

Limulus amoebocyte lysate assay in the diagnosis of peritonitis in patients receiving continuous ambulatory peritoneal dialysis

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Abstract

Aims: To evaluate the *Limulus* amoebocyte lysate (LAL) assay for differentiating Gram positive from Gram negative peritonitis in patients receiving continuous ambulatory peritoneal dialysis (CAPD).

Methods: One hundred and six patients with suspected peritonitis were studied. LAL assay was performed by adding 0.1 ml of CAPD fluid to 0.1 ml of LAL reagent and incubating in a heating block for 60 minutes at 37°C. The sensitivity of the reaction was determined by: (i) diluting endotoxin in distilled water and used (filter sterilised) peritoneal dialysis fluid; and (ii) diluting a broth culture of *E coli* used in peritoneal dialysis fluid. A positive LAL assay was defined as the constant stability of the clot through an inversion of 180°.

Results: Compared with bacterial culture, the LAL assay had a sensitivity of 65% and a specificity of 98%. The sensitivity of microscopy compared with culture of Gram negative organisms was 76%; overall sensitivity of microscopy in comparison was 80%.

Conclusions: The Gram stain was more sensitive than has previously been reported; the LAL assay was specific but insensitive for the diagnosis of CAPD peritonitis. There was a correlation between reduced leucocyte count and culture; this was reduced in cases from which Gram negative organisms had been isolated. It is recommended that laboratories evaluate their Gram stain procedure to improve its sensitivity because the LAL assay is not a satisfactory substitute.

Gram negative or Gram positive organisms.^{3,4} The emphasis has been directed towards rapid diagnosis to exclude the presence of Gram negative bacteria to facilitate more specific treatment, possibly avoiding the exposure of patients to potentially ototoxic aminoglycosides. This approach has been suggested due to the lack of sensitivity of the Gram stain for the rapid diagnosis of CAPD peritonitis. This report describes an evaluation of the LAL (gel clot) assay in differentiating peritonitis due to either Gram negative or Gram positive organisms. We compared the sensitivity of the LAL assay and direct microscopy with culture for the rapid diagnosis of Gram negative peritonitis. We also assessed the theoretical sensitivity of the LAL assay for detecting Gram negative bacilli in used, sterile peritoneal dialysis fluid.

Methods

Peritoneal dialysis fluids from patients with suspected peritonitis who attended the renal unit at Sir Charles Gairdner Hospital, a 700-bed general hospital, were examined in the study. Fluids were stored at 4°C until routine bacteriological investigation; the delay in processing specimens was never more than two hours. If the LAL assay was not performed immediately the specimen was frozen at -20°C until assayed.

On receipt of the specimen in the laboratory, 50 ml was removed aseptically with a sterile syringe. A 20 ml aliquot was centrifuged at 3000 rpm for 10 minutes in a bench centrifuge and the deposit resuspended in 1.0 ml. One drop (0.1 ml) was used to inoculate a horse haematin agar (HH) plate, a cysteine lactose electrolyte deficient (CLEED) agar plate, two blood agar plates, and a blood agar plate containing saponin (SBA). The SBA, one blood agar, and HH agar plates were incubated at 35°C for 48 hours in an atmosphere containing 5% carbon dioxide; the CLEED plate was incubated aerobically at 35°C for 24 hours, and the remaining blood agar plate was incubated in an anaerobic chamber (Don Whitley Scientific Ltd) at 35°C for 48 hours. A Gram stained smear was also prepared from the centrifuged deposit. A 5 ml sample of the neat fluid was also inoculated into aerobic and anaerobic blood culture bottles (Bactec 6A and 7A) which were incubated at 35°C for five days.

