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The clinical diagnostic accuracy of rapid detection of healthcare-associated bloodstream infection in intensive care using multipathogen real-time PCR technology

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ABSTRACT

Background: There is growing interest in the potential utility of real-time PCR in diagnosing bloodstream infection by detecting pathogen DNA in blood samples within a few hours. SeptiFast is a multipathogen probe-based real-time PCR system targeting ribosomal DNA sequences of bacteria and fungi. It detects and identifies the commonest pathogens causing bloodstream infection and has European regulatory approval. The SeptiFast pathogen panel is suited to identifying healthcare-associated bloodstream infection acquired during complex healthcare, and the authors report here the protocol for the first detailed health-technology assessment of multiplex real-time PCR in this setting.

Methods/design: A Phase III multicentre double-blinded diagnostic study will determine the clinical validity of SeptiFast for the rapid detection of healthcare-associated bloodstream infection, against the current service standard of microbiological culture, in an adequately sized population of critically ill adult patients. Results from SeptiFast and standard microbiological culture procedures in each patient will be compared at study conclusion and the metrics of clinical diagnostic accuracy of SeptiFast determined in this population setting. In addition, this study aims to assess further the preliminary evidence that the detection of pathogen DNA in the bloodstream using SeptiFast may have value in identifying the presence of infection elsewhere in the body. Furthermore, differences in circulating immune-inflammatory markers in patient groups differentiated by the presence/absence of culturable pathogens and pathogen DNA will help elucidate further the patho-physiology of infection developing in the critically ill.

Ethics and dissemination: Ethical approval has been granted by the North West 6 Research Ethics Committee (09/H1003/109). Based on the results of this first non-commercial study, independent recommendations will be made to The Department of Health (open-access health technology assessment report) as to whether SeptiFast has sufficient clinical diagnostic accuracy to move forward to efficacy testing during the provision of routine clinical care.

ARTICLE SUMMARY

Article focus

- To highlight the unmet need for accurate and rapid infection diagnostics in the setting of life-threatening infection.
- To describe the systematic plans of a clinical diagnostic validity study of a new real-time PCR technology, designed to detect circulating pathogen DNA associated with bloodstream infection.
- To describe the clinical standards for sepsis and healthcare-associated infection diagnosis and identify how these standards will be utilised to determine the clinical validity of the new real-time PCR test in critically ill patients.

Key messages

- The study will provide the first independent, systematic, clinical validity study of real-time PCR technologies in the focused setting of suspected life-threatening healthcare-associated infections during the provision of routine emergency critical care.
- Based on the results of this study, independent recommendations will be made to the UK's Department of Health as to whether the real-time PCR technology has sufficient clinical diagnostic accuracy to move forward to efficacy testing during the provision of routine clinical care.

BACKGROUND

Healthcare-associated infection (HCAI) is a major cause of mortality and morbidity and represents a massive burden on resources with the national audit office in England alone estimating annual costs in excess of £1

ARTICLE SUMMARY

Strengths and limitations of this study

- The study is focused on a carefully delineated clinical cohort at significant risk of developing life-threatening infection.
- The study is non-commercial and has been planned systematically by a multidisciplinary team of experts and patient representatives, working on behalf of the key stakeholders within a nationalised healthcare system.
- Current clinical infection diagnosis standards may not have a high diagnostic accuracy in all settings and with all infections.
- There is a documented high rate of broad-spectrum antimicrobial therapies delivered to critically ill patients empirically which could confound the comparison between culture methods and pathogen DNA-detection methods.

