Correlation of Cerebrospinal Fluid Endotoxinlike Activity with Clinical and Laboratory Variables in Gram-Negative Bacterial Meningitis in Children

TERRY L. DWELLE,†Lisa M. DUNKLE, and LAURA BLAIR
Department of Pediatrics, St. Louis University School of Medicine, and Division of Infectious Disease, Cardinal Glennon Memorial Hospital, St. Louis, Missouri 63104

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Detection of endotoxinlike activity in cerebrospinal fluid by Limulus amebocyte lysate gelation has been suggested as a useful technique for the diagnosis of gram-negative bacterial meningitis. We prospectively screened 1,503 cerebrospinal fluid specimens with a Limulus amebocyte lysate microassay. The limit of sensitivity of the assay was 0.01 ng/ml. All specimens that were positive for endotoxinlike activity were subjected to confirmatory retesting, after which 38 (86%) remained positive. Comparison with available culture results revealed that 33 of 38 specimens (86%) were culture positive; 3 of the 5 culture-negative specimens were from patients on therapy for gram-negative bacterial meningitis, and 1 was from a neonate. The overall specificity of confirmed positive tests was 99.5%, with a positive predictive value of 97.3%. There was one false-negative specimen, giving an overall sensitivity of 97.3% and a negative predictive value of 99.9%. Endotoxinlike activities of \( \geq 150 \text{ ng/ml} \) correlated with present illness of less than 2 days' duration \( (P = 0.024) \), elevated cerebrospinal fluid protein \( (P < 0.05) \), and seizures \( (P = 0.004) \); levels of \( \geq 3,000 \text{ ng/ml} \) correlated with neutropenia \( (P = 0.032) \), and levels of \( \geq 3.2 \times 10^6 \text{ ng/ml} \) correlated with death \( (P = 0.001) \). We conclude that the Limulus amebocyte lysate microslide gelation test has prognostic value as a sensitive, specific, simple, inexpensive screening test for gram-negative bacterial meningitis.

The Limulus amebocyte lysate (LAL) test is sensitive in detecting gram-negative bacterial meningitis, with generally less than 1% false-negative results (1, 9, 11, 20, 25, 27, 28, 30; Whittaker M. A. Bioproducts, Quantitative Chromogenic LAL Third Generation Pyrogen Testing [product information booklet], p. 18, 1986). By counterimmune electrophoresis correlations between antigen levels, convulsions, and bacteremia were demonstrated (13), yet in one quantitative study no correlation could be shown between initial cerebrospinal fluid (CSF) endotoxin levels and clinical or laboratory variables (1). A small, preliminary study from our laboratory in 1978 demonstrated an association of CSF endotoxinlike activity (ELA) of \( > 1,200 \text{ ng/ml} \) with shock, cyanois, and death in patients with gram-negative bacterial meningitis. This study was undertaken to clarify this association as well as to evaluate the sensitivity of a modified LAL microassay.

MATERIALS AND METHODS

Specimens. A total of 1,503 consecutive specimens of CSF submitted to the Microbiology or Infectious Disease Laboratory were tested without prior knowledge of culture results. These were collected in sterile endotoxin-free plastic containers and refrigerated (4°C) until delivery to the laboratory, at which time they were frozen (−20°C) until assay.

LAL microslide gelation test. All glassware (Acupipettes, 10, 20, 50, and 100 μl, and latex agglutination slides; Dade Diagnostic, Inc.) was rendered endotoxin free by heating to 175°C for 2 to 3 h. Clinical samples (20 μl) were added to 20 μl of Limulus reagent (E-Toxate, Sigma Chemical Co., St. Louis, Mo.; Pyrotell, Associates of Cape Cod, Woods Hole, Mass.) on an endotoxin-free latex agglutination slide. Negative and positive controls (Pyrotrol; Difco Laboratories, Detroit, Mich.) were tested in a similar fashion. Air bubbles were eliminated by using a needle that was previously heated red hot and allowed to cool. The slides were then incubated for 60 min in a moisture chamber at 37°C and observed for gel formation from a horizontal position via gentle tilting.

