Publisher: Taylor & Francis & Informa UK Limited, trading as Taylor & Francis Group

Journal: *Expert Opinion on Drug Metabolism & Toxicology*

DOI: 10.1080/17425255.2019.1655540

Pharmacokinetic modelling and Bayesian estimation-assisted decision tools to optimize vancomycin dosage in neonates: only one piece of the puzzle

Karel Allegaert^{1,2}, Robert Flint^{1,3} and Anne Smits^{2,4}

¹Department of Pediatrics, Division of Neonatology, Erasmus MC Sophia Children's Hospital, University Medical Center Rotterdam, the Netherlands

²Department of Development and Regeneration, KU Leuven, Belgium

³Department of Pharmacy, Erasmus University Medical Center, Rotterdam, The Netherlands

⁴Neonatal Intensive Care Unit, University Hospitals Leuven, Belgium

Corresponding author:

Karel Allegaert

Department of Development and Regeneration, KU Leuven, Neonatal Intensive Care Unit, University Hospital, Herestraat 49, 3000 Leuven, Belgium

Tel.: +32-16-343850

Fax: +32-16-343209

Email: karel.allegaert@uzleuven.be

Accepted Manuscript

Abstract

Introduction: Vancomycin is commonly administered to neonates, while observational data on therapeutic drug monitoring (TDM, trough levels) suggest that vancomycin exposure and dosage remain substandard.

Area covered: Data on vancomycin pharmacokinetics (PK) and its covariates are abundant. Consequently, modelling is an obvious tool to improve targeted exposure, with a shift from TDM trough levels to area under the curve (AUC_{24h}) targets, as in adults. Continuous administration appeared as practice to facilitate AUC_{24h} target attainment, while Bayesian model-supported targeting emerged as novel tool. However, the AUC_{24h}/MIC (minimal inhibitory concentration) target itself should consider neonate specific aspects (bloodstream infections, coagulase-negative staphylococci, protein binding, underexplored causes of variability, like assays, preparation and administration inaccuracies, or missing covariates).

Expert opinion: To improve targeted exposure in neonates, initial vancomycin prescription should be based on 'a priori model based individual dosing' using validated dosing regimens, followed by further tailoring by dosing optimization applying Bayesian estimation-assisted TDM. Future research should focus on feasibility to integrate these tools (individualized dosing, Bayesian models) in clinical practice, and to perform PK/PD studies in the relevant animal models and human neonatal setting (coagulase-negative staphylococci, blood stream infections).

Keywords: vancomycin, therapeutic drug monitoring, newborn, pharmacokinetics, Bayesian model-supported targeting, coagulase-negative staphylococcus

Article highlights

- Coagulase-negative staphylococci (CoNS) are the most frequently isolated pathogen in blood cultures for neonatal late onset sepsis. Consequently, vancomycin is the 5th most commonly prescribed drug in neonatal intensive care (91 exposed, 150 courses/ 1000 admissions). Data on toxicity (renal, hearing) are reassuring, but limited.
- A scoping review on vancomycin PK reflects maturational (age, weight), and non-maturational (disease, co-medication) covariates. These models show relevant unexplained variability, population PK and Bayesian models can support initial empiric dose selection and – after therapeutic drug monitoring – improve decision making process to attain targeted exposure.
- Targeted exposure has commonly been based on trough levels (intermittent 5-15; dose interval related, continuous 10-25 mg/l), while the AUC_{24h}/MIC (400) is more accurate. However, this target is extrapolated from adult *S. aureus* pneumonia data, different from blood culture positive CoNS in neonates.
- Continuous vancomycin administration is emerging as practice in neonates, since the relation between trough (steady state) and AUC_{24h} (trough x 24h) is easy to calculate. The evidence is summarized, and limitations (loading dose, compatibility, impact on resistance) applicable to neonates are discussed.
- A stepwise approach to improve targeted vancomycin exposure is suggested (improve empiric exposure>improve targeted exposure>confirm targeted exposure). Future research should focus on feasibility to integrate these tools (individualized dosing, Bayesian estimation-assisted TDM decision making) in clinical practice, and to perform PK/PD studies driven by valid animal models to explore the PK/PD relationship in neonates (CoNS, blood stream infection).

1. Introduction

Clinical pharmacology uses pharmacokinetics (PK) and pharmacodynamics (PD) to predict (side)effects of drugs, including their variability [1,2]. Very specific to newborns are the fast maturational changes with weight and age as main drivers, resulting in extensive in part unexplained variability in PK and subsequent uncertainty [3,4]. Non-maturational changes further add to this variability. Observed non-maturational changes relate to co-medication (ibuprofen, indomethacin), pharmacogenetics, co-morbidity (sepsis, asphyxia, patent ductus arteriosus) or treatment modalities (extra-corporeal membrane oxygenation (ECMO), whole body cooling) [5-7].

The need for more rational drug use obviously also applies to antibiotics, like vancomycin [8]. The importance of antibiotics is reflected in the latest *Pediatrics* overview on most commonly used drugs, with 3 antibiotics in the top 5 (ampicillin (1th), gentamicin (2th), and vancomycin (5th)). This resulted in vancomycin 91/1000 exposed (150 courses) neonates [9]. This is because neonatal sepsis is a major cause of mortality and morbidity, with a pathogen distribution specific for early- or late-onset sepsis (EOS, LOS). A key factor to improve outcome is optimal (when, what and how) antibiotic use. Decisions when to prescribe are still not based on robust decision tools, choices (what) on empiric regimens should depend on the anticipated pathogens, and the data on efficacy and safety. Finally, progress has been made on how (including vancomycin) to dose [8,10,11].

We applied a generic quality improvement approach for sepsis treatment in neonates as described by van Ronge et al. to LOS with specific emphasis on vancomycin (**Figure 1**) [8]. The first step (**Figure 1a**) relates to drug selection (what). Based on the pathogens (type, resistance) isolated for LOS, the antibiotic regimen is an empiric decision (either or not including vancomycin). Once the blood culture and antibiogram are known, a more targeted antibiotic regimen is used (either or not including vancomycin). **Figure 1b** relates to drug exposure (how). Based on data in a cohort with similar characteristics (e.g. preterm, renal function), an empiric dose is selected, aiming to reach as soon as possible the target range. Therapeutic drug monitoring (TDM) should subsequently be used to attain and sustain a preferably narrowed therapeutic target range, avoiding under- and overexposure [7,12,13].

This review focusses on how to improve vancomycin exposure in neonates and therefore relates to the second part (drug exposure, dose selection and concentration attainment) of such a quality improvement approach. As a backbone for this review, the basic rules to consider the clinical relevance of TDM were applied to vancomycin in neonates (**Box 1**) [7,12,13].

In section 2., we summarize the information on vancomycin PK and its covariates (2.1), and highlight some toxicity data (2.2.). Section 3. discusses the commonly used 'targets' (trough or area under the curve over 24h, AUC_{24h}) and their limitations specific to neonates (3.1.), and the use of continuous vancomycin administration (available evidence, potential issues) (3.2.). Section 4. subsequently discusses Bayesian model-supported TDM to improve vancomycin exposure. Sections 5. and 6. discuss 'knowledge gaps' or poorly explored aspects of vancomycin AUC_{24h} targets in neonates. Section 5. provides information on the characteristics of *coagulase-negative staphylococci* (CoNS) commonly isolated as blood stream infection pathogen in neonates (epidemiology, resistance patterns, does this affect clinical practice). Section 6. discusses underexplored causes of unexplained variability of vancomycin PK. In the subsequent discussion and conclusion (7.) and expert opinion section (8.), we aim to integrate these pieces of information into the earlier mentioned quality cycle approach [8].

2. Vancomycin pharmacokinetics and its toxicity in neonates: a scoping review

2.1. Pharmacokinetics

The main conclusions of the recent (2012) systematic search on vancomycin PK in neonates still hold [14]. Vancomycin clearance displays extensive variability, with age (postmenstrual, postnatal), weight (birth or current weight) and creatinine as most commonly reported covariates of explained variability [14]. The distribution volume is higher (l/kg), driven by body composition (body fluid, albumin) and lower plasma protein binding capacity (albumin) in neonates [3,15]. This combination results in a further prolonged elimination half-life and extended time to reach steady state [16]. Finally, vancomycin penetrates rather poorly (7-42%) into the cerebrospinal fluid [14]. The overall driver of maturational vancomycin clearance is the renal clearance capacity maturation.[17]. Consequently, ibuprofen or indomethacin co-administration result in decreased clearance (-16% and -55% respectively) [18]. However, there is

likely also renal tubular handling. An interesting observation that suggested the relevance of renal tubular activity has been reported by Marqués-Minamä et al. In their population-PK weight-normalized analysis in 70 neonates, postmenstrual age, but also amoxicillin-clavulanic acid co-administration resulted in a significant *increase* of clearance [19]. This observation might be explained by competitive phenomena at the renal tubular cell surface [19].

