Laboratory Indices of Clinical Peritonitis: Total Leukocyte Count, Microscopy, and Microbiologic Culture of Peritoneal Dialysis Effluent

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Total leukocyte count, microscopy, and conventional bacteriologic culture (10-ml sediment) of dialysis effluent were assessed for their ability to detect peritonitis in patients on peritoneal dialysis. A total of 73 patients were surveyed over a 17-month period. Laboratory findings included an examination of 1,774 dialysate samples and culture results from blood, wounds, indwelling catheters, and other specimens. Of 90 peritonitis events, 72 were culture positive. Gram-stained films were positive in no more than 14% of the dialysates collected during periods of clinical peritonitis. Factors which adversely affected the microscopic or cultural detection of microorganisms in effluent included the concentration of organisms in dialysate, antibiotic therapy, and growth medium used. Seeding of the peritoneum with organisms originating from other sites of infection or colonization was documented, although infrequent, yet bacteremia secondary to peritonitis was not seen. Because of the frequent isolation of microorganisms from dialysates in the absence of clinical peritonitis, culture-positive findings were a poor predictor of peritonitis without other evidence of infection. Detection of peritonitis by total leukocyte count (without a differential count) of dialysate specimens was adversely affected by the overlap in cell counts between dialysates collected either during or in the absence of peritonitis. This was attributed in part to nonspecific increases in dialysate cell count in the absence of peritonitis and was associated with intermittent dialysis and extraperitoneal infection.

Peritoneal dialysis is used as short-term therapy for acute renal failure (19) and as long-term treatment for end-stage renal disease (15); however, a frequent complication of peritoneal dialysis is peritonitis (21, 22, 27, 29). The diagnosis and effective treatment of peritonitis depends upon clinical evaluation of the patient and correlation with examination of the dialysate. The latter routinely includes the determination of total leukocyte count of the dialysate and the recovery and identification of microorganisms from dialysis effluent. Previous investigations have demonstrated problems associated with the diagnosis of peritonitis based solely on these parameters (5, 18, 22). Various techniques have been used to facilitate the recovery of microorganisms from dialysate, such as the use of selected broth media (13, 22, 23, 30), the processing of large volumes of dialysis effluent by concentration techniques (22, 28) or total volume (dialysis bag) culture (4), chemical or physical disruption of phagocytes in dialysate sediment for recovery of sequestered organisms (26), and provisions for the removal of antibiotics from the dialysate (28, 30).

Dialysate specimens from patients seen at the Erie County Medical Center were submitted to the clinical microbiology laboratory for determination of total leukocyte count, microscopic examination, and bacteriologic culture of dialysate sediment (18). The efficacy of each dialysate parameter to detect peritonitis was assessed among dialysis patients according to the type of dialysis performed and the infectious status of the patient (e.g., extraperitoneal infections), which included culture results for other specimens, i.e., blood, lines and catheters, and wound sites. An attempt was made to discern those factors which might adversely affect the ability of each parameter to predict peritonitis in peritoneal dialysis patients. We report our findings on the use of total cell count determination, microscopy, and bacteriologic culture of dialysate effluent; we describe those factors which contribute to abnormal dialysate findings.

MATERIALS AND METHODS

A total of 73 patients on peritoneal dialysis were surveyed over a 17-month period. They included 13 patients on temporary dialysis for acute renal failure (5 men and 8 women, of whom 6 were diabetics), and 60 patients on long-term therapy for end-stage renal disease (28 men and 32 women, of whom 19 were diabetics). Total time on dialysis was 551 patient-months. The dialysis routine consisted of four dialysis exchanges every 24 h (continuous ambulatory peritoneal dialysis) or intermittent dialysis, which was performed twice weekly for 20 or 24 h or once a week for 40 to 50 h. During treatment for peritonitis, patients normally on an intermittent schedule were dialyzed on a daily basis.