Quantitation of ELA. All specimens, where volume permitted, were quantitated for ELA. Dilutions of specimens were performed in sterile plastic tubes (Falcon, 10 by 75 mm; Becton Dickinson Labware, Oxnard, Calif.) with pyrogen-free water (10-ml Dossette ampoules, nonbacteriostatic water; Elkin-Sinn, Inc.) that had been previously demonstrated negative for ELA. Serial twofold dilutions were performed to a gelation endpoint and compared with an Escherichia coli standard (Pyrotrol) diluted to the final endpoint. A quantitative calibration was performed: (dilutions to final endpoint) \( \times \) (endotoxin quantity in endpoint of standard) = (ELA of sample).

Record review. Medical records were reviewed for clinical and laboratory parameters, including age, duration of illness, previous antibiotic therapy, initial vital signs, physical examinations, peripheral blood count, CSF cell count and chemistries, serum electrolytes, bacterial cultures of blood and CSF, radiographic and computed tomographic studies, presence or absence of seizures, subdural effusions, neurologic or other sequelae, total duration of fever, and results of electroencephalograms and audiograms. The statistical significance was determined by the chi-square or Fisher's exact test as applicable.

* Corresponding author.
† Present address: Department of Pediatrics, University of North Dakota School of Medicine, Bismarck, ND 58501.
Bacteriologic analysis. CSF and blood were cultured in the Microbiology Laboratory by standard microbiologic procedures (18, 19, 26).

RESULTS

Of 1,503 CSF specimens evaluated, 44 contained ELA. Comparison with the culture results available for 1,491 specimens revealed a sensitivity of 97.3%, a specificity of 99.5%, a false-negative rate of 2.7%, and a false-positive rate of 0.5% on initial testing. These results yielded a positive predictive value of 83.7% and a negative predictive value of 99.9%. On retesting of all positive specimens (same sample) the false-positive rate fell to 0.06%, with a positive predictive value of 97.3%. The sensitivity of LAL lots varied from 0.01 to 0.35 ng of *E. coli* endotoxin standard per ml.

Sufficient volume was available for 33 specimens from patients with gram-negative meningitis (29 *Haemophilus influenzae* type b, 2 *Neisseria meningitidis*, 1 *Pseudomonas aeruginosa*, 1 *E. coli*) to allow ELA quantitation. ELA values ranged from 0.1 ng/ml to 512 mg/ml, with a mean of 101 mg/ml and a median of 1,028 mg/ml. An ELA of ≥150 mg/ml was significantly associated with present illness of less than 2 days' duration (10 of 10 less than 2 days versus 9 of 17 greater than 2 days; P = 0.024), CSF protein of >150 mg/100 ml (12 of 15 >150 mg/100 ml versus 6 of 14 <150 mg/100 ml; P < 0.05), and seizures during the hospital course (10 of 10 with seizures versus 10 of 21 without seizures; P = 0.004). An ELA of <1,000 ng/ml was significantly associated with peripheral leukocytosis (8 of 19 with leukocytosis versus 0 of 12 without leukocytosis; P = 0.020), whereas an ELA of ≥3,000 ng/ml was associated with neutropenia (4 of 12 with neutropenia versus 0 of 19 without neutropenia; P = 0.032). An ELA of ≥3.2 × 10^6 ng/ml was significantly associated with death (3 of 4 died with an ELA of ≥3.2 × 10^6 ng/ml versus 0 of 29 with an ELA of <3.2 × 10^6; P = 0.001).

The following other clinical laboratory parameters evaluated were not significant (P > 0.05): leukopenia, decreased hemoglobin, thrombocytosis or thrombocytopenia, CSF leukocytosis, CSF glucose of <40 mg/100 ml, bacteremia, serum sodium, etiologic agent, fever, shock, and neurologic sequelae.

Seven patients, aged 1 day to 18 months, with CSF ELA values from 0.4 × 10^6 to 512 × 10^6 ng/ml were noted. All three patients who died during the study period were from this group. Two of the four survivors in this group have no apparent sequelae, one has a seizure disorder, and one has hearing impairment and hemiparesis.

