

Rapid detection of health-care-associated bloodstream infection in critical care using multipathogen real-time polymerase chain reaction technology: a diagnostic accuracy study and systematic review

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Declared competing interests of authors: Geoffrey Warhurst, Paul Dark, Ronan McMullen and Daniel McAuley have received research funding from the Technology Strategy Board to develop point-of-care diagnostics for sepsis. Paul Chadwick has received lecture honoraria from Novartis and funding from Pfizer and AstraZeneca to support local and regional research meetings in Clinical Microbiology.

Published May 2015

DOI: 10.3310/hta19350

Scientific summary

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Health Technology Assessment 2015; Vol. 19: No. 35

DOI: 10.3310/hta19350

NIHR Journals Library www.journalslibrary.nihr.ac.uk

Scientific summary

Background

Health-care-associated infections impose a significant burden on health-care systems worldwide, with bloodstream infections particularly problematic in terms of hospital costs, increased length of stay and mortality, especially in the critically ill and when associated with sepsis. Blood culture (BC) is the current service standard for bloodstream infection diagnosis but is insufficiently time critical to assist in early management decisions. International guidelines for the early management of sepsis advocate initiating antibiotic therapy within 1 hour of initial clinical suspicion, usually involving administration of high potency, broad-spectrum antibiotics as a 'safety first' strategy. Although this approach is life-saving in patients with severe sepsis, an inevitable consequence of the lack of early diagnostic confirmation is a wasteful and potentially dangerous overuse of antimicrobial chemotherapy. This is associated with the spread of antibiotic-resistant species and susceptibility to superinfections such as *Clostridium difficile*. There is growing interest in the potential of real-time polymerase chain reaction (PCR) to address this problem based on the ability to detect minute amounts of pathogen deoxyribonucleic acid (DNA) in patient blood samples within a few hours, allowing more informed use of early antibiotic therapy. SeptiFast (Roche Diagnostics GmbH, Mannheim, Germany) is a multipathogen, probe-based real-time PCR system targeting ribosomal DNA sequences of bacteria and fungi to detect and identify 25 of the commonest pathogens causing bloodstream infection. The SeptiFast panel is suited to identifying health-care-associated bloodstream infection acquired during complex health care and has European regulatory approval. To date, there has been no formal health technology assessment of the performance of SeptiFast in this setting.

Objective

This report investigates the diagnostic accuracy of the LightCycler® (Roche Diagnostics GmbH, Mannheim, Germany) SeptiFast multipathogen real-time PCR platform for detection of suspected health-care-associated sepsis compared with BC in the setting of critical care.

Methodology

Systematic review

A systematic review and meta-analysis of clinical diagnostic accuracy studies involving SeptiFast (from January 2006 to November 2012) was conducted. The protocol for systematic review was published in advance by *BMJ Open* and is available from <http://bmjopen.bmj.com/content/2/1/e000392.long>. A comprehensive literature search strategy was used to identify studies that incorporated SeptiFast as the index test for the detection and identification of pathogens in blood samples of patients with suspected sepsis when compared with BC as the reference standard test. We searched the following databases: Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects, the Health Technology Assessment (HTA) database, the NHS Economic Evaluation Database, The Cochrane Library, MEDLINE, EMBASE, ISI Web of Science, Bioscience Information Service (BIOSIS) Previews, Medion and the Aggressive Research Intelligence Facility database. This systematic review considered only publications from 2006 onwards. A standard set of data was searched for and extracted including clinical setting, features of included population, reference standard and index test methodologies, and reported diagnostic accuracy metrics. A specific checklist adapted from the quality assessment of diagnostic accuracy studies tool was used to assess the quality of the selected studies by independent assessors. Statistical analysis and data synthesis including subgroup analyses were performed by an independent external statistician using bivariate meta-regression analysis of assay sensitivity and specificity.