Definitions. The status of each patient was assessed on the basis of various clinical and laboratory findings and was categorized as follows: clinical peritonitis (cloudy dialysate with or without abdominal pain, plus or minus rebound tenderness, or diffuse abdominal pain with rebound tenderness), peritonitis event (minimum period encompassing clinical or laboratory evidence of peritonitis [a reoccurrence of clinical peritonitis associated with recovery of the same microorganism or microorganisms from dialysate represented a separate peritonitis event]), extraperitoneal infection (evidence of infection other than clinical peritonitis),
and no infection (no evidence of clinical peritonitis or other infection).

**Specimen collection and processing.** A total of 1,959 dialysate samples were collected from patients during the 17-month survey period as part of routine monitoring or during periods of suspected peritonitis. Total leukocyte counts and culture results were available from 1,774 of the 1,959 dialysates. These 1,774 specimens were included in the present evaluation. At least 10 ml of dialysate was received by the laboratory. A sample was removed for determination of total leukocyte count with a hemacytometer. The remaining fluid was handled as previously described (18). The dialysate was centrifuged, and the sediment was used for gram-stained film and inoculation of the following media: chocolate and 5% sheep blood tryptic soy agar (Scott Laboratories, Inc., Fiskeville, R.I.), MacConkey agar, thioglycolate broth, and 5% human blood agar containing vitamin K, yeast extract, and hemin for anaerobic growth, which were prepared in-house.

Additional information was obtained from other sources and specimens submitted for culture. These included indwelling venous and other catheters (in thioglycolate broth), blood (radiometric detection of bacterial growth in aerobic, hypertonic, and anaerobic blood culture media; BACTEC [Johnston Laboratories, Inc., Towson, Md.]), and wounds, including peritoneal catheter (exit-tunnel) site swab samples (microscopy and bacteriologic culture done as for dialysis effluent; with inclusion of phenylethyl alcohol blood agar [Scott Laboratories, Inc.]).

All cultures were incubated and examined daily for 7 days. Identification of isolates was determined by standard methods.

**Dialysate isolates.** Organisms recovered from dialysis effluent were categorized as peritonitis related (isolates recovered from dialysate during clinical peritonitis or isolates recovered from dialysate just before or immediately after peritonitis and identical to organisms recovered from dialysate during peritonitis) or peritonitis unrelated (organisms recovered from dialysate, not associated with clinical peritonitis, with or without concomitant recovery from other body sites or indwelling lines and catheters).

**Statistical analyses.** When appropriate, data were analyzed by chi-square analysis (2).

**RESULTS**

During the 17-month period, there were 90 events of clinical peritonitis. A total of 72 events were culture positive, of which 71 were bacterial in origin. One event was associated with the recovery of *Candida tropicalis* from dialysis effluent. Eighteen of these peritonitis events were culture negative.

Laboratory findings for all dialysate specimens are summarized in Table 1. Specimens are listed according to the presence or absence of peritonitis, total leukocyte count, and antimicrobial therapy. No more than 14% of dialysates collected during clinical peritonitis were positive by Gram stain. In the absence of peritonitis, 8 of 1,255 dialysates were smear positive, but only 1 of 8 dialysates yielded organisms identical to those later recovered during peritonitis.

During clinical peritonitis but before the start of antimicrobial therapy, all culture-positive specimens (73 dialysates) yielded organisms related to the onset of peritonitis (Table 1). After initiation of therapy, some isolates (7 of 76) yielded organisms unrelated to the peritonitis event or to infection or colonization of other body sites or indwelling catheters; the remaining 69 isolates were identical to organisms recovered before the start of therapy.

In the absence of peritonitis, up to 13% of the specimens were culture positive; this was related to the degree of leukocytosis and antimicrobial therapy, if any (Table 1).

