Biofilms on Right Heart Flow-directed Catheters*

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This study was designed to detect biofilm and bacteria on right heart flow-directed catheters using scanning electron microscopy and culture following scraping and dispersion of biofilm by sonication. We examined 20 consecutive catheters removed from 18 critically ill patients, an average of 2.6 days after insertion. On scanning electron microscopy, all catheters were found to be covered by a biofilm, with bacteria visible on 50 percent of them. Cultures of specimens from 40 percent of the catheters grew skin organisms (Staphylococcus warneri, Diphtheroid), anaerobes (Propionibacterium), and other potential pathogens (Proteus vulgaris, Enterobacter cloacaes). Combination of the two techniques produced a bacterial detection rate of 75 percent. This study demonstrates that the presence of biofilm with bacterial adherence is common on right heart flow-directed catheters. The phenomenon could play a significant role in endogenous infection in critically ill patients.

When used judiciously, flow-directed pulmonary artery catheters may be of assistance in the management of critically ill patients; however, the enthusiasm of the 70s and early 80s for invasive central monitoring has been tempered by the recognition of multiple associated complications, such as ventricular arrhythmias, pulmonary artery rupture, pulmonary infarction, and systemic or local infections.1 Using semiquantitative bacterial recovery techniques, previous studies reported pulmonary artery catheter infection rates from 5.6 percent (of 170 catheters)9 to 16 percent (of 37 catheters).3 On postmortem examination, Rowley et al4 found evidence of right-sided infective endocarditis in four of 55 (7 percent) patients previously catheterized.

It is not surprising that these intravascular foreign materials can become colonized with bacteria. Contamination can occur at the time of insertion due to poor aseptic technique with secondary bacterial migration from the skin after catheter placement, from hematogenous spread from distant infected foci, or from infected intravenously administered fluids, tubing or transducers. Once a catheter is colonized it may become a nidus for disseminated infection, particularly in the seriously ill and immunocompromised host.

Scanning electron microscopy (SEM) techniques have demonstrated bacteria in biofilms on the surface of intravascular catheters both in vitro5 and in vivo.6 Recently the phenomenon of bacterial adhesion has been extensively reviewed7 and the resultant bacteria-glycocalyx biofilms have been suggested to play a major role in quiescent or recurrent bacterial infection associated with prostheses.8 This study was designed to examine the incidence of biofilms on flow-directed right heart catheters in an active intensive care unit setting, and to correlate the phenomenon with clinical observations and cultures obtained by a special sonication technique.

METHODS

Patient Selection

Indications for insertion and removal of the pulmonary artery catheters were determined by attending physicians in charge of patients in a closed multisystem failure intensive care unit and a postoperative open heart cardiovascular unit, and conformed to those commonly accepted.9 Twenty consecutive flow-directed right heart catheters from 18 patients were included in the study. Brief clinical descriptions of the patients from whom the catheters were collected are given in Table 1.

Processing of the Catheters

After removal of the catheter dressing, the insertion site was swabbed to obtain a specimen for culture. Blood was drawn through the catheters and placed in Bectec (Becton, Dickinson and Company) culture vials, NR6A and NR7A, for aerobic and anaerobic
Table 1—Clinical Data on 18 Patients From Whom 20 Right Heart Flow-Directed Catheters Were Studied

