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

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.

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. 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; ELISA, enzyme immunoassay units; LTA, lipoteichoic acid

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Central venous catheters (CVCs) are widely used in clinical practice. However, the major complication associated with CVCs continues to be infection, which has considerable associated costs. Catheter related sepsis (CRS) is difficult to diagnose, owing to its non-specific clinical presentation, resulting in the unnecessary removal of the catheters 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 precautionary prophylactic measure. 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.

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. However, a positive blood culture cannot clearly distinguish between catheter contamination, colonisation, or associated sepsis. The roll plate method is also widely used in many routine laboratories because it is simple and economical, but it requires catheter removal and lacks specificity. Other more recent approaches include the use of the Gram stain and acridine orange cytospin, and the application of an endoluminal brush to sample the internal lumen of a catheter.

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.

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

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.
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 (Bradsure 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 anti-human 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 sulfoxide 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
resulting optical density was read at 450 nm.

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, 99939</td>
</tr>
<tr>
<td>Mean (EIU)</td>
<td>10429</td>
<td>20175</td>
</tr>
<tr>
<td>SD</td>
<td>2703</td>
<td>20175</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th></th>
<th>CVC related sepsis</th>
</tr>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>70%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>100%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>77%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>85%</td>
</tr>
</tbody>
</table>

The lipid S ELISA

Patients

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.

Serology

The serum IgG titres of patients with CRS were raised and
were significantly higher than the control group (p < 0.0001).
Table 2 shows the diagnostic parameters of the lipid S ELISA.

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 labora-
tories do not offer this investigation because the method is
time consuming and the results can be complicated by micro-
bial 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
colleagues16 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 consider-
ably when investigated by another group, and were also
shown to be influenced by the duration of patient
catheterisation.18 More recently, an endoluminal brush tech-
nique for the diagnosis of CRS has been combined with an
acidine orange leucocyte cytopsin (AOLC) test.17 The
doluminal 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 microor-
gisms. In combination, the endoluminal brush significantly
improved the yield of the AOLC test. However, both these
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.

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

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gisms. 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 tuer 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 reagents, 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 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.

**ACKNOWLEDGEMENT**

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**REFERENCES**

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