The types and frequencies of organisms recovered from dialysates and their association with peritonitis is depicted in Table 2. Coagulase-negative staphylococci (C-NS) were the most prevalent organism group recovered from dialysates in

### TABLE 1. Laboratory results of 1,774 dialysate specimens collected during this study

<table>
<thead>
<tr>
<th>No. of leukocytes/mm³</th>
<th>Peritonitis</th>
<th>Nonperitonitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture positive</td>
<td>Smear positive</td>
</tr>
<tr>
<td>Without antimicrobial therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤100</td>
<td>22 [20]</td>
<td>15 (68)</td>
</tr>
<tr>
<td>101–499</td>
<td>24 [22]</td>
<td>9 (38)</td>
</tr>
<tr>
<td>≥500</td>
<td>65 [58]</td>
<td>49 (75)</td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>73 (66)</td>
</tr>
<tr>
<td>With antimicrobial therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤100</td>
<td>241 [59]</td>
<td>23 (10)</td>
</tr>
<tr>
<td>101–499</td>
<td>97 [24]</td>
<td>16 (16)</td>
</tr>
<tr>
<td>≥500</td>
<td>70 [17]</td>
<td>37 (53)</td>
</tr>
<tr>
<td>Total</td>
<td>408</td>
<td>76 (19)</td>
</tr>
</tbody>
</table>

* Values in brackets indicate the percent distribution within cell range.
TABLE 3. Recovery of peritonitis-related and -unrelated microorganisms from dialysate by conventional culture

<table>
<thead>
<tr>
<th>Dialysate isolate</th>
<th>No. (%) recovered by time (h) of incubation</th>
<th>No. (%) recovered by medium type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤48</td>
<td>&gt;48</td>
</tr>
<tr>
<td>Peritonitis related</td>
<td>170</td>
<td>132 (78)</td>
</tr>
<tr>
<td>Unrelated</td>
<td>107</td>
<td>54 (50)</td>
</tr>
<tr>
<td>Total</td>
<td>277</td>
<td></td>
</tr>
<tr>
<td></td>
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</tbody>
</table>

Each category and accounted for 36% (26 of 72) of culture-positive peritonitis events. Staphylococcus aureus was the second most frequent organism associated with culture-positive peritonitis (15 of 72 events); they accounted for the largest number (7 of 19) of smear-positive dialysates and were associated with 6 of 10 catheter site infections seen in our patients. Of the 10 peritonitis events attributed to gram-negative bacilli, Pseudomonas aeruginosa was the single most frequent gram-negative bacilli and accounted for 3 of the 10 events. Yeast (Candida tropicalis) accounted for only one case of culture-positive peritonitis. Recovery of anaerobic organisms was limited to diphtheroids. These organisms were not associated with evidence of clinical peritonitis or other body sites of infection or colonization.

The characteristics of bacterial recovery by a conventional bacteriologic culture method of established efficacy (18) are presented in Table 3. In addition, results of blood cultures were assessed. No bacterial growth was detected in 63 blood culture sets obtained during 25 peritonitis events.

The distribution of dialysates by total leukocyte count is shown in Table 1; it includes all specimens collected from patients with and without peritonitis. A more detailed analysis of total leukocyte count from specimens collected in the absence of peritonitis is given in Table 4. Results for nonperitonitis dialysates collected during or in the absence of antimicrobial therapy were not statistically different (P > 0.05) and were combined. Dialysates are listed by patient status and dialysis schedule. Selected dialysates collected just before or immediately after clinical peritonitis were defined as preperitonitis and postperitonitis specimens, respectively, on the basis of their containing increased numbers (>100/mm²) of leukocytes, yielding organisms identical to those recovered from dialysates during peritonitis, or both. A small number of dialysis effluents was associated with extraperitoneal infections, that is, infections other than peritonitis. These infections were sometimes associated with peripheral leukocytosis and consisted of the following: cellulitis, 2; pneumonia, 1; meningitis, 1; catheter exit site infection, 4; catheter exit site infection with bacteremia (unrelated to dialysis) and urinary tract infection, 1; gangrene of the foot and catheter exit site infection, 1; abdominal wound, 1; and bacteremia (in the absence of clinical peritonitis) with recovery of the same organism from blood and dialysis effluent, 1.

