

ORIGINAL ARTICLE

A rapid ELISA for the diagnosis of intravascular catheter related sepsis caused by coagulase negative staphylococci

T Worthington, P A Lambert, A Traube, T S J Elliott

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See end of article for authors' affiliations

Correspondence to:
Professor T S J Elliott,
Department of Clinical
Microbiology, University
Hospital Birmingham,
Birmingham B15 2TH, UK;
tom.elliott@
university-b.wmids.nhs.uk

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Aim: To develop and evaluate a rapid enzyme linked immunosorbent assay (ELISA) for the diagnosis of intravascular catheter related sepsis caused by coagulase negative staphylococci.

Methods: Forty patients with a clinical and microbiological diagnosis of intravascular catheter related sepsis and positive blood cultures, caused by coagulase negative staphylococci, and 40 control patients requiring a central venous catheter as part of their clinical management were recruited into the study. Serum IgG responses to a previously undetected exocellular antigen produced by coagulase negative staphylococci, termed lipid S, were determined in the patient groups by a rapid ELISA.

Results: There was a significant difference ($p = < 0.0001$) in serum IgG to lipid S between patients with catheter related sepsis and controls. The mean antibody titre in patients with sepsis caused by coagulase negative staphylococci was 10 429 (range, no detectable serum IgG antibody to 99 939), whereas serum IgG was not detected in the control group of patients.

Conclusions: The rapid ELISA offers a simple, economical, and rapid diagnostic test for suspected intravascular catheter related sepsis caused by coagulase negative staphylococci, which can be difficult to diagnose clinically. This may facilitate treatment with appropriate antimicrobials and may help prevent the unnecessary removal of intravascular catheters.

Central venous catheters (CVCs) are widely used in clinical practice.¹⁻³ However, the major complication associated with CVCs continues to be infection,^{4,5} which has considerable associated costs.^{6,7} Catheter related sepsis (CRS) is difficult to diagnose, owing to its non-specific clinical presentation, resulting in the unnecessary removal of the catheters^{4,8} or the use of unwarranted antibiotic treatment, which could encourage the emergence of bacterial resistance. Indeed, because the diagnostic criteria are non-specific, many intensive care units routinely remove catheters as a pre-emptive prophylactic measure.⁸ However, we recommended that CVCs should remain in situ as long as regular clinical and microbiological surveillance based on well defined criteria are carried out.⁹ Several microbiological methods are currently available to support the clinical diagnosis of CRS with blood cultures being the standard approach. Comparative times to positivity of blood cultures obtained via the CVC and from a peripheral venepuncture have also been shown to be of value.¹⁰ However, a positive blood culture cannot clearly distinguish between catheter contamination, colonisation, or associated sepsis. The roll plate method¹¹ is also widely used in many routine laboratories because it is simple and economical, but it requires catheter removal and lacks specificity.^{12,13} Other more recent approaches include the use of the Gram stain and acridine orange cytospin,¹⁴ and the application of an endoluminal brush to sample the internal lumen of a catheter.^{15,16} However, there is no specific simple serological test available for the diagnosis of CRS. This would be of particular value in facilitating the interpretation of positive blood cultures resulting from CRS by distinguishing between contamination, colonisation, and sepsis. The anti-staphylolysin test, which is a widely used serodiagnostic assay, may assist in making the diagnosis of infections caused by *Staphylococcus aureus*, but not for coagulase negative staphylococci, the principal cause of CRS.¹⁷

We have developed a new serological approach to assist in the interpretation of positive blood cultures possibly associ-

ated with CRS and caused by coagulase negative staphylococci. An indirect enzyme linked immunosorbent assay (ELISA), taking 24 hours to perform and using a newly discovered antigen with a sensitivity and specificity of 70% and 90%, respectively, for the diagnosis of CRS has been described recently.¹⁸ The antigen, a glycerophospholipid (lipid S), is an exocellular short chain form of the cellular lipoteichoic acid (LTA) and is produced by coagulase negative staphylococci.¹⁹ Lipid S shares common antigenic determinants with LTA but differs in chain length, containing only six glycerophosphate units compared with 40-42 in whole cell LTA. In our present study, the value of the assay, which has been optimised to provide a rapid test, was assessed for the diagnosis of CRS caused by coagulase negative staphylococci.

