SIEMENS

White Paper

Beta-trace Protein Quantification for Diagnosis of CSF Leakage Syndrome

Dr. Luca Bernasconi,[1] Dr. Carola Wagner,[2] Prof. Dr. Andreas Huber[1]

1.Kantonsspital Aarau, 5001 Aarau, Switzerland
2.Siemens Healthcare Diagnostics Products GmbH, 35001 Marburg, Germany

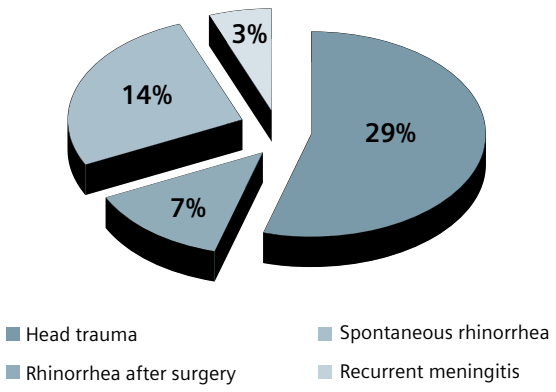
Beta-trace Protein Quantification for Diagnosis of CSF Leakage Syndrome

Introduction

Cerebrospinal fluid (CSF) leakage can arise through head trauma and skull-based surgery as well as nontraumatic conditions, including inflammation, tumor, and congenital malformation, or may occur spontaneously (Figure 1).[1,2]

It is essential to identify and manage such leakage because of the risk of CSF contamination by passage of intranasal and paranasal bacteria directly into the cranium, potentially leading to bacterial meningitis. There is an estimated mortality rate of 20–50%, with approximately 20% of bacterial meningitis cases arising from CSF leakage.[3] Failure to perform dural repair in acute traumatic CSF leakage led to a cumulative risk of bacterial meningitis >85% after 10 years.[4] Even after surgical intervention, there is a 22.5% risk of recurrence of CSF leakage,[5] hence the need for continued monitoring.

Figure 1: Clinical symptoms and etiology-based distribution in 53 patients with suspicion of CSF leakage.[2]



Confirming CSF Leakage

Identification of the leakage site is necessary for surgical intervention. Although high-resolution computed tomography (CT) and magnetic resonance imaging (MRI) may localize a suspected leak involving a bony fracture or tumor with erosion of the skull base, they could still fail to demonstrate an actual CSF leakage. In addition, such examinations are expensive and availability of the equipment is limited. The use of radioactive tracers in CT scanning and scintigraphy can confirm leakage at the time of examination but would fail to diagnose intermittent CSF leakage,[6] and such tracers are potentially dangerous. Sodium fluorescein allows identification of CSF leakage and localization of fistulas. However, its application into the subarachnoid space by lumbar puncture has associated trauma and potential complications. Less invasive is its topical application, although this still requires endoscopic insertion.

Therefore, CSF leakage should first be verified prior to using such methodologies to identify the actual leakage site. CSF leakage can be confirmed from the compositional difference of CSF from other bodily secretions. Therefore, a simple screening test for a CSF-specific marker is required that is sensitive, noninvasive, and rapid.

Determination of CSF Leakage Using CSF Marker Proteins

The first CSF marker applied for this indication was β2-transferrin,[7] which is nearly absent from plasma, nasal mucus, tears, and mucosal discharge, and a noninvasive determination of β2-transferrin by gel electrophoresis was introduced to identify CSF leakage.

However, β2-transferrin (also known as carbohydrate-deficient transferrin, or CDT) is increased in the plasma of patients with chronic alcohol abuse, while saliva contains isoforms with similar electrophoretic mobility.[8] When there is contamination with blood, the hemoglobin must be removed by column chromatography. Interpretation of the gel may be difficult, with possible overlap of the application area and the detection point of β2-transferrin, while a high transferrin concentration may interfere with the β2-transferrin band.[9] Because β2-transferrin gel electrophoresis is time-consuming and expensive, it is unsuitable for emergencies or single-case determinations. Furthermore, the various pieces of equipment needed may not be available in clinical laboratories.