DISCUSSION

The types and frequencies of microorganisms recovered from dialysates (Table 2) were consistent with those in previous studies for specimens collected during (14, 22, 29) and in the absence of (3, 22) peritonitis. In most instances, detection of microorganisms was based on culture findings, since few dialysates were positive by gram-stained film (Table 1). However, S. aureus accounted for the largest number (7 of 19) smear-positive dialysates. Microscopy is a fairly insensitive test (5, 13, 18, 22), but it is still useful in those instances in which the bacterial concentration in effluent is high enough (10⁶ CFU/ml) for detection by this method.

Microorganisms are not always recovered from dialysates during peritonitis. Up to one-third of reported (1, 8, 14, 22) cases of peritonitis have been culture negative. During the 17-month period of this study, culture-negative peritonitis in our patients, accounted for 18 (21%) of 90 peritonitis events seen, which included both clear and cloudy dialysates by definition. The frequency with which bacteria can be recovered from peritoneal effluents collected from patients with suspected peritonitis can vary with the evaluation criteria used—cloudy effluent, diffuse abdominal pain, etc. (30). Sterile peritonitis must be differentiated from culture-negative peritonitis. Low pH (20), the presence of endotoxin (12) or chemical irritants (16) in dialysis fluid, retrograde menstruation (8), pseudomembranous colitis (9), and eosinophilia (25) have been implicated in sterile peritonitis. Culture-negative peritonitis is largely attributed to low numbers of organisms present in dialysate as a result of sequestration of bacteria in phagocytes (26). They can escape detection by conventional bacteriologic methods. Culture-negative peritonitis can occur if steps are not taken for removal of antibiotics that may be present in the effluent (29, 30). Of the 18 events of culture-negative peritonitis in our patients, 4 were associated with ongoing antimicrobial therapy initiated before specimen collection. Indeed, antimicrobial therapy had a significant impact on our recovery of microorganisms from all dialysates collected during and in the absence of peritonitis (Table 1) (our culture protocol did not include steps for removal of antibiotics from the effluent).
Peritonitis-related isolates were more readily recovered on all media and usually required less than 48 h of incubation time unlike peritonitis-unrelated isolates (Table 3). This pattern of growth was suggestive of higher colony counts for microorganisms associated with peritonitis, compared with isolates of the peritonitis-unrelated class. Differences in bacterial counts between these two classes of isolates have been demonstrated, although there may be considerable overlap in numbers (3, 22). Recovery of all organisms from dialysates was affected by the growth medium used (Table 3). Use of an enriched broth medium, such as thioglycolate broth, was shown to be more effective in recovery of microorganisms from dialysis effluent than the use of conventional plate media (13). Our results confirmed this finding.

On nine occasions, microorganisms recovered from dialysates of patients without peritonitis were identical to organisms recovered from other body sites or indwelling catheters. They were identified as: C-NS, 7; and mixed cultures with C-NS, 2. The other sites of recovery included: blood, 2; and peritoneal catheter site, 7. Seeding of the peritoneum by organisms originating from other sites may have occurred in our patients. Yet there appeared to be no spread of microorganisms from the infected peritoneum to other areas; in those patients from whom blood cultures were taken, bacteremia secondary to peritonitis was not seen. Indeed, hematogenous spread is rare (1, 29). This has been a key feature of dialysis-associated peritonitis, which distinguishes it from surgical peritonitis, in surgical peritonitis, up to 30% of patients develop bacteremia (29).

