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Reply

Beta-trace protein concentration in nasal secretion: discrepancies and flaws in recent publications

Dear Sirs,

I would like to reply to Bachmann and Petereit: 'Beta-trace protein as sensitive marker for liquorrhea.' *Acta Neurologica Scandinavica* 2004; 110: 337–338.

A number of recent publications (1–3) deal with the beta-trace protein concentration in nasal secretions as a sensitive marker for cerebrospinal fluid (CSF) rhinorhea and CSF otorhea.

In their letter to the editor (4) Bachmann and Petereit discussed our report (1) on the reference range of normal beta trace values and in particular the cut-off value reported for discrimination between a normal nasal secretion and a secretion contaminated with CSF.

We appreciate and share their concerns to find a clinically relevant cut-off value, which is high enough to avoid false-positive interpretations. We also agree that a determination of the clinically relevant cut-off value must be based on values of clinically defined cases. But the data of a control group with normal beta trace values in the nasal secretion (normal reference range) is also a mandatory information to get a reliable cut-off value. Unfortunately none of the reports, Bachmann et al. (4) refer to, i.e. their own data (2) and the data of Arrer et al. (3, 5) (Table 1) present reliable data for a normal reference range.

This reply comments therefore primarily on the accurate determination of the reference range for beta trace protein of normal controls and the discrepancies between different reports (Table 1), which are obviously due to analytical flaws.

Our recent publication (1) reports reference values of nasal secretions from normal controls, (range: 0.003–0.12 mg/l, median: 0.016 mg/l), which are significantly different from the results reported by Arrer et al. (3, 5) with a 100-fold higher reference range 0.219–1.69 mg/l (mean: 0.39 mg/l). Both groups used the same commercial assay (*N*-Latex β-trace protein; BNA® Nephelometer or BN Pro Spec; Dade Behring, Germany) with a sensitivity of 0.002 mg/l (according to the assay supplier and confirmed by (1) and (2)). The main difference between our application of the assay (1) and that of Arrer et al. (3) originate from the use of undiluted (1) versus 1:100 prediluted (3, 5) secretion samples. Petereit et al. (2) applied undiluted samples but analysed the samples with a 1:100 default dilution.

Our routine analysis (1) of CSF and serum samples was performed according to the proposal of the supplier (Dade Behring). Undiluted CSF samples were analysed with a default dilution (automated dilution in the machine) of 1:400 and undiluted serum samples with an automated default dilution of 1:100.

Nasal secretion samples of patients were analysed undiluted, first using the automated default dilution of 1:100. This step helps to avoid possible errors in the antigen excess range as observed for values > 50 mg/l. But usually a reliable value for nasal secretions (in particular with values in the reference range) is only obtained for undiluted samples and a default dilution of 1:1 (modified automated measuring process using 1:1 instead of 1:100 default

Table 1 Reference ranges of beta-trace protein concentration in nasal secretion (NS), serum, lumbar CSF (L-CSF) and ventricular CSF (V-CSF)

	NS (mg/l)	Serum (mg/l)	L-CSF (mg/l)	V-CSF (mg/l)	Cut-off proposed (mg/l)
Reiber et al. (1, 8)	<0.003–0.12[1]	0.38–0.86	9.4–29.2	1.5[7]	0.35
Arrer et al.(3, 5)	<0.219–1.69[2]	0.117–1.44	11.5–32.6		1.31
Schnabel et al. (6)	<0.25[3]		16.3[6]		1.0
Petereit et al. and Bachmann et al. (2, 7)	<3[4]	0.5[5]	11[5]		6.0

[1] 9/29 values were <0.003

[2] 90/160 values measured as <0.219 (5)

[3] Detection limit of the assay, reported for $n = 7$ volunteers.

[4] Values <3 mg/l were reported as β-trace protein negative (7).

[5] Mean values.

[6] Mean from a CSF pool.

[7] Mean β-trace protein concentration in ventricular CSF, V-CSF (8).

dilution). Only highly viscous samples and samples with a volume too small for routine analysis have been slightly prediluted with a defined volume of 0.9% sodium chloride solution (1:2 to maximally 1:10), again using the default dilution 1:1.

