Gel clot LAL assay in the initial management of peritoneal dialysis patients with peritonitis: a retrospective study

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Abstract

**Background.** Indiscriminate use of broad-spectrum antibiotic treatment of peritonitis in peritoneal dialysis patients may have either unwanted side-effects or contribute to the development of antibiotic resistance. This may be avoided by improved diagnosis at presentation. The *Limulus* amoebocyte lysate assay is a convenient test detecting bacterial endotoxins or fungal beta glucans. This study evaluates a qualitative *Limulus* amoebocyte lysate test as a diagnostic tool used at presentation of a peritoneal dialysis patient with peritonitis.

**Methods.** One-hundred and eleven episodes of peritonitis in peritoneal dialysis patients have been analysed retrospectively. *Limulus* amoebocyte lysate results at presentation were compared with culture results. A *Limulus* amoebocyte lysate assay was performed using a commercial kit by incubating a mixture of dialysate effluent and *Limulus* amoebocyte lysate reagent at 37 °C. The development of a stable solid clot was considered positive. The specificity and sensitivity of the test were calculated.

**Results.** The specificity of the *Limulus* amoebocyte lysate assay was found to be 98% and the sensitivity 74%. *Limulus* amoebocyte lysate assay was false-negative in 13 cases of Gram-negative peritonitis (22%). *Limulus* amoebocyte lysate was positive in three of seven cases of fungal peritonitis. The study included one case each with false-positive *Limulus* amoebocyte lysate and with culture-negative peritonitis.

**Conclusions.** The *Limulus* amoebocyte lysate assay is a convenient and valuable diagnostic tool for excluding Gram-positive peritonitis in peritoneal dialysis patients. This allows more specific antibiotic treatment at presentation and may avoid the development of bacterial resistance. A negative *Limulus* amoebocyte lysate test is not reliable for the exclusion of Gram-negative peritonitis. In the absence of a positive culture result 48 h after presentation, accompanied by a delayed response to treatment, a positive *Limulus* amoebocyte lysate assay may indicate the presence of fungus. This justifies early empiric antifungal treatment before definitive culture results are made available. Routine *Limulus* amoebocyte lysate assay of dialysate effluent from continuous ambulatory peritoneal dialysis patients presenting with peritonitis is recommended.

**Keywords:** fungus; Gram-negative bacteria; Gram-positive bacteria; *Limulus* amoebocyte lysate (LAL) assay; peritoneal dialysis; peritonitis

Introduction

Peritonitis is the most frequent complication of peritoneal dialysis (PD). Drop-outs from PD due to peritonitis have been reduced by improved connection techniques. Early and effective treatment of peritonitis preserves the peritoneum and prevents the development of fibrosis and adhesions eventually leading to dialysis failure. Uncontrolled use of broad-spectrum antibiotics may, however, have unwanted long-term effects such as ototoxicity and reduction of residual renal function by aminoglycosides, or the development of resistant bacterial strains such as the vancomycin-resistant strains of *Enterococci* (VRE) [1]. The significance of an accurate diagnosis of the organism, therefore, goes beyond the immediate benefit for the patient presenting with peritonitis.

The early diagnosis of peritonitis is traditionally based on clinical signs of peritoneal irritation, the appearance of a cloudy peritoneal effluent or an increased leucocyte count in the fluid. Antibiotic treatment has to be initiated before an accurate diagnosis has been established based on bacterial culture of the dialysate effluent. Hence, treatment protocols initially recommend a combination of antibiotics covering both Gram-negative and Gram-positive organisms [2]. Reported sensitivities of Gram-staining vary between 9 and 80% in dialysate effluents and are, therefore, not widely used for the early diagnosis of peritonitis [3]. Small amounts of bacterial endotoxin (lipopolysachar-
ide) can be detected in biological fluids by a 1-h test known as the Limulus amoebocyte lysate (LAL) assay. LAL assays are the most sensitive tests currently known for endotoxin, with a theoretical detection limit of 500 Gram-negative bacteria/ml in peritoneal dialysate effluent [4,5]. The LAL test also yields positive results when a fungus is the infective organism [6]. Previous studies evaluating LAL testing in peritonitis of CAPD patients included 3–17 episodes of Gram-negative peritonitis. Reported sensitivities vary between 65 and 100% [3,5,7]. This retrospective study was conducted to assess the value of the qualitative gel clot LAL test in the early diagnosis of peritonitis in CAPD patients.

