A new nephelometric assay for β-trace protein (prostaglandin D synthase) as an indicator of liquorhooea

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Abstract

Objectives—To determine the sensitivity and specificity of a nephelometric β-trace protein assay for the diagnosis of liquorhooea.

Methods—One hundred and forty clinical samples with suspected liquorhooea were analysed by a newly developed nephelometric assay. An established electroimmunoassay served as a reference method. The sensitivity and specificity of the β-trace nephelometric assay were calculated by a 2x2 contingency table for 10 different versions of a dichotomised nephelometric variable. In 52 patients (79 samples), the nephelometric findings were validated by referring to the clinical diagnosis based on the course of the disease, imaging techniques, and surgical inspection.

Results—Given a specificity of 100%, a β-trace protein concentration of 6 mg/l or higher in a sample indicated liquorhooea with a sensitivity of 92% compared with the reference method and of 93% compared with the clinical evaluation. The relation between the electroimmunoassay and the nephelometric assay was highly significant (p<0.001).

Conclusions—The nephelometric β-trace protein assay is a simple and rapid method for the detection of liquorhooea with high sensitivity and specificity and may facilitate the diagnosis of fistulas leaking CSF.

Keywords: β-trace protein; fistulas leaking CSF; liquorhooea

The early diagnosis of fistulas leaking CSF is of great importance as they may lead to bacterial meningitis, which is a life threatening disease with a mortality rate of 25%–50%. About 20% of cases of bacterial meningitis are due to a CSF leak. Fistulas leaking CSF are sequelae of skull fractures or surgical procedures at the anterior or middle cranial fossa, the nasal sinus, or the temporal bone. Their frequency after temporal bone fracture was found to be 15%. The aetiology of liquorhooea remains obscure in up to 39% of cases, including those with spontaneous liquorhooea. Rare conditions leading to fistulas leaking CSF are intracranial hypertension, sphenoidal sinusitis, bromocriptine therapy of macroprolactinoma, and malformations of the inner ear. Such fistulas should be suspected in patients presenting with intermittent or permanent water-like secretion of the nose or—to a lesser extent—of the ear, which may be confounded with rhinitis or otitis. In some patients, orthostatic headache may be present as well. The first aim of the diagnostic procedures is to prove the presence of CSF in a given sample. The second step is the location of the fistula using either high resolution CT, CT cisternography, MRI, endoscopy, or detection of radioactive tracer or fluorescein dye previously injected into the lumbar subarachnoidal space. In a third step the dural defect has to be closed by surgery.

Felgenhauer et al demonstrated that an ideal marker for liquorhooea should be present in normal CSF but not in other body fluids and should be detectable rapidly with routine laboratory methods. β-Trace protein was introduced by Felgenhauer et al as a marker for liquorhooea because its concentration in normal CSF is 35-fold higher than in serum. The protein is absent in tear fluid or nasal secretion. This protein belongs to the lipocalin family, a group of carrier proteins with enzymatic function. After determination of its amino acid sequence, it became evident that β-trace protein is identical with prostaglandin D-synthase. The physiological role of β-trace protein, which is produced in the meninges, the choroid plexus, and to a lesser extent in astrocytes, is not known. Animal experiments point to a role in sleep regulation and nociception. β-Trace protein in CSF has been studied in various CNS diseases but is of limited value except for the differential diagnosis of blood-brain barrier disturbances. By contrast, β-trace protein electroimmunoassay is an important tool with a sensitivity of 97% and specificity of 100% in the diagnosis of fistulas leaking CSF. Here we present a study of 140 liquid samples suspected of containing CSF which were investigated for β-trace protein using a new nephelometric assay.

Methods

SAMPLIES

Samples (140) from 100 patients from neurological (9%), neurosurgical (19%), otolaryngological (57%), and other (15%) departments with clinically suspected liquorhooea were collected during 2 years and investigated with electroimmunoassay and nephelometric assay. Sampling of ear or nose secretion, ear or nose tamponades, intraoperative secretion suspected of containing CSF, or postoperative
hygroma was performed. Sensitivity and specificity of the nephelometric assay were calculated referring to the electroimmunoassay results.