METHODS

Preparation of the lipid S ELISA plates

The lipid S antigen was prepared from seven strains of coagulase negative staphylococci isolated from patients with confirmed CRS. Gel permeation chromatography (Superose 12) was used to recover the antigen from the culture medium.^{18,19} The antigen was diluted in sodium carbonate/bicarbonate buffer (0.05M, pH 9.6) and 100 µl, containing 0.125 µg/ml of antigen, was used to coat each well of a microtitre plate (Immulon 2; Dynatech Laboratories, Chantilly, Virginia, USA). The plates were kept at 4°C for 18 hours to allow the antigen to bind, after which they were washed in TBS/Tween (0.01M Tris/HCl, pH 7.4, 0.9% wt/vol NaCl, 0.3% vol/vol Tween 20). Unbound sites were blocked by incubation

Abbreviations: AOLC, acridine orange leucocyte cytospin; CRS, catheter related sepsis; CVC, central venous catheter; EIU, enzyme immunoassay units; ELISA, enzyme linked immunosorbent assay; LTA, lipoteichoic acid

Table 1 Patient demographics

	Control group	CVC related sepsis group
Mean age (years)	55	45
Range	24–81	18–82
Male	26	23
Female	14	17

CVC, central venous catheter.

Table 2 Serum IgG titres to lipid S in each patient group

	Control group	CVC related sepsis group
Number	40	40
Range (EIU)	ND	ND, 99939
Mean (EIU)		10429
SD		20175

EIU, enzyme immunoassay units; ND, no detectable antibody.

at 4°C for one hour in fresh buffer. After blocking, the buffer was removed and the plates were dried and stored in sealed containers at –20°C until required.

The lipid S ELISA

Patients' sera were diluted to 1/6400 in TBS/Tween buffer and 100 µl was added to each well of a microtitre plate. Positive and negative control sera were also tested in duplicate on each plate. The positive control serum was obtained from a patient with a clinical diagnosis of CRS who had a titre of 1/100 000,¹⁸ the negative control serum was normal human serum (Bradshure Biologicals, Loughborough, UK). After incubation at 37°C for two hours, excess serum was removed and the plates were washed with TBS/Tween. Bound IgG was detected by the addition of antihuman IgG conjugate (Sigma, Poole, Dorset, UK; diluted 1/5000 in TBS/Tween), which was incubated at 37°C for one hour. The conjugate was removed by washing with TBS/Tween and 100 µl of chromogenic substrate was added to each well. The substrate contained 10 mg of 3,3',5,5'-tetramethylbenzidine (Sigma) dissolved in 1 ml dimethyl sulphoxide and made up to 100 ml with sodium acetate/citrate buffer (0.1M, pH 6.0) incorporating 50 µl of H₂O₂ (5% vol/vol). After 25 minutes at 37°C, the reaction was stopped by the addition of 100 µl sulphuric acid (1M) and the optical density was read at 450 nm. Control wells contained no sera, but all other reagents were included as the blank. Enzyme immunoassay units (EIU) were calculated based on a previously described formula.²⁰

Patients

Catheter related sepsis

Serum samples were obtained from 40 patients over the age of 18 years with a clinical diagnosis of septicaemia associated with CRS. All of the patients had a CVC inserted into the internal jugular vein. No patients received total parenteral nutrition. Half of the patients had a short term CVC in situ for up to seven days and the remainder had long term Hickman catheters. A systemic infection associated with a CVC was diagnosed by the presence of a local infection at the insertion site, low grade fever (37.5–38.5°C), positive blood cultures taken via the catheter and by separate peripheral venepuncture, and no other focus of infection.³ The diagnosis was supported by the isolation of the same coagulase negative staphylococcus from blood culture samples obtained from each patient.

Control group

Serum samples were collected from 40 patients over the age of 18 years admitted for coronary arterial bypass grafting. The

Table 3 Parameters of the lipid S enzyme linked immunosorbent assay for the diagnosis of central venous catheter (CVC) related sepsis

	CVC related sepsis
Sensitivity	70%
Specificity	100%
Positive predictive value	100%
Negative predictive value	77%
Accuracy	85%

patients had no clinical signs or symptoms of sepsis including catheter associated infection. These patients constituted the negative control group. The samples of blood were collected immediately after insertion of a CVC into the patient.

Local research ethical committee approval and informed patient consent were obtained before entry into our study.

Statistics

Mean values were compared using the Mann-Whitney U test and p values of ≤ 0.01 were regarded as significant.

RESULTS

Patients

Table 1 presents the characteristics of the patient groups. None of the patients was immunocompromised.

