Irrigation-Aspiration for Culturing Draining Decubitus Ulcers: Correlation of Bacteriological Findings with a Clinical Inflammatory Scoring Index

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Biopsy of infected decubitus ulcers for culture disrupts tissues and may disseminate infection. Antimicrobial prophylaxis to prevent dissemination of infection may adversely affect biopsy culture results. Irrigation-aspiration to obtain submarginal specimens from draining decubitus ulcers was studied as an atraumatic, noninvasive culturing technique to serve as an alternative to biopsy in research activities. Two aspirates were obtained serially from 32 subjects; in 12 subjects, biopsies were also performed immediately. A median of 4.5 bacterial species was recovered per ulcer by irrigation-aspiration. Recent antimicrobial treatment had no evident effect on the recovery of bacterial species in general or, specifically, on the recovery of Bacteroides species. Concordance of results for both aspirates was 97.6% for aerobes and 91.8% for anaerobes, indicating no interactive methodological effect of the first irrigation-aspiration on the second. Compared with biopsy isolates for one aspirate, the sensitivity was 93% and the specificity was 99.0%; for another aspirate, the sensitivity was 94.7% and the specificity was 99.5%. The positive predictive value for either aspirate was ≥93.9%. A weighted clinical index to score inflammatory ulcer characteristics was devised (score range, 0 to 15). In the absence of anaerobes in 15 subjects, the mean score was 6.1 ± 3.5; in the presence of anaerobes in 17 subjects, the mean score was 9.4 ± 3.2 (P = 0.008). The presence of aerobic gram-positive or gram-negative species did not significantly affect scores. Irrigation-aspiration for culture and clinical scoring of inflammation should permit independent serial measures of bacteriological and clinical courses of draining decubitus ulcers without patient risk or discomfort.

The generally accepted method for culturing draining decubitus ulcers is by biopsy or percutaneous needle aspiration (11, 16, 20). National authorities use results of needle aspiration or biopsy cultures of decubitus ulcers in the definition of decubitus ulcer infection (8). However, the efficacy of needle aspiration to obtain a specimen for culture from the central or leading edges of lesions found in a variety of inflammatory skin conditions, including cellulitis complicating decubitus ulcers, is controversial; needle aspiration is not necessarily a reliable method of recovering pathogenic bacteria (14). Moreover, in the presence of active infection, an invasive method of obtaining specimens from decubitus ulcers for culture is a potentially dangerous procedure. For an individual with an infected decubitus ulcer, adverse consequences of the trauma of surgical biopsy may include direct extension of infection. Surgical manipulation of necrotic ulcers is also regarded as being likely to induce bacteremia; in fact, antimicrobial therapy to prevent endocarditis is recommended under these circumstances (1). Bacteremia arising from infected decubitus ulcers is a well-described complication (4, 5, 7, 13). Thus, when done for research purposes, biopsy of decubitus ulcers for culture poses potential risks and raises important ethical issues. Conversely, use of antimicrobial prophylaxis to promote safety may adversely alter bacteriological findings. A safe, nontraumatic method for culturing draining decubitus ulcers to obtain a representative sample of bacteria from infected tissues would be highly desirable as an aid to bacteriological investigations of decubitus ulcers. Hence, a study was undertaken to explore the efficacy of recovering bacteria from beneath the margins of draining decubitus ulcers by a different method: gentle irrigation and aspiration of saline under the ulcer borders, which is an atraumatic noninvasive procedure.

In the present study, the irrigation-aspiration technique was compared with the surgical biopsy method for obtaining bacterial cultures of draining decubitus ulcers, to determine the sensitivity, specificity, and predictive values of the new method. Two irrigation-aspirations were done sequentially to seek a possible enhancing interaction of the first procedure on the second. The presence or absence of specific bacterial species was sought in order to make comparisons with reported findings of biopsied ulcers. Since the growth, survival, and virulence of aerobic and facultative bacterial species in mixed aerobic-anaerobic infections appear to be promoted by the simultaneous presence of anaerobic bacteria, especially Bacteroides species (3, 9, 15, 16), particular attention was paid to the recovery of anaerobes.