2.2. Nephro- and ototoxicity

The evidence on vancomycin nephro- and ototoxicity in neonates has recently been summarized [20]. The claimed mechanism relates to dose-dependent intracellular oxidative damage at the level of the renal tubular and cochlear sensory hair cells [20]. Letsner et al. concluded that nephrotoxicity occurs in 1-9% of neonates receiving the 'recommended' doses (although commonly in a setting of subtherapeutic exposure), but is more common in neonates treated with concomitant nephrotoxic (e.g. aminoglycosides, ibuprofen/indomethacin) drugs. The data on the overall low incidence of vancomycin nephrotoxicity in neonates have been confirmed in more recent reports. In the Leroux prospective validation study (continuous), only 2/190 cases displayed nephrotoxicity, both associated with hemodynamic instability and vasopressor treatment with subsequent normalization [21]. Germovsek et al. (continuous and intermittent dosing) observed nephrotoxicity in 4/77 cases (3 stage 1, 1 stage 3 renal injury) [22]. For both more recent studies, target attainment (vancomycin exposure, $AUC_{24h} > 400$) was common, so better reflecting contemporary target exposure.

This compares to 10-15% of nephrotoxicity in adults, and is also exposure dependent. Vancomycin trough levels > 15 mg/l had a higher risk (Odd Risk 3.12) of nephrotoxicity in adults and children, also after adjustment for other covariates. Duration of treatment was another predictor of renal toxicity [23]. Along the same line, Zasowski et al. observed that there was a 3- to 4-fold increase in renal impairment (consecutive creatinine increase $+ 0.5$ mg/l and $> 50\%$ from baseline) with increasing AUC_{24h} estimates, with best predictive performance between 600 and 800 mg.h/l [24]. It is unclear if these differences reflect differences in drug exposure, or in developmental toxicity.

Ototoxicity is another issue. Oto-acoustic emissions or auditory brainstem responses are used to assess hearing performance as part of outcome assessment of neonatal intensive care graduates. In essence, the available data provide at best a very poor

association with vancomycin administration [20]. The current paradigm is that hearing impairment is the outcome of synergism of noise, drug (vancomycin, aminoglycosides, diuretics) exposure and pharmacogenetics [25,26].

3. Targets for vancomycin exposure, and how to aim for these targets

3.1. Trough or AUC_{24h}

The area under the curve over 24h/the minimal inhibitory concentration (AUC_{24h}/MIC) is the best biomarker for vancomycin efficacy, while trough concentrations serve as surrogate marker. This is because the correlation between trough concentrations and AUC_{24h} is poor, since depending on dose, PK, but also dosing interval [27,28].

Using Monte Carlo simulations (n=76 neonates, mainly beyond 2 weeks postnatal age, birth weight range 320-3655 g) the minimal trough concentration (from q24h to q6h dosing regimens) to reach AUC_{24h} >400 was 8-8.9 mg/l, troughs between 10 and 14.9 mg/l resulted in almost no (<0.1%) AUC_{24h} values >700, while 15-20 mg/l trough target resulted in AUC_{24h} overexposure in 24.5% [29]. Interesting, an increasing trough level with age was observed, reflecting the impact of the decreasing dosing interval (from q24h to q6h) with increasing age on the trough level, not necessarily correlated with the AUC_{24h} value [29]. Chen also recently reported on a 'trough to AUC_{24h} conversion' in neonates, but the clinical use of this tool cannot be simply extrapolated beyond this specific dosing regimen (10-15 mg/kg.q12h to q8h pending on postmenstrual and postnatal age) [30]. Overall, the trough (5-15 mg/l) vancomycin range was still rather large [30]. Finally, Tseng et al. (10-15 mg/l trough target) documented only a poor correlation (r=0.53) between trough and AUC [29].

The poor correlation and dosing interval dependent pattern has also been confirmed in another recent paper in a population PK meta-analysis of individual data on vancomycin (1631 neonates, 4894 vancomycin levels) [31]. An optimal intermittent infusion dosing to reach target AUC_{24h} has hereby been developed [31]. To reach this AUC_{24h} target as soon as possible, a loading dose (25 mg/kg) precedes maintenance dosing (15 mg/kg.q12h if <35 weeks, 15 mg/kg.q8h if ≥35 weeks postmenstrual age) [31]. Using Monte Carlo simulations with this dosing, 89% of the neonates were on target, while 33% are >700. These AUC_{24h} targets relate to median (5th-95th centile) vancomycin trough levels of 6 (1.1-14.7), 12.3 (4.1-28.3) and 17.2 (5.6-46) mg/l in neonates of <29, 29-35 or >35 weeks of postmenstrual age [31].

The adult AUC_{24h} range commonly aimed for is between 400 and 700 mg/l.h to maximize the likelihood of bacterial clearance, while avoiding (renal) toxicity [32]. However, this target is based on *S. aureus* pneumonia [33] and renal toxicity data in adults respectively, likely not reflecting the neonatal setting (CoNS, blood stream infection, maturational toxicity) [16,34].

In essence, this means that we should shift from 'simple trough levels' driven decisions to AUC_{24h} estimate based dosing, but still have to consider the neonatal aspects [35]. This is because if trough target levels are considered, the targets should be dose interval specific, since a same trough target after either a 24, 12, 8 or 6 h interval will reflect another AUC_{24h} value [27,31]. If you consider the use of trough levels without Bayesian models, AUC target attainment (steady state 'trough' x 24 h = AUC_{24h}) can be facilitated by continuous administration (cf 3.2). Otherwise, at least a two point (peak/intermediate-trough) AUC_{24h} estimate or - preferred - Bayesian model approaches are needed (cf 4.). Besides better targeting, we should also consider the AUC_{24h} target to aim for in neonates (5.).

3.2. Continuous administration of vancomycin in neonates

In adults, there is meta-analytical evidence that continuous vancomycin administration results in improved vancomycin target attainment and reduced TDM sampling. In contrast, the evidence on more relevant benefits (e.g. nephrotoxicity, efficacy) is limited. Although debated, there is a trend to reduced nephrotoxicity, likely because other risk factors as co-medication or disease severity interact [36,37].

3.2.1. The available experience reported in neonates

To provide an overview on the experience with continuous vancomycin administration in neonates, we conducted a systematic search (24 May 2019, search terms 'vancomycin', 'continuous' and 'newborn, preterm or infant') on PubMed. This resulted in 45, 6 and 71 hits respectively. Titles and abstracts were assessed on relevance and screened for references and citations (**Table 1**). We hereby retained 10 studies (1994-2019), suggesting that the concept of continuous administration in neonates is not really new. Some studies were retrospective (n=3), other prospective (n=7). We only retrieved two randomized controlled trials (continuous versus intermittent dosing) [38,39]. Loading doses varied (range 7-15 mg/kg, over 1-2 hours), and was even not used in two studies [40,41]. Maintenance doses also varied (range 10-60 mg/kg/day, as mg/kg is commonly used in neonates) with creatinine and age (postnatal, postmenstrual, postconceptional) as covariates. The vancomycin

target concentration aimed for during continuous administration varied between 10 and 30 mg/l, and obviously differs from the trough levels (range 5-20 mg/l) in the comparative (intermittent dosing) studies. Compared to intermittent (prospective or retrospective) dosing, target achievement was more common (85 vs 45% [39]; 52.8 to 34.1% [42]; 68 to 33.6% [38]; 62 to 28% [43]) at first measurement when continuous administration was used. This first measurement was aimed to be at 'steady' state (18-48 h), while Zhao et al. collected the first TDM observation 6-12 h after vancomycin administration. These time ranges also illustrate the potential advantage of flexibility of sampling times. The Zhao et al. individualized continuous dosing regimen (**cf Table 1**) has subsequently been evaluated by Leroux et al., confirming increased target attainment rate (42 to 72%) [21,44]. In contrast, a simplified dosing regimen (loading 15 mg/day + 30 mg/kg/day for all) in 75 preterm (median gestational age 27 weeks) neonates resulted in target attainment (20-30 mg/l in their study, but equal to AUC_{24h} of 480-720) in only 30% of cases [45] (**Table 1**) [21,38-47].

This overview also unveiled some limitations, including the limited number of studies that assessed efficacy and aspects of clinical implementation. Fewer dose adaptations were the most commonly reported observation in case of continuous administration [39]. Renal toxicity was either similar to intermittent administration. Along the same line, trends in creatinine with increasing age were neither different [21,39,46]. Differences in the number of manipulations, in the need for interruption (incompatibility with other infusions, none of the papers reported on their practices) or additional venous access, or in the incidence of dosing errors were poorly reported. Finally, we noticed that the vancomycin assay characteristics for vancomycin (n=4) or creatinine (n=1) were only very rarely provided, although assays matter (*cf* 6.1) [48].

3.2.2. Issues to consider when deciding to go for continuous or intermittent dosing

Based on the above summarized evidence, there seems to be equipoise for either continuous or intermittent dosing in neonates. In line with findings in adults, it is more likely to attain 'trough' targets with continuous administration, but data on other burdens/benefits are limited, so that decisions can be based on preferences. However, we still would like to highlight 3 specific issues when continuous administration is considered.

