Lactic Acid in Cerebrospinal Fluid: Evaluation and Application of an Automated Enzymatic Assay

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ABSTRACT

The DuPont ACA lactic acid procedure was evaluated for use on cerebrospinal fluid (CSF). The method was linear to at least 13.9 mmol per L, day-to-day precision ranged from 3 to 9 percent (CV), and recovery of lactate added to cerebrospinal fluid averaged 104 percent. The method correlated closely with a centrifugal analyzer procedure; r = 0.996, SEy,x 0.16 mmol per L. The reference range, based on patient CSF samples, was 0.8 to 2.3 mmol per L. Elevated CSF lactate concentrations were found in patients with bacterial, but not viral, meningitis. Less marked elevations occurred with head trauma, seizures, strokes, or hemorrhage in the central nervous system. The results suggest that this method is useful in the early diagnosis of bacterial meningitis.

Introduction

Recent reports suggest that cerebrospinal fluid (CSF) lactate measurements could be useful in the early diagnosis of bacterial meningitis. Cerebrospinal fluid lactate is elevated even in the early stages of bacterial meningitis and in partially treated bacterial meningitis. The false negative rate for CSF lactate in bacterial meningitis has been reported to be less than 3 percent. These reports notwithstanding, lactic acid determinations have not gained widespread use which, in part, reflects the problem of providing results rapidly enough for the clinician to use the data at the time of decision. Additionally, there is controversy whether or not the CSF lactate concentration can be used to distinguish bacterial from viral meningitis. Thus, it is not clear whether or not small overlaps between viral and bacterial meningitis in some studies were due to the poor precision of the assay.

Our evaluation of the DuPont ACA for CSF lactate determinations is described.
This analytical instrument is designed to provide rapid results and is especially useful for STAT requests. This lactic acid method appears, in our opinion, to provide the accuracy and precision required for diagnostic studies of CSF. Using this method, the clinical findings of previous workers have been confirmed and extended.

Materials and Methods

Apparatus

The instrument used was a DuPont ACA III.* The centrifugal analyzer used was a Rotochem IIa/36† with a Rotofill II dilutor for sample, reagent, and diluent delivery.

Reagents

Centrifugal analyzer reagent‡ is prepared and used as described previously.¹ DuPont ACA III Reagent uses LA test packs as provided.

Standards

Stock lactate standard (100 mmol/L). Exactly 4.80 g of lithium L(+)-lactate§ are transferred to a 500-ml volumetric flask containing approximately 300 ml of distilled water. The lactate is dissolved and diluted to volume with distilled water. This standard solution is stable for six months when stored in an amber-colored bottle at 4°C. This standard is diluted with distilled water to provide samples for the aqueous linearity study.

DuPont calibrator solutions. Solutions are obtained from DuPont Instruments Co. to provide a three-point calibration. This calibration is performed whenever the lot number of the reagent changes.

Cerebrospinal Fluid Specimens

The CSF specimens were obtained from a patient who had died of a myocardial infarction and who had an elevated CSF lactate concentration and from a patient with low back pain undergoing a mylogram whose CSF lactate was low. These specimens were filtered through 0.47 micron filters and aliquots were stored at 4°C in 8 x 30-mm glass tubes covered by four layers of Parafilm. Lactate in these pools was stable for at least four months. These samples were used for linearity and precision studies. For the comparison study of the ACA and the Rotochem, CSF samples submitted for routine protein and glucose measurements were used.

Procedures

DuPont ACA III Method. This method involves the oxidation of L-lactate by a molar equivalent of nicotinamide adenine dinucleotide (NAD⁺) in the presence of lactate dehydrogenase giving pyruvate and nicotinamide adenine dinucleotide, reduced form (NADH). The reaction mixture contains hydrazine to react with the pyruvate forcing the indicator reaction to completion. The absorbance due to NADH is directly proportional to the lactate concentration and is measured using a two-filter (340 to 383 nm) technique. The instrument is calibrated and operated according to the manufacturer's instructions. All patient samples were analyzed in duplicate to verify that an adequate volume of CSF was sampled. The first of the duplicate results was used for all statistical analyses.

Centrifugal analyzer method. The procedure used was the one described by Al­derman and Cross.¹
TABLE I
Precision Studies*

<table>
<thead>
<tr>
<th>Method</th>
<th>1 mmol/L</th>
<th>2 mmol/L</th>
<th>5 mmol/L</th>
<th>S.D.</th>
<th>C.V. (Per-</th>
<th>S.D.</th>
<th>C.V. (Per-</th>
<th>S.D.</th>
<th>C.V. (Per-</th>
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<tr>
<td>DuPont ACA</td>
<td>0.07</td>
<td>3.6</td>
<td>0.07</td>
<td>1.4</td>
<td>0.09</td>
<td>0.76</td>
<td>1.15</td>
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<tr>
<td>Centrifuge</td>
<td>0.08</td>
<td>4.4</td>
<td>0.19</td>
<td>3.6</td>
<td>0.14</td>
<td>1.15</td>
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| *n = 20 for each result.

Results
LINEARITY

It was established that the DuPont ACA method was linear up to 15 mmol per L using sequential dilution of the stock lactate standard with distilled water. Linearity with CSF also was established by diluting a sample of CSF containing a high lactate concentration (13.9 mmol per L) with a sample containing only 1.9 mmol per L. Plots of both linearity studies intersected the origin.