Clinical diagnostic accuracy study

A Phase III multicentre, double-blinded, clinical diagnostic accuracy study was performed using SeptiFast real-time PCR in patients from four large NHS hospital trusts in the north-west of England. The protocol agreed by the Trial Steering Committee for the Phase III study was published in advance by the National Institute for Health Research (NIHR) HTA (available from www.nets.nihr.ac.uk/projects/hta/081316) and, subsequently, by *BMJ Open* (available from <http://bmjopen.bmj.com/content/1/1/e000181.long>). Designed using the standards for the reporting of diagnostic accuracy studies (STARD) criteria, in an adequately sized and defined adult critical-care population, the study aimed to determine the accuracy of SeptiFast real-time PCR for rapid detection and identification of suspected sepsis-related health-care-associated bloodstream infection when compared with BC as the reference standard. Patient inclusion was based on meeting the Systemic Inflammatory Response Syndrome criteria, developing at least 48 hours after hospital admission. Evidence that pathogen DNA detection in the bloodstream using SeptiFast has value in detecting infection elsewhere in the body was also sought using an enhanced reference standard, defined as any positive BC and/or cultures from other specimens taken 48 hours either side of the primary research blood sample contributing to an independently adjudicated infection episode during this period. Summary measures of diagnostic accuracy, with their 95% confidence intervals (CIs), including sensitivity, specificity, predictive values and likelihood ratios were performed at the level of event (positive/negative result) and pathogen species concordance for SeptiFast real-time PCR against both BC and the enhanced reference standard. Statistical evaluation of the potential impact of error in the BC gold standard was undertaken using latent class modelling. Impact on diagnostic performance of the commonly used infection biomarker procalcitonin (PCT) as an instrumental variable was also considered.

Results

Description and quality of the available evidence on SeptiFast real-time polymerase chain reaction

The literature searches identified 2129 citations in total, and following full-text review 37 studies were included in the final analysis. Summary sensitivity and specificity for SeptiFast real-time PCR compared with BC from the included studies, estimated using a bivariate model, were 68% (95% CI 62% to 74%) and 86% (95% CI 84% to 89%) respectively. This suggests that a positive SeptiFast test at genus/species level in blood samples from patients with suspected sepsis could have higher diagnostic utility (rule in) than a negative test (rule out) compared with BC. However, study quality was judged to be variable, with important deficiencies overall in study design and reporting. The reference standard was not always adequately described and no consistent standards for reporting of BC results were followed, giving likelihood of misclassification errors when comparing reference and index tests. Incorporation bias was also likely due to universal lack of reported blinding of reference standard and index test. Lack of uniform reporting made classification of studies difficult, with a variety of care settings, outcomes and alternative clinical reference standards reported alongside direct comparison of SeptiFast real-time PCR with BC. Overall, independent review indicated serious deficiencies in the included studies impacting on the derived diagnostic accuracy metrics with none, as reported, meeting the STARD criteria in full.

Findings of the clinical diagnostic accuracy study of SeptiFast in detection of sepsis-related health-care-associated bloodstream infection

A total of 922 new episodes of suspected health-care-associated bloodstream infection from 795 patients were analysed. Median age was 58 years with a 60% : 40% gender distribution in favour of males. Patients were recruited across a range of primary specialties suggesting a generalisable study cohort. Organ support activities at time of blood sampling were higher than those recorded nationally during the study period but mean 28-day survival and survival to hospital discharge compared favourably with national audit figures for sepsis outcomes. A large majority of patients (86%) were receiving antimicrobial drugs, often delivered in combination, within the 48 hours prior to the suspected sepsis episode.

Summary diagnostic accuracy metrics of SeptiFast real-time PCR against culture-proven bloodstream infection at event level across all hospital sites showed sensitivity of 58.8% (95% CI 47.2% to 69.6%) and specificity of 88.5% (95% CI 86.1% to 90.6%) with a prevalence of 8.7%. Pathogen concordance is likely to be a more robust indicator of the potential clinical utility of SeptiFast real-time PCR, and here both sensitivity 50.0% (95% CI 39.1% to 60.8%) and specificity 85.8% (95% CI 83.3% to 88.1%) were lower. Some variation in diagnostic metrics was observed across hospital sites. These data suggest SeptiFast has better diagnostic rule-in than rule-out potential. However, consideration of mean likelihood ratios indicates significant limitations in diagnostic utility of SeptiFast. For example, at pathogen level, the positive likelihood ratio overall was only 3.5 (95% CI 2.7 to 4.5) with a post-test probability following a positive SeptiFast test of 26.3% (95% CI 19.8% to 33.7%). The negative likelihood ratio was only 0.69 (95% CI 0.50 to 0.73), with post-test probability of 5.6% (95% CI 4.1% to 7.4%). The probability of a patient having culture-proven bloodstream infection following a positive SeptiFast test would therefore be no greater than 33.7%, a low level to confidently rule in a diagnosis. Following a negative SeptiFast test the probability of a culture-proven bloodstream infection would be no greater than 7.4%.