Serology

The serum IgG titres of patients with CRS were raised and were significantly higher than the control group ($p < 0.0001$) (table 2). Antibody to lipid S was not detected in the serum samples obtained for control patients by the single absorbance ELISA, whereas 70% of the samples from patients with CRS had raised titres. Table 3 shows the diagnostic parameters of the lipid S ELISA.

DISCUSSION

The accurate diagnosis of CRS continues to offer a diagnostic challenge because clinical presentation is often silent or non-specific.³ There are also difficulties in accurately interpreting the results of microbiological investigations. The current standard method is the examination of blood cultures taken either via a peripheral venepuncture or the CVC. However, positive blood cultures may be the result of contamination or colonisation, rather than sepsis. Quantitative analysis of paired blood cultures is also available, although many laboratories do not offer this investigation because the method is time consuming and the results can be complicated by microbial colonisation of the catheter rather than systemic infection.¹³ It is also uncommon in clinical practice for both sets of cultures to be obtained. The recent method of Blot and colleagues¹⁰ compared the differential time to positivity between paired blood cultures taken via the CVC and a peripheral venepuncture. The differential time to positivity method had a sensitivity of 94% and specificity of 91% for the diagnosis of CRS, although these parameters varied considerably when investigated by another group, and were also shown to be influenced by the duration of patient catheterisation.²¹ More recently, an endoluminal brush technique for the diagnosis of CRS has been combined with an acridine orange leucocyte cytospin (AOLC) test.¹⁶ The endoluminal brush sampled the internal lumen of the CVC for microorganisms in situ, whereas in the AOLC test blood taken from the CVC was examined for the presence of microorganisms. In combination, the endoluminal brush significantly improved the yield of the AOLC test. However, both these

Take home messages

- The lipid S enzyme linked immunosorbent assay is a simple, economical, and rapid diagnostic test for suspected intravascular catheter related sepsis caused by coagulase negative staphylococci
- It may be useful for optimising patient management and may help prevent the unnecessary removal of intravascular catheters

methods require specialist techniques and may not distinguish colonisation of the internal lumen of catheters and luer connectors from infection.

The results of our study highlight the potential of the lipid S ELISA as a rapid test for the diagnosis of CRS caused by staphylococci. In the prototype lipid S assay, the patients' sera were titrated and compared with a standardised positive serum sample. This achieved a sensitivity of 75% and specificity of 90%.¹⁸ In comparison, the rapid ELISA had a sensitivity and specificity of 70% and 100%, respectively. This improved specificity was achieved by the optimisation of the assay reactants, including lipid S concentration, incubation conditions, and the ELISA methodology. In the modified rapid assay, optical densities of patients' sera were also compared directly with a reference negative control serum sample of known antilipid S IgG titre,²⁰ which effectively screened out patients with negative titres, resulting in high ELISA specificity.

The rapid ELISA also compares favourably with other diagnostic methods, including paired quantitative blood cultures and quantitative catheter segment culture.¹² The clinical diagnosis often relies heavily on the results of single blood cultures, which may be misleading. The lipid S ELISA may facilitate the full interpretation of such positive blood culture results with coagulase negative staphylococci and prove a useful adjunct for the diagnosis of CRS. The combination of negative serology and positive blood cultures may reflect catheter colonisation rather than CRS, whereas positive serology and blood cultures strongly suggest sepsis. Thus, the serological test may be of clinical value in distinguishing between colonisation and infection.

The lipid S ELISA has several other advantages over currently available methods for the diagnosis of CRS. The assay is rapid, with results available within four hours, and does not require unnecessary catheter removal. The assay is also relatively economical, using readily available laboratory equipment, and requires minimal training to perform. The serological test may also aid in a more accurate interpretation of the microbiological investigations of catheter tips, blood cultures, and blood drawn through contaminated luers for staining. In conclusion, we present a sensitive and specific indirect ELISA for the rapid serodiagnosis of CRS, which may assist in optimising patient management. Further prospective clinical trials are required to confirm the clinical value of the assay in this setting.

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Authors' affiliations

T Worthington, A Traube, T S J Elliott, Department of Clinical Microbiology, University Hospital, Edgbaston, Birmingham B15 2TH, UK
P A Lambert, Department of Pharmaceutical and Biological Sciences, Aston University, Aston Triangle, Birmingham, UK

This work was performed at the Department of Clinical Microbiology, University Hospital, Edgbaston, Birmingham B15 2TH, UK

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