To provide a quantitative clinical description of draining decubitus ulcers for comparison of the extent of ulcer inflammation with the bacteriological findings, a scoring index was devised. The index was weighted to emphasize manifestations of inflammation.

Specimens were obtained from subjects living in nursing homes in the Miami area. Debridements and surgical biopsies were ordered by attending physicians as part of usual patient care activities. A total of 30 individuals whose cultures yielded bacterial growth was sought.

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MATERIALS AND METHODS

Subjects: entry criteria. Persons were selected for irrigation-aspiration cultures on the basis of having a draining decubitus ulcer and a willingness to participate in the study. In persons with more than one decubitus ulcer, a single ulcer was arbitrarily chosen for culture, based on its accessibility. No one from whom an irrigation-aspiration specimen for culture was obtained was excluded from analysis.

Clinical descriptions of ulcers: weighted inflammatory scoring index. Ulcers were inspected and clinical findings were recorded at the time of culture and prior to biopsy. Clinical assessments included amount and character of drainage, extent of cellulitis, presence of foul odor, and presence of any necrosis in ulcer margins or bases. Findings were scored as follows: drainage on dressing only, 0; minimal, moderate, and copious, drainage in ulcer bed, 1, 2, and 4, respectively; serous, sanguinous, sanguineo-purulent, and purulent, drainage, 0, 1, 3, and 4, respectively; widest cellulitis width of <1 cm, ≥1 to <2 cm, ≥2 to <3 cm, ≥3 to <4 cm, and ≥4 cm, 0, 1, 2, 3, and 4, respectively; no foul odor, 0; foul odor, 1; no necrosis, 0; necrosis in ulcer base, 1; necrosis in ulcer margin, 1; and necrosis in both base and margin, 2. The total score of an individual ulcer was the sum of the scores described above and ranged from 0 to 15.

Culturing techniques. To obtain a specimen from an ulcer by irrigation-aspiration to be used for culturing, at least 1 ml of sterile 0.085% NaCl without preservative was aseptically irrigated under the ulcer margin with a needleless 1- or 5-ml sterile syringe. The syringe tip was placed beneath the disrupted skin margin. Gentle irrigation was done in four submarginal sites approximately 45 degrees apart. Fluid was irrigated to the limit of tissue resistance without inducing subject discomfort. Excess fluid was removed with a sterile gauze pad. The ulcer margins were gently massaged circumferentially with a sterile cotton swab. By using a fresh syringe, a second irrigation of at least 1 ml of saline was applied under the ulcer margins as described above, and the ulcer was again massaged. At least 0.25 ml of fluid was then aspirated and placed into a Port-A-Cul vial (BBL Microbiology Systems) for aerobic and anaerobic cultures. Specimens were transported to the laboratory for plating within 2 h. For duplicate specimens, the full irrigation-aspiration procedure was repeated in an identical manner between 30 min and 2 h after the first one. Cultures were processed as follows. For aerobic cultures, the aspirate was plated onto the following media: Trypticase soy agar (BBL) with 5% sheep blood, MacConkey agar, and phenylethyl alcohol agar with 5% sheep blood. For anaerobic cultures, the aspirate was plated onto CDC anaerobic blood agar, CDC anaerobic kanamycin-vancomycin laked blood agar, and CDC anaerobic blood agar with phenylethyl alcohol. In addition, anaerobic specimens were inoculated into 8 ml of enriched thioglycolate broth supplemented with vitamin K and hemin.

Biopsies were done at the patient's bedside by an attending surgeon. Biopsy specimens were obtained on the same day as the aspiration-irrigations were done, immediately after both aspirations were recovered, and prior to administration of any antimicrobial drug ordered in conjunction with the biopsy. Biopsied tissues were transported to the laboratory in an icebox and plated within 2 h. The tissue specimens were ground with a disposable tissue grinder, and a representative sample was removed and processed for recovery of aerobic and anaerobic bacteria in the same manner as described above for the aspirates.