3.2.2.1. Do not forget the loading dose

In the setting of continuous administration without loading dose, the time to reach this target concentration will be significantly prolonged. This is even more the case in newborns, because the distribution volume (V_d) is significantly higher (lower peak concentrations) and clearance significantly lower (lower maintenance doses). Based on the prolonged vancomycin half-life, preterm neonates need 30-50 h to reach steady state [16,49]. This loading dose concept is not unique to continuous administration, but should also be considered in intermittent dosing regimens [50]. The relevance of a loading dose has recently also been modelled by Germovsek et al [22]: if vancomycin was administered as continuous infusion and a loading dose was not given or was <15 mg/kg, the AUC_{24h}/MIC remained <400 , particularly in the first 24 h of therapy. The formula [target (20 mg/l) $\times V_d$, and $V_d = 0.79 \times (\text{weight}/1416)^{0.898}$] to calculate the loading dose as suggested by Zhao et al. hereby reflects maturational changes in body composition (body water content, albumin) in neonates with an even higher (l/kg) V_d in the most immature preterms, similar to aminoglycosides [17,44].

3.2.2.2. Consider issues related to drug (in)compatibility

Data on vancomycin stability and compatibility following dilution are available. Vancomycin itself remained stable over a relevant period of time (up to 72 h). In contrast, incompatibility (assessed by visual inspection, particle analyser, and vancomycin quantification) was observed with β -lactams, but not with aminoglycosides. Amino acids, midazolam or morphine were compatible, while propofol, intravenous lipids (increased particle size), phenytoin, theophylline (methylxanthine, caffeine not assessed), furosemide or methylprednisolone were incompatible [51,52]. Although conversion of these findings [e.g. only one vancomycin concentration (10 mg/ml) tested, infusion rate, osmolarity] to neonates has its issues, it is relevant to consider these aspects. Related to this, Drouet et al. assessed the endothelial cell toxicity of continuous vs intermittent administration, using a human umbilical vein endothelial cell model. Endothelial cell death related to concentration and time, resulting in higher cell toxicity during continuous dosing [53].

3.2.2.3. There is some experimental basis for a PK/PD target for CoNS

In an experimental rabbit model with a foreign body implant, Ramos-Martin et al. characterized vancomycin PK/PD for CoNS (*S. epidermidis* and *S. capitis*) and investigated optimal exposure. Taking the limitations into account to translate these experimental findings to neonates who commonly suffer from CoNS infections, this

study supports the use of intermittent dosing, since continuous infusion may be associated with a higher likelihood of antimicrobial resistance [54].

4. Bayesian estimation-assisted TDM decision making

Bayesian estimation-assisted TDM decision making combines a given population model with the data from an individual patient to “update” the model to an individualized, “Bayesian posterior” version and related dosing advice. Until present, there are data on improved target attainment (70 versus 19 %) and reduced nephrotoxicity (2 versus 8 %) after introduction of Bayesian estimation-assisted AUC-guided TDM in adults [55]. An additional benefit is the reduced number of required blood samples to forecast the optimal dosage to maintain target attainment, as the model incorporates the covariates for clearance and the need for individual dosage adjustments during treatment.

Next to the reduced blood volume drawn from the neonate, the bloodstream infection risk is lower due to less manipulations of venous accesses. Furthermore, AUC-guided TDM does not require trough concentrations, enabling the use of scavenged samples. Moreover, this flexibility enables individual AUC target attainment based on one (or two, for a more accurate estimation) sample collected after the first (loading) dose or a sample collected within the first hours after start of continuous infusion, so sooner compared to the current practice. Rapid quantification of vancomycin followed by AUC-guided TDM will lead to an immediate individualized treatment optimization.

Moreover, AUC-guided TDM and more frequent dosing results in lower median trough concentrations. Neely et al. found that 60 % of the adults already reach the 400 AUC target with trough level < 15 mg/l, despite their general target range was 15-20 mg/l, making the point that Bayesian tool are a more effective approach to attain targeted treatment [56].

The influence of the dosing interval on the trough concentrations achieved with the same AUC_{24h}/MIC 400 mg/l.h can be appreciated in **Figure 2A-D** for a neonate (GA 29 weeks, postnatal age 10 days, current weight 1170 g) following different dosing regimens (q24h to q6h, with the same (28-28.4 mg) 24h dose) [17].

5. Characteristics of CoNS as pathogen to use vancomycin in neonates: does this affect the target to aim for?

5.1. Epidemiology and resistance patterns

CoNS are the most commonly isolated pathogens during LOS in neonates [10,57]. A recent paper on CoNS sepsis confirmed the overall low mortality and limited short term morbidity associated with this pathogen when treated, although there are concerns on long term morbidity [57,58,59]. *S. epidermidis* (63%) is the most frequently retrieved isolate, while *S. haemolyticus* (28%), or other CoNS (9%) (*S. hominis*, *S. warnerii*, *S. saprophyticus*, or *S. capitis*) are also isolates from blood cultures collected in neonates [34,60,61]. However, *S. capitis* became the main CoNS pathogen in several units in France, with subsequent dissemination to Western Europe and Australia [62]. This turned out to be a specific strain, with a resistance profile adapted to antibiotics commonly used in neonatal intensive care (resistant to β -lactams, aminoglycosides, not quinolones). Even more relevant, this strain has reduced vancomycin susceptibility, associated with a more severe LOS compared to 'routine' CoNS [63,64].

Methicillin resistance in CoNS exceeds 70% in most centers [16,34]. This relates to carriage of *mecA* in CoNS. This results in low affinity for β -lactam antibiotics to the penicillin-binding protein (PBP)-2a. This is especially for methicillin or oxacillin, while an important part of these CoNS remain susceptible to cefazolin (92%) or amoxicillin/clavulanic acid (88%) [65]. Consequently, a relevant portion of centers will administer vancomycin as part (combined with either aminoglycosides, or 3rd generation cephalosporins) of their empiric LOS treatment [16,66]. For LOS, a recent German survey described that 48% of the units use vancomycin empirically before any methicillin-resistant Gram-positive pathogen is detected [66].

In an attempt to provide an overview on variability in MIC values for vancomycin in isolates of neonates, we have pooled data on 318 blood culture CoNS isolates (2006-2017) reported from France, the Netherlands, Estonia, Australia and Belgium (**Table 2**) [15,34,39,46,60]. In essence, the majority (75-80%) have a MIC value ≤ 2 , with a relevant minority of more (MIC > 2) resistant CoNS isolates, while >4 mg/l is the currently used MIC breakpoint for CoNS [16]. However, such targets (2-4 x >400, so 800 to 1600) can never be reached without disproportional toxicity.

5.2. How can we translate these findings to clinical practice?

CoNS infections are common, resistance patterns vary, and a relevant portion has higher MIC values for vancomycin. The relevance to clinical practice was converted to 3 questions: *do we need 'empiric' vancomycin, what if vancomycin fails, and how confident can we be on the currently used vancomycin target ?*

5.2.1. Do we need 'empiric' vancomycin?

One may question the need for vancomycin as part of empiric LOS treatment modality, and even to use vancomycin once the blood culture confirms CoNS. Related to the observations on the difference in resistance between different β -lactams [65], there are reports on cefazolin use [67]. Of 163 infants with proven CoNS sepsis, 142 were treated with cefazolin (121 cefazolin susceptible, 21 resistant since MIC ≥ 32 mg/l). 21/142 were switched to vancomycin, but the most relevant indicator of failure was the removal (yes/no, failure=22 vs 81%) of the central venous catheter [67].

5.2.2. What if vancomycin fails?

Persistent CoNS bacteremia (positive blood culture >48-72 h of appropriate treatment) occurs in up to 40% of very-low-birth-weight neonates [68]. In a cohort of 58 cases, persistent CoNS bacteremia was associated with hypothermia at presentation, transient raised creatinine, endocarditis or thrombus presence, while breastfeeding was protective. Others reported on the link with the presence of a central venous catheter [67,69]. Bacteremic infants experience fewer infection-related complications when the central catheter was removed promptly, and persistent CoNS bacteremia warrants removal [69]. Rifampicin co-treatment (with vancomycin) has been suggested as strategy for persistent CoNS bacteremia after central line removal (18 neonates) [70]. Linezolid treatment is another option and this drug can also be administered enteral. Using either 30 mg/kg/day intravenous (continuous infusion), or 3 x 10 mg/kg enteral, MIC target values were reached [71]. In a retrospective analysis on 89 cases treated with linezolid, compared to 56 treated with vancomycin as definitive treatment of heterogeneously resistant vancomycin-intermediate CoNS, effectiveness (early or late recurrence, mortality, central line-associated bloodstream infection duration) was similar [72].

5.2.3. How much confidence should we have on the AUC_{24h}/MIC 400 vancomycin target in neonates?

It seems that there is a discrepancy between the target ($AUC_{24h}/MIC > 400$) commonly aimed for and the range of MIC values for vancomycin observed in blood culture isolates in neonates. As mentioned earlier, the currently advocated $AUC_{24h}/MIC > 400$ target is based on data in adults with *S. aureus* pneumonia [33], while neonatal CoNS sepsis are in general less invasive (biofilm related) and rarely cause other infections than blood stream infections [33,34]. This is also reflected in the CoNS

confirmed sepsis cohorts reported by Sinkeler (n=112) [60] and Pandari (n=57) [34]. The 400 target was only reached in 25% (trough, collected before the fifth dosing, so perhaps not yet on steady state, Sinkeler) or 25-40% (AUC_{24h}/MIC , Padari) of neonates, without any link between AUC_{24h}/MIC and outcome (treatment failure) [34,60].

