Early detection of peritonitis in continuous ambulatory peritoneal dialysis patients by use of chemiluminescence: evaluation of diagnostic accuracy by receiver-operating characteristic curve analysis

Gönül Dalaman,1 Goncağül Haklar,1 Armand Sipahi,2 Çetin Özener,2 Emel Akoğlu,2 and A. Süha Yalcın1*

Continuous ambulatory peritoneal dialysis (CAPD) is now a widely accepted treatment for end-stage renal disease. However, the high incidence of peritonitis is a major complication of CAPD. Polymorphonuclear leukocytes (PMNs) play a major role in antimicrobial response of the host. During phagocytosis, the PMNs undergo a striking increase in oxidative metabolism, known as the respiratory burst, and emit light as chemiluminescence (CL). CL is thus a sensitive measure of PMN oxidative potential and correlates well with antimicrobial activity. In view of the observation of increased susceptibility to infection in CAPD patients, we have studied lucigenin- and luminol-enhanced CL in peritoneal fluids of these patients and assessed the diagnostic accuracy of these tests by ROC curve analysis. ROC curves showed diagnostic accuracies for both tests that were superior to counts of PMNs in the dialysis fluid ($P < 0.001$). At selected cutoff values of 150,000 cpm/vial for lucigenin CL and 600,000 cpm/vial for luminol CL, sensitivities were 100%. Specificities for lucigenin and luminol CL were 89% and 80%, respectively. Our results suggest that CL measurements can be used as an early marker for the presence of infection in CAPD patients.

Continuous ambulatory peritoneal dialysis (CAPD)3 is a widely accepted treatment for end-stage renal disease that may be caused by chronic glomerulonephritis, pyelonephritis, hypertension, some immunological diseases, and toxic or ischemic damage to the kidney (1–3). However, the frequent occurrence of peritonitis, which is associated with high risk of mortality and morbidity, is a major complication of CAPD (1, 4–7). The diagnosis and effective treatment of peritonitis depends on clinical evaluation of the patient and correlation of this with laboratory examination of the dialysate. The latter routinely includes the determination of total leukocyte count and the recovery and identification of microorganisms (7–9). Previous reports have demonstrated problems associated with the diagnosis of peritonitis based solely on these indicators (4, 7, 10). Various techniques have been used to facilitate the recovery of microorganisms from dialysate, among them are the use of selected broth media, processing of large volumes of dialysate effluent by concentration techniques or total volume culture, chemical or physical disruption of phagocytes in dialysate sediment for recovery of sequestered organisms, and the removal of antibiotics from dialysate (7). However, microorganisms are not always recovered from dialysate during peritonitis, and up to one-third of reported cases of peritonitis have been culture-negative (4, 7, 10).

The major role of phagocytes in antimicrobial response by the host has encouraged the study of phagocyte function in CAPD patients. During phagocytosis, the polymorphonuclear leukocytes (PMNs) undergo a striking increase in oxidative metabolism, which is known as the respiratory burst. Superoxide anion ($O_2^-$) is produced during the burst and is bactericidal either directly or by conversion to other reactive metabolites via metal cata-
lyzed reactions (11). Production of $O_2^-$ is accompanied by light emission (chemiluminescence, CL) (12, 13). Therefore, CL is a sensitive measure of the oxidative potential of phagocytes and correlates well with antimicrobial activity.

In view of the observation of increased susceptibility to infection in CAPD patients and on seeing the low sensitivity and specificity of the methods available for the diagnosis of peritonitis, we have attempted to study CL formation in dialysates of CAPD patients. We have evaluated the diagnostic accuracy of CL in peritonitis by using ROC analysis of data.

Patients and Methods

PATIENTS

Patients (n = 55) attending the nephrology outpatient clinic of Marmara University Hospital were included in the study. These were 27 women and 28 men, with an age distribution of 4–72 years, established on CAPD for 2 months or longer. All patients gave informed consent for the study, which was approved by the ethical committee. Patients who had an underlying disease known to be associated with abnormal PMN function, such as diabetes mellitus, tuberculosis, or systemic lupus erythematosus, were excluded. Plasma creatinine concentrations of the patients were 628 ± 221 μmol/L (7.1 ± 2.5 mg/dL), and blood urea nitrogen concentrations were 31.7 ± 11.8 mmol urea/L (88.9 ± 33.0 mg/dL). The dialysis fluids contained NaCl (5.7 g), sodium lactate (3.9 g), CaCl$_2$ (257 mg), MgCl$_2$ (152 mg), and glucose (13.6, 22.7, and 38.6 g/L) with an osmolality range of 275-494 mOsm/L. All patients were trained in aseptic technique, and none had infection at the time of study or had received antibiotics for 1 month before the study. Dialysate fluids (n = 112) were collected from 55 patients, which included both noninfected patients and those presenting with acute peritonitis. When symptoms such as cloudy fluid, fever, abdominal pain, and rebound tenderness were present, diagnosis of peritonitis was suspected; manual PMN counts >100/mm$^3$ in the peritoneal dialysate effluent and positive culture proved the diagnosis.