Arrer et al. (3) also used the default solution 1:1 but used 1:100 prediluted samples. The analytical range of an undiluted sample with a default dilution 1:1 (1) is 0.0025–0.16 mg/l. In case of a 1:100 predilution with a default dilution 1:1 (3, 5) the lowest concentration detectable is 0.25 mg/l (e.g. a β-trace protein concentration of 0.01 mg/l in a nasal secretion, if diluted 1:100 would be 0.0001 mg/l in the assay probe. In this case the assay protocol reports a value of < 0.0025 mg/l. If this value is multiplied with the dilution factor 100, the result of the measurement would be obtained with < 0.25 mg/l. This detection limit is indeed identical with the lowest values of the reference range reported by Arrer et al. (3) (Table 1). In their later publication with a graphical presentation of the control values [Fig. 2 in ref. (5)] they clearly demonstrate that most of the control samples were below the detection limit ($> 60\%$ of their reference values were in the range < 0.3 mg/l).

With this data distribution [Fig. 2 in ref. (5)] the calculation of a mean [0.39 mg/l in ref. (3)] is not acceptable. Together with the restricted sensitivity, the predilution also leads to a high imprecision for the measurable values (> 0.25 mg/l) of normal controls. With these biased data their reported cut-off value (1.3 mg/l) is not reliable. In contrast to nasal secretions, the reported data for lumbar CSF and serum concentrations of beta-trace protein (Table 1) match very good between both groups (1, 3), as there is no methodological bias involved.

Petereit et al. (2) discussed the option to use a lower default dilution on the nephelometer than 1:100, but did not realize it in their study, i.e. the lowest values they could measure were 0.25 mg/l. This is why they report (2) that β-trace protein is absent in tear fluid or nasal secretion.

So, the reference range of β-trace protein in nasal secretion reported by us (1) is still the most accurately measured set of data available to characterize a reliable reference range.

The discussion about the clinically relevant cut-off value in the actual letter of Bachmann and Petereit (4) suffers from the deficit that the authors referred to an unreliable report on the normal reference range (3) and did not measure own values in the reference range (2).

As a second, most important aspect for the discussion of a clinically relevant cut-off value Bachmann et al. (2, 4) did not consider the possible occurrence of a subclinical liquorrhea as stated by Schnabel et al. (6): 'Diagnosing CSF fistulas is always challenging. Particularly, in subclinical liquorrhea with a loss of small quantities of CSF, the fistula may not be detectable by radiologic imaging.' This aspect is completely ignored by Bachmann et al. (4, 7) when they state, based only on radiological and clinical information that a nasal secretion with < 3 mg/l beta trace protein has to be regarded as negative for CSF traces, and only β-trace protein concentration with > 6 mg/l as positive, i.e. indicating the presence of CSF in nasal secretion (7).

By this definition the sensitivity of the β-trace protein assay would be reduced to the sensitivity of the radiological, imaging techniques. According to our reported data (1) there are definitely patients with a CSF contamination in nasal secretion with β-trace values in the range 0.36–1 mg/l.

I accept the critic that we did not publish sufficient information about neurosurgical intervention in our cases of a clinically confirmed rhinorhea, but we offered enough data from serial analysis before (0.98 mg/l) and after (0.11 mg/l) neurosurgical intervention or data from patients where the right and left nostrils have been to allow a statement that the nasal secretion was definitely contaminated with brain derived CSF. Regarding our lower cut-off value of 0.35 mg/l we have to mention that the data from nasal secretion samples with increased β-trace concentrations refer to samples, which had no blood contamination. This was analysed after visual inspection by haemoglobin analysis. That means that we had no problem to accept a cut-off value for CSF contamination in nasal secretion (0.35 mg/l) below the normal serum concentrations of β-trace protein (Table 1). Of course, in case of serious blood contamination in the secretion sample the cut-off value for CSF contamination should be above the serum values. This approach would match the cut-off value reported by Schnabel et al. (6) with 1 mg/l.

There remains an important clinical aspect to be considered. We have a very sensitive test (1) to detect a pathological CSF contamination in nasal secretions. But can the decision for a neurosurgical intervention depend only on a β-trace protein concentration in nasal secretion? Is this only a question of a reliable cut-off value? As described by Schnabel et al (6) an intermittent rhino-liquorrhea, i.e. diagnosis of CSF leakage, does not mean automatically a decision for a neurosurgical intervention.

In this context it might also be relevant to recognise that the absolute value of β-trace protein concentration depends on the source of CSF. The ventricular CSF concentration of β-trace protein

(Table 1) with a mean of 1.5 mg/l is much lower than that for the 11-fold higher value in lumbar CSF (8).

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