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ABBREVIATIONS AND DEFINITIONS OF TERMS

°C	Degrees centigrade
AE	Adverse event
AKI	Acute kidney injury
Anchoring dose	Dose of vancomycin preceding performance of TDM/PK sampling
AUC	Area under the curve
CHOP	Children's Hospital of Philadelphia
CHPS	Center for Human Phenomic Science
CITI	Collaborative Institutional Training Initiative
C_{min}	Minimum blood plasma concentration
CysC	Cystatin C
DOB	Date of Birth
ECMO	Extracorporeal Membrane Oxygenation
ELISA	Enzyme-Linked Immunosorbent Assay
GFR	Glomerular Filtration Rate
ICU	Intensive Care Unit
IRB	Institutional Review Board
IV	Intravenous
Kg	Kilogram
KIM-1	Kidney Injury Molecule-1
mg	Milligram
mL	Milliliter
min	Minute
MIC	Minimum Inhibitory Concentration
MRN	Medical Record Number

NGAL	Neutrophil Gelatinase-Associated Lipocalin
NIH	National Institutes of Health
PD	Pharmacodynamics
PI	Principle Investigator
PICU	Pediatric Intensive Care Unit
PIM-3	Pediatric Index of Mortality-3
PK	Pharmacokinetics
PK sampling	Vancomycin concentrations drawn for research
PopPK	Population PK
REDCap	Secure web application for building and managing online surveys and databases
ROC	Receiver Operating Characteristic
SAN	Storage Area Network
SCr	Serum Creatinine
SIRS	Systemic Inflammatory Response Syndrome
t½	Elimination half-life
TDM	Therapeutic Drug Monitoring
TDM samples	Vancomycin concentrations drawn for clinical care
UCr	Urine Creatinine

ABSTRACT

Context:

Vancomycin is the drug of choice for treatment of serious gram-positive infections, exhibiting area under the curve (AUC_{24})-dependent efficacy, as well as toxicity. Currently, vancomycin dosing is based on a child's weight and renal function, determined by measurement of serum creatinine (SCr). But, the ability of SCr to guide vancomycin dosing is poor in critically ill children because of the dynamic nature of critical illness. Cystatin C (CysC) is a recently available plasma biomarker that better estimates kidney function and drug clearance than SCr. By using population pharmacokinetic (popPK) models as prior information, Bayesian dose adaptation can incorporate a patient's characteristics (i.e. age, renal function, etc.), dosage history, and optimally timed drug concentrations to estimate patient-specific PK parameters and facilitate personalized vancomycin dosing that improves therapeutic target attainment. Through prior work, we have developed popPK models that can be used to improve vancomycin dosing in critically ill children through Bayesian estimation approaches.

Objectives:

The objective of the study is to evaluate the ability of Bayesian dose adaptation based on our previously developed popPK models for intravenous (IV) vancomycin to accurately predict vancomycin AUC_{24} in critically ill children.

Study Design:

Observational study.

Setting/Participants:

Participants will be recruited from the Children's Hospital of Philadelphia. A total of 20 evaluable participants aged 1-17 years receiving standard of care IV vancomycin for a suspected or confirmed infection will be enrolled.

Study Interventions and Measures:

Study intervention(s): Dosing of vancomycin will be determined by clinical teams. We will use Bayesian dose adaptation software that incorporates our previously developed vancomycin popPK model(s) to predict each subject's vancomycin AUC_{24} based on a single, optimally-timed vancomycin concentration.

Study measures include: urine samples, blood samples, demographics, anthropomorphic and clinical data.

TABLE 1: SCHEDULE OF STUDY PROCEDURES

Study Phase	**Screening**	**Visit 1[a]**	**Visit 2[a]**
Study Days	**1**	**1-2**	**2-5**
Review inclusion/exclusion criteria	X		
Informed consent	X		
Medical records review	X	X	X
Weight[b]	X		
Urine samples		X	X
Blood samples		X	X

[a] Study visits will be based on the timing of therapeutic drug monitoring (TDM) performed for clinical care. The first instance of TDM after enrollment will serve as Visit 1 for the study; Visit 2 will be the second instance of TDM after enrollment. If TDM will not be repeated by the clinical team, Visit 2 will be specified by the study team and occur within 72 hours of Visit 1.

[b] Weight will be collected if one is not available from within 48 hours of vancomycin initiation. Weight can be collected at any time during study participation.

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Sepsis is a leading cause of death in children with an in-hospital mortality of 25%.[1,2] Broad-spectrum antibiotics are necessary and the timely initiation of effective therapy improves survival.[3] Vancomycin is a commonly used antibiotic in children with suspected or proven sepsis in the pediatric intensive care unit (PICU),[4] exhibiting area under the curve (AUC_{24})-dependent efficacy,[5-7] as well as toxicity.[8] Increased vancomycin concentrations can lead to acute kidney injury (AKI),[8-12] which is an independent risk factor for PICU mortality,[12-16] and critically ill children sustain AKI in approximately 25% of vancomycin treatment courses.[10,11,13]

At CHOP, vancomycin therapeutic drug monitoring (TDM) involves measurement of serum creatinine (SCr), a traditional marker of kidney function, and collection of two vancomycin serum concentrations, which are used to calculate AUC_{24}. These two vancomycin concentrations must be collected at specific times, which can be difficult for clinical teams to coordinate, and collection of two blood samples is often uncomfortable for patients. While SCr is traditionally used as a marker of kidney function, its ability to guide vancomycin dosing in critically ill children, who often have dynamic physiology, is poor.[17-19] Newly discovered biomarkers, such as plasma cystatin C (CysC), may better estimate kidney function and drug clearance than SCr.[20-24]

By using population pharmacokinetic (popPK) models as prior information, Bayesian dose adaptation can incorporate a patient's characteristics (i.e. age, renal function, etc.), dosage history, and drug concentrations to estimate patient-specific PK parameters. This information can then be used to facilitate personalized vancomycin dosing that improves therapeutic target attainment and reduces AKI.[25] Most available popPK models are not specific to critically ill children and are therefore insufficient to guide dosing in this population via Bayesian dose adaption.