Pretreatment with antibiotics was commonly associated with lower ELA values (5 of 11 with an ELA of <100 ng/ml versus 5 of 20 with an ELA of ≥100 ng/ml); however, this association did not reach statistical significance (P > 0.10).

Similarly, there was a suggestion that an ELA of >50,000 ng/ml was associated with an age of less than 6 months (4 of 9 <6 months versus 3 of 22 ≥6 months; P > 0.10) and that an ELA of ≥3,000 ng/ml was associated with hypoglycorrachia (CSF glucose, <40 mg/100 ml) (9 of 19 with hypoglycorrachia versus 1 of 10 without hypoglycorrachia; P = 0.051).

DISCUSSION

Minute quantities of endotoxin have been shown to produce gelation of blood from *Limulus polyphemus* (22), and numerous studies have confirmed the utility of the LAL assay in the diagnosis of gram-negative bacterial meningitis, generally approaching a sensitivity of 99.5%, specificity of 99.8%, positive predictive value of 99.9%, and negative predictive value of 99.0% (1, 11, 20, 25, 26, 30). The ELA value of 0.01 to 0.35 ng of *E. coli* endotoxin standard per ml, versus than 2 days' duration (10 of 10 less than 2 days versus 9 of 17 with death (3 of 4 died with an ELA of >3.0 ng/ml)) was significantly associated with peripheral leukocytosis (8 of 19 with leukocytosis versus 0 of 12 without leukocytosis; P = 0.020), whereas an ELA of ≥3,000 ng/ml was associated with neutropenia (4 of 12 with neutropenia versus 0 of 19 without neutropenia; P = 0.032). An ELA of ≥3.2 × 10^6 ng/ml was significantly associated with death (3 of 4 died with an ELA of ≥3.2 × 10^6 ng/ml versus 0 of 29 with an ELA of <3.2 × 10^6; P = 0.001). The following other clinical laboratory parameters evaluated were not significant (P > 0.05): leukopenia, decreased hemoglobin, thrombocytosis or thrombocytopenia, CSF leukocytosis, CSF glucose of <40 mg/100 ml, bacteremia, serum sodium, etiologic agent, fever, shock, and neurologic sequelae.

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>50,000 ng/ml and that pretreatment with oral antibiotics was associated with an ELA of <100 ng/ml.

The role of CSF endotoxin in peripheral effects is unclear, inasmuch as the blood-brain barrier may be impermeable to endotoxin (5). In this study, peripheral leukocytosis was associated with a CSF ELA of <3,000 ng/ml, and neutropenia was associated with an ELA of ≥3,000 ng/ml, even though other parameters classically associated with endotoxia (thrombocytopenia, proteinuria, hynpanetria, elevated blood urea nitrogen, hypothermia, and hypertension) were not significantly correlated. Concomitant endotoxia or endotoxin mediators may explain this result.

The sensitivity of the LAL microslide gelation test (0.01 to 0.35 ng/ml) is similar to that of the tube gelation assay (0.1 to 1.0 ng/ml) (4, 21) but generally less than that of the chromogenic substrate assay (0.001 to 0.2 ng/ml) (2, 23; product information booklet, Whittaker M. A. Bioproducts).

That the CSF ELA in patients with meningitis ranged from 0.1 ng/ml to 512 mg/ml (mean ELA, 1.028 ng/ml) and the sensitivity of LAL lots varied from 0.01 to 0.35 ng/ml may explain the one negative LAL assay with subsequent positive culture, although this should not occur commonly, as reflected in the sensitivity of 97.3%. The microslide, tube gelation, and chromogenic substrate assays cost approximately $0.40, $2.00, and $1.33, respectively.

In summary, the LAL microslide gelation test exhibits prognostic value as a sensitive, specific, simple, inexpensive screening test for gram-negative bacterial meningitis. Specimens exhibiting positive gelation should be retested for confirmation.

LITERATURE CITED