Detection of CSF Proteins Using LC-MS/MS:

Measurement of Beta-Trace Protein as an Indicator of a CNS Breach

Kari M. Gurtner[1], David R. Barnidge[1], Melissa R. Snyder[1], Maria Alice V. Willrich[1], Erin K. Obrien[2], and David L. Murray[1]

[1]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN

[2]Department of Otorhinolaryngology, Mayo Clinic, Rochester, MN

Introduction: Detection of cerebrospinal fluid (CSF) in clinical specimens is the most sensitive method for diagnosis of a breach of the central nervous system (CNS). Currently, two separate methods are widely employed to detect CSF: immunofixation electrophoresis (IFE) to identify Beta2-transferrin (ß2-Trf) and nephelometric quantification of beta-trace protein (prostaglandin H2 D-isomerase (PH2D)). Both methods rely on the detection of a single marker for CSF and have limitations in the presence of serum contamination. The staining pattern of ß2-Trf on an IFE gel can be overwhelmed by non-CSF proteins or affected by bacteria within the sample. With nephelometry, small amounts of beta-trace from serum can be misinterpreted as coming from CSF. To overcome these limitations, monitoring proteotypic peptides by LC-MS/MS in both CSF abundant beta-trace and serum abundant complement component C3 provide accurate detection of CSF along with a marker of serum contamination. Peptide analysis by LC-MS/MS is not affected by non-CSF protein contamination or bacterial deglycosylation. Measuring beta-trace by LC-MS/MS adds diagnostic value when run concurrently with ß2-Trf detection by IFE.

Methods: Residual CSF and serum were blended to compare the sensitivity between two methods of CSF fluid detection. Body fluids routinely submitted for CSF detection were analyzed by IFE for ß2-Trf and beta-trace by LC-MS/MS. The ß2-Trf was measured by a validated method using an agarose gel immunofixed with an antiserum to human transferrin. The LC-MS/MS detection of the beta-trace and C3 peptides was accomplished using 10 microliters of body fluid suspended in 50 mM ammonium bicarbonate, treated with 2,2,2–Trifluroethanol, and reduced with dithiothreitol. Samples were then alkylated with iodoacetamide, and digested with trypsin for 2 hours. Proteotypic peptides from beta-trace were monitored by SRM on a Thermo TLX-2 LC system coupled to an AB-SCIEX API- 5000.

Results were qualitatively reported as positive or negative compared to a 30:70 pooled CSF:serum standard.

Results: From the 104 fluids tested, 100 body fluids (96%) were in agreement between the two assays and four were discrepant (4%). Of those four discrepant fluids, one was negative by LC-MS/MS and positive by IFE. It had been extracted from a cotton swab with saline prior to testing, falsely decreasing the final beta-trace concentration to be below the positive cutoff level. The three other discrepant samples tested were positive by LC-MS/MS and negative by IFE. One of these samples had a significantly increased C3 peptide peak area, suggesting serum contamination; as for another sample, the patient had returned to the operating room and a CSF leak was confirmed visually. These three fluids demonstrate the increased sensitivity of the LC-MS/MS methodology.

Conclusion: Detection of beta-trace by LC-MS/MS shows superior sensitivity in body fluids containing non-CSF proteins over ß2-Trf detection by IFE. Measurement of beta-trace by LC-MS/MS along with detection of ß2-Trf by IFE can confirm the diagnosis of a cerebrospinal fluid leak with increased sensitivity and specificity compared to using IFE alone.