Beta-trace Protein as a Marker for CSF Leakage

A solution to the drawbacks of β2-transferrin was provided by beta-trace protein (BTP), a 25-kDa protein identified as prostaglandin D synthase. At almost 20 mg/L, it is the second-most abundant CSF protein after albumin, with a CSF-to-serum ratio of 33, the highest of all CSF-specific proteins.[10,11] Together with a low BTP concentration in nasal secretion (NS) and its absence in tears, BTP is an ideal marker for CSF leakage, even when there may be some post-trauma bleeding. Initially, immunoelectrophoresis was applied to determine BTP in CSF.[12] This technology has been surpassed by a nephelometric assay for BTP that overcomes the drawbacks of time, cost, and necessary equipment associated with immunoelectrophoresis but has at least equivalent if not greater sensitivity and specificity compared to β2-transferrin analysis (Table 1).

The high sensitivity of BTP in reflecting CSF admixture to nasal secretion also has been demonstrated in a mixing exercise of nasal secretions and CSF, with the result that a CSF contamination of 5% (v/v) CSF or greater is indicated by a BTP ratio of 2.0 or greater.[13]

Table 1: Sensitivity and specificity of β2-transferrin and BTP for detection of CSF leakage (95% confidence range in brackets)

Authors	B2-Transferrin Electrophoresis		BTP Nephelometric Assay	
	Sensitivity	Specificity	Sensitivity	Specificity
Petereit 2001[14]	-	-	93%	100%
Arrer 2002[10]	93% (78–99%)	97% (94–99%)	100% (88–100%)	100% (98–100%)
Bachmann 2002[1]	-	-	80%	~100%
Schnabel 2004[9]	-	-	100% (78–100%)	100% (97–100%)
Risch 2005[15]	84% (70–92%)	100% (97–100%)	93% (81–98%)	100% (86–100%)
McCudden 2013[16]	87.4% (75.6–97.4%)	93.8% (78.6–100%)	100%	85.7%

Factors Influencing BTP Levels

Like other small plasma proteins, serum concentrations of BTP depend on renal clearance. As a consequence, serum BTP levels increase with severity of kidney disease, with the highest levels seen in patients under hemodialysis.[2]

BTP levels in CSF have been found to be reduced in patients with normal pressure hydrocephalus but elevated in patients with spinal canal stenosis.[17,18] In patients with meningitis, BTP levels in CSF are significantly reduced, a condition that is reversed with successful antibiotic therapy.[2]

N Latex BTP Assay for Detection of CSF Leakage

The N Latex BTP assay is designed for the quantitative determination of BTP in human serum, heparinized and EDTA plasma, urine, CSF, and CSF containing nasal or ear secretions using the BN™ Systems from Siemens Healthineers.

For secretion sample collection, a tampon is placed in the nose or ear for about 4–6 hours, followed by centrifugation to extract the secretion.[2] The supernatant obtained is then diluted using N Sample Diluent buffer.

The nephelometric assay, in addition to taking considerably less time than ß2-transferrin electrophoresis, is more precise in that a direct quantification is obtained. In contrast, visual result interpretation is necessary in ß2-transferrin electrophoresis, with the inherent problems of observer error, being less readily repeatable, and providing only a qualitative yes/no result compared to the automated, quantitative nephelometric BTP method.

Table 2: N Latex BTP assay reference ranges in mg/L.

Sample Type	N	2.5th %	Median	97.5th %
CSF*	178	8.89	15.9	25.9 mg/L
Serum*	166	0.30	0.50	0.77 mg/L
CSF/serum ratio*	162	62.5	30.3	17.0 mg/L
Nasal secretion†	160	Not reported	0.39	1.31 mg/L

*N Latex BTP validation study at University Ulm, Germany.
†Meco 2003.[2]

Sensitivity and Specificity of the N Latex BTP Assay for Detection of CSF Leakage

The first study was published in 2001 in patients with rhinorrhea or otorrhea (secretions from nose or ear) and showed high sensitivity and specificity for the detection of CSF leaking from nose or ear (Table 1). Applying a cutoff of 1.31 mg/L, the sensitivity and specificity of the BTP assay in 187 patients with clinically suspected CSF rhinorrhea were both superior to those of the β2-transferrin analysis, with positive predictive value (PPV) and negative predictive value (NPV) of 100% and 97.1%, respectively.[10] Further studies confirmed these findings.[1,9]

In the study by Risch,[4] BTP testing allowed good discrimination between the presence or absence of CSF leakage, with an AUC of 0.98 in ROC analysis (N = 176 samples from 105 patients). At a decision limit of 0.68 mg/L BTP, sensitivity for exclusion of CSF leakage was 100% with 91% specificity, whereas at a level of 1.11 mg/L BTP, the best discrimination between positive and negative cases was obtained, with a sensitivity and specificity of 93% and 100%, respectively.