Against the enhanced reference standard that accounts for infection present at other body sites in addition to the bloodstream, SeptiFast real-time PCR sensitivity was markedly lower being 31.1% (95% CI 25.6% to 37.0%) at event level and 18.9% (95% CI 14.9% to 23.4%) at species level. Specificity against the enhanced reference standard was maintained, being 90.8% (95% CI 88.3% to 92.9%) and 85.4% (95% CI 82.6% to 88.0%) for event and pathogen concordance respectively. Using likelihood ratios to derive post-test probabilities, the probability of a patient having a culture-proven infection following a positive SeptiFast test would be 47.4% or less, and as high as 35.5% following a negative test.

Challenging the assumption that laboratory-confirmed diagnosis of bloodstream infection is an error-free gold standard: statistical modelling using latent class analysis

Using latent class analysis of our new study data, BC appears to have a worryingly low sensitivity in the setting of suspected sepsis-related health-care-associated bloodstream infection. The sensitivity of the SeptiFast real-time PCR test is also less than ideal for a useful diagnostic test but appears much better than BC. Blood samples identified as positive by either BC or SeptiFast real-time PCR in our study population have a high probability of having infection. Data from additional body sites and from circulating biomarkers such as PCT appear to be additional indicators of infection and a dichotomised PCT measurement appears to be as sensitive as BC and only marginally less specific. Preliminary investigations suggest that combinations of these biomarkers show promise in terms of achieving high diagnostic accuracy in our patient cohort.

Conclusions

Our systematic review indicated that the SeptiFast test could have higher diagnostic rule-in utility than rule-out utility compared with BC. However, study quality was variable with serious deficiencies in impacting on diagnostic accuracy metrics. Accordingly, our diagnostic accuracy study showed the sensitivity of the SeptiFast real-time PCR assay was poor and may have little value as a 'rule-out' test in suspected sepsis-related health-care-associated bloodstream infection. Overall, sensitivity of BC and SeptiFast real-time PCR was less than ideal for a useful diagnostic test in the setting of sepsis-related health-care-associated bloodstream infection. Measurement of additional biomarkers from other body sites shows good potential for accurately diagnosing infection in critically ill patients with suspected sepsis.

Implications for practice

When compared with NHS service culture standards, the clinical diagnostic accuracy of SeptiFast real-time PCR appears unlikely to result in sufficient diagnostic utility in the setting of suspected sepsis-related health-care-associated infection, despite its potential to deliver results more rapidly.

Using preliminary analyses that take into account the possibility that culture standards are not completely error free, SeptiFast real-time PCR may have a greater ability to rule in infection than may be apparent from conventional analyses; however, its diagnostic sensitivity remains inadequate such that clinical utility may remain significantly compromised.

In further preliminary analyses, other circulating biomarkers, such as procalcitonin, when used in combination with SeptiFast real-time PCR, may improve clinical diagnostic accuracy to levels where clinical utility is far more likely.

Based on the present study, we found no evidence that SeptiFast real-time PCR should replace traditional BC but the assay could be added to the diagnostic test battery (together with data on infections from other sites and levels of biomarkers such as procalcitonin). Algorithms for the optimal use of such a test battery should be the subject of further work.

We do not know if our findings are relevant to patients with suspected community-acquired sepsis as they were not investigated and there is a lack of high-quality diagnostic evidence in relation to this setting.