Besides observations in human neonates, there are also some animal experimental data in support of a lower target range. Leiva et al. developed a model for successful growth of *S. epidermidis* in a neutropenic mouse thigh infection model without foreign body, more reflecting the newborn setting with blood stream infection. For CoNS related infections in this animal model, the vancomycin AUC_{24h}/MIC target was 150, not 400 [55,73]. In contrast, for infections treated with a catheter *in situ* rabbit model, the AUC_{24h}/MIC target was 520 [54]. This likely relates to biofilm creation, more pronounced in the presence of a foreign body [16].

6. Underexplored causes of unexplained variability of vancomycin PK

The primary goal of clinical pharmacology is to understand the determinants of the dose-concentration-effect relationship to tailor dosing [74]. For vancomycin dosing in neonates, there are still knowledge gaps related to the concentration-effect (target) relationship (section 5.), while TDM may assist to further individualize treatment. Underexplored causes of vancomycin variability relate to assay variability, preparation, stability and compatibility, and uncertainties on clinical covariates.

6.1. Vancomycin assay related variability

Immuno-assays are still commonly used to quantify vancomycin. Such assays can be affected by plasma composition (IgM) or vancomycin degradation products (accumulation in patients with renal impairment) [75]. Compared to liquid chromatography-tandem mass spectrometry (LC-MS/MS), 3 commonly used immunoassays showed a mean proportional difference within 10%, while another assay showed a substantial (>20%) mean proportional difference. This resulted in clinical discordant interpretation in 6.1 to 22.2% [76]. There is also relevant between-lab variability, highlighted in audit reports from Belgium (2014) and Poland (2011-2018) [13,77]. Finally, there are some papers on the interference of vancomycin crystalline degradation products, most relevant in patients with renal failure, resulting in vancomycin overestimation [75]. This may be of relevance in extreme preterm or critically ill neonates with renal failure.

Findings on the relevance of a given assay have also been reported in neonates. Using the same vancomycin dosing regimens and confirming similarity in clinical characteristics, vancomycin trough concentrations measured by PETINIA (2011-2012, n=400) were 20% lower, with a mean difference of 1.93 mg/l compared to COBAS (2012-2014, n=352) [78]. Furthermore, Zhao et al. quantified the predictive performance of 6 published vancomycin PK models when applied to a new dataset, and observed that performance in part related to the vancomycin assay [48].

6.2. Preparation, stability and compatibility

While calculation errors during preparation of vancomycin can be largely eliminated using protocols on standard drug concentrations, this does not exclude accuracy error rates [79]. In an audit in 10 Spanish neonatal intensive care units on 444 preparations, 6 (1.4%) calculation and 243 (55%) accuracy (+/- 10%) errors were observed. Specific for vancomycin (n=238/444) and aiming for a 50 mg/ml solution (according to manufacturer instructions), 125/218 samples were classified as inaccurate, irrespective of who (nurse, pharmacist) prepared the formulation. Underdosing was 4x more common [79]. A similar observation has been reported by a French group. In their unit, there was variability (3.33-60 mg/ml) of the intended vancomycin concentrations, and measured concentrations were on average 7% (75.8-120.4%) lower [80].

In contrast, studies on vancomycin stability documented that formulations (10 to 83 g vancomycin/l, in glucose 5%) remain stable (>93% recovery) for up to 72 h at 37°C [51]. Incompatibility (assessed by visual inspection, particle analyser, and vancomycin quantification) was observed with β -lactams, propofol, lipids (increased particle size), phenytoin, theophylline (methylxanthine, caffeine not assessed), furosemide, midazolam and methylprednisolone [16,51,52]. This incompatibility is often caused by the low pH (3-5) of vancomycin premixed infusions.

6.3. Issues related to clinical covariates

Relevant clinical covariates may be missing (e.g. current weight), underexplored (co-medication) as not incorporated in the PK model, or may display assay-related differences (e.g. creatinine).

Body weight is one of the commonly observed covariate for PK of many drugs - including vancomycin - and dramatically changes over the first weeks of life. Although growth charts based on real world data exist, it still results in additional uncertainty for the individual prescription [81]. Even when clinical characteristics of the relevant

covariates for a given dosing regimen developed in a specific unit are similar to another unit, it is still possible that covariates not retained in the initial model are relevant, like disease characteristics (patent ductus, growth restriction), or co-medication (ibuprofen or indomethacin, amoxi-clavulanic acid, spironolactone) [18,19]. The creatinine assay also matters, with higher creatinine values when Jaffe assays are compared to enzymatic assays [82]. The creatinine assay also affected the predictive performance of 6 existing models on an independent dataset [48].

6.4. Vancomycin protein binding: not total, but free concentrations matter

Vancomycin binds moderately to human serum albumin and immunoglobulin A in plasma. This binding is reversible and may affect PK/PD [83]. However, TDM commonly uses total drug concentrations, while only the free, unbound concentration is pharmacologically active and available for clearance. In a cohort of 33 neonates, median (interquartile range) 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 0.9 (0.77-0.98), with total vancomycin concentration and albumin as most relevant covariates of unbound vancomycin concentration [15]. This unbound vancomycin fraction in neonates is higher compared to older children and adults [76]. This is in line with observations for other compounds like cefazolin, flucloxacillin, diazepam and cyclosporine [84,85,86]. Less albumin binding places (hypoalbuminemia, competing substances) as well as altered binding affinity contribute to this [87].

To achieve safe and effective, 'unbound' vancomycin exposure, protein binding needs to be integrated in PK/PD studies. De Cock et al documented improved target attainment (83% versus 54%) using PK/PD indices based on unbound compared to total vancomycin concentrations in critically ill children [88]. When aiming for a similar unbound vancomycin exposure in neonates as in adults, a total $AUC_{24h}/MIC \geq 267$ target (400×0.67) should be applied, derived from an unbound fraction of 0.67 in adults versus 0.9 in neonates. PK simulations hereby observed that 54.2% of neonates achieved the $AUC_{24h}/MIC \geq 400$ target, while 91.2% achieved the $AUC_{24h}/MIC \geq 267$ target (unbound target approach) [89]. Besides between-population differences, a large variability in vancomycin protein binding is observed within the neonatal population [15]. Therefore, using individual instead of fixed unbound fractions is advised in future PK/PD (validation) studies. Taking into account the

maturational aspects of vancomycin protein binding will also influence dosing regimens in neonates [89].

7. Discussion and conclusions

CoNS are the most frequently isolated pathogens in blood cultures for LOS. Consequently, vancomycin is the 5th most commonly prescribed drug in neonatal intensive care (91 exposed or 150 courses/1000 admissions). Vancomycin PK in neonates display extensive variability, combining reduced clearance with a higher distribution volume, resulting in a prolonged duration to reach steady state. This setting can at best be covered by a loading dose, both for intermittent or continuous administration (**Figure 3 A-E**) [21,22,44,50]. Data on the subsequent intermittent or continuous maintenance dosing illustrate that 'simple' dosing regimens fail to attain sufficiently well the target, because such dosing regimens do not fully cover the variability of clearance in neonates [31,45,50]. Rapid quantification of vancomycin shortly following initiation of treatment, followed by AUC-guided TDM will lead to an immediate individualized treatment optimization.

Both **Figures 2 and 3** illustrate that the choice of a dosing interval of a certain daily dose leading to a target AUC affects the corresponding trough concentration. This requires different defined trough concentrations per dosing interval or - even better - AUC-guided TDM based on the calculated AUC. Furthermore, the immediate target attainment following a loading dose versus target attainment not earlier than 48 hours without loading dose, is illustrated in **Figure 3**.

At present, targeted exposure is commonly been based on trough levels, despite the fact that vancomycin trough concentrations are only poor surrogate markers and possible misleading for the 'golden' target, i.e. AUC_{24h}/MIC . This is also the case in neonates, and is an important message for clinicians, since this is a paradigm shift and is the basis for the need to shift to PK modelling and Bayesian estimation-assisted TDM decision making to improve targeted vancomycin exposure [29,30,31]. Bayesian methods estimates are in general more accurate dosage regimen tools. However, this require additional resources, skills and knowledge. This includes information technology, staff with PK training, but there are also other benefits. In adults, these benefits relate to the time until being on target, the number of samples to be collected, and duration of hospitalization [49]. An understressed aspect when introducing these models to neonates is that we should not only work towards

improved empiric exposure and subsequent TDM supported target exposure, but - because of their fast maturational changes - also verified sustained target exposure. This structured approach on integration is graphically summarized in **Figure 4**.

8. Expert opinion

Initial vancomycin prescription should be based on 'a priori model based individual dosing', followed by tailoring by 'posterior dosing optimization' after TDM [16]. When applying Bayesian estimation-assisted decision making, TDM can even be shifted to earlier sampling (**Figure 4**).