Aerobic culture plates were incubated at 36°C in 5% CO₂ for 48 h. The anaerobic medium was placed into an anaerobic GasPak jar (BBL) and incubated at 37°C for 48 h prior to examination. The anaerobic medium was reincubated and observed thereafter for a total of 7 days. Identification and determination of aerobic and anaerobic bacteria to the species level were done by standard techniques (12). For aerobic gram-positive bacteria (AGPB), identification was based on conventional morphological and biochemical methods, for aerobic gram-negative bacteria (AGNB) the API 20E system (Analytab Products) was used, and for anaerobes the Minitek Anaerobe II system (BBL) and the API An-Ident system (Analytab Products) were used. Susceptibilities of isolates to antibiotics were determined by the broth microdilution susceptibility method by using standard interpretive criteria for MICs (10, 19). Frozen microdilution trays (Prepared Media Laboratories) were used for susceptibility testing. Bacterial isolates characterized to either the species or the genus level were biotyped by microscopic and colonial morphology along with biochemical and hemolytic reactions. These findings and the antibiograms were used to determine the identities of isolates recovered from the same subject.

Postirrigation-aspiration clinical evaluation. Patients' ulcers were inspected and their medical records were reviewed weekly for a period of 3 weeks after irrigation-aspiration to seek indications of new local or systemic manifestations to suggest dissemination of infection; indications included orders for new bacteriological studies or antimicrobial treatments possibly related to new decubitus ulcer infection.

Data analysis. Student's t test was used for statistical analysis. To determine the sensitivity of the irrigation-aspiration method, the bacterial species recovered in each biopsy specimen were compared with those found in corresponding aspirates. To determine specificity, bacterial isolates recovered from biopsy specimens of other patients but absent from the biopsy specimen of the patient under study were compared with those in the corresponding aspirates.

RESULTS

Characteristics of study subjects. Specimens for culture were obtained from 32 patients with draining decubitus ulcers. Their mean age was 82 ± 5.8 years. There were 9 men and 23 women. Sixteen subjects had received no systemic antimicrobial therapy within 4 weeks prior to culture, and 16 had received some antimicrobial therapy within 24 h before a specimen for culture was obtained. None of the patients had received local antibiotic therapy during the 24 h before a specimen for culture was obtained. In nine of the patients who had received antimicrobial therapy, the drugs included agents capable of inhibiting growth of Bacteroides species (amoxicillin-clavulinate, cefoxitin, clindamycin, piperacillin, ticarcillin-clavulanate). The extents of the ulcers according to a standard anatomic characterization (18) were class II-9, class III-16, and class IV-7. The body distributions were as follows: foot (including heel), 9 patients; hip, 11 patients; sacrum, 12 patients. In 5 ulcers drainage was serous, in 3 ulcers it was sanguineous, in 11 ulcers it was sanguineo-purulent, and in 13 ulcers it was purulent. In 12 patients biopsies were done; none of the patients required preoperative antimicrobial prophylaxis for endocarditis.

Characteristics of bacterial isolates. Bacteria were recovered in both aspirates from 30 patients and in all patients who had biopsies. In two subjects, neither aspirate yielded organisms. Mixed aerobic-anaerobic bacterial flora were
present in 17 patients, and aerobic flora alone were present in 13 patients. The number of individual bacterial species recovered from an ulcer ranged from 0 to 10, with a median of 4.5. Recovery of any bacterial species from aspirates of either recently treated or untreated patients was similar (Table 1). The frequency of recovery of Bacteroides species among patients who had or had no received antimicrobial therapy that could have inhibited these bacteria was also similar.