Through a previous study (IRB 18-014851), we have developed robust population PK models for vancomycin in critically ill children, incorporating newer biomarkers (plasma CysC and urinary NGAL). We will test the ability of our model(s) to predict vancomycin AUC_{24} using Bayesian dose adaptation in this pilot study.

1.2 Relevant Literature and Data

Vancomycin is the drug of choice for treatment of serious gram-positive infections in children: Vancomycin is a glycopeptide antibiotic with excellent activity against gram-positive bacteria. Due to the rising prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) infections, current clinical practice guidelines from the Infectious Diseases Society of America (IDSA) recommend its use empirically in children with suspected serious infections such as bacteremia and meningitis.[26] At our institution, gram-positive organisms account for nearly 75% of all isolates causing bacteremia, with *S. aureus* the most common pathogen,[27] and vancomycin is administered empirically in all patients with suspected sepsis.

Current dosing and monitoring strategies for vancomycin are inadequate in critically ill children: Therapeutic drug monitoring (TDM) is used primarily to minimize drug

toxicity and, to a lesser extent, maximize therapeutic efficacy. In adults with MRSA infections, a ratio of the vancomycin area under the curve over 24 hours (AUC_{24}) to the minimum inhibitory concentration (MIC) of the bacteria >400 is the therapeutic target associated with improved clinical outcomes.[5-7,28] Direct measurement of AUC_{24} is technically difficult, however, requiring multiple blood draws, and is not routinely performed in clinical practice. Thus, vancomycin trough concentrations (C_{min}; levels obtained immediately prior to a dose) had been traditionally used as surrogate therapeutic targets for AUC_{24}. Although simple to interpret, a single trough measurement does not capture the inter-individual variability in PK present in most populations and the correlation between troughs and AUC_{24} in children is poor.[17,18,29,30] As a result, newer guidelines call for estimation of vancomycin AUC_{24} in all children and adults.[31]

Critically ill patients frequently undergo physiologic changes that alter the distribution and clearance of medications.[32] Altered fluid balance leads to an increased volume of distribution and lower plasma drug concentrations, while organ dysfunction, including acute kidney injury (AKI), may impair drug clearance.[32] Higher doses of vancomycin are generally needed in critically ill children to achieve therapeutic concentrations.[33-35] As a result of these physiologic alterations, a one-size-fits-all weight-based dosing strategy for antibiotics is suboptimal.

Vancomycin has a narrow therapeutic index and exhibits AUC-dependent nephrotoxicity.[8] Due to several factors (decreased renal perfusion, receipt of concurrent nephrotoxic medications), critically ill children sustain AKI during 15-25% of vancomycin courses,[10-13] which is associated with increased mortality and development of chronic kidney disease.[13-15,36-38] Meanwhile, delayed attainment of vancomycin therapeutic targets increases the risk of treatment failure,[5,6,39,40] and sub-inhibitory concentrations can contribute to selection of resistant organisms.[41-43] Considering the tenuous balance between vancomycin efficacy and toxicity in critically ill children, and their complex physiology, clinicians need reliable approaches to attain target AUC_{24} to maximize efficacy, minimize toxicity, and prevent the development of resistance.

The current approach to TDM of vancomycin at CHOP involves measurement of two serum concentrations obtained 1-1.5 hours after infusion of a dose and immediately prior to the next dose. These two concentrations are then used to calculate AUC_{24} using the Sawchuk-Zaske method. Limitations to this approach are: a) collection of two samples can be uncomfortable for patients, b) obtaining two samples at these specific times can be difficult for clinical teams, c) mistimed samples may be uninformative with this method, and d) samples must be collected at steady-state, which is not always possible in critically ill patients, who often have dynamic physiology.

Novel acute kidney injury biomarkers can improve estimation of vancomycin AUC_{24} and clearance: Vancomycin is renally eliminated and total body clearance (CL) is related to glomerular filtration rate (GFR).[44] Direct GFR measurement in the critical care setting is impractical and clinicians utilize surrogate markers such as SCr to estimate GFR (eGFR). Creatinine is a suboptimal marker of kidney function in children as values can be affected by age, gender, medications, and muscle mass.[45] Changes in SCr resulting from AKI are also not evident until significant renal mass has been affected.[46]

Cystatin C (CysC) is an endogenous cysteine protease inhibitor widely expressed by nucleated cells and produced at a constant rate in the body.[47] It is freely filtered across the glomerular membrane, not secreted by renal tubules, and, therefore, is felt to be a good marker of GFR.[47] Studies have described its superiority over SCr for GFR estimation and earlier AKI detection in critically ill children including those with sepsis.[48-54] In previous popPK work, we have found that CysC is a more informative covariate on vancomycin CL than SCr in critically ill children.[55]

Neutrophil gelatinase-associated lipocalin (NGAL) is another promising biomarker for the early detection of AKI in critically ill children.[54,56,57] Plasma and urinary NGAL increase prior to changes in SCr in critically ill children with sepsis.[54,58] In a study of 21 adult patients with severe burns receiving vancomycin, NGAL served as a better predictor of vancomycin concentrations than creatinine clearance: $R^2 = 0.58$ vs. 0.30, respectively.[23]

Given the known limitations of SCr in children, the clinical use of novel AKI biomarkers can improve estimation of kidney function and vancomycin CL, detect children at risk for subsequent AKI, and promote individualized vancomycin dosing.