In a *first step (improve empiric exposure)*, an initial dosage guideline should be as accurate as possible. There are quite some different population PK models, and subsequent model-derived dosing regimens. It is hereby of relevance to select a valid dosing regimen. Such a validation is at best based on internal validation, external validation, and prospective validation as reported for amikacin [26], but also for vancomycin, either based on subpopulations and covariates driven dosing [50,90], or model-based patient-tailored vancomycin dosing [21]. Furthermore, clinicians should realize that such a validation only applies when case and setting is similar to the cases and setting as used for model development and validation. This may apply to patient-specific disease characteristics like weight, age, or extracorporeal membrane oxygenation (ECMO) [91,92] or indomethacin or ibuprofen co-administration [18], but also other aspects like e.g. vancomycin or creatinine assays further affect performance of individual models [48]. An elegant go-between to facilitate access to the best validated, applicable to local practices, is the development of a national formulary. The stepwise approach taken to develop a national pediatric formulary for the Netherlands, with specific interest in (pre)term neonates serves as an example, and its methodological approach has been reported elsewhere [93].

In *step 2 (improve targeted exposure)* and following Bayesian estimation-assisted TDM, targeting can be further tailored. These decisions should at best be based on the similar models already mentioned in step 1, but with integration of TDM. In this way, there is a shift from a poorly controlled decision process on vancomycin dosing based on (trough) concentrations to a Bayesian driven decision. Obvious disadvantages are the need for additional training, and the need for a software platform that has been validated in a patient cohort with similar characteristics [27].

This approach has proven to be effective in adults [55,56], but ‘uptake’ of this practice in neonatal care is warranted. However, further validation and critical reflection remain relevant. In a recent validation effort, Turner et al. assessed the performance of 5 Bayesian dose-optimizing software programs (Adult and Pediatric Kinetics [APK], BestDose, DoseMe, InsightRx, and Precise PK) and two first-order PK equations, based on rich sampling data in 19 adult patients, to enable the calculation of a ‘true’ AUC_{24h} . Utilizing only the trough, accuracy (range 0.79-1.03) and bias (range 5.1-21.2%) of the Bayesian dose-optimizing software were variable. The addition of a second vancomycin level to the trough improved the accuracy and bias for DoseMe and InsightRx, but not for Precise PK and BestDose [94]. At least, this reflects the need to carefully consider the benefits, limitations and challenges of the methodologies of AUC_{24h} estimation before implementation [94]. The limitations relate - among others - to the need of similarities in clinical characteristics of patients or assays.

Related to the uptake of this practice, a recent cross-sectional survey in 124 academic medical centers on the readiness to implement vancomycin monitoring based on AUC_{24h} curve, showed that only a minority (23%) performed AUC_{24h} based TDM, mainly 2-points PK, with only a small fraction using either Bayesian software or population-based PK. The main barrier to implement this was unfamiliarity with the approach [95]. This suggests that teaching and knowledge diffusion (‘implementation’) remain an obvious need.

The 3rd step is crucial and very relevant to neonates, since ‘*sustained targeted exposure*’ should not be taken for granted because of their very fast changes in (patho)physiology and the related (non)maturational covariates (mainly renal clearance, and body composition + albumin). In essence, it is crucial that target exposure is sustained over time. To do so, Bayesian techniques can again be very supportive [81,96].

Once this targeted approach is sufficiently well established, further research should also cover PK/PD studies - including safety - in relevant animal models and human neonatal setting (CoNS, blood stream infections) to further explore PK/PD patterns of vancomycin in (pre)term neonates. The neutropenic mice and ‘device-implant’ rabbit models illustrate the feasibility and relevance [54,73].

Funding

This paper was not funded.

Declaration of interests

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Accepted Manuscript

References

Papers of special note have been highlighted as:

** of interest*

*** of considerable interest*

1. Allegaert K. Rational use of medicines in neonates: current observations, areas for research and perspectives. *Healthcare* 2018;6(3):E115.
2. Smits A, Kulo A, de Hoon JN, et al. Pharmacokinetics of drugs in neonates: pattern recognition beyond compound specific observations. *Curr Pharm Des.* 2012;18(21):3119-3146.
3. Allegaert K, Mian P, van den Anker JN. Developmental pharmacokinetics in neonates: maturational changes and beyond. *Curr Pharm Des.* 2017;23(38):5769-5778.
4. Ward RM, Benjamin D, Barrett JS, et al. Safety, dosing, and pharmaceutical quality for studies that evaluate medicinal products (including biological products) in neonates. *Pediatr Res.* 2017;81(5):692-711.

**** recent overview with guidance on neonatal drug development**

5. Allegaert K, Simons SHP, Tibboel D, et al. Non-maturational covariates for dynamic systems pharmacology models in neonates, infants, and children: Filling the gaps beyond developmental pharmacology. *Eur J Pharm Sci.* 2017;109S:S27-S31.
6. Krekels EHJ, van Hasselt JGC, van den Anker JN, et al. Evidence-based drug treatment for special patient populations through model-based approaches. *Eur J Pharm Sci.* 2017;1092:S22-S26.
7. Young TE. Therapeutic drug monitoring – the appropriate use of drug level measurement in the care of the neonate. *Clin Perinatol.* 2012;39(1):25-31.
8. van Donge T, Bielicki JA, van den Anker J, et al. Key components for antibiotic dose optimization of sepsis in neonates and infants. *Front Pediatr.* 2018;6:325.

***recent review on antibiotic dose optimization for neonatal infection, applied to vancomycin in this review**

9. Hsieh EM, Hornik CP, Clark RH, et al. Medication use in the neonatal intensive care unit. *Am J Perinatol.* 2014;31(9):811-821.
10. van den Anker J, Allegaert K. Rational use of antibiotics in neonates: still in search of tailored tools. *Healthcare* 2019;7(1):E28.

11. Rivera-Chaparro ND, Cohen-Wolkowicz M, Greenberg RG. Dosing antibiotics in neonates: review of the pharmacokinetic data. *Future Microbiol.* 2017;12:1001-1016.

***extensive review on the pharmacokinetic data of the different individual antibiotics published in neonates, with related to this, dosing regimens.**

12. Mian P, Flint RB, Tibboel D, et al. Therapeutic drug monitoring in neonates: what makes them unique ? *Curr Pharm Des.* 23(38):5790-5800.

13. Pauwels S, Allegaert K. Therapeutic drug monitoring in neonates. *Arch Dis Child.* 2016;101(4):377-381.

14. Pacifici GM, Allegaert K. Clinical pharmacokinetics of vancomycin in the neonate: a review. *Clinics* 2012;67(7):831-837.

15. Smits A, Pauwels S, Oyaert M, et al. Factors impacting unbound vancomycin concentrations in neonates and young infants. *Eur J Clin Microbiol Infect Dis.* 2018;37(8):1503-1510.

16. Jacqz-Aigrain E, Leroux S, Zhao W, et al. How to use vancomycin optimally in neonates: remaining questions. *Expert Rev Clin Pharmacol.* 2015;8(5):635-648.

17. De Cock RF, Allegaert K, Brussee JM, et al. Simultaneous pharmacokinetic modeling of gentamicin, tobramycin and vancomycin clearance from neonates to adults: towards a semi-physiological function for maturation in glomerular filtration. *Pharm Res.* 2014;31(10):2643-2654.

18. Cristea S, Allegaert K, Falcao AC, et al. Larger dose reductions of vancomycin required in neonates with patent ductus arteriosus receiving indomethacin vs. ibuprofen. *Antimicrob Agents Chemother.* doi: 10.1128/AAC.00853-19.

19. Marqués-Miñana MR, Saadeddin A, Peris JE. Population pharmacokinetic analysis of vancomycin in neonates. A new proposal of initial dosage guideline. *Br J Clin Pharmacol.* 2010;70(5):713-720.

20. Lestner JM, Hill LF, Heath PT, et al. Vancomycin toxicity in neonates: a review of the evidence. *Curr Opin Infect Dis.* 2016;29(3):237-247.

***recent overview on vancomycin toxicity in neonates.**

21. Leroux S, Jacqz-Aigrain E, Biran V, et al. Clinical utility and safety of a model-based patient-tailored dose of vancomycin in neonates. *Antimicrob Agents Chemother.* 2016;60(4):2039-2042.

***recent model development effort specific to vancomycin dosing in neonates.**

22. Germovsek E, Osborne L, Gunaratnam F, et al. Development and external evaluation of a population pharmacokinetic model for continuous and intermittent administration of vancomycin in neonates and infants using prospectively collected data. *J Antimicrob Chemother.* 2019;74(4):1003-1011.

***very recent model development effort specific to vancomycin dosing in neonates.**

23. van Hal SJ, Paterson DL, Lodise TP. Systematic review and meta-analysis of vancomycin-induced nephrotoxicity associated with dosing schedules that maintain troughs between 15 and 20 milligrams per liter. *Antimicrob Agents Chemother.* 2013;57(2):734-744.

24. Zasowski EJ, Murray KP, Trinh TD, et al. Identification of vancomycin exposure-toxicity thresholds in hospitalized patients receiving intravenous vancomycin. *Antimicrob Agents Chemother.* 2017;62(1):e01684-17.