PROCEDURES

CL measurements. Fresh specimens (3 mL) were transferred to counting vials. CL was measured using a scintillation counter (Tricarb 1500, Packard Instruments) in out-of-coincidence mode with a single active photomultiplier tube. CL was recorded after the addition of luminol or lucigenin at a final concentration of 0.2 mmol/L to the vials at room temperature. Each vial was observed continually for 60 min at 1-min intervals. After subtracting the appropriate control values, peak CL was expressed as cpm per vial.

Additional quantitation and contribution of hypochlorite, hydroxyl radical (OH), and hydrogen peroxide ($H_2O_2$) to luminol CL was determined in samples with high CL (arbitrarily defined as >20 000 cpm/vial). Inhibitors, enzymes, or scavengers affecting luminol-enhanced CL were added to the vials, and the samples were recounted. Effects of inhibitors and scavengers were expressed as the percentage of inhibition of CL.

Spectrophotometric measurements. Measurement of $O_2^-$ and myeloperoxidase (MPO) in dialysis effluents was made by spectrophotometric methods. $O_2^-$ determination was based on reduction of ferricytochrome C (14). MPO activity was determined by using $H_2O_2$-dependent oxidation of 3,3',5,5'-tetramethylbenzidine as substrate (15).

Bacteriological methods. The injection ports were disinfected with methanol and allowed to dry for 2 min. Dialysate (50 mL) was then aspirated into a sterile container and centrifuged at 2000g at room temperature. The sediment was used for gram-stained film and inoculated to the following media: blood agar, MacConkey agar, chocolate agar, thioglyconate broth, and blood culture medium. All cultures were incubated and examined daily for 7 days. Identification of isolates was determined by standard methods.

Statistical analysis. Numerical data were expressed as mean ± SD. The significance of differences between the experimental groups was estimated by the Mann–Whitney U-test, and the differences were considered significant when the probability was $P <0.05$.

ROC curves. To assess the diagnostic accuracy of lucigenin- and luminol-enhanced CL, we used ROC curves (16) and calculated the areas under curves (AUCs) for comparison. ROC curves were generated by plotting the relationship of the true positivity (sensitivity) and false positivity (1 − specificity) at various cutoff points of the tests. An AUC of 1.0 is characteristic of an ideal test, whereas 0.5 indicates a test of no diagnostic value (17). Cutoff values of lucigenin- and luminol-enhanced CL for diagnosis of peritonitis were selected from experimental data and were the values that maximized the sum of sensitivity and specificity.

RESULTS

PERITONEAL DIALYSATE LUCIGENIN AND LUMINOL CL CONCENTRATIONS IN PATIENTS WITH AND WITHOUT PERITONITIS

As shown in Fig. 1, the mean lucigenin CL was 16 179 ± 14 916 cpm/vial in patients without peritonitis (n = 81) and 1 389 554 ± 806 046 cpm/vial ($P <0.05$) in patients with peritonitis (n = 41), whereas that of luminol CL was 11 780 ± 7 111 and 5 501 879 ± 2 839 440 cpm/vial ($P <0.05$), respectively.

PERCENTAGE OF INHIBITION OF LUMINOL CL

Luminol CL was 98.5% inhibited by 0.1 mmol/L sodium azide, which is an inhibitor of phagocyte MPO. The inhibition with 1570 U/vial catalase (an enzyme that breaks down $H_2O_2$) was 98.3% and that of 50 mL/L...
dimethyl sulfoxide (a scavenger of \( \cdot \text{OH} \) radicals) was 63.2%.

**Spectrophotometric Measurements**

Table 1 shows the results obtained from \( \text{O}_2^\cdot \) and MPO determinations. These indicators were significantly increased in patients with peritonitis compared with non-infected patients.

**ROC Curves**

Figure 2 shows that the ability of peritoneal dialysate lucigenin and luminol CL to differentiate patients with peritonitis from noninfected cases exceeds that of PMN counts (AUC, 0.937 vs 0.774 for lucigenin CL vs PMN count, and 0.928 vs 0.774 for luminol CL vs PMN count, \( P < 0.001 \)). There was no significant difference between lucigenin and luminol CL (AUC, 0.937 vs 0.928). The selected cutoff values for diagnosis of peritonitis were 150 000 and 600 000 cpm/vial (lucigenin and luminol CL, respectively). Table 2 shows the sensitivities and specificities of each test for the diagnosis of peritonitis.

**Discussion**

CAPD, brilliantly conceived by Popovich et al. (18), should be an ideal form of dialysis treatment for acute and chronic renal failure because it provides continuous, steady renal substitution with good biochemical control, more liberal dietary and fluid intake, improvement in anemia, and freedom from machines (19). Although it can be easily performed at home, the relatively high incidence of peritonitis is the major problem of CAPD. The incidence of peritonitis was 5.2–7.5 episodes of per patient per year of dialysis in late 1968 (20). The risk has reduced to 1 case in 24–36 months with improvement of the technique (21), which was later confirmed by a multicenter trial in Canada (22). The most commonly encountered microorganism is coagulase-negative *Staphylococcus* (30–40%), whereas *S. aureus* (20%), *Streptococcus* sp. (10–15%), and other organisms, including fungi, have also been isolated (1, 23–25). Every peritonitis attack damages the peritoneum and decreases peritoneal diffusion capacity (25).

