Guidewire Catheter Change in Central Venous Catheter Biofilm Formation in a Burn Population*

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This study was designed to assess the risk of colonization and biofilm formation of central venous catheters left in situ for seven days vs those changed over a guidewire at three days and removed at seven days. Colonization was determined using scanning and transmission electron microscopy and compared to a special scraping/sonication culture method. Thirty-one catheters were examined, and no difference was found between catheters left in situ (9 of 16 colonized) and those changed over a guidewire (11 of 15 colonized). Colonization rates rose significantly from 4 of 15 catheters at the time of guidewire change to 11 of 15 at 7 days (p<0.001). Of the catheters defined as colonized by SEM, the special culture technique showed bacterial growth in only 35 percent, making a negative culture result of dubious value in ruling out catheter colonization. No beneficial effect of guidewire changes in reducing colonization could be demonstrated. (Chest 1991; 100:1090-95)

CVC = central venous catheter; SEM = scanning electron microscopy; TEM = transmission electron microscopy

The use of central venous catheters (CVC) in the care of burn patients is now standard practice. Local infection, bacteremia, and thrombophlebitis are complications associated with these catheters, and to reduce these risks, regular catheter changes over a guidewire have become common practice.

Sepsis remains the most serious complication related to central lines. Reported catheter colonization rates range from 6 to 40 percent,1 and catheter-related sepsis rates range from 5 to 16 percent of patients with CVC.2,3 Scrupulous care of the insertion site and frequent site changes are thought to be essential in reducing CVC septic complications. Insertion site asepsis is particularly difficult to achieve in burned patients who undergo daily therapeutic tub baths with wetting of the site. The problem is often compounded by the lack of unburned sites for new line placement. These issues raise concerns about the ability to minimize risk of sepsis stemming from CVC insertion and maintenance.

Catheter placement over a guidewire has become a routine technique for easily changing a malfunctioning catheter or for substituting a new one for an existing CVC after an arbitrarily chosen period of time. Inserting a guidewire through the existing catheter, removing the catheter, and threading a new catheter over the wire through the existing site generally precludes insertion complications, but the role of guidewire changes in the prevention and treatment of catheter-related sepsis remains unclear. Guidewire changes with tip cultures of removed catheters have been advocated as being safe and useful in the diagnosis of catheter-related sepsis,4 and in fact, guidewire changes have been advocated as a method of treatment for catheter sepsis.5 Recent studies in our laboratory using a sheep model have demonstrated rapid recolonization of the replacement catheter, remote seeding of catheters elsewhere in the cardiovascular system, and establishment of bacterial growth on right heart valves and throughout the lungs. Interestingly, blood cultures from these animals were uniformly negative.

This study was undertaken to determine the incidence of bacterial colonization and biofilm formation on CVC changed over a guidewire compared to those left in place in a population of burn patients.

**Materials and Methods**

**Patients**

A prospective, restricted randomization study was conducted at the University of Alberta Hospitals Burn Unit, Edmonton, Alberta, from February to December 1987. Informed consent was obtained from the patient or family prior to entry into the study, and the protocol was approved by the Ethics Committee of the University of Alberta Faculty of Medicine. The CVCs were inserted at the discretion of attending medical staff. Standard sterile technique employed gloves, masks, and gowns, and all catheters were inserted

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Using the Seldinger catheter-over-wire technique,

Processing of Catheters

Before removal of the catheter, the insertion site was examined for pus or redness, and a culture was obtained if there was any drainage. The site was cleansed with povidone and rinsed with saline. The CVC was gently withdrawn and placed on a sterile metal tray and then divided into segments.

Specimens were taken from the tip and the portion in the subcutaneous tunnel at least 2 cm from the point at which the catheter exited the skin. From each area, a 4-cm segment of catheter was obtained and then randomly divided into four equal 1-cm long segments. These were assessed by special scraping/sonication microbiologic methods (vide infra) and scanning and transmission electron microscopy (Fig 1). The presence of bacteria on the luminal surface of the catheter tip as seen by scanning electron microscopy was considered to be evidence of colonization. Figure 2 shows a catheter with no adherent bacteria. Figure 3 represents a catheter with adherent bacteria and biofilm. Transmission electron microscopy was used to confirm that the bacteria visualized on SEM were ultrastructurally intact and thus viable (Fig 4).

Quantitative Microbiology

The number of adherent bacteria on the catheter segments was estimated using an adaptation of the technique of Cleri et al. The number was assessed by the following methodology (Fig 1). The number of adherent bacteria on the catheter segments was estimated using an adaptation of the technique of Cleri et al.* The outside of a 1-cm segment of catheter was scraped with a sterile scalpel blade and then split longitudinally. Blade, scrapings, and catheter segment were placed in tubes containing 1.0 ml of nutrient broth. Scrapings were dispersed using a vortex mixer and by gentle ultrasonic disruption in a cooled ultrasonic bath for 30 min.

