Detection of Catheter-Related Bloodstream Infections by the Differential-Time-to-Positivity Method and Gram Stain-Acridine Orange Leukocyte Cytospin Test in Neutropenic Patients after Hematopoietic Stem Cell Transplantation


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For febrile neutropenic patients who received hematopoietic stem cell transplantation, the Gram stain-acridine orange leukocyte cytospin (AOLC) test and the differential-time-to-positivity method (DTP) were performed. As a diagnostic tool for catheter-related bloodstream infections in these patients, the Gram stain-AOLC test has a lower sensitivity than does the DTP method but acceptable positive and negative predictive values.

Management of patients undergoing hematopoietic stem cell transplantation (SCT) often entails the use of central venous lines for administration of drugs and blood products. Such intravascular devices are associated with a significant risk for bacteremia or candidemia (11). Little is known about the frequency of catheter-related bloodstream infection (CRBSI) in neutropenic patients after SCT. Conventional methods to diagnose CRBSI require removal of the catheter for quantitative catheter-tip culture. Only 20% of central venous catheters (CVCs) removed because of suspected infection actually prove to be infected, and the diagnosis is always retrospective (5, 14, 16, 18, 20). Recently, it has been shown that CRBSI can be detected by the Gram stain and acidine orange leukocyte cytospin (AOLC) test or the differential-time-to-positivity (DTP) method without catheter removal (2, 3, 9, 15, 19). The DTP method has been shown to be highly sensitive and specific for CRBSI, even in neutropenic patients (12, 15, 19). The aim of the present study was to determine the rate of CRBSI in SCT patients and whether the Gram stain-AOLC test could offer accuracy comparable to that of DTP for the diagnosis of CRBSI in this patient group.

Between August 2002 and May 2003, we prospectively monitored all patients admitted to the SCT unit at the Division of Hematology, Medical University of Graz, Graz, Austria. Patients eligible for the study had to receive SCT for treatment of a hematologic malignancy as primary disease. They also had to have neutropenia (absolute neutrophil count of <500/μl), a central venous access in place, and fever as defined previously (8). Most of the patients received high-dose chemotherapy followed by autologous or allogeneic SCT. Some patients had reduced-intensity conditioning followed by autologous or allogeneic SCT. Stem cells were always obtained from peripheral blood. Anti-infectious prophylaxis, hygiene and diagnostic measures, and empirical antimicrobial therapy were performed as previously published (1). Defervescence was defined as a decline in body temperature to <37.5°C for >24 h. The following tests were done immediately after onset of fever in all patients: a 1-ml sample of blood (treated with EDTA) was drawn from every lumen of the catheter for Gram stain and the AOLC test. Afterwards blood was drawn through every lumen of the catheter for one pair of aerobic and anaerobic blood culture bottles each (hub-blood culture) and through a peripheral vein for an additional pair of aerobic and anaerobic blood culture bottles. The EDTA-blood samples and blood cultures were processed, and the DTP was calculated and expressed as previously described (4, 10, 12). CRBSI was defined by the presence of fever and a positive DTP result. CRBSI was defined as clinically confirmed if defervescence occurred within 24 h after catheter removal or inactivation of the Port-A-Cath (e.g., locked with teicoplanin) and lasted for at least 3 days without changes in a potentially effective antimicrobial regimen. In the case of CRBSI and/or at the clinician’s discretion, the CVC was removed, and Port-A-Caths were inactivated. Removed CVCs were processed by the method of Brun-Buisson et al. (5). If insertion of a new central venous access posed a greater risk than maintaining the catheter (for example, high risk of bleeding due to thrombocytopenia), at the clinician’s discretion the central venous access could be preserved. To determine the relatedness of coagulase-negative staphylococci (CNS) derived from peripheral blood cultures, hub-blood cultures, Gram stain and AOLC control cultures, and Brun-Buisson cultures, pulsed-field gel electrophoresis (PFGE) was performed using standard methods with minor modifications (13, 21).

Fifty-one patients (32 males and 19 females; median age, 61
years; range, 29 to 69 years) were included in the study. Thirty-
two had a CVC without subcutaneous tunnel, 2 had a tunneled
CVC, and 17 had totally implanted CVCs (Port-A-Caths). Six-
teen of the 51 patients (31%) had CRBSI as determined by
the DTP method. In 11 of 16 patients (69%) with a positive DTP
result, the Gram stain-AOLC test was positive, providing a
positive and negative predictive value of 100 and 88%, respec-
tively. In 13 patients with a positive DTP result, CVCs were
removed or Port-A-Caths were inactivated. In 10 of these 13
patients, CRBSI was clinically confirmed. None of the patients
with CRBSI and inactivation of Port-A-Cath relapsed. The
remaining three patients who did not defervesce despite cath-
eter removal or inactivation had concomitant infectious com-
lications (n = 2) or died (pulmonary hemorrhage) before
evaluation was possible (n = 1). In the three patients with
CRBSI for whom the catheter was not removed or inactivated,
fever continued >24 h (Table 1). In another three patients, the
CVC was removed at the clinician’s discretion despite a nega-
tive DTP and/or negative Gram stain-AOLC test. The subse-
quent Brun-Buisson tests were negative in all cases. In addi-
tion, there was no defervescence after catheter removal,
indicating that CRBSI was not present. In 13 of 16 patients
with CRBSI, CNS were cultured. By the use of PFGE one
patient was found to have two distinct clones in hub-blood
cultures and AOLC and Gram stain control cultures. In the
remaining patients, the CNS strains from corresponding cul-
tures had identical PFGE patterns, indicating that the positive
results from these methods (e.g., DTP and Gram stain-AOLC
and its control culture and Brun-Buisson method) were due to
the same strain.

