

# Beta-trace protein as sensitive marker for CSF rhinorhea and CSF otorhea

Reiber H, Walther K, Althaus H. Beta-trace protein as sensitive marker for CSF rhinorhea and CSF otorhea.

Acta Neurol Scand 2003; 108: 359–362. © Blackwell Munksgaard 2003.

**Objective** – Beta-trace protein concentrations in cerebrospinal fluid (CSF), serum and nasal secretions are investigated with a new quantitative, immunonephelometric assay. **Results** – The mean beta-trace concentration of normal lumbar CSF (18.4 mg/l) and normal serum (0.59 mg/l), from  $n = 132$  control patients, were 10% higher than reported earlier for smaller control groups. The reference range of beta-trace protein in nasal secretions is very low (median: 0.016 mg/l, range < 0.003–0.12 mg/l, for  $n = 29$  controls). Clinically confirmed cases of CSF rhinorhea ( $n = 20$ ) showed beta-trace concentrations between 0.36 and 53.6 mg/l, with a median of 2.4 mg/l. We propose a cut-off value of 0.35 mg/l above which a CSF contamination in the secretion is plausible. A clinically confirmed CSF otorhea had a value of 1.75 mg/l. **Conclusion** – This new beta-trace protein assay offers a fast, sensitive and reliable routine method to detect a CSF rhinorhea or otorhea.

**H. Reiber<sup>1</sup>, K. Walther<sup>1</sup>,  
H. Althaus<sup>2</sup>**

<sup>1</sup>Neurochemisches Labor, Universität Göttingen, Göttingen, Germany; <sup>2</sup>Dade Behring Marburg GmbH, Marburg, Germany

Key words: beta trace protein; cerebrospinal fluid; serum; secretions; body fluids; rhinorhea; otorhea

Hansotto Reiber, Neurochemisches Labor, Universität Göttingen, Robert-Koch-Str. 40, D-37075 Göttingen, Germany

Tel.: +49 551 39 6619

Fax: +49 551 39 2028

e-mail: hreiber@med.uni-goettingen.de

Accepted for publication March 28, 2003

A cerebrospinal fluid (CSF) fistula with a CSF drainage into nose or ear are observed because of pathological connections between CSF space and exterior after head trauma, skull-base surgeries or occur spontaneously (1). The sensitive detection of a CSF leakage, for example, in nasal secretions (CSF rhinorhea), is actually based on two methods: the immunoblot for  $\beta_2$ -transferrin (2–4) and the immunonephelometric assay of beta-trace protein (5). Total protein or glucose determinations are unreliable methods for differentiation (2).

The report of Felgenhauer et al. (5) that beta-trace protein is a reliable marker for detection of CSF in nasal secretions encouraged Dade Behring to develop an assay that is now commercially available for research use.

Beta-trace is a brain-specific protein with a prostaglandin synthase activity (prostaglandin-H2 D-isomerase; EC 5.3.99.2) (6, 7). The major site of biosynthesis are the leptomeninges and to some extent the choroid plexus. In particular, the analysis of the ventricular–lumbar concentration gradient (8) was the clue to decide between the controversial reports (7, 9, 10).

The low ventricular CSF concentration (1.5 mg/l) of beta-trace (8), which originates from choroid plexus, increases along the CSF flow way in the

subarachnoid space because of a steady release from the leptomeninges up to a mean lumbar CSF concentration of 16.6 mg/l (8, 10).

With an earlier version of the current assay we made several investigations (10, 11) to characterize the diagnostic relevance of this protein for diagnosis of neurological diseases. These investigations had more relevance for supporting the molecular flux/CSF flow theory (12) to establish the actual view of the blood CSF barrier dysfunction as a reduced CSF flow rate (8, 12) than a diagnostic relevance for differential diagnosis of neurological diseases.

Now, with the latest version of the beta-trace assay, we investigated the reference ranges of lumbar CSF, serum and nasal secretions from a larger group of patients as a base for the sensitive and specific detection of a CSF rhino- or otorhea. In a very recent publication (13) with the same assay, extremely different values for nasal secretions (100-fold higher than ours) were reported.