Serial dilutions were made by sequentially transferring 0.1 ml of the solution to 0.9 ml of fresh broth, mixing, then transferring 0.1 ml of the diluted solution to the next tube to make dilutions of 10⁻¹ to 10⁻⁴. Each dilution of 0.1 ml was spread over the surface of brain-heart infusion agar plates for aerobic cultures and with added sheep blood for anaerobic cultures. The inoculated plates were incubated at 37°C for 24 to 72 hours. All different colony types were

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identified and counted. Plates showing greater than or equal to 10⁶ colonies were designated as a positive quantitative culture.

Maki Culture Methods

A 1-cm segment of each catheter was placed on the surface of a 100-mm diameter blood-agar plate. The segment was rolled four times across the agar surface with application of downward pressure. The plates were then incubated at 35°C in air for 24 to 48 hours. All colony types appearing on the plates were counted and organisms identified according to standard microbiologic methods. Plates showing 15 or more colonies were designated to be a positive semiquantitative culture.¹

Scanning Electron Microscopy

Solutions were prepared as follows:

1. Cacodylate buffer: cacodylic buffer (0.1 M) was made up to contain 0.15 percent ruthenium red and adjusted to pH 7.0.

2. Fixation solution: one vial (2 ml) of glutaraldehyde was added to 26 ml of ruthenium red-cacodylate buffer for a final glutaraldehyde concentration of 5 percent.

Procedure for fixation of electron microscopy specimens consisted of immersion of the catheter segments in fixation solution for 2 h at room temperature or 24 h at 4°C. Specimens were washed by removing the fixative solution and replacing it with ruthenium red-cacodylate buffer; the procedure was repeated three times at 10-min intervals.

Specimens were bisected longitudinally and washed twice in cacodylate buffer (0.1 M, pH 7.0) containing no ruthenium red. They were then dehydrated in ethanol 30, 50 and 70 percent air dried and "sputter coated" with gold and palladium. Specimens were examined by means of a scanning electron microscope.⁴ The entire luminal and outer surface areas were scanned to eliminate any sampling error when determining presence of bacteria.

Transmission Electron Microscopy

Adherent material was scraped from a small area (less than 0.5 cm²) of the inner and outer surface of selected microscopy specimens using a sterile scalpel blade. This material was enrobed in agar, washed twice in cacodylate buffer (0.1 M, pH 7.0) containing 0.15 percent ruthenium red and then postfixed in 2 percent osmium tetroxide in the same buffer.

Following five washes in the ruthenium red-cacodylate buffer, the specimens were dehydrated through a series of acetone washes, and then in propylene oxide before embedding in Spurr resin.⁶ Sections were cut using an ultramicrotome stained with uranylacetate and lead citrate,⁷ reinforced with evaporated carbon before examination using a transmission electron microscope at an acceleration voltage of 60 kV.⁸

Analysis

Data were analyzed using Fisher's exact, chi-square and Student's t-tests, and relationships were determined by Pearson correlations. Upon removal at day 7, each catheter was treated as a separate event for data analysis. Catheter exchanges at day 3 over a guidewire were not treated as separate events. All tests of significance were at the 0.05 level.

RESULTS

There were 31 catheters inserted in 19 patients. Nine patients with 16 catheters were placed in group 1; ten patients with 15 catheters were placed in group 2. Five patients in group 1 and six patients in group 2 had only one CVC inserted, and only one patient in each group required more than two catheters to be inserted at different sites during their hospital stay. One patient in group 1 required five catheters and one patient in group 2 required three catheters (Table 1). All catheters were changed to a new site at day 7.

There was no significant difference between the groups' baseline characteristics as measured by chi-square and Student's t-tests (Table 1). These included sex, catheter insertions, age, percentage total body
Table 1—Demographic and Clinical Characteristics by Catheter Grouping*

<table>
<thead>
<tr>
<th></th>
<th>Group 1 In Situ</th>
<th>Group 2 Guidewire</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Catheters (n)</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Insertions/pt -1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>≥2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Age (X±SD)</td>
<td>30±20</td>
<td>35±17</td>
</tr>
<tr>
<td>TBSA % Burn (X±SD)</td>
<td>31±24</td>
<td>31±14</td>
</tr>
<tr>
<td>Sex (m/f)</td>
<td>9/0</td>
<td>7/3</td>
</tr>
<tr>
<td>Insertion site:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subclavian</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Femoral</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Internal Jugular</td>
<td>3</td>
<td>2</td>
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*Values were not statistically significant.

The catheter tip occurring in group 1. There were three positive blood cultures in two patients: one each of *K. pneumoniae*, *Candida albicans*, and *P. aeruginosa*. In all instances, the organisms were different from those isolated from catheters. There were no episodes of catheter-related sepsis in 228 catheter-days.

Table 4 summarizes the combined culture and SEM results for the two groups. Only 19 percent of the tips from group 1 (seven days) cultured positive for bacteria, but using SEM and TEM, biofilm and bacteria were present on 56 percent of the catheters. In group 2, 13 percent of the catheter tips cultured positive at the time of the guidewire change (three days), and bacteria and biofilm were seen on 26 percent of the catheter tips. After guidewire change, 26 percent of the catheters removed at seven days showed bacterial growth on culture, but 73 percent of the catheters were positive using SEM and TEM. The difference between special culture and SEM results was significant (p<0.05).