<table>
<thead>
<tr>
<th>Catheter Placement No.</th>
<th>Clinical Diagnosis*</th>
<th>Days in Situ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mitral valve replacement, anterosetal myocardial infarction, pulmonary edema; S</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Same as catheter 1 (right heart catheterization on two separate occasions)</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Elective CAGB; C</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Elective tricuspid valve replacement</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Elective for unstable angina</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Elective aortic valve replacement complicated by myocardial infarction, cerebrovascular accident, sepsis, ARDS, renal failure; S, C</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>Elective CAGB; C</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>Elective aortic valve replacement and tricuspid anuloplasty, complicated by renal failure and pneumonia; S, C</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>Postoperative bleeding gastric ulcer, perioraoperative myocardial infarction, cerebrovascular accident</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>Same as catheter 6 (right heart catheterization on two separate occasions); S, C</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>Multiple rib fractures complicated by ARDS; S</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>Septic shock from perforated small bowel, pneumonia; S</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>Elective CAGB</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>Upper gastrointestinal bleeding complicated by myocardial infarction, renal failure; S, C</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td>Mitral and tricuspid valve prostheses, surgery for hip fracture</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>CAGB for unstable angina complicated by sternal infection and mediastinitis, myocardial infarction; S</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>Chronic obstructive pulmonary disease, perforated duodenal ulcer, congestive heart failure, renal failure, pneumonia; C</td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td>Elective aortobifemoral graft; C</td>
<td>2</td>
</tr>
<tr>
<td>19</td>
<td>Myocardial infarction complicated by superior mesenteric artery occlusion and bowel resection; S</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>CAGB for unstable angina</td>
<td>3</td>
</tr>
</tbody>
</table>

*Mean: 2.6

**S** = septic, defined as positive culture from distant focus; C = catheter colonization-infection, ie, positive culture from catheter; CAGB = coronary artery bypass graft; ARDS = adult respiratory distress syndrome.

culture. The insertion site was then prepared with a povidone-iodine solution. The flow-directed thermolysis catheters (Swan-Ganz, Edwards laboratories) and their sheaths (American Pharmaseal Company) were removed simultaneously without moving the catheter in relation to its sheath, to reduce the possibility of scraping adherent material from the outer catheter surface. Catheters were divided into four segments (A to D), each 4 cm in length. Segment A was the tip of the catheter, B was at the 20-cm mark on the catheter, C was at the proximal port, and D was the portion of the catheter within the sheath. All segments were divided in two, one part to be used for microbiology studies and the other for SEM.

Microbiology

The following method was used to estimate the number of adherent bacteria on the catheters when they were removed:

1. The inside and the outside of each segment was scraped with a sterile scalpel blade. The blade, scrapings and catheter segment were placed in tubes containing 1.0 ml of nutrient broth.

2. The scrapings were dispersed by vigorous mixing with a Vortex mixer and by gentle ultrasonic disruption in a cooled ultrasonic bath for 30 min.

3. 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 new diluted solution to the next tube to make dilutions ranging from $10^0$ to $10^{-4}$.

4. 0.1 ml of each dilution was spread over the surface of brain-heart infusion agar plates for aerobic cultures and to the same amount sheep blood was added for anaerobic cultures.

5. The inoculation plates were incubated at 37°C for 24 to 72 h.

6. All different colony types were subcultured and identified.

7. Cultures were considered positive if they grew greater than 30 colonies at any of the dilutions: $10^0$, $10^{-1}$ or $10^{-2}$.

Fixation for Electron Microscopy

Solutions were prepared as follows:

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

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

The procedure of fixation for electron microscopy consisted of:

1. Immersion of the catheter segments in the fixative solution for 2 h at room temperature or 24 h at 4°C.

2. Specimens were then washed by removing the fixative solution and replacing it with the ruthenium red-cacodylate buffer, the procedure was repeated three times at ten-minute intervals. Specimens were then processed for SEM or transmission electron microscopy (TEM).

Scanning Electron Microscopy

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 finally “sputter-coated” with gold and palladium. The specimens were examined using a Hitachi S450 (Hitachi Company) scanning electron microscope. Extent of biofilm coverage of the catheter surface was semiquantitatively assessed by examination of ten randomly chosen fields on both the luminal and outer surfaces.

Transmission Electron Microscopy

Adherent material was scraped from a small area (less than 0.5 sq cm) 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 (Electron Microscopy Sciences Company). Sections were cut using an LKB ultramicrotome, stained with uranyl acetate and lead citrate (Electron Microscopy Sciences Company). Sections were then processed for SEM or transmission electron microscope (Hitachi, model H600) at an acceleration voltage of 60 kV.

RESULTS

Over a two-month period, 20 consecutive pulmo-
nary artery catheters were collected and studied: 12 catheters from patients in a general system failure intensive care unit and eight catheters from a postoperative cardiovascular unit.

