

Bedside model-informed precision dosing of vancomycin in severely ill neonates and children in Belgium (the BENEFICIAL trial): a multicentre, randomised controlled trial

Pieter A De Cock, Roos Colman, Anne Smits, Fiammetta Piersigilli, Marleen Renard, An Van Damme, Alexander Clarysse, Evelyn Dhont, Sophie Vanhaesebrouck, Victoria Bordon, Lidvine Boland, Bram De Wilde, Anca Amza, Lieselot Vanlanduyt, Thi Van Anh Nguyen, Filip Cools, Jutte van der Werff Ten Bosch, Daphne Vens, Frank Derriks, Laurent Houtekie, Reiner Mauel, Dimitri Van der Linden, Petra Schelstraete, for the BENEFICIAL study group*

Lancet Child Adolesc Health 2026

Published Online April 10, 2026 [https://doi.org/10.1016/S2352-4642\(25\)00385-2](https://doi.org/10.1016/S2352-4642(25)00385-2)

See Online/Comment [https://doi.org/10.1016/S2352-4642\(26\)00018-0](https://doi.org/10.1016/S2352-4642(26)00018-0)

Lancet Child and Adolescent Health homepage: <https://www.thelancet.com/journals/lanchi/home>

*Members listed at the end of the Article

Faculty of Medicine and Health Sciences, Department of Basic and Applied Medical Sciences, Ghent Precision Dosing Unit, Ghent University and Department of Pharmacy, Ghent University Hospital, Ghent, Belgium (Prof P A De Cock PhD)

Faculty of Medicine and Health Sciences, Biostatistics Unit, Ghent University, Ghent, Belgium (R Colman Msc)

Neonatal Intensive Care Unit, University Hospitals Leuven, Leuven, Belgium (Prof A Smits MD)

Department of Development and Regeneration, KU Leuven, Leuven, Belgium (Prof A Smits)

Department of Neonatology, Cliniques Universitaires Saint-Luc, Brussels, Belgium (F Piersigilli MD)

Department of Paediatric Haemato-Oncology, University Hospitals Leuven, Leuven, Belgium
(Prof M Renard MD)

Department of Pediatric Haematology and Oncology, Cliniques Universitaires Saint-Luc, Brussels, Belgium
(Prof A Van Damme MD)

Department of Neonatal Intensive Care, AZ, Sint-Jan Bruges, Bruges, Belgium (A Clarysse MD)

Pediatric Intensive Care Unit, Department of Intensive Care Medicine, Ghent University Hospital & Faculty of Medicine and Health Sciences, Department of Basic and Applied Medical Sciences, Ghent Precision Dosing Unit, Ghent University Ghent, Belgium (E Dhont MD)

Department of Neonatal Intensive Care, Ghent University Hospital, Ghent, Belgium (Prof S Vanhaesebrouck PhD)

Department of Pediatric Hematology, Oncology and Stem Cell Transplantation, Ghent University Hospital, Ghent, Belgium (V Bordon MD, Prof B De Wilde MD)

Department of Laboratory Medicine, Cliniques Universitaires Saint-Luc, Brussels, Belgium (L Boland PharmD)

Department of Emergency Medicine, Ghent University Hospital, Ghent, Belgium (A Amza RN); Health, Innovation and Research Institute, Ghent University Hospital, Ghent, Belgium (L Vanlanduyt BSc)

Department of Basic and Applied Medical Sciences, Ghent Precision Dosing Unit, Ghent University and Department of Pharmacy, Ghent University Hospital, Ghent, Belgium (T V A Nguyen MSc)

Department of Neonatology, Brussels University Hospital, Brussels, Belgium (Prof F Cools MD, F Derriks MD)

Department of Paediatric Haemato-Oncology, Brussel University Hospital, Brussels, Belgium (Prof J van der Werff Ten Bosch MD)

University Children's Hospital Reine Fabiola, Department of Pediatric Intensive Care, Brussels, Belgium (D Vens MD)

Department of Neonatology, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium (F Derriks)

Department of Pediatric Intensive Care Unit, Cliniques universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium (L Houtekie MD)

Department of Pediatric Intensive Care, Brussels University Hospital, Brussels, Belgium (R Mauel MD)

Paediatric Infectious Diseases Unit, Specialized Paediatrics Service, Department of Paediatrics, Cliniques universitaires Saint-Luc, Brussels, Belgium (Prof. Dimitri Van Der Linden MD)

Department of Pediatric Infectious Diseases, Ghent University Hospital, Ghent, Belgium (Prof. Petra Schelstraete MD)

Correspondence to: Prof. P.A. De Cock, Faculty of Medicine and Health Sciences, Department of Basic and Applied Medical Sciences, Ghent Precision Dosing Unit, Ghent University and Department of Pharmacy, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium. Tel: +32-9-332-29-69; E-mail: Pieter.decock@uzgent.be

Summary

Background

Vancomycin is widely used to treat serious Gram-positive infections but is difficult to dose given its narrow therapeutic index and risk of acute kidney injury at high doses. We aimed to study whether model-informed precision dosing (MIPD) of vancomycin, compared with standard-of-care therapeutic drug monitoring (TDM), increases pharmacokinetic and pharmacodynamic target attainment, is safe, and reduces vancomycin-associated acute kidney injury in children with severe illness.

Methods

The BENEFICIAL trial is a pragmatic, individually randomised, controlled superiority trial done in 14 paediatric or neonatal intensive care and haemato-oncology units in seven hospitals in Belgium. Critically ill patients younger than 18 years initiating intravenous vancomycin for suspected or confirmed Gram-positive infection were eligible. Key exclusion criteria were extracorporeal support, severe acute kidney injury, chronic kidney disease, and imminent death. The intervention combined the use of an MIPD dosing calculator for starting and follow-up doses, with extra sampling for TDM in the first hours of treatment compared with standard-of-care TDM. Patients were randomly assigned (1:1) to MIPD or standard-of-care TDM of vancomycin using stratified permuted blocks by ward type. Allocation occurred via a secure web interface; patients, families, and the biostatistician were masked, but treating physicians and pharmacist staff were not. The intervention used Bayesian software with early sampling to estimate AUC. The primary outcome was the proportion of patients with a 24-h AUC-to-MIC ratio of 400–600 mg·h/L, assuming a minimum inhibitory concentration of 1 mg/L, 24–48 h after treatment initiation. A key secondary outcome was the proportion of patients with new or worsening acute kidney injury or death. These outcomes were assessed in the intention-to-treat (ITT) population (all randomly assigned patients who provided informed consent). Safety was evaluated in all patients who received at least one dose of vancomycin. This trial is registered with ClinicalTrials.gov (NCT04666948) and the EU Clinical Trials register (EudraCT 2019–004538–40), and is closed to recruitment.

Findings

Between Dec 28, 2020, and Dec 14, 2023, 332 patients aged between 1 day and 18 years were randomly assigned, 165 to the standard-of-care group and 167 to the intervention group. 18 participants were excluded from the analysis when their deferred consent was not followed by informed consent; therefore 314 patients (179 male and 135 female) were included in the analysis. Target AUC-to-MIC ratio attainment at 24–48 h was found in 82 (53.9%) of 152 patients with available data in the standard-of-care group and 112 (71.8%) of 156 in the MIPD group (absolute difference 18.9% [1.7 to 34.7]). The proportion of patients with acute kidney injury or all-cause mortality was numerically but not significantly lower in the intervention group (26 [16.9%] of 154 vs 19 [12.4%] of 153; absolute difference –4.5% [95% CI –11.6 to 3.5]). Serious adverse events occurred in eight (5%) of 158 patients in the standard-of-care group and eight (5%) of 156 patients in the intervention group. One patient in the intervention group died due to a serious adverse event at least possibly related to the vancomycin administration method.

Interpretation

AUC-based MIPD improves AUC-to-MIC ratio-based target attainment and has a low risk of harm. Given that elevated vancomycin AUC is a well established driver of acute kidney injury, the lower cumulative exposure observed in this trial support the use of MIPD in severely ill children, particularly in those who require long treatment durations or present with additional acute kidney injury risk factors.

Funding Belgian Federal Knowledge Centre Trials Programme.

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Research in context

Evidence before this study

Vancomycin has a narrow therapeutic index, with efficacy requiring a ratio of area under the concentration–time curve (AUC) to minimum inhibitory concentration (MIC) of 400 or higher; however, AUC values of 600 mg·h/L or higher increase the risk of acute kidney injury. Acute kidney injury risk is also increased by higher or cumulative doses, intensive care admission, long-term treatment, and concomitant nephrotoxins, and is associated with substantial morbidity, mortality, and health-care burden. Numerous, mainly retrospective, paediatric studies show suboptimal AUC target attainment with standard-of-care initial dosing, with several days often needed to reach therapeutic targets through standard therapeutic drug monitoring (TDM). In 2020, international guidelines therefore endorsed AUC-based model-informed precision dosing (MIPD) in children; however, uptake is limited by a paucity of high-quality evidence, particularly prospective multicentre RCTs. We searched PubMed from database inception to May 15, 2025, using the terms “vancomycin”, “AUC”, “Area-under-the-Curve”, “Area-under-the-Concentration-Time Curve”, “model-informed precision dosing”, “Bayesian”, “children”, “paediatrics”, and “randomised controlled trial”, with English as a language restriction. We found one open-label, single-centre RCT in children older than 3 months. This RCT showed improved AUC-to-MIC ratio-based target attainment with AUC-based vancomycin dosing using MIPD and reported no safety concerns. However, the generalisability of this study is limited by a small sample size of 99 patients, a narrowly defined population with a median age of 4.5 years (range 0.2–17.4), the exclusive use of continuous infusion, the use of standard-of-care starting dosing regimens, and the use of MIPD only after 6 h of treatment.

Added value of this study

The BENEFICIAL trial is the first multicentre RCT to evaluate whether early AUC-based MIPD of vancomycin improves AUC-to-MIC ratio-based target attainment, clinical efficacy, and safety in neonates and children with severe illness across a heterogeneous, real-world cohort. The intervention significantly improved AUC-to-MIC ratio-based target attainment between 24 h and 48 h after start of treatment and at subsequent timepoints compared with standard-of-care dosing. Although the absolute reduction in proportion of patients with vancomycin-associated acute kidney injury and time to clinical cure in the intervention group did not reach statistical significance, cumulative doses and AUCs were significantly lower with MIPD. The

intervention had a low risk of harm and was feasible to implement across multiple hospital settings, with active involvement from dedicated multidisciplinary teams. Importantly, no increased patient burden with regard to number of required blood samples was observed.

Implications of all the available evidence

These findings provide evidence supporting the use of AUC-based MIPD in severely ill children with longer treatment duration and additional risk factors for acute kidney injury. The observed improvements in AUC-to-MIC ratio-based target attainment and reduced vancomycin exposure support MIPD as a strategy with an acceptable safety profile for personalised vancomycin dosing in this vulnerable population. This study fills an important evidence gap and should inform future clinical guidelines and antimicrobial stewardship initiatives that aim to optimise vancomycin therapy in children.

Introduction

Vancomycin is widely used to treat neonates and children with serious Gram-positive infections, particularly hospital-acquired central-line-associated bloodstream infections and sepsis, which are associated with substantial morbidity and mortality of up to 12% and prolonged hospitalisation.^{1,2} The efficacy of vancomycin is linked to a 24-h area under the concentration–time curve (AUC) to minimum inhibitory concentration (MIC) ratio of 400 or higher in patients with methicillin-resistant *Staphylococcus aureus* infections;³ however, vancomycin has a narrow therapeutic index. Acute kidney injury occurs in up to 26% of critically ill children receiving vancomycin therapy,^{4,5} and a 24-h AUC of 600 mg·h/L or higher is independently associated with vancomycin-induced acute kidney injury.^{3,6} Additional risk factors include high or cumulative dosing, prolonged vancomycin courses, mechanical ventilation, vasoactive support, intensive care stay, and concomitant nephrotoxins.^{4,7,8} Even mild acute kidney injury contributes to increased morbidity, mortality, length of hospital stay, and healthcare costs.^{9–11}

Vancomycin treatment is typically initiated with weight-based intermittent or continuous dosing, followed by therapeutic drug monitoring (TDM) 18–24 h after the start of treatment, to guide dose adjustment using a target concentration range as a surrogate for AUC; however, this approach has several limitations. Target concentration attainment rates are low in critically ill children, and reaching therapeutic concentrations often takes several days.¹² Delays in appropriate therapy in patients with sepsis are known to increase mortality and morbidity.¹³ Subtherapeutic exposure might promote antimicrobial resistance.¹⁴ Furthermore, the use of a target concentration range as a surrogate for AUC is controversial, as single-point measurements have high variability and poor correlation with actual AUC.³ Repeated blood sampling for TDM can result in iatrogenic anaemia, increased infection risk, and additional patient burden and healthcare costs.^{15,16} Last, incorrect timing of standard-of-care TDM sampling can lead to inaccurate dosing decisions.¹²

Model-informed precision dosing (MIPD) uses a validated population pharmacokinetic model and Bayesian forecasting to individualise dosing by combining pharmacokinetic knowledge with each patient's real-time clinical data and eventually measured concentrations to provide an estimate of the full 24-h AUC. In 2020, international consensus guidelines recommended replacing traditional TDM-guided dosing with AUC-based MIPD for vancomycin in adults and children.³ Proposed benefits in adults include faster pharmacokinetic and pharmacodynamic target attainment, reduced acute kidney injury rates, lower exposure to vancomycin, fewer blood draws, and shorter treatment duration than TDM, without compromising efficacy.¹⁷ MIPD is also considered more patient-friendly and caregiver-friendly, as it allows AUC estimation from a single, conveniently timed or leftover sample.³ Despite these proposed advantages, uptake has been slow in paediatric clinical care, mainly due to the absence of randomised controlled trials (RCTs) that support its use and the resource demands of implementation.^{18,19} Robust evidence is needed to guide practice.

Here, we report results from the BENEFICIAL trial, a pragmatic, multicentre, individual RCT comparing vancomycin AUC-based MIPD versus standard-of-care dosing and TDM on pharmacokinetic and pharmacodynamic target attainment, efficacy, and safety in critically ill children.

Methods

Study design

We conducted a pragmatic, individually randomised, controlled superiority trial in 14 neonatal intensive care units (NICUs), paediatric intensive care units (PICUs), and paediatric haemato-oncology (PHO) wards from seven hospitals in Belgium. Sites were selected on the basis of their interest in the trial, feasibility in terms of vancomycin use, absence of interference with other trials in terms of patient recruitment, and trial team capacity. The study protocol was published and approved by the central ethics committees of Ghent University Hospital (BC-05429) for all participating sites and the respective competent authorities. Patients were involved in the trial design and reporting of the trial. The full research protocol has been published previously.²⁰ The statistical analysis is presented in the appendix (pp 20–64). The study was registered with EudraCT (2019–004538–40) and ClinicalTrials.gov (NCT04666948) and is closed to recruitment.

Patients

Eligible patients were neonates and children younger than 18 years who were admitted to a NICU, PICU, or PHO ward with a suspected or confirmed Gram-positive infection and were planned to start on intravenous intermittent or continuous infusion vancomycin treatment. Eligible patients were considered critically ill. Patients were excluded if they had extracorporeal treatment at inclusion, a neonatal or paediatric acute kidney injury categorised as failure at inclusion (according to the modified Risk Injury Failure Loss End-Stage [RIFLE] assessment criteria; appendix p 7), or a known chronic kidney disease as defined by the Kidney Diseases Improving Global Outcomes (KDIGO) criteria (appendix p 8). Patients were also excluded if death was imminent and inevitable. A patient restarted on vancomycin within the 30-day study period was not newly enrolled; they had the same treatment that was assigned for the first treatment episode. Written informed consent for all trial participants was obtained by discussing with the parents or legal representatives the nature, objectives, and the possible adverse events associated with their participation in the study. Maximum effort was made to obtain written informed consent before inclusion (ie, a priori consent), or whenever not possible due to a matter of clinical urgency, within 24 h after the start of therapy (ie, deferred consent). A substantial amendment to the research protocol was made when it became apparent that the initial sample size could not be met within the project timelines. An analysis indicated an overestimation of contamination rates in the initial sample size calculation; on the basis of this information, the contamination rates were lowered. Details on sample size recalculation are described in the published protocol.²⁰ For participants 12 years or older, a separate written assent form was obtained, after informing them using a separate information sheet. Sex data were collected from medical records. Race and ethnicity data were not collected.

Randomisation and masking

Patients were randomly assigned to the standard-of-care (TDM) group or intervention (MIPD) group at inclusion, with an allocation ratio of 1:1. Randomisation was stratified by ward unit and hence by ward unit type (NICU, PICU, or PHO). Random permuted blocks were created with a computer random number generator with variable block sizes (2, 4, 6, and 8). The allocation sequence was generated in SAS version 9.4 by the statistician and imported into a web-based encrypted interface. At each participating site, the ward unit investigator, clinical research coordinator, or a delegated person did the randomisation. Allocation concealment was applied. Patients and their legal representatives were masked to group assignment until the end of the study. Treating physicians and pharmacist staff (ie, the MIPD software end-users) were not masked, due to the nature of the study. Patients and their representatives were informed that the assigned treatment group would not be disclosed until completion of the study. Treating physicians and the nursing team were instructed not to communicate the purpose of the sampling procedures or the

allocated treatment group to the patients or their representatives. The biostatistician was masked for data analysis. Success of masking was not assessed.

Procedures

In the standard-of-care group, vancomycin starting doses were prescribed according to institutional guidelines and adjusted, either empirically or by using dose-adjustment nomograms, on the basis of TDM. Steady-state TDM samples were typically taken before the third dose for intermittent dosing regimens and around 24 h after the start of the loading dose for continuous dosing regimens (timing was dependent on the institutional or local ward unit protocol). Repeat sampling after dose adjustment was often done at similar timepoints. Whenever therapeutic concentrations were reached, repeat samples were typically taken every 3–7 days, or sooner in the case of changing renal function, therapy failure, or major surgery (depending on local ward unit protocol).

The intervention combined the use of a cloud-based MIPD dosing calculator (InsightRX Nova web application versions 1.26 and higher; InsightRX, San Francisco, CA, USA) for starting and follow-up vancomycin dose calculation, in combination with extra sampling for TDM in the first hours of treatment as compared with standard of care. A target 24-h AUC-to-MIC ratio was set to between 400 and 600, assuming an MIC of 1 mg/L. Details on institutional standard-of-care initial dosing and dose adjustment guidelines, dose calculation workflow, sampling regimens, and dose limits are described in the appendix (pp 3–4).

Vancomycin dosing and sampling details were recorded in both groups by the attending nurse on electronic administration and sampling registration sheets. Weight was measured at least once weekly for patients younger than 1 month and at least once every 2 weeks for patients 1 month and older. Serum creatinine measurements were collected on a regular basis as per standard of care to allow acute kidney injury category assessment using serum creatinine and estimated glomerular filtration rate during vancomycin treatment. Urine output, if measured, was also used to allow acute kidney injury category assessment. When serum creatinine or urine output criteria resulted in different stages of acute kidney injury, the highest stage was chosen. Concomitantly administered nephrotoxic drugs, vasopressors, inotropes, and antibiotics, date of hospital and ward unit discharge and reason, isolated pathogens, site of infection, and reported sensitivity of the pathogen were recorded. The list of possible protocol deviations was predefined.

Outcomes

The primary endpoint was the proportion of patients with the target 24-h AUC-to-MIC ratio of 400–600 between 24 h and 48 h after treatment initiation. 24-h to 48-h AUC was estimated for patients in both study groups using Colin and colleagues' pharmacokinetic model in NONMEM (version 7.5.1, GloboMax, Hanover, MD, USA), using all available concentration measurements per patient over the 30-day study period.²¹ The NONMEM script is available in the appendix (pp 5–6). A flattened prior (30%) in the NONMEM \$PRIOR subroutine was used, giving most weight (70%) to the individual concentration measurements for individual AUC estimation.²² A weighting approach was used giving more importance to more recent observations, as described in the appendix (pp 5–6). The NONMEM scripts were run on the whole dataset by TVAN and PADC. A blinded dataset with individual AUC estimations was transferred to the biostatistician.

The key secondary outcome to assess safety was the proportion of patients with new or worsening acute kidney injury (ie, an increase in acute kidney injury category) during vancomycin treatment per modified RIFLE criteria (appendix p 7) or 30-day all-cause mortality. The other secondary outcomes were the proportion of patients with the target 24-h AUC between 48 h and 72 h after start of treatment, time to clinical cure, ward unit length of stay, and hospital length of stay. Tertiary outcomes that were collected were the number of blood samples and additional blood samples (ie, a sample for vancomycin TDM taken at a timepoint other than for routine biochemical monitoring) taken during treatment, the cumulative number of blood samples taken in a subgroup of patients with clinical cure, 24-h AUC between 72 h and 96 h after the start of treatment, number of dose adjustments, cumulative vancomycin dose during treatment, cumulative AUC during treatment, the number of sampling time errors in case of intermittent dosing, and the proportion of patients with new or worsening acute kidney injury during vancomycin treatment using

modified KDIGO criteria (appendix p 8). Time to clinical cure was defined as the time interval in days from start to completion of the vancomycin treatment, without recommencement of antibiotics for the same indication within 48 h after stopping. De-escalation or escalation of vancomycin for the same indication was considered as part of the antibiotic treatment course. A sampling time error (measured in the standard-of-care group only) was defined as a sample taken outside the 30-min interval before the administration of a new dose. An additional blood sample was defined as a sample for vancomycin concentration measurement taken at another timepoint of routine biochemical monitoring sampling.

Reportable adverse events were safety issues (including death) at the discretion of the investigator thought to be at least possibly related to the vancomycin dosing method, categorisation as neonatal or paediatric RIFLE class failure, and vancomycin flushing syndrome. Safety assessments were collected on a continuous basis until 48 h after the last study-related vancomycin administration within the 30-day study period. Adverse events were assessed in all patients who received at least one dose of vancomycin.

Statistical analysis

The sample size calculation was based on the assumption that the proportion of patients reaching the therapeutic target 24-h AUC-to-MIC ratio between 24 h and 48 h after start of treatment would be 50% in the standard-of-care group and 70% in the AUC-to-MIC ratio-based study group. Because patients were individually randomly assigned, patients of both study groups were treated in the same ward unit. Potentially, a learning effect could cause contamination. When assuming 15% contamination from intervention to control, and 3.75% contamination from standard-of-care to intervention, a dilution of the odds ratio (OR) was expected from 2.33 (70% vs 50%) to 2.00 (69.25% vs 53.00%). In total, 281 participants were required for 80% power at a two-sided significance level of 5% to detect an OR of 2.00 ($b=0.69$) when the proportion in the standard-of-care group is 53.00% (odds=1.13, intercept=0.12) using logistic regression. Assuming a drop-out rate of 15%, a total sample size of 332 patients was required.

The primary analysis for the binary outcome of reaching the target 24-h AUC-to-MIC ratio between 24 h and 48 h after starting vancomycin treatment was performed on the intention-to-treat (ITT) analysis set, which consisted of all randomly assigned patients, except when randomisation of a patient was based on a deferred consent that was not followed by informed consent for use of already collected data. Observed values in patients with available data are presented as well as the estimated effect sizes based on the ITT analysis set. To account for missing values, a multiple imputation model was applied separately for each randomisation group; further details are in the appendix (p 11). The intervention effect was presented as an OR conditional on type of ward (main estimand) and an absolute risk difference (additional estimands). The estimated proportions, the relative risk, and the absolute risk difference were calculated with the plug-in estimator, as described by Freedman, by averaging the predicted probabilities based on the logistic regression model.²³ The Boot MI method (with 2000 bootstraps and 100 imputations) as described by Schomaker and Heumann was used to compute the 95% bias-corrected and accelerated bootstrap confidence interval.²⁴

The secondary binary outcomes of new or worsening acute kidney injury and 30-day all-cause mortality were examined using a logistic regression model, similar to that used for the primary analysis. For this endpoint, the composite strategy with death as an unfavorable outcome was used, in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guideline addendum on estimands in clinical trials (appendix p 10).²⁵ For time to clinical cure, a Cox proportional hazards model stratified for type of ward unit was used. The proportional hazards assumption for a Cox regression was tested. Ward unit and hospital length-of-stay were evaluated using a plug-in estimator with negative binomial regression. The secondary and tertiary outcomes related to AUC (24-h AUC at 48–72 h and at 72–96 h) were estimated using a similar method as described for the primary outcome, as was the proportion of patients with new or worsening acute kidney injury during vancomycin treatment using modified KDIGO criteria. Cumulative AUC was estimated from the start of vancomycin treatment to 48 h after stopping treatment. For the number of additional blood samples taken, the number of dose adjustments during vancomycin treatment, and the number of sampling time errors, a negative binomial regression was applied, incorporating randomisation group (intervention group vs standard-of-

care group) and ward type (NICU, PICU, or PHO) as categorical predictor variables. The standardised mean outcome per randomisation group was estimated using the plug-in method, with missing data included in the population for which the standardised estimate was derived. Bootstrapping was used to generate 95% CIs for the mean per randomisation group, as well as for the absolute risk difference and relative risk. Similarly, the cumulative vancomycin dose and cumulative AUC during vancomycin treatment were analysed with linear regression.