Methods

Patient population

Records of all CAPD patients treated at our centre between 1995 and 1998 were reviewed. All episodes of peritonitis were recorded. Abdominal pain, cloudy peritoneal effluent or more than 100 leucocytes/mm² in the effluent were considered positive criteria for the clinical diagnosis of peritonitis. The diagnosis was further confirmed by laboratory analysis of peritoneal effluent solutions.

At presentation, peritoneal effluent samples were obtained from dialysate bags using a sterile 18-gauge needle and syringe from the alcohol-swabbed stopper port. Effluent was saved at 4°C for the LAL assay, which was performed the same day, and was also incubated in bacteriological culture media (Bactec, Becton Dickinson, MD, USA).

The LAL assay was performed in duplicate using commercial LAL reagent containing Limulus amoebocyte lysate in its haemolymph (Charles River Endosave, Charleston, SC, USA). The assay was performed in pyrogen-free test tubes to which 0.1 ml of peritoneal effluent solution and 0.1 ml of LAL reagent were added. Following 1 h of incubation at 37°C, the test tubes were examined by 180° inversion for the presence of a stable solid clot. A clotted incubation mixture was considered to be a positive result. Endotoxin standard (Escherichia coli strain O55:B5, 0.125 EU/ml) and pyrogen-free LAL reagent water, both provided by the manufacturer, were used as a control. A labelled LAL reagent sensitivity of 0.125 EU/ml was confirmed by serial dilutions prior to use.

Statistical analysis

The results of the LAL assay are presented in relation to bacteriological culture results. Sensitivity, specificity, positive and negative predictive values were calculated. Effluent specimens with Gram-positive or sterile culture results were expected to be LAL negative. Effluent specimens with Gram-negative or fungal culture results were expected to be LAL positive. Where appropriate, values are reported as mean ± SE.

Results

Data from 111 episodes of peritonitis in 54 patients (30 males, 24 females) were reviewed. The average age was 57.8 ± 2.6 years (56.3 ± 3.6 for males, 59.5 ± 3.6 for females). The incidence of peritonitis during the period of observation was one peritonitis episode per 15.1 treatment months.

We compared bacteriological culture results with the respective results of the LAL assay. A Gram-positive agent was identified in 40% of cases (44/111) and a Gram-negative agent in 53% of cases (59/111). Fungal peritonitis was diagnosed in 5% of cases (7/111) and one episode of peritonitis was culture negative.

The LAL assay was false-negative in 15% of cases. This included 13 cases of Gram-negative peritonitis and four cases of fungal peritonitis. Thus, 22% (13/59) of Gram-negative organisms and 57% (4/7) of fungi were associated with false-negative results. No specific pattern could be discerned in the distribution of Gram-negative species yielding false-negative results. Only one false-positive result was found, caused by Streptococcus sanguinis (1%) (Table 1).

Overall specificity and positive predictive value were both 98%, whereas sensitivity was found to be 74% and negative predictive value 72%.

Discussion

This retrospective study underscores the high specificity (98%) and positive predictive value (98%) of the qualitative gel clot LAL test in the diagnosis of peritonitis in PD patients. Thus, a Gram-positive infection may be excluded with a high degree of certainty if the LAL test is positive. According to our study 45% (50/111) of all patients with peritonitis have a positive LAL test at presentation. In 49 of 50 cases the LAL test is true positive. This finding is significant because in these patients, based on LAL testing, antibiotic treatment against Gram-positive organisms may be safely omitted from their initial empirical management. If relying only on culture results treatment would be adjusted and narrowed with an average delay of 48 h.

