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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 acridine 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 $<500/\mu l$), 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

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TABLE 1. Patients with CRBSI

Patient	Type of catheter	DTP result	Gram stain-AOLC result	Microorganism	Brun-Buisson test result	Defervescence within 24 hours
1	CVC	Positive	Positive	CNS	10^6 CFU/ml	Yes
2	CVC	Positive	Positive	CNS	10^6 CFU/ml	Yes
3	Port-A-Cath	Positive	Positive	CNS	Not done	Yes
4	CVC	Positive	Positive	*Streptococcus mitis*	Negative	Yes
5	Port-A-Cath	Positive	Positive	CNS	Not done	Yes
6	CVC	Positive	Positive	CNS	Negative	Yes
7	CVC	Positive	Positive	CNS, *Escherichia coli*	Not done	Yes
8	Port-A-Cath	Positive	Positive	CNS	Not done	Yes
9	CVC	Positive	Negative	CNS	Negative	Yes
10	Port-A-Cath	Positive	Positive	CNS	Not done	Yes
11	CVC	Positive	Positive	CNS	3.9×10^2 CFU/ml	No (concomitant infection)
12	CVC	Positive	Positive	CNS	10^6 CFU/ml	No (concomitant infection)
13	Port-A-Cath	Positive	Negative	CNS	Not done	No (patient died prior to assessment)
14	CVC	Positive	Negative	*Pseudomonas aeruginosa*	Not done	No (CVC not removed)
15	CVC (Hickman)[a]	Positive	Negative	*Streptococcus mitis*	Not done	No (CVC not removed)
17	Port-A-Cath	Positive	Negative	CNS	Not done	No (Port-A-Cath not inactivated)

[a] CVC with subcutaneous tunnel.

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). Sixteen 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%, respectively. 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 catheter removal or inactivation had concomitant infectious complications ($n = 2$) 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 negative DTP and/or negative Gram stain-AOLC test. The subsequent Brun-Buisson tests were negative in all cases. In addition, 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 cultures 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 microorganisms/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 peripheral 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 approximately 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 defervesced 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 antimicrobial therapy does not lead to misclassification of CRBSI based on the DTP method in neutropenic patients with hematologic malignancies and SCT. We believe that, due to their different advantages, the two methods tested in this study should be performed together when CRBSI is suspected in neutropenic patients after SCT: the Gram stain-AOLC test is time efficient, provides rapid results, and so has a great clinical impact, and the DTP method is simple to perform and has been validated in previous studies (3, 12, 15, 19).

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