Additionally, the clinical records of 79 of the 140 samples were reviewed for radiological or surgical findings indicating fistulas and compared with the results of the β-trace protein assay. These 79 samples were from 52 patients, 32 men and 20 women, with a mean age of 48 years and a range between 8 and 85 years. All patients had a serum creatinine within the normal range.

Twenty CSF samples of patients presenting with tension headache who showed a normal CSF analysis were analysed for β-trace protein, as well as serum samples from 34 normal volunteers. The mean age (SD) of the 20 patients was 45 (15.8) years. The mean cell count was 1 (SD 0.9)/µl and the mean serum/CSF albumin ratio was 223.9 (SD 84.3).

**β-TRACE PROTEIN ELECTROIMMUNOASSAY**

Anti-human β-trace protein antibody production was induced in rabbits and detection of β-trace protein was performed by electroimmunoassay as described previously. Briefly, Gelbond plates (Sigma, Munich, Germany) were coated with gel containing 1.5% Litex agarose (Litex, Denmark), 1% polyethylene glycol 6000 (Merck, Darmstadt, Germany), 0.02 M barbital buffer pH 8.6, and 1% antibody; 5 µl of sample and a positive and negative control were placed in preformed holes. Samples of CSF served as positive controls, serum samples as negative controls. Electrophoresis was performed with a 0.02 M barbital buffer pH 8.6 for 3 hours at 4°C and 250 V. Complexes of β-trace protein and its antibodies were detected by 0.5% Coomassie blue (Serva Heidelberg, Germany). The detection limit of the method is 5 mg/l. A sample was judged as positive if β-trace protein-protein antibody complexes were present in the typical rocket formation (fig 1).

**β-TRACE PROTEIN NEPHELOMETRIC ASSAY**

For determination of β-trace protein a newly developed nephelometric research assay (N latex βTP) was used. N Latex βTP (Dade Behring, Marburg Germany) is a lyophilised reagent for Behring nephelometer (BN) systems. It contains polystyrene particles coated with immunoaffinity purified polyclonal antibodies from rabbits against human β-trace protein, which are agglutinated in the presence of β-trace protein. The increase in light scattering caused by agglutination is measured by the BN.

Samples (5 µl) are diluted to a total volume of 500 µl with diluens buffer (Dade Behring, Marburg, Germany) and measured on a nephelometer. Twelve minutes after addition of N latex βTP light scattering is measured again. The reaction kit consists of 50 µl polystyrene particles coated with 1.8 mg/100 mg immunoaffinity purified polyclonal rabbit anti-human β-trace protein antibodies, 15 µl supplement to avoid interference with anti-IgG-antibodies (Dade Behring, Marburg, Germany), and diluens buffer. The concentration of β-trace protein is calculated by the BN software using a seven point standard curve which is automatically from a single calibrator containing native human β-trace protein. Standardisation of the N Latex βTP assay is based on highly purified β-trace protein from CSF characterised by amino acid sequencing and quantified by quantitative amino assay analysis. The measuring range is 0.25 to 15.8 mg/l for the original dilution of 1:100. Samples with higher or lower β-trace content are automatically measured again with an appropriate dilution. The detection limit for a 1:1 diluted sample is 2.5 µg/l. The analytical imprecision of the assay is 2.3%-6.5%.

**STATISTICS**

To validate the nephelometric assay for β-trace protein in the diagnosis of liquorhœa the well established electroimmunoassay was used as an external validation criterion. The reference method is coded in the form of a dichotomous variable with the possible outcome 1 (negative) and 2 (positive). The tested method is a continuos variable with a minimum score of 0.2 and a maximum score of 53.9 mg/l. Because the emphasis is on avoiding false positive predictions the critical value for the nephelometric assay was determined for the specificity which reaches 100%. Accordingly, 2×2 contingency tables were determined for 10 different versions of a dichotomised nephelometric variable. The nephelometric β-trace protein value was recorded to 1 (negative) and 2 (positive) by varying the critical value for a negative diagnosis from 1 mg/l to 10 mg/l. Sensitivity and specificity for each critical value are summarised in figure 2. Additionally, χ² tests (or Fisher’s exact tests) were applied to test for statistical significance of the relation between the two methods.

**Results**

**ELECTROIMMUNOASSAY AS REFERENCE METHOD**

Of 140 samples of clinically suspected liquorhœa, 36 were found to be positive for β-trace protein detected by electroimmunoassay. The
Table 1 Clinical characteristics of patients with CSF leak. The diagnostic procedures that proved the diagnosis are given in bold.