Bayesian dose adaptation facilitates rapid attainment of evidence-based therapeutic targets in individual patients: The use of Bayesian dose adaptation can provide robust data about drug exposure and ensure that pharmacokinetic/pharmacodynamic (PK/PD) targets are attained in individual patients.[32] Briefly, Bayesian dose adaptation is a tool to more accurately estimate the probability distribution of PK parameter values (i.e. clearance, volume of distribution) in individual patients based on how the drug has behaved in prior patients.[30] By using population PK models as prior information (Bayesian prior), Bayesian approaches incorporate a patient's characteristics (i.e. age, gender, renal function, etc.), dosage history, and drug concentrations to estimate patient-specific PK parameters (Bayesian posterior) that more accurately reflect drug behavior in that specific patient.[25] The individual Bayesian posterior model can then be used to predict a drug concentration following a dose or calculate the dose needed to attain a target concentration (Bayesian adaptive control).[25] Importantly, Bayesian methods do not require waiting for steady-state concentrations and drug sampling can occur at any time. Therefore, more rapid attainment of therapeutic goals can be achieved.[25] And, an important potential benefit of a Bayesian approach to patients, compared to the Sawchuk-Zaske method, is that vancomycin AUC_{24} can be estimated from a single drug concentration (i.e limited sampling).

We have developed population PK models for vancomycin in critically ill children to inform Bayesian approaches: Through a previous study (IRB 18-014851), we have developed robust population PK models for vancomycin in critically ill children. Briefly, we enrolled 50 critically ill children treated with IV vancomycin and collected up to 5 PK samples and urinary and plasma biomarkers on each. We performed nonparametric popPK modeling for vancomycin using data from 30 subjects (model development group). During popPK model development, cystatin C (CysC) was a superior renal function estimator (i.e covariate on clearance) compared to serum creatinine (Akaike Information Criteria [AIC] 793.3 vs. 807.2). And, inclusion of urinary NGAL (uNGAL) as a covariate on vancomycin clearance further improved the model compared to the model with only CysC (AIC 785.0 vs 793.3).

We then sought to evaluate the ability of popPK models to estimate AUC_{24} using Bayesian adaptation in the other 20 subjects (validation group). We utilized 3 separate popPK models: 1) the model that incorporated urinary NGAL and plasma cystatin C (uNGAL model), 2) a model that incorporated only plasma cystatin C (CysC model), and 3) a model that incorporated only serum creatinine (SCr model). We evaluated the accuracy of these 3 models to estimate AUC_{24} using limited sampling (1 or 2 optimal sampling times) compared to non-compartmental AUC_{24} from 4 or 5 vancomycin measurements. Our *a priori* target for model validation was bias <10% and correlation >0.9, compared to non-compartmental AUC_{24}.

We found that the uNGAL model and the CysC model performed similarly in terms of AUC_{24} estimation. When using a single, optimally timed sample, the uNGAL model estimated AUC with a median bias of 3.5% and had a correlation with non-compartmental analysis AUC_{24} of 0.868. The bias and correlation of the CysC model were 2.5% and 0.891, respectively. Meanwhile, the bias and correlation of the SCr model were -0.9% and 0.825, respectively. When using the 2 optimally timed samples, the bias and correlation of the uNGAL model, CysC model and SCr model were: 0.9% and 0.924, 1.8% and 0.928, and -1.2% and 0.860, respectively. Thus, we concluded that the CysC and uNGAL models could estimate AUC_{24} using 2 optimally timed samples with adequate accuracy and that these two models performed similarly.

Upon further evaluation, the 20 subjects included in the validation analyses differed slightly from the 30 subjects used during model development. The validation population was slightly heavier (37.6 vs 25.9 kg) and had better kidney function (estimated GFR based on Cystatin C: 156 vs 130 ml/min/1.73 m^2) at vancomycin initiation. Urinary NGAL was also slightly lower in the validation cohort (35.5 vs 59.8 ng/mg urine creatinine), suggesting less kidney injury. These differences may have contributed to the suboptimal correlation between AUCs when using a single optimal sample, especially given the small sample size of our validation cohort (n=20).

We next combined data from all 50 subjects enrolled in the project (IRB 18-014851) and developed new popPK models. As before, CysC was the most informative covariate on vancomycin CL. And, as before, uNGAL was also an informative covariate on vancomycin CL. Interestingly, in this population of 50 subjects, plasma NGAL (pNGAL) led to a similar reduction in AIC as uNGAL, with similar bias and imprecision. Through this process, we have developed a new CysC model, as well as a new uNGAL model and a pNGAL model. Inclusion of all 50 subjects in our model(s) improved the bias and precision of parameter estimation and should improve the generalizability of our model(s) across a spectrum of illness severity. These models will serve as the focus of the current observational study.

Because the uNGAL and CysC models performed similarly in terms of AUC_{24} estimation during our initial model development and validation analyses, the CysC model will be the primary model of interest for the current study. This is because: a) CysC is a clinically validated and available test, b) CysC results can be available in real-time to inform dosing via Bayesian estimation at the bedside, and c) the CysC model is simpler (i.e. has fewer covariates) and will be easier to implement clinically in the future. The pNGAL and uNGAL models will serve as the basis for exploratory analyses (see Section 5.2).

1.3 Compliance Statement

This study will be conducted in full accordance with all applicable Children's Hospital of Philadelphia Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46. All episodes of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent (unless a waiver is granted), and will report unanticipated problems involving risks to subjects or others in accordance with Children's Hospital of Philadelphia IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2 STUDY OBJECTIVES

The purpose of the study is to evaluate the feasibility of Bayesian dose adaptation based on our CysC model and a single optimally timed PK sample to predict vancomycin AUC_{24} in critically ill children.

Secondary objectives are to:

- Determine the accuracy of Bayesian AUC estimation using an optimally timed PK sample compared to Bayesian estimation using ≥2 samples.
- Compare AUC_{24} derived from Bayesian estimation using a single, optimally timed sample to AUC_{24} calculated using standard clinical methods (Zaske-Sawchuk method).
- Assess the ability of our model to predict Visit 2 AUC_{24} using Bayesian estimation.
- Compare AUC_{24} derived from Bayesian estimation using the uNGAL and pNGAL models to the CysC model (exploratory set of analyses).

