Effect of Direct-from-blood Bacterial Testing on Antibiotic Administration and Clinical Outcomes

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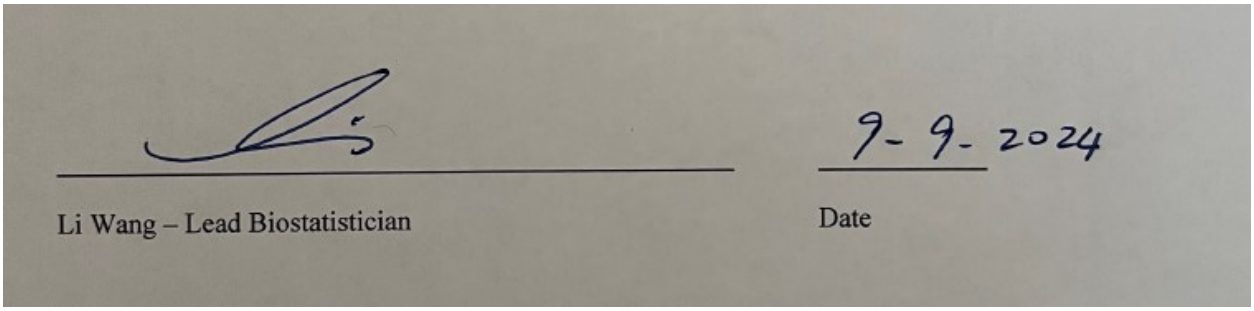
PIs: David Gaston, MD, PhD, and Romney Humphries, PhD, D(ADBMM), M(ASCP)

Effect of direct-from-blood bacterial testing on antibiotic administration and clinical outcomes: A learning healthcare pragmatic randomized trial

Statistical Analysis Plan

August 26, 2024



Change log:

- Version 1.0- November 15, 2023-Original version
- Version 1.1- August 26, 2024
    - Intervention group procedures updated to reflect protocol amendment approved on January 24, 2024.
    - Exclusion criteria updated to reflect protocol amendment approved on February 6, 2024
    - Updated 1) sample size considerations 2) main and additional analyses of the primary outcome 3) exploratory outcome and effect modification analyses and 4) clarifications regarding the handling of missing data to match manuscript submission to *BMJ Open* on June 20, 2024

Introduction

Bacterial blood stream infections are common and life-threatening. Bloodstream infections have historically been identified using blood cultures, which often take 24-72 hours to result and are imperfectly sensitive. Because of these limitations, there has been increasing interest in using rapid diagnostic tests that identify bacteria directly from whole blood samples without relying on growth in culture, referred to as "direct-from-blood" tests, in addition to standard blood cultures, to guide early therapeutic management of patients with suspected bloodstream infections.

Early administration of antimicrobial therapy is a fundamental component of the management of adults presenting to the hospital with a suspected bloodstream infection or sepsis. Current guidelines recommend the use of empiric intravenous vancomycin as coverage for methicillin-resistant *S. aureus* (MRSA) bacteremia. However, Vancomycin requires careful monitoring due to its narrow therapeutic range and high risk of toxicity.

Before considering implementation of direct-from-blood testing across the health system, Vanderbilt seeks to better understand how the use of direct-from-blood testing for bacterial

pathogens impacts use of antimicrobials, antimicrobial stewardship, health system resources, and costs. We will conduct a pragmatic, randomized clinical trial examining the effect of using direct-from-blood testing for bacterial pathogens, compared to using blood cultures alone, on antimicrobial receipt and clinical outcomes for adults presenting to the hospital with suspected infection who have been initiated on empiric therapy with intravenous vancomycin.

Population and design considerations

Study Population:

500 adult patients presenting to the Emergency Department at Vanderbilt University Medical Center with suspected infection for whom collection of blood cultures and empiric initiation of intravenous vancomycin are ordered within 12 hours of presentation to the Emergency Department.

Inclusion Criteria

- Patient is located in the Emergency Department at Vanderbilt University Hospital
- ≤ 12 hours from patient presentation to the Emergency Department at Vanderbilt University Hospital
- Age ≥ 18 years
- Clinician has ordered blood cultures
- Clinician has ordered intravenous vancomycin

Exclusion Criteria

- Patient is known to be a prisoner

- Patient is known to be pregnant
- Patient is known to have received more than 2 doses of intravenous vancomycin since presentation to the Vanderbilt ED
- Patient is known to have a positive bacterial culture in the previous 7 days
- Patient is known to have an infection for which at least 7 days of intravenous vancomycin would routinely be administered regardless of bacterial testing results (e.g., skin and soft tissue infection, etc.)