Recommendations for future research

Clinical research

1. Develop new strategies for assessing the clinical validity of diagnostic tests that do not rely exclusively on evidence derived from traditional analyses of diagnostic accuracy where reference standards are known to be prone to error (e.g. BC in the setting of high antimicrobial use).
2. Explore further the application of multivariate modelling techniques (e.g. latent class analysis) in diagnostic accuracy studies to assess the potential value of additional biomarkers and/or account for error in reference standards.
3. Validate diagnostic pathways in stratified populations of patients with health-care-associated and community-acquired sepsis.
4. Conduct further analyses using diagnostic and therapeutic data from stratified populations, using techniques such as risk–benefit analyses and decision analyses, to develop an understanding of the likely effectiveness of SeptiFast real-time PCR and other emerging rapid diagnostic tests in the clinical setting of sepsis.
5. Based on the current study data and outcomes from the above recommendations, consider future intervention studies based on the potential for SeptiFast as a rapid ‘rule-in’ test for pathogens in blood samples in the setting of:
 - i. suspected sepsis-related bloodstream infection asking what is the impact on antimicrobial stewardship in the setting of a positive SeptiFast test?
 - ii. interventional studies investigating bloodstream infection (e.g. a clinical trial investigating the effectiveness of different durations of antimicrobial therapy) asking what is the utility of identifying patient cohorts rapidly based on SeptiFast testing rather than relying solely on the results of BC?
6. Develop collaborative guidelines and funding initiatives with NHS stakeholders, including the NIHR, to co-ordinate biobanking of samples and, crucially, clinical information/phenotypes from patients with sepsis. This will put the NHS in a prime position to lead on co-ordinating HTA of the vast array of technologies emerging in this field aimed at meeting the Chief Medical Officer’s challenges laid out in her recent report on infection and antibiotic resistance (Davies PD. *Infections and the Rise of Antimicrobial Resistance*. London: Department of Health; 2013).
7. Encourage better adherence to internationally recognised guidelines in the reporting of results of diagnostic accuracy studies.

Technology development

Each of the following generic recommendations are informed by our considerable experience with SeptiFast and a range of other assays, and are aimed at improving the accuracy of nucleic acid amplification tests, particularly focused on improving clinical diagnostic sensitivity.

1. Increase analytical sensitivity of nucleic acid amplification assays through more efficient pathogen DNA extraction techniques and/or increasing the volume of blood extracted.
2. Widen pathogen coverage or develop tests in which the pathogen panel can be more easily modified to account for differences in pathogen spectrum in particular settings.
3. Explore alternative paradigms for use of different nucleic acid amplification tests to support clinical decision-making at different stages of patient management, for example development of rapid, low-cost screening tests capable of detecting a broad range of bacterial and fungal DNA that could be performed more frequently. Such tests could be used to support, for instance, rule-out decisions at an early stage. There is also the potential to combine nucleic acid amplification tests with other circulating biomarkers to improve diagnostic accuracy. This approach could inform selective deployment of higher-cost molecular platforms designed to subsequently identify pathogen species and microbial resistance genes.

Study registration

This study is registered as PROSPERO CRD42011001289 and is available from www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42011001289.

Funding

Funding for this study was provided by the Health Technology Assessment programme of the National Institute for Health Research. Professor Daniel McAuley and Professor Gavin D Perkins contributed to the systematic view through their funded roles as codirectors of the Intensive Care Foundation (UK).

ISSN 1366-5278 (Print)

ISSN 2046-4924 (Online)

Impact factor: 5.116

Health Technology Assessment is indexed in MEDLINE, CINAHL, EMBASE, The Cochrane Library and the ISI Science Citation Index and is assessed for inclusion in the Database of Abstracts of Reviews of Effects.

This journal is a member of and subscribes to the principles of the Committee on Publication Ethics (COPE) (www.publicationethics.org/).

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This report

The research reported in this issue of the journal was funded by the HTA programme as project number 08/13/16. The contractual start date was in May 2010. The draft report began editorial review in November 2013 and was accepted for publication in December 2014. The authors have been wholly responsible for all data collection, analysis and interpretation, and for writing up their work. The HTA editors and publisher have tried to ensure the accuracy of the authors' report and would like to thank the reviewers for their constructive comments on the draft document. However, they do not accept liability for damages or losses arising from material published in this report.

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