measured unbound vancomycin concentration of − 0.008 (95% CI − 0.92–0.91) mg/L. Limits of agreement are provided in Fig. 2a. Figure 2b presents the Passing-Bablok regression line $y = 0.58 + 0.95\ x$, with residual standard deviation (RSD) = 1.74 (± 1.96 RSD interval, − 3.42–3.42) and $R^2 = 0.935$ (95% CI 0.867–0.970). Cusum test documented no significant deviation from linearity. This indicates adequate predictive performance of the equation formula for unbound vancomycin concentration.

Discussion

This is the first study that documents that the median unbound vancomycin fraction in neonates and young infants (0.90) is higher compared to reported values in children (median 0.71 to 0.81) [10, 16] and adults (mean (± SD) 0.45 ± 0.09 up to 0.73 ± 0.12) [13, 15, 17–20]. For adults with different degrees of renal function, end-stage renal failure, and burn injuries, mean reported values were 0.70 ± 0.07, 0.82 ± 0.12, and 0.71 ± 0.06 respectively [21–23]. In Table 3, we compared the current data in neonates and young infants with an earlier published dataset on vancomycin protein binding in children and adults of Oyaert et al., since the same validated vancomycin assay was used in all datasets [10].

A comparable trend (lower binding in neonatal compared to adult plasma) was previously described for the glycopeptide teicoplanin (in vitro analysis) [24], as well as for compounds of other antimicrobial classes, e.g., cefazolin and flucloxacillin [25, 26]. Since only the unbound drug is pharmacologically active and available for elimination, protein binding can directly influence vancomycin PK parameters (volume of distribution (Vd) and clearance (Cl)) as well as PD targets in neonates. In general, for hydrophilic antibacterial agents like glycopeptides, for which distribution is limited to the extracellular space, reduced protein binding results in increased Vd and is associated with increased clearance by glomerular filtration, because only the unbound drug is filtered [27, 28]. Since a higher unbound vancomycin fraction is documented in neonates, it is reasonable to hypothesize that vancomycin clearance is proportionally higher compared to that in adults when extrapolated based on maturation of renal clearance capacity. However, the combination of less effective renal tubular functions and an overall low glomerular filtration rate makes it

Table 1 Demographic, clinical, and biochemical characteristics of neonates and young infants included in the study. Data are provided by number, or median and interquartile range

Characteristics	Number or median (interquartile range)	
Demographics		
Gestational age (weeks)	35	(29–39)
Postnatal age (days)	14	(8–29)
Postmenstrual age (weeks)	38	(33–41)
Birth weight (g)	2200	(981–3222)
Current weight (g)	2681	(1722–3295)
Clinical characteristics		
Blood culture proven infection	9/37	
Any culture proven infection	17/37	
Biochemical characteristics		
Total protein (g/L)	49	(46–56)
Albumin (g/L)	29.90	(27.6–34.20)
Creatinine (mg/dL)	0.42	(0.32–0.53)
Total bilirubin concentration (mg/dL)	4.28	(0.71–7.13)
Indirect bilirubin concentration (mg/dL)	3.70	(0.36–6.05)

difficult to predict the effect of protein binding on drug clearance in neonates [25]. Therefore, integration of the protein binding data in available vancomycin population PK models is needed, to explore the impact of protein binding on vancomycin PK parameters and dosing (both intermittent and continuous administration) in neonates.

The concept of protein binding should also be considered in future vancomycin PD studies, especially since the optimal vancomycin target in neonates is still unknown. First, in the currently used target of $AUC_{24}/MIC > 400$, the AUC_{24} value is calculated based on total vancomycin concentrations. If only the unbound vancomycin is used to calculate AUC_{24}, this will result in an altered target for a given MIC [29]. Since the unbound vancomycin fraction in adults differs compared to that in neonates, population-specific vancomycin PD targets are requested. Second, the target of 400 is extrapolated from analyses in adults suffering from MRSA pneumonia. Because neonates more often display bacteremia-related CoNS infections, we anticipate that respective MIC values will also determine the final PD target. In the search for the optimal neonatal vancomycin efficacy target, we have to be aware that an increase in MIC breakpoints for vancomycin over time has been observed [7]. In our neonatal unit, CoNS MIC ranges ≤ 0.50–2 mg/L, with 81.6% between 1 and 2 mg/L. This is in line with Sinkeler et al. (most 1.5–2 mg/L), Padari et al. (median 1, range 0.2–3 mg/L), and the European committee on antimicrobial susceptibility testing (EUCAST) data (93% between 1 and 2 mg/L) [30–32]. However, the same