**Concordance between first and second aspirate cultures.** Comparison of findings of both aspirates revealed an overall concordance of aerobic bacterial species of 83 of 85 (97.6%) isolates. For groups of AGPB isolates there was concordance in 46 of 48 (95.8%) aspirate pairs (Table 2). In one instance each, an enterococcal or staphylococcal isolate was recovered from only one aspirate. For AGNB isolates, there was concordance in all 37 pairs (100%). For anaerobic species, there was an overall concordance of 45 of 49 pairs (91.8%). In three instances a peptostreptococcal isolate was present in one aspirate; in one instance a Bacteroides species was present in one aspirate. For anaerobic gram-positive bacteria (AAGPB) isolates there was concordance in 27 of 30 (90%) aspirate pairs, and for anaerobic gram-negative bacteria (AAGNB) isolates there was concordance in 18 of 19 (94.7%) pairs.

**Comparison of biopsy and irrigation-aspiration cultures.** Results of biopsy cultures were compared with the findings from both irrigation-aspiration cultures to determine the sensitivity and specificity and the positive and negative predictive values (Table 3). For AGPB in aspirate 1 there was concordance of 15 of 19 isolates present in biopsy cultures (sensitivity, 78.9%) and concordance of 39 of 41 isolates absent from biopsy cultures (specificity, 95.1%). For AGPB in aspirate 2, there was concordance of 16 of 19 isolates present in biopsy cultures (sensitivity, 84.2%) and concordance of 40 of 41 AGPB isolates absent from biopsy cultures (specificity, 97.6%). In aspirate 1 one isolate each of corynebacterial, enterococcal, and staphylococcal species and Staphylococcus aureus that was recovered in the biopsy cultures of three subjects was absent, and one isolate each of enterococcal and staphylococcal species that was not found in biopsy cultures of two subjects was present. In aspirate 2 one isolate each of corynebacterium and enterococcal species and S. aureus that was recovered in biopsy cultures of three subjects was absent; these isolates were not present in aspirate 1 from these same three subjects. In aspirate 2, one isolate of staphylococcal species that was not recovered in a biopsy specimen was present; for the individual who yielded that isolate, the isolate was also present in aspirate 1.

**For AGNB there was complete concordance of both aspirate cultures for the 16 isolates found in biopsy cultures (sensitivity, 100%) and the absence of the 48 isolates that were not present in biopsy cultures (specificity, 100%). For anaerobes there was complete concordance of both aspirate cultures with biopsy cultures for the 15 AAGPB isolates that were present in the biopsy cultures and the 57 AAGPB isolates that were absent (sensitivity, 100%; specificity, 100%) and for the 7 AAGNB isolates that were present and the 41 AAGNB isolates that were absent (sensitivity, 100%; specificity, 100%). Positive predictive values were ≥93.9%**
TABLE 3. Sensitivity, specificity, and predictive values of first and second irrigation-aspiration cultures of draining decubitus ulcers compared with biopsy cultures

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No. of isolates on biopsy culture</th>
<th>First irrigation-aspiration culture</th>
<th>Second irrigation-aspiration culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Aerobic bacteria&lt;sup&gt;b&lt;/sup&gt; Aspirate culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>31</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>Absent</td>
<td>4</td>
<td>107</td>
<td>3</td>
</tr>
<tr>
<td>Anaerobic bacteria Aspirate culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>22</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
<td>98</td>
<td>0</td>
</tr>
<tr>
<td>All bacteria Aspirate culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>53</td>
<td>2</td>
<td>54</td>
</tr>
<tr>
<td>Absent</td>
<td>4</td>
<td>205</td>
<td>3</td>
</tr>
</tbody>
</table>

<sup>a</sup> The sensitivities, specificities, positive predictive values, and negative predictive values in the first and second irrigation cultures were 98.6, 98.2, 93.8, and 94.4% and 91.4, 99.1, 97.0, and 97.3%, respectively, for aerobic bacteria; 100% for all calculations for anaerobic bacteria; and 93, 99, 96.4, and 98.15% and 94.7, 99.5, 98.2, and 98.6%, respectively, for all bacteria.

<sup>b</sup> Includes facultative anaerobic species.

and negative predictive values were ≥96.4% for either aspirate.