25. Zimmerman E, Lahav A. Ototoxicity in preterm infants: effects of genetics, aminoglycosides, and loud environmental noise. *J Perinatol.* 2013;33:3-8.

26. Smits A, Kulo A, van den Anker J, et al. The amikacin research program: a stepwise approach to validate dosing regimens in neonates. *Expert Opin Drug Metab Toxicol.* 2017;13(2):157-166.

27. Drennan PG, Begg EJ, Gardiner SJ, et al. The dosing and monitoring of vancomycin: what is the best way forward ? *Int J Antimicrob Agents* 2019;53(4):401-407.

28. Biagi MJ, Butler DA, Wenzler E. AUC-based monitoring of vancomycin: closing the therapeutic window. *J Appl Lab Med.* 2019;3(3-4):743-746.

29. Tseng SH, Lim CP, Chen Q, et al. Evaluating the relationship between vancomycin trough concentration and 24-hour area under the concentration-time curve in neonates. *Antimicrob Agents Chemother.* 2018;62(4):e01647-17.

30. Chen Y, Wu D, Dong M, et al. Population pharmacokinetics of vancomycin and AUC-guided dosing in Chinese neonates and young infants. *Eur J Clin Pharmacol.* 2018;74(7):921-930.

31. Jacqz-Aigrain E, Leroux S, Thomson AH, et al. Population pharmacokinetic meta-analysis of individual data to design the first randomized efficacy trial of

vancomycin in neonates and young infants. J Antimicrob Chemother. 2019 doi: 10.1093/jac/dkz158.

***very recent model development effort specific to vancomycin dosing in neonates.**

32. Men P, Li HB, Zhai SD, et al. Association between the AUC₀₋₂₄/MIC ratio of vancomycin and its clinical effectiveness: a systematic review and meta-analysis. PLoS One. 2016;11(1):e0146224.
33. Moise PA, Forrest A, Bhavnani SM, et al. Area under the inhibitory curve and a pneumonia scoring system for predicting outcomes of vancomycin therapy for respiratory infections by Staphylococcus aureus. Am J Health Syst Pharm. 2000;57(Suppl 2):S4-S9.
34. Padari H, Oselin K, Tasa T, et al. Coagulase negative staphylococcal sepsis in neonates: do we need to adapt vancomycin dose or target ? BMC Pediatr. 2016;16(1):206.
35. Dao K, Guidi M, André P, et al. Optimisation of vancomycin exposure in neonates based on the best level of evidence. Pharmacol Res. 2019 doi: 10.1016/j.phrs.2019.104278.
36. Echeverria-Esnal D, Marin-Casino M, Retamero A, et al. Can we guarantee less nephrotoxicity when vancomycin is administered by continuous infusion ? Int J Antimicrob Agents 2016;48(1):116-117.
37. Dong H, Zhong Y. Response to: 'continuous versus intermittent infusion of vancomycin in adult patients: a systematic review and meta-analysis. Int J Antimicrob Agents 2016;48(1):114-115.
38. Patel AD, Anand D, Lucas C, et al. Continuous infusion of vancomycin in neonates. Arch Dis Child. 2013;98(6):478-479.
39. Gwee A, Cranswick N, McMullan B, et al. Continuous versus intermittent vancomycin infusions in infants: a randomized controlled trial. Pediatrics 2019;143(2):e20182179.
40. Borderon JC, Laugier J, Chamboux C, et al. Continuous infusion of vancomycin during the neonatal period. Pathol Biol. 1994;42(5):525-529.
41. Plan O, Cambonie G, Barbotte E, et al. Continuous-infusion vancomycin therapy for preterm neonates with suspected or documented Gram-positive infections: a new dosage schedule. Arch Dis Child Fetal Neonatal Ed. 2008;93(6):F418-F421.

42. Demirel B, Imamoglu E, Gursoy T, et al. Comparison of intermittent versus continuous vancomycin infusion for the treatment of late-onset sepsis in preterm infants. *J Neonatal Perinatal Med.* 2015;8(2):149-155.
43. Whitticase L, Holder G, Cawsey M, et al. An audit of vancomycin plasma concentrations when vancomycin is administered by continuous intravenous infusion in neonates. *Arch Dis Child.* 2016;101(9):e2
44. Zhao W, Lopez E, Biran V, et al. Vancomycin continuous infusion in neonates: dosing optimisation and therapeutic drug monitoring. *Arch Dis Child.* 2013;98(6):449-453.
45. Tauzin M, Cohen R, Durrmeyer X, et al. Continuous-infusion vancomycin in neonates: assessment of a dosing regimen and therapeutic proposal. *Front Pediatr.* 2019;7:188.
46. Oudin C, Vialet R, Boulamery A, et al. Vancomycin prescription in neonates and young infants: toward a simplified dosage. *Arch Dis Child Fetal Neonatal Ed.* 2011;96(5):F365-F370.
47. Pawlotsky F, Thomas A, Kergueris MF, Debillon T, Roze JC. Constant rate infusion of vancomycin in premature neonates: a new dosage schedule. *Br J Clin Pharmacol.* 1998;46(2):163-167.
48. Zhao W, Kaguelidou F, Biran V, et al. External evaluation of population pharmacokinetic models of vancomycin in neonates: the transferability of published models to different clinical settings. *Br J Clin Pharmacol.* 2013;75(4):1068-1080.
- model effort specific to vancomycin dosing in neonates that illustrate the relevance of assays (vancomycin, creatinine) in neonates.**
49. Monteiro JF, Hahn SR, Gonçalves J, et al. Vancomycin therapeutic drug monitoring and population pharmacokinetic models in special patient subpopulations. *Pharmacol Res Perspect.* 2018;6(4):e00420.
50. Janssen EJ, Välitälo PA, Allegaert K, et al. Towards rational dosing algorithms for vancomycin in neonates and infants based on population pharmacokinetic modeling. *Antimicrob Agents Chemother.* 2015;60(2):1013-1021.
- *very recent model development effort specific to vancomycin dosing in neonates.**

51. Raverdy V, Ampe E, Hecq JD, et al. Stability and compatibility of vancomycin for administration by continuous infusion. *J Antimicrob Chemother.* 2013;68(5):1179-1182.
52. Omotani S, Aoe M, Esaki S, et al. Compatibility of intravenous fat emulsion with antibiotics for secondary piggyback infusion. *Ann Nutr Metab.* 2018;73(3):227-233.
53. Drouet M, Chai F, Barthélémy C, et al. Influence of vancomycin infusion methods on endothelial cell toxicity. *Antimicrob Agents Chemother.* 2015;59(2):930-934.
54. Ramos-Martin V, Johnson A, Livermore J, et al. Pharmacodynamics of vancomycin for CoNS infection: experimental basis for optimal use of vancomycin in neonates. *J Antimicrob Chemother.* 2016;71(4):992-1002.
- **recent report on an animal model developed to study PK/PD of CoNS in the presence of a foreign body.**
55. Neely MN, Kato L, Youn G, et al. Prospective trial on the use of trough concentration versus area under the curve to determine therapeutic vancomycin dosing. *Antimicrob Agents Chemother.* 2018;62(2):e02042-17.
56. Neely MN, Youn G, Jones B, et al. Are vancomycin trough concentrations adequate for optimal dosing ? *Antimicrob Agents Chemother.* 2014;58(1):309-316.
57. Zonnenberg IA, van Dijk-Lokkart EM, van den Dungen FAM, et al. Neurodevelopmental outcome at 2 years of age in preterm infants with late-onset sepsis. *Eur J Pediatr.* 2019;178(5):673-680.
58. Cantey JB, Anderson KR, Kalagiri RR, et al. Morbidity and mortality of coagulase-negative staphylococcal sepsis in very-low-birth-weight infants. *World J Pediatr.* 2018;14(3):269-273.
59. Alshaikh B, Yee W, Lodha A, et al. Coagulase-negative staphylococcus sepsis in preterm infants and long-term neurodevelopmental outcome. *J Perinatol.* 2014;34(2):125-129.
60. Sinkeler FS, de Haan TR, Hodiamont CJ, et al. Inadequate vancomycin therapy in term and preterm neonates: a retrospective analysis of trough serum concentrations in relation to minimal inhibitory concentrations. *BMC Pediatr.* 2014;14:193.