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

This is an observational pilot study. We will identify 20 evaluable subjects receiving vancomycin for standard of care in the CHOP PICU. For each subject, we will measure CysC upon enrollment and at the time of any clinical SCr measurement following enrollment. We will use the MMOpt function of Pmetrics™ to determine the optimal sampling time to estimate AUC_{24} for each subject. At the time of clinical TDM (Visit 1), we will collect a single PK sample at the optimal sampling time. We will then utilize Bayesian dosing software (InsightRx, Inc.), informed by our CysC popPK model, to estimate each subject's PK parameters (clearance, volume of distribution, etc.) and vancomycin AUC_{24}. We will compare each subject's Visit 1 AUC_{24} using the optimally timed PK sample to: a) AUC_{24} estimated using the Bayesian software and all available vancomycin measurements (TDM + PK samples; primary outcome), and b) AUC_{24} calculated for clinical care via TDM (secondary outcome). This will provide information about the accuracy and feasibility of real-time Bayesian estimation based on optimal sampling. If the subject has only a single vancomycin concentration obtained for clinical TDM, we will draw the optimally timed

sample as well as a "peak" concentration, which will allow us to calculate AUC_{24} as is done clinically (Zaske-Sawchuk method).

We will then repeat the process of optimal sampling time determination and TDM/PK sampling 24-72 hours later (Visit 2). We will use the Bayesian dosing software and data available prior to Visit 2 to predict AUC_{24} at Visit 2. We will compare the predicted AUC_{24} to the measured $AUC_{24;}$ the measured AUC_{24} will incorporate vancomycin concentrations collected at Visit 2.

As an exploratory analysis, we will also collect urine samples twice daily from enrollment through Visit 2 for measurement of urinary NGAL. Further, we will collect any residual plasma samples available in the CHOP lab from clinical chemistry tests for measurement of plasma NGAL. We will then repeat the process of Bayesian AUC_{24} estimation using the Bayesian software and the uNGAL and pNGAL models. Measurement of NGAL will be performed after study enrollment is completed.

3.2 Study Duration, Enrollment and Number of Sites

3.2.1 Duration of Study Participation

Enrolled subjects will be followed for the duration of their hospitalization or 30 days, whichever is shorter.

3.2.2 Total Number of Study Sites/Total Number of Subjects Projected

All subjects will be enrolled at the Children's Hospital of Philadelphia.

We will plan to enroll 20 evaluable subjects. Subjects will be considered evaluable if they provide CysC and PK samples at both study visits (Visits 1 and 2). Subjects that are not evaluable may be replaced.

Because administration of vancomycin may be transient or stopped for clinical care reasons prior to PK sampling, we anticipate that 40 subjects will provide consent/assent in order to enroll 20 evaluable subjects.

3.3 Study Population

3.3.1 Inclusion Criteria

1) Males and females 1-17 years of age,
2) Administered intravenous vancomycin via intermittent infusion,
3) Eligible for vancomycin therapeutic drug monitoring, per the subject's clinical team, and
4) Parental/guardian permission (informed consent).

3.3.2 Exclusion Criteria

1) Receipt of renal replacement therapy, plasmapheresis, or extracorporeal membrane oxygenation (ECMO), or

2) Unable to provide urine and blood samples.

Subjects that do not meet all of the enrollment criteria may not be enrolled. Any violations of these criteria must be reported in accordance with IRB Policies and Procedures.

4 STUDY PROCEDURES

4.1 Screening Visit

- Informed Consent.
- Review of inclusion/exclusion criteria – following consent and prior to performance of any study procedures.

4.2 Observational Period

A schematic for study procedures is shown in Appendix A.

Following informed consent, study subjects will complete two study visits within 4 days to provide blood and urine samples. No more than a teaspoon (3mL) of blood will be collected on any given day of study participation, and no more than two teaspoons (9mL) of blood will be collected over the course of the study from a given participant. After their active participation is complete, their data will continue to be collected from the EMR through the end of their follow-up period.

4.2.1 Visit 1

At Visit 1 subjects will provide 1 blood sample (0.5 – 1.0 mL each) for the measurement of vancomycin in serum in the CHOP clinical lab.

Subjects will undergo TDM for clinical care. This involves collection of 1 or 2 blood samples for measurement of vancomycin concentrations in serum in the CHOP clinical lab. Vancomycin concentrations ordered by the clinical team for TDM will be referred to as "TDM samples." The timing and ordering of all TDM sampling in enrolled subjects will be directed by the clinical team. Because TDM samples are drawn for clinical care, they will not count towards blood volume limits for research.

The optimal sample collection time to estimate AUC using Bayesian estimation will be determined by using our CysC model and each subject's dosing information and informative covariates (i.e. weight, age, CysC). We will use the MMOpt function of Pmetrics™, a library package within R used for population modeling and simulation,[59] to determine the optimal sampling time. The timing of this sample collection may or may not align with one of the TDM samples. If timing does not align, a research-only blood sample will be collected, called the "PK sample". If the optimal sample collection time does align with one of the TDM samples, a PK sample will not be collected. The results of the PK sample will not be reported in the patient's electronic medical record to avoid confusion to learners and providers who may not be fully acquainted with AUC_{24} calculations and TDM practices. If the subject has only a single vancomycin concentration obtained for clinical TDM, we will draw the optimally timed sample as well as a "peak" concentration, which will allow us to calculate AUC_{24} as is done clinically (Zaske-Sawchuk method).

