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Value of Direct Catheter Staining in the Diagnosis of Intravascular-Catheter-Related Infection

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Ninety-nine intravascular catheters were evaluated by a semiquantitative culture and Gram and acridine orange direct stains. A diagnosis of catheter-related infection was determined by a retrospective review of clinical records. Compared with the culture method, direct examination of catheters lacked sensitivity. Surprisingly, for some patients, a positive stain for yeasts not recovered by culture was considered significant. The culture correlated with bacteremia but failed to predict infection in 42% of patients.

Catheter-related bacteremias can complicate as many as 0.08% of peripheral venous catheterizations in adult-care hospitals (11). This complication represents up to 33% of endemic nosocomial infections (7). The diagnosis of these infections relies on blood and catheter cultures (1, 3, 4, 8, 9, 12). The semiquantitative culture (SQC) method devised by Maki and colleagues (8) replaced the less specific broth culture by reliably discriminating colonization from infection (3). Cooper and Hopkins (5) described the direct Gram staining of catheters, obviating the need for awaiting conventional bacterial culture results. Acridine orange is a fluorescent dye (10) not yet described for this purpose. We report a prospective evaluation of direct Gram and acridine orange staining of catheters compared with the SQC method. Patient records were retrospectively reviewed, permitting the analysis of discrepancies between these methods.

MATERIALS AND METHODS

This study was performed at Notre-Dame Hospital, an adult tertiary care facility of 984 beds also provided with a nursery and a neonatal intensive care unit. In this study, intravascular catheters were taken predominantly from patients in the adult medical and surgical services (92), especially from the intensive care units, while a few catheters (7) came from neonates. Over a 3-month period, we prospectively studied all intravascular catheter distal segments received in the laboratory from patients at risk for catheter-related infection (CRI). All catheters were withdrawn aseptically and cut with sterile scissors by trained nurses and physicians, according to a written protocol. They were then brought in less than 2 h by hospital transport in a sterile container to the laboratory. After being processed as suggested for a SQC (8), the catheter distal segment was then immersed and fixed in 95% methanol in a sterile tube for 1 min. It was air dried, kept afterwards at 4°C, and stained in the next 24 h.

The Gram and acridine orange stains were performed by standard methods (6, 10). The staining procedures were done in a series of different sterile tubes, each containing a different solution, without cross contamination between catheters. Sterile forceps were used to transfer the segments between solutions. Opaque catheters were cut in half longitudinally. First, the catheter was immersed in 0.01% acridine orange for 2 min. After being air dried, both ends of the catheter were firmly fixed on a glass slide with appropriately molded plastic. This enabled us to examine freely almost the entire length of the catheter, initially at ×400 and then under oil immersion at ×1,000 with an epifluorescence microscope. The plastic could easily be removed and the catheter reused for the Gram stain. For that staining, the catheter was immersed in crystal violet for 1 min, rinsed under tap water, put in Lugol solution for 1 min, rinsed under tap water, briefly decolorized in ethanol-acetone, and stained with safranin O for 1 min. It was then air dried and examined under oil immersion at ×1,000, after being fixed as described above with molded plastic, with a microscope with a conventional light source.

For both stains, 50 fields were examined in 15 min; after bacterial counts from the culture were compared with those of the direct examinations, a threshold for positivity of at least 5 organisms per 50 fields was selected as compared with the criterion of 1 organism per 20 fields used by Cooper and Hopkins (5).

Each procedure was interpreted by a different observer in a double-blind fashion. All patient records were retrospectively reviewed without knowledge of the results of the three diagnostic methods.

RESULTS

A total of 99 catheters (72 central, 22 peripheral, 5 unknown) from 53 patients were collected and originated from the following places: the subclavian, jugular, or femoral vein, 50 (Sorenson 1-lumen catheter [18.4 cm]; Abbott Laboratories, North Chicago, Ill. [the majority]; five Cordis introducer catheters); peripheral vein, 15 (Argyle short venous catheter [5 cm]; Sherwood Medical); pulmonary artery, 11 (Swan-Ganz catheters; American Edwards Laboratory); radial artery, 8 (Argyle short venous catheter); umbilical artery, 7 (Argyle umbilical vessel catheter [41 cm]; Sherwood Medical); femoral artery, 3 (various catheters); and unknown origin, 5.