Study Design:

Single-center, parallel-group, non-blinded, pragmatic, randomized clinical trial comparing antibiotic administration and clinical outcomes between the blood culture group (usual care) and the direct-from-blood test group (intervention group).

Randomization:

Eligible patients will be randomized in a 1:1 ratio to the direct-from-blood test group or the blood culture alone group. A series of study group assignments will be generated by computerized randomization using EPIC functionality. Simple randomization without stratification, permuted blocks, or minimization will be used.

Sample Size Considerations:

The planned sample size for this trial is 500 patients (250 patients per group). The planned follow up duration for each patient for the primary outcome is 14 days. Prior data indicate that the median time to the primary outcome in the control group will be approximately

48 hours. If the true median times to the primary outcome in the control and intervention groups are 48 and 36 hours, respectively, we will be able to reject the null hypothesis that the experimental and control survival curves are equal with probability (power) .895. The Type I error probability associated with this test of this null hypothesis is 0.05.

Interventions

Blood Culture Group (Usual Care)

For patients assigned to the blood culture group, the blood cultures ordered at the time of eligibility assessment will be collected, performed, and reported as in routine clinical care. The blood culture testing will be performed in the clinical laboratory at Vanderbilt. At Vanderbilt, blood culture results are updated intermittently as results become available. Negative blood cultures, similar to other tests without a critical result, are uploaded in the electronic health record for review by the clinician. Following a gram stain for a positive blood culture, the ePlex/Roche test is used. This tests for 95% of the most common bacterial pathogens recovered in blood cultures. The results of the ePlex/Roche test on the blood culture are reported in the electronic health record along with an electronic notification (alert) to the clinical team. Identification of selected organisms (e.g., *S. aureus*, *Enterococcus* and *S. lugdunensis*) are also alerted to the Infectious Disease fellows. Blood containing bacteria is plated onto culture media to definitively identify organisms and characterize antibiotic susceptibility, if indicated. These results are also communicated to the clinical team in the electronic health record. Patients will have access to these results in their MyHealth at Vanderbilt portal.

Direct From Blood Test Group (Intervention Group)

For patients assigned to the direct-from-blood test group, in addition to having blood cultures collected, performed, and reported in the process described above, direct-from-blood testing for bacterial pathogens will be performed. The order for blood cultures will be accompanied by an order for direct-from-blood testing for bacterial pathogens. As the blood is being collected for blood cultures, clinical personnel will collect an additional 4 mL for direct-from-blood testing for bacterial pathogens. If an adequate direct-from-blood test sample is not drawn (e.g. incorrect tube or insufficient sample volume, missed or cancelled lab order, etc.) additional attempts to collect the sample for direct-from-blood testing may be made for up to 12 hours following randomization. Direct-from-blood testing for bacterial pathogens will be performed in the clinical laboratory. Direct-from-blood testing will use the T2Bacteria® Panel. Results of direct-from-blood testing for bacterial pathogens will be reported in the following ways:

1. The results will be reported in the electronic health record in the same section on microbiology in which blood culture results are reported. The report will contain a description of the performance characteristics of the direct-from-blood testing and an interpretation of the findings. Patients will have access to these results in their MyHealth at Vanderbilt portal.
2. The results will be sent to the treating clinicians on the patient's primary team via a text page at the time the results populate into the patient's electronic health record. The text page will include the results for all five pathogens identified by the panel. Treating clinicians will also be directed to information regarding interpreting the direct-from-blood testing results.
3. For patients with direct-from-blood testing that is negative for *S. aureus* who continue to have an active order for intravenous vancomycin at 7am, an interruptive best practice

alert (BPA) will remind treating clinicians of the result of the direct-from-blood testing, , and prompt the clinician to select a reason for continuing vancomycin if continued (e.g., known or suspected infection with a pathogen for which vancomycin is standard of care; critical illness; severe or serious allergy to alternative antimicrobials; surgical prophylaxis; other). This BPA does not result in any automatic action and does not limit the autonomy of the care provider in the clinical decision-making process. All aspects of the patient's treatment remain at the clinician's discretion.