When possible, blood samples will be drawn in conjunction with clinical care to minimize blood draws. Blood will be collected via an existing central venous, arterial or peripheral venous catheter, separate and distinct from the infusing catheter, when possible. If a separate catheter for blood collection is not present, a peripheral venous catheter for blood collection

may be placed, if permission given by the subject's legal guardian and treating PICU physician. If arterial or venous access is not possible, blood may be collected via capillary stick.

Samples will be collected by qualified study staff or CHOP nurses.

4.2.2 Visit 2

At Visit 2 subjects will provide 2 blood samples (0.5 – 1.0 mL) for the measurement of vancomycin in serum in the CHOP clinical lab

If dosing adjustments are made by the clinical team after Visit 1, TDM may be repeated to confirm the adequacy of the dosing change. If 2 TDM samples are obtained for clinical care, no additional PK samples will be drawn. Otherwise, we will collect 2 PK samples. The timing of these PK samples will be determined using the MMOpt function of Pmetrics™. The results of the PK samples will not be reported in the patient's electronic medical record.

When possible, blood samples will be drawn in conjunction with clinical care to minimize blood draws. Blood will be collected via an existing central venous, arterial or peripheral venous catheter, separate and distinct from the infusing catheter, when possible. If a separate catheter for blood collection is not present, a peripheral venous catheter for blood collection may be placed, if permission given by the subject's legal guardian and treating PICU physician. If arterial or venous access is not possible, blood may be collected via capillary stick.

Samples will be collected by qualified study staff or CHOP nurses.

4.2.3 Daily blood and urine samples

- From the time of enrollment through completion of Visit 2, we will measure CysC at least once daily. This test will be performed using the same sample used for SCr measurement or another clinical blood test, thus no additional blood will be collected. On the day of enrollment, a residual sample from a clinical test performed earlier in the day can be used. In the event that a subject does not have labs performed clinically on a given day, we will collect 1 mL for measurement of CysC; we will also keep residual plasma from this sample. No more than 5 mL/kg may be drawn for research purposes in a single day and no more than 9.5 mL/kg may be drawn over any eight-week period for children.

- If available, we will collect or utilize residual blood samples stored in the CHOP Clinical Labs from clinical tests performed from the start of the subject's vancomycin course through the end of active study participation. These samples will be used for CysC or NGAL measurement, and for future research if the subject consents to future use.

- From the time of enrollment through completion of Visit 2, we will collect urine samples twice daily for measurement of urinary NGAL in a research lab. The goal time for these urine samples will be 6-10am and 3-7pm, although we will allow for collection outside of these windows if necessary. Urine samples will be collected via

indwelling urinary catheter (if present), clean intermittent catheterization (if performed for clinical care), urine cup or urine bag.

4.2.4 Weight measurement

Weight will be measured if is one not available from clinical care within 48 hours of vancomycin initiation. Weight can be measured at any time during study participation.

4.2.5 Medical record review

Data/information will be collected. We will collect demographic, anthropomorphic and clinical data, including diagnoses, laboratory and imaging results, and concomitant medications. Outcomes will be tracked including infection outcomes, PICU length of stay and mortality.

4.3 Subject Completion/Withdrawal

Subjects may withdraw from the study at any time without prejudice to their care. They may also be discontinued from the study at the discretion of the investigator due to change in diagnosis, treatment, or lack of adherence to study schedule.

Subjects will be considered evaluable if they provide CysC and PK samples at both study visits (Visits 1 and 2). Subjects that are not evaluable may be replaced.

5 STUDY EVALUATIONS AND MEASUREMENTS

5.1 Screening and Monitoring Evaluations and Measurements

5.1.1 Screening for eligible subjects

Utilizing a daily roster of PICU patients, eligible subjects will be identified each day by a study team member through screening of the PICU census, discussions with the PICU research team, review of electronic medical records and discussion with clinical team.

5.1.2 Informed consent

Subjects' parents or legal guardians will be consented and subjects will be enrolled following initiation of IV vancomycin.

5.1.3 Medical record review

Medical records will be reviewed for collection of data from 48 hours prior to the start of vancomycin therapy through hospital discharge or 30 days from initiation of vancomycin, whichever is shorter:

- Demographics
- Anthropomorphic measures (weight, body surface area)
- Vital signs
- Diagnoses
- Vancomycin dosing information
- Vancomycin TDM information (including calculated AUC_{24} according to clinical team)
- Microbiology results*
- Laboratory results**
- Severity of illness scores
- Respiratory support
- Medications
- Infection outcomes at 14 and 30 days after vancomycin initiation
- PICU and hospital length of stay
- Discharge status (alive/dead)

* Data will be included from vancomycin initiation through 30 days post-treatment to identify infection outcomes.

** Data will be included from 90 days prior to vancomycin initiation through initiation of vancomycin treatment to generate baseline data such as renal function.

5.1.4 Cystatin C measurement

CysC will be measured via particle-enhanced turbidimetric immunoassay (PETIA) in the CHOP Chemistry Laboratory. Results of the CysC measurement will be reported in a subject's electronic medical record.

Up to 5 mL blood will be collected for CysC measurement for this study.

5.1.5 NGAL measurement

Urine samples will be collected via indwelling urinary catheter, clean intermittent catheterization (if performed for clinical care), or urine cup/bag. Urine volumes will be recorded and samples will be kept on ice or refrigerated at 4°C until centrifugation (3000 RPM at 4°C for 15 minutes). The supernatants will be divided and stored at -80°C.

Each urine sample, along with any residual plasma collected for the study, will be used to quantify NGAL via ELISA in the Translational Core Laboratory. Urinary creatinine will also be measured on each urine sample, to correct for urinary volume.

5.1.6 Physical examination

Weight may be measured for research purposes if these measurements were not completed as standard of care. Weight will be collected if one not available from within 48 hours of vancomycin initiation.