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Aetiology of CSF leak</th>
<th>Diagnostic procedures</th>
<th>Observation period (months)</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68</td>
<td>Female</td>
<td>Unknown</td>
<td>CT, scintigraphy</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>Female</td>
<td>Post-traumatic</td>
<td>CT, cisternography, intrathecal fluorescein, endoscopic surgery</td>
<td>5</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>58</td>
<td>Male</td>
<td>Sinusitis sphenoidalis</td>
<td>CT, surgery</td>
<td>9</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>73</td>
<td>Male</td>
<td>After craniotomy</td>
<td>CT, surgery</td>
<td>7</td>
<td>Meningitis</td>
</tr>
<tr>
<td>5</td>
<td>72</td>
<td>Female</td>
<td>After ventriculotomy</td>
<td>CT, surgery</td>
<td>31</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>83</td>
<td>Male</td>
<td>Frontobasal fracture</td>
<td>CT, surgery</td>
<td>1,5</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>58</td>
<td>Female</td>
<td>Unknown</td>
<td>CT, surgery</td>
<td>32</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>57</td>
<td>Male</td>
<td>Post-traumatic</td>
<td>CT, surgery</td>
<td>37</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>46</td>
<td>Male</td>
<td>After craniotomy</td>
<td>CT, surgery</td>
<td>38</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>19</td>
<td>Male</td>
<td>Frontobasal fracture</td>
<td>CT, MRT, intrathecal fluorescein, endoscopic surgery</td>
<td>40</td>
<td>Recurrence which led to revision surgery, none</td>
</tr>
</tbody>
</table>

Our results indicate a high sensitivity and specificity of the nephelometric β-trace protein.

Nephelometric assay for β-trace protein showed 33 positive samples. None of the electroimmunoassay negative samples was positive in the nephelometric assay. Under the premise of a low rate of falsely positively predicted cases (a specificity of 100%) the critical value of the nephelometric assay could be quantified with 6 mg/l or more of protein. If sensitivity was considered simultaneously, the critical value of 6 mg/l was the best choice leading to a sensitivity of 92%. The relation between the electroimmunoassay and the nephelometric assay was highly significant in any chosen critical value (p<0.001).

CLINICAL EVALUATION AS A REFERENCE METHOD

In the group of 52 patients (79 samples), 13 samples from 10 patients were positive for β-trace protein with both methods. In three patients measurements were repeated. In one patient two positive results in two samples were found and in another patient three positive results in five samples were found. The two negative samples of this patient were taken after successful closure of a CSF leak. In the third patient—a child of 8 years with a combined temporal bone and sphenoid sinus fracture—the first sample taken from the nose was positive for β-trace protein. Therefore endoscopic sinus surgery was performed with closure of the sphenoid fracture line. Two postoperative controls from nasal secretion were negative. When the patient developed a bacterial meningitis 8 months later, a sample of nasal secretion was taken and was found negative for β-trace protein. Cranial CT showed signs of fluid in the mastoid cells at the side of the temporal bone fracture. An explorative mastoidectomy and tympanoscopy was performed which showed a dural prolapse with a clear CSF leak. The defect was closed the next day by a temporal approach. It is possible that the nasal secretion from the anterior parts of the nose did not contain any CSF as this time the CSF fistula was located temporally. Although the negative result of this β-trace analysis was due more to inadequate sampling than lack of sensitivity of the method, we judged this analysis as a false negative result. This evaluation gave a sensitivity of 93% for the nephelometric β-trace assay.

In all 10 patients, the clinically suspected liquorrohea and the positive β-trace protein result could be confirmed by other techniques.
In our series, the results of \( \beta \)-trace protein measurement in serum and CSF in the control group are in line with previous results.14 Whereas the serum values were identical with those given in the literature, the CSF values were slightly lower, which might be explained by the lower age and serum/CSF albumin ratio in our control group. The samples negative for \( \beta \)-trace protein showed low mean \( \beta \)-trace protein concentrations, comparable with those measured in the serum of the control group (fig 3). The positive samples showed mean \( \beta \)-trace protein concentrations that were expected for CSF. In a few samples, \( \beta \)-trace protein concentration was unexpectedly high. Together with the broad range of the SD in the group of positive samples, this might reflect the various methods of sampling. These will result in a dilution of the sample containing CSF by other body fluids in most of the cases, but may lead to a higher concentration of the CSF within the sample in isolated cases as well.