4. For patients receiving intravenous vancomycin for whom the pharmacokinetics services is consulting, the pharmacokinetics service will incorporate the results of the direct-from-blood testing into their recommendations and communications with the treating clinicians (as they would standard blood culture results as well).

Clinicians and patients may use these results, in addition to information about the patient's demographics, chronic conditions, acute conditions, vital signs, other laboratory results, and concurrent treatments to help inform decisions. Neither the blood cultures nor the direct-from-blood test will serve as the sole basis for diagnosis, treatment, or other management decisions.

Co-Interventions: Management of Vancomycin by Pharmacokinetics Service

For patients in both trial groups of the current study, management of intravenous vancomycin by the pharmacokinetics service will occur as it does in routine clinical care. At Vanderbilt, adult patients with an active order for intravenous vancomycin are followed by the pharmacokinetics service to determine the dosing regimen of vancomycin to maximize safety and therapeutic efficacy. The typical workflow consists of:

- Assessing patient information necessary to dose and monitor the antibiotic.
- Entering order for serum creatinine if one has not been obtained.
- Entering order for dosage regimen based on pharmacokinetic principles.
- Entering order for peak, trough, and/or random levels, as applicable.
- Performing follow-up monitoring and/or dose adjustments, as needed.
- Documenting a consult note in the electronic medical record upon initial consultation and daily until the protocol is discontinued.
- Notifying the provider for further instructions if:
    - Adverse reactions or evidence of hypersensitivity.
    - Systemic concentration of specified medication that is two times above the upper limit or if the concentration is significantly below the lower limit of the therapeutic range as defined by VUMC.
    - Increases in serum creatinine two times the patient's baseline serum creatinine value.

Endpoints

Primary Endpoint

The primary outcome is time to last dose of intravenous vancomycin, defined as the time between randomization and the start time for the last dose of intravenous vancomycin received by the patient within 14 days of randomization.

Secondary Endpoint(s)

The secondary outcome is time to last dose of systemic anti-pseudomonal beta-lactam antibiotic, defined as the time between randomization and start time of the last dose of systemic, anti-pseudomonal, beta-lactam antibiotic received by the patient within 14 days of randomization.

Exploratory Endpoint(s)

Exploratory Antimicrobial Stewardship Outcomes:

1. Total number of doses of intravenous vancomycin received between randomization and 14 days after randomization.
2. Total number of days on which gram-positive antibacterial therapy was received between randomization and 14 days after randomization.
3. Total number of days on which gram-negative antibacterial therapy was received between randomization and 14 days after randomization.
4. Time to receipt of antibacterial therapy with effective coverage for blood stream infections identified by final blood culture results collected prior to or at the same time as randomization, assessed at 14 days after randomization.
5. Proportion of patients who experienced *Clostridioides difficile* infection between randomization and hospital discharge or 28 days after randomization, whichever occurs first.

Exploratory Safety Outcomes:

1. Proportion of patients who experienced an allergic reaction to antimicrobial therapy between randomization and 14 days after randomization

2. Proportion of patients for whom vancomycin was discontinued between randomization and 72 hours for whom any culture from the 24 hours prior to or 24 hours after randomization grew methicillin resistant *S. aureus.*
3. Proportion of patients for whom all anti-staphylococcal therapy was discontinued between randomization and 72 hours for whom any culture from the 24 hours prior to or 24 hours after randomization grew *S. aureus.*
4. Proportion of patients for whom all anti-pseudomonal therapy was discontinued between randomization and 72 hours for whom any culture from the 24 hours prior to or 24 hours after randomization grew *P. aeruginosa.*

Exploratory Clinical Outcomes:

1. Highest stage of acute kidney injury by KDGIO creatinine criteria between randomization and 14 days after randomization
2. Receipt of renal replacement therapy between randomization and 14 days after randomization
3. Lowest platelet count between randomization and 14 days after randomization
4. Hospital-free days to day 28, defined as the number of calendar days alive and free of hospitalization between randomization and 28 days after randomization with outcome assessment censored at hospital discharge
5. ICU-free days to day 28, defined as the number of calendar days alive and free of intensive care unit admission between randomization and 28 days after randomization with outcome assessment censored at hospital discharge
6. All-cause, in-hospital mortality to day 28