A detailed description of the sensitivity analysis of the primary endpoint, protocol-compliant estimands, subgroup analyses, and handling of intercurrent events and missing values is in the appendix (pp 8–11). Statistical analysis was performed using R and R studio version 4.3.1. As this trial was categorised as a low-risk interventional trial, no data safety monitoring board was deemed necessary. A chief medical advisor held clinical oversight of the safety of participants in the trial.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Dec 28, 2020, and Dec 14, 2023, 332 patients were randomly assigned, with 165 patients in the standard-of-care group and 167 in the intervention group. Enrolment by site is shown in the appendix (p 13). For 203 patients, a priori informed consent was collected; for 129 patients, a deferred consent approach was used. 18 patients (nine in each group) were excluded from the analysis when deferred consent was not followed by informed consent. The ITT population consisted of 314 patients, with 156 in the standard-of-care group and 158 patients in the intervention group (figure 1). 30 patients in each group had an intercurrent event for the primary outcome (appendix p 11). For the composite secondary outcome of new or worsening acute kidney injury or all-cause mortality, five patients in the standard-of-care group and four patients in the intervention group had an intercurrent event (appendix p 11). The safety population consisted of the same patients as in the ITT population, as all participants in the ITT population had at least one dose of vancomycin. Protocol violations are summarised in the appendix (pp 18–19).

At baseline, of the 314 patients in the ITT population, 185 (59%) were admitted to the NICU, 55 (18%) were admitted to the PICU, and 74 (24%) were admitted to the PHO unit (table 1). Patients were aged between 1 day and 18 years. The most common indication for vancomycin treatment was bloodstream infection, found in 113 patients (36%). 158 (50%) patients had a documented infection at baseline. Gram-positive organisms were identified at baseline in 124 patients (40%) and are listed in the appendix (p 14). Vancomycin resistance as defined by the European Committee on Antimicrobial Susceptibility Testing Breakpoints²⁶ was reported in one patient with *Bacillus cereus*. More details on the start of vancomycin treatment, starting dose, TDM, and reasons for cessation of vancomycin are in the appendix (pp 12–13).

82 (53.9%) of 152 patients in the ITT population with available data in the standard-of-care group and 112 patients (71.8%) of 156 patients with available data in the intervention group had the target 24-h AUC 24–48 h after the start of treatment (absolute difference in proportion 18.9% [95% CI 1.7–34.7]; table 2; figure 2). The protocol-compliant estimand of the primary outcome was also significantly in favour of the intervention group, with 79 (54.5%) of 145 patients with available data in the standard-of-care group reaching the primary outcome target versus 100 (73.0%) of 137 patients with available data in the intervention group (absolute difference in proportion 18.3% [95% CI 0.3–33.5]), as was the case for the two sensitivity analyses (appendix p 15). Predefined subgroup analysis showed that the proportions of patients from NICU wards and of patients on intermittent infusion who had the target AUC-to-MIC ratio 24–48 h after the start of treatment were significantly higher in the intervention group compared with the standard-of-care group (table 2).

The proportion of patients with acute kidney injury or all-cause mortality according to modified RIFLE criteria was numerically, but not significantly, lower with 19 (12.4%) of 153 patients with available data in the intervention group versus 26 (16.9%) of 154 patients with available data in the standard-of-care group

(absolute difference in proportion -4.5% [95% CI -11.6 to 3.5]; table 2). 30-day all-cause mortality was low overall (three patients [1.9%] in the intervention group vs six patients [3.8%] in the standard-of-care group) and was not statistically different between both groups (absolute difference in proportion -1.9% [95% CI -5.8 to 1.6]; table 2). Median time to clinical cure was 9 days (8–10) in the intervention group versus 9 days (8–11) in the standard-of-care group (HR 1.26 [95% CI 0.99 to 1.60]; table 2; figure 3). The assumption of proportional hazards was fulfilled (Cox proportional hazards test $p=0.89$). Ward unit and hospital length of stay were not statistically different between the two groups (table 2). In line with a significantly lower dose, mean cumulative AUC was significantly lower in the intervention group (3429.4 mg·h/L [SD 2544.3]) than in the standard-of-care group (4099.1 mg·h/L [SD 3509.9]; absolute difference in mean -710.6 [95% CI -1496.9 to -109.5]; table 2). Other tertiary outcomes are outlined in table 2 and the appendix (pp 16–17).

Results of sex-based analyses are presented in the appendix (p 15). The proportion of male children who had the target AUC-to-MIC ratio 24–48 h after the start of treatment was significantly higher in the intervention group compared with the standard-of-care group; in female children this proportion was only numerically higher. Serious adverse events were reported in eight (5%) of 158 patients in the standard-of-care group and eight (5%) of 156 patients in the intervention group (appendix p 17). One patient in the intervention group died due to a serious adverse event at least possibly related to the vancomycin administration method.

Discussion

The BENEFICIAL trial showed that AUC-based MIPD was superior to standard-of-care dosing and TDM in reaching predefined AUC-based targets. Although the proportion of patients with acute kidney injury or all-cause mortality was numerically lower in the intervention group, this difference did not reach statistical significance. MIPD was deemed to have a low risk of harm in this patient population. Notably, patients in the intervention group required significantly lower cumulative vancomycin doses and had reduced cumulative AUCs.

The intervention in this trial consisted of an MIPD tool to guide starting and follow-up vancomycin dosing, combined with early pharmacokinetic sampling in the first hours of treatment. The first 48 h—often referred to as the golden window for antibiotic therapy in severe infections—are crucial for reaching pharmacokinetic and pharmacodynamic targets.¹³ In our study, MIPD significantly increased the proportion of patients with AUC-to-MIC ratio target attainment at 24–48 h, 48–72 h, and 72–96 h intervals after starting treatment, compared with standard-of-care dosing and TDM. Achieving such precision in target attainment in severely ill patients with highly dynamic pathophysiological changes that affect drug pharmacokinetics is not straightforward. Two recent trials in critically ill adults did not show improved pharmacokinetic and pharmacodynamic target attainment using MIPD for vancomycin and β -lactam antibiotics.^{27,28} One reason cited for the lack of effect in adult MIPD trials was the use of pharmacokinetic models that were not validated in the target population.²⁹ In our trial, we used a fit-for-purpose population pharmacokinetic model, including data from 559 neonates and 118 children, which captured both interpatient and inpatient variability.²¹ Another proposed explanation is the omission of MIPD-based individualisation of starting doses and early post-initiation adjustments in the adult trials—elements that were fully integrated into our intervention.

The overall proportion of exposures falling outside the therapeutic range was higher in the standard-of-care group than in the intervention group, probably due to the poor correlation between single-point concentrations and AUC in severely ill children.³⁰ In our study, patients in the NICU in particular showed a low early (24–48 h) target attainment under standard care, mainly from underdosing. Despite updated guidelines, early-phase underdosing—and to a lesser extent, overdosing—remains problematic in paediatric medicine.³¹ Waiting for steady-state delayed dose adjustments, while relying on fixed nomograms or evidence-based guesses from physicians could have additionally contributed to target attainment delays. By contrast, the MIPD software provided model-informed recommendations for both starting and follow-up doses, enabling pre-steady-state adjustments and reducing clinical uncertainty. Beyond the precision of model-based individualisation, this time advantage of earlier dose adaptation could itself be an equally relevant benefit of MIPD, particularly during the critical early treatment phase. The

benefit of MIPD persisted, shifting over time from preventing underdosing to preventing overdosing. Safety caps on initial and follow-up dose increases in our study might have underestimated the true effect of MIPD. Our findings align with a previous single-centre, open-label RCT in children using Bayesian MIPD for continuous infusion.³² Although methodologically similar, this previous study included only children older than 3 months, started with a standard-of-care dosing regimen, used MIPD only 6 h after the start of treatment, and used a broader target AUC-to-MIC ratio range (400–800) within the 0–24 h interval. By contrast, only 12% of our participants received continuous infusion, and no clear MIPD effect was observed in this subgroup. Whether this absence of MIPD effect in the subgroup who received continuous infusion reflects a true difference or limited power remains uncertain. Intermittent dosing remains standard due to practical barriers, such as drug incompatibility and poor line access, although continuous infusion use is increasing. Our findings warrant more research into the applicability and optimisation of MIPD for continuous infusion.

The observed lower proportion of patients with acute kidney injury in the intervention group versus the standard-of-care group is considered clinically meaningful given the long-term consequences of acute kidney injury, although the difference was not statistically significant. One possible explanation for the lack of statistical significance is our pragmatic approach to acute kidney injury assessment, based on routinely collected serum creatinine and urine output data. The sensitivity of RIFLE and KDIGO criteria to detect acute kidney injury in critically ill children—particularly in neonates, the largest subgroup in our study—is subject to ongoing debate, and alternative biomarkers are under investigation.³³ As this was a pragmatic study, we did not influence the frequency of serum creatinine monitoring. In neonates especially, creatinine was not measured daily, which could have limited the detection of acute kidney injury. Additionally, acute kidney injury in the standard-of-care group was lower than previously reported in similar populations (range 17–26%).^{4,5,34} We speculate that the lower incidence of acute kidney injury in the standard-of-care group reported in our trial versus others might reflect a trend towards shorter treatment durations driven by antimicrobial stewardship initiatives.^{35,36} Despite not finding a statistically significant difference in acute kidney injury or all-cause mortality, we observed significantly higher cumulative doses and corresponding AUCs in the standard-of-care group compared with the intervention group (mean differences of 94.8 mg/kg and 710.6 mg·h/L, respectively). For context, a typical starting dose for a 1-month-old infant is 60 mg/kg per day, and the recommended 24-h AUC target is 400–600 mg·h/L. A similar observation of increased dose requirements in standard care was reported in an explanatory, retrospective, single-centre study of hospitalised children.³⁷ Notably, increased dose, cumulative AUC, and 24-h AUC of higher than 600 mg·h/L have consistently been described as risk factors for development of acute kidney injury in critically ill children receiving vancomycin.^{3,4,6–8}

Time to clinical cure was not significantly different between groups. As most patients were empirically treated, we applied a uniform efficacy threshold of AUC-to-MIC ratio of higher than 400, in line with current guidelines for *S aureus*.³ This single-target approach reflects routine practice but is based on little and inconsistent evidence in children, particularly for non-meticillin-resistant *S aureus* pathogens. A large proportion of our cohort had infections with coagulase-negative staphylococci, typically associated with a milder course. Emerging data suggest lower AUC-to-MIC thresholds could suffice in infants with Gram-positive infections other than *S aureus*.³⁸ Additionally, neonates and young infants show reduced plasma protein binding, which results in higher unbound vancomycin exposure and could in turn permit lower total AUCs while maintaining efficacy.³⁰ This observation might also partly explain the high clinical cure rates observed in the standard-of-care group—especially among patients in NICUs—despite frequent subtherapeutic AUC-to-MIC ratios. Whether current AUC-to-MIC targets should be refined by age and pathogen warrants more investigation.

AUC-based MIPD was considered to have a low risk of harm in this vulnerable population of severely ill children because serious adverse events were infrequent, expected, and similar between groups. The most common event, vancomycin flushing syndrome, is linked to rapid infusion rather than drug exposure, which aligns with findings from the previously described RCT comparing AUC-based MIPD for continuous infusion to standard-of-care in children.³² A 2022 non-inferiority trial in infants with late-onset sepsis noted a potential safety signal for hearing loss with intensified dosing.³⁹ Cumulative exposure to vancomycin has

been described as a risk factor for ototoxicity in infants with very low birthweight.⁴⁰ In our pragmatic trial, formal audiological assessments were not performed, but given the lower overall vancomycin doses in the intervention group, increased ototoxicity risk is considered unlikely.

Judicious use of invasive procedures, such as blood sampling, improves quality of life, and minimising blood loss reduces the risk of iatrogenic anaemia.¹⁵ Despite the MIPD group requiring one additional early sample on average, total sampling did not differ significantly between groups. Although MIPD supports flexible timing and the use of leftover samples, these benefits were probably underused, as clinicians appeared to follow routine ward-based remonitoring schedules (eg, every 24 h). The added complexity of coordinating opportunistic sampling might have limited uptake. Sampling time errors, which were common in the standard-of-care group during intermittent dosing, can lead to uninterpretable TDM results and repeat testing, if detected; this result is consistent with previous findings in both paediatric and adult populations.^{12,17}

To our knowledge, the BENEFICIAL trial is the first multicentre RCT to evaluate the prospective use of AUC-based MIPD of vancomycin in severely ill children. Key strengths of the trial include the use of MIPD-guided dose optimisation to its full potential—targeting the optimal starting dose and subsequent doses—which proved both feasible and effective in a patient population with highly variable pharmacokinetics, treated in a complex and dynamic clinical setting. Moreover, the study's pragmatic design and the inclusion of a heterogeneous cohort enhance the generalisability of the findings to a broad paediatric population. From an operational perspective, we showed that constant use of MIPD dosing software across multiple hospitals, supported by large, interdisciplinary teams, is both feasible and has a low risk of harm. Looking ahead, routine implementation will probably require organisational support, including integration with electronic health records, alignment with existing clinical workflows, and identification of local champions to coordinate its use; such measures can facilitate adoption, reduce workload, and reduce the risk of error. In addition, any dosing software used should hold appropriate regulatory labelling, such as compliance with medical device regulations in the EU.

This study has several limitations. First, a fixed MIC value of 1 mg/L was used to calculate AUC-to-MIC ratios, as the causative pathogen was often unknown and repeated MIC determinations are not feasible in routine clinical practice. Second, vancomycin exposure was assessed in serum or plasma, which might not accurately reflect drug concentrations at the site of infection. In critically ill children with sepsis, impaired tissue penetration can result in subtherapeutic concentrations at the infection site and potential treatment failure, even when systemic exposure appears adequate. Third, only the study statistician and parents were masked; treating clinicians were aware of the group allocation, which could have influenced dose titration in the standard-of-care group during the study via the learning effect; however, we expect such risk of bias to be low as we accounted in the sample size calculation for contamination from the intervention group to the standard-of-care group and vice versa. Fourth, follow-up data on hearing were not collected, which limited our ability to assess ototoxicity, a known, albeit rare, adverse effect of vancomycin. Fifth, interpretation of the time to clinical cure outcome parameter is limited by the uncertainty regarding the need for vancomycin in patients in whom vancomycin was started empirically, as well as by the heterogeneity of patients and infectious disease syndromes. Sixth, as described previously, the timing of baseline creatinine could have affected acute kidney injury outcome assessments.⁴¹ Seventh, the use of different serum creatinine assays across sites might have introduced variability in detection and staging of acute kidney injury. Eighth, Colin and colleagues' pharmacokinetic model used in InsightRX was, at the time of its development, the first meta-analysis pharmacokinetic model covering the entire age range represented in our trial, pooling data from premature neonates to older children and adults. Although the model included the largest neonatal dataset at the time, representation of children aged between 1 year and 3.5 years was somewhat sparse, and this age corresponds to an important period of clearance maturation. This sparsity could have introduced additional uncertainty in model-based clearance and AUC estimations for this specific age band; however, in our study this uncertainty was substantially mitigated through Bayesian updating using individual concentration measurements, which were given greater weight than the population prior. Finally, the study period was limited to 30 days, which resulted in censored data on ward and hospital length of stay for a substantial proportion of patients in NICUs.

In summary, AUC-based MIPD significantly improved pharmacokinetic and pharmacodynamic target attainment during treatment, reduced cumulative vancomycin dose and AUC, and had a low risk of harm. Sampling errors were frequent in the standard-of-care group with intermittent dosing. The intervention group had a non-significantly smaller proportion of patients with acute kidney injury. Given the serious long-term consequences of acute kidney injury, we recommend routine implementation of AUC-to-MIC ratio-based MIPD for intermittent vancomycin dosing in severely ill children with additional risk factors for acute kidney injury who require long-term treatment.

BENEFICIAL Study Group

Pieter A De Cock, Roos Colman, Anne Smits, Fiammetta Piersigilli, Marleen Renard, An Van Damme, Alexander Clarysse, Evelyn Dhont, Sophie Vanhaesebrouck, Victoria Bordon, Lidvine Boland, Bram De Wilde, Anca Amza, Lieselot Vanlanduyt, Thi Van Anh Nguyen, Filip Cools, Jutte van der Werff Ten Bosch, Daphne Vens, Frank Derriks, Laurent Houtekie, Reiner Mauel, Dimitri Van der Linden, Petra Schelstraete, Peter De Paepe, Phebe Debouver, Dominique Biarent, Valerie Godart, Olivier Danhaive, Koenraad Smets, Alexandra Zecic, Linde Goossens, Kris De Coen, Annelies Keymeulen, Lara Garabedian, Julie De Meulemeester, Naessens Pauline, Tom Schepens, Emma Beel, Jef Willems, Annick de Jaeger, Ann Verrijckt, Tienne Bauters, Nele Clottens, Sarah Mertens, Fleur Camfermann, Michael Sonnaert, Julie Lefevere, Barbara De Bisschop, Floortje Krechting, Lissa De Potter, James d'Haese, Marit Sijmons, Tine Francois, Xavier Beretta-Piccoli, Shancy Rooze, Alfredo Vicinanza, Vanessa Guy Viterbo, Montserrat Sierra Colomina, Laura Slegers, Zoe Vander Elst, Anneleen Dereymaker, An Eerdeken, Liesbeth Thewissen, Maissa Rayyan, Laurien Vanbuggenhout, Marie Julie Debuf, Sarah Verbeeck, Karlijn van Damme, Anne Uyttebroeck, Veerle Labarque, Heidi Segers, Katrien Cosaert, Lotte Vander Elst, Eve Vanlaer, Isabelle Ceuterick, Olga Chatzis, Matthieu Deltombe, Bastien Tossens, Arnaud Nevraumont, Louise Guillaume, Martin Vanderdonck, Meryem Benamour, Mohammad Panahandeh, Veerle Mondelaers, Leen Willems, Leentje Peetermans, Astrid Haenecour, Maëlle de Ville de Goyet, Bénédicte Brichard, Manon Le Roux, Evelien Snauwaert, Charlotte Clauwaert, Hanife Kokur, Hans De Pla, Stefanie De Buyser.

Contributors

PADC was the chief investigator and sponsor's representative. PS was the coordinating medical principal investigator. PADC, RC, AA, and PS conceived and designed the study. RC was the lead statistician. AA managed the trial. LV was data manager. PADC, RC, AS, LB, AA, and DVdL were members of the trial steering committee. AS, FP, MR, AVD, AC, ED, SV, VB, BDW, FC, JvdWTB, DV, FD, LH, RM, and DVdL were ward unit investigators involved in participant recruitment and data collection. LB was involved in data collection. PADC, RC, and LV developed the statistical analysis plan and PADC, LV, AA, and TVAN curated the data. DVdL was chief medical advisor. PADC and RC wrote the first draft of the manuscript. PADC, RC, and TVAN have directly accessed the data and verified the underlying data reported in the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

PADC reports a non-restricted academic grant to their institution from Eumedica for an unrelated study. AVD reports honoraria paid to their institution for lecture preceptorship from Roche, the participation on an advisory board on emicizumab (Roche), travel support from Sobi, and stock options in Bluetrees via the institution. All other authors declare no competing interests.

Data sharing

Sharing of individual pseudonymised data will be considered with publication on the basis of a detailed proposal which should include aims, methods, and a statistical analysis plan. Proposals should be addressed to the corresponding author and sent via email and will be evaluated by the sponsor. Requests will be checked for compatibility with regulatory and ethics committee requirements and parents' informed consent forms.

Acknowledgments

The study was funded by the Belgian Federal Knowledge Centre KCE Trials Program grant number 18–1128. KCE Trials is a funding programme for non-commercial clinical trials financed by the Belgian public authorities. These clinical trials address issues that are usually neglected by industry despite their high societal importance. KCE is responsible for the selection and funding of the clinical trials, but does not conduct them itself. Hospitals, universities, or non-commercial research institutions take on the sponsorship and coordination responsibilities. We thank the parents and guardians of participants, all investigators from participating centres, precision dosing specialist Rob ter Heine (Radboud University Medical Center, Nijmegen, Netherlands) and patient representatives from the Vlaamse Vereniging voor Couveusekinderen for their input in designing the study.

The research activities of AS are supported by a Senior Clinical Investigatorship of the Research Foundation - Flanders (18E2H24N).

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Tables

	Standard-of-care group (n=156)	Intervention group (n=158)
Postnatal age, months	0.84 (0.4–26.8; 0.1–216)	0.74 (0.3–32.1; 0.1–208)
Sex		
Female	63 (40%)	72 (46%)
Male	93 (60%)	86 (54%)
Weight, kg	3.5 (1.3–12.3; 0.60–71.6)	3.1 (1.2–14.3; 0.4–62.0)
Height, cm	51.5 (41.0–92.0; 30.0–180.0)*	49.0 (28.5–176.0; 28.5–176.0)†
Serum creatinine concentration, mg/L	0.41 (0.30–0.53; 0.2–1.2)	0.42 (0.3–0.62; 0.2–1.5)
Ward		
NICU	93 (60%)	92 (58%)
PICU	28 (18%)	27 (17%)
PHO	35 (22%)	39 (25%)
Vasoactive support	18 (12%)	17 (11%)
Mechanical ventilation	49 (31%)	42 (27%)
Number of concomitant nephrotoxic drugs		
0	94 (60%)	107 (68%)
1	40 (26%)	28 (18%)
2	13 (8%)	17 (11%)
3	5 (3%)	4 (3%)
≥4	4 (3%)	2 (1%)
Indication for vancomycin treatment		
Bloodstream infection	51 (33%)	62 (39%)
Systemic inflammatory response syndrome with no specific focus	57 (37%)	53 (34%)
Febrile neutropenia or other form of manifestation of infection in immunocompromised patient with no clear anatomical site	12 (8%)	17 (11%)
Pulmonary infection	11 (7%)	10 (6%)
Gastrointestinal infection	9 (6%)	9 (6%)
Skin and soft tissue infection	6 (4%)	3 (2%)
Infection of the central nervous system	5 (3%)	1 (1%)
Other	5 (3%)	3 (2%)

Documented infection†	84 (54%)	74 (47%)
Documented infection with Gram-positive pathogen	63 (40%)	61 (39%)
Concomitant antibiotics	142 (91%)	146 (92%)

Data are median (IQR; range) or n (%). PICU=paediatric intensive care unit. NICU=neonatal intensive care unit. PHO=paediatric haemato-oncology ward unit. *Data missing for 48 patients. †Data missing for 56 patients. ‡Defined as an infection with a pathogen grown in blood or sterile site or an abscess or volume of infected tissue (eg, pneumonia or soft tissue).