Other CSF specific proteins, such as \( \beta_2 \)-transferrin, have been suggested as indicators of liquorrhoea.15 However, compared with \( \beta \)-trace protein, the clinical evaluation of \( \beta_2 \)-transferrin showed it to have lower sensitivity and specificity. In earlier studies, a sensitivity of 79% was found.20 Other findings report a higher sensitivity, of nearly 100%, but a reduced specificity of 95%.27 Reasons for a falsely positive \( \beta \)-transferrin test are underlying hepatic diseases with \( \beta \)-transferrin being detectable in the serum, also, and alleric variants of the serum transferrin that cannot be distinguished from \( \beta_2 \)-transferrin by the electroimmunoassay.28 A further disadvantage of \( \beta_2 \)-transferrin assay is that it is time consuming; it takes 3.5 hours even in its latest improved version.29

A historical marker of liquorrhoea—glucose—has not been considered as it is present abundantly in serum and blood. Even in water-like secretions from the nose or ear it is of limited use as reference values have not yet been established and data on sensitivity and specificity of the method are lacking.

In conclusion nephelometric \( \beta \)-trace protein detection is rapid and highly valid for the diagnosis of a CSF leak and should help to facilitate the management of patients with suspected liquorrhoea.
HISTORICAL NOTE

Ammon’s horn and the hippocampus

The word hippocampus comes from late Latin: hippocampus, derived from the Greek words for a horse + sea monster. In mythology it was a sea horse, having two forefeet, with the body ending in a dolphin’s or fish’s tail, represented as drawing the vehicle of Neptune on a hippocampus, with his Tritons and Nêreides” (Drummond of Hawthornden Let. Wks. English Dictionary) was in 1606, cited as tune the sea God. The earliest use (Oxford English Dictionary) was in 1606, cited as “Drummond of Hawthornden” Let. Wks. (1711) 232. “Stately pageants. that of Cheapside was of Neptune on a hippocampus, with his Tritons and Néréides”.

Neurologists recognise it as each of two elongated eminences (hippocampus major and minor) on the floor of each lateral ventricle of the brain; so called from their supposed resemblance to the fish.

With its base in ancient classical history, neuroanatomy provides several metaphors that relate the gods and the brain. One is Ammon’s horn. The term Cornua Ammonis, or Ammon’s horn, is a well known description of the whorled chambered shells of a fossil genus of Cephalopods. They were once supposed to be coiled snakes petrified, and of the whorled chambered shells of a fossil genus of Cephalopods. They were once supposed to be coiled snakes petrified, and hence called “snake-stones” from their resemblance to the involuted horn of Jupiter Ammon.

The hippocampus received its name from the Italian Julius Caesar Arantius in the late 16th century. Less than two centuries later, the hippocampus was called Ammon’s horn. An early, anatomical use is in the 1742 book of a felicitously named surgeon René Jacques Croissant de Garregeot. In 1732 Jacques Benigne Winslow used the term ram’s horn. Thus Ammon’s horn was probably not in use at this date. Albrecht von Haller, the anatomist, indicated that the term Ammon’s horn was already used in a paper of the Oeconomische Abhandlung of 1755 (Haller, 1774–7, vol 2 p 507).

The term Ammon’s horn is a metaphor that refers to the ram shaped horns on the head representing the Egyptian God Amun who protected the Pharaoh Taharqa in the temple of Kawa. Many temples were dedicated to Amun. The Greek form of the name was Ammon, the Libyan Jupiter whom the Greeks identified with Zeus. King David conquered a Jordanian tribe, the Ammonites, who were descendants of Lot, by the son of his younger daughter.

It is of interest that the related hippocampal commissure together with the crura of the fornix, is sometimes termed the “psalterium” or “lyra Davidis”. Psalterium and lyra are both harps.

To add to the confusion, French neuroanatomists refer to the horn shaped lateral part of the fourth ventricle with its choroid plexus leaving the foramen like a posy of flowers as the “corne d’abondance” (horn of plenty, or cornucopia).

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