Of the 99 catheters stained, 14 (14%) were uninterpretable by the Gram stain (12 opaque and 2 with artifacts), which left 85 to be compared with culture results. High nonspecific diffuse fluorescence of 9 catheters left 90 catheters to be studied with acridine orange. The majority of the catheters were made with translucent polyvinyl chloride. Cordis introducer and Swan-Ganz catheters were opaque, and both were implicated in Gram and acridine orange staining artifacts (29 and 22%, respectively). For 6 of 53 records, no conclusion

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TABLE 1. Relationship between direct catheter staining with the Gram stain or acridine orange and results obtained with the SQC

Stain	No. of culture results		Sensitivity[a] (%)	Specificity[b] (%)	PPV[c] (%)	NPV[d] (%)
	Positive	Negative				
Gram						
+	8	6	44	91	57	86
−	10	61				
Acridine orange						
+	12	17	71	77	41	92
−	5	56				

[a] Sensitivity = true-positive/(true-positive + false-negative).
[b] Specificity = true-negative/(true-negative + false-positive).
[c] PPV, Positive predictive value = true-positive/(true-positive + false-positive).
[d] NPV, Negative predictive value = true-negative/(true-negative + false-negative).

on the presence of a CRI could be drawn, leaving the following to be compared with clinical diagnosis of CRI: 93 catheters for the SQC, 84 with acridine orange, and 79 with Gram stain.

A diagnosis of CRI was made for 19 catheters on the basis of these criteria: (i) positive venous blood cultures with local signs of infection for six; (ii) positive venous blood cultures with a clinical response to catheter withdrawal without another source for sepsis for four; (iii) local signs of infection for six; (iv) clinical response to catheter removal with at least two positive catheter exams for three. Overall, 21% (4 of 19) of the CRIs were related to peripheral catheters, which represented 23% (22 of 94) of the total peripheral and central catheters.

The results of the direct stains are compared in Table 1 with the SQC. In Table 2, direct stains and SQC are compared with clinical evidence of CRI. Results obtained with intraarterial versus intravenous and peripheral versus central catheters were similar and were pooled together. The agents recovered were nine *Staphylococcus epidermidis*, five *Candida albicans*, two gram-positive cocci, one *Staphylococcus aureus*, one *Acinetobacter calcoaceticus* var. *lwoffii*, one yeast form. All yeast infections (six cases) were found in patients with a severe underlying disease but without neutropenia. All were treated with broad-spectrum antibiotics. Two patients received total parenteral nutrition. Two patients had positive blood cultures. Notably, two cases (one with a positive blood culture) had a negative SQC, but yeast forms were seen in one or both of the stains. For the diagnosis of fungal CRIs, the Gram stain had a sensitivity of 33% (1 of 3) and a specificity of 97% (77 of 79), the acridine orange stain had a sensitivity of 80% (4 of 5) and a specificity of 98% (82 of 84), while the SQC had a sensitivity of 66% (4 of 6) and a specificity of 96% (89 of 93).

DISCUSSION

When compared with the catheter SQC, both direct stains had a poor sensitivity. This is in contrast with data from a previous study (5). In that study, the direct Gram staining of 330 catheters had a sensitivity of 100% and a specificity of 96.9%. The higher sensitivity reached with acridine orange here was still below these values and resulted in a loss of specificity. The differences in the results of both studies could be related to the higher proportion of peripheral catheters included in our report (23% versus 2.4%). But in our study, results with peripheral catheters were similar to those obtained with central catheters. In both reports, similar methods were used except for some details which will be further discussed here and could explain the differences in the results.

Cooper and Hopkins (5) examined at least 200 oil immersion fields. In 15 min, we could screen only 50 and found it difficult to perform the test faster. The threshold of 5 organisms per 50 fields was chosen after analysis of various possibilities (unpublished data) including 10 and 5 organisms per 50 fields; for Gram staining, sensitivity compared with SQC for a threshold of 10 and 5 organisms was identical at 44% and specificities were 94 and 91%, respectively. For acridine orange staining, sensitivity for a threshold of 10 and 5 organisms was 65 and 71% and specificity was 84 and 77%, respectively. We selected the threshold with better sensitivity for both staining methods. When examining a curved specimen, frequent fine focus adjustments were mandatory. Cutting the catheter in half longitudinally helped with the analysis of most opaque specimens. This report supports doubts cast by some (2) about the cost and time performance of the test. This time-consuming method did not allow us to examine more fields in a reasonable amount of time, which could have optimized our results.