61. Brzychczy-Wloch M, Borszewska-Kornacka M, Gulczynska E, et al. Prevalence of antibiotic resistance in multi-drug resistant coagulase-negative staphylococci isolated from invasive infection in very low birth weight neonates in two Polish NICUs. *Ann Clin Microbiol Antimicrob*. 2013;12:14.
62. Ben Said M, Hays S, Bonfils M, et al. Late-onset sepsis due to *Staphylococcus capitis* 'neonatalis' in low-birthweight infants: a new entity ? *J Hosp Infect*. 2016;94(1):95-98.
63. Butin M, Martins-Simões P, Rasigade JP, et al. Worldwide endemicity of a multidrug-resistant *staphylococcus capitis* clone involved in neonatal sepsis. *Emerg Infect Dis*. 2017;23(3):538-539.
64. Ehlersson G, Hellmark B, Svartström O, et al. Phenotypic characterization of coagulase-negative staphylococci isolated from blood cultures in newborn infants, with a special focus on *Staphylococcus capitis*. *Acta Paediatr*. 2017;106(10):1576-1582.
65. Fler A, Hemels MA, Paauw A, et al. Reduced expression of PBP-2A by neonatal *mecA*-positive coagulase-negative staphylococci (CoNS) blood isolates: β -lactams are useful first-line agents for the treatment of neonatal CoNS sepsis, restricting the use of vancomycin. *J Antimicrob Chemother*. 2012;67(7):1616-1618.
66. Litz JE, Goedicke-Fritz S, Härtel C, et al. Management of early- and late-onset sepsis: results from a survey in 80 German NICUs. *Infection*. 2019, 10.1007/s15010-018-1263-9.
67. Hemels MA, van den Hoogen A, Verboon-Macielek MA, et al. A seven-year survey of management of coagulase-negative staphylococcal sepsis in the neonatal intensive care unit: vancomycin may not be necessary as empiric therapy. *Neonatology* 2011;100(2):180-185.
68. Linder N, Hernandez A, Amit L, et al. Persistent coagulase-negative staphylococci bacteremia in very-low-birth-weight infants. *Eur J Pediatr*. 2011;170(8):989-995.
69. Benjamin DK Jr, Miller W, Garges H, et al. Bacteremia, central catheters, and neonates: when to pull the line. *Pediatrics* 2001;107(6):1272-1276.
70. van der Lugt NM, Steggerda SJ, Walther FJ. Use of rifampin in persistent coagulase negative staphylococcal bacteremia in neonates. *BMC Pediatr*. 2010;10:84.

71. Sicard M, Launay E, Caillon J, et al. Pharmacokinetics of linezolid treatment using intravenous and oral administrations in extremely premature infants. *Eur J Clin Pharmacol*. 2015;71(5):611-615.
72. Blanchard AC, Fortin E, Laferrière C, et al. Comparative effectiveness of linezolid versus vancomycin as definitive antibiotic therapy for heterogeneously resistant vancomycin-intermediate coagulase-negative staphylococcal central-line-associated bloodstream infections in a neonatal intensive care unit. *J Antimicrob Chemother*. 2017;72(6):1812-1817.
73. Leiva ML, Imbett S, Gómez PJ, et al. Successful Growth of *Staphylococcus epidermidis* in the Neutropenic Mouse Thigh Infection Model (NMTIM) without the Use of a Foreign Body. *Pap Present 54th Intersci Conf Antimicrob Agents Chemother*. Washington: ICAAC; 2014.
- *report on an animal model developed to study PK/PD of CoNS in the absence of a foreign body.**
74. Holford NH, Buclin T. Safe and effective variability-a criterion for dose individualization. *Ther Drug Monit*. 2012;34(5):565-568.
75. Somerville AL, Wright DH, Rotschafer JC. Implications of vancomycin degradation products on therapeutic drug monitoring in patients with end-stage renal disease. *Pharmacotherapy* 1999;19(6):702-707.
76. Oyaert M, Peersman N, Kieffer D, et al. Novel LC MS/MS method for plasma vancomycin: comparison with immunoassays and clinical impact. *Clin Chim Acta*. 2015;441:63-70.
77. Kowalski R, Cwiklinska A, Lizakowski M, et al. Insufficient harmonization of antibiotics assays - Polish experience with an external quality assessment program in the years 2011-2018. *Clin Biochem*. 2019;66:91-94.
78. Samardzic J, Smits A, Spriet I, et al. Different vancomycin immunoassays contribute to the variability in vancomycin trough measurements in neonates. *Biomed Res Int*. 2016;2016:1974972.
79. Campino A, Arranz C, Unceta M, et al. Medicine preparation errors in ten Spanish neonatal intensive care units. *Eur J Pediatr*. 2016;175:203-210.
80. Popescu M, Vialet R, Loundou A, et al. Imprecision of vancomycin prepared for intravenous administration at the bedside in a neonatal intensive care unit. *Ann Fr Anesth Reanim*. 2011;30(10):726-729.

81. Smits A, De Cock P, Vermeulen A, et al. Physiologically based pharmacokinetic (PBPK) modeling and simulation in neonatal drug development: how clinicians can contribute. *Expert Opin Drug Metab Toxicol.* 2019;15(1):25-34.
82. Allegaert K, Kuppens M, Mekahli D, et al. Creatinine reference values in ELBW infants: impact of quantification by Jaffe or enzymatic method. *J Matern Fetal Neonatal Med.* 2012;25(9):1678-1681.
83. Schmidt S, Gonzalez D, Hartmut D. Significance of protein binding in pharmacokinetics and pharmacodynamics. *J Pharm Sci.* 2010;99(3):1107-1122.
84. Smits A, Kulo A, Verbesselt R, et al. Cefazolin plasma protein binding and its covariates in neonates. *Eur J Clin Microbiol Infect Dis.* 2012; 31:3359-3365.
85. Pullen J, Stolk LM, Degraeuwe PL, et al. Protein binding of flucloxacillin in neonates. 2007; *Ther Drug Monit*:29:279-283.
86. Sethi PK, White CA, Cummings BS, et al. Ontogeny of plasma proteins, albumin and binding of diazepam, cyclosporine, and deltamethrin. *Ped Research.* 2016;79(3): 409-415.
87. Notarianni LJ. Plasma protein binding of drugs in pregnancy and in neonates. *Clin Pharmacokinet.* 1990;18(1):20-36.
88. De Cock PA, Desmet S, De Jaeger A, et al. Impact of vancomycin protein binding on target attainment in critically ill children: back to the drawing board? *J Antimicrob Chemother.* 2017;72(3):801-804.
89. Leroux S, van den Anker J, Smits A, et al. Maturation changes in vancomycin protein binding affect vancomycin dosing in neonates. *Br J Clin Pharmacol.* 2019;85(5):865-867.
90. Kim J, Walker SA, Iaboni DC, et al. Determination of vancomycin pharmacokinetics in neonates to develop practical initial dosing recommendations. *Antimicrob Agents Chemother.* 2014;58(5):2830-2840.
91. Cies JJ, Moore WS 2nd, Nichols K, et al. Population pharmacokinetics and pharmacodynamic target attainment of vancomycin in neonates on extracorporeal life support. *Pediatr Crit Care Med.* 2017;18(10):977-985.
92. Wildschut ED, Ahsman MJ, Allegaert K, et al. Determinants of drug absorption in different ECMO circuits. *Intensive Care Med.* 2010;36(12):2109-2116.

93. van der Zanden TM; de Wildt SN, Liem Y, et al. Developing a paediatric drug formulary for the Netherlands. Arch Dis Child. 2017;102(4):357-361.

94. Turner RB, Kojiro K, Shephard EA, et al. Review and validation of Bayesian dose-optimizing software and equations for calculation of the vancomycin area under the curve in critically ill patients. Pharmacotherapy 2018;38(12):1174-1183.

***Validation effort of different Bayesian dose-optimizing software program to calculate the vancomycin AUC in adults.**

95. Kufel WD, Seabury RW, Mogle BT, et al. Readiness to implement vancomycin monitoring based on area under the concentration-time curve: a cross-sectional survey of a national health consortium. Am J Health Syst Pharm. 2019, 10.1093/ajhp/zxz070.

96. Abduljalil K, Jamei M, Rostami-Hodjegan A, Johnson TN. Changes in individual drug-independent system parameters during virtual paediatric pharmacokinetic trials: introducing time-varying physiology into a paediatric PBPK model. AAPS J. 2014;16(3):568-576.

Legends of boxes, tables and figures

Box 1: The basic rules to perform therapeutic drug monitoring, applied to vancomycin in neonates.

Table 1: Overview on studies on continuous vancomycin administration in neonates as retrieved in the PubMed search (chronological) [21,38-47].

Table 2: An overview on vancomycin MIC values of *coagulase-negative staphylococci* (CoNS) isolates in blood cultures of neonates as reported in recently published cohorts [15,34,39,46,60].

Figure 1: Conceptual approach for sepsis treatment in neonates, applied to late onset sepsis with emphasis on vancomycin. **Figure A** relates to drug selection, **Figure B** relates to dose selection, and subsequent drug exposure.

Figure 2: Population predicted vancomycin concentration-time profiles aiming for target attainment (AUC_{24h} 400 mg/l.h) following different dosing intervals. Population predicted vancomycin concentrations with the population PK model developed by De Cock et al. without interpatient variability were predicted for one neonate (GA 29 weeks, PNA 10 days, current body weight 1170 g) following different dosing regimens (q24h to q6h, with the same (28-28.4 mg) 24h dose) [17]. The maintenance daily dose was divided into one (A), two (B), three (C) and four (D) administrations per day. The influence of dosing interval on the required daily vancomycin dosage and the differences in trough concentrations to achieve an AUC_{24h}/MIC 400 mg/l.h can be appreciated in **Figure 2A-D**.