Table 1. Baseline characteristics of the intention-to-treat population

	Standard-of-care group (n=156)	Intervention group (n=158)	Absolute difference in mean or proportion, % (95% CI)	OR, HR, or mean ratio (95% CI)	p value for OR, HR, and mean ratios
Primary outcome					
24-h AUC-to-MIC target reached 24–48 h after start of treatment	82/152 (53.9%)	112/156 (71.8%)	18.9% (1.7 to 34.7)	OR 2.29 (1.41 to 3.72)	0.0007
Protocol-compliant estimand	79/145 (54.5%)	100/137 (73.0%)	18.3% (0.3 to 33.5)	OR 2.42 (1.45 to 4.05)	0.0007
Subgroup analyses of the primary outcome					
Type of ward unit					
NICU	50/91 (54.9%)	70/90 (77.8%)	23.5% (1.8 to 43.4)	OR 2.92 (1.51 to 5.64)	0.0013*
PICU	15/28 (53.6%)	20/27 (74.1%)	21.3% (–13.6 to 53.9)	OR 2.48 (0.78 to 7.90)	0.12*
PHO	17/33 (51.5%)	22/39 (56.4%)	7.6% (–27.2 to 40.6)	OR 1.32 (0.51 to 3.39)	0.56*
Type of dosing regimen at start treatment					
Intermittent infusion	72/135 (53.3%)	101/135 (74.8%)	22.3% (4.4 to 39.0)	OR 2.73 (1.61 to 4.63)	0.0002†
Continuous infusion	10/17 (58.8%)	11/21 (52.4%)	–2.5% (–48.6 to 45.7)	OR 0.83 (0.22 to 3.15)	0.78†
Patients with documented infection	47/82 (57.3%)	50/74 (67.6%)	11.6% (–13.0 to 33.1)	OR 1.66 (0.84 to 3.25)	0.14
Secondary outcomes					
Patients with new or worsening AKI or all-cause mortality (modified RIFLE criteria)	26/154 (16.9%)	19/153 (12.4%)	–4.5% (–11.6 to 3.5)	OR 0.67 (0.34 to 1.33)	0.25
Protocol-compliant estimand	26/146 (17.8%)	16/140 (11.4%)	–6.3% (–11.6 to 3.5)	OR 0.53 (0.26 to 1.09)	0.079
30-day all-cause mortality	6/156 (3.8%)	3/156 (1.9%)	–1.9% (–5.8 to 1.6)	OR 0.49 (0.12 to 2.06)	0.32
24-h AUC-to-MIC target reached 48–72 h after start of treatment	89/152 (58.6%)	120/155 (77.4%)	20.5% (4.5 to 36.9)	OR 2.54 (1.52 to 4.26)	0.0004
Median time to clinical cure, days	9 (95% CI 8–11)	9 (95% CI 8–10)	NA	HR 1.26 (0.99 to 1.6)	0.056
Length of ward stay, days	21.6 (10.4); 30 (11–30)	21.7 (10.1); 28.5 (13–30)§	0.3 (–1.6 to 2.3)	1.02 (0.93 to 1.11)‡	0.94
Length of hospital stay, days	24.1 (8.8); 30 (18–30)	23.1 (9.4); 30 (16–30)§	–0.8 (–2.5 to 1.1)	0.97 (0.90 to 1.05)‡	0.32
Tertiary outcomes					

Number of blood samples taken during vancomycin treatment	4.2 (3.9); 3 (2–6)	4.9 (3.5); 4 (2.75–6)§	0.6 (–0.3 to 1.4)	1.15 (0.95 to 1.36)‡	NA
Number of additional blood samples taken during vancomycin treatment	2.9 (2.9); 2 (1–4)	3.4 (2.6); 3 (2–5)§	0.5 (–0.1 to 1.1)	1.18 (0.97 to 1.42)‡	NA
Cumulative number of blood samples taken in patients with clinical cure (n=290)¶	3.9 (3.2); 3 (2–5)	4.9 (3.5); 4 (2.25–6)	0.9 (0.1 to 1.6)	1.18 (0.97 to 1.46)‡	NA
Number of dose adjustments during vancomycin treatment	2.8 (2.8); 2 (1–4)	2.2 (2.1); 2 (1–3)§	–0.6 (–1.2 to –0.1)	0.78 (0.63 to 0.97)‡	NA
24-h AUC-to-MIC target reached 72–96 h after start of treatment	92/152 (60.5%)	127/155 (81.9%)	22.2% (6.8 to 37.8)	OR 3.18 (1.85 to 5.47)	NA
Cumulative dose during vancomycin treatment, mg/kg	412.2 (421.5); 258.4 (136.1–538.1)	324.7 (333.6); 241.4 (113.2–422.9)§	–94.8 (–183.6 to –17.7)	NA	NA
Cumulative AUC during vancomycin treatment, mg·h/L	4099.1 (3509.9); 3191.9 (1606.1–5146.8)	3429.4 (2544.3); 2822.5 (1397.7–4595.5)**	–710.6 (–1496.9 to –109.5)	NA	NA
Number of sampling time errors	1.7 (2.2); 1 (0–2)	NA	NA	NA	NA
Patients with new or worsening AKI or all-cause mortality (KDIGO criteria)	23/154 (14.9%)	15/154 (9.7%)	–4.7% (–11.1 to 3.1)	OR 0.61 (0.3 to 1.24)	NA

Data are n/N (%) or mean (SD); median (IQR) unless otherwise specified. The proportions in the standard-of-care group and intervention group columns are based on participants who had available data for that particular outcome. For the data in the subsequent columns, multiple imputation was used to account for these missing data, so these results apply to all randomly assigned participants (ie, the ITT population).

AKI=acute kidney injury. AUC=area under the concentration–time curve. ITT=intention-to-treat. KDIGO=Kidney Diseases Improving Global Outcomes. MIC=minimal inhibitory concentration. NA=not applicable. RIFLE=Risk Injury Failure Loss End-Stage. *p value for type of ward unit × randomisation group 0.20. †p value for type of dosing regimen × randomisation group 0.098. ‡Mean ratio. §n=2 with missing data. ¶140 patients in the standard-of-care group and 150 in the intervention group had a clinical cure. ||n=6 with missing data. **n=5 with missing data.

Table 2. Primary, secondary, and tertiary outcomes

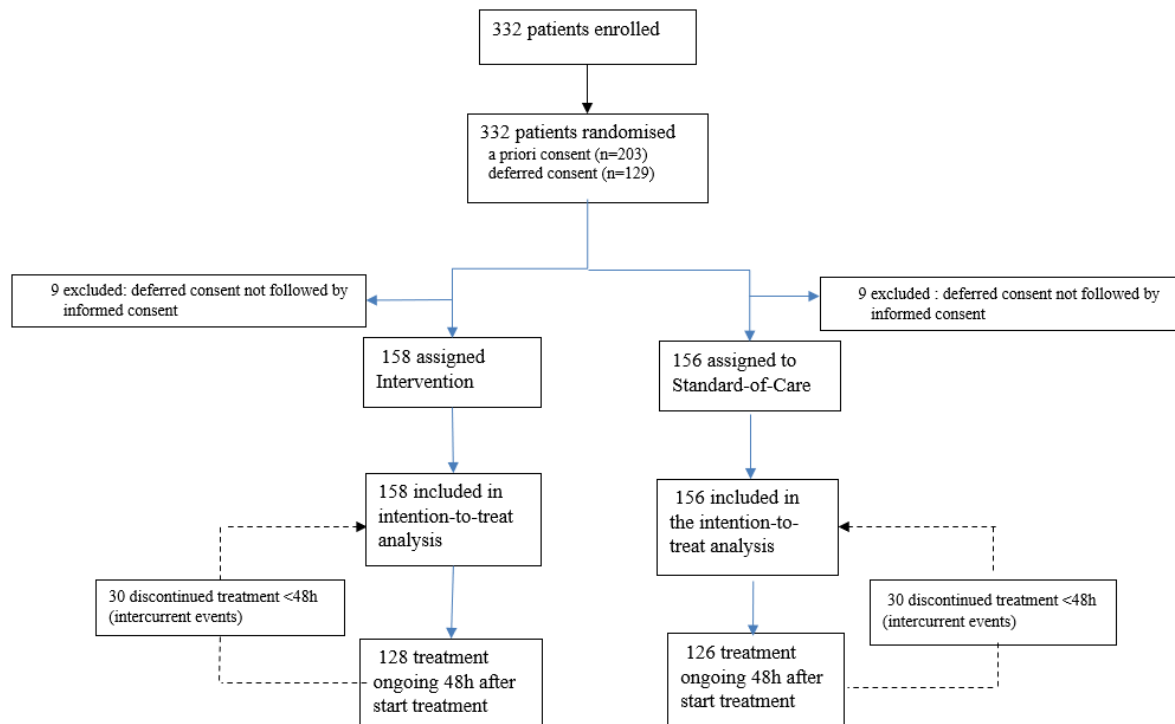


Figure 1 : Trial profile

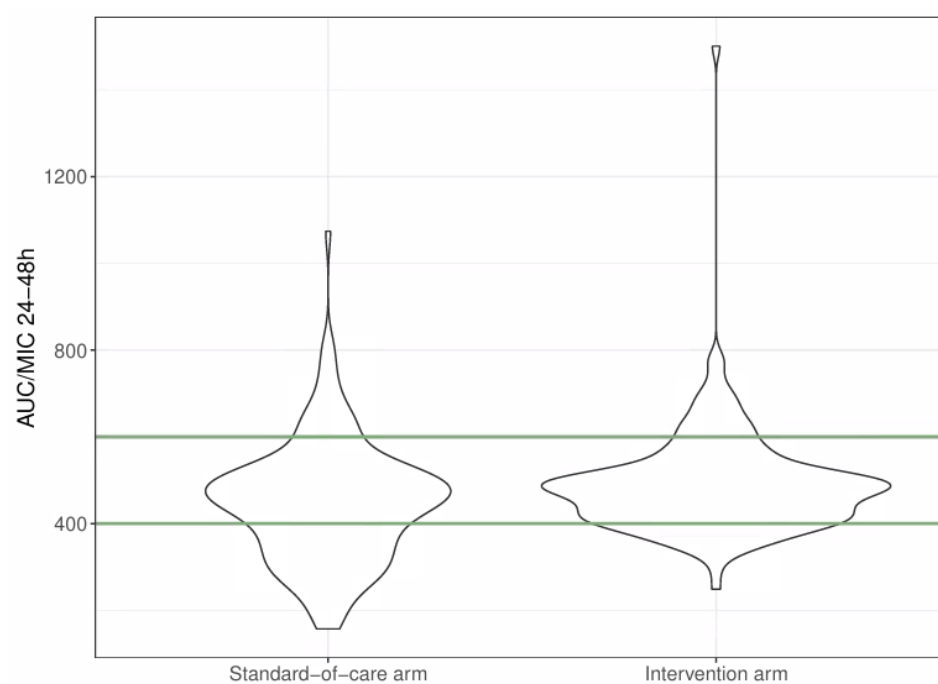


Figure 2: Distribution of AUC-to-MIC ratios (assuming a MIC of 1 mg/L)

Violin plots showing the distribution of the AUC-to-MIC ratios 24–48 h after the start of treatment in each group; the width of each curve corresponds to the approximate frequency of datapoints. The horizontal red lines represent the target AUC-to-MIC ratio range of 400–600. AUC=area under the concentration–time curve. MIC=minimum inhibitory concentration.

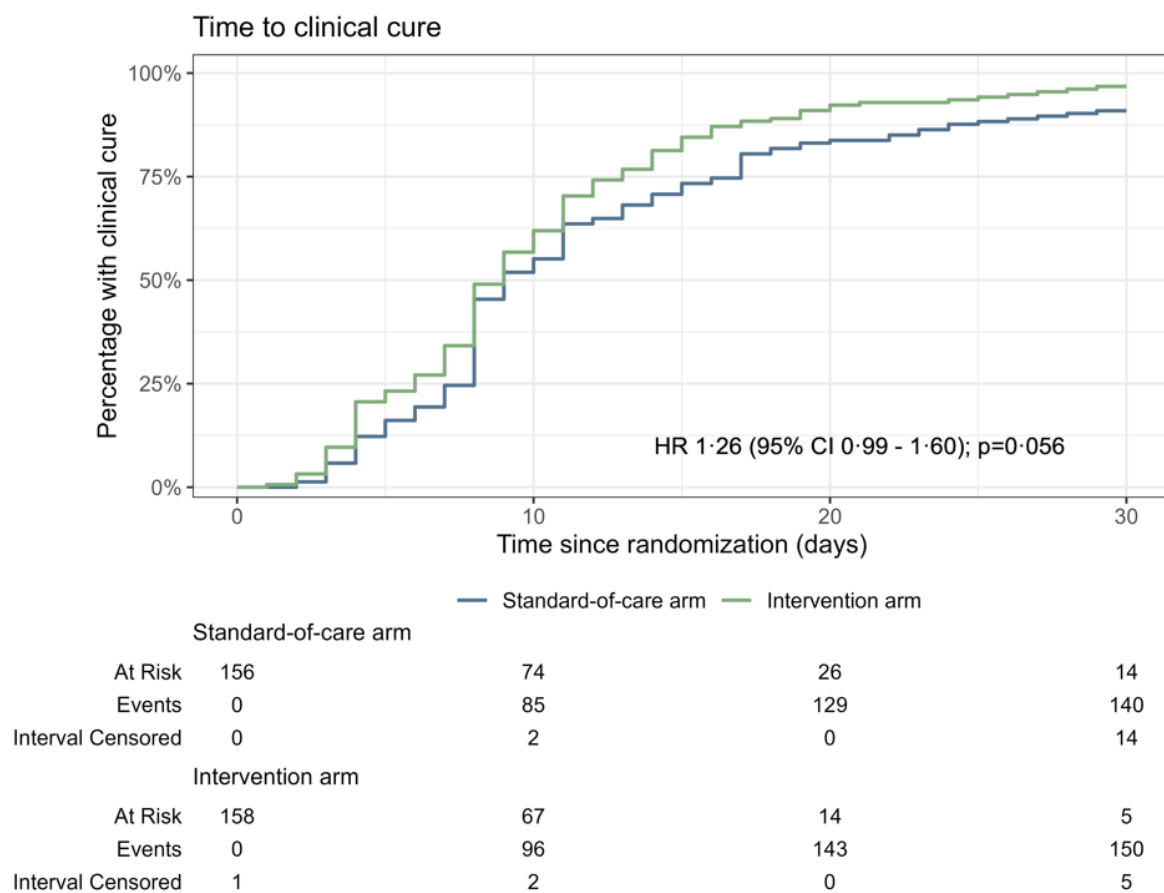


Figure 3 : Estimates of the cumulative incidence of the time to clinical cure for the intention-to-treat population

APPENDIX

A pragmatic, multicentric, randomised controlled clinical trial to study the impact of bedside model-informed precision dosing of vancomycin in critically ill children – BENEFICIAL trial.

Pieter A. De Cock, Roos Colman, Anne Smits, Fiammetta Piersigilli, Marleen Renard, An Van Damme, Alexander Clarysse, Evelyn Dhont, Sophie Vanhaesebrouck, Victoria Bordon, Lidvine Boland, Bram De Wilde, Anca Amza, Lieselot Vanlanduyt, Thi Van Anh Nguyen, Filip Cools, Jutte van der Werff Ten Bosch, Daphne Vania Vens, Frank Derriks, Laurent Houtekie, Reiner Mauel, Dimitri Van der Linden, Petra Schelstraete, for the BENEFICIAL study group *

Lancet Child Adolesc Health 2026

Published Online April 10, 2026 [https://doi.org/10.1016/S2352-4642\(25\)00385-2](https://doi.org/10.1016/S2352-4642(25)00385-2)

See Online/Comment [https://doi.org/10.1016/S2352-4642\(26\)00018-0](https://doi.org/10.1016/S2352-4642(26)00018-0)

Lancet Child and Adolescent Health homepage: <https://www.thelancet.com/journals/lanchi/home>

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Methods: Participants

Patients were excluded with a known chronic kidney disease as defined by KDIGO: structural or functional abnormalities of the kidney regardless of glomerular filtration rate for <3 months in duration or GFR <60 ml/min/1.73 m² for ≥ 3 months in durations. eGFR is estimated using the modified Schwartz equation.

Written informed consent (IC) for all trial participants was obtained, by discussing with the parents or legal representatives the nature, objectives, and the possible adverse events associated with their participation in the study. Maximum effort was made to obtain written IC prior to inclusion (a priori consent), or whenever not possible due to a matter of clinical urgency, within 24h after the start of therapy (deferred consent). For participants older than 12 years or older, a separate written assent form was obtained, after informing them using a separate information sheet. Additional consent (optional) from parents or legal representatives was asked for collection and storage of the national insurance number, sharing personal data with the Federal Healthcare Knowledge Centre (funder) and other European research institutions for public health for supplementary research, use of the participant data to optimize PK/PD models for vancomycin.

Patients were categorised as receiving mechanical ventilation at baseline, if they received: any mode of invasive mechanical ventilation via an endotracheal or nasotracheal or tracheostomy tube, with or without positive end expiratory pressure, High Frequency Oscillation ventilation, continuous positive airway pressure ventilation delivered through a tracheostomy or endotracheal tube.

Methods: Procedures

Dosing details according to institutional guidelines in the standard-of-care arm

Vancomycin starting doses were prescribed according to institutional guidelines within the range:

- (i) neonates ≤28 days with an intermittent dosing regimen: loading dose of 16 to 26 mg per kg (administered in 3 units), intermittent dosing regimen of 15 to 60 mg per kg per day depending on the patient's birth weight, postmenstrual age, postconceptional age, gestational age, postnatal age, ibuprofen use, serum creatinine and/or current weight;
- (ii) neonates ≤28 days postnatal age with a continuous infusion: loading dose of 15 to 35 mg per kg and continuous infusion of 20 to 35 mg per kg per day, depending on gestational age and serum creatinine;
- (iii) children >28 days with an intermittent dosing regimen: loading dose of 20 to 30 mg per kg (in 1 ward unit), intermittent dosing regimen of 20 to 60 mg per kg per day, depending on serum creatinine ;
- (iv) children >28 days postnatal age with a continuous infusion: loading dose of 15 to 25 mg per kg and continuous infusion of 15 to 60 mg per kg per day, depending on serum creatinine.)

Dose adjustments were performed empirically or using dose adjustment nomograms, based on TDM. For intermittent dosing regimens, dose adjustments included shortening or prolongation of the dose interval (+/- 2 to 4 hours), an increase or decrease in dose amount (range in dose adjustment nomograms : - 50 to + 150%) or a postponement of administering the next dose (+ 6 to 24 hours). For dose adjustment of continuous infusions, an increase or decrease in infusion rate (-33 to +300%), eventually in combination with a repeat loading dose (if the measured TDM concentration was below the institutional threshold for efficacy) or temporary infusion stop (range of 2 to 12 hours if the measured TDM concentration was above the institutional upper threshold) was applied.

Sampling

Extra sampling as compared to standard-of-care in the intervention group:

- for intermittent dosing regimens, a minimum of one and maximum (but ideally) of two samples were taken after administration of the starting dose, to allow for early and accurate AUC-based dose adjustment on subsequent doses. Guidance was given whenever 1 or 2 samples were taken.
 - o If one sample could be drawn, it was suggested to combine this sample with other biochemical testing during the dosing interval. If not feasible, it was suggested to draw the sample in the second half of the dosing interval for intermittent dosing regimens.
 - o If two samples could be drawn, it was suggested to combine as much as feasible, these samples with routine biochemical testing. If not feasible, it was suggested to draw one of these samples

in the first half of the dosing interval and the other in the second half of the dosing interval with at least one hour in between.

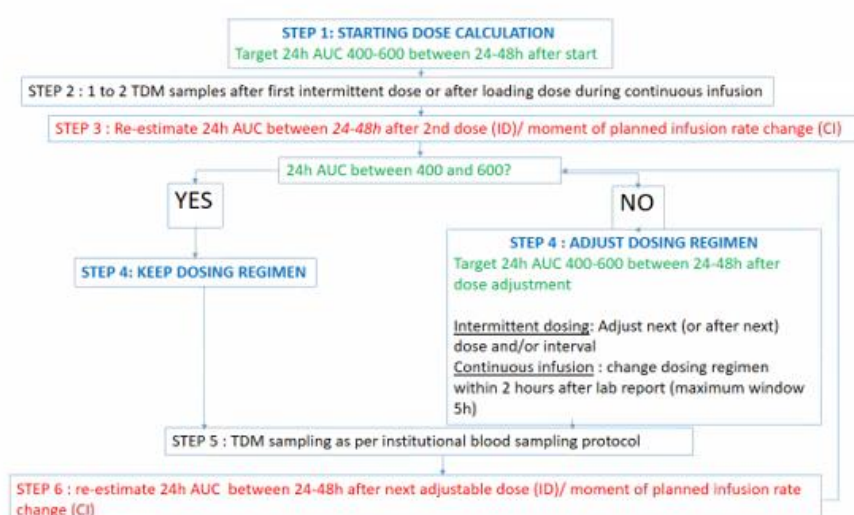
- for continuous infusions, a minimum of one and maximum (but ideally) of two samples were taken after administration of the loading dose, to allow for early and accurate AUC-based dose adjustment on subsequent supplementary loading doses and continuous infusion rates. The decision to take one or two samples was at the discretion of the treating physician. The sampling window was within 6h after start and with at least 1 hour in between, if two samples were drawn.

Hereafter, the sampling standard-of-care principles (as described in the main paper) were also applicable to the intervention group patients. In the interventional arm, a deviation from the standard-of-care sampling time point (i.e. intermittent dosing: just before the intermittent dose; continuous dosing: standard-of-care timepoint depending on the local ward unit protocol) was allowed within an interval of 3h before the standard-of-care sampling timepoint, in case this sample could be combined with a scheduled biochemical sample or another sampling timepoint that was more convenient for the patient and/or nurse.

Dose calculation and dose limits

The vancomycin pooled PK model by Colin et al. was used within this MIPD software package. Calculated starting doses could deviate a maximum of 50% of institutional dosing guidelines. A maximum upward dose adjustment of 33% in total daily dose was allowed for both intermittent and continuous dosing regimens. Dosing regimens were suggested by MIPD software end-users and implemented by the attending physician. The choice for an intermittent or loading dose/continuous infusion dosing regimen was at the discretion of the attending physician/site preference. MIPD software end-users and attending physicians were allowed to be the same person. Intermittent starting dosing intervals were restricted to 4 hourly, 6 hourly, 8 hourly, 12 hourly, 18 hourly, and 24h hourly intervals. A dose suggestion for continuous dosing regimens was required to include a loading dose, administered over a 1 to 2h interval, immediately followed by a continuous infusion; The maximum allowed loading dose was 30 mg per kg. It was allowed to round the MIPD suggested dose to the nearest manageable dose for administration with a maximum allowed deviation of 10% of the minimum or maximum suggested dose to attain a 24h AUC between 400 and 600.

Dosing regimens were suggested by the MIPD software end-users and implemented by the attending physician for the starting dose regimen and follow-up dosing regimens, based on AUC assessment, every time a TDM concentration became available. The choice for an intermittent or loading dose/continuous infusion dosing regimen was at the discretion of the attending physician/site preference.