Frequent recovery of microorganisms from dialysates of patients without peritonitis has been documented (24). Of 1,255 dialysates collected from our patients in the absence of suspected peritonitis, 128 (10%) specimens were culture positive (Table 1). A majority of these dialysates yielded organisms not associated with any other infection or site of colonization. However, 28 of these dialysates were culture positive for pathogens; these included 23 of 29 preperitonitis dialysates and 5 of 15 postperitonitis dialysates. Like Vites et al. (31), we could frequently distinguish peritonitis-associated isolates from contaminants by the successive recovery of the former from multiple dialysates collected before the onset of peritonitis. Yet this pattern of recovery was not always seen with these pathogens, and so a distinction between contaminants and peritonitis-associated isolates could not be readily made by the laboratory without other evidence of infection.

Our results (Table 1) confirmed previous observations (3, 5, 7, 22) of an overlap in cell count between dialysates collected either during or in the absence of peritonitis. This was attributed, in part, to nonspecific increases in cell count that we observed (Table 4) in patients on intermittent dialysis (11) if the effluent was collected at the start of dialysis (3). Dialysate leukocytosis has also been associated with noninfectious conditions, such as eosinophilia (13, 25), which has already been discussed. These nonspecific rises in effluent cell count have been differentiated by others (3, 11, 13, 22) from a true inflammatory response to infection by performance of a differential count. Polymorphonuclear leukocytes predominate during peritonitis, whereas in the absence of infection, the normal cellular response is mononuclear (7, 10).

Among dialysis patients with peritonitis, Hurley et al. (10) observed peripheral leukocytosis only among patients with concomitant, extraperitoneal infections, e.g., an infected shunt, pericarditis, or pneumonia. They suggested that "inflammation of the peritoneal cavity is contained locally without systemic response" in dialysis patients. Others (6, 22, 29) have observed a low incidence of blood leukocytosis in dialysis patients with peritonitis. Extraperitoneal infection of the exit site, abdominal surgery, catheter leakage, and unexplained abdominal pain or diverticulitis have been associated with an increased number of leukocytes and an altered differential (polymorphonuclear?) in dialysates (17). We could demonstrate a significant (P < 0.05) increase in the proportion of dialysates with elevated leukocyte counts from patients with extraperitoneal infections compared with specimens collected in the absence of infection (no infection) (Table 4). However, during this study, differential counts were not routinely performed. These infections were sometimes associated with peripheral leukocytosis.

Increased numbers of leukocytes were more characteristic of (and helped to define) pre- and postperitonitis dialysates (Table 4), but this was of little diagnostic value. Few preperitonitis specimens had elevated leukocyte counts, and whereas postperitonitis dialysates often had increased cell counts, we could not document that they were followed by a reoccurrence of peritonitis.

It is evident that none of the laboratory tests discussed herein were adequate for detecting peritonitis in dialysis patients. Detection of microorganisms in effluent is dependent on the bacteriologic method and growth medium used. Culture-positive findings are a poor predictor of peritonitis because of the frequency of microorganisms in dialysates from patients without evidence of clinical peritonitis. Inclusion of total leukocyte count is useful, but there is no absolute correlation of dialysate cell count with peritonitis. Nonspecific leukocytosis of the effluent is seen in patients on intermittent dialysis and can arise from the presence of chemical irritants or other substances in dialysis fluid, trauma, or extraperitoneal infection. Consequently, performance of a differential count is recommended. Our findings indicated that other conditions, e.g., extraperitoneal infections, dialysis schedule, and bacterial seeding from other body sites or indwelling catheters, can alter the bacteriologic and cytological composition of dialysis fluid.

Clearly, our experience indicates that no one test is diagnostic for peritonitis; clinical symptoms and signs in conjunction with laboratory data need to be applied. The microbiology laboratory should be prepared to make a presumptive determination of peritonitis based on differential leukocyte count and a Gram stain of the dialysate sediment. Recovery of potential pathogens from peritoneal effluent is dependent on the volume cultured (at least 10 ml should be used) and the primary recovery medium used. Bacteremia is rarely associated with peritonitis.

LITERATURE CITED