The elimination of antibiotics in the initial phase of peritonitis will be more pronounced in patient populations with higher incidences of Gram-negative peritonitis. In our patient population, the incidence of Gram-negative peritonitis was 53%, contrasting with other centres that report incidences of ~15% [8]. The distribution of organisms may vary greatly between different centres and at different periods of observation in the same centre. Thus, in a previous survey over 1984–1987 at our institution, the incidence of Gram-negative peritonitis was 32%, whereas Korbet et al. report an incidence of 14% for 1981–1993 [8,9]. The shift in distribution of bacteria at the same institution has been attributed to the reduced incidence of Staphylococcus epidermidis due to the introduction of disconnect systems [8,10]. Thus, during 1984–1987, 33% of episodes at our institution were caused by S. epidermidis, whereas in the present study S. epidermidis was the cause of only 13% of peritonitis episodes. Racial and educational differences may also contribute to differences in peritonitis rates [8,11].
Narrowing the choice of empirical treatment at presentation is likely to lower the incidence of resistance to antibiotics. Because of emerging strains of VRE and *S. aureus* [12] current recommendations include first-generation cephalosporins, rather than vancomycin, in the initial treatment of peritonitis [2]. The indiscriminate use of first-generation cephalosporins may contribute to the selection of ceftazidime-resistant Gram-negative bacilli, although their use has not been shown to lead to antibiotic resistance in a PD population [13,14]. Also, substitution of cephalosporin for vancomycin may not be implemented in institutions in which the rates of methicillin-resistant *S. aureus* or *S. epidermidis* are high. Reported rates of methicillin resistance are 15–20% for *S. aureus* and 30–40% for *S. epidermidis* [15,16]. Reliable and convenient LAL testing, as used in this study can, therefore, contribute significantly to clinical management of the patient.

The LAL assay used here is not specific for Gram-negative bacteria since fungal infections may also cause positive results due to (1→3)-beta glucans which form a major structural component of fungal cell walls. Endotoxin and glucan activate C and G compounds, respectively, which are clotting enzymes contained in amoebocyte granules. Several specific assays containing only G compound have been developed to detect glucan without interference from endotoxin [17]. In our study, three of seven episodes of fungal peritonitis were LAL positive at presentation and initially culture-negative. In these patients, specific antifungal therapy was delayed for up to 11 days until culture results were available. Empirical antifungal therapy may be indicated in patients with a LAL-positive test and initially negative culture not responding to conventional antibiotic treatment. This will ensure specific treatment within 48 h of presentation and possibly lower dropouts from CAPD due to membrane failure.

Originally, LAL assays were expected to be useful for the early detection of Gram-negative infections. They have been applied to the diagnosis of meningitis, intra-amniotic or biliary infections [18–20], as well as to the diagnosis of bacterial peritonitis in CAPD patients [3,5,21]. These studies included between 3 and 17 patients with Gram-negative peritonitis and reported sensitivities of up to 100%. However, in a prospective study of septic patients, a quantitative chromogenic LAL assay was disappointing for the diagnosis of Gram-negative septicemia [6]. Our study shows that the test is not sensitive enough to exclude Gram-negative infection if the LAL test is negative. Sterilized dialysate effluent has been shown to reduce the sensitivity of the LAL test from 0.12 EU/ml to 0.5 EU/ml compared with distilled water [3]. The LAL test may also be false-negative because it does not detect small molecular mass endotoxins [22]. In our study, false-negative results of the LAL assay could not be attributed to a specific organism. Indeed, different bacteria and strains of the same bacteria differ in their ability to release endotoxins from their surfaces [23]. This is likely to affect the LAL assay since cell-bound endotoxin has a lower *Limulus* activity than shedded endotoxin [24].

Whereas 22% of Gram-negative infections were associated with LAL-negative results, 57% of fungal infections were LAL-negative. This difference in sensitivity is consistent with the reported lower *in vitro* sensitivity of LAL tests to LAL-reactive glucans than to endotoxins [25]. The fungal cell-wall content of beta glucans is variable. It depends on other cell-wall components and is regulated by genes [26,27]. Also, fungal beta 1,3 chains of d-glucan have variable degree of 1,6 branching which affects LAL reactivity [28].

The gel clot LAL assay used in this study is a practical tool, in that the time required for incubation is 1 h. The cost per assay is $3, whereas expenses for controls add $7. The surplus investment for assays of PD effluents is negligible in a laboratory where LAL assays are routinely performed for the quality control of the haemodialysis water supply.

In conclusion, the gel clot LAL assay is a reliable and convenient diagnostic tool to exclude Gram-positive peritonitis in CAPD patients because of its high specificity of 98%. A positive test justifies selective treatment with aminoglycosides only. If culture results turn out to be negative and the patient does not respond to the empirical treatment, the addition of specific antifungal therapy should be considered. A negative test does not exclude Gram-negative infection and warrants broad-spectrum antibiotic treatment until culture results are available. Routine LAL testing of peritoneal effluents from CAPD patients presenting with peritonitis is advised.

**References**

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Table 1. Qualitative gel-clot LAL assay and bactriologic culture results of peritoneal effluent from CAPD patients presenting with peritonitis
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