ID, intermittent dosing regimen; CI, continuous infusion

Figure 1S. Workflow for AUC-based dose calculation and dose adjustment in the intervention arm

NONMEM script for AUC calculation

The script below describes how individual vancomycin AUC were estimated using Bayesian method using the previously published Colin et al. (1) population PK model in NONMEM v 7.5, Icon Development Solutions, USA. The Colin model ¹ was modified in the \$OMEGA block to have flattened prior of 30%, which gives more weight (70%) to the individual concentration measurements from patients in the Beneficial trial.

```
; modified model, with:
; - prior flattened to 30%
; - weights for observations within:
; - 0 – 4 days of treatment: 100%
; - 4 – 10 days of treatment: linear interpolation from 100% -> 20%
; - 10 – 30 days of treatment: linear interpolation from 20% -> 10%
;
$PROBLEM AUC calculation Beneficial trial with prior 30
$DATA 20240621_input_nm_v2.csv IGNORE=@
$INPUT ID1 ID TIME AMT RATE TOD DV MDV EVID CR AGE WT PMA AUCF
; the AUCF (flag column) identifies the integrated AUC at timepoints of interest for further processing (see below)
;CR: serum creatinine
;AGE: age(days)
;PMA: Post Menstrual Age (weeks)
; $SUBROUTINE ADVAN3 TRANS4
$SUBROUTINE ADVAN6 TOL=8
$MODEL NCOMP=3
COMP=(CENTR)
COMP=(PERIPH)
COMP=(AUC)
$PK
PMA_R = 35+(40/52)
PMA_YRS = PMA/52
HEEL = 0
CANCER = 0
DEG50 = EXP(THETA(5))
MAT50 = EXP(THETA(6))
GAM = EXP(THETA(7))
GAM2 = -EXP(THETA(8))
EXPO2 = EXP(THETA(9))

LnSCRstd = -1.22839 + LOG10(PMA_YRS) * 0.67190 + 6.27017 * EXP(-3.10940 * PMA_YRS)
SCRstd = EXP(LnSCRstd)
Fsize = WT/70
Fmat = PMA_YRS ** GAM / (PMA_YRS ** GAM + MAT50 ** GAM)
Fmat_R = PMA_R ** GAM / (PMA_R ** GAM + MAT50 ** GAM)
Fage = PMA_YRS ** GAM2 / (PMA_YRS ** GAM2 + DEG50 ** GAM2)
Fage_R = PMA_R ** GAM2 / (PMA_R ** GAM2 + DEG50 ** GAM2)
Fscr = EXP(-EXPO2 * (CR-SCRstd)) ; For a man of 35 yr, 70 kg and GA 40 wks, SCRstd = 0.84 mg/dL
TVV1 = EXP(THETA(2)) * Fsize * (1-HEEL*THETA(10))
V1 = TVV1 * EXP(ETA(2))
TVV2 = EXP(THETA(3)) * Fsize
V2 = TVV2 * EXP(ETA(3))
RV1 = EXP(THETA(2))
TVCL = EXP(THETA(1)) * (V1/RV1) ** 0.75 * Fmat/Fmat_R * Fage/Fage_R * Fscr * (1 + CANCER * THETA(12))
CL = TVCL * EXP(ETA(1))
RV2 = EXP(THETA(3))
TVQ = EXP(THETA(4)) * (V2/RV2)**0.75 * (1-HEEL*THETA(11))
Q = TVQ
S1=V1
; Rewritten original model to ODE model to allow AUC estimation
$DES
DADT(1) = -(CL/V1)*A(1) + (Q/V2)*A(2) - (Q/V1)*A(1)
DADT(2) = - (Q/V2)*A(2) + (Q/V1)*A(1)
DADT(3) = (A(1)/V1)
$ERROR
AUC = A(3)
IPRED = F
PROPvar = 0.046
ADDvar = 1.52
; MODIFIED: weighting of data over time
WEIGHT = 1.0 ; from t=0 to t=4 days
```

```

IF(TIME.GT.96.AND.TIME.LT.240) THEN ; from t=4 to t=10 days
WEIGHT = 1.0 - (TIME-96) * (0.8/144)
ENDIF
IF(TIME.GT.240.AND.TIME.LT.720) THEN ; from t=10 to t=30 days
WEIGHT = 0.2 - (TIME-240) * (0.1/480)
ENDIF
IF(WEIGHT.LT.0.1) WEIGHT=0.1 ; fallback, if any datapoints >30 days, set to 10%
W = SQRT(IPRED**2 * PROPvar + ADDvar)
Y = IPRED + (W/WEIGHT) * ERR(1) ; Weight-factor added
IRES = DV-IPRED
$THETA
(1,1.67,2.5) FIX ; CLstd, L/h
(2.77,3.75,4.5) FIX ; V1std, L
(2.78,3.73,4.5) FIX ; V2std, L
(0.5,1.17,2.5) FIX ; Qstd, L/h
(3.5,4.12,4.8) FIX ; DEG50
(-0.69,-0.113,0.40) FIX ; MAT50
(-0.69,1.06,1.5)FIX ; GAM
(-0.69,0.807,1.5) FIX ; GAM2
(-1.5,-0.433,-0.01) FIX ; EXPO2: SCR~CL
(0,0.312,1) FIX ; HEEL ~ V1
(0,0.597,1) FIX ; HEEL ~ Q2
(0,0.294,1.5) FIX ; CANCER ~ C
; MODIFIED: omega modified to have flattened prior (30%)

$OMEGA
; 0.0746 FIX ; IIV_CL
0.2487 ; = 0.0746 / 0.3
; 0.0716 FIX ; IIV_V1
0.2387 ; = 0.0716 / 0.3
; 0.684 FIX ; IIV_V2
2.28 ; = 0.684 / 0.3
$SIGMA
1 FIX
$EST METHOD=0 POSTHOC MAXEVAL=0
$TABLE ID1 ID TIME TOD DV PRED IPRED EVID AUC CL V1 Q V2 PMA_YRS AUCF
FILE=nmout2.tab NOPRINT NOAPPEND
; the AUC column in the nmout2.tab output file contains per row the cumulative AUC from start to timepoint
; the AUCF (flag column) identifies the integrated AUC at timepoints of interest for further processing (see below)

```

For the first sensitivity analysis of the primary endpoint, this model code was modified in the \$OMEGA block code to have a flattened prior of 50%, which gives equal weight to the model as to the individual concentration measurements from patients in the Beneficial trial.

Class	Paediatric		Class	Neonatal	
	Creatinine based classification	Urine output		Urine output	
<i>Risk</i>	1.5 to 2 fold rise in baseline serum creatinine or eGFR decrease by 25%	<0.5ml/kg/h for 8 h	<i>Risk</i>	<1.5 ml/kg/h for 24 h	
<i>Injury</i>	2 to 3 fold rise in baseline serum creatinine or eGFR decrease by 50%	<0.5 ml/kg/h for 16h	<i>Injury</i>	<1 ml/kg/h for 24h	
<i>Failure</i>	>3 fold rise in baseline serum creatinine or eGFR decrease by 75%	<0.3 ml/kg/h for 24h or anuria for 12h	<i>Failure</i>	<0.7 ml/kg/h for 24h or anuria for 12h	
<i>Loss</i>	Failure >4 weeks		<i>Loss</i>	Failure >4 weeks	
<i>ESRD</i>	persistent failure > 3 months		<i>ESRD</i>	persistent failure > 3 months	

ESRD, end-stage renal disease

Baseline serum creatinine was defined, as follows:

- If patients were not categorized in p RIFLE category risk or injury at baseline: serum creatinine and calculated eGFR were compared to the most recent baseline value just before start of vancomycin treatment (max 120h interval to start vancomycin) to evaluate RIFLE class during vancomycin treatment.
- If patients were categorized in p RIFLE category risk or injury at inclusion: age-, assay- and gender-specific reference values for serum creatinine were used as baseline to evaluate RIFLE class during vancomycin treatment

(Worsening) AKI during vancomycin treatment was defined as increase in AKI category during vancomycin treatment.

Urine output was assessed according to local practice, most commonly through 24h collections. In neonates without an indwelling urinary catheter, diapers were weighed to estimate urine output. In children older than 1 month without a urinary catheter, urine output was not consistently available, and AKI classification using the pRIFLE criteria was therefore based solely on serum creatinine. eGFR is estimated using the modified Schwartz equation.

Table 1S. Modified neonatal and paediatric Risk Injury, Failure, Loss (RIFLE) AKI criteria

Serum creatinine criteria			Urine output criteria	
Stage	Paediatric definition	Neonatal definition	Paediatric definition	Neonatal definition
1	≥ 0.3 mg/L rise within 48 h or ≥ 1.5 – $1.9 \times$ rise from baseline	≥ 0.3 mg/L rise within 48 h or ≥ 1.5 – $1.9 \times$ rise from baseline	< 0.5 ml/kg/h for 8 h	< 1.5 ml/kg/h for 24 h
2	≥ 2 – $2.9 \times$ rise from baseline	Unchanged	< 0.5 ml/kg/h for 16h	< 1 ml/kg/h for 24h
3	$\geq 3 \times$ rise from baseline or ≥ 4.0 mg/L or eGFR < 35 ml/min per 1.73 m ² or RRT initiation	$\geq 3 \times$ rise from baseline or ≥ 2.5 mg/L or RRT initiation	< 0.3 ml/kg/h for 24h or anuria for 12h	< 0.7 ml/kg/h for 24h Or anuria for 12h

RRT, renal replacement therapy

eGFR is estimated using the modified Schwartz equation.

Table 2S. Modified Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury criteria

Statistical analysis methods – protocol-compliant estimands

The protocol-compliant estimand was estimated as the unconditional relative risk and absolute risk difference of reaching the primary endpoint under the presumptions that the following intercurrent events would not have occurred: early discontinuation of MIPD calculator; treatment switch between MIPD and standard treatment (or vice versa); serious protocol deviation with impact on primary endpoint, and that these patients had followed the pattern of the other patients in the protocol-compliant sample (hypothetical strategy).

The protocol-compliant estimand for (worsening) AKI was estimated as the unconditional relative risk and absolute risk difference of (worsening) AKI under the presumptions that the following intercurrent events would not have occurred: early discontinuation of MIPD calculator; treatment switch between MIPD and standard treatment (or vice versa); serious protocol deviation with potential impact on AKI, and that these patients had followed the pattern of the other patients in the protocol-compliant sample (hypothetical strategy).

Statistical analysis methods: sensitivity analysis for the primary endpoint

A sensitivity analysis for the main estimand of the primary outcome was performed using a flattened prior of 50% for AUC estimation, which gives more weight to the Colin PK model for individual AUC estimation. Due to the nature of the intervention being the use of an MIPD calculator in combination with one or two extra early samples, the number of available samples was higher in the intervention group compared to the control group.

Another sensitivity analysis therefore included an AUC estimation, in which early samples (< 6 hours of treatment) were omitted to avoid potential imbalances in accurate AUC estimation between both groups. Prespecified subgroup analysis for type of ward unit, type of dosing regimen, patients with documented bacterial infection and a non-predefined sex-based analysis were also performed for the primary outcome and/or secondary safety outcomes. A documented infection was defined as an infection with a pathogen grown in blood or sterile site or an abscess or volume of infected tissue e.g. pneumonia, soft tissue.

To assess the robustness of our results against the assumption of independent observations, a third sensitivity analysis which takes into account the possibly correlated outcomes of children “nested within twins or triplets”

was planned. Since subjects that are part of a twin were randomized individually, the power of the study is not impacted by the presence of twins. The following sensitivity analysis was planned in case ten percent of the subjects or more have a twin or triplet sibling included in the study: a logistic generalized estimating equation (GEE) with randomization group (interventional versus the standard-of-care arm) as categorical predictor of interest, ward type as categorical predictor and an exchangeable working correlation structure.

Statistical analysis methods: intercurrent events

The ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials was taken into account when defining the strategy of handling intercurrent events.³⁶ The intercurrent events for the primary outcome and corresponding strategy for data analysis were summarized in Table 3S.

Primary outcome: Intercurrent events (if occurring within 48h after start vancomycin)	
Death	Composite strategy – Subjects who die will be considered to have an unfavorable outcome
Discontinuation of vancomycin treatment because of clinical cure	Composite strategy – Subjects will be considered to have a favorable outcome
Discontinuation of vancomycin treatment because of adverse event	Composite strategy – Subjects will be considered to have an unfavorable outcome
Discontinuation of vancomycin treatment because of clinical failure	Composite strategy – Subjects will be considered to have an unfavorable outcome
Patient treatment focus changed to palliation	While on treatment strategy – Estimated AUC prior to the discontinuation of vancomycin treatment The “while on treatment strategy” is not expected to cause bias because 1) The occurrence of the intercurrent event is not expected to differ between the treatments being compared (the occurrence of this ICE will be reported by treatment arm) 2) Reaching the target earlier than the 24h to 48h interval is more difficult
Discontinuation of vancomycin treatment because of change in antimicrobial therapy within 48h after start vancomycin	While on treatment strategy – Estimated AUC prior to the discontinuation of vancomycin treatment The “while on treatment strategy” is not expected to cause bias because 1) The occurrence of the intercurrent event is not expected to differ between the treatments being compared (the occurrence of this ICE will be reported by treatment arm) 2) Reaching the target earlier than the 24h to 48h interval is more difficult
Deferred consent not followed by an informed consent to continue the study, but with an informed consent for use of already collected data.	Hypothetical strategy – What would have happened if these subjects did not have this IE?
Treatment switch (intervention and standard-of-care or vice versa)	Treatment policy strategy – Observed outcomes are used regardless of whether or not this IE occurred

Early discontinuation of calculator	Treatment policy strategy – Observed outcomes are used regardless of whether or not this IE occurred
Major protocol deviation with possible impact on primary endpoint	Treatment policy strategy – Observed outcomes are used regardless of whether or not this IE occurred

Table 3S. Intercurrent events for the primary outcome and corresponding strategy for data analysis

The secondary endpoint “Proportion of patients reaching target 24h AUC (400-600 mg·h/L) between [48 to 72] h after start treatment” and the tertiary endpoint “Proportion of patients reaching target 24h AUC (400-600 mg·h/L) between [72 to 96] h after start treatment” were analysed similarly as described in Table 3S. The intercurrent events and corresponding strategy for the other secondary endpoints are summarized in Table 4S.

Estimand	Strategy for accounting intercurrent events	Endpoints
Treatment policy	Treatment policy strategy (the data collected for the variable of interest are used regardless of whether or not this intercurrent event occurs): All intercurrent events for which the endpoint could be observed Hypothetical strategy (censoring) (What would have happened if these subjects did not have this Intercurrent event?): Deferred consent not followed by an informed consent to continue the study, but with an informed consent for use of already collected data. Composite strategy: Death (unfavorable outcome) For the key secondary endpoint “time to clinical cure”, the intercurrent event “Patient-treatment focus changed to palliation” is treated as a competing risk (censoring is applied).	-All cause 30-day mortality
		-Time to clinical cure
		-Ward unit length-of-stay -Hospital length-of-stay
While on treatment	While on treatment strategy: Discontinuation of vancomycin treatment Composite strategy: Death (unfavorable outcome) Treatment policy strategy: Treatment switch (MIPD and standard-of-care or vice versa) Early discontinuation of calculator Hypothetical strategy: Deferred consent not followed by an informed consent to continue the study, but with an informed consent for use of already collected data.	-Proportion of patients with (worsening) AKI during vancomycin treatment

Table 4S. The intercurrent events and corresponding strategy for the other secondary endpoints

Statistical analysis methods: missing values

To handle the missing values for the primary outcome, a multiple imputation model was applied separately for each randomization group, including the following predictors: type of ward unit, type of consent, indication for vancomycin treatment, mechanical ventilation, nephrotoxic medication, serum creatinine, age, weight, cumulative vancomycin dose (mg/kg) within 48h-interval, time since start of the study in that specific ward unit; the intercurrent events “Discontinuation of vancomycin treatment because of change in antimicrobial therapy within 48h after start vancomycin” (Yes/No) and “Treatment switch/Early discontinuation of calculator /Serious protocol deviation with possible impact on primary endpoint” (Yes/No). Following the imputation of missing values, a logistic regression model was applied with randomisation group (interventional versus the standard-of-care arm) as categorical predictor of interest. The stratification factor “type of ward unit” was added as a covariate in the model.

Statistical methods for (worsening) AKI were similar to those described for the primary outcome, but with different predictors of the multiple imputation model: type of ward, duration of vancomycin treatment (log-transformed) and number of nephrotoxic medications at baseline.

Statistical analysis methods –subgroup analysis

For the primary endpoint, subgroup analyses for interaction were predefined for ward type (NICU, PICU, PHO) and for dosing regimen (continuous versus intermittent). A subgroup analysis on the subset of patients with confirmed infection at baseline was also predefined.

Posthoc gender-based analyses for the primary outcome and key secondary outcomes for safety were also performed.

	Standard-of-Care arm (n=156)	Intervention arm (n=158)
Discontinuation of vancomycin treatment because of clinical cure	5 (3.2%)	7 (4.4%)
Discontinuation of vancomycin treatment because of adverse event	1 (0.6%)	2 (1.3%)
Patient treatment focus changed to palliation	1 (0.6%)	1 (0.6%)
Discontinuation of vancomycin treatment because of change in antimicrobial therapy within 48h after start vancomycin treatment	18 (11.5%)	17 (10.8%)
Deferred consent not followed by informed consent to continue the study, but with an informed consent for use of already collected data	0 (0%)	1 (0.6%)
Other	5 (3.2%)	2 (1.3%)

Data are n (%).

Table 3S. Reporting of intercurrent events for the primary endpoint

	Standard-of-Care arm (n=156)	Intervention arm (n=158)
Proportion of patients with (worsening) AKI		
Deferred consent not followed by informed consent to continue the study, but with an informed consent for use of already collected data	0 (0%)	2 (1.3%)
Mortality	5 (3.2%)	2 (1.3%)
30-day all-cause mortality		
Deferred consent not followed by informed consent to continue the study, but with an informed consent for use of already collected data	0 (0%)	2 (1.3%)

Data are n (%).

Table 4S. Reporting of intercurrent events for the key secondary endpoints for safety

AUC 48 to 72h		
	Standard-of-Care arm (n=156)	Intervention arm (n=158)
Discontinuation of vancomycin treatment because of clinical cure	12 (7.7%)	24 (15.2%)
Discontinuation of vancomycin treatment because of adverse event	1 (0.6%)	2 (1.3%)
Patient treatment focus changed to palliation	1 (0.6%)	1 (0.6%)
Discontinuation of vancomycin treatment because of change in antimicrobial therapy within 72h after start vancomycin treatment	31 (19.9%)	26 (16.5%)
Deferred consent not followed by informed consent to continue the study, but with an informed consent for use of already collected data	-	2 (1.3%)
Other	8 (5.1%)	2 (1.3%)
AUC 72 to 96h		
	Standard-of-Care arm (n=156)	Intervention arm (n=158)
Discontinuation of vancomycin treatment because of clinical cure	17 (10.9%)	32 (20.3%)
Discontinuation of vancomycin treatment because of adverse event	1 (0.6%)	2 (1.3%)
Patient treatment focus changed to palliation	1 (0.6%)	1 (0.6%)
Discontinuation of vancomycin treatment because of change in antimicrobial therapy within 72h after start vancomycin treatment	31 (23.7%)	31 (19.6%)
Deferred consent not followed by informed consent to continue the study, but with an informed consent for use of already collected data	-	2 (1.3%)
Other	9 (5.8%)	3 (1.9%)

Table 5S. Reporting of intercurrent events for the secondary endpoints 48-72h 24h AUC and 72-96h 24h AUC

	Standard-of-Care arm (n=156)	Intervention arm (n=158)
Duration of first episode of vancomycin treatment	146 (119); 119 (57-176)	143 (113);122 (55-183);2*
Proportion of patients with>1 episode of treatment	41 (26.3%)	27 (17.3%);2
Vancomycin method of administration		
Intermittent (%)	139 (89.7%)	137 (86.7%)
Continuous (%)	17 (10.9%)	21 (13.3%)
Cumulative starting dose in first 24h (mg/kg)	54 (19); 53 (41-64)	52 (22);47 (34-66)
Switch during treatment from intermittent to continuous infusion	5 (3.2%)	3 (1.9%);2
Number of TDM samples during treatment	4.2 (3.9); 3(2-6)	4.9 (3.5); 4(2.5-6);2
Time to first TDM sample since start vancomycin treatment	22.7 (15.7); 23.2 (15.6-24.0); 6	4.3 (3.5); 3.3 (1.9-5.5); 3
Time to first dose adjustment since start vancomycin treatment	35.7 (83.0); 18.0 (7.2-30.9); 21	23.7 (64.8); 8.0 (6.0-13.3); 27

Data are mean (SD), median (IQR); missing or n (%).

Table 6S. Vancomycin treatment, dosing and sampling details for therapeutic drug monitoring; TDM, therapeutic drug monitoring.*only loading dose

	Standard-of-Care arm (n= 154)	Intervention arm (n=155)
Reasons for stop vancomycin		
clinical cure	78 (50.6%)	93 (60.0%)
change in antimicrobial therapy	61 (39.6 %)	53 (34.2%)
adverse event	1 (0.7%)	3 (1.9%)
patient treatment focus changed to palliation	1 (0.7%)	1 (0.6%)
Other	13 (8.4%)	5 (3.2%)

Data are n (%).

Table 7S. Reasons for stop vancomycin treatment

Site	Standard-of-Care group (n=164)	Intervention arm (n=168)
AZ Sint-Jan, Bruges, NICU	10	10
Hopital Erasme, NICU	3	2
Hopital universitaire Reine Fabiola, PICU	8	7
Cliniques universitaires de Saint-Luc, NICU	17	16
Cliniques universitaires de Saint-Luc, PHO	10	11
Cliniques universitaires de Saint-Luc, PICU	3	4
UZ Brussel, NICU	11	12
UZ Brussel, PHO	3	3
UZ Brussel, PICU	3	3
UZ Gent, NICU	30	31
UZ Gent, PHO	12	13
UZ Gent, PICU	15	13
UZ Leuven, NICU	28	30
UZ Leuven, PHO	11	13

NICU, neonatal intensive care unit; PHO, paediatric hemato-oncology ward unit; PICU, paediatric intensive care unit

Table 8S. Enrolment by site

Pathogen	Standard-of-Care group (n=156)	Intervention arm (n=158)
<i>Coagulase negative Staphylococcus</i>	36	30
<i>Methicillin-Sensitive Staphylococcus aureus</i>	17	13
<i>Viridans Group Streptococci</i>	0	6
<i>Enterococcus spp.</i>	3	2
<i>Bacillus spp.</i>	2	2
<i>Beta-haemolytic Streptococci (Group A, B, C or G)</i>	2	2
<i>Streptococcus pneumoniae (or Pneumococcus)</i>	0	3
<i>Staphylococcus aureus (not further specified)</i>	1	1
<i>Methicillin Resistant Staphylococcus aureus</i>	1	0
<i>Lactaseibacillus Rhamnosus</i>	1	0
<i>Staphylococcus spp (not further specified)</i>	0	1
<i>Corynebacterium amycolatum</i>	0	1

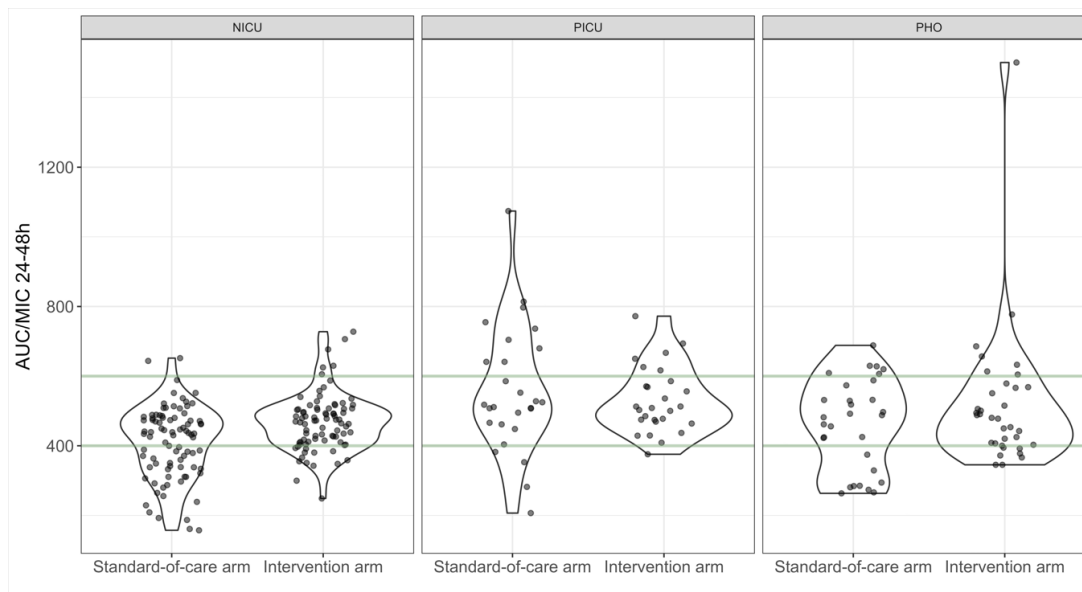
Table 9S: Identified pathogens at baseline

Outcome	Standard-of-Care arm (n=156)	Intervention arm (n=158)	Odds ratio (95% CI)	P value
Primary outcome: proportion of patients reaching 24h AUC/MIC target between 24-48h after start of treatment				
Sensitivity analysis 1 : flattened prior 50%	82 (53·9%); 4	111 (71·2%); 2	2·21 (1·36 to 3·59)	0·0011
Sensitivity analysis 2 : exclusion early samples	82 (53·9%); 4	100 (64·9%); 4	1·66 (1·04 to 2·66)	0·031
Sex-based subgroup analysis** :				P value Sex x Randomization arm: 0·13
Boy (n=179)	52 (57·1%); 2	66 (77·6%); 1	2·81 (1·43 to 5·55)	0·0025
Girl (n=135)	30 (49·2%); 2	46 (64·8%); 1	1·96 (0·96 to 4·01)	0·062
Secondary outcome: proportion of patients with (worsening) AKI* or death				
Sex-based subgroup analysis**:				P value Sex x Randomization arm: 0·59
Boy (n=179)	15(16·1%)	9 (10·8%);3	0·55 (0·21 to 1·42)	0·21
Girl (n=135)	11 (18·0%);2	10 (14·3%);2	0·80 (0·30 to 2·17)	0·66
Secondary outcome: 30-day all-cause mortality				
Sex-based subgroup analysis**				P value Sex x Randomization arm: 0·41
Boy (n=179)	4 (4·3%)	1 (1·2%); 1	0·26 (0·03 to 2·49)	0·23
Girl (n=135)	2 (3·2%)	2 (2·8%); 1	0·90 (0·12 to 6·91)	0·92

Data are presented as n (%); number of missing values. Sensitivity analysis 1: 50% weight is given to the PK model for 24hAUC estimation; Sensitivity analysis 2 : early samples are omitted for 24hAUC estimation; Sensitivity analysis 3: The number of subjects that have a twin or triplet sibling included in the study is 16, which corresponds with 5.1 % of the dataset. Therefore, this sensitivity analysis was not performed.