Clinical Observations

Catheters were removed from one to seven days following insertion depending on the need for right heart hemodynamic monitoring and on the presence of clinically apparent sepsis and suspected catheter infection (Table 1). While nine of the 20 catheters were in place, the patients had infection documented by positive cultures from other sites, whereas the remaining 11 had no evident clinical infection during the time the right heart catheters were in place. All of those patients who had clinical evidence of sepsis received antibiotics. Eight of the 11 catheters were unassociated with apparent infection; for patients from whom these were removed, prophylactic antibiotics were prescribed for perioperative coverage. In the remaining three cases, no antibiotics were used. Therefore, 17 of the catheters were associated with antibiotic coverage.

Sonication/Culture

Eight of the 20 catheters grew a variety of organisms by sonication-culture technique (bacterial detection rate of 40 percent).

In the nine catheter placements in which infection was documented at sites remote from the flow-directed catheter and in which therapeutic antibiotics were used, four catheters grew organisms. The organism cultured from the catheter was the same as that from the distant site of infection in two catheter placements (No. 6—Enterobacter cloacae; No. 10—Proteus vulgaris) and in these catheter placements the organism from the catheter was different from that at the skin site (No. 6—no growth; No. 10—E aerogenes). In the other two catheter placements the organisms cultured from the catheter were the same as those from the skin site (No. 8—Diphtheroids and Staphylococcus warneri; No. 14—Propionibacterium), while the organisms were different at the distant site (No. 8—S aureus from blood, and Streptococcus viridans and microaerophilic Streptococcus from bronchial brushings; No. 14—S aureus and Strep pneumoniae from sputum).

In the 11 catheter placements without clinically apparent infection, four catheters grew organisms. Staphylococcus warneri was cultured from the catheter in all four (No. 3, 7, 17 and 18) but only in No. 3 and 7 was the S warneri grown from both skin site and catheter. A prophylactic antibiotic (Cephalothin) was used in two of these four cases of catheter-positive culture but without clinically apparent infection.

There were six positive cultures from swabs of the skin insertion site. In all six catheter placements, bacteria were detected on the catheters by culture of the scrapings or on SEM. The organisms cultured from the skin insertion sites included Diphtheroid, S warneri, Propionibacterium, Enterococcus and E aerogenes. In the four catheter placements in which the same organism was cultured from the skin site and from the catheter, positive cultures were obtained from catheter segments A and D in two catheter placements and from segments A, B and C in two catheter placements. In the latter two instances, cultures of the catheter sheaths also were positive for the same organism.

Two blood cultures drawn through the catheters before removal were positive (No. 6 and 8). The organisms were E cloacae and P vulgaris in No. 6 and E cloacae in No. 8. In the former situation the same organisms also were cultured from the catheter, sputum and blood taken from another site. In the latter instance, the organisms cultured from the catheter, skin and distant sites were different from the organism at the skin site.

Serial dilutions of the samples of catheter scrapings were variable in their production of bacterial colonies but showed a general trend toward decreasing colony counts with increasing dilution.

Scanning Electron Microscopy

Scanning electron microscopy of the pulmonary artery catheters revealed the presence of biofilms on all, including those four removed within 24 h of insertion. Bacteria were detected by SEM on ten of the 20 catheters producing a 50 percent bacterial detection rate. In contrast, control catheters had clear surfaces prior to their utilization. Figures 1 and 2 are representative scanning electron micrographs of the right heart flow-directed catheters showing the presence of biofilms with embedded bacteria. The smooth areas (arrows) in Figure 1, top, represent the uncoated catheter surface, and the amorphous biofilm can be seen to cover the remainder except where it is interrupted by dehydration cracks. Spherical bacteria are present at the surface of this biofilm and partly buried within it (Fig 1, center). Transmission electron microscopy of a section of ruthenium red-stained preparation from a different catheter shows bacteria embedded in an amorphous matrix (Fig 1, bottom). Figure 2, top, demonstrates another typical biofilm containing spherical bacterial cells at or near the surface, while Figure 2, bottom, shows a continuous biofilm with host blood cells on the surface.