5.1.7 PK sample collection

Following the Visit 1 anchoring dose for TDM, we will collect a single 0.5-1.0 mL blood sample for quantification of vancomycin in serum. This PK sample will be collected at the optimal time to estimate vancomycin AUC_{24} via Bayesian estimation. If the timing of the PK sample coincides with a TDM sample, the PK sample will not be drawn. If the subject has only a single vancomycin concentration obtained for clinical TDM, we will draw the optimally timed sample as well as a "peak" concentration, which will allow us to calculate AUC_{24} as is done clinically (Zaske-Sawchuk method).

Within 72 hours of Visit 1, we will collect 2 additional PK samples. The timing of these specimens will be informed by MMOpt and used to estimate vancomycin AUC_{24} via Bayesian estimation. If a subject undergoes clinical TDM within 72 hours of Visit 1, no additional samples will be collected.

Vancomycin concentrations will be measured by chemiluminescent microparticle immunoassay (Abbott Diagnostics) in the CHOP Chemistry Lab. Results of vancomycin concentrations drawn specifically for our study (i.e. PK samples) will not be reported in the patient's medical chart.

Up to 3 mL will be collected for vancomycin concentration measurement for this study.

5.1.8 Future use of stored samples

The consent form will include a section to ask for permission to retain any specimens collected for possible use in future research studies, such as analyses of other biomarkers. We will plan to retain samples indefinitely following the conclusion of the study.

There are no benefits to subjects in the collection, storage and subsequent research use of specimens. Reports about future research done with subjects' samples will not be kept in their health records, but subjects' samples may be kept with the study records or in other secure areas. Subjects can decide if they want their samples to be used for future research or have their samples destroyed at the end of the study. A subject's decision can be changed at any time prior to the end of the study by notifying the study personnel in writing. However, if a subject consents to future use and samples have already been used for research purposes, the information from that research may still be used.

5.2 Bayesian AUC_{24} estimation

We have developed nonparametric population PK models for vancomycin using Pmetrics™, a library package within R used for population modeling and simulation.[59] As described in section 1.2, we have developed three popPK models in our previous work that have performed similarly in terms of their accuracy and precision for AUC_{24} estimation. The first is based on plasma CysC as a renal function biomarker (CysC model) and the others include urinary NGAL (uNGAL model) and plasma NGAL (pNGAL model) on vancomycin CL. The CysC model is our primary model of interest while the NGAL models are exploratory models.

For each subject enrolled, we will utilize the Multiple Model optimal (MMopt) sampling algorithm in Pmetrics™ to identify the single optimal sampling time to estimate AUC_{24}.[60] MMopt finds the optimal times based on when all the PK curves generated by the support points in the nonparametric model, in this case the CysC model, are most separated (e.g. the time points that are most informative). Thus, MMopt will inform the optimal sampling time for each subject, based on the subject's dosing information, covariate data (i.e. CysC result), and anticipated sampling window.

Through a Collaborative Research Agreement with InsightRx, Inc. (2021-1898), the CysC and NGAL models can be incorporated into the InsightRx clinical dosing software program for Bayesian estimation in our study. Data will be de-identified prior to entry into their web-based interface. If integration of our models into InsightRx software cannot be accomplished, we will use BestDose™ or Pmetrics™, which are freely available, R-based PK software programs.

For each subject, we will enter the relevant vancomycin dosing information and Visit 1 covariate data (e.g. CysC concentration, weight, age, etc.) into the program. We will additionally enter their single, optimally timed, Visit 1 vancomycin concentration. From this, we will generate estimates of each subject's PK (CL, volume of distribution, elimination rate, etc.) and their Visit 1 vancomycin AUC_{24}, which will be recorded in REDCap. We will then add any additional Visit 1 vancomycin concentrations obtained for TDM to the software, re-estimate the subject's PK and AUC_{24}, and record these values in REDCap. We will also calculate each subject's PK and AUC_{24} using TDM samples via the Zaske-Sawchuk method, as will be performed clinically.

We will then use Bayesian estimation to predict each subject's Visit 2 AUC_{24} based on the relevant dosing information, covariate data, and optimally timed Visit 1 PK. We will also predict each subject's Visit 2 vancomycin concentrations, based on the time that TDM or PK samples are collected.

Finally, we will re-estimate the Visit 2 PK and AUC_{24} incorporating all relevant dosing information, covariate data, and vancomycin concentrations.

The uNGAL and pNGAL models will be utilized retrospectively, since measurement of urinary and plasma NGAL will only take place after completion of enrollment.

6 STATISTICAL CONSIDERATIONS

6.1 Primary Endpoints

Vancomycin AUC_{24} at Visit 1 estimated using Bayesian estimation and the single, optimally timed vancomycin PK sample ($AUC_{optimal}$).

6.2 Secondary Endpoints

Vancomycin AUC_{24} at Visit 1 estimated using Bayesian estimation and all available vancomycin concentrations (AUC_{full_1})

Vancomycin AUC_{24} at Visit 1 calculated using Zaske-Sawchuk method and TDM vancomycin concentrations ($AUC_{clinical\_1}$)

Predicted vancomycin AUC_{24} at Visit 2 using Bayesian estimation and the single, optimally timed vancomycin PK sample from visit 1 ($AUC_{predicted}$).

Vancomycin AUC_{24} at Visit 2 estimated using Bayesian estimation and all available vancomycin concentrations (AUC_{full_2})

Vancomycin AUC_{24} at Visit 2 calculated using Zaske-Sawchuk method and TDM or PK vancomycin concentrations ($AUC_{clinical\_2}$)

Vancomycin concentrations at Visits 1 and 2

6.3 Control of Bias and Confounding

Subjects will be identified based on eligibility criteria defined above. Among those eligible for participation, selection of subjects will not be influenced by subjects' cultural background, age, or socioeconomic status. We will minimize information bias by collecting data from the medical record in a systematic manner using a common data abstraction tool. All efforts will be made to record data accurately.