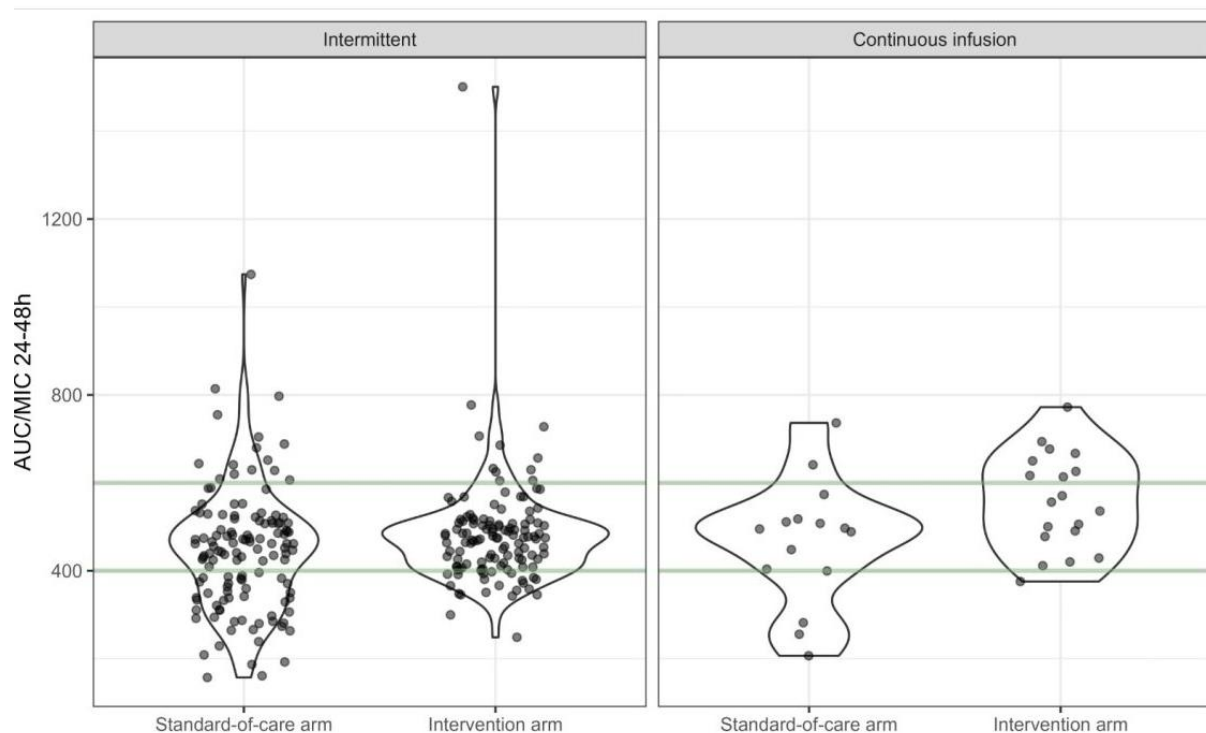
AUC, Area-Under-the-Concentration Time curve; MIC, Minimal Inhibitory Concentration; AKI, acute kidney injury; *according to modified Risk Injury Failure Loss End-Stage (RIFLE) assessment criteria; ** not predefined analysis

Table 10S. Sensitivity and subgroup analysis of primary outcome and key secondary outcomes for safety



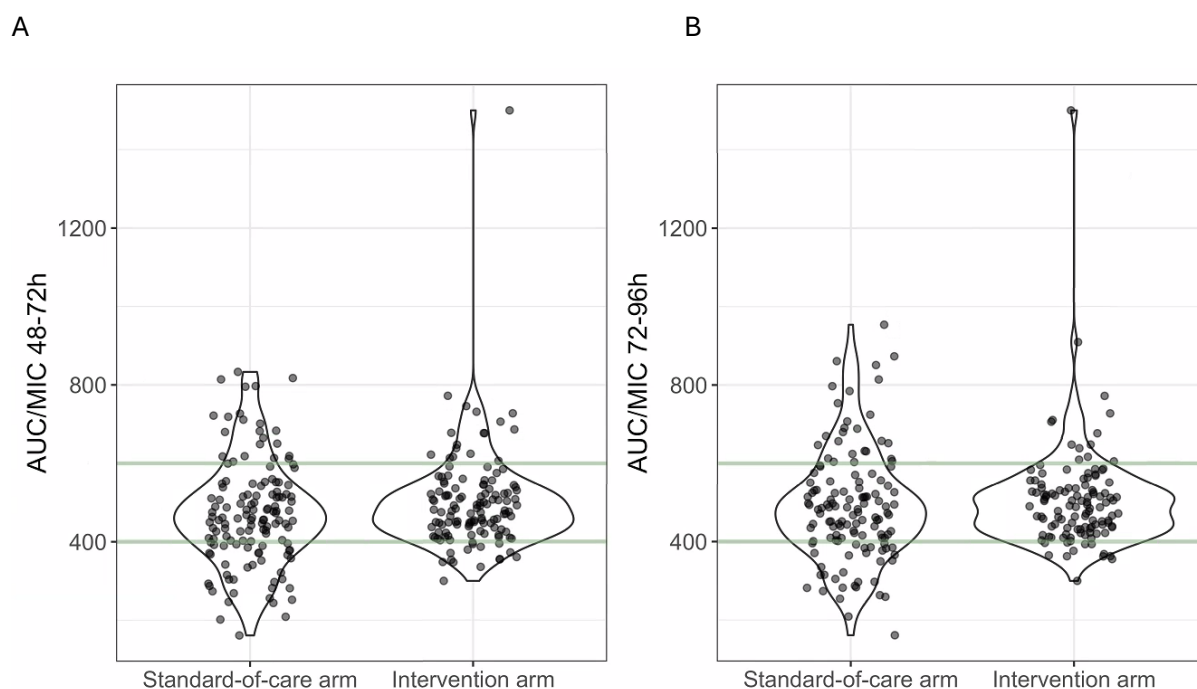
NICU, neonatal intensive care unit; PICU, paediatric intensive care unit; PHO, paediatric haemato-oncology ward unit

Figure 2S. Violin plot for standard-of-care and intervention arm per ward type showing distribution of AUC/MICs (assuming a MIC of 1 mg/L) in the interval 24h to 48 h after start of treatment



The solid horizontal green line represents the target range of 24hAUC/MIC 400-600.

Figure 3S. Violin plot for standard-of-care and intervention arm per type of dosing regimen showing distribution of AUC/MICs (assuming a MIC of 1 mg/L) in the interval 24h to 48h after start of treatment



The solid horizontal green line represents the target range of 24hAUC/MIC 400-600.

Figure 4S. Violin plot for standard-of-care and intervention arm showing distribution of AUC/MICs (assuming a MIC of 1 mg/L) in the interval (A) 48-72h after start of vancomycin treatment, (B) 72-96 h after start of vancomycin treatment

Results: other tertiary endpoints

Mean number of blood samples during vancomycin treatment were 4.2 (SD: 3.9) and 4.9 (SD: 3.5) in the standard-of-care group and intervention group, respectively (absolute difference in mean: 0.6 [95% CI: -0.3 to 1.4]; OR 1.15 [95% CI 0.95 to 1.36]) ; mean number of additional blood samples during vancomycin treatment were 2.9 (SD: 2.9) and 3.4 (SD: 2.6) in the standard-of-care group and the intervention group, respectively.

Mean number of sampling time errors in the standard-of-care group for intermittent infusions was 1.7 (SD: 2.2).

	Standard-of-Care arm (n=156)	Intervention arm (n=158)
Neonatal or paediatric RIFLE class Failure	1 (12)	2 (25)*
Vancomycin flushing syndrome	7 (88)	6 (75)
Other significant safety issue at the discretion of the investigator thought to be at least possibly related to the vancomycin dosing method	/	/

Data are n (%). RIFLE, modified Risk Injury Failure Loss End-Stage assessment criteria; *one patient died

Table 11S. Severe reportable adverse events

Deviation	Standard-of-Care group (n=158)	Intervention group (n=223)
Treatment deviation	78 (49.4%)	47 (21.1%)
Major	8	7
Minor	70	40
Incorrect or incomplete data entry in dosing calculator	1 (0.6%)	79 (35.4%)
Major	-	1
Minor	-	78
Informed consent	50 (31.6%)	54 (24.2%)
Major	4	5
Minor	46	49
Trial assessments	18 (11.4%)	10 (4.5%)
Major	1	-
Minor	17	10
Noncompliance with randomised arm	2 (1.3%)	15 (6.7 %)
Major	-	14
Minor	2	1
Eligibility criteria not met	3 (1.9%)	6 (2.7 %)
Major	3	6
Safety reporting	2 (1.3%)	2 (0.9 %)
Major	2	2
Randomisation	-	1 (0.4 %)
Minor	-	1
Other	4 (2.5%)	9 (4.0 %)
Major	-	1
Minor	4	8

Table 12S. Summary of protocol violations

Major Deviation	Standard-of-Care group (n= 17)	Intervention group (n=36)
Treatment deviation	8 (47 %)	7 (19.4 %)
intermittent dosing: the patient received wrong dose(s) outside the following limits : calculated dose plus or minus 21% or higher	2	1
intermittent dosing: the dosing interval period between infusions deviated more than 2h from the planned time (+ >2h or - >2h) and no clinical reason is documented.	2	1
intermittent dosing or loading dose in continuous infusion: the duration of the infusion was less than 1 hour, and more specific less than 30min.	2	2
intermittent dosing: the patient received wrong dose(s) within the following limits : calculated dose plus or minus 11 to 20%.	1	1
intermittent dosing: dose(s) was not administered to the patient and no clinical reason was documented (Major)	1	-
continuous infusion: no loading dose was administered in the intervention arm	-	2
Incorrect or incomplete data entry in dosing calculator	-	1 (2.7 %)
Not all baseline information was entered correctly in the dosing calculator.	-	1
Informed consent	4 (23.5 %)	5 (13.8 %)
No written ICF was obtained within the first 24h of study participation, and no oral consent was documented.	3	2
Assent not signed by the subject.	1	1
The original signed ICF is missing on site.	-	2
Trial assessments	1	-
The serum creatinine sample used as baseline was taken outside the allowed time window of 120h before planned start of vancomycin and no symptoms of acute or chronic kidney injury documented	1	-
Noncompliance with randomised arm	-	14 (38.8 %)
Subject that was restarted on vancomycin on one of the participating ward units within the 30 day study period did not receive the same study assigned method as assigned for the first period.	-	4
The dosing calculator was not used for a subject randomized in the intervention arm while in a participating ward unit	-	10
Eligibility criteria not met	3 (17.6 %)	6 (16.6 %)
Inclusion criterion "not previously enrolled in this trial" failed but the subject was randomized to the same study arm as on the first occasion.	1	-
Inclusion criterion "planned to start on intravenous intermittent (INT) or continuous infusion (CI) vancomycin treatment" failed.	2	6
Safety reporting	2 (11.7 %)	2 (5.5 %)
The SAE was not reported within the timelines as per protocol i.e. 24h after site staff awareness	2	2
Randomisation	-	-
Other	-	1 (2.7 %)
Continuous dosing: the patient received a wrong infusion rate with an AUC calculation less than 400mg/L. Only the first dose had an AUC estimation between 400-600mg/L.	-	1

Table 13S. Details on major protocol violations

Statistical Analysis Plan

BENEFICIAL: Statistical analysis plan

Administrative information

TRIAL FULL TITLE	A multicentric, randomized controlled clinical trial to study the impact of bedside model-informed precision dosing of vancomycin in critically ill children
SAP VERSION	1.0
SAP VERSION DATE	August 12 th 2024
PROTOCOL VERSION	5.0
PROTOCOL VERSION DATE	October 2 nd 2023
EUDRACT NUMBER	
CLINICALTRIALS.GOV IDENTIFIER	NCT04666948
TRIAL STATISTICIAN	Roos Colman, Biostatistics Unit, Faculty of Medicine and Health Sciences, Ghent University
TRIAL CHIEF INVESTIGATOR	Prof Dr Pieter De Cock, Department of Pharmacy, Ghent University Hospital
SAP AUTHOR	Roos Colman, Biostatistics Unit, Faculty of Medicine and Health Sciences, Ghent University
SAP REVIEWERS	Prof Dr Pieter De Cock, Department of Pharmacy, Ghent University Hospital

SAP Revision History

The statistical analysis plan (SAP) was written after the last patient had completed the study, but prior to the database being locked and final analysis beginning and prior to any comparative outcome analyses carried out. The SAP is not a standalone document and should be read in conjunction with the corresponding version of the protocol.

Version	Date	Edited by	Comments / justification	Timing in relation to unblinding for the final analyses
0.1	27/06/2024	RC	First draft	Prior

0.2	06/08/2024	RC	Second draft after meeting with the Chief Investigator and the Data Manager, and after discussions with the statistical team	Prior
1.0	12/08/2024	RC	Final version after meeting with the Chief Investigator and the Data Manager	Prior

Signature page

Signature of the SAP author: Roos Colman, senior statistician

Signature of trial chief investigator: Prof Dr Pieter De Cock

Abbreviations

AKI	Acute kidney injury
AUC	Area under the concentration-time curve
CE	Conformité Européenne (European conformity)
CI	Confidence interval
IE	Intercurrent event
ITT	Intention-to-treat
MIC	Minimum inhibitory concentration
MIPD	Model-informed precision dosing
OR	Odds Ratio
PMM	Predictive Mean Matching
SAE	Serious Adverse Event
SCr	Serum Creatinine

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Introduction

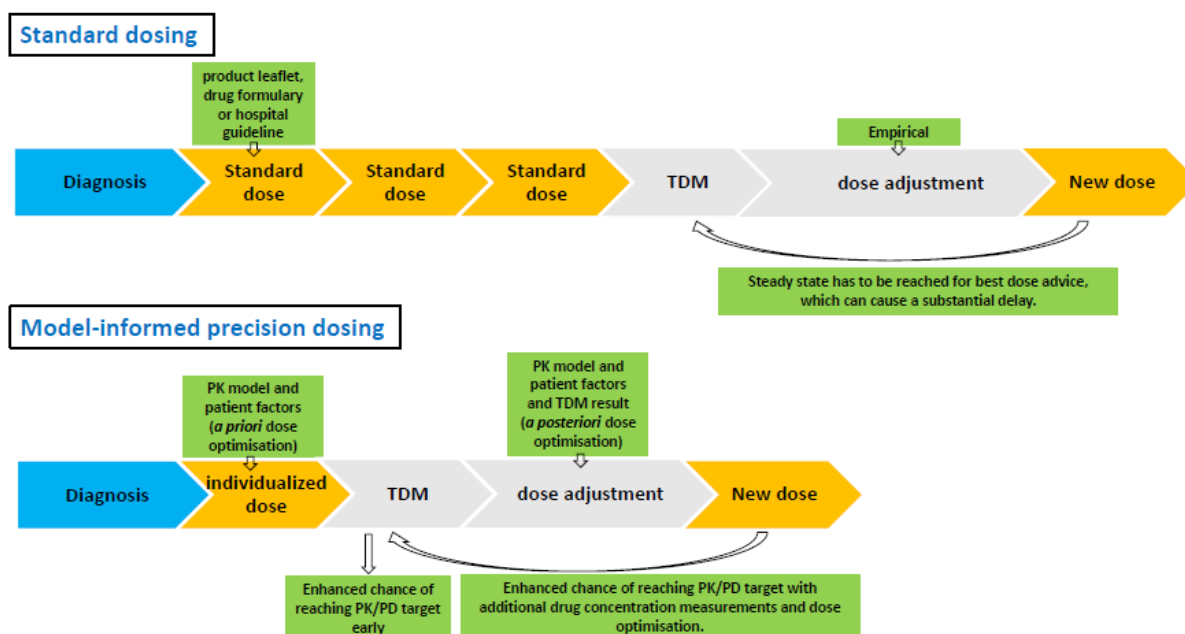
Background and rationale

Vancomycin is a commonly prescribed antibiotic to treat serious Gram-positive infections in children,

Efficacy of vancomycin is known to be directly related to the pharmacokinetic/pharmacodynamic (PK/PD) index of area under the concentration-time curve (AUC) divided by the minimal inhibitory concentration (MIC) of the pathogen (AUC/MIC). To date, the advocated steady-state 24-hour AUC/MIC for favorable clinical outcome in humans with methicillin-resistant *Staphylococcus aureus* and Enterococci infections is at least 400.⁶ Instead, a 24-hour AUC of 600 µg/L·h is known to be independently associated with increased risk of acute kidney injury (AKI), indicating its narrow therapeutic index.⁷ AKI is reported in up to 23.4% of critically ill children treated with vancomycin, with higher risks in those receiving mechanical ventilation and concomitant vaso-active and nephrotoxic drugs.⁸ It is further known that (even mild) AKI is linked to significantly increased morbidity, mortality, hospital length of stay and costs.^{9,10}

Growth, development and critical illness (including cancer) are known to have a massive impact on antibiotic PK/PD and complicate optimal drug dosing.¹¹ To date, vancomycin is commonly initiated, on a mg per kg basis, according to institutional guidelines. Both intermittent dosing and loading dose/continuous infusion dosing regimens are used. After reaching steady-state conditions (i.e. after the third or fourth dose for intermittent dosing regimens or 24h after start of a continuous infusion), therapeutic drug monitoring (TDM) is performed to ensure attainment of target concentrations.⁶ Since traditional AUC/MIC calculations cause much patient burden and are labour- and cost-intensive, in routine practice, trough (C_{min}) concentrations are measured as a 'surrogate' single-point parameter for target AUCs, just before the next intermittent dose or during infusion for continuous dosing regimens.⁶ If necessary, empiric dose adjustments are performed, aiming at a C_{min} concentration range of 10 to 15 mg/L for intermittent infusions and a concentration range of 15 to 20 mg/L for continuous infusions. (Figure 1).¹²

Figure 1 : Standard versus model-informed precision dosing (adapted from Tängden et al. Intensive Care Med. 2017;43(7):1021-1032)



The current dosing approach has a number of drawbacks. First, reported Cmin target attainment rates in critically ill children are abominable (after start: 5.7 to 62%; after dose adjustment: 44 to 63%), with several days needed to reach target concentrations.¹³⁻²³ Delay in appropriate antibiotic therapy in patients with severe sepsis and septic shock leads to increased morbidity and mortality.^{24,25} Growing evidence exists that subtherapeutic antibiotic dosing can lead to emergence of resistance of colonising bystander organisms.²⁶ Second, Cmin level monitoring as surrogate for target AUCs has its limitations as recent studies have documented a high degree of variability as well as therapeutic discordance between both.^{27, 28} In essence, current evidence suggests that Cmin monitoring in children frequently results in supratherapeutic AUCs, and increased risk of nephrotoxicity.^{6,7} Third, multiple blood drawings for TDM and biochemical tests may lead to iatrogenic anemia, increased patient burden, infection risk and healthcare costs.^{29,30} Fourth, poor adherence to proper Cmin timing of sampling is common and may lead to erroneous dose adjustments, therapy failure and toxicity.³¹

PK modelling involves the development of mathematical models to describe a drug PK behavior. Once available, these models can be used to predict in the drug PK in the individual patient. In the Bayesian approach, estimates of an individual patient's PK parameters (e.g. clearance and volume of distribution), are provided using the PK model and patient characteristics (e.g. weight, age, renal function) known as prior information. Then, after obtaining a single level, a revision of PK parameter estimates is provided. These PK estimates, referred to as the Bayesian conditional posteriors, can be used to estimate a patient-specific AUC.

In recent decades, a priori Bayesian (without TDM) and a posteriori (in combination with TDM measurement(s)) model-informed precision dosing (MIPD) has extensively been used to ensure efficacious antibiotic treatment with significant success (Figure 1).^{12,31} For vancomycin, Bayesian AUC guided MIPD in adults has been shown to lead to significantly decreased nephrotoxicity, lower total vancomycin doses, reduced per-patient blood sampling, and shorter length of therapy,

without compromising efficacy, when compared to trough-only monitoring.^{6,7,32} In critically ill neonates, MIPD guided starting doses increased early target attainment from 41 to 72% without any case of vancomycin-related toxicity.³³

Besides improved clinical outcome, most modern Bayesian MIPD is more “patient- and caregiver-friendly” by allowing AUC estimation using one blood sample taken at a most convenient time point. Moreover, this approach implies that vancomycin concentrations can be measured on left-over samples obtained from daily clinical care (i.e. opportunistic or scavenged sampling).^{6,34}

Despite vancomycin dosing in critically ill children largely meets the criteria to result in high-impact MIPD using an AUC/MIC targeted strategy (Figure 2), its adoption at point-of-care (POC) is still lacking in Belgium.^{31,42} This low uptake is due to the lack of high-level clinical evidence on its clinical impact and the absence of validated, easy-to-use, clinician proved software tools.⁶

Objectives

Primary objective

To assess if AUC/MIC based vancomycin dosing, using a model-informed precision dosing calculator, is superior compared to standard-of-care dosing regimens with therapeutic drug monitoring

with respect to the proportion of patients with favorable outcomes, defined as reaching the therapeutic target 24h AUC/MIC (400-600 mg*h/L) between [24 to 48] h after start of treatment,

in critically ill children, who are admitted to neonatal and pediatric intensive care and pediatric hemo-oncology ward units across Belgium and planned to start on intravenous intermittent or continuous infusion vancomycin treatment.

Secondary objectives

This study also aims to test the following secondary hypotheses in critically ill children, who are admitted to neonatal and pediatric intensive care and pediatric hemo-oncology ward units across Belgium and planned to start on intravenous intermittent or continuous infusion vancomycin treatment.

- AUC/MIC based vancomycin dosing, using a model-informed precision dosing calculator, reduces the proportion of patients with (worsening) acute kidney injury during treatment with vancomycin, when compared to the use of standard-of-care dosing regimens with therapeutic drug monitoring;
- AUC/MIC based vancomycin dosing, using a model-informed precision dosing calculator, increases the proportion of patients reaching the therapeutic target 24h AUC/MIC (400-600) between [48-72] hours after start of treatment, when compared to the use of standard-of-care dosing regimens with therapeutic drug monitoring;

- AUC/MIC based vancomycin dosing, using a model-informed precision dosing calculator reduces the time to clinical cure, when compared to the use of standard-of-care dosing regimens with therapeutic drug monitoring;
- AUC/MIC based vancomycin dosing, using a model-informed precision dosing calculator reduces the ward unit length-of-stay, when compared to the use of standard-of-care dosing regimens with therapeutic drug monitoring;
- AUC/MIC based vancomycin dosing, using a model-informed precision dosing calculator reduces the hospital length-of-stay, when compared to the use of standard-of-care dosing regimens with therapeutic drug monitoring;
- AUC/MIC based vancomycin dosing, using a model-informed precision dosing calculator reduces all cause 30-day mortality, when compared to the use of standard-of-care dosing regimens with therapeutic drug monitoring;

Tertiary objectives

This study also aims to test the following tertiary hypotheses drug in critically ill children, who are admitted to neonatal and pediatric intensive care and pediatric hemo-oncology ward units across Belgium and planned to start on intravenous intermittent or continuous infusion vancomycin treatment:

- AUC/MIC based vancomycin dosing, using a model-informed precision dosing calculator reduces the number of (additional) blood samples, when compared to the use of standard-of-care dosing regimens with therapeutic drug monitoring;
- AUC/MIC based vancomycin dosing, using a model-informed precision dosing calculator reduces the cumulative number of (additional) blood samples during treatment in patients with clinical cure, when compared to the use of standard-of-care dosing regimens with therapeutic drug monitoring;
- AUC/MIC based vancomycin dosing, using a model-informed precision dosing calculator, increases the proportion of patients reaching the therapeutic target 24h AUC/MIC (400-600) between [72-96] h after start of treatment, when compared to the use of standard-of-care dosing regimens with therapeutic drug monitoring;
- AUC/MIC based vancomycin dosing, using a model-informed precision dosing calculator reduces the number of dose adjustments (24h AUC/MIC between 400-600 in the interventional arm; target concentrations for intermittent and continuous dosing regimens according to institutional guidelines) during treatment, when compared to the use of standard-of-care dosing regimens with therapeutic drug monitoring;
- AUC/MIC based vancomycin dosing, using a model-informed precision dosing calculator reduces the cumulative vancomycin dose and corresponding AUC during vancomycin treatment, when compared to the use of standard-of-care dosing regimens with therapeutic drug monitoring;
- AUC/MIC based vancomycin dosing, using a model-informed precision dosing calculator, reduces the proportion of patients with (worsening) acute kidney injury as assessed using KDIGO criteria, during treatment with vancomycin, when compared to the use of standard-of-care dosing regimens with therapeutic drug monitoring;

Finally, we also aim to measure a trough sampling error rate in the comparator arm.