Table 2 Overview of the univariate linear regressions of unbound vancomycin concentration versus available clinical and biochemical characteristics

Parameter	Equation	Coefficient (slope)	SE	95% CI	*t*	R^2	*P*
Total vanco concentration (mg/L)	$y = -0.005 + 0.929\ x$	0.929	0.04349	0.840 to 1.017	21.352	0.936	< 0.0001*
Gestational age (weeks)	$y = -17.763 + 0.955\ x$	0.955	0.358	0.226 to 1.684	2.671	0.187	0.012*
Birth weight (g)	$y = 5.887 + 0.004\ x$	0.004	0.002	0.001 to 0.007	2.647	0.184	0.013*
Current weight (g)	$y = 4.819 + 0.004\ x$	0.004	0.002	0.0003 to 0.007	2.273	0.143	0.030*
PNA (days)	$y = 15.628 + -0.031\ x$	−0.0314	0.073	−0.179 to 0.117	−0.433	0.006	0.668
PMA (weeks)	$y = -18.015 + 0.870\ x$	0.870	0.393	0.068 to 1.672	2.214	0.137	0.034*
Creatinine (mg/dL)	$y = 18.181 + -7.399\ x$	−7.399	7.336	−22.402 to 7.605	−1.009	0.034	0.322
Total protein (g/L)	$y = 30.936 + -0.338\ x$	−0.338	0.293	−0.939 to 0.263	−1.153	0.047	0.259
Albumin (g/L)	$y = 50.397 + -1.169\ x$	−1.169	0.427	−2.046 to −0.293	−2.737	0.217	0.011*
Bilirubin, total (mg/dL)	$y = 16.023 + -0.336\ x$	−0.336	0.710	−1.801 to 1.128	−0.474	0.009	0.640
Bilirubin, indirect (mg/dL)	$y = 15.284 + -0.177\ x$	−0.177	0.737	−1.699 to 1.345	−0.2401	0.002	0.812

SE, standard error; *CI*, confidence interval; R^2, coefficient of determination; *t*, *t* value; *p*, *p* value; *PNA*, postnatal age; *PMA*, postmenstrual age

*p value < 0.05 is considered statistically significant

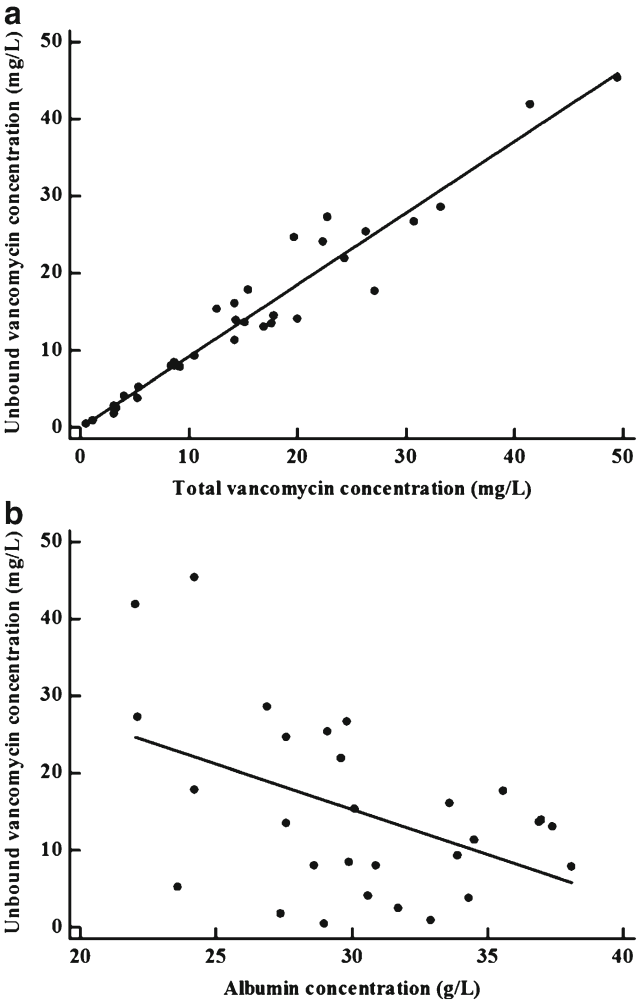

Fig. 1 Linear regression of the unbound vancomycin concentration as a function of the total vancomycin concentration (**a**, $y = -0.005 + 0.93\ x$), and as a function of the albumin concentration (**b**, $y = 50.39 - 1.1$ x)

