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Original Contribution

Retrospective analysis of clinical data associated with patients enrolled in a molecular diagnostic feasibility study highlights the potential utility for rapid detection of bloodstream infection☆,☆☆,☆☆☆,★,★★

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ABSTRACT

Background: Measurement of pathogen DNA polymerase activity by enzymatic template generation and amplification (ETGA) has shown promise in detecting pathogens in bloodstream infection (BSI). We perform an in-depth analysis of patients with clinical BSI enrolled in ETGA feasibility experiments.

Methods: In addition to hospital blood cultures, 1 study aerobic culture bottle was drawn from patients with suspected BSI. The study bottle was split into 2 bottles and was additionally subjected to ETGA analysis. Enzymatic template generation and amplification sensitivity/specificity for BSI detection was determined against the Centers for Disease Control BSI definition. When split cultures were both positive, time course analysis was performed to determine time to detection. The records of patients with BSI were reviewed for presence of systemic inflammatory response syndrome, antibiotic timing and appropriateness, and organism identification.

Results: Of 307 enrollees, 38 met the Centers for Disease Control BSI definition. Seventy-four percent met systemic inflammatory response syndrome criteria on admission. Antibiotic coverage was adequate in 76% of patients. Antibiotics were more often delayed in afebrile patients (odds ratio, 5).

Twenty-seven of the split study culture bottles were positive in at least 1 sample, and ETGA detected microbes within all samples (sensitivity/specificity, 70.3%/99.3%). Of these, 22 were culture positive in both split study bottles and underwent ETGA time course analysis. Enzymatic template generation and amplification detected microbes within these 3-fold faster than culture.

Conclusions: Patients with BSI often have diagnostic and treatment delays. Enzymatic template generation and amplification provides clinically meaningful data more rapidly than cultures. Future development should focus on real-time application of assays that detect microbes at the molecular level.

☆ Conflict of interest statement: The authors listed as being from Research and Development, Zeus Scientific, are the developers of the ETGA technology discussed in this article, and they performed the assay for the purposes of the study.

☆☆ The authors from St Luke's Hospital and Samaritan Hospital received no compensation from Zeus Scientific and have no conflicts of interest to report.

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★ This study was funded by Zeus Scientific, the developers of ETGA technology. The authors listed as being from Zeus were responsible for transportation of specimens and off-site testing and storage including study sample splitting, study sample culture, and performance of ETGA assay. They were responsible for maintaining and transmitting these data to the clinical site investigators (primarily RJ and JM). They supplied materials for the study, including blind-coded labels, the study refrigerators, and empty aerobic blood culture bottles. In addition to the above, the authors from Zeus Scientific were involved in study design and IRB submission. They reviewed and approved the manuscript.

★★ JA, JR, JM, and RJ were involved in study design, institutional review board submission, and drafting and review of the manuscript. JM and RJ were additionally responsible for gathering all patient data, review of medical records, statistical analyses and interpretation, and had access to all data from both Zeus Scientific and the clinical site.

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1. Introduction

1.1. Background

Based on epidemiology studies, sepsis affects between 650 000 and 750 000 Americans each year, resulting in more than 200 000 deaths [1,2]. Current recommendations from the Surviving Sepsis Campaign endorse obtaining blood cultures (BCs) before initiation of antibiotics in septic patients, although clear supporting evidence is lacking [3]. Indeed, since Osler first described BCs in the late 19th century, they have become the standard of care for evaluating patients with suspected serious infectious conditions and are recommended by numerous panels of medical experts including the Surviving Sepsis Campaign, the Infectious Disease Society of America, and the American Thoracic Society.

1.2. Importance

In spite of this, BCs are a problematic criterion standard for true bloodstream infection (BSI) and provide only limited clinical utility. One of the major reasons for this is that BSI and sepsis can progress rapidly, over the course of hours, whereas BCs can take days to identify responsible pathogens [4]. Because of this, medical providers often start patients on empiric broad spectrum antimicrobial agents, with the hope to narrow coverage when a pathogen species is identified. Unfortunately, this practice produces selection pressure and likely contributes to the problem of multidrug-resistant organisms and superinfections [5,6].

In addition to the delay to useable data, BCs are limited by their interpretation. There is no consensus as to when a BC should be considered positive, as a given microbe may be a contaminant for one individual but a pathogen for the next. Blood cultures are also often contaminated or may fail to detect a pathogen that is present in low concentrations. To overcome these limitations, it is customary to draw a paired set of cultures from 2 different sites on the same individual, decreasing the likelihood of false-positive or false-negative BCs. This results in added cost and patient discomfort.