The collected data are available for continuous PK/PD model refinement if necessary.

Endpoints

Primary endpoint

Reaching a 24h AUC/MIC between 24h to 48h after start of treatment that falls within the therapeutic target range of 400-600 mg*h/L.

The 24h AUC/MIC between 24h to 48h after start of treatment is estimated using a model with low prior weight 0.3 fitted and using all available blood samples.

Sensitivity endpoints

Reaching a 24h AUC/MIC between 24h to 48h after start of treatment that falls within the therapeutic target range of 400-600 mg*h/L:

- The 24h AUC/MIC between 24h to 48h after start of treatment is estimated using a model with prior weight 0.5 and using all available blood samples.
- The 24h AUC/MIC between 24h to 48h after start of treatment is estimated using a model with low prior weight 0.3 fitted and using all available blood samples collected after the first 6 hours after start of the vancomycin treatment.

Secondary endpoints

Secondary endpoints for safety:

- (Worsening) acute kidney injury during treatment with vancomycin
- 30-day all-cause mortality

Key secondary endpoints:

- Time to clinical cure, defined as the time in days from randomization to completion of the vancomycin treatment, without recommencement of antibiotics for the same indication within 48h after stop. Patients who do not experience clinical cure, will be censored at 30 days, the pre-defined follow-up time. Patients who die before having experienced clinical cure (while receiving antibiotic therapy for the initial indication as vancomycin was started or where antibiotic therapy is ceased in the setting of palliative care), will also be censored at 30 days.
- Reaching a 24h AUC/MIC between 48h to 72h after start of treatment that falls within the therapeutic target range of 400-600 mg*h/L.

Other secondary endpoints:

- Ward unit length-of-stay, defined as the number of days between randomisation and discharge to another ward unit
- Hospital length-of-stay, defined as the number of days between randomisation and hospital discharge

Exploratory endpoints

- Number of (additional) blood samples during vancomycin treatment
- Cumulative number of (additional) blood samples during treatment in patients with clinical cure
- Proportion of patients reaching target 24h AUC/MIC between [72-96]h after start vancomycin treatment
- Number of dose adjustments during vancomycin treatment

- Cumulative vancomycin dose during vancomycin treatment
- Cumulative AUC during vancomycin treatment
- (worsening) AKI using KDIGO criteria during vancomycin treatment
- Number of trough sampling time errors during vancomycin treatment (in the comparator arm)

Harms

The following SAEs occurring until 48h after last vancomycin study administration (maximum D30+48h in case vancomycin is administered on day 30 after randomisation):

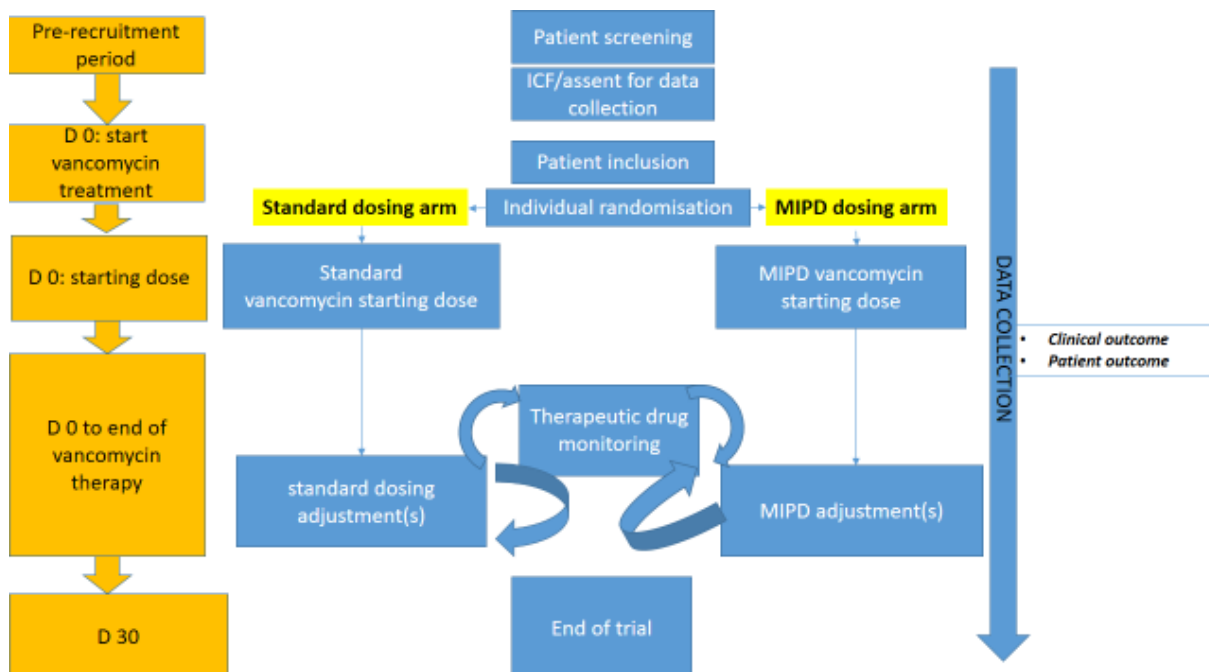
- Neonatal or pediatric RIFLE class category failure
- vancomycin flushing syndrome (also known as red man syndrome)
- Other significant safety issues (including death) at the discretion of the investigator thought to be at least possibly related to the vancomycin dose calculation

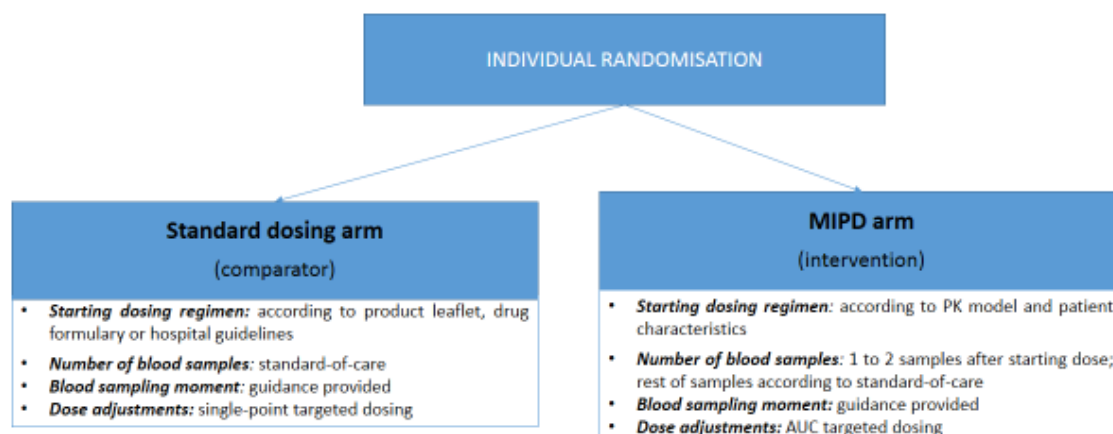
Study Methods

Trial design

- Prospective
- Multicentric: 14 ward units in university and large non-university hospitals in Belgium
- Parallel group
- Randomized: randomization occurs at the subject level with a 1:1 allocation ratio
- Superiority

Figure 2: Schematic trial design:





Brief description of the intervention (MIPD arm):

AUC/MIC-based model-informed precision dosing of vancomycin using a CE labelled dosing calculator.

Randomization

Patients were randomly allocated to the intervention arm or standard-of-care arm at inclusion. The study is a parallel-group RCT with a 1:1 allocation ratio. Randomization was stratified by ward unit and hence by ward unit type (NICU, PICU, PHO). The allocation table was created in SAS v9.4. Random permuted blocks with variable sizes were used to avoid that the treatment allocation could be predicted.

The stratified randomization was performed using the randomization module of REDCap (Research Electronic Data Capture). REDCap is a secure, web-based application designed to support data capture for research studies. This web-based software programme enables randomization by site and other stratification factors. The REDCap software randomized patients based on the allocation table.

Before a patient was randomized, the baseline characteristics (at least the stratification factor ward unit) of the patient were entered in REDCap.

Sample size

Primary outcome: Proportions of patients reaching the therapeutic target 24h AUC/MIC (400-600 mg*h/L) between [24 to 48] h after start of treatment

Sample size calculation for the primary analysis was performed in SAS version 9.4 using the power procedure.

The sample size calculation was based on the assumption that the proportion of patients reaching the therapeutic target 24h AUC/MIC (400-600 mg*h/L) between [24 to 48] h after start of treatment is 50% in the standard-of-care dosing group and 70% in the AUC/MIC based vancomycin dosing

group. Since patients are randomized individually, patients of both groups (standard-of-care and AUC/MIC based vancomycin dosing) will be treated in the same ward. Potentially, a learning effect will cause contamination.

As suggested in Torgerson DJ (2001) sample size was increased by taking into account some contamination. When assuming 20% contamination from intervention to control, and 5% contamination from control to intervention, a dilution of the odds ratio (OR) is expected from 2.33 (70% versus 50%) to 1.90 (69% versus 54%).

When it became apparent that the sample size corresponding to this diluted OR could not be met within the project timelines, an analysis was performed after inclusion of 299 patients, to assess the initial contamination rates stated above. This analysis indicated there was an overestimation of contamination rates in the initial sample size calculation. We observed only 4% contamination from intervention to control, and 2.3% contamination from control to intervention. Based on these observations, we lowered the contamination rates assumed for the sample size with 25%, taking into account uncertainty around the observed contamination rates and the potential learning effect, without impacting power of the study. Hence, the assumed contamination rates were lowered from 20% to 15% for intervention-to-control contamination, and from 5% to 3.75% for control-to-intervention contamination.

When assuming 15% contamination from intervention to control, and 3.75% contamination from control to intervention, a dilution of the OR is expected from 2.33 (70% versus 50%) to 2.0 (69.25% versus 53%).

In total, 281 participants are required to achieve 80% power at a two-sided significance level of 5%, to detect an OR of 2.0 ($b = 0.69$) when the proportion in the control group is 53% (odds=1.13, intercept= 0.12) using logistic regression. An assignment ratio of 1 will be applied.

Assuming a drop-out rate of 15%, a total sample size (both groups) of 332 patients is required.

Proportion of patients with (worsening) AKI during vancomycin treatment

The heterogeneity in reported AKI rates in patients treated with vancomycin is high but prevalences of 23.4% in critically ill children treated with vancomycin have been reported, with higher risks in those receiving mechanical ventilation and concomitant vaso-active and nephrotoxic drugs (Bonazza (2018)). Furthermore, it is known that trough concentrations >15 mcg/L and $AUC \geq 800$ mcg*h/L are independently associated with a >2.7 -fold increased risk of AKI (Fiorito (2018); Le (2014)). We expect a large proportion of our patients in the control arm to have trough concentrations >15 mcg/L and $AUC \geq 800$ mcg*h/L.

When assuming 15% contamination from intervention to control, and 3.75% contamination from control to intervention, the OR is expected to dilute from 0.219 (4% versus 16%) to 0.281 (4.45% versus 14.2%). A total sample size of 281 participants yields 84% power at the two-sided 5% significance level, to detect an OR of 0.28 ($b = -1.27$) when the proportion in the control group is 14.2% (odds=0.166, intercept= -1.80) using logistic regression. An assignment ratio of 1 will be applied.

Framework

This is a superiority trial to compare the intervention arm (MIPD) with the standard-of-care arm.

Statistical interim analyses and stopping guidance

No interim analysis was planned for this study and no efficacy interim analysis was performed. As described in the protocol and the sample size section of this document, initial assumed contamination rates were reassessed and sample size was adjusted.

Timing of final analysis

Final analyses will be performed after:

- All subjects completed the study as described in the protocol
- Data cleaning has been completed and final database is locked by the Data Manager
- All criteria for unblinding the randomization codes to the statistician have been met.

Timing of outcome assessments

Timing of outcome and other assessments:

Table 1: Timing of trial procedures

	Screening	Baseline	Day 0 (randomization date)→ end of vancomycin treatment	Day 0→ D30
Eligibility assessment	X			
Informed consent	X			
Patient characteristics		X		
Pathogen characteristics				X
Vancomycin dosing			X	
Concomitant nephrotoxic and vasopressor medication			X	
Concomitant antibiotic use				X
Blood sampling			X	
Weight			X	
Urine output			X	
Serum creatinine			X	
pRIFLE category	X		X	
Time to clinical cure				X
Ward unit stay				X
Hospital length of stay				X

Mortality				X
Cost item assessments			X	
Safety assessments			X	X
Compliance	X	X	X	X
End of trial assessments				X

Statistical Principles

Confidence intervals and P values

Analyses are performed at the two-sided 5% significance level. All reported confidence intervals (CIs) will be 95% CIs.

If the primary analysis is significant, the results of the analyses for the secondary endpoints will be reported, including the p-values and estimates with 95% CIs. The significance level of the analyses for the secondary endpoints will be adjusted using the Hochberg procedure for multiple testing, except for the secondary outcomes for safety.

If the primary analysis is not significant, the analyses of the secondary endpoints (except the secondary outcomes for safety) shall be considered as hypotheses-generating (not conclusive) and interpreted with care. In this case, only estimates with 95% CIs will be provided, but no p values will be reported.

The results of the analyses of the secondary outcomes for safety will be reported, including the p-values and estimates with 95% CIs, regardless of the results of the primary analysis. No adjustments for multiplicity are planned for “(worsening) acute kidney injury” and “30-day all-cause mortality”. They will be considered as distinct outcome comparisons, as strong effects on safety outcomes must be taken seriously, regardless of the number of comparisons performed. Individual interpretations for each comparison will be made (Parker 2022).

The analyses of the tertiary outcomes will be considered as exploratory.

Adherence and Protocol deviations

As stipulated in the protocol, protocol deviations are documented and reported on the protocol deviation log. Any deviation that potentially interferes with and/or affects the efficiency and/or quality conduct of the study is discussed by the monitor with the PI and will be documented on the protocol deviation log as well on the monitoring report including a proposed plan of action for resolution if applicable.

The number of major and minor protocol deviations concerning Eligibility criteria, Informed Consent, Randomization, Trial assessments, Treatment deviation, Noncompliance with randomized arm, Incorrect or incomplete data entry in the dosing calculator, Withdrawal criteria, Safety reporting and others will be summarized will be reported by allocated treatment arm.

Analysis sets

Intention-to-treat analysis set

The intention to-treat (ITT) analysis set consists of all patients randomized, except when randomization of a patient was based on referred consent which was not followed by an informed consent and no subject information may be used. Subjects are analyzed according to the allocated treatment group irrespective of their compliance with the planned course of treatment. The ITT analysis set is considered the main analysis set for analysis of efficacy data and the secondary endpoints for safety to assess superiority.

Protocol-compliant analysis set

All subjects of the ITT analysis set are included in the protocol-compliant analysis set except those for whom any serious deviation with major impact on safety or the primary endpoint was reported. As described in the analysis section, the analysis in the protocol-compliant analysis set will be replaced by the Protocol – compliant estimand analysis.

Safety analysis set

The safety analysis set consists of all subjects who received at least one dose of vancomycin. For the analysis of adverse events, the subjects in the safety analysis set are grouped by received treatment. The safety analysis set is considered the main analysis set for analysis of adverse events.

Intercurrent events (IEs)

The following intercurrent events (IE) are under consideration:

- Death
- Discontinuation of vancomycin treatment because of clinical cure
- Discontinuation of vancomycin treatment because of adverse event
- Discontinuation of vancomycin treatment because of clinical failure
- Discontinuation of vancomycin treatment because of change in antimicrobial therapy within 48 after start vancomycin
- Patient-treatment focus changed to palliation
- Deferred consent not followed by an informed consent to continue the study, but with an informed consent for use of already collected data.
- Treatment switch between MIPD and standard treatment (or vice versa)
- Early discontinuation of calculator
- Major protocol deviation with possible impact on primary endpoint: only considered as an IE for the primary objective, considered irrelevant for the other objectives (equivalent to treatment policy)

Estimands

The ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials was not taken into account when the protocol was written and is not reflected in the study protocol. However, the SAP was written taking this guideline into account and the estimand for the primary outcome is defined in this SAP even though it was not defined in the protocol.

Estimands for the primary objective

Three estimands will be estimated for the primary objective.

Main estimand and additional estimand

The main estimand and additional estimand only differ from each other in their measure to compare the likelihood of a favorable outcome between treatment conditions: a conditional OR will be used for the main estimand and an unconditional relative risk for the additional estimand.

Protocol – compliant estimand

The “protocol-compliant estimand” will estimate the unconditional relative risk of reaching the primary endpoint under the presumptions that the following intercurrent events would not have occurred:

- Early discontinuation of calculator
- Treatment switch between MIPD and standard treatment (or vice versa)
- Serious protocol deviation with impact on primary endpoint

and that these patients had followed the pattern of the other patients in the protocol-compliant sample (hypothetical strategy). This analysis will be performed instead of the analysis in the protocol-compliant sample, which was defined in the protocol.

Table 2. Attributes of the estimands for the primary objective

	Main estimand	Additional estimand	Protocol – compliant estimand
Population	Critically ill children, who are admitted to neonatal and pediatric intensive care and pediatric hemo-oncology ward units across Belgium and planned to start on intravenous intermittent or continuous infusion vancomycin treatment = all randomized subjects with written consent to start or continue the study or to use the data already collected		
Outcome variable	Favorable outcome = Reaching a 24h AUC/MIC between 24h to 48h after start of treatment that falls within the therapeutic target range of 400-600 mg*h/L		
Treatment	Comparator = standard-of-care dosing regimens Experimental = AUC/MIC based vancomycin dosing		
Comparison between treatment conditions	Conditional Odds Ratio	Unconditional Relative Risk	Unconditional Relative Risk
Intercurrent events (if Occurring within 48h after start vancomycin)			
Death	Composite strategy – Subjects who die will be considered to have an unfavorable outcome		
Discontinuation of vancomycin treatment because of clinical cure	Composite strategy – Subjects will be considered to have a favorable outcome		
Discontinuation of vancomycin treatment because of adverse event	Composite strategy – Subjects will be considered to have an unfavorable outcome		

Discontinuation of vancomycin treatment because of clinical failure	Composite strategy – Subjects will be considered to have an unfavorable outcome	
Patient treatment focus changed to palliation	While on treatment strategy – Estimated AUC prior to the discontinuation of vancomycin treatment The “while on treatment strategy” is not expected to cause bias because 1) The occurrence of the intercurrent event is not expected to differ between the treatments being compared (the occurrence of this ICE will be reported by treatment arm) 2) Reaching the target earlier than the 24h to 48h interval is more difficult	
Discontinuation of vancomycin treatment because of change in antimicrobial therapy within 48h after start vancomycin	While on treatment strategy – Estimated AUC prior to the discontinuation of vancomycin treatment The “while on treatment strategy” is not expected to cause bias because 1) The occurrence of the intercurrent event is not expected to differ between the treatments being compared (the occurrence of this ICE will be reported by treatment arm) 2) Reaching the target earlier than the 24h to 48h interval is more difficult	
Deferred consent not followed by an informed consent to continue the study, but with an informed consent for use of already collected data.	Hypothetical strategy – What would have happened if these subjects did not have this IE?	
Treatment switch (MIPD and standard-of-care or vice versa)	Treatment policy strategy – Observed outcomes are used regardless of whether or not this IE occurred	Hypothetical strategy – What would have happened if these subjects did not have this IE?
Early discontinuation of calculator	Treatment policy strategy – Observed outcomes are used regardless of whether or not this IE occurred	Hypothetical strategy – What would have happened if these subjects did not have this IE?
Serious protocol deviation with possible impact on primary endpoint	Treatment policy strategy – Observed outcomes are used regardless of whether or not this IE occurred	Hypothetical strategy – What would have happened if these subjects did not have this IE?

Estimands for the secondary objectives

The secondary endpoint “Proportion of patients reaching target 24h AUC (400-600 mg*h/L) between [48 to 72] h after start treatment” and the tertiary endpoint “Proportion of patients reaching target 24h AUC (400-600 mg*h/L) between [72 to 96] h after start treatment” are analyzed analogously as described in Table 2.

The attributes of estimands for further objectives are described below (Table 3). Depending on the endpoint, discontinuation of vancomycin treatment will be handled using the treatment policy strategy or the while on treatment strategy.

Table 3: Estimands for the secondary objectives

Estimand	Population	Strategy for accounting intercurrent events	Endpoints	Population summary
Treatment policy	Critically ill children, who are admitted to neonatal and pediatric intensive care and pediatric hematology ward units across Belgium and planned to start on intravenous intermittent or continuous infusion vancomycin treatment	Treatment policy strategy (the data collected for the variable of interest are used regardless of whether or not this intercurrent event occurs): - All intercurrent events for which the endpoint could be observed Hypothetical strategy (censoring) (What would have happened if these subjects did not have this IE?): - Deferred consent not followed by an informed consent to continue the study, but with an informed consent for use of already collected data. Composite strategy: - Death (unfavorable outcome) For the key secondary endpoint “time to clinical cure”, the intercurrent event “Patient-treatment focus changed to palliation” is treated as a competing risk. For “All cause 30-day mortality”, “Ward unit length-of-stay” and “Hospital length-of-stay”, treatment policy is applied.	Secondary endpoint for safety: - All cause 30-day mortality	Odds Ratio conditional on ward type and unconditional Relative Risk
			Key secondary endpoint: - Time to clinical cure	Hazard Ratio
			Secondary: - Ward unit length-of-stay - Hospital length-of-stay	Relative difference

While on treatment		While on treatment strategy: - Discontinuation of vancomycin treatment Composite strategy: - Death (unfavorable outcome) Treatment policy strategy: - Treatment switch (MIPD and standard-of-care or vice versa) - Early discontinuation of calculator Hypothetical strategy: - Deferred consent not followed by an informed consent to continue the study, but with an informed consent for use of already collected data.	Secondary endpoint for safety: - AKI Tertiary: - AKI using KDIGO criteria	Odds Ratio conditional on ward type and unconditional Relative Risk (for AKI)
		While on treatment strategy: - Discontinuation of vancomycin treatment - Death Treatment policy strategy: - Treatment switch (MIPD and standard-of-care or vice versa) - Early discontinuation of calculator Hypothetical strategy: - Deferred consent not followed by an informed consent to continue the study, but with an informed consent for use of already collected data.	- Number of (additional) blood samples during vancomycin treatment - Cumulative number of (additional) blood samples during treatment in patients with clinical cure - Number of dose adjustments during vancomycin treatment - Cumulative vancomycin dose and AUC during vancomycin treatment	Relative difference

	Critically ill children, who are admitted to neonatal and pediatric intensive care and pediatric hematology ward units across Belgium and planned to start on intravenous intermittent or continuous infusion vancomycin treatment randomized to the standard treatment.	While on treatment strategy (while on standard treatment): - Discontinuation of vancomycin treatment - Death Hypothetical strategy: - Treatment switch - Deferred consent not followed by an informed consent to continue the study, but with an informed consent for use of already collected data. Not applicable (only standard treatment arm is considered): - Early discontinuation of calculator	- Number of trough sampling time errors during vancomycin treatment	Estimated count in control group
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Trial Population

Screening data

Screening data were not collected.