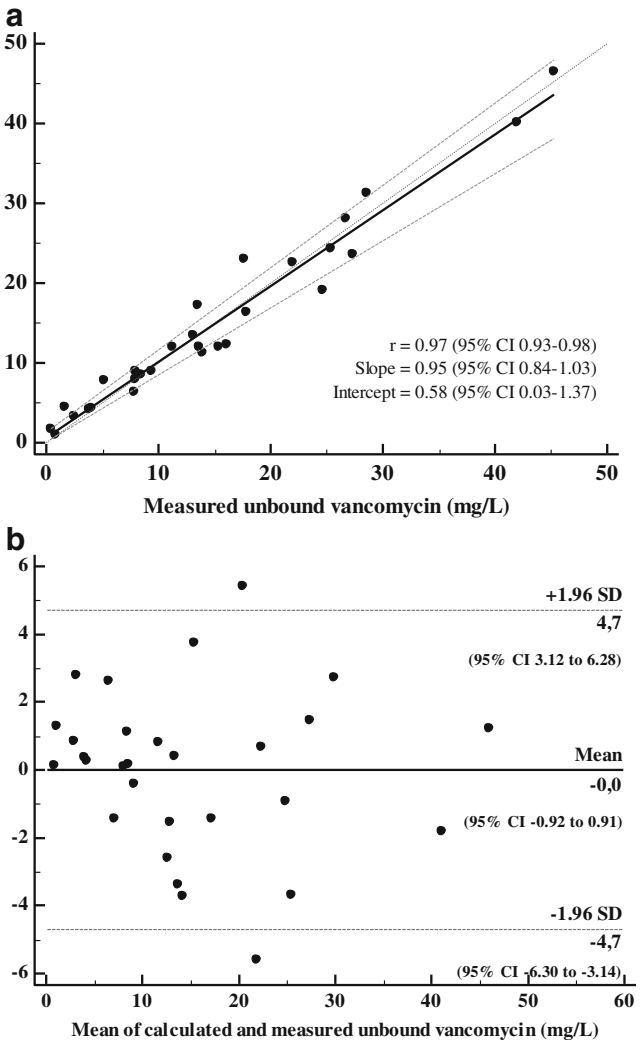

Fig. 2 **a** Passing-Bablok regression analysis of the calculated unbound vancomycin concentration and the measured unbound vancomycin concentration. The full line represents the regression equation, the dashed lines the 95% CI. **b** Bland-Altman analysis of the absolute difference between calculated and measured unbound vancomycin concentration (mg/L) plotted against the mean of the calculated and measured unbound vancomycin concentrations (mg/L). Full horizontal line presents the mean difference (95% confidence interval, CI) and dashed lines present the limits of agreement (95% CI)

EUCAST database reports a species-specific epidemiological cutoff (ECOFF) for vancomycin at 2 mg/L for MRSA and 4 mg/L for CoNS [7, 33]. Also outside the European region, comparable values can be found. Bhongsatiern et al. refer to the CLSI MIC values for MRSA (2 mg/L) and CoNS (4 mg/L), while their own vancomycin MIC breakpoints for both CoNS and *S. aureus* ranged from ≤0.5 to 4 mg/L, with a breakpoint of ≤2 mg/L most common (approximately 70%) in both CoNS and *S. aureus* [5]. The combination of high unbound vancomycin fraction and increasing MIC makes it difficult to predict optimal vancomycin dose and target in neonates and requires more sophisticated modeling and subsequent validation. Third, age-specific risk factors for infection (e.g., immune status) in neonates may require altered PD targets. Besides concerns about the optimal target "value," early target achievement is needed to avoid resistance [7].