## Material and methods

### Patients

Lumbar CSF and serum samples originated from patients of the Department of Neurology,

University Göttingen. All samples were taken for routine analysis with the informed consent of the patients. The CSF was normal, that is, no signs of inflammation (no oligoclonal IgG, normal cell count) and normal blood-CSF barrier function. The patients had no kidney failure, which would have increased the serum concentration of beta-trace.

Samples from nasal secretions (and some few ear secretions) together with corresponding serum samples were obtained from the neurosurgery department in Göttingen and different hospitals with the request to exclude or confirm a CSF contamination. In the group of patients with normal values of nasal secretions the blood values of beta-trace were normal (not reported).

#### Preanalytical treatment of secretion samples

We obtained the secretions collected directly into a tube. Most of the samples could be analysed without any preanalytical treatment. A few samples were obtained by absorption in cotton gauzes. These samples were centrifuged (2). Highly viscous samples and samples with a volume too small for routine analysis were diluted with a defined volume of 0.9% sodium chloride solution (1:2 or 1:4, in one case 1:10).

#### Beta-trace protein assay

For determination of beta-trace protein an immunonephelometric research assay (N Latex  $\beta$ -Trace Protein) was used. N Latex  $\beta$ -Trace Protein (Dade Behring Marburg, Germany) is a lyophilized reagent for BN<sup>TM</sup> Systems. It contains polystyrene particles coated with immunoaffinity-purified polyclonal antibodies from rabbit against human beta-trace protein, which are agglutinated in the presence of beta-trace protein. The increase in light scattering caused by aggregation is measured by the BN<sup>TM</sup> System. The total assay time is 12 min for a single determination. The concentration of beta-trace protein is calculated by the BN<sup>TM</sup> software using a seven-point reference curve prepared automatically from a single calibrator containing native human beta-trace protein. Standardization of the N Latex  $\beta$ -Trace Protein assay is based on highly purified beta-trace protein from cerebrospinal fluid, characterized by amino acid sequencing and quantified by quantitative amino acid analysis. The measurement range for an undiluted sample is 0.25–15.8 mg/l if the automated default serum dilution of 1:100 is used in the nephelometer. Samples with higher or lower beta-trace content are automatically re-measured with an appropriate

automatic dilution. The lowest detection limit for a sample measured without any default dilution in the nephelometer is 0.0025 mg/l. The total analytical imprecision (intra-assay plus interassay;  $n = 40$ ) of the assay, calculated from two control materials and three serum samples with concentrations of 1.51–7.89 mg/l (measured in automated default dilution of 1:100), was between 2.3 and 6.5% as specified by Dade Behring.

#### Routine analysis

CSF samples (automated default dilution of 1:400) and serum samples (automated default dilution of 1:100) as well as the secretion sample (automated default dilution of 1:100) were analysed on a BN<sup>TM</sup> A Nephelometer or a BN Pro Spec® (Dade Behring). The sample volume for the analyser is 200  $\mu$ l. The reference curve was generated with N Protein Standard UY (OQLV) from Dade Behring with a concentration of 1.36 mg/l. With each analytical run a control sample was analysed [N/T Protein Control LC (OQLW)] with a concentration of 1.63 mg/l. Dilutions in the assay were performed with the N Diluent (OUMT) from Dade Behring.

The interassay imprecision of the control samples was 2.3% ( $n = 10$ ) or 3.4% including an outlier of 10%. The mean values between two different calibrations changed from 1.65 to 1.69 mg/l.

#### Secretions

All secretion samples were started undiluted in the automated default dilution of 1:100 to avoid possible errors in the antigen excess range as observed for values  $>50$  mg/l. But usually a reliable value for secretion (in particular with values in the reference range) is obtained for samples undiluted or for manual 1:10 predilution, detected without an automated default dilution in the automated re-measuring process. Samples with a too small volume are prediluted and started in the nephelometer with a correspondingly smaller default dilution.

## Results

#### CSF, serum and nasal secretion

The mean of the beta-trace concentrations in this improved assay (Table 1) in normal lumbar CSF (18.4 mg/l) and normal serum (0.59 mg/l), analysed for  $n = 132$  control patients, were 10% higher than that reported earlier (10, 11) for smaller groups of controls.