billion.[1] Improved measures to prevent and treat HCAI are currently among the very highest priority areas for many health services worldwide. The critically ill, despite significant advances in their supportive care, remain at high risk of developing severe infections and organ failure, which carry a high mortality and healthcare costs.[2 3] Confirmation of infection in this setting depends on identification of live micro-organisms (pathogens) by microbiological culture of samples such as blood.[4] However, culture routinely takes several days before a positive result is available and at least 5 days to determine that a specimen is culture-negative.[5] This temporal separation between initial clinical suspicion and confirmation of infection routinely results in the early and sustained application of potent broad-spectrum antibiotics and antifungal agents aimed at covering the most likely pathogens as a 'safety first' strategy because a delay in appropriate antimicrobial therapy is associated with increased mortality.[6] The increased and inappropriate use of antibiotics, which is an inevitable consequence, is associated with the development of multiresistant strains of bacteria (eg, MRSA) and superinfection with *Clostridium difficile*.[1] By the nature of complex healthcare, overwhelming inflammation of the body is a common occurrence in critically ill patients and is not always caused by infection[7] (eg, it may be caused by trauma, blood transfusion or pancreatitis), leading further to the inappropriate use of antibiotics owing to the lack of time-critical biomarkers of infection. There is therefore an urgent need to develop techniques that can provide appropriate diagnostic accuracy within hours of clinical signs appearing and so allow more informed use of antibiotic therapy at an early stage.[8 9]

There is growing interest in the potential of real-time PCR technology to address this problem based on the ability of this technique to detect minute amounts of pathogen DNA in patient blood samples with results available within 4–6 h.[10] Proof-of-concept studies have focused on two approaches, either the use of PCR with universal probes to show the presence of bacterial or fungal DNA, with species identification dependent on later sequencing of the PCR products, or using strain-specific probes that provide a direct readout of the pathogen species present.[10] Intuitively, the latter approach would seem to have the greatest clinical utility. While the analytical sensitivity and specificity of these approaches for the detection of pathogen DNA in blood are recognised, there remains an acknowledged lack of clinically driven research to assess their clinical validity. This has been due in part to the lack of standardised technology platforms that meet accepted regulatory standards for clinical diagnosis. SeptiFast, manufactured by Roche Diagnostics, and run on their real-time PCR instrument (the LightCycler 2), was the first real-time PCR-based system to gain a Conformité Européenne (CE) mark for pathogen detection in suspected bloodstream infection.[11] The system uses a multiplex approach in a single blood sample which allows the detection of 25 of the most common pathogen species causing bloodstream infection internationally (table 1), and this panel is particularly well matched to the common pathogens responsible for bloodstream HCAI in critical care.[12] Identification of the pathogens is based on the use of species-specific probes targeting the internal transcribed region between the 16S and 23S ribosomal DNA sequence of bacteria and the 18S and 5.8S regions of the fungal genome. As part of the first stage of health-technology assessment (analytical validity testing), SeptiFast has been extensively assessed at the laboratory level on clinical isolates and shown to have excellent analytical specificity.[11] Subsequently, data (unpublished) from an EU registrational study, undertaken as part of the CE-marking process, investigating 278 ICU patients from Denmark, Germany and Italy, claimed

Table 1 Pathogens detectable using the LightCycler SeptiFast test

Gram-negative bacteria	Gram-positive bacteria	Fungi
Escherichia coli	*Staphylococcus aureus*	*Candida albicans*
Klebsiella (pneumoniae/oxytoca)	Coagulase-negative staphylococci*	*Candida tropicalis*
Serratia marcescens	*Streptococcus pneumonia*	*Candida parapsilosis*
Enterobacter (cloacae/aerogenes)	*Streptococcus* spp†	*Candida glabrata*
Proteus mirabilis	*Enterococcus faecium*	*Candida krusei*
Acinetobacter baumanni	*Enterococcus faecalis*	*Aspergillus fumigatus*
Pseudomonas aeruginosa		
Stentrophomonas maltophilia		

*Single probe detects a group of staphylococcal pathogens including *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*.
†Single probe detects a group of streptococcal pathogens including *Streptococcus pyogenes, Streptococcus agalacticae* and *Streptococcus mitis*.

a high diagnostic specificity and a three- to 10-fold higher sensitivity for real-time PCR compared with conventional culture in patients with sepsis. More recently, a number of multicentre investigator-led commercial clinical observational studies have commenced reporting on SeptiFast,[13 14] but to date no studies have investigated systematically the clinical diagnostic accuracy of SeptiFast in the setting of suspected HCAI.

The aim of this study, therefore, is to determine the clinical validity of the use of real-time PCR-based diagnostic technology for the rapid detection and identification of healthcare-associated bloodstream infection when compared with the current service standard of microbiological culture, in an appropriately sized population of adult intensive care patients being treated within a regional Critical Care Network in the UK.