Process Measures

Antimicrobial Stewardship Process Measures:

1. Time from randomization to a positive test for bacteria in the blood (either direct-from-blood test or bacterial culture)
2. Time from randomization to direct-from-blood test result (in direct-from-blood test group)
3. Time from randomization to blood culture results (in blood culture and direct-from-blood-testgroup)
4. Concordance between direct-from-blood result and blood culture result (in direct-from-blood test group)
5. Receipt of non-vancomycin antimicrobial therapy for MRSA bacteremia in the 14 days following randomization
6. Number of consultations to Infectious Disease in the 14 days following randomization
7. Number of supratherapeutic vancomycin levels in the 14 days following randomization
8. Number of patients followed by the Pharmacokinetics team.

Analysis dataset

The analysis for the trial will use an intent-to-treat approach to answer the effectiveness question posed. That is, participants will be evaluated by treatment group as assigned regardless of what was delivered. All eligible participants will be included. There is no plan to restrict the analysis to a per protocol set.

Statistical Approach

Analyses will be conducted following reproducible research principles using R (R Foundation for Statistical Computing, Vienna, Austria). Categorical variables will be presented as number and percentage. Continuous variables will be presented as mean ± SD or median and IQR. A two-sided P-value of < 0.05 will define a statistically significant between-group difference in the primary outcome. With a single primary outcome, no adjustment for multiplicity will be made. For secondary, safety, and exploratory analyses, emphasis will be placed on the magnitude of differences between groups with 95% confidence intervals rather than statistical significance.

Descriptive Analysis

To characterize the study sample, baseline demographic and clinical data will be described overall and by group. Categorical variables will be described using frequencies and proportions, and continuous variables will be described using means and standard deviations, as well as medians and interquartile ranges. Missingness will be reported for each variable. Graphical summaries using box plots, violin plots, and/or histograms may be used to describe the data graphically.

We will describe all of the primary outcome, secondary outcomes, exploratory outcomes, exploratory safety outcomes, and exploratory clinical outcomes overall and grouped by study arm using the same approach as for the demographic data. Summary statistics and graphical representations may be displayed, and missingness will be reported for each variable. No statistical comparisons between groups will be done for this descriptive analysis.

Main Analysis of the Primary Outcome

The primary outcome is time to last dose of intravenous vancomycin, defined as the time between randomization and the start time for the last dose of intravenous vancomycin received by the patient within 14 days of randomization. The main analysis will be an unadjusted, intention-to-treat comparison of the primary outcome between patients randomized to the direct-from-blood testing group versus the usual care group. Patients who are discharged on or prior to 14 days after randomization will be assumed to not receive vancomycin after discharge. Estimation and inferences of the intervention effect will be made using an unadjusted Cox proportional hazard model with the dependent variable of time to last dose of intravenous vancomycin and the independent variable of trial group assignment. The model results will be presented as a hazard ratio with 95% confidence interval.

Additional Analyses of the Primary Outcome

Sensitivity Analyses – We will perform the following sensitivity analyses:

1. We will repeat the primary analysis but will assign patients who died on or prior to day 14 after randomization a value of 15, higher than the worst possible value for the outcome among patients who did not die. The aim of this sensitivity analysis is to assess whether any observed difference between groups in the time to last dose of intravenous vancomycin is not a result of a difference between groups in the incidence of death, after which additional doses of vancomycin cannot be received.
2. We will compare the primary outcome of time to last dose of intravenous vancomycin between the trial groups using the Fine-Gray subdistribution hazard model that models both the risk of the primary outcome and the competing risk of death. Patients who survive for at least 48 hours after the final dose of vancomycin will be considered to have had vancomycin discontinued while alive and patients who died within 48 hours after the

final dose of vancomycin will be considered to have died without having had vancomycin discontinued.

3. We will compare the primary outcome of time to last dose of intravenous vancomycin between trial groups using a proportional odds model. Each patient will receive a value between 1 day (last dose of vancomycin on the day of enrollment) to 14 days (last dose of vancomycin on day 14). Patients who died within 14 days of enrollment will receive a value of 15 (worse than the longest possible duration of vancomycin therapy).
4. We will repeat the main analysis of the primary outcome in two subsets of the overall trial population defined by receipt of key co-interventions:
    a. Among only patients for whom blood cultures were successfully drawn in the 12 hours prior to or 12 hours after randomization;
    b. Among only patients who received one or more doses of vancomycin in the 12 hours prior to or 12 hours after randomization.