The two independent covariates explaining the unbound vancomycin concentration in neonates, total vancomycin concentration and albumin, are in line with findings in pediatric and adult patients [10, 16]. Butterfield et al. documented total vancomycin concentration as the strongest predictive factor of unbound concentration in a cohort of 50 adults [13]. In 32 intensive care unit children, De Cock et al. reported total vancomycin and total protein concentration as significant covariates of unbound vancomycin concentration [16]. The add-on value of measuring unbound vancomycin concentration was questioned by Stove et al. since it could reliably be predicted by the total concentration [15]. Surprisingly, others reported that vancomycin protein binding in adults was dependent on protein concentration but independent of the total vancomycin concentration, due to a dissociation constant larger than therapeutic drug concentrations [18, 19]. Overall, vancomycin is

Table 3 Demographic, clinical, and biochemical characteristics in neonates and young infants (italicized) compared to earlier reported observations in adults and children (adapted from Oyaert et al.) using the same validated vancomycin assay [10]. Data are provided by number or median and interquartile range

Characteristics	ICU adults	Hematology adults	Orthopedic adults	Children[a]	Neonates and young infants
Patients (number)	33	22	24	11	*33*
Samples (number)	51	33	44	18	*37*
Total protein (g/L)	55 (51.6–61.6)	60.5 (53.2–64.0)	61.2 (57.3–65.2)	52 (49.3–56.4)	*49 (46–56)*
Albumin (g/L)	28.4 (25.0–32.2)	29.4 (28.0–32.3)	35 (32.0–38.5)	28.9 (27.1–32.2)	*29.9 (27.60–34.20)*
Creatinine (mg/dL)	1.3 (0.7–3.8)	0.6 (0.6–0.8)	1.0 (0.8–1.1)	0.2 (0.15–0.28)	*0.42 (0.32–0.53)*
Total vancomycin concentration (mg/L)	17.8 (10.8–26.2)	11.8 (7.9–14.8)	13 (11.0–17.2)	10.7 (5.9–16.3)	*14.2 (7.4–20.6)*
Unbound vancomycin concentration (mg/L)	10.9 (7.0–14.5)	7.7 (4.6–9.5)	7.4 (5.9–9.7)	8.2 (4.6–14.2)	*13.6 (7.2–22.5)*
Unbound vancomycin fraction	0.62 (0.55–0.65)	0.61 (0.58–0.67)	0.56 (0.53–0.64)	0.81 (0.70–0.87)	*0.90 (77–98.50)*

[a] Children: age 6 months to 14 years

considered as moderately bound to albumin [34]. Lower protein binding in neonates compared to that in adults has also been reported for different drugs and is in part attributed to quantitative and qualitative characteristics of albumin [35, 36]. Albumin levels in neonates depend on GA and increase rapidly during the first days of life [36]. Competition for albumin binding sites by (non)-endogenous agents, as well as altered binding affinity due to modification of the conformational state of the albumin molecule, may influence the drug binding capacity of albumin. In adults, co-administration of highly bound drugs was not a significant covariate of vancomycin protein binding [10]. In neonates, decreased albumin binding due to drug displacement by bilirubin (e.g., phenytoin, ampicillin) or elevated free fatty acids (e.g., diazepam, propranolol, cloxacillin) are reported [36].

Although this prospective study reports vancomycin protein binding in a relevant number of observations in neonates and young infants, there are some limitations. First, we did not measure IgA levels. Since IgA was not a predictor of unbound vancomycin concentration in children, we a priori decided not to collect these additional samples in the neonatal cohort [10]. This was because the IgA mean reference values are much lower in neonates (< 10 mg/dL for preterms in the first week of life) compared to those in children (> 100 mg/dL at 4–5 years) and in adults (reaching almost 200 mg/dL), making it very likely a between-cohort covariate, but not of relevance within the neonatal population [37–39]. Second, patients with a variable nature of infection were included. However, the pathogens isolated are in line with literature reports on neonatal late-onset sepsis [40]. In addition, since maturational covariates mainly influence neonatal vancomycin disposition, we do not consider infection variation as a relevant confounder [2, 3]. Third, alterations in vancomycin protein binding due to (pre-)analytical variabilities (pH, temperature, centrifugal forces (pressure effect), reagent and consumable lots, volumes) have been reported [13, 18]. This limits comparison of published data using different methodology. To bypass this method-related variability, the same methodology and equipment as Oyaert et al.'s was used in the current study (Table 3) [10, 14].

We conclude that the unbound vancomycin fraction in neonates is higher compared to that in children and in adults. In line with other populations, total vancomycin concentration and albumin are the most important predictors of neonatal unbound vancomycin concentration. Integration of the protein binding data in future PK/PD analyses is needed to optimize vancomycin dosing and to determine population-specific vancomycin PD targets in neonates.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed parental consent was obtained for all included participants.

Data availability statement The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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