METHODS

Study objectives

The primary objectives are to determine the clinical validity of the use of real-time PCR-based diagnostic technology for the rapid detection and identification of healthcare-associated bloodstream infection against the current service standard of microbiological culture in a population of adult intensive-care patients within the Greater Manchester Critical Care Network, UK, and to assess further the preliminary evidence that the detection of pathogen DNA in the bloodstream using SeptiFast may have value in detecting the presence of HCAI elsewhere in the body. The secondary objective is to profile differences in circulating immune-inflammatory biomarkers in patients with or without circulating culturable pathogen and/or micro-organism DNA identified by real-time PCR.

Study design and sample size

This Phase III diagnostic trial[15] will evaluate the clinical validity of SeptiFast for the identification of healthcare-associated bloodstream infection in the setting of UK adult intensive-care practice when compared with the current blood-culture standards. The study will recruit from at least two intensive care units of the University-affiliated tertiary referral centres within The Greater Manchester Critical Care Network (Salford Royal NHS Foundation Trust and The University Hospital of South Manchester). Laboratory records show that, on average, over 1200 requests are made in total from these two intensive-care units for microbiological culture of blood per annum, and our clinical pilot study indicated that approximately 12% (95% CI 6% to 16%) will be diagnosed as having culture-confirmed bloodstream infection.[16] Based on this event rate, a sample-size estimation was performed, after Buderer,[17] which indicated that a minimum sample size of 600 patients will be required to be 95% sure that the pathogen-specific molecular test has at least a 95% specificity and sensitivity when compared with a culture-proven diagnosis. It is anticipated that this study can be delivered within the agreed 2-year funding period.

Patient selection

Inclusion criteria

Patients being managed in a designated intensive-care bed, 16 years or older, developing a clinical suspicion of bloodstream infection based a priori on meeting two or more Systemic Inflammatory Response Syndrome (SIRS) criteria,[18] developing at least 48 h after admission/recent exposure to hospital care (defining 'healthcare-associated'[19]) will be eligible for inclusion in the study. In the present study, the SIRS definition will be met if two or more of the following clinical signs are present: body temperature greater than 38°C or less than 36°C, heart rate greater than 90 beats/min, high respiratory rate (more than 20 breaths/min) or, on blood gas, a $Paco_2$ less than 32 mm Hg for spontaneously breathing patients; or requirement for mechanical ventilation in established critical illness, or white blood cell count <4000 cells/mm^3 or $>12\,000$ cells/mm^3 or the presence of more than 10% immature neutrophils.

Exclusion criteria

A patient will be excluded if they have been recruited already into this study, or they are on a Care of the Dying Pathway, or if consent from the patient is declined or, where appropriate, assent from next of kin or Independent Mental Capacity Advocate is not obtained. All such patient exclusions will be recorded in a site-specific screening log and summarised in the final report.

Clinical research methodology

Identification and recruitment of participants

Potential participants will be identified to the study research nurse through the normal clinical surveillance of patients by the multidisciplinary critical care teams. Intensive-care patients, 16 years or older, developing clinical suspicion of healthcare-associated bloodstream infection as indicated by meeting the predetermined SIRS criteria related to changes in heart rate, body temperature, ventilation and white blood cell count will be potential participants (as described above).

Since blood sampling for infection diagnosis is taken as part of routine emergency care of these patients, under international guidelines the sample has to be taken within 1 h of suspecting infection.[4] Under these circumstances, unless the patient has the capacity to provide consent, we will adopt a process of deferred assent where the research blood sample is taken as part of routine care and permission sought from the consultee or the Independent Mental Capacity Advocate at each recruitment site for inclusion into the study.

In many cases, the potential participants will lack capacity at study inception owing to the combination of their overwhelming illness and therapeutic interventions such as sedation. Therefore, assent will be sought from their family members/friends (consultees). Every effort will be made to seek out and consult the patient's designated consultee and conduct a formal assent as part of the process which will include a participant/consultee

information sheet, a formal interview by the clinical research team and signed assent. A patient will not be recruited if the designated consultee believes it is likely to be against the views and wishes of that patient. It is our experience that we can locate a designated consultee for the majority of patient's in our critical care network within 72 h. However, from time to time, this may prove impossible, and under such circumstances we will approach the appropriate designated Independent Mental Capacity Advocate.