The lower sensitivity of the Gram stain in this study could be explained by a loss of organisms during the acridine orange procedure, since the Gram stain followed it. Cooper and Hopkins (5) report that unpublished data confirm that there are still enough bacteria left to be seen with the Gram stain after the catheter has been rolled on agar. It would be surprising that after the catheter was fixed in methanol, we would encounter a significant loss of bacteria after the few manipulations required for the acridine orange stain. But still, the design of our study could have resulted in some loss of sensitivity for the Gram stain. When we compared the first stain made after SQC in both studies (Gram stain for the study of Cooper and Hopkins [5] and acridine orange stain for our study), the sensitivity of the acridine orange stain remained lower. Even the combination of both stains did not alter our results (data not shown).

Better visualization of organisms could have been obtained with a microscope with a direct incident light source instead of with a conventional microscope. However, the former is not used in routine clinical microbiology laboratories. Finally, an important proportion of stained catheters were uninterpretable because of artifacts (14% with the

TABLE 2. Correlation of the results of Gram and acridine orange staining and SQC of catheters with the clinical diagnosis of catheter infection[a]

Test	No. of culture results		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative				
Culture						
+	11	10	58	87	52	89
−	8	64				
Gram						
+	7	8	50	88	47	89
−	7	57				
Acridine orange						
+	12	13	75	81	48	93
−	4	55				

[a] Sensitivity, specificity, PPV, and NPV are as defined in Table 1, footnotes *a* through *d*.

Gram stain and 9.1% for acridine orange). A shorter immersion time in the staining solutions by Cooper and Hopkins (5) (5 s) when compared with ours (60 s) could explain why in their study no artifacts interfered. In our study, despite a low positive predictive value of direct stains when compared with culture, a higher negative predictive value around 90% gave a better confidence in negative results of catheter stains.

As previously stated (4, 8), the SQC was significantly predictive of bacteremia, since bacteremias were found in 8 of 23 positive cultures compared with 2 of 76 negative cultures ($P < 0.001$, chi-square test with Yates' correction). Overall, the sensitivity of the three methods to detect CRI was low. The most sensitive was acridine orange staining, which again had the lowest specificity. With a poor positive predictive value, each test could predict infection in only 50% of the cases.

The analysis of the eight false-negative SQCs revealed that seven patients were receiving antibiotics. All had at least one positive direct examination. Two had positive blood cultures indicating a significant infection. Three cultures yielded low colony counts of less than 5 CFU per plate, and five were negative. Even newly suggested criteria (4) would not have modified our results. As noticed by others (5), a positive yeast stain with a negative culture can be significant, as for two cases in this study. The direct staining of catheters can be useful to diagnose fungal CRI, especially with acridine orange. The finding of a positive examination for yeasts was highly significant, since all tests had a high specificity. The most sensitive examination for this purpose was the acridine orange stain. Cooper and Hopkins (5) found that 9 of 330 catheters had a false-positive Gram stain compared with culture. Seven of these nine false-positive results revealed yeast forms, and two of them were associated with candidemia. Direct catheter stains could be useful to detect fungal infections of intravascular lines, especially when cultures are negative.

Laboratory diagnosis of CRI is difficult with one technique. In our hands, the direct stains did not provide good results. They are laborious, and a significant minority of catheters are uninterpretable. This is the first report of acridine orange utilization in the diagnosis of CRI. Acridine orange staining is easier and microscopic examination at ×400 is faster than Gram staining. Nevertheless, it correlates imperfectly with infection. The retrospective nature of this clinical review weakens our conclusions on the predictiveness of clinical infection, but still casts some doubts on the usefulness of the direct catheter stains. Further studies are necessary to clarify the role and value of methods of rapid diagnosis of CRI. However, direct catheter staining could prove to be useful in the diagnosis of fungal CRI.

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