The LAL assay was performed by adding 0.1

Continuous ambulatory peritoneal dialysis (CAPD) is becoming more widely used as an alternative to haemodialysis in patients with end-stage renal disease, but bacterial peritonitis remains a major complication.¹ Although isolation and identification of the causative organism remains the priority of the clinical bacteriology laboratory in the diagnosis of peritonitis in patients receiving CAPD, one aspect that has received attention recently is the use of the *Limulus* amoebocyte lysate (LAL) assay.² The LAL assay has been reported to differentiate reliably peritonitis due to either

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ml of CAPD fluid to 0.1 ml of LAL reagent (Pyrogen, Whittakers Bioproducts; Walkersville, Maryland, USA) and incubating it in a heating block at 37°C for 60 minutes. The reaction was carried out in 10 × 75 mm glass tubes that had been depyrogenated by heating in a hot air oven at 180°C for four hours. A positive control containing 0.12 EU/ml (*Escherichia coli* O55:B5 endotoxin, Whittakers Bioproducts) and a negative control (pyrogen free water) were used with each LAL assay. A positive LAL result was defined as a tube showing clot formation that remained intact after inversion of the tube through 180 degrees.

The relative sensitivity of the LAL reaction in used peritoneal dialysis fluid was determined by two methods. The first involved dilution of endotoxin in distilled water and used (filter sterilised) peritoneal dialysis fluid followed by the standard LAL assay. The second was by dilution of a broth culture of *E coli* (NCTC 10418) in used peritoneal dialysis fluid. Each dilution was then assayed by LAL, and viable counts were performed by dilution in physiological saline and plating 0.1 ml volumes on to CLED agar plates.

Results

One hundred and six CAPD fluids were examined during the study. Of these, 69 were culture positive—17 with Gram negative bacteria and 54 with Gram positive bacteria (two specimens yielded both Gram negative and Gram positive organisms). Of the 17 fluids that yielded a Gram negative organism, 11 were positive in the LAL assay, while of the 91 fluids that did not yield a Gram negative organism on culture, two were positive in the LAL assay. In

Table 1 Comparison of LAL assay with culture for diagnosis of CAPD peritonitis

LAL	Culture (Gram negative bacteria)	
	Positive	Negative
Positive	11	2*
Negative	6	89
Totals	17	91

*Gram negative organisms seen at microscopy in one case.

Table 2 Association between culture, microscopy, and LAL assay in laboratory diagnosis of CAPD peritonitis

Organism	Number	Microscopy		LAL	
		Positive	Negative	Positive	Negative
<i>E coli</i>	4	4	0	4	0
<i>Pseudomonas</i> spp	4	4	0	3	1
<i>Serratia</i> spp	2	1	1	1	1
<i>E cloacae</i>	2	1	1	1	1
<i>Klebsiella</i> spp	3	3	0	2	1
<i>Compylobacter jejuni</i>	1	0	1	0	1
<i>Bacteroides catarrhalis</i>	1	0	1	0	1
Totals	17	13	4	11	6
<i>S epidermidis</i>	31	25	6	0	31
<i>viridans Streptococci</i>	6	6	0	0	6
<i>S aureus</i>	5	5	0	1	4
<i>Corynebacterium</i> spp	4	2	2	0	4
<i>Candida albicans</i>	4	4	0	0	4
<i>S hominis</i>	2	1	1	0	2
<i>S warneri</i>	1	1	0	0	1
<i>Enterococcus faecalis</i>	1	0	1	0	1
Totals	54	44	10	1	53

Table 3 Sensitivity of LAL assay in water and peritoneal fluid

Endotoxin concentration EU/ml (pg/ml)	Diluent	
	Water	Used peritoneal fluid
1.0 (100)	+	+
0.5 (50)	+	+
0.25 (25)	+	—
0.12 (12)	+	—
0.06 (6)	—	—
Negative control	—	—

one of these fluids, however, Gram negative bacilli were seen at microscopy but failed to grow on culture. The remaining LAL positive (culture negative for Gram negative bacteria) fluid yielded *Staphylococcus aureus* only and did not show the presence of Gram negative bacteria at microscopy. All 37 culture negative cases were negative in the LAL assay. These results are summarised in table 1. Therefore the sensitivity and specificity of the LAL assay with respect to culture were 65% and 98%, respectively.

Of 17 Gram negative isolations, 13 were detected at microscopy. Therefore the sensitivity of microscopy compared with culture was 76%. Of 54 Gram positive isolations, 44 were detected at microscopy. Overall, 57 of the 71 isolates were detected at microscopy (sensitivity 80%). These results, together with the respective LAL assays, are shown in table 2.