Should the participant regain capacity during their acute care hospitalisation, informed consent for inclusion in the study will also be sought.

Laboratory-confirmed diagnosis of bloodstream infection

Key to the success of this study will be the culture-based laboratory confirmed diagnosis of bloodstream infection in our target population. We will use the consensus definition of bloodstream infection utilised in our Network for prevalence studies of HCAI.[19]

Definition: Laboratory culture confirmed bloodstream infection must meet at least one of the following criteria:
Criterion 1: patient has a recognised pathogen cultured from one or more blood cultures
Criterion 2: patient has systemic signs of infection (defined as meeting the SIRS criteria in the present study—see 'Inclusion criteria') and a common skin contaminant (eg, diptheroids, *Bacillus* spp, *Propionibacterium* spp, coagulase-negative staphylococci or micrococci) as cultured from two or more blood cultures drawn on separate occasions. Contamination of the blood culture will be defined when these common skin species are otherwise present in culture outwith Criterion 2.

Having identified a patient in the target population, two blood samples of at least 20 ml each will be taken sequentially from two separate sites.[5] Importantly, blood from indwelling catheters must be avoided where possible for the purposes of SeptiFast PCR analysis and the associated blood culture, but may be used for the analysis of other biomarkers of infection if necessary. Any breach of this protocol will be recorded in the Clinical Record Form (see below). An NHS-approved aseptic non-touch technique will be used (http://www.antt.org.uk/). These blood samples will be inoculated in turn into paired culture bottles, labelled and processed as per standard clinical practice in the participating hospitals. Local audit, in association with quality-improvement initiatives within the study sites, shows that by using these methods we can keep blood-culture contamination rates to below 5%. The blood-collection process and automated culture methods used in each centre, the quality-assurance accreditation from Clinical Pathology Accreditation (UK) Ltd and any change in methodology during the study period will be recorded and reported. All culture bottles will enter the standard clinical pathway in the respective services in the participating hospitals with subsequent reporting to clinical service.

Research blood amples

A sample of whole blood equivalent to no more than 30 ml over and above that required for routine clinical investigation will be sampled using an NHS-approved non-touch technique (http://www.antt.org.uk/) and collected into closed tubes containing ethylene diamine tetra acetic acid (EDTA). This research sample and routine sampling for microbiological culture analysis will be performed by suitably trained clinical service staff under the supervision of the senior clinical investigators, and will be taken simultaneously. Each study participant will contribute a single suspected bloodstream-infection episode to the research study. Whole blood will be collected using a closed system. Initial plasma separation and blood storage will be performed near-patient (10 ml of this whole blood will be used for plasma isolation using standard techniques). All research samples will be stored for up to a maximum of 72 h in a locked refrigerator within the individual critical care unit prior to being transported to the Biomedical Facility research laboratories at Salford Royal Hospital for further analysis. Collection and transportation of samples will be coordinated by the research nurse at each site so that a maximum storage time of 72 h at 4°C is not exceeded prior to sample analysis and/or freezing. Timings of this process will be recorded with the samples to facilitate quality-assurance measures for analysis.

Recording of clinical information

All potential participants who meet the study inclusion criteria will be identified in a screening log in each participating centre. Each included patient will be given a unique study identification number (ID). Any immediately identifiable patient details will be recorded once, on a Patients Identification Form. Once a study ID is assigned, a Case Record Form (CRF) will be opened and the ID number copied to all pages in the CRF. Once the ID number is entered on the CRF, it becomes a confidential document and must conform to the specified Data Management procedures of the Study Sponsor (Salford Royal NHS Foundation Trust). The CRF consists of seven sections in order to record all the clinical information required for the completion of the study and acts as a point of reference for the research nurse to ensure all clinical study stages are complete. These are:

1. Recruitment: requires confirmation that the study participant meets the inclusion criteria and summarises the patient's admission details including the last 7 days.
2. Trial sample data: summarises the sample data allowing tracking of sample from the site from which it was taken to transportation to the research laboratory.
3. Consent/assent: acts as a checklist and point of reference for the acquisition of consent or assent.
4. Patient observations:
   - day 0: records a detailed snap-shot of the patient's clinical condition and treatment at the time of the samples being taken. This includes general

and specific infection-based clinical observations, calculation of the critical care minimum dataset and a focused overview considering the past 7 days;
- antibiotics: a record of any antibiotics given from 7 days prior to sample being taken to study day 6;
- microbiology results: a record of any microbiology results from the date the sample was taken;
- summary records of clinical progress of suspected infection episode.