An average of 40 percent of the inner surface of the catheters was covered by biofilm while comparatively more of the outer surfaces (75 percent) were biofilm-coated (Table 2). There was no significant difference in the amount of biofilm on the inner surface of catheter segments A to D. Segments A, B and C had 77, 81, and 78 percent of the outer surface covered with biofilm.
Segment D, the segment closest to the skin and within the sheath, had somewhat less biofilm coverage (64 percent). As shown in Table 2, biofilms were present on the catheters removed early after insertion and the surface area covered did not appear to increase significantly with increasing duration of dwelling time.

Table 2—Duration Catheter Indwelling vs Percentage of Catheter Surface Covered With Biofilm

<table>
<thead>
<tr>
<th>Days in Situ</th>
<th>No. Catheters</th>
<th>Inner Surface, %</th>
<th>Outer Surface, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>51</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>42</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>36</td>
<td>63</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>55</td>
<td>86</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>70</td>
<td>75</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>Means: 40</td>
<td>75</td>
</tr>
</tbody>
</table>

**Combined SEM and Culture**

The detection of bacteria by sonication-culture or by SEM was eight out of nine in the catheter placements with documented infection at other sites and seven out of 11 in the catheter placements with no clinically apparent infection. Using the combination of sonication-culture and SEM, bacteria were detected on 15 of the 20 catheters (75 percent rate of bacterial detection). The following tabulation gives the breakdown of bacterial detection by culture or by SEM:

- Bacterial detection by culture or on SEM in cases of infection documented by positive cultures from other sites. All nine cases were under therapeutic antibiotic coverage.
  - SEM positive, culture negative: four
  - SEM positive, culture positive: one
  - SEM negative, culture negative: one
  - SEM negative, culture positive: three

Bacterial detection by culture or on SEM in cases...
Figure 2. Top, low magnification SEM of outer surface of segment B of catheter from same patient illustrated in Figure 1, top and center. Note presence of bacterial cells of several morphological types at surface of biofilm or partially buried (arrows) in its amorphous matrix (bar = 5 μm). Bottom, very low magnification SEM of outer surface of catheter that had been in situ for four days. This thick biofilm is traversed by numerous cracks as a consequence of dehydration and crenated host blood cells are observed on its surface (bar = 5 μm).

without clinically apparent infection. Eight of eleven cases received perioperative prophylactic antibiotic coverage.

- SEM positive, culture negative: three
- SEM positive, culture positive: two
- SEM negative, culture negative: four
- SEM negative, culture positive: two

The next tabulation shows the rate of bacterial detection by culture or by SEM in relation to the dwelling time of the catheter:

<table>
<thead>
<tr>
<th>Days in situ</th>
<th>Rate of bacterial detection by culture or on SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>one of four</td>
</tr>
<tr>
<td>2</td>
<td>eight of nine</td>
</tr>
<tr>
<td>3</td>
<td>two of three</td>
</tr>
<tr>
<td>4</td>
<td>two of two</td>
</tr>
<tr>
<td>5</td>
<td>one of one</td>
</tr>
<tr>
<td>7</td>
<td>one of one</td>
</tr>
</tbody>
</table>

Total: 15 of 20

Although the number of catheters for each dwelling time was small, there was bacterial detection for all catheters in place for four or more days.

Discussion

Right heart flow-directed catheters have been used extensively and perhaps abused in recent years. The potential for abuse becomes more evident as increasing numbers of complications are recognized and benefits to patient outcome questioned. We have focused our attention on the phenomenon of biofilm production on right heart flow-directed catheter surfaces and on the problem of bacterial detection within this coagulum using SEM and sonication-culture, techniques that have not previously been applied to right heart flow-directed catheters separately or in combination.