As basal levels in the secretion might vary depending on the plasma level, the ratio of the BTP concentration in the secretion to the serum or plasma concentration was investigated as well. When applying the secretion-to-serum ratio, a ratio of <1.02 could exclude CSF leakage at 74% specificity, while a ratio of >4.9 confirmed a leak at 92% sensitivity.[15]

Diagnostic Algorithm for BTP Interpretation

Recommended BTP decision levels for CSF leakage according to Bernasconi 2015:

BTP in secretion

<0.70 mg/L:	Exclusion of CSF leakage with high certainty
0.70–1.30 mg/L:	Consider ratio
>1.30 mg/L:	CSF leakage very likely*

BTP secretion/serum ratio

≥2.0:	CSF leakage confirmed
<2.0:	Presence of CSF unlikely; repeated testing on later sample recommended

*If ratio <1.0, elevation may be unspecific, e.g., due to blood contamination or passive transfer; repeated sampling recommended.

Table 3: Results of BTP validation study.

		Clinical CSF Leakage Status		
		Positive	Negative	**Total**
BTP Interpretation	Positive	58	7	**65**
	Negative	1	167	**168**
	Total	**59**	**174**	**233**

Table 4: Sensitivity, specificity, PPV, and NPV values.

Interpretation, Cutoff	Sensitivity	Specificity	PPV	NPV
BTP ≥1.3 mg/L	91.5	97.7	93.1	97.2
BTP ≥0.7 mg/L	98.3	80.2	62.4	99.3
Ratio ≥2	96.5	95.3	87.3	98.8
Risch algorithm	93.2	96.0	88.7	97.7
Bernasconi algorithm	98.3	96.0	89.2	99.4

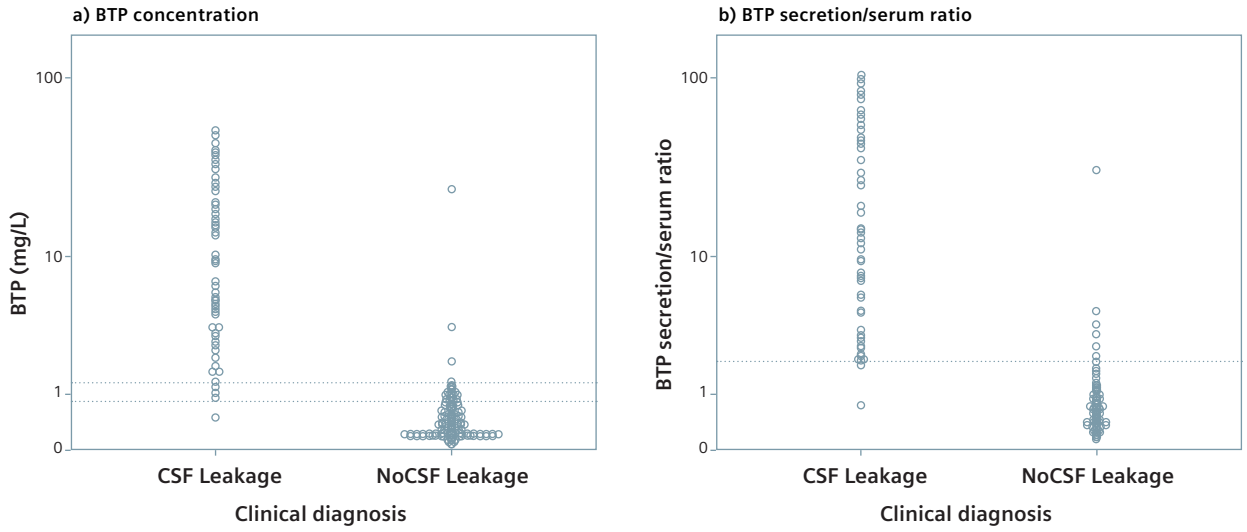
To validate the above proposed diagnostic algorithm, the Kantonsspital Aarau, Switzerland, performed a retrospective study of all samples submitted for BTP testing to determine CSF leakage between 2004 and 2012 (and for which the final, clinical diagnosis was available). BTP results were evaluated versus the final clinical diagnosis established.[19]

During this time, 747 test requests from 409 patients were recorded. 233 samples (from 121 patients) fulfilled the inclusion criteria regarding availability of both BTP results and clinical patient evaluation. Mean patient age was 48 years (±18), 98% of samples were nose secretion, and most samples were from neurosurgery/surgery (52%) and otorhinolaryngology (35%).