**Discussion**

This study demonstrates that biofilm formation and bacterial colonization occur rapidly on CVCs in burn patients and that changing the catheters over a guidewire does not seem to confer any advantage in terms of the colonization process. Assessment of bacterial colonization of catheters by culture methods has been fraught with problems. Since Maki et al's original publication, his method has become widely accepted as the means of identifying catheter-related sepsis or infection. Unfortunately, bacterial adherence within biofilm layers and antimicrobial therapy make interpretation of negative culture results difficult. Furthermore, the relatively low rate of catheter-related sepsis makes it virtually impossible to assess, by the Maki approach, techniques to reduce sepsis, particularly if an attempt is made to correlate the septic syndrome with bacterial colonization of the catheter tips.
catheter. Our data on culture and SEM results for catheter tips underscore the difficulty in obtaining and reliably interpreting microbiology of intravascular devices. Both quantitative and semiquantitative methods were created to be predictors of sepsis or at least of local infection (a probable sepsis prerequisite). Sensitivity of catheter cultures was found to be poor, with predictive value of a positive culture being as low as 8.3 percent. A few studies reported episodes of catheter-related sepsis with colony counts below 15 on semiquantitative cultures. Nevertheless, the majority of positive catheters (with infections or bacteremias) were associated with heavy growth, far exceeding the recommended 15 colonies. Culture methods are, by design, supposed to predict catheter-related sepsis or local infection but not catheter colonization. It is, therefore, difficult to compare their performance to SEM.

Electron microscopy is essentially a qualitative descriptive tool unable to precisely distinguish a low level of colonization from a higher, possibly "prebacteremic" one, and yet, it could alert physicians early to the potential dangers of prosthetic device infections. The role of biofilm in bacterial adherence to prosthetic devices and protection of bacteria from host defenses has been well established, and this relationship increases the problem of obtaining accurate cultures. This is largely due to the difficulty in dislodging catheter-adherent organisms onto a culture plate, and consequently, SEM is probably superior in detecting catheter colonization. Its value as a clinical test is uncertain at this time. To us, however, it appears to be superior to culture methods in studying the pathogenesis of catheter-related infections.

Survival of bacteria in the face of host resistance and antibiotic treatment is enhanced by biofilm. Biofilm or bacterial extracapsular secretions can be identified as a polysaccharide outside the cell wall by use of specific stains for polysaccharides. A layer of biofilm aids in adhesion to the surface by affecting the charge and surface tension of the substrate. It also allows growth in a protected environment in which soluble nutrients are more easily trapped and utilized, resulting in increased bacterial metabolic activity and growth. Adherent organisms may be less susceptible to clearance by blood flow, phagocytosis, antibodies, and antimicrobials. Higher rates of arterial flow may explain the difference in colonization rates between arterial and venous catheters previously documented. Higher minimum inhibitory concentrations for antibiotics have been demonstrated for biofilm-encased bacteria as opposed to free floating organisms, which probably account for the increased difficulty in eradicating prosthetic device-associated infections. These infections tend to persist, often in a subclinical state, until the foreign material is removed.

The indolence of biofilm-associated infections is likely related to the decreased opportunity for encased bacteria to be washed off the catheter and spread hematogenously. Of interest is the parenthetic observation that biofilm was actually present on 94 percent (15 of 16) of the catheters in group 1 and 80 percent (12 of 15) in group 2. The discrepancy between bacterial identification on SEM-TEM and the simple presence of biofilm is related to the thickness of the film which can obscure embedded bacteria. Despite this apparently high incidence of biofilm presence, culture-positive catheter tips in a burn population have ranged from only 10 to 34 percent.

Skin organisms, intraluminal fluid path contamination, and hematogenous seeding have been reported as sources of CVC colonization and infection. However, there have been no studies to determine the association between guidewire and catheter colonization. Our data indicate that guidewire changes do not decrease the rate of catheter colonization. The most likely explanation for this observation is that bacteria and biofilm adhere to the guidewire with subsequent inoculation of the new catheter during the over-the-guidewire maneuver. This interpretation is in keeping with our observations in the sheep model alluded to earlier.

Contrary to previous reports listing a secondary source of infection as a risk factor for CVC sepsis, our observations failed to show a relationship between urinary, pulmonary, or burn wound infections and colonization. There was no evidence of intraoperative seeding as a source of colonization, as no association between colonization and operative debridement could be found. This, combined with the increased colonization after guidewire changes, raises the possibility that the insertion site skin is playing a role as a bacterial colonization source.

Based on these findings, there would be little to be gained in the prevention of CVC bacterial colonization and biofilm formation from routine over-guidewire catheter changes. The precise role of catheter biofilm colonization in the generation of catheter-associated sepsis remains speculative, but its putative role deserves ongoing study.

REFERENCES
4. Pettigrew RW, Lang SDR, Haydock DA, Parry BR, Bremmer DA, Hill GL. Catheter related sepsis in patients on intravenous nutrition: a prospective study of quantitative catheter cultures...
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