Biofilm was present on all catheters examined and
was evident as early as 24 h after insertion. The biofilm covered a large percentage of both inner and outer surfaces of the catheters. Several studies have demonstrated this almost universal phenomenon of bacterial-biofilm growth on any foreign material exposed to human tissues: pacemakers, intrauterine contraceptive prostheses, and endotracheal tubes. Bacteria utilize their capacity for adherence and coating as a strategy to survive in potentially hostile natural (sloughs), industrial (oil pipelines) and medical (dental plaque) environments. Microorganisms produce a glycocalyx matrix that allows them to adhere to available surfaces forming a biofilm that binds together microcolonies of bacteria within a fibrous exopolysaccharide matrix. This protective biofilm permits the exchange of nutrient molecules while isolating the bacteria from phagocytic cells, antibodies and antibiotics. An example of this phenomenon has recently been described in which Pseudomonas aeruginosa, growing in a biofilm on a urinary catheter, was demonstrated to be resistant to tobramycin despite the free-floating bacteria being susceptible to much lower concentrations of the antibiotic. The concept of biofilm providing a protected environment for bacteria is supported by our ability to detect viable bacteria by the sonication-culture technique despite the presence of antibiotic coverage.

To break up the biofilm, we used the sonication technique for aerobic and anaerobic cultures, resulting in eight positive cultures in 20 catheters (40 percent). This represents both colonization or infection, or both, of these catheters, infection being defined as positive catheter tip culture plus positive blood culture. By this definition, catheter placement No. 6 was considered to be infected, the others being colonized. Initial studies using cultures of catheter tips in broth reported rates of 19 to 30 percent of catheter infection-contamination. Using the semiquantitative method of Maki et al to differentiate infection from contamination, Myers et al reported an incidence of 5.8 percent of positive catheter tips cultured. Although the semiquantitative technique of catheter tip culture and the sonication technique have not been systematically compared, it seems likely that sonication will recover bacteria buried in the biofilm that would not have otherwise been recovered by simply rolling the catheter segment on an agar plate. Similarly, Gram-staining of the catheter tip would be anticipated to only identify surface bacteria.

We isolated Propionibacterium in anaerobic culture from three catheters. Although the clinical significance of this organism is unclear, it emphasizes the necessity of obtaining both aerobic and anaerobic cultures. The sonication technique has allowed a higher recovery rate of positive anaerobic cultures than previously reported.

The low incidence of culture positivity from "drawback" blood cultures in this study calls into question the value of this common clinical sampling technique.

Although bacterial contamination of intravascular catheters may be presumed to be definitively detected by culture, we found that bacterial detection by SEM was the more productive of the two techniques. The discrepancy between bacterial detection by SEM and sonication-culture, as shown in the first of two tabulations mentioned earlier, is likely related to at least four factors, including (1) the inability of SEM to distinguish between living and dead bacteria, (2) the effect of antibiotics on culture techniques, (3) the patchy nature of the biofilm phenomenon with consequent sampling variability, and (4) the fact that bacteria are commonly buried deep within the biofilm and therefore are not visible on the surface.

The high recovery rate (75 percent) of bacteria using both sonication for microbiologic studies and SEM indicates that colonization of intravascular catheters is more commonplace than previously realized. What is more worrisome is that all patients are at risk of catheter-related infection whether or not they have active infection and despite the assumption of protection offered by the presence of antibiotics. We have shown that most right heart flow-directed catheters become invested with biofilms. The clinical impact of this universal phenomenon will depend on the host-foreign body interaction. We may speculate on the potential role of pulmonary artery catheters covered with biofilms as a reservoir of bacteria awaiting a break in defense mechanisms to invade the host. The precise role, if any, of these organisms in the pathogenesis of disease in critically ill patients remains to be elucidated. In this study, the incidence of clinically apparent catheter sepsis was low compared with the incidence of catheter colonization as detected by combined SEM and sonication-culture. It is clear, however, that the biofilm phenomenon provides a protected environment in which bacteria may thrive. In seriously ill, immunologically compromised hosts, concern over intravascular catheters as a focus for bacterial hematologic seeding must be raised. The purpose of reporting this study is to raise the flag of awareness and to reemphasize the importance of considering the risk-benefit ratio to the individual patient before using invasive monitoring routinely in the intensive care unit. We recommend more critically selective use of pulmonary artery catheters with the suspicion that identifying catheter biofilm on SEM may only be the tip of the iceberg.

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