The diagnosis of peritonitis in CAPD patients is established when any two of the following three criteria are

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**Table 1. Superoxide Radical (\( \text{O}_2^\cdot \)) and MPO Concentrations in Dialysate Effluents.**

<table>
<thead>
<tr>
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<th>Noninfected CAPD patients* (n = 8)</th>
<th>CAPD patients with peritonitis* (n = 5)</th>
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<tbody>
<tr>
<td>( \text{O}_2^\cdot ), ( \mu \text{mol/L} )</td>
<td>0.66 ± 0.53</td>
<td>1.14 ± 0.74b</td>
</tr>
<tr>
<td>MPO, IU/L</td>
<td>0.08 ± 0.01</td>
<td>1.16 ± 0.63b</td>
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* Values represent mean ± SD.

b \( P < 0.001 \) compared with noninfected patient.
The major role played by phagocytes in the host antimicrobial response largely depends on oxidative reactions known as the respiratory burst (31). The respiratory burst consists of “four increases”: increase in oxygen uptake, \( \text{O}_2 \) production, \( \text{H}_2\text{O}_2 \) production, and hexose monophosphate shunt activity. The initial radical (\( \text{O}_2^- \)) is dismutated to \( \text{H}_2\text{O}_2 \), which is then converted to hypochlorous acid by the action of phagocyte MPO when \( \text{Cl}^- \) is available, or to \( \cdot\text{OH} \) in the presence of metals (32). CL is a phenomenon that is related to respiratory burst and light emission by phagocytes and has proved to be useful as a test of phagocyte function (13, 32–34).

Luminol and lucigenin are widely used CL probes that differ in selectivity and enhance the sensitivity of native CL. Luminol quantifies a group of reactive species, including \( \text{H}_2\text{O}_2 \), \( \cdot\text{OH} \), hypochlorite, and peroxynitrite, but lucigenin is mostly selective for \( \text{O}_2^- \) (35). We have observed increases in both lucigenin and luminol CL in effluents of patients having peritonitis. We have added inhibitors and scavengers of free radicals and determined that the phagocytes were functionally intact by all means. The functional well-being of dialysate-elicited peritoneal macrophages was also reported by others (36). Oxygen concentrations within the peritoneal cavity were sufficient to maintain adequate respiratory burst, even during acute peritonitis (37).

We have examined additional indicators to validate CL increases. In agreement with lucigenin CL, spectroscopic determination showed that \( \text{O}_2^- \) production was higher in patients with peritonitis compared with the noninfected group. MPO, which catalyzes the conversion of \( \text{H}_2\text{O}_2 \) to hypochlorous acid for bactericidal action, was again significantly increased (\( P < 0.05 \)).

To compare the performance of CL tests with other diagnostic measures of peritonitis, we have used ROC plots, which provide pure indices of accuracy (16). Both lucigenin and luminol CL exhibited greater observed accuracy than PMN count for the diagnosis of peritonitis. Statistical comparison of the AUCs showed that the differences in accuracy were highly significant. The ROC plot AUC is the most convenient global way to quantify the diagnostic accuracy of a test (17). The AUC values for lucigenin CL indicated that in 93.7% of the cases, a randomly selected patient with peritonitis will have a higher lucigenin CL than will a randomly selected noninfected patient. This value is 92.8% for luminol CL. Because positive culture was used for the estimation of peritonitis in constructing the plot, comparison of diagnostic accuracy of CL with microbiologic techniques was not possible. However, we had two patients with negative culture results but with above-cutoff CL values, and they were later diagnosed with fungal peritonitis.

The use of ROC curve analysis to assess the diagnostic accuracy of a biological marker for use in patient management requires the selection of a decision threshold. The large number of peritoneal fluids used in our study allowed us to calculate with precision the cutoff values of lucigenin and luminol CL as 150,000 and 600,000 cpm/vial, respectively. We have selected cutoff values that maximized the sum of sensitivity and specificity assuming that, at present, both sensitivity and specificity are equally important in diagnosing patients with peritonitis.

CAPD has proved to be a safe and effective alternative to hemodialysis for patients with end-stage renal disease.

### Table 2. Diagnostic performance of lucigenin and luminol CL at selected cutoff points in patients with peritonitis.

<table>
<thead>
<tr>
<th>Cutoff point</th>
<th>Lucigenin CL</th>
<th>Luminol CL</th>
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<tbody>
<tr>
<td>Sensitivity, %</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>89</td>
<td>80</td>
</tr>
<tr>
<td>Lucigenin CL</td>
<td>150,000 cpm/vial</td>
<td>600,000 cpm/vial</td>
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Despite improvements in technology, a major complication associated with this procedure is peritonitis. Detection of etiologic agent relies heavily on culture techniques, but the results have not been satisfactory. Our results show that CL measurements can be used as an early marker for the presence of infection in CAPD patients. In addition, decisions on treatment effectiveness and duration can be easily made if CL values are considered.

References