**Table 1** Reference ranges of beta-trace protein concentrations in normal lumbar CSF, serum and nasal secretions of control patients

|                    | Normal beta-trace protein concentrations |           |                    |                  |
|--------------------|--|-----------|--------------------|------------------|
|                    | Lumbar CSF                               | Serum     | Nasal secretion    | CSF Rhinorhea    |
| Number of patients | 132                                      | 132       | 29                 | 20               |
| Mean (mg/l)        | 18.4                                     | 0.59      | 0.016 <sup>1</sup> | 2.4 <sup>2</sup> |
| CV (%)             | 22.6                                     | 16.5      | –                  | –                |
| Range (mg/l)       | 9.4–29.2                                 | 0.38–0.86 | <0.003–0.12        | 0.36–53.6        |

<sup>1</sup> Median from *n* = 29 data with nine values <0.003 mg/l.

<sup>2</sup> Median.

The reference range of beta-trace protein in nasal secretions (Table 1) is very low (median: 0.016 mg/l, range <0.0024–0.12 mg/l, for total *n* = 29 controls). A few samples (*n* = 9) were below the detection limit of the assay (0.0024 mg/l) for undiluted samples.

CSF rhinorhea

The clinically confirmed cases of CSF rhinorhea (*n* = 20) showed beta-trace concentrations in the material collected from nose to be between 0.36 and 53.6 mg/l.

The median was 2.4 mg/l. The largest values with 31.2, 51.9 and 53.6 mg/l were clearly above the range of the normal lumbar CSF (<29.2 mg/l). These largest values were obtained from patients after neurosurgery.

For six patients with values between 0.26 and 0.33 mg/l, we could not get a clinical confirmation of a rhinorhea.

Particular cases

In one patient we obtained three subsequent samples: two with 0.98 mg/l before and one after neurosurgical intervention with a normal value of 0.11 mg/l.

Two patients had rather constantly increased values in samples collected over 2 or 3 weeks: 0.59/0.64 and 19.2/17.8 mg/l, respectively.

From two patients we obtained, for comparison, the secretions separately collected for the right and left nostrils. One pathological case had a combination where the value from one nostril was normal and the value from the other nostril was increased (0.11/0.39 mg/l, respectively) and one control had the same low values on both sides (0.011/0.012 mg/l).

Oto-liquorrhea

Amongst three samples with secretions from the ear, we found two as normal with values of 0.015

and <0.008 mg/l and one case with a CSF contamination (1.75 mg/l) in the secretion. This patient had a clinically confirmed postsurgery CSF otorhea.

Discussion

The new beta-trace protein assay offers a fast, sensitive and reliable method to detect a CSF rhino- or otorhea. This quantitative assay can be performed in a few minutes on a routine nephelometer. This is a basic advantage compared with the qualitative assay for β<sub>2</sub>-transferrin (2, 13). It is sufficient to analyse the secretion sample without particular comparison with the blood values of beta-trace protein, another advantage compared to the β<sub>2</sub>-transferrin immunoblot. A moderate increase of blood values because of kidney failure of the patient would not significantly contribute to the much higher CSF values of beta-trace protein (8). But for the rare case of a patient with extremely high blood values [like in haemodialysis patients (13) combined with a questionable CSF leakage] a possible interpretation problem should be kept in mind.

The high precision and reproducibility of the results in this assay are convincing in particular as shown by the comparison of data from left vs right nostrils (13) and the invariability of values in cases where samples have been taken over 2–3-week periods (see above results).

Cut-off value

There is a clear discrimination of pathological beta-trace values due to CSF leakage from those in normal secretions. We propose 0.35 mg/l of beta-trace protein as a cut-off value for the presence of CSF in a secretion.

There was a certain grey zone between 0.26 and 0.35 mg/l in which we might find also pathological values, but we have so far no clear clinical indication of a CSF rhinorhea in the six cases reported. But these values are clearly above the reference range of a normal nasal secretion of 0.12 mg/l. This should be kept in mind for cases with a clinical indication of a CSF rhinorhea, but low beta-trace protein values. In these samples with intermediate beta-trace protein concentrations the analysis of β<sub>2</sub>-transferrin seems not to be helpful because of a lower specificity and sensitivity for detection of asialo-transferrin in the immunoblot (13).

The extremely high beta-trace concentrations in nasal secretions of some postsurgical patients, with values larger than the humoral CSF values, need a

further investigation. Obviously, there are spaces at the brain surface (like cortical CSF space), which have still higher concentrations than lumbar CSF (<30 mg/l) and in particular in ventricular CSF (<3mg/l) (8).