The results obtained from aspirate 2 were arbitrarily selected for correlation with the weighted clinical score. Comparisons of the means of the weighted inflammatory scores of draining ulcers by the presence or absence of various bacterial groups are given in Table 4. There appeared to be an association between the score and the presence of anaerobes in the aspirate, as manifested by a significantly greater score when either AAGPB or AAGNB species were found. In the absence of either group of anaerobes, with a total possible score of 15, the mean score was 6.1 ± 3.5; in the presence of either group of anaerobes the mean score was 9.4 ± 3.2 (P = 0.008). The presence of AGBP or AGBN isolates, by themselves, did not appear to influence inflammatory scores significantly.

Postirrigation-aspiration culture evaluation. In the 20 subjects who were not surgically treated, the 3-week follow-up observations after irrigation-aspiration revealed no clinical, diagnostic, or therapeutic finding to suggest direct extension of infection at the ulcer site or the occurrence of bacteremia.

**DISCUSSION**

In this study, a submarginal irrigation-aspiration method to obtain aerobic and anaerobic bacteria from draining decubitus ulcers for culture provided bacteriological results in two aspirates that were similar to each other, with 97.6% concordance for the recovery of aerobes and 91.8% concordance for the recovery of anaerobes. These findings did not indicate that the first irrigation-aspiration induced bacterial contamination of the second to any great extent or that the bacterial yield in the second irrigation-aspiration was substantially enhanced because of some effect of the first. The bacteriological results of both irrigation-aspirations were comparable to results of cultures of tissues obtained by biopsy from 12 patients. For the first aspirate, by using biopsy cultures as the standard, the overall sensitivity was 93.0% and the specificity was 99.0%; for the second aspirate, the overall sensitivity was 94.7% and the specificity was 99.5%. The positive predictive value for either aspirate was ≥93.9%; the negative predictive value was ≥96.4%.

A different number and variety of bacterial species have been reported to be present in biopsy cultures of decubitus ulcers as compared with swab cultures of the ulcers' surfaces (11, 16). Results of biopsy cultures are generally considered to be more meaningful than swab culture results, and the former have been taken as a standard for definition of decubitus ulcer infection by national authorities (8). The potential risks of biopsy to obtain specimens for culture in the presence of active decubitus ulcer infection or necrosis and the potential limitations of biopsies in serial studies because of ulcer trauma, however, have not been addressed as concerns by advocates of biopsy culture. In contrast to biopsy, the irrigation-aspiration culture method caused no tissue disruption. Furthermore, irrigation-aspiration did not result in any clinical outcomes suggesting dissemination of infection. As a noninvasive atraumatic procedure, this method has no inherent risk of dissemination of infection or causation of discomfort and thus is appropriate for research purposes.

There was 100% sensitivity and 100% specificity for AGBN species and for all anaerobic isolates for the irrigation-aspiration culture method in comparison with the biopsy findings. However, for recovery of the AGBP group of isolates, the sensitivities were 78.9% for aspirate 1 and 84.2% for aspirate 2; the specificities were 95.1 and 97.6%, respectively. Some AGBP isolates present in an individual's biopsy specimen were absent from both aspirate cultures; this may indicate that these AGBP isolates were present in such diminished numbers in the periphery of the ulcer that they were not represented in the submarginal areas. This is consonant with the observation of variability in culture results obtained with biopsy specimens from both peripheral and central areas of the same ulcer (16). On the other hand, the AGBLN isolates recovered from a biopsy specimen but not found in both aspirates may have reflected coloniza-

**TABLE 4. Comparisons of mean weighted inflammatory index scores of draining decubitus ulcers in relation to recovery of various bacterial groups in irrigation-aspiration culture**