5. Patient surveillance: provides a continuous record of relevant patient care up to and including study day 6. This includes any significant clinical events, general observations and calculation of critical care minimum dataset.
6. Patient surveillance summary: details the patients outcome and any study related adverse incidences. This section is also concluded with nurse signature of completion.
7. Clinical adjudication: utilises the criteria set out in the Prevalence Survey of Healthcare-associated Infections[19] to conclude and summarise the clinical opinion regarding each suspected infection episode. The Trial Steering Committee has agreed that this adjudication will be performed in each participating centre by two senior clinical practitioners who have governance responsibility within their relevant service for observing and reporting HCAI. No between-centre adjudication will be performed, as this clinical validity study is designed to observe routine clinical practice (Phase 3 diagnostic study).

Laboratory research methodology

Once received at the research laboratory, blood samples will be processed by one of two research scientists who have received approved training by Roche Diagnostics UK for this purpose as follows: one portion of the whole blood (5 ml) will be used for pathogen DNA extraction and analysis by SeptiFast real-time PCR for the identification of pathogen DNA. The procedures for pathogen DNA extraction, performing multipathogen PCR and subsequent data analysis are set out in the SeptiFast CE-marked kit (Roche Diagnostics) and will be followed precisely. To ensure the validity of the data, the SeptiFast system contains several built-in quality-assurance processes. The results of each SeptiFast analysis will not be returned to routine clinical service and will not be associated with any clinical or culture detail until completion of the data-collection period. The research laboratories within our Biomedical Facility are not physically or operationally part of routine clinical service, allowing research and clinically service to be double-blinded during this study.

The plasma sample will be stored at −80°C for subsequent analyses of inflammatory-immune biomarkers, including pro- and counter-regulatory cytokine profiling and procalcitonin assays. The final portion of whole blood (about 10−15 ml, depending on patient volume yield) will be stored at 80°C for future reanalysis of pathogen DNA using alternative real-time PCR technologies that are currently under development by ourselves and others (a stated requirement of the study funding body).

Data-analysis plan

Summary diagnostic accuracy measures will be computed for the SeptiFast PCR test compared with laboratory culture-proven bloodstream infection, including sensitivity, specificity, predictive values and likelihood ratios, including 95% CIs. A similar analysis will be performed using an 'enhanced' reference standard including culture-based identification of infection from clinical samples other than blood. All analyses of diagnostic accuracy measures will be performed for all participating hospitals combined, but allowing for hospital as a stratifying factor (failure to allow for hospital differences might lead to biased results owing to confounding). In addition, sensitivity analyses will be performed to investigate the possibility that blood culture may not be an adequate reference standard for infection diagnosis in these critically ill patients and will include analyses using instrumental variables[20] such as replicate-independent cultures (and clinically relevant cultures from other body sites), patient treatment/response factors and circulating immune-inflammatory biomarkers. Finally, the immune-inflammatory biomarker responses will be investigated in this cohort of critically ill patients with the results of blood culture, clinically relevant culture from other body sites and circulating pathogen DNA as co-factors.

Sample and data security

Clinical samples

Detailed procedures are in place for the recording, labelling and tracking of clinical samples in the clinical and laboratory environments. Each sample will be anonymised by labelling with a unique identifier number that will remain with the sample throughout the subsequent laboratory analysis and storage procedures. The PCR research team will be blinded from all culture results and clinical practice. Standard procedures for transporting samples from the clinic to the laboratory are in place. Laboratory processing of the samples will be undertaken in the Biomedical Facility at Salford Royal, which is a secure facility with restricted access. Where required, samples will be stored in a dedicated −80°C freezer which is fully alarmed and has both manual and automatic monitoring systems in place. All stored blood and plasma samples will be destroyed at the end of a 5-year period as agreed as part of the ethical review.