In contrast to previous studies we focused on patients who
had undergone SCT and compared the DTP technique with
the Gram stain and AOLC tests; these have not yet been
evaluated in neutropenic patients. The Gram stain-AOLC test
requires 30 min to complete and therefore has a great impact
on clinical practice, e.g., for the decision to remove a catheter
and/or change antimicrobial therapy. Whereas the Gram stain-
AOLC test has an absolute threshold of 1,000 microorgan-
isms/ml of blood (9; P. Kite, Leeds, United Kingdom, personal
communication), the DTP method has a relative threshold
since it measures the difference in the microbial load of pe-
ripheral and hub-blood cultures. Compared to the Gram stain-
AOLC test, the DTP method may therefore indicate CRBSI in
an earlier stage of the disease in which the microorganisms in
catheter blood have not yet reached the threshold of 1,000
organisms/ml of blood. The rate of CRBSI in our patients with
hematologic malignancies and SCT was 31%, which is approxi-
matley the rate of patients with hematologic malignancies
without SCT (3). All of our patients with CRBSI received an
antimicrobial regimen that was potentially effective against the
offending pathogen, but they did not respond to therapy. After
catheter removal or inactivation of the port without changes in
the antibiotic regimen, 10 of 13 patients with CRBSI de
deferced within 24 h. In the remaining three patients evaluation
was not possible due to concomitant infection and death. In
three patients with CRBSI, the catheter remained in place or
the Port-A-Cath was not inactivated, and the patients showed
no clinical improvement. Our results suggest that prior anti-
microbial therapy does not lead to misclassifica

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Laplanche, C. Brun-Buisson, and C. Tancrede. 1999. Diagnosis of catheter-
related bacteremia: a prospective comparison of the time to positivity of

TABLE 1. Patients with CRBSI

<table>
<thead>
<tr>
<th>Patient</th>
<th>Type of catheter</th>
<th>DTP result</th>
<th>Gram stain-AOLC result</th>
<th>Microorganism</th>
<th>Brun-Buisson test result</th>
<th>Defervescence within 24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CVC</td>
<td>Positive</td>
<td>Positive</td>
<td>CNS</td>
<td>10⁶ CFU/ml</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>CVC</td>
<td>Positive</td>
<td>Positive</td>
<td>CNS</td>
<td>10⁶ CFU/ml</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>Port-A-Cath</td>
<td>Positive</td>
<td>Positive</td>
<td>CNS</td>
<td>Not done</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>CVC</td>
<td>Positive</td>
<td>Positive</td>
<td>Strep. mitis</td>
<td>Negative</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Port-A-Cath</td>
<td>Positive</td>
<td>Positive</td>
<td>CNS</td>
<td>Not done</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>CVC</td>
<td>Positive</td>
<td>Positive</td>
<td>CNS</td>
<td>Not done</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>CVC</td>
<td>Positive</td>
<td>Positive</td>
<td>CNS, E. coli</td>
<td>Not done</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>Port-A-Cath</td>
<td>Positive</td>
<td>Positive</td>
<td>CNS</td>
<td>Not done</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>CVC</td>
<td>Positive</td>
<td>Negative</td>
<td>CNS</td>
<td>Negative</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>Port-A-Cath</td>
<td>Positive</td>
<td>Positive</td>
<td>CNS</td>
<td>Not done</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>CVC</td>
<td>Positive</td>
<td>Positive</td>
<td>CNS</td>
<td>3.9 × 10⁵ CFU/ml</td>
<td>No (concomitant infection)</td>
</tr>
<tr>
<td>12</td>
<td>CVC</td>
<td>Positive</td>
<td>Positive</td>
<td>CNS</td>
<td>10⁵ CFU/ml</td>
<td>No (concomitant infection)</td>
</tr>
<tr>
<td>13</td>
<td>Port-A-Cath</td>
<td>Positive</td>
<td>Negative</td>
<td>CNS</td>
<td>Not done</td>
<td>No (patient died prior to assessment)</td>
</tr>
<tr>
<td>14</td>
<td>CVC</td>
<td>Positive</td>
<td>Negative</td>
<td>Pseudomonas aeruginosa</td>
<td>Not done</td>
<td>No (CVC not removed)</td>
</tr>
<tr>
<td>15</td>
<td>CVC (Hickman)</td>
<td>Positive</td>
<td>Negative</td>
<td>Strep. mitis</td>
<td>Not done</td>
<td>No (CVC not removed)</td>
</tr>
<tr>
<td>16</td>
<td>Port-A-Cath</td>
<td>Positive</td>
<td>Negative</td>
<td>CNS</td>
<td>Not done</td>
<td>No (Port-A-Cath not inactivated)</td>
</tr>
<tr>
<td>17</td>
<td>Port-A-Cath</td>
<td>Positive</td>
<td>Negative</td>
<td>CNS</td>
<td>Not done</td>
<td>No (Port-A-Cath not inactivated)</td>
</tr>
</tbody>
</table>

* CVC with subcutaneous tunnel.