6.4 Statistical Methods

6.4.1 Baseline Data

Baseline and demographic characteristics will be summarized by standard descriptive summaries (e.g. means and standard deviations for continuous variables such as age and percentages for categorical variables such as sex).

6.4.2 Analysis of Primary Outcome of Interest

The primary analysis will compare the Visit 1 AUC_{24} estimated using Bayesian estimation and the single, optimally timed vancomycin PK sample ($AUC_{optimal}$) to the Visit 1 AUC_{24} estimated using Bayesian estimation and all available vancomycin concentrations (AUC_{full}). Since we have shown through our previous work that vancomycin AUC_{24} can be accurately estimated with $\geq$2 concentrations, Bayesian estimation from all available concentrations (PK sample + TDM samples) will serve as the primary comparator of interest.

We will determine the bias [($AUC_{optimal}$ – AUC_{full} / AUC_{full}) * 100] and imprecision [absolute value of ($AUC_{optimal}$ – AUC_{full} / AUC_{full}) * 100] for each subject, and, we will summarize these measures across subjects. Bias <10% will be considered excellent and <20% will be considered good.

A series of secondary comparisons will also be performed:

- $AUC_{optimal}$ to $AUC_{clinical\_1}$
- $AUC_{predicted}$ to AUC_{full_2}
- $AUC_{predicted}$ to $AUC_{clinical\_2}$
- Estimated Visit 1 vancomycin concentrations after Bayesian estimation with the optimally timed PK sample to measured vancomycin concentrations
- Predicted Visit 2 vancomycin concentrations after Bayesian estimation with the optimally timed PK sample to measured vancomycin concentrations
- Comparison of PK parameters derived from Bayesian estimation vs clinical calculations

All comparisons will be made using the CysC model as the model embedded in the Bayesian estimation software.

As an exploratory analysis, we will embed the Bayesian software with the uNGAL and pNGAL models and repeat all AUC estimations. Each AUC estimation ($AUC_{optimal}$, AUC_{full_1}, and AUC_{full_2}) will be compared to the estimate generated using the CysC model, as well as to $AUC_{clinical\_1}$ and $AUC_{clinical\_2}$, as appropriate.

6.5 Sample Size and Power

This is a pilot study with the primary objective to demonstrate the feasibility of real-time Bayesian estimation for vancomycin at the bedside using CysC. Sample size was not determined by power calculations since no statistical comparisons are planned for this study. Results of this pilot study will serve as preliminary data to inform future grant applications and large-scale studies that will investigate the impact of AUC-driven dosing on clinical outcomes.

7 SAFETY MANAGEMENT

7.1 Clinical Adverse Events

Vancomycin will be administered by the CHOP clinical team. All routine administration and monitoring practices will be followed by the CHOP clinical team. Decisions regarding initiation, dosing, monitoring, and discontinuation of vancomycin will be solely at the discretion of the clinical team. Monitoring of adverse events (AEs) will not be conducted as part of this observational study. Results of vancomycin concentrations drawn for research purposes will not be made available to the clinical team. Results of Bayesian estimation will also not be shared with the clinical team.

7.2 Adverse Event Reporting

Since the study procedures are not greater than minimal risk, SAEs are not expected. If any unanticipated problems related to the research involving risks to subjects or others happen during the course of this study (including SAEs) these will be reported to the IRB in accordance with CHOP IRB SOP 408: Unanticipated Problems Involving Risks to Subjects. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

8 STUDY ADMINISTRATION

8.1 Data Collection and Management

Data entry and storage will be facilitated using REDCap, which enables secure capture of research data as coded, data-dictionary-compliant observations through web-based electronic case report forms and surveys. Real-time transactional data such as variables associated with an updated clinical encounter in the EHR can be captured programmatically through the REDCap application-programming interface. Whenever possible, study data will be entered directly into REDCap to minimize the use of paper forms.

Research urine samples, and any residual plasma samples, will be labeled with a unique Subject ID and sample number. Paper forms may be used to collect research information related to sample collection such as collection date/times, collection site, and other pertinent collection, storage, and sample processing information. All paper forms will be coded with a Subject ID and direct patient identifiers (name, DOB, MRN) will be omitted. It is possible that research samples will need to be temporarily labeled with a clinical patient label, but this label will be removed and destroyed prior to any longer-term storage in the Clinical Pharmacology Lab in Abramson Research Center.

Vancomycin and CysC samples will be sent to the CHOP Chemistry Laboratory and labeled with patient stickers, as is routine clinical care. Results of CysC will be made available in the patient's EMR. Results of vancomycin concentrations drawn specifically for the study will not be made available, since these PK samples are not part of routine care.

Pharmacokinetic data and research test results will be stored on a secure Research SAN. Subject ID numbers will be used in lieu of direct patient identifiers (name, DOB, MRN), although these files may contain dates and times.

All data entered into InsightRx will be de-identified. Dates will be shifted prior to entry into the InsightRx interface to ensure confidentiality of the data.

1. Confidentiality

    - All research materials are inaccessible to anyone other than the investigators and other project personnel approved for the conduct of this research by the IRB of the Children's Hospital of Philadelphia.

    - The REDCap project will link PHI and Subject ID numbers. Paper data forms will be coded with a Subject ID number; these files will also contain dates. Electronic data will be stored in REDCap or on the CHOP Research SAN. Electronic files will be coded and will be stored on the CHOP Research SAN. No identifying information about the subjects will be extracted or reproduced on collection forms or labels.

    - Electronic files will be stored on the CHOP Research SAN accessible only to CHOP research staff who have completed their CITI training.

2. Security

- Electronic files will be stored on the CHOP secure network that is backed up at least daily.