Eligibility

The following patient eligibility criteria are defined in the protocol:

Inclusion criteria

- age: 0-18 years
- admitted to ICU or PHO unit
- suspected or confirmed Gram positive infection
- planned to start on intravenous intermittent (INT) or continuous infusion (CI) vancomycin treatment (if the patient was treated with vancomycin before inclusion: the minimum interval to previous vancomycin treatment episode is 48 hours)
- informed consent signed by parents or legal representatives
- not previously enrolled in this trial. note: a patient restarted on vancomycin within the 30-day study period is considered not a new enrollment and should receive the treatment assigned for the first episode)

Exclusion criteria

- extracorporeal treatment at inclusion (extracorporeal membrane oxygenation, dialysis, body cooling)
- n or p RIFLE category failure at inclusion (Day 0)
- Known chronic kidney disease as defined by the KDIGO definition as: structural or functional abnormalities of the kidney regardless of GFR for < 3 months in duration or GFR < 60ml/min/1.73m² for ≥ 3 months in duration. eGFR is estimated using the modified Schwartz equation
- patient death is deemed imminent and inevitable

Recruitment and flow diagram

The CONSORT flow diagram will contain the following information:

Enrollment:

- Number of subjects randomized
- Number of subjects by type of consent at randomization:
 - o A priori consent
 - o Deferred consent

Allocation (by randomization arm):

- Allocated to intervention (n=)

- Received allocated intervention (n=)
- Did not receive allocated intervention (n=)
 - o Reasons for not receiving allocated intervention (n=)
 - Withdrawal of informed consent (n=)
 - Protocol deviation (n= , specify)

Withdrawal / Follow-up

Discontinuation of the intervention:

The number of subjects for which discontinuation of the intervention occurred within 48 after start of vancomycin treatment will be reported by randomization arm, together with the reason for discontinuation:

- Death
- Discontinuation of vancomycin treatment because of clinical cure
- Discontinuation of vancomycin treatment because of adverse event
- Discontinuation of vancomycin treatment because of clinical failure
- Discontinuation of vancomycin treatment because of change in antimicrobial therapy within 48 after start vancomycin
- Patient-treatment focus changed to palliation
- Early discontinuation of calculator:
 - o Moved to another ward unit not participating in the study
 - o Patient transferred to a ward unit in a hospital not participating in the study
 - o Patient discharged from hospital to home
- Patient requiring renal replacement therapy
- Patient requiring extracorporeal membrane oxygenation treatment
- Patient developing acute kidney injury RIFLE class failure
- Treating physician's decision due to other safety reasons than kidney injury
- Deferred consent not followed by an informed consent to continue the study, but with an informed consent for use of already collected data.
- Other reason (will be specified)

Lost to follow-up

The number of subjects for which the primary endpoint could not be calculated will be reported, together with the reason for not calculating the primary endpoint.

Analysis

For the statistical analyses for the primary and secondary objectives, the number of subjects in each intercurrent event category (as described in Table 2 and Table 3) will be reported.

Baseline patient characteristics

Summary of baseline data and flow of patients

The following variables will be summarised at baseline by treatment group:

Table 4: Baseline characteristics at ward unit level

Type of ward (NICU, PICU PHO)	Categorical
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Table 5: Demographic data at patient level

- Age of the participant (years, weeks, days) - for children <6 month (182 days) of age: gestational age if retrievable (otherwise it will be replaced to a gestational age of 39 weeks if needed for MIPD dose calculation) and postnatal age; - for children ≥6 months (183 days) of age: postnatal age	Continuous
- Weight (kg, g): - birth weight and current weight for children <1 month (30 days) of age; - current weight for children ≥1 month (31 days) of age	Continuous
Height (cm)	Continuous
Gender (M, V)	Categorical

Table 6: Baseline characteristics at patient level

Date and time of entering the trial	Date
Admission diagnosis	Categorical
Date of admission to hospital	Date
Date of admission to ward unit	Date
Mechanical ventilation support	Categorical
Vaso-active medication support	Categorical
Indication for vancomycin treatment	Categorical
Documented infection at baseline	Dichotomous

Summary tables (descriptive statistics and/or frequency tables) will be provided for all baseline variables, efficacy variables, and safety variables. Continuous variables will be summarized with descriptive statistics (n, mean, standard deviation (except for age), range, median, p25 and p75).

Dates will be reported as min, max and median. Frequency counts and proportions of subjects within each category will be provided for categorical data.

No formal comparison of baseline characteristics between randomization arms will be performed, as significant differences will most likely be the result of chance.

Analysis

Outcome definitions

Primary outcome

The primary outcome is “reaching the therapeutic target 24h AUC/MIC (400-600 mg*h/L) between [24 to 48] h after start of treatment”

Or, more precisely:

“Reaching the therapeutic target 24h AUC/MIC (400-600 mg*h/L) between [24 to 48] h after start of treatment, or, if the vancomycin treatment is discontinued earlier, reaching the therapeutic target in the latest available interval”.

When the duration of the first vancomycin episode is at least 48 hours:

Scenario 1. For patients with a duration of the first vancomycin episode of at least 48 hours, the exact integrated AUC between 24 and 48 hours is used.

When the duration of the first vancomycin episode is less than 48 hours, and the while on treatment strategy is applied (intercurrent events “early discontinuation of vancomycin treatment” and “Patient treatment focus changed to palliation”):

- **Scenario 2 (while on treatment).** For patients with a duration of the first vancomycin episode between 24h and 48h: take the duration of the first vancomycin episode and look back 24 hours (duration of the first vancomycin episode minus 24 hours): the exact integrated AUC between those two time points is used.
- **Scenario 3 (while on treatment).** For patients with a duration of the first vancomycin episode less than 24 hours:
 - For intermittent dosing, the last known dose interval before <24 hours is used. For continuous infusion, the last 6 hours of infusion are used.
 - The 24h AUC is then calculated as described in “Vancomycin AUC24 Explained” (Sanford Guide):
 - Calculate the integrated AUC within that dosing interval
 - Recalculate AUC to a 24h interval: For example, for a 6 hours interval: $AUC_{24} = \frac{AUC_6}{6} \times 24$

The duration of first vancomycin episode is calculated as:

- The duration from the start of the first vancomycin dose to the start time of the last dose (of the first episode) + 6 hours in the case of intermittent dosing.

- The duration from start of the first vancomycin dose to the stop time of infusion of last dose (of the first episode) in the case of continuous infusion.

The AUC is calculated using the population PK model from in Colin et al (2019). A flattened prior of 30% is applied, which gives more weight (70%) to the individual concentration measurements from patients in the Beneficial trial. Appendix A contains the coding and a more detailed description of the AUC calculation.

Primary outcome definition for MIPD sensitivity analysis 1

For the primary analysis, low model prior weight (0.3) will be used to estimate the AUC. To assess the sensitivity of the results to this choice, a first MIPD sensitivity analysis with a model prior weight of 0.5 will be performed.

Primary outcome definition for MIPD sensitivity analysis 2

Due to the nature of the intervention being the use of an MIPD calculator in combination with early sampling, the number of available early samples will be higher in the intervention group compared to the control group.

While these samples provide useful information to estimate the primary endpoint (reaching the target AUC/MIC between 24h and 48h after start vancomycin) more precisely, this availability of samples will be imbalanced between both treatment groups and holds a risk for possible bias. Therefore, a second MIPD sensitivity analysis will be performed where all early samples are omitted. Early samples are considered as samples taken within 6 hours after the start of the vancomycin treatment.

(worsening) AKI during vancomycin treatment

AKI classes are defined according to the neonatal (n) and pediatric (p) Risk Injury, Failure, Loss (RIFLE) criteria as shown in Table 7.

The nRIFLE are used for children <1 month of age (30 days); pRIFLE criteria are used in children ≥1 month of age (31 days). For eGFR, the modified bedside Schwartz formula is used: $0.413 * (\text{height/serum creatinine})$ (in mL/min/1.73m²).

AKI is categorized in children 1 month and older if at least one of the criteria on serum creatinine, eGFR or urine output is attained. When the three criteria result in different stages of AKI, the higher stage is chosen. If height could not accurately be collected, only the fold rise criterion for creatinine is to be used (and not the eGFR decrease criterion).

Table 7: Neonatal and pediatric Risk Injury, Failure, Loss AKI criteria

Legend: ESRD, end-stage renal disease, fold rise is calculated whenever the most recent value of serum creatinine is higher than the baseline value using the formula Y/X with X the original value and Y the most recent value

p RIFLE			n RIFLE	
Class	Creatinine based classification	Urine output	Class	Urine output
<i>Risk</i>	1,5-to-2-fold rise in baseline serum creatinine or eGFR decrease by 25%	<0.5mL/kg/h for 8 h	<i>Risk</i>	<1.5 mL/kg/h for 24 h

<i>Injury</i>	2-to-3-fold rise in baseline serum creatinine or eGFR decrease by 50%	<0.5 ml/kg/h for 16h	<i>Injury</i>	<1 ml/kg/h for 24h
<i>Failure</i>	3-fold rise in baseline serum creatinine or eGFR decrease by 75%	<0.3 ml/kg/h for 24h or anuria for 12h	<i>Failure</i>	<0.7 ml/kg/h for 24h or anuria for 12h
<i>Loss</i>	Failure >4 weeks		<i>Loss</i>	Failure >4 weeks
<i>ESRD</i>	persistent failure > 3 months		<i>ESRD</i>	persistent failure > 3 months

30-day mortality

Time frame: date of randomization – 30 days after randomisation

Time to clinical cure

Time to clinical cure is defined as the time interval (in days) from randomization to completion of the vancomycin treatment, without recommencement of antibiotics for the same indication within 48h after stop. De-escalation or escalation of vancomycin for the same indication is considered as part of the antibiotic treatment course.

Definition of de-escalation:

- (1) Discontinuation of one or more antimicrobial of the empirical combination therapy which were considered to be no (or no longer) necessary for treatment of the infection (e.g., stopping vancomycin on day 2 following initial treatment of piperacillin-tazobactam combined with vancomycin)
- (2) Replacement of an antimicrobial agents by another drug with the intention to narrow the spectrum of activity (replacement of vancomycin by flucloxacillin)

Patients who do not experience clinical cure, will be censored at the 30 days, the maximum follow up time. Patients who die before having experienced clinical cure (while receiving antibiotic therapy for the initial indication as vancomycin was started or where antibiotic therapy is ceased in the setting of palliative care), will also be censored at 30 days.

Timeframe: date of randomization – stop date treatment or 30 days after randomization (what comes first)

Reaching target 24h AUC (400-600 mg*h/L) between [48 to 72] h after start treatment

More precisely this outcome was defined as:

“Reaching the therapeutic target 24h AUC/MIC (400-600 mg*h/L) between [48 to 72] h after start of treatment, or, if the vancomycin treatment is discontinued earlier, reaching the therapeutic target in the latest available interval”.

This outcome is calculated analogously to the primary outcome. No MIPD sensitivity analysis will be performed for this outcome.

Ward unit length-of-stay

Number of days from randomization to discharge from ward unit.

Patients who are not discharged from ward unit within 30 days will be censored at 30 days, the maximum follow up time . Patients who die before discharge from ward unit will also be censored at 30 days.

Timeframe: date of randomization– date of discharge to another ward unit, with a maximum of 30 days

Hospital length-of-stay

Number of days from randomization to hospital discharge.

Patients who are not discharged from hospital within 30 days will be censored at 30 days, the maximum follow up time .

Patients who die before hospital discharge will also be censored at 30 days.

Timeframe: date of randomization – date of hospital discharge, with a maximum of 30 days

Number of (additional) blood samples to during vancomycin treatment

Both the total number of blood samples and the additional number of blood samples will be analyzed.

An additional blood sample is defined as a sample for vancomycin TDM taken at another timepoint of routine biochemical monitoring samples.

Timeframe: start date treatment – stop date treatment

Cumulative number of (additional) blood samples during treatment in patients with reported clinical cure

An additional blood sample is a sample on which no other biochemistry measurements performed on the vancomycin TDM sample - same time or same sample ([blood_lb1tp] = '0').

Timeframe: start date treatment – end date of treatment

Reaching target 24h AUC (400-600 mg*h/L) between [72 to 96] h after start treatment

More precisely this outcome was defined as:

“Reaching the therapeutic target 24h AUC/MIC (400-600 mg*h/L) between [72 to 96] h after start of treatment, or, if the vancomycin treatment is discontinued earlier, reaching the therapeutic target in the latest available interval”.

This outcome is calculated analogously to the primary outcome. No MIPD sensitivity analysis will be performed for this outcome.

Number of dose adjustments

A dose adjustment is defined as:

- A dose that differs more than 5 % compared to the previous dose (applicable to both intermittent dosing and continuous infusion)
- A dosing interval that differs at least 2 hours compared to the previous dosing interval (only applicable to intermittent dosing)
- A switch from intermittent dosing to continuous infusion or vice versa is also considered as a dose adjustment.

Loading doses are not considered as dose adjustments.

Timeframe: start date treatment – stop date treatment

Cumulative vancomycin dose during vancomycin treatment

Sum of all doses (both for intermittent dosing and continuous infusion), expressed as mg/kg.

Timeframe: start date treatment – stop date treatment

Cumulative AUC during vancomycin treatment

The cumulative AUC is calculated using the population PK model (Colin et al., 2019) as used for the main estimand for the primary objective: a flattened prior of 30% is applied, which gives more weight (70%) to the individual concentration measurements from patients in the Beneficial trial (Appendix A).

Timeframe: start date and time treatment – stop date and time treatment + 48h to allow for vancomycin to be eliminated.

Number of trough sampling time errors during vancomycin treatment

The number of trough sampling time errors during vancomycin treatment can only be derived for the control group. Definition: trough concentrations are defined as samples obtained 23.5 to 24h, 17.5 to 18h, 11.5 to 12 h, 7.5 to 8h, 5.5 to 6h, 3.5 to 4h after the previous dose for respectively 24 hourly, 18 hourly, 12hourly, 8hourly, 6hourly and 4hourly dosing regimens.

(worsening) AKI during vancomycin treatment using KDIGO criteria

AKI classes are defined according to the neonatal (n) and pediatric (p) Kidney Disease: Improving Global Outcomes (KDIGO) criteria.

The nKDIGO are used for children <1 month of age (30 days); pKDIGO criteria are used in children ≥1 month of age (31 days).

Table 8: Proposed neonatal AKI definition modifications from KDIGO pediatric AKI definition, using SCr and urine output criteria. From: Developing a neonatal acute kidney injury research definition: a report from the NIDDK neonatal AKI workshop (Zappitelli (2017))

Serum creatinine criteria (in mg/dl)		
Stage	Pediatric definition	Neonatal modification: 2015–2016
1	≥0.3 Rise within 48 h or ≥1.5–1.9 × rise from baseline* within 7 days	≥0.3 rise within 48 h or ≥1.5–1.9 × rise from baseline (previous lowest value) within 7 days
2	≥2–2.9 × rise from baseline	Unchanged
3	≥3 × rise from baseline or ≥4.0 or eGFR<35 mL/min per 1.73 m ² or RRT initiation	≥3 × rise from baseline or ≥2.5 or RRT initiation

*Baseline SCr: no clear guideline on how to define pediatric baseline SCr. In the literature, baseline SCr has most commonly been defined as the lowest SCr measured in the previous 3 months.

In the BENEFICIAL trial, Baseline SCr corresponds to the serum creatinine measured at baseline.

Analysis methods

Primary analysis/estimator for the main estimand for the primary objective

A logistic regression model will be applied, with “reaching the target 24h AUC/MIC between 24h and 48h after start vancomycin” as binary outcome variable and with randomization group (interventional versus the standard-of-care arm) as categorical predictor of interest. The stratification factor “Type of ward” will be added as a covariate in the model.

The primary analysis will be performed on the ITT analysis set, because this avoids bias associated with non-random loss of participants. All randomized patients will be included. Intercurrent events will be handled as described in Table 2. Imputation techniques will be used for outcome data that are missing (see further below).

The intervention effect will be expressed by using the OR conditional on Type of ward.

Multiple imputation

Multivariate imputation by fully conditional specification (FCS) will be used to apply multiple imputation of missing data. More specifically, the mice package in R will be used to create 100 imputed datasets using FCS.

The missing values for 24h AUC/MIC between 24h and 48h after start vancomycin will be imputed using PMM (Predictive Mean Matching) to ensure plausible values. The imputation model will be applied separately by randomization group. The imputation model will include:

- Type of ward (NICU/ PICU/PHO)
 - Type of consent (A priori consent; Deferred consent at randomization)
 - Indication for vancomycin treatment: indication categorized in
 - o Bloodstream infection/ bacteraemia
 - o Systemic inflammatory response syndrome with no specific focus & Febrile neutropenia or other form of manifestation of infection in immunocompromised host (e.g., HIV, chemotherapy, etc.) with no clear anatomical site
 - o Other
- Combined with variable Sepsis (Yes/No)
- Mechanical ventilation support at randomization (Yes/No)
 - Number of nephrotoxic medications at baseline
 - Age, serum creatinine (mg/dL) and weight at baseline; the cumulative vancomycin dose (mg/kg) within 48h-interval and duration of treatment.
 - Time since start of the study in that specific ward unit, to take into account the potential learning effect
 - The intercurrent events “Discontinuation of vancomycin treatment because of change in antimicrobial therapy within 48 after start vancomycin” (Yes/No) and “Treatment switch/Early discontinuation of calculator /Serious protocol deviation with possible impact on primary endpoint” (Yes/No)

When randomization of a patient was based on deferred consent not followed by an informed consent to continue the study, but with an informed consent for use of already collected data, the available data will be used to apply multiple imputation.

Once the missing AUCs are imputed, the obtained values are converted to the binary outcome variable “reaching the target 24h AUC/MIC between 24h and 48h after start vancomycin”.

Results of the logistic regression model with categorical predictors randomization group (predictor of interest) and “Type of ward” (stratification factor) will be pooled using the pool() function of the mice R-package.

Sensitivity analysis 1/estimator for the main estimand for the primary objective: MIPD sensitivity analysis 1

To assess the sensitivity of the results to the choice of model prior weight, an MIPD sensitivity analysis with a model prior weight of 0.5 will be performed. The statistical analyses will be the same as the analysis for the estimator for the main estimand for the primary objective, and the additional estimand for the primary objective.

Sensitivity analysis 2/estimator for the main estimand for the primary objective: MIPD sensitivity analysis 2

In this sensitivity analysis, the primary endpoint is considered as missing for the subjects for which the AUC/MIC estimate is solely based on early samples. Early samples are considered as

samples taken within 6 hours after the start of the vancomycin treatment. (See outcome definitions/primary outcome).

The statistical analyses will be the same as the analysis for the estimator for the main estimand for the primary objective, and the additional estimand for the primary objective.

Sensitivity analysis 3/estimator for the main estimand for the primary objective: GEE analysis

To assess the robustness of our results against the assumption of independent observations, we will perform a sensitivity analysis which takes into account the possibly correlated outcomes of children “nested within twins or triplets”.

Since subjects that are part of a twin will be randomized individually, the power of the study is not impacted by the presence of twins. The following sensitivity analysis will be performed: a logistic generalized estimating equation (GEE) with randomization group (interventional versus the standard-of-care arm) as categorical predictor of interest, ward type as categorical predictor and an exchangeable working correlation structure will be applied to account for the presence of twins. This analysis will be performed if ten percent of the subjects or more have a twin or triplet sibling included in the study. Only if convergence is reached, the results will be reported.

Additional analysis/estimator for the additional estimand for the primary objective

The unconditional Relative Risk and the proportion of patients reaching primary endpoint will be estimated in line with the FDA guidance “Adjusting for Covariates in Randomized Clinical Trials for Drugs and Biological Products” (2023). The unconditional treatment effect for the primary outcome will be calculated using the plug-in estimator as described in (Freedman 2008) by averaging predicted probabilities based on the logistic regression model.

In order to reduce the variability of the estimation of the treatment effect and thus obtain narrower CIs and more power, the model will correct for the following potentially prognostic baseline covariates (these covariates are a selection of the baseline covariates included in the imputation model):

- Type of ward
- Baseline serum creatinine (mg/dL)
- Weight
- Age
- Mechanical ventilation support at randomization (Yes/No)
- Indication for vancomycin treatment

95% CIs around the proportions and the unconditional Relative Risk will be computed using the Boot MI method as described in Schomaker (2018):

10000 bootstrap samples (including missing data) D_b^* will be drawn, and each of them will be imputed 5 times using the same imputation model as described for the “Primary analysis/estimator for the main estimand for the primary objective”. Thus, there will be 5 imputed data sets for each bootstrap sample D_b^* . Point estimates for the Relative Risk are obtained for each of these 10000 bootstrap samples. The set of ordered point estimates can then be used to construct the 95% CI for the unconditional Relative Risk.

Additional analysis/estimator for the protocol-compliant estimand for the primary objective

To answer the research question “What would have happened if no major protocol deviations occurred.”, the unconditional relative risk and the proportions of patients reaching the primary endpoint will be estimated in line as described in “Additional analysis/estimator for the additional estimand for the primary objective”, but for the subjects that do not belong to the protocol-compliant analysis set because one of the following intercurrent events occurred:

- Treatment switch
- Early discontinuation of calculator
- Major protocol deviation with possible impact on primary endpoint

the outcome will be made missing. As such, a hypothetical approach is applied, answering the research question “What would have happened if the intercurrent event did not occur.”

Predefined subgroup analyses

- A subgroup analysis will be performed for the type of ward (NICU, PICU and PHO). The statistical method will be analogous to those described in ‘Primary analysis’. Type of ward unit will be added as a categorical predictor variable. An interaction term between type of ward unit and randomization group (intervention arm versus standard-of-care arm) will be included in the model.
- A subgroup analysis on the patients with confirmed infection at baseline. Statistical methods will be the same as to those described in ‘Primary analysis’, but applied only on the subgroup with confirmed infection.
- A subgroup analysis will be performed for the type of dosing regimen (continuous versus intermittent). Statistical method will be analogous to those described in ‘Primary analysis’. Type of dosing regimen and ward type will be added as categorical predictor variables. An interaction term between dosing regimen and randomization group (intervention arm versus standard-of-care arm) will be included in the model.

Analyses for the secondary objectives

Intercurrent events are treated analogously as described in Table 2 “Treatment of intercurrent events for primary outcome: Proportion of patients reaching the therapeutic target 24h AUC/MIC (400-600 mg*h/L) between [24 to 48] h after start of treatment” for the outcome “Proportion of patients reaching target 24h AUC/MIC between [48-72]h after start vancomycin treatment” and treated as described in Table 3 “Treatment of intercurrent events for secondary outcomes” for the other outcomes.

Proportion of patients with (worsening) acute kidney injury during vancomycin treatment:

Statistical methods will be similar to those described in ‘Primary analysis/estimator for the main estimand for the primary objective’. Except that no extra conversion step is needed, since imputed missing values for “(worsening) acute kidney injury during vancomycin treatment” will already be binary.

The predictors of the multiple imputation model are:

- Type of ward
- Duration of vancomycin treatment (log-transformed)
- Number of nephrotoxic medications at baseline

We will express the intervention effect by using the OR conditional on type of ward.

The unconditional Relative Risk and the estimated proportion per group will be calculated as described in ‘Additional analysis/estimator for the additional estimand for the primary objective’. The baseline prognostic factors in this model are: type of ward and number of nephrotoxic medications at baseline.

Additional analysis/estimator for the protocol-compliant estimand

The unconditional Relative Risk and the proportions of patients with (worsening) acute kidney injury will be estimated in line as described in “Additional analysis/estimator for the protocol-compliant estimand for the primary objective”.

For the subjects that do not belong to the protocol-compliant population because one of the following intercurrent events occurred:

- Treatment switch
- Early discontinuation of calculator
- Serious protocol deviation with possible impact on AKI

the outcome will be considered as missing. As such, a hypothetical approach is applied, answering the research question “What would have happened if the intercurrent event did not occur.”

30-day all-cause mortality

Statistical methods will be similar to those described in ‘Primary analysis/estimator for the main estimand for the primary objective’. We will express the intervention effect by the OR conditional on type of ward.

The unconditional Relative Risk and the estimated proportion per group will be calculated as described in ‘Additional analysis/estimator for the additional estimand for the primary objective 1’.

Since the limited number of events that is expected only type of ward will be considered as covariate. If no convergence can be reached, this term will be omitted.

Proportion of patients reaching target 24h AUC/MIC between [48-72]h after start vancomycin treatment

Statistical methods will be analogous to those described in ‘Primary analysis/estimator for the main estimand for the primary objective’, including the multiple imputation model. We will express the intervention effect by the OR conditional on type of ward.

The unconditional Relative Risk and the estimated proportion per group will be calculated as described in ‘Additional analysis/estimator for the additional estimand for the primary objective 1’.

Time to clinical cure

A Cox proportional hazards model with randomization group (intervention arm versus standard-of-care arm) and ward type (NICU, PICU, PHO) as categorical predictor variables will be used to compare the hazard.

Patients who deceased while receiving antibiotic therapy for the initial indication as vancomycin was started or where antibiotic therapy is ceased in the setting of palliative care, will be

considered as a competing event, and will be censored at the time of the decision. They will be censored at day 30 (duration of follow up).

Subjects with missing data due to deferred consent not followed by an informed consent to continue the study, but with an informed consent for use of already collected data will be censored at the time of withdrawal.