A novel methodology was recently developed that allows early detection of viable microbes in blood. This technology uses differential cell lysis and subsequent microbe detection by measuring their endogenous DNA polymerase activity through enzymatic template generation and amplification (ETGA). Details of the methodology have been published previously [7]. Experiments with simulated BSI (using blood spiked with known quantities of bacteria) have demonstrated detection of blood-borne pathogens 3-fold earlier with EGTA as compared with standard BC incubation flip times [7,8]. Experiments using blood samples taken from patients with clinical BSI have also demonstrated detection of pathogens 3-fold earlier [9].

1.3. Goals of this investigation

We sought to perform an in-depth analysis of the patients with clinical BSI from this ETGA feasibility experiment. We further explore the clinical applicability of this technology in patients with suspected BSI.

2. Materials and methods

2.1. Study design

The study was a prospective cohort design of patients presenting to the emergency department (ED) or intensive care unit (ICU) with suspected BSI. We used blood samples from these patients for culture and ETGA. The patient's medical record was queried by 2 research investigators at the clinical site for clinical data. Off-site BC and ETGA were funded and performed by the developers of ETGA. The participation in this study posed no additional cost, change in standard of care, harm, nor benefit to the patients enrolled, as the clinicians caring for the patients did not have access to study results, and off-site personnel received no patient identifying information.

2.2. Study setting and population

The study site is a level 1 community trauma center with an ED census of 75 000 and houses 37 ICU beds. A convenience sample of adult patients ($\geq$18 years) in the ED and ICU were screened for eligibility over an 11-month period in 2011 to 2012. Patients were deemed eligible if they presented with signs and symptoms suspicious for serious infection at the discretion of the enrolling physician and if their clinical evaluation included ordering BCs. A nonexhaustive list of signs and symptoms used in this decision include hypotension, tachycardia, fever, chills, cough, tachypnea, shortness of breath, abdominal pain, and oliguria. Subjects were enrolled after written informed consent was obtained from the patient or health care proxy. The study was approved by the institutional review board at the clinical site.

2.3. Study protocol and measurements

The detailed protocol for blood handling is described elsewhere [9]. In brief, each enrolled patient had 2 paired BC bottles (2 aerobic and 2 anaerobic) drawn via standard hospital protocol. In addition, a third aerobic bottle was drawn without additional needle stick and was designated the off-site test sample. This sample was always last in the draw sequence. Although manufacturer guidelines recommend 8 to 10 mL of blood per culture bottle, no attempt was made to standardize blood volumes drawn.

The paired hospital BC bottles were incubated in a BD BACTEC automated BC system (BD Company, Franklin Lakes, NJ). After 5 days of incubation without growth, a sample was recorded as negative. In cases of positive samples, the flip time, Gram stain result, and final speciation of organism were recorded in a standardized Excel spreadsheet. The BC incubator spectrophotometry growth curve of the organism was also obtained when available.

The study BC bottle was blind coded and placed in designated temperature-controlled refrigerators set at 4°C in the ED and ICU. Twice weekly, these were transported 50 miles to the off-site laboratory and split into 2 culture bottles. These underwent independent incubation and culture as well as testing with the ETGA assay. Using a time course sampling described elsewhere [7], aliquots were taken from the culture bottle in a time course fashion and refrigerated. All BCs were tested at 24 and 48 hours with ETGA assay. The time course aliquots were tested with ETGA assay to determine the earliest time to pathogen detection in any specimen in which both of the split study culture bottles were resulted as positive. Because ETGA assay yields pathogen genomic DNA for further speciation, gene-specific polymerase chain reaction was used to identify 4 common BSI pathogens in these blood samples. The details of this are reported elsewhere [9].

The ETGA assay was resulted as either “positive” or “negative” for detection of any viable microbe activity. For positive samples in which both of the split study culture bottles were also positive, the spectrophotometric growth curve from the hospital specimen was used to compare the time to detection in hours of microbe presence in hospital cultures vs the earliest time course positive ETGA assay for that matched sample. Off-site BCs were also used as a reference standard. This was done to serve as a marker for internal validity because there was concern that handling might decrease microbe viability in the study specimen. Therefore, the study culture represents results from the specimen after being subjected to refrigeration for up to 3 days and transported off-site. Both results are reported here.