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>No. of decubitus ulcers</th>
<th>Mean ± SD decubitus inflammatory index score&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGBP Absent</td>
<td>13</td>
<td>6.3 ± 3.6</td>
</tr>
<tr>
<td>AGBP Present</td>
<td>19</td>
<td>8.8 ± 3.4</td>
</tr>
<tr>
<td>AGBN Absent</td>
<td>4</td>
<td>6.0 ± 4.3</td>
</tr>
<tr>
<td>AGBN Present</td>
<td>28</td>
<td>8.0 ± 3.5</td>
</tr>
<tr>
<td>AAGPB Absent</td>
<td>18</td>
<td>6.3 ± 3.2</td>
</tr>
<tr>
<td>AAGPB Present</td>
<td>14</td>
<td>9.7 ± 3.4</td>
</tr>
<tr>
<td>AAGNB Absent</td>
<td>19</td>
<td>6.2 ± 3.5</td>
</tr>
<tr>
<td>AAGNB Present</td>
<td>13</td>
<td>10.1 ± 2.6</td>
</tr>
<tr>
<td>Total anaerobes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>15</td>
<td>6.1 ± 3.5</td>
</tr>
<tr>
<td>Present</td>
<td>17</td>
<td>9.4 ± 3.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Weighted inflammatory score derivations are defined in the text.

<sup>b</sup> Significance of difference in scores of ulcers when anaerobic bacterial species were absent or present by Student's t test (two-tail; P < 0.01). Differences of scores of ulcers when aerobic bacterial species were absent or present were not statistically significant.
tion isolates that were present on the biopsied surface of the ulcer but not in deeper tissues or the submarginal areas. By the same token, an AGPB isolate found in both irrigation-aspirates in one person but not in the biopsy culture may reflect either variability in bacterial distribution in infected tissues or, alternatively, submarginal colonization.

Systemic antimicrobial therapy within 24 h of obtaining the specimens, including treatment that might have inhibited growth of Bacteroides species, did not appear to diminish recovery of aerobes or anaerobes (Table 1). This is not necessarily an unexpected finding. In one study, cefazolin was not detected in the debrided tissues of decubitus ulcers (2). The clinical implications of these findings remain to be explored.

The variety and number of isolates of individual bacterial species that were present in the aspirate cultures were similar to the variety and number of species of bacteria reported in blood cultures of patients with infected decubitus ulcers (4, 5, 7, 13). This indicates that irrigation-aspiration cultures yielded the bacterial species with a true potential for tissue invasion.

A noteworthy finding of results of invasive methods for obtaining bacterial specimens from decubitus ulcers for culture has been the recovery of a wide range of aerobic and anaerobic species (11, 16). As the healing process occurs, there is a progressive decrease in the number of aerobic and anaerobic species found in biopsied tissues (16). Thus, the number of isolated species is an important comparison measure for irrigation-aspiration and invasive methods. The median recovery of 4.5 bacterial species per ulcer found by irrigation-aspiration is in keeping with reported results of invasive methods, in which means of 4.8 to 5.8 species per biopsied ulcer have been described (16, 17).

A scoring index, which was weighted to magnify manifestations of inflammation, was used to quantify clinical observations of inflammation in the draining ulcers. There was a direct correlation with the score and the presence of anaerobes in aspirate 2, as manifested by a significantly greater score when anaerobes were present. Recovery of anaerobic isolates in a variety of infections correlates with an active or enhanced inflammatory process (3, 15, 16); as described previously (16) anaerobes virtually disappear from decubitus ulcers during the healing process. Of a total possible score of 15, the mean ulcer score in the absence of anaerobic species was 6.1 ± 3.5; in the presence of anaerobes, the mean score was 9.4 ± 3.2 (P = 0.008). The presence of either AGPB or AGNB species, by contrast, did not appear to influence scores significantly. However, in view of the small number of ulcers that did not yield AGNB, a type II error is possible. The weighted scoring method offers a more precise means by which decubitus ulcer inflammation may be defined than does the generally used qualitative clinical description.

As others have pointed out, the microbiology of infected decubitus ulcers has not yet been fully studied (16). The irrigation-aspiration culturing method, along with the inflammatory scoring index, may serve to provide meaningful serial observations in experimental therapeutic studies of both bacteriological and clinical findings of draining decubitus ulcers.

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LITERATURE CITED