PRECISION

Replicate measurements were performed on three CSF pools and the results of within-run and day-to-day precision are given in table I. For the within-run studies, 60 samples, 20 of each level, were analyzed sequentially on the DuPont ACA. Day-to-day precision was studied over a period of five months. The precision estimated from duplicate analyses of patient samples (N = 221) over a three month period was 0.13 mmol per L (S.D.).

RECOVERY

Recovery was determined by adding aqueous stock lactate standard to five 0.5 ml aliquots of CSF pool. The amount of lactate added provided a range to 11 mmol per L lactate.

The amount of lactate recovered was 104 ± 2 percent of expected.

COMPARISON STUDY

Lactic acid results obtained with the DuPont ACA were compared with the results obtained using the centrifugal analyzer method. Measurements were performed on 102 patient samples spanning a concentration range of 0.6 to 9.0 mmol per L, having a correlation coefficient of 0.996, a y intercept of the regression line of 0.18 mmol per L and a slope of 0.95. The standard error between x and y was 0.16 mmol per L lactate.

REFERENCE RANGE

Using the DuPont ACA method, the lactate concentrations in CSF were measured from 36 patients with no evidence of meningitis, head trauma, stroke, seizure, or hemorrhage in the central nervous system* and the results are given in figure 1. These data appear to approximate a gaussian distribution. The mean ± 2 S.D. was 0.6 to 2.4 mmol per L while the central 95 percent of values were in the range 0.8 to 2.3 mmol per L.

CSF LACTATE LEVELS IN CNS DISORDERS

The CSF lactate levels on 38 patients were grouped by diagnosis and are shown in figure 2. Markedly elevated results were seen in all patients with bacterial meningitis. Less marked elevations were seen in other conditions. Circled points in figure 2 indicate patients that died owing to the diagnoses listed.2 It was unclear from the medical records if the patient who died during seizures and the patient

* These patients had the following diagnoses: 16 low-back pain, myelogram, or herniated disc; 2 premature births; 2 metastatic Ca of prostate; one each of Crouzon’s disease, oat cell carcinoma, earache and sore throat, viral pneumonia, vasovagal syncope, acute lymphocytic leukemia, chronic lymphocytic leukemia, cerebral palsy, diarrhea secondary to Salmonella enteritis, right arm weakness, upper G. I. bleed, normal pressure hydrocephalus, “Flu,” paresthesias of chest and arm, Parkinson’s disease, and progressive systemic sclerosis.
who died of a stroke had undergone anaerobic brain death at the time the CSF sample was obtained.

Discussion

Several methods have been used for the determination of lactic acid in clinical samples. Gas chromatography and manual enzymatic methods were used in the exploratory studies which established the potential clinical usefulness of CSF lactate levels.\(^2,3,4,5,7,8,9,10,11,12\) In most of these studies, sample volumes of 0.5 to 1.0 ml were required. A centrifugal analyzer method has also been described\(^1\) and was used as our reference method. None of these early methods was designed to analyze a single sample on a STAT basis. The DuPont ACA procedure is suited for such a STAT service and has the added advantage of using only 40 \(\mu\)l of CSF.

The DuPont ACA procedure for CSF lactate was found to be precise and linear within the necessary range and to correlate with a well-established centrifugal analyzer procedure. The reference range agreed with the studies of Brook et al\(^3\) and Knight et al\(^9\) who concluded that CSF lactate concentrations below 2.8 mmol per L were compatible with either viral or no meningitis.

As shown in figure 2, CSF lactate concentrations above 9 mmol per L were found in bacterial meningitis. Intermediate levels were found in head trauma, stroke, and seizures. These three conditions are important in the differential diagnosis of bacterial meningitis. However, head trauma and stroke are usually identifiable from history, physical examination, and laboratory findings. Of potentially greater importance are seizures in children, which may be benign (febrile) or indicative of other disease. Fortunately, CSF lactic acid concentrations are not increased after first febrile convulsions lasting less than 30 minutes,\(^2,13\) so that an elevated CSF lactate in such a setting should prompt a search for other causes.\(^14\)

In the diagnosis of bacterial meningitis, the sensitivity and specificity of CSF lactate measurements appear excellent. In the present study and previous studies,\(^2,3,5,6,7,9,10,11,12\) 203 patients with bacterial meningitis have been studied. Of these, 197 or 97 percent have had increased CSF lactic acid concentrations, whereas 138 of 147 (94 percent) patients with viral meningitis had normal concentrations. The sensitivity for tuberculous meningitis appears similar to that for bacterial meningitis (95 percent, \(n = 21\)). Much lower sensitivities have been reported in cryptococcal (33 percent) and coccidioidal (25 percent) meningitis, but published reports include only a few pa-

\(\dagger\) Deaths were due to cardiopulmonary arrest secondary to status epilepticus, massive stroke, hemophilus influenzae meningitis (2), and pneumococcal meningitis.
patients (6 and 4, respectively). These results must be interpreted in light of the fact that most studies have not included data on the accuracy and precision of their assays. Acceptable accuracy and precision have been demonstrated by the present authors of the DuPont ACA CSF lactate assay, which should permit more extensive use of CSF lactate as a diagnostic aid in suspected bacterial meningitis.

Acknowledgments

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References


