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

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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.
Table 1 Patient demographics

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>CVC related sepsis group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>Range</td>
<td>24–81</td>
<td>18–82</td>
</tr>
<tr>
<td>Male</td>
<td>26</td>
<td>23</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>17</td>
</tr>
</tbody>
</table>

CVC, central venous catheter.

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

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>CVC related sepsis group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Range (EIU)</td>
<td>ND</td>
<td>ND (ND, 99939)</td>
</tr>
<tr>
<td>Mean (EIU)</td>
<td>10429</td>
<td>20175</td>
</tr>
<tr>
<td>SD</td>
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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 1/100 000.16 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.26

Patients

Catheter related sepsis
Serum samples were obtained from 40 patients over the age of 18 years with a clinical diagnosis of septicemia 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.1 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 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 absorbence 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 CRSContinue to offer a diagnostic challenge because clinical presentation is often silent or non-specific.1 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 colleagues14 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.15 More recently, an endoluminal brush technique for the diagnosis of CRS has been combined with an acidine orange leucocyte cytoplasm (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
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%. 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, 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. 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 luer 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.

ACKNOWLEDGEMENT

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REFERENCES