Modified Intention to Treat

We will repeat the primary analysis among all patients for whom blood cultures resulted and, within the intervention group, for whom the direct-from-blood test resulted.

Analysis of the Secondary outcome

The secondary outcome is time to last dose of systemic anti-pseudomonal beta-lactam antibiotic, defined as the time between randomization and start time of the last dose of systemic, anti-pseudomonal, beta-lactam antibiotic received by the patient within 14 days of randomization. The analysis of the secondary outcomes will use the same approach as for the primary outcome.

Analysis of Exploratory outcomes

For each exploratory outcome, we will perform intention-to-treat analyses comparing patients randomized to each of the two trial groups. For categorical outcomes, we will use the Chi-square test for unadjusted comparisons and a binary or multinomial logistic regression model for adjusted comparisons. For continuous or ordinal outcomes, we will use the Wilcoxon rank sum test for unadjusted comparisons or a proportional odds logistic regression model for adjusted comparisons. For time-to-event analyses, we will use a Cox proportional hazards model. All model results will be summarized with point estimates and 95% confidence intervals, which will be emphasized over P values when reporting the results for exploratory outcomes. No adjustments for multiplicity will be made.

Effect Modification

We will examine whether prespecified baseline variables modify the effect of study group assignment (direct-from-blood testing group vs usual care group) on the primary outcome using a formal test of statistical interaction in a Cox proportional hazards model with the primary outcome as the dependent variable and fixed effects of trial group, the prespecified proposed effect modifier and the interaction between the two. For categorical variables, we will present the HR and 95% CIs within each prespecified subgroup. Continuous variables will not be dichotomized for analysis of effect modification but may be dichotomized for data presentation. All continuous variables will be modelled assuming a non-linear relationship to the outcome using restricted cubic splines with between 3 and 5 knots.

In accordance with the Instrument for assessing the Credibility of effect Modification Analyses (ICEMAN) recommendations, we have prespecified the following baseline variables as potential effect modifiers and hypothesized the direction of effect modification for each:

1) Presence of End Stage Kidney Disease on Renal Replacement Therapy (yes/no). We hypothesize that the presence of ESKD on RRT will not modify the effect of study group assignment on the primary outcome.
2) Sepsis (yes/no for meets Sepsis-3 criteria). We hypothesize that the presence of sepsis will not modify the effect of study group assignment on the primary outcome.

3) Severity of illness (SOFA score). We hypothesize that severity of illness will not modify the effect of study group assignment on the primary outcome.
4) Suspected source of infection (lung, intra-abdominal, genitourinary, other, unknown). We hypothesize that the suspected source of infection will modify the effect of study group assignment on the primary outcome, with a greater difference between trial groups in the time to final dose of vancomycin among patients with non-pulmonary sources of infection compared to among patients with a pulmonary source of infection.
5) Transplant recipient (yes/no for history of solid organ or stem cell transplant). We hypothesize that whether a patient is a transplant recipient will not modify the effect of study group assignment on the primary outcome.
6) Neutropenia (yes/no for absolute neutrophil count less than 1500 cells/mcL). We hypothesize that the presence of neutropenia will modify the effect of study group assignment on the primary outcome, with a greater difference between trial groups in the time to final dose of vancomycin among patients without neutropenia than among patients with neutropenia. This hypothesis is supported by prior evidence suggesting that patients with neutropenia are more likely to receive longer durations of antimicrobial therapy in the absence of data demonstrating the presence of a bacterial infection.

Missing Data

We anticipate that no patients will be lost to follow up before assessment of the primary outcome . because outcome ascertainment occurs only during the index hospitalization. Missing data will not be imputed for the primary outcome or for any of the secondary or exploratory

outcomes. In adjusted analyses, missing data for baseline covariates will be imputed using multiple imputations.

Summary

The analysis approach we describe is selected based on the trial's pragmatic nature and the intent to understand the effectiveness of direct-from-blood testing. The results of this study will help to determine the effects of direct-from-blood testing to blood cultures alone on the de-escalation of empirical antibiotic therapy, transition to targeted antimicrobial therapy, antimicrobial stewardship efforts, and clinical outcomes.