Figure 3: The impact of a loading dose (30 minutes) to attain target exposure faster. We hereby used the same the population PK model developed by De Cock et al. without interpatient variability for one neonate (GA 29 weeks, PNA 10 days, current body weight 1170 g) [17]. A daily maintenance dose of 28 mg (q24h to q6h, A-D, or continuous, E) was required to attain an AUC_{24h}/MIC of 400 mg/l.h, which was calculated for the time interval between 96 and 120 hours after start of therapy. The immediate target attainment following a start with a loading dose, versus target

attainment not earlier than 48 hours after start without loading dose is illustrated for each plot (A-E). The trough concentration at steady state was determined at T=120 hours.

Figure 4: Structured approach to improve vancomycin exposure: the initial vancomycin dose selection should improve empiric exposure (step 1), followed by improved targeted exposure based on therapeutic drug monitoring, supported by Bayesian software (step 2), and with subsequent follow up - because of the fast (non)maturational changes - to ensure sustained targeted exposure (step 3).

Box 1: The basic rules to perform therapeutic drug monitoring, applied to vancomycin in neonates [7,12,13].

There is only a weak correlation between dose and concentration reached (poor predictability) (2.1.), and concentration relates more closely to effect or toxicity (2.2.) than dose = *vancomycin pharmacokinetics in neonates display extensive (un)explained variability (dose-concentration). Exposure (AUC, trough) relates closer to the concentration vs dose (3.).*

A quantitative relationship between concentration (plasma) and (side)effect is anticipated. The therapeutic concentration range is narrow. There are consequences associated with under- or overexposure (poorer outcome, toxicity) = *for neonates, there is still quite some 'extrapolation' from adult data on target (efficacy, safety) range (2., 3. and 5.).*

The pharmacokinetics and its covariates are sufficiently well known to support a decision = *a relevant amount of population models have been reported and quantify the impact of covariates (age, weight, co-medication) on variability (2.1.). Bayesian models are emerging to support decisions (4.)*

The analytical technique is sufficiently specific, precise and accurate in the population of interest, and cost-effective = *There are still issues on the vancomycin assay, and one of underexplored causes of unexplained variability (6.).*

Table 1: overview studies on continuous vancomycin administration in neonates (chronological)[21,38-47]

reference	characteristics	patients	loading dose	maintenance dose	main findings
Gwee 2019 [39]	<p>prospective RCT in young infants (<90 days). continuous (n=53) or intermittent (n=51).</p> <p>target, early steady state continuous: 15-25 mg/l intermittent: 10-20 mg/l vancomycin TDM at 18-30 h (continuous) or before 3rd dose (intermittent).</p> <p>assay not mentioned, neither for creatinine.</p>	<p>34 wks gestational age, 23 days postnatal age, median weight 2271 g at inclusion. 23/92 (25%) positive blood cultures (CoNS n=13; two different CoNS n=3; <i>Enterococcus</i> spp n=2; <i>S. aureus</i> n=1; <i>Streptococcus mitis</i> n=1; gram-negative n=2).</p>	yes, in the continuous dosing group (15 mg/kg) over 1 hour.	<p>20 mg/kg/day (<i>crea</i>>60 μmol/l, any age); 30 mg/kg/day (<i>crea</i> 40-60, any age); 40 mg/kg/day (<i>crea</i><40, corrected age <40 wks); 50 mg/kg/day (<i>crea</i><40, corrected age $\geq$40 wks).</p> <p><u>intermittent:</u> 15 mg/kg/dose, q6-8-12, or 24h (corrected age either <29,29-35, 36-44,>44 wks).</p>	<p>achievement of the target in early steady state was more common (85 vs 45%) the continuous (15-25 mg/l) vs intermittent (trough 10-20 mg/l).</p> <p>fewer dose adaptations in the continuous group.</p> <p>no differences in toxicity.</p>
Tauzin 2019 [45]	<p>retrospective audit on continuous vancomycin in preterm neonates. target: 20-30 mg/l assay: turbimetric assay creatinine assay not mentioned.</p>	<p>75 neonates, 99 courses. Median (IQR) 27 (26-30.5) gestational weeks, birth weight 815 (685-1240) g.</p>	yes, 15 mg/kg, in all cases	30 mg/kg/day, in all cases.	<p>a simplified dosing regimen did not achieve therapeutic ranges (30.8 % on target with first sample) sufficiently well in preterm neonates.</p> <p>an adapted maintenance dosing regimen was suggested.</p>
Whitticase 2016 (abstract) [43]	<p>retrospective audit on continuous vancomycin in (pre)term neonates. target: 15-25 mg/l. assay: not mentioned, neither for creatinine.</p>	<p>49 (pre)term neonates, 115 vancomycin data.</p>	yes, 15 mg/kg, in all cases/	<p>maintenance 20-50 mg/kg/day, according to corrected age and creatinine.</p>	<p>target reached in 62%, better compared to historical data on intermittent (28%). Off-target not related to age (gestational, postnatal) or creatinine.</p>
Demirel	retrospective audit study	blood culture	yes, in the	<u>intermittent:</u> according	target assessment at 48 h,

	assay: not mentioned, neither for creatinine.			during audit, 60 mg/kg/day omitted).	target, 5% >25 mg/l. creatinine decreased during exposure.
Oudin 2011 [46]	prospective cohort study in 47 patients, 83 treatments, 108 vancomycin observations. target: 10-30 mg/l assay: RxL immuno-assay, creatinine enzymatic.	blood culture positive in 42/83 (50%); 39 CoNS infections (oxacillin resistant) gestational age 29.5 (23-41 wks); postnatal age 21.6 (4-169) days; weight 1.5 (0.58-4.6) kg.	7 mg/kg, over 2 hours <u>modified regimen (based on popPK analysis of the dataset):</u> 20 mg/kg over 2 hours, (suggestion, not validated).	30 mg/kg/day, in all cases (crea >120 µmol/l contra-indication) <u>modified regimen:</u> same dosing (30 mg/kg/day), for the first day, followed by TDM.	target assessment at 24-48 h, in the 'actual' dosing group (108 vanco measurements), target was attained in 89%, 5.8% was <10 mg/l, 5% was >30 mg/l. prescription errors occurs in 12% (10 of 83 treatments), creatinine remained stable (-7 to -10 µmol/l) throughout treatment.
Plan 2008 [41]	prospective cohort study in 145 preterms (<34 wks), using two consecutive dosing regimens (target seeking). target: 10-25 mg/l. assay: not mentioned, neither for creatinine.	blood culture positive in 82/145 (56%); 80 CoNS infections. cohort 1 vs 2: 11 vs 10 days postnatal age, 28 vs 27.5 wks: postconception age, weight at inclusion: 0.94 vs 0.87 kg.	no loading dose	15-25 mg/kg/day in the first cohort, 20-30 mg/kg/day in the second cohort. For both cohorts, dose selection was based on creatinine (90 µmol/l).	target assessment at 48 h [adaptations, stepwise: +/- 5 mg/kg/day when below 10 or above 25 mg/l respectively]. cohort 1 vs 2: median vancomycin 13 (10-17) vs 19 (15-23) mg/l. target >25 mg/l: 1.4 vs 19%; target <10 mg/l: 24 vs 5%; no difference in efficacy between both regimens.
Pawlotsky 1998 [47]	prospective evaluation of 2 different constant rate vancomycin infusions in preterms (2nd cohort to improve target attainment) target steady state: 12 mg/l (3 x MIC), target range 10-30 mg/l. assay not mentioned.	stepwise approach, dose seeking, 13/53 (24%) proven infection. cohort 1 vs 2: 29.2 vs 30.5 wks gestational age, 4.3 vs 3.4 wks postnatal age, weight at inclusion: 1.5 vs 1.8 kg.	yes, but only in the second cohort: 7 mg/kg, over 1 hour.	cohort 1: 10-30 mg/kg/day, 26->45 wks corrected age cohort 2: 10-40 mg/kg/day (dose increase for each 2 wks age, 11 classes).	mean vancomycin TDM at steady state (24-48 h) was 11 (SD 3.1) and 15.4 (SD 6.2) mg/l in cohort 1 and 2. target range (10-30 mg/l): 56 to 88% target <10 mg/l: 42 to 10%.

Borderon 1994 [40]	description of a cohort, dose seeking. target: 20-25 mg/l. assay: fluorescence polarization, TDx, creatinine not mentioned.	15/25 (pre)term neonates (27-35 wks, 7-30 days).	no	10-45 mg/kg/day, as starting dose.	25-40 mg/kg/day, crea<70 µmol/l, 10-30 mg/kg/day, crea >90 µmol/l.
-----------------------------------	--	--	----	---------------------------------------	---

Table 2: an overview on vancomycin MIC (<1, 1 to 2, or >2) values of *coagulase negative staphylococcus* isolates in blood cultures of neonates as reported in different recently reported cohorts [15,34,39,46,60].

country	time interval	number	< 1	1 to ≤2	> 2
France	2006-2008	39	8	23	11
Netherlands	2009-2012	19	2	15	2
Estonia	2010-2012	187	54	105	28
Australia	2014-2017	13	0	7	6
Belgium	2016	60	11	49	0
		318	75	199	47
			23%	61%	16%

Figure 1

Figure 2

Figure 3

Figure 4