Data

Anonymised clinical and laboratory data for each patient sample are recorded in CRF and Laboratory Case Record forms respectively. Completed forms are photocopied once, and both documents are stored in a locked filing cabinet in secure locations at Salford Royal Foundation Trust (Trials Office) and University Hospital of

South Manchester (filing cabinet in AICU store room). Electronic data (eg, SeptiFast PCR data) will be stored in a password-protected folder with electronic backup of the contents of the folder to an encoded portable hard drive on the last working day of each month. Paper copies of key electronic data will also be stored as above.

Serious adverse events

The procedure of collecting an additional blood sample during routine sampling for blood culture analysis is not usually associated with any significant increased risk to patients or their carers. Standard operating procedures are in place to cover all aspects of blood sampling, storage, transportation and laboratory analysis to ensure no significant risk to any of the research staff associated with this study. During the period of clinical data monitoring (7 days), any serious adverse incident reported within each participating centre's statutory reporting system will be reported to the Trial Sponsor by the Principal Investigator at each site in association with the Chief Investigator.

Regulatory/ethical approval

The Medicines and Healthcare products Regulatory Agency has agreed that this study does not require their formal approval, because it involves the observation of a CE-marked assay performance during routine clinical care, and that the results of the assay within this study will not be used to influence clinical care. In addition, this study has received favourable ethical opinion from the North West 6 Research Ethics Committee—Reference No: 09/H1003/109. R&D approval has been granted by the study Sponsor, Salford Royal NHS Foundation Trust (215ETt (25733/GM)).

Trial Steering Committee

A Trial Steering Committee has been appointed by the National Institute for Health Research Health Technology Assessment programme, including an independent chair and representation from patients recovering from critical illness, the Investigators and Trial Sponsor (Salford Royal NHS Foundation Trust), and have already met in advance of the study commencement, approving this protocol. Future regular meetings have been planned during the course of this study to oversee progress and adherence to regulatory and governance research frameworks within the NHS.

Peer-review and funding

This study has been peer-reviewed and supported through the Health Technology Assessment programme of the National Institute of Health Research; grant number and trial registration National Institute for Health Research Health Technology Assessment 08/13/16.

DISCUSSION

Blood-culture technology is at the centre of evidence-based guidelines for the investigation and treatment of critically ill patients with suspected bloodstream infection. While culture has been refined over the last century, it remains insufficiently time-critical and cannot assist with early management decisions, inevitably resulting in wasteful and potentially dangerous overtreatment with antimicrobial chemotherapy. PCR-based technologies have become standard laboratory technologies over the last two decades and could deliver real opportunity in terms of sensitivity and speed in the clinical setting of life-threatening infection in critical care. Unfortunately, there is a paucity of clinical translation, and formal health-technology assessment is lacking. SeptiFast is the first CE-marked assay that has yet to be independently assessed in a systematic study involving a clearly defined clinical population of critically ill patients suspected of developing healthcare-associated bloodstream infection. This protocol describes the first independent clinical validity study of SeptiFast in the setting of suspected healthcare-associated sepsis in critical care. Based on the results of this non-commercial study, independent recommendations will be made to National Health Service providers as to whether SeptiFast has sufficient clinical diagnostic accuracy to move forward to efficacy and effectiveness testing during the provision of routine critical care. The present study therefore represents a crucial phase of detailed independent health technology assessment of the first multiplex real-time PCR technique aimed at helping deliver more effective care to critically ill patients internationally.

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Competing interests None.

Ethics approval Ethics approval was provided by the North West 6 Research Ethics Committee.

Contributors PD and GW initiated the project; GD and DY assisted on study design and sample size estimation; PC assisted on microbiological diagnosis of healthcare-associated infection; GC and AB assisted on the clinical

perspectives of sepsis diagnosis and management in general; PD and GW drafted the protocol, and all authors critically reviewed it. All authors are trial investigators, GW is principal (laboratory scientific) investigator, and PD is principal (clinical scientific) investigator.

Provenance and peer review Not commissioned; peer reviewed for ethical and funding approval prior to submission.

Data sharing statement We are happy to consider depositing our data in Dryad data repository when the dataset has been collected.

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