The recently published (13) values for normal nasal secretions (0.219–1.69 mg/l) are about 100-fold higher than our values for the same assay and analyser but the mean values for serum with 0.59 mg/l are identical to our findings and the mean CSF value for a smaller, undefined control group in Ref. (13) is also quite similar to our mean CSF value (Table 1). The values for the normal secretions are implausible and probably biased by a methodological error for the 1:100 prediluted samples.

The measurement of a generally 1:100 prediluted secretion (and no automated default dilution in the nephelometer) would not allow measuring a large fraction of the normal controls.

In the reference range (e.g. 0.1 mg/l) a 1:100 prediluted sample would have a concentration of 0.001 mg/l, which is below the detection limit of the assay (0.0025 mg/l). In fact, this detection limit is reflected by the (false) lowest reference values (0.22 mg/l) reported in Ref. (13).

In conclusion, the immunonephelometric beta-trace protein assay could be proposed as the best routine method to detect a CSF rhino- or otorhea.

## References

1. HAR-EL G. What is 'spontaneous' cerebrospinal fluid rhinorhea? Classification of cerebrospinal fluid leaks. *Ann Otol Rhinol Laryngol*, 1999;**108**:323–6.
2. THOMAS L. Cerebrospinal fluid or nasal secretion. In: THOMAS L, ed. *Clinical laboratory diagnostics*, 1st edn. Frankfurt/Main: TH books Verlagsgesellschaft, 1998; 1344–46.
3. DELAROCHE O, BORDURE P, LIPPERT E, SAGNIEZ M. Perilymph detection by  $\beta_2$  transferrin immunoblotting assay. Application to the diagnosis of perilymphatic fistulae. *Clin Chim Acta* 1996;**245**:93–104.
4. FREEK WC, ROELANDE N, VAN DER ZWART JHD, VAN LOON J, SOUVERIJN JHM. Detection of CSF leakage by isoelectric focussing on polyacrylamide gel, direct immunofixation of transferrins and silver staining. *Clin Chem* 1998; **44**:351–3.
5. FELGENHAUER K, SCHÄDLICH HJ, NEKIC M. Beta trace protein as marker for cerebrospinal fluid fistula. *Klin Wochenschr* 1987;**65**:764–8.
6. HOFFMANN A, CONRADT HS, GROSS G, NITZ M, LOTTSPICH F, WURSTER U. Purification and chemical characterization of  $\beta$ -trace protein from human cerebrospinal fluid: its identification as prostaglandin D synthase. *J Neurochem* 1993;**61**:451–6.
7. URADE Y, KATAHAMA K, OHISHI J, KANEKO T, MIZUNO N, HAYAISHI O. Dominant expression of m-RNA for prostaglandin D-synthetase in leptomeninges, choroid plexus and oligodendrocytes of the adult rat brain. *Proc Natl Acad Sci USA* 1993;**90**:9070–4.
8. REIBER H. Dynamic of brain proteins in cerebrospinal fluid. *Clin Chim Acta* 2001;**310**:173–86.
9. BLÖDORN B, MAEDER M, URADE Y, HAYAISHI O, FELGENHAUER K, BRÜCK W. Choroid plexus: the major site of mRNA expression for the  $\beta$ -trace protein (prostaglandin D synthetase) in human brain. *Neurosci Lett* 1996;**209**: 117–20.
10. TUMANI H, REIBER H, NAU R et al. Beta-trace protein concentration in cerebrospinal fluid is decreased in patients with bacterial meningitis. *Neurosci Lett* 1998;**242**:5–8.
11. TUMANI H, NAU R, FELGENHAUER K.  $\beta$ -Trace protein in cerebrospinal fluid: a blood–CSF barrier-related evaluation in neurological diseases. *Ann Neurol* 1998;**44**:882–9.
12. REIBER H. Flow rate of cerebrospinal fluid (CSF) – a concept common to normal blood–CSF barrier function and to dysfunction in neurological diseases. *J Neurol Sci* 1994;**122**:189–203.
13. ARRER E, MECO C, OBERASCHER G, PIOTROWSKI W, ALBEGGER K, PATSCH W.  $\beta$ -Trace protein as a marker for cerebrospinal fluid rhinorhea. *Clin Chem* 2002;**48**:939–41.