3. Anonymization, de-identification or destruction

    - A coded data set will be used to replace the name, MRN and other readily identifiable fields with a unique Subject ID number. No identifying information about the subjects will be extracted or reproduced on collection forms or labels. Each subject in the study will be assigned a unique Subject ID to be used on all data forms and labels. The link between direct identifiers and Subject ID numbers will be maintained in REDCap. Only the investigators and the IRB approved project staff at CHOP will have access to this information. All personally identifiable information will be kept strictly confidential and on secure servers at CHOP.

8.2 Confidentiality

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy, and the Investigator and other site personnel will not use such data and records for any purpose other than conducting the study. Safeguards to maintain confidentiality have been described above.

No identifiable data will be used for future study without first obtaining IRB approval. The investigator will obtain a data use agreement between provider (the PI) and any recipient researchers (including others at CHOP) before sharing a limited dataset (dates and zip codes).

8.3 Regulatory and Ethical Considerations

8.3.1 Data and Safety Monitoring Plan

The Principal Investigator will be responsible for data and safety monitoring. The only risks anticipated to patients are those from blood draws and potential loss of confidentiality.

8.3.2 Risk Assessment

The study is minimal risk for all subjects and potential for minor discomfort is possible.

Risks associated with collection of blood:

Blood samples will be collected primarily via pre-existing vascular catheters. For subjects that do not have adequate access, investigators may place a research IV catheter to collect blood samples. Placing an IV may cause some pain, and bleeding or bruising at the spot where the needle enters your body. Rarely, it may cause fainting. The longer an IV catheter is left in place, the more common it is for redness or infection to develop. If placement of a research IV is not possible and clinically-placed central lines or arterial lines are not available, blood samples may be collected via venipuncture, which may cause some pain, bleeding or bruising at the spot where the needle enters your body. If arterial or venous access is not possible, blood may be collected via capillary stick. Rarely, taking blood may cause fainting or infection. To reduce risk, bedside nurses will be used to obtain samples and

follow all CHOP protocols. Phlebotomists and/or accredited/approved research staff may draw blood samples via venipuncture.

Risks associated with collection of urine:

Urine samples introduce no additional risk due to collection from pre-existing urinary catheter, clean intermittent catheterization, urine cup, or urine bag.

To minimize loss of confidentiality, study files containing PHI will be password protected and only accessed by the CHOP study team.

8.3.3 Potential Benefits of Study Participation

There is no direct benefit to study participation, but patients involved in the study may incur an indirect benefit from participation. CysC results will be made available to a subject's treating physicians. Measurement of CysC concentrations could facilitate identification of individuals with impaired renal function and/or promote needed dose adjustments or renally eliminated drugs, such as vancomycin.

Participation in this study may also help future children treated with IV vancomycin. This study will demonstrate the feasibility of Bayesian estimation based on a robust population PK model of IV vancomycin in critically ill children. Results from this study will inform future studies and may inform clinical care of future children.

8.3.4 Risk-Benefit Assessment

The proposed research is justified, considering that the risk associated with participation is minimal compared to the potential and anticipated benefits. With experienced healthcare providers and trained staff conducting blood draws, and appropriate confidentiality measures in place, the risk to the subjects should be negligible.

8.4 Recruitment Strategy

Eligible subjects will be identified through daily screening of the PICU census by study staff. Further, study staff will be notified by Antimicrobial Stewardship team, clinical pharmacists, or via an EPIC alert, when a patient in the PICU is receiving IV vancomycin and eligible for clinical AUC_{24} monitoring. Principal Investigator or qualified research staff will approach the eligible subjects and their parent(s)/legal guardian(s) in the PICU for consent. The screening for this study qualifies for a waiver of HIPAA authorization since it is no more than minimal risk and it will not be feasible to obtain consent and HIPAA authorization prior to identification of eligible subjects (initiation of vancomycin).

8.5 Informed Consent/Assent and HIPAA Authorization

In obtaining consent, research staff will explain the risks and benefits of participation to the child and their parent/legal guardian. The family will be able to ask any study-related questions and will be given time to consider their decision. If requested, the research staff will leave the family to consider study participation and come back later. After all study-related questions are answered and families have had time to consider their decision, written informed consent and HIPAA authorization (using a combined form) will be obtained by the

research staff. Assent will be obtained from children, as possible. We expect that the majority of eligible patients will have altered mental status due to the severity of their infections that necessitate a level of care provided in the PICU, in which case assent will be deferred.

The informed consent will cover the child's participation including collection of biological samples and electronic health data. The in-person consent process will take place at Children's Hospital of Philadelphia.

Because procedures involved in the study are time-sensitive, and parents/guardians may not be present in a patient's room at all times, we may verbally consent the subject's parent/guardian over the phone. Subjects' parents/guardians will be provided comprehensive information using the verbal consent and qualified staff will go over the study procedures with them and answer any questions that they might have. Documentation of consent will be made by the person consenting by phone. A copy of the completed verbal consent form will be provided to the parent/guardian of the study subject.

8.6 Payment to Subjects/Families

The subject's family will be provided with a $20 gift card upon study completion for their time and effort associated with completion of all study procedures for the study.

9 PUBLICATION

We plan to publish the results in a peer-reviewed journal. No individually identifiable PHI will be published.

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APPENDIX

Figure 1. Schematic of study procedures. Subjects will be enrolled following initiation of vancomycin. A blood sample (for CysC) will be collected following enrollment and then with each SCr clinical measurement (at least daily). At the time of therapeutic drug monitoring (TDM), an additional, optimally timed PK sample will be collected following the same dose used for TDM. Within 72 hours of initial TDM, additional blood (for CysC) will be obtained, prior to repeat performance of vancomycin TDM. If TDM sampling will not occur a second time, 2 samples will be drawn for measurement of vancomycin concentrations for purposes of the study. We will also collect urine samples twice daily, as well as residual plasma from clinical samples, from enrollment through Visit 2, which will be used for measurement of NGAL after completion of enrollment.