Ward unit length of stay

Negative binomial regression with randomization group (intervention arm versus standard-of-care arm) and ward type (NICU, PICU, PHO) as categorical predictor variables will be applied. The standardized mean ward unit length-of-stay per randomization group will be calculated using the plug-in method. Subjects with missing data will be part of the population for which the standardized estimate is calculated. The intervention effect will be expressed as a percentage increase or reduction of the number of days in ward unit. Bootstrapping will be used to create 95% CIs for the mean ward unit length-of stay and the percentage change in number of days in ward unit.

Hospital length of stay

Negative binomial regression with randomization group (intervention arm versus standard-of-care arm) and ward type (NICU, PICU, PHO) as categorical predictor variables will be applied. The standardized mean hospital length-of-stay will be calculated using the plug-in method. Subjects with missing data will be part of the population for which the standardized estimate is calculated. The intervention effect will be expressed as a percentage increase or reduction of the number of days in ward unit. Bootstrapping will be used to create 95% CIs for the mean hospital length-of stay and the percentage change in number of days in hospital.

Statistical analysis for tertiary objectives

Outcome measure	Statistical method
<i>Number of (additional) blood samples during vancomycin treatment</i> <i>Cumulative number of (additional) blood samples during treatment in patients with clinical cure</i> <i>Number of dose adjustments during vancomycin treatment</i>	Negative binomial regression with randomization group (intervention arm versus standard-of-care arm) and ward type (NICU, PICU, PHO) as categorical predictor variables will be applied. The standardized mean outcome per randomization group will be calculated using the plug-in method. Subjects with missing data will be part of the population for which the standardized estimate is calculated. The intervention effect will be expressed as a percentage increase or reduction of

	the outcome for Intervention versus control. Bootstrapping will be used to create 95% confidence intervals for the mean per randomization group and the percentage difference in the outcome.
<i>Proportion of patients reaching target 24h AUC/MIC between [72-96]h after start vancomycin treatment</i>	Statistical methods will be similar to those described in 'Primary analysis/estimator for the main estimand for the primary objective'. We will express the intervention effect using the OR conditional on type of ward.
<i>Cumulative vancomycin dose during vancomycin treatment</i> <i>Cumulative AUC during vancomycin treatment</i>	Linear regression with randomization group (intervention arm versus standard-of-care arm) and ward type (NICU, PICU, PHO) as categorical predictor variables will be applied. The standardized mean outcome per randomization group will be calculated using the plug-in method. Subjects with missing data will be part of the population for which the standardized estimate is calculated. The intervention effect will be expressed as the mean difference in outcome for Intervention versus control. Bootstrapping will be used to create 95% confidence intervals for the mean per randomization group and the estimated difference.

Missing data

Handling of missing data is described in the analysis section.

Harms

The following SAEs occurring until 48h after last vancomycin study administration (maximum D30+48h in case vancomycin is administered on day 30 after randomisation):

- Neonatal or pediatric RIFLE class category failure
- Vancomycin flushing syndrome (also known as red man syndrome)
- Other significant safety issues (including death) at the discretion of the investigator thought to be at least possibly related to the vancomycin dose calculation

Based on the safety analysis set, a listing for all adverse events considered the event as serious will be created separately by received treatment containing the following information:

- The subject id
- Days since start treatment
- Severity (Mild/Moderate/Severe)
- Reportable event
- Description of reportable event
- Relationship to IMP (vancomycin)
- Was the reportable event expected to IMP?
- Relationship to vancomycin dose calculation (SOC/MIPD tool)
- Was the reportable event expected to IMP dose calculation (SOC/MIPD tool)?
- The number of days from start of the adverse event to the date resolved / resolved with sequelae

Moreover, secondary endpoints for safety will be compared between randomization arms as described in the analysis section based on the ITT analysis set.

- (worsening) acute kidney injury
- 30-day all-cause mortality

Changes to the statistical analysis planned in the protocol

The ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials was not taken into account when the protocol was written and is not reflected in the study protocol. However, the SAP was written taking this guideline into account and the estimand for the primary outcome is defined in this SAP even though it was not defined in the protocol. Moreover a “protocol-compliant estimand” was added to replace the Per-Protocol analysis defined in the protocol. In pharmacological studies, this estimand is called the “Trial Product Estimand” (Gogtay 2021).

Admission diagnosis was not included in the imputation model because of the high number of separate categories, both for NICU and PICU type of ward unit. - Age, serum creatinine (mg/dL) and weight at baseline; the last documented cumulative vancomycin dose (mg/kg) within 48h-interval and duration of treatment were used instead of a priori AUC in the multiple imputation model, to avoid assumptions on timing of hypothetical future doses. The tertiary endpoints “Number of (additional) blood samples to attain target ranges during vancomycin treatment” and “Number of dose adjustments to attain target concentrations during vancomycin treatment” were changed to “Number of (additional) blood samples during vancomycin treatment” and “Number of dose adjustments during vancomycin treatment” respectively.

Statistical software

- Statistical analysis will be performed using R and RStudio version 4.3.1 or later.

References

References – Introduction

See protocol version 5.0.

References related to statistical methods

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APPENDIX A: AUC calculation

A. *R SCRIPT for creating Nonmem file for AUC calculation*

An R script was written to transform the Beneficial study data from the Redcap study database to the required format to estimate AUCs in NONMEM, v 7.5, Icon Development Solutions, USA

A.1 Description of the start and end times of the relevant interval for the primary endpoint (see SAP)

Based on the ADSL dataset (Analysis Dataset on Subject Level), the start and end times of the relevant interval are calculated as described in the SAP. When an intercurrent event occurred on which the composite strategy is applicable, no AUC was calculated.

When the duration of the first vancomycin episode is at least 48 hours:

- **Scenario 1.** For patients with a duration of the first vancomycin episode of at least 48 hours, the exact integrated AUC between 24 and 48 hours is used.

Scenarios with intercurrent event “early discontinuation of vancomycin treatment” or “while on treatment strategy”:

- **Scenario 2 (while on treatment).** For patients with a duration of the first vancomycin episode between 24h and 48h: take the duration of the first vancomycin episode and look back 24 hours (duration of the first vancomycin episode minus 24 hours).
- **Scenario 3 (while on treatment).** For patients with a duration of the first vancomycin episode less than 24 hours:
 - For intermittent dosing: the last known dose interval before <24 hours is used.
 - For continuous infusion: the last 6 hours are used.

The duration of first vancomycin episode is calculated as:

- the duration from the start of the first vancomycin dose to the start time of the last dose (of the first episode) + 6 hours in the case of intermittent dosing.
- the duration from start of the first vancomycin dose to the stop time of infusion of last dose (of the first episode) in the case of continuous infusion.

A.2 R-script to obtain start and end times of the relevant interval for the primary endpoint:

Variables used in the R code

- nbloodsamples: number of blood samples available to estimate the AUC
- AUCVCDUR: Duration of first episode of the vancomycin treatment
- AUCVCDURO: Observed duration of first episode of the vancomycin treatment (Equals AUCVCDUR except when consent is withdrawn)

- ICE_primary_composite: value of the primary endpoint when composite strategy is applied (0=unfavorable; 1=favorable)
- end_AUCepi1: end of first episode of vancomycin treatment; end_AUCepi_intermittent: end of first episode of vancomycin treatment in case of intermittent dosing; end_epi1_continuous: end of first episode of vancomycin treatment in case of continuous infusion.

R-script code

```
#start times first interval (primary endpoint):
AUC_calc_24_48_start <- ADSL %>%
# if no blood samples are available, AUC cannot be estimated and is missing
filter(!is.na(nbloodsamples))%>%
# if a primary endpoint is reached before 48 hours for which a composite strategy is applied, then AUC should not be
estimated
filter(is.na(ICE_primary_composite))%>%
#for all other subjects with an AUCVCDUR of for which an observed AUCVDUR of more than 48 hours is available
filter(!is.na(AUCVCDUR)|AUCVCDURO>=48)%>%
#start times interval 24h - 48h:
#scenario 3 intermittent--> last known interval
mutate(TIME=case_when(AUCVCDUR <= 24 & end_AUCepi1==end_AUCepi1_intermittent ~ start_lastknowndosing,
# scenario 3 continuous --> go back 6 hours
AUCVCDUR <= 24 & end_AUCepi1==end_epi1_continuous ~ AUCVCDUR - 6,
#scenario 2
AUCVCDUR > 24 & AUCVCDUR < 48 ~ AUCVCDUR-24,
#scenario 1, also in the case of withdrawal of consent later than 48 hours
AUCVCDURO >= 48 ~ 24))%>%
mutate(AUCF24H48H=1)%>%
select (subjid,IDNONMEM, TIME,AUCF24H48H)

#end times first interval 24h - 48h (primary endpoint):
AUC_calc_24_48_end <- ADSL %>%
# if no blood samples are available, AUC can not be estimated and is missing
filter(!is.na(nbloodsamples))%>%
# if a primary endpoint is reached before 48 hours for which a composite strategy is applied, then AUC should not be
estimated
filter(is.na(ICE_primary_composite))%>%
#for all other subjects with an AUCVCDUR of for which an observed AUCVDUR of more than 48 hours is available
filter(!is.na(AUCVCDUR)|AUCVCDURO>=48)%>%
#Scenario 3 intermittent--> last known interval
mutate(TIME=case_when((AUCVCDUR <= 24) & (end_AUCepi1 == end_AUCepi1_intermittent) ~ end_lastknowndosing,
#Scenario 3 continuous
(AUCVCDUR <= 24) & (end_AUCepi1 == end_epi1_continuous) ~ AUCVCDUR, #Scenario 3
intermittent~VCDUR,
#Scenario 2
(AUCVCDUR > 24) & (AUCVCDUR < 48) ~ AUCVCDUR,
#Scenario 1, also in the case of withdrawal of consent later than 48 hours
(AUCVCDURO >= 48) ~ 48))%>%
mutate(AUCF24H48H=1)%>%
select (subjid,IDNONMEM, TIME,AUCF24H48H)
#start and end times for the primary endpoint are saved
AUC_calc_24_48 <- bind_rows(AUC_calc_24_48_start,AUC_calc_24_48_end )
```

Start and end times for the intervals 48h-72h (secondary endpoint) and 72h-96 (tertiary endpoint) are calculated analogously.

A. AUC estimation

The primary outcome is “reaching the therapeutic target 24h AUC/MIC (400-600 mg*h/L) between [24 to 48] h after start of treatment”

Or more precise, if the vancomycin treatment is discontinued earlier, reaching the therapeutic target in the latest available interval will be taken into consideration.

Different scenarios are described in detail:

Scenario 1. For patients with a duration of the first vancomycin episode of at least 48 hours, the exact integrated AUC between 24 and 48 hours is used.

Scenario 2 (while on treatment). For patients with a duration of the first vancomycin episode between 24h and 48h: take the duration of the first vancomycin episode and look back 24 hours (duration of the first vancomycin episode minus 24 hours): the exact integrated AUC between those two time points is used.

Scenario 3 (while on treatment). For patients with a duration of the first vancomycin episode less than 24 hours:

- i. For intermittent dosing, the last known dose interval before <24 hours is used.
For continuous infusion, the last 6 hours of infusion are used.
- ii. *Calculate the integrated AUC within that dosing interval*
- iii. *Recalculate AUC to a 24h interval*
For example for a 6 hours interval: $AUC_{24} = AUC_{6/6} \times 24$

- The script below describes how individual vancomycin AUC were estimated using Bayesian method using the previously published Colin et al. (1) population PK model in NONMEM v 7.5, Icon Development Solutions, USA. The Colin model was modified in the \$OMEGA block to have flattened prior of 30%, which gives more weight (70%) to the individual concentration measurements from patients in the Beneficial trial.

```
; modified model, with:
; - prior flattened to 30%
; - weights for observations within:
; - 0 – 4 days of treatment: 100%
; - 4 – 10 days of treatment: linear interpolation from 100% -> 20%
; - 10 – 30 days of treatment: linear interpolation from 20% -> 10%
;

$PROBLEM AUC calculation Beneficial trial with prior 30
$DATA 20240621_input_nm_v2.csv IGNORE=@
$INPUT ID1 ID TIME AMT RATE TOD DV MDV EVID CR AGE WT PMA AUCF
; the AUCF (flag column) identifies the integrated AUC at timepoints of interest for further processing (see below)
;CR: serum creatinine
;AGE: age(days)
;PMA: Post Menstrual Age (weeks)

; $SUBROUTINE ADVAN3 TRANS4
$SUBROUTINE ADVAN6 TOL=8

$MODEL NCOMP=3
COMP=(CENTR)
COMP=(PERIPH)
COMP=(AUC)
```

```

$PK
PMA_R = 35+(40/52)
PMA_YRS = PMA/52

HEEL = 0
CANCER = 0
DEG50 = EXP(THETA(5))
MAT50 = EXP(THETA(6))
GAM = EXP(THETA(7))
GAM2 = -EXP(THETA(8))
EXPO2 = EXP(THETA(9))

LnSCRstd = -1.22839 + LOG10(PMA_YRS) * 0.67190 + 6.27017 * EXP(-3.10940 * PMA_YRS)
SCRstd = EXP(LnSCRstd)

Fsize = WT/70
Fmat = PMA_YRS ** GAM / (PMA_YRS ** GAM + MAT50 ** GAM)
Fmat_R = PMA_R ** GAM / (PMA_R ** GAM + MAT50 ** GAM)

Fage = PMA_YRS ** GAM2 / (PMA_YRS ** GAM2 + DEG50 ** GAM2)
Fage_R = PMA_R ** GAM2 / (PMA_R ** GAM2 + DEG50 ** GAM2)

Fscr = EXP(-EXPO2 * (CR-SCRstd)) ; For a man of 35 yr, 70 kg and GA 40 wks, SCRstd = 0.84 mg/dL

TVV1 = EXP(THETA(2)) * Fsize * (1-HEEL*THETA(10))
V1 = TVV1 * EXP(ETA(2))

TVV2 = EXP(THETA(3)) * Fsize
V2 = TVV2 * EXP(ETA(3))

RV1 = EXP(THETA(2))
TVCL = EXP(THETA(1)) * (V1/RV1) ** 0.75 * Fmat/Fmat_R * Fage/Fage_R * Fscr * (1 + CANCER * THETA(12))
CL = TVCL * EXP(ETA(1))

RV2 = EXP(THETA(3))
TVQ = EXP(THETA(4)) * (V2/RV2)**0.75 * (1-HEEL*THETA(11))
Q = TVQ

S1=V1

; Rewritten original model to ODE model to allow AUC estimation
$DES
DADT(1) = -(CL/V1)*A(1) + (Q/V2)*A(2) - (Q/V1)*A(1)
DADT(2) = - (Q/V2)*A(2) + (Q/V1)*A(1)
DADT(3) = (A(1)/V1)

$ERROR
AUC = A(3)
IPRED = F
PROPvar = 0.046
ADDvar = 1.52

; MODIFIED: weighting of data over time
WEIGHT = 1.0 ; from t=0 to t=4 days
IF(TIME.GT.96.AND.TIME.LT.240) THEN ; from t=4 to t=10 days
  WEIGHT = 1.0 - (TIME-96) * (0.8/144)
ENDIF
IF(TIME.GT.240.AND.TIME.LT.720) THEN ; from t=10 to t=30 days
  WEIGHT = 0.2 - (TIME-240) * (0.1/480)
ENDIF
IF(WEIGHT.LT.0.1) WEIGHT=0.1 ; fallback, if any datapoints >30 days, set to 10%

W = SQRT(IPRED**2 * PROPvar + ADDvar)
Y = IPRED + (W/WEIGHT) * ERR(1) ; Weight-factor added
IRES = DV-IPRED

```

```

$THETA
(1,1.67,2.5) FIX ; CLstd, L/h
(2.77,3.75,4.5) FIX ; V1std, L
(2.78,3.73,4.5) FIX ; V2std, L
(0.5,1.17,2.5) FIX ; Qstd, L/h
(3.5,4.12,4.8) FIX ; DEG50
(-0.69,-0.113,0.40) FIX ; MAT50
(-0.69,1.06,1.5)FIX ; GAM
(-0.69,0.807,1.5) FIX ; GAM2
(-1.5,-0.433,-0.01) FIX ; EXPO2: SCR~CL
(0,0.312,1) FIX ; HEEL ~ V1
(0,0.597,1) FIX ; HEEL ~ Q2
(0,0.294,1.5) FIX ; CANCER ~ C

```

; MODIFIED: omega modified to have flattened prior (30%)

```

$OMEGA
; 0.0746 FIX ; IIV_CL
0.2487 ; = 0.0746 / 0.3
; 0.0716 FIX ; IIV_V1
0.2387 ; = 0.0716 / 0.3
; 0.684 FIX ; IIV_V2
2.28 ; = 0.684 / 0.3

```

```

$SIGMA
1 FIX

```

```

$EST METHOD=0 POSTHOC MAXEVAL=0
$TABLE ID1 ID TIME TOD DV PRED IPRED EVID AUC CL V1 Q V2 PMA_YRS AUCF
FILE=nmout2.tab NOPRINT NOAPPEND

```

; the AUC column in the nmout2.tab output file contains per row the cumulative AUC from start to timepoint

; the AUCF (flag column) identifies the integrated AUC at timepoints of interest for further processing (see below)

- Data processing NONMEM output in R to calculate integrated AUC within interval of interest [24h to 48h] or, if vancomycin treatment was discontinued earlier, in the latest available interval.

```

##### Calculate differences 24H48h

AUCs24H48H <- nmout2 %>%

#select the relevant AUCs for the primary endpoint

filter(as.numeric(AUCF24H48H)==1)%>%

#calculate by patient

group_by(ID) %>%

#calculate time interval

mutate(Diff_Time = Time - lag(Time)) %>%

#calculate difference in AUC

mutate(Diff_AUC = AUC - lag(AUC)) %>%

# recalcultate AUC to a 24h interval

mutate(AUC_24H48H=Diff_AUC*24/Diff_Time)%>%

```

```
filter(!is.na(Diff_Time))%>%
select(ID_ORI,ID,AUC_24H48H )
```

- Sensitivity analysis:

- For the first sensitivity analysis of the primary endpoint, the model code was modified in the \$OMEGA block code to have a flattened prior of 50%, which gives equal weight to the model as to the individual concentration measurements from patients in the Beneficial trial.

```
;; modified model, with:
; - prior flattened to 50%
; - weights for observations within:
; - 0 – 4 days of treatment: 100%
; - 4 – 10 days of treatment: linear interpolation from 100% -> 20%
; - 10 – 30 days of treatment: linear interpolation from 20% -> 10%
;

- $PROBLEM AUC calculation Beneficial trial with prior 50
- $DATA 20240621_input_nm_v2.csv IGNORE=@
- $INPUT ID1 ID TIME AMT RATE TOD DV MDV EVID CR AGE WT PMA AUCF
-
- ; $SUBROUTINE ADVAN3 TRANS4
- $SUBROUTINE ADVAN6 TOL=8
-
- $MODEL NCOMP=3
- COMP=(CENTR)
- COMP=(PERIPH)
- COMP=(AUC)
-
- $PK
- PMA_R = 35+(40/52)
- PMA_YRS = PMA/52
-
-
- HEEL = 0
- CANCER = 0
- DEG50 = EXP(THETA(5))
- MAT50 = EXP(THETA(6))
- GAM = EXP(THETA(7))
- GAM2 = -EXP(THETA(8))
- EXPO2 = EXP(THETA(9))
-
- LnSCRstd = -1.22839 + LOG10(PMA_YRS) * 0.67190 + 6.27017 * EXP(-3.10940 * PMA_YRS)
- SCRstd = EXP(LnSCRstd)
-
- Fsize = WT/70
- Fmat = PMA_YRS ** GAM / (PMA_YRS ** GAM + MAT50 ** GAM)
- Fmat_R = PMA_R ** GAM / (PMA_R ** GAM + MAT50 ** GAM)
-
- Fage = PMA_YRS ** GAM2 / (PMA_YRS ** GAM2 + DEG50 ** GAM2)
- Fage_R = PMA_R ** GAM2 / (PMA_R ** GAM2 + DEG50 ** GAM2)
-
- Fscr = EXP(-EXPO2 * (CR-SCRstd)) ; For a man of 35 yr, 70 kg and GA 40 wks, SCRstd = 0.84 mg/dL
-
- TVV1 = EXP(THETA(2)) * Fsize * (1-HEEL*THETA(10))
- V1 = TVV1 * EXP(ETA(2))
-
- TVV2 = EXP(THETA(3)) * Fsize
- V2 = TVV2 * EXP(ETA(3))
-
- RV1 = EXP(THETA(2))
- TVCL = EXP(THETA(1)) * (V1/RV1) ** 0.75 * Fmat/Fmat_R * Fage/Fage_R * Fscr * (1 + CANCER * THETA(12))
- CL = TVCL * EXP(ETA(1))
-
```

```

- RV2 = EXP(THETA(3))
- TVQ = EXP(THETA(4)) * (V2/RV2)**0.75 * (1-HEEL*THETA(11))
- Q = TVQ
-
- S1=V1
-
- ; Rewritten original model to ODE model to allow AUC estimation
- $DES
- DADT(1) = -(CL/V1)*A(1) + (Q/V2)*A(2) - (Q/V1)*A(1)
- DADT(2) = - (Q/V2)*A(2) + (Q/V1)*A(1)
- DADT(3) = (A(1)/V1)
-
- $ERROR
- AUC = A(3)
- IPRED = F
- PROPvar = 0.046
- ADDvar = 1.52
-
- ; MODIFIED: weighing of data over time
- WEIGHT = 1.0 ; from t=0 to t=4 days
- IF(TIME.GT.96.AND.TIME.LT.240) THEN ; from t=4 to t=10 days
-   WEIGHT = 1.0 - (TIME-96) * (0.8/144)
- ENDIF
- IF(TIME.GT.240.AND.TIME.LT.720) THEN ; from t=10 to t=30 days
-   WEIGHT = 0.2 - (TIME-240) * (0.1/480)
- ENDIF
- IF(WEIGHT.LT.0.1) WEIGHT=0.1 ; fallback, if any datapoints >30 days, set to 10%
-
- W = SQRT(IPRED**2 * PROPvar + ADDvar)
- Y = IPRED + (W/WEIGHT) * ERR(1) ; Weight-factor added
- IRES = DV-IPRED
-
- $THETA
- (1,1.67,2.5) FIX ; CLstd, L/h
- (2.77,3.75,4.5) FIX ; V1std, L
- (2.78,3.73,4.5) FIX ; V2std, L
- (0.5,1.17,2.5) FIX ; Qstd, L/h
- (3.5,4.12,4.8) FIX ; DEG50
- (-0.69,-0.113,0.40) FIX ; MAT50
- (-0.69,1.06,1.5) FIX ; GAM
- (-0.69,0.807,1.5) FIX ; GAM2
- (-1.5,-0.433,-0.01) FIX ; EXPO2: SCR~CL
- (0,0.312,1) FIX ; HEEL ~ V1
- (0,0.597,1) FIX ; HEEL ~ Q2
- (0,0.294,1.5) FIX ; CANCER ~ C
-
- ; MODIFIED: omega modified to have flattened prior (50%)
-
- $OMEGA
- ; 0.0746 FIX ; IIV_CL
- 0.2487 ; = 0.0746 / 0.5
- ; 0.0716 FIX ; IIV_V1
- 0.2387 ; = 0.0716 / 0.5
- ; 0.684 FIX ; IIV_V2
- 2.28 ; = 0.684 / 0.5
-
- $SIGMA
- 1 FIX
-
- $EST METHOD=0 POSTHOC MAXEVAL=0
- $TABLE ID1 ID TIME TOD DV PRED IPRED EVID AUC CL V1 Q V2 PMA_YRS AUCF
- FILE=nmout2.tab NOPRINT NOAPPEND

```

- For the second sensitivity analysis of the primary endpoint, the NONMEM input file did not contain individual concentration measurements <6h after start of treatment. In this analysis, a flattened prior of 30% was used.

REFERENCE

- (1) Colin PJ, Allegaert K, Thomson AH, Touw DJ, Dolton M, de Hoog M, Roberts JA, Adane ED, Yamamoto M, Santos-Buelga D, Martín-Suarez A, Simon N, Taccone FS, Lo YL, Barcia E, Struys MMRF, Eleveld DJ. Vancomycin pharmacokinetics throughout life: results from a pooled population analysis and evaluation of current dosing recommendations. Clin Pharmacokinet. 2019 Jun;58(6):767-780. doi: 10.1007/s40262-018-0727-5.
- (2) www.sanfordguide.com/vanco_auc/, consulted 27th of June 2024

References

1. Colin P., Allegaert K., Thomson A., et al. Vancomycin pharmacokinetics throughout life: results from a pooled population analysis and evaluation of current dosing recommendations. Clin Pharmacokinet 2019: 767-780.