Cerebrospinal Fluid Protein Determination

Methods

Cerebrospinal fluid (CSF) protein concentration at Moffitt/Long Hospital and the VA Hospital is usually measured on the DuPont automated clinical analyzer (ACA) by a turbidometric procedure using trichloroacetic acid (TCA). The method used at SFCH is a dye binding assay using Coomassie Brilliant Blue G-250 (CBB). The Ames urine dipsticks (Albustix, Multistix) contain a protein reagent which may be useful as a rapid screening test for clinically significant elevation of CSF protein.

The ACA uses TCA to precipitate CSF proteins, and light scattering from this precipitate is measured by decreased transmission at 340 nm. The reagent pack contains 195 microliters of 4 M TCA, to which is added 300 microliters of CSF and 4.7 ml of water. The reaction is complete within 40 seconds, the result is printed to the nearest mg/dl, and the method is claimed linear from 10 to 200 mg/dl (1). The reference range is 15-50 mg/dl for adults, and is probably accurate for children older than 3 months. Neonates have an upper normal limit as high as 150 mg/dl, which has been attributed to immaturity of the blood-CSF barrier.

The CBB method utilizes the "protein error of indicators" phenomenon of dye-protein complex formation. When a pH indicator dye is properly buffered, it will exhibit its typical pH-dependent color change after protein binding at constant pH (2). The buffered CBB reagent in the kit (3) is prepared by mixing the dye and a stabilizer reagent, is stable for four months, and can also be used for urine protein measurement. Fifty microliters of CSF is mixed with four ml of working reagent, incubated for 20 minutes at 37 degrees C, and the resulting steel gray color is read at 610 nm. The method is claimed to be linear from 0-100 mg/dl, and the reference range is 10-45 mg/dl. A CBB kit from a different manufacturer is used as the backup manual method at Moffitt/Long.

The Ames urinary dipstick can be used as a screening test for elevated CSF protein. The protein reagent contains 3,3',5,5'-tetrabromophenol-sulfonphthalein (tetrabromophenol blue), heavily buffered to pH 3.5 so that the "protein error of indicators" principle applies (4). The CSF is applied to the reagent pad (by dipping or use of a pipet), excess fluid is wiped off, and the color is compared to printed standards. The standard colors have numerical values and the protein concentration is estimated as follows: 1+, 30 mg/dl; 2+, 100 mg/dl; and 3+, 300 mg/dl.
Technical Limitations

CSF protein measurement can be difficult because the concentration of protein is low, and the concentration of inorganic ions and potentially interfering nonprotein substances is relatively high (5). The ACA and CBB methods have sufficient sensitivity at the expected protein concentrations and are not affected by other substances normally found in CSF. The ACA eliminates temperature-induced variations in protein particle size which plagued TCA assays, and there are few interfering substances. Intrathecal methacillin elevates the measured protein level, but this is not a significant problem with systemic therapy (6). Turbid, hemolyzed, and xanthochromic specimens can also cause false elevation (but are obvious to the technologist), and the radiographic contrast agent metrizamide can also produce a false positive. The CBB method described here is more demanding of temperature control and time than the original assay (2), but has better linearity in the upper range. Significant false elevations have been reported with acetone and some detergents, so glassware must be clean. The assay cannot be done in plastic tubes (to which the dye adheres) and the specimen must not be frozen (causing falsely decreased values). Both the TCA and CBB methods are affected by the albumin/globulin ratio of the sample. CBB is about twice as sensitive to albumin, and TCA is about 20% more sensitive to globulins. The problem is minimized by calibrating with protein standards similar to CSF (normal albumin range 56-76%). If the standard has 70% albumin and 30% globulin, the CBB method will have a maximum error of 10% if the sample has 45-97% albumin (7). Most CSF specimens have an A/G ratio within or close to the normal range because the selectivity of the blood-CSF barrier is not significantly changed by most pathologic processes (although the permeability is altered, causing elevated total protein).

Clinical Applications and Limitations

The CSF protein measurement is a traditional part of the STAT test battery ordered when acute infectious meningitis is suspected. The recommended STAT tests include cell count, glucose, protein, and Gram stain and can usually establish or rule out the diagnosis and suggest treatment (8). Acute meningitis is a medical emergency and housestaff are encouraged to perform lumbar puncture "at the slightest suspicion of meningitis," (9) which may result in a low yield of positives (13.9% in this series). Since most lumbar puncture patients do not have meningitis, the CSF tests chosen must have a high level of sensitivity. The specificity is less important, since the consequences of untreated bacterial meningitis outweigh any problems due to antibiotic treatment of patients without disease. Thus, although CSF testing can contribute substantially to the STAT laboratory workload (over 150 specimens monthly at SFGH), clinical considerations are more important when selecting the optimal test strategy.

The CSF protein determination, although traditional, may add little information to that obtained by other tests. Several studies have calculated the sensitivity and specificity of various CSF tests, with particular regard for the problem of Gram-stain-negative acute bacterial meningitis (10-13). The "gold standard" was a positive culture (or, less commonly, bacterial antigen detection), and results were as follows:
The diagnostic value of CSF protein testing is limited because the protein is less than 100 mg/dl in up to 28% of bacterial meningitis cases, and a critical value only slightly above the reference range is needed to obtain a high sensitivity. The positive predictive value is then too low to be clinically useful, and the deceptively high negative predictive value merely reflects the fact that bacterial meningitis patients seldom have normal CSF protein. Raising the critical value to 100 mg/dl markedly improves the specificity, since few cases of viral or "aseptic" meningitis have this much protein; but the corresponding decrease in sensitivity means many cases of meningitis will be missed. Sensitivity is a problem with several other CSF tests because the usual aggressive approach to patients with meningitis symptoms probably leads to many "taps" in the early stages of infection, when CSF constituent levels are likely to be only slightly abnormal. The cell count and differential are the best tests for meningitis screening if the Gram stain is negative, and have the added advantage of speed and simplicity. The protein can be nearly as effective if the critical value is set at 100 mg/dl, although the results of the former tests would be available first in laboratories without an automated protein method. A CSF protein level of 200 mg/dl or greater could conceivably be used to give a 100% positive predictive value, but would miss many cases and provide information already available with the more specific glucose and CSF-serum glucose ratio.

Rapid Screening of CSF Protein

The coincidence of the 100 mg/dl protein level causing a color change with the Ames dipstick protein reagent, as well as being the best value for meningitis screening, suggested a comparative study. Three hundred forty-two CSF specimens at SFGH were tested for protein using both the CBB method and the dipstick during August to November 1984 (14). With a CBB protein level of 100 mg/dl or greater taken to represent "disease", the sensitivity (dipstick result of 2+ or greater) was 93.5%. The specificity (dipstick result of 1+ or...
less) was 91.2%. The prevalence of CSF proteins of 100 mg/dl or greater in adult patients was 9.8%, so the positive predictive value was 53.6%, and the negative predictive value was 99.2%. Since 80% of CSF specimens had a dipstick reading of 1+ or less, the use of this screening technique theoretically eliminates the need for STAT protein assays on the majority of specimens. This can reduce the laboratory workload considerably when an automated method is not available, providing that an agreement can be worked out with the various clinical services to accept the dipstick results.

References