To determine the sensitivity of the LAL assay in peritoneal dialysis fluid compared with water, serial dilutions of an endotoxin standard were prepared in sterilised, distilled water and used, filter sterilised peritoneal dialysis fluid. The LAL assay achieved the stated sensitivity of the lysate batch (0.12 EU/ml) in water, but there was a fourfold decrease in sensitivity to 0.5 EU/ml using peritoneal dialysis fluid (table 3). Tenfold dilutions of a broth culture of *E coli* NCTC 10418 were prepared in distilled water and used, filter sterilised peritoneal dialysis fluid, and each dilution was then subjected to LAL assay and a count for viability. The LAL assay detected 1000 CFU *E coli*/ml in both distilled water and used peritoneal dialysis fluid.

Discussion

The primary aim of this study was to evaluate the application of the LAL assay in differentiating Gram positive from Gram negative peritonitis. During the study, however, other important aspects of the laboratory diagnosis of peritonitis in patients with CAPD became apparent and warrant comment.

The Gram stain was more sensitive than has been previously reported. Knight *et al*⁵ reported the sensitivity of Gram stain to be 16% overall, and 19% when compared with culture. Other workers have reported sensitivities ranging from 9% to 50%.⁶ In our study 57 of 71 (80%) culture positive episodes of peritonitis were detected by Gram stain.

Another interesting feature that emerged was the relation between culture and leucocyte count. The mean leucocyte count in cases of

peritonitis with Gram positive organisms was 4200/ μ l compared with 380/ μ l from cases with Gram negative organisms (results not shown). In one case of peritonitis from which *Enterobacter cloacae* was isolated the cell count was only 10 white cells/ μ l. This reduced leucocyte response may be related to the reportedly increased morbidity associated with Gram negative CAPD peritonitis.⁷

Our study showed that the LAL assay was specific but insensitive for the diagnosis of CAPD peritonitis. This contrasts with the results of other investigators. Smalley *et al*⁴ used a chromogenic assay and reported a sensitivity of 100%, but five of 63 culture negative fluids were LAL positive (specificity 92%), and nine of 49 fluids which contained Gram positive organisms only were also LAL positive (specificity 82%). Using a gel-clot assay, Clayman *et al*² reported a sensitivity of 100% and a specificity of 86% and, similarly, McCartney *et al*³ reported a sensitivity and specificity of 100%. These were small studies, however, with only three cases of Gram negative peritonitis in each.

The favourable sensitivity figure (0.01 EU/ml) reported by Clayman *et al*² may have been due, in part, to the criterion they used to define a positive LAL assay. They defined a positive LAL assay as one that formed a partially stable clot, whereas the more widely accepted criterion for a positive LAL assay requires the clot to be stable through a 180° inversion of the tube. Their sensitivity was roughly 10 times that which we and McCartney *et al*³ achieved (0.12 EU/ml). The sensitivity achieved by Clayman *et al*² in used peritoneal dialysis fluid (0.20 EU/ml), however, was similar to ours (0.50 EU/ml) and to that of McCartney *et al* (0.25 EU/ml).³

We determined the sensitivity of the LAL assay to be 1000 bacteria/ml using a viable count assay, or 0.5 EU/ml (50 pg/ml) using a

diluted endotoxin standard. This is consistent with the observation that a single Gram negative bacterium contains 40 fg of endotoxin.⁸ This is similar to the finding of McCartney *et al*,³ who reported a theoretical detection limit of 500 bacteria/ml based on their assay sensitivity of 0.25 EU/ml (25 pg/ml) and each bacterium containing 40 fg of endotoxin. Our findings, however, do not support the comments by McCartney *et al*³ that 100 000 bacteria/ml are required before the Gram stain is able to detect them.

In our study the sensitivity of the Gram stain was 80%; this is dependent on volume centrifuged, resuspension volume, time and speed of centrifugation and, to some extent, the experience and time spent by the examiner. Accordingly, we recommend that laboratories evaluate their Gram stain procedure in an effort to improve sensitivity, and suggest that the LAL assay is not a satisfactory substitute for a well performed Gram stain.

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