Patients with clinically confirmed CSF leakage had a significantly higher mean BTP concentration (median 6.8 mg/L; mean 13.4 ±14.0 mg/L) than without leakage (median 0.3 mg/L, mean 0.6 ±1.8 mg/L; $p < 0.001$) (Figure 2). Both BTP concentration and BTP secretion/serum ratio provide an AUC of 0.98 in ROC analysis.

This retrospective study confirmed previous findings, showing the BTP assay's ability to distinguish between patients who are positive and negative for CSF leakage and provide high sensitivity and specificity with outstanding operational performance (PPV/NPV).

Figure 2: BTP secretion in cases with and without clinically confirmed CSF leakage. Horizontal lines represent the cutoffs according to the diagnostic algorithm.



Advantages and Limitations of BTP Determination for Detection of CSF Leakage

N Latex BTP determination in nasal or ear secretions is a noninvasive technique able to confirm or exclude CSF leakage with high sensitivity and specificity, which may not be possible using expensive magnetic imaging methods. The N Latex BTP assay avoids the trauma of lumbar puncture and potential complications associated with sodium fluorescein imaging and may reduce the number of patients in whom this technique must be applied.

The N Latex BTP assay shares the advantages of the β2-transferrin electrophoresis assay. While determination of β2-transferrin is a labor-intensive method requiring isoform separation by electrophoresis and specific equipment, BTP testing is far more rapidly accessible[2] and automated on the BN Systems from Siemens, which are widely available in clinical routine labs. Evaluation of β2-transferrin is performed manually, requiring experience in interpreting borderline results, whereas BTP determination directly by the nephelometer rules out observer error, making the test readily repeatable and reproducible. The N Latex BTP assay requires as little as 5 µL of sample.[13] The lower cost of the N Latex BTP assay,[2] combined with a fast turnaround time, allows for regular checks in case of intermittent CSF leakage and to confirm the long-term success of fistula surgery, particularly when considering the relatively high risk of leakage recurrence.[5]

Table 5: Comparison of the β2-transferrin and BTP assays.

β2-Transferrin Electrophoresis	BTP Nephelometric Assay
Noninvasive	Noninvasive
Applicable to comatose patients	Applicable to comatose patients
Up to 24-hour turnaround	Assay results within 15 minutes
Visual evaluation	Direct reading by device
Partly automated	Fully automated, allowing 24/365 use
Very costly if single sample evaluation	Cost-efficient random-access testing

Summary

A sensitive and reliable test is vital to identify CSF leakage because of the high risk of bacterial meningitis and its associated significant mortality rate. After surgical intervention, CSF leakage may recur;[5] thus, repeated testing in such patients is indicated. The high concentration of BTP in CSF versus its low concentration in plasma results in a CSF-serum ratio of about 35—the highest observed for any CSF protein—a feature that qualifies BTP as an ideal marker to identify CSF in other body fluids.

The N Latex BTP assay allows identification of CSF leakage with equivalent or better sensitivity and specificity than β2-transferrin and is similarly noninvasive. In addition, determination of the BTP level is far more rapid, cost-efficient, and easier than that of β2-transferrin, particularly because of the BN Systems' automation and single-sample STAT capability.[20]

The definition of positive (>1.3 mg/mL) and negative (<0.70 mg/mL) BTP cutoff values in nasal secretions provides a ready interpretation of the data. In the grey area between these values, a ratio of NS-to-serum BTP ≥2.0 indicates a confirmation of leakage. When results are inconsistent, they must be discussed, taking into consideration both the clinical and laboratory data and further follow-up.

In 2009, the EFNS (European Federation of Neurological Societies) included BTP testing in their guidelines on disease-specific CSF investigation for detection of CSF admixture in rhino- and otorrhea.[21] Thus, BTP testing should be the first choice to confirm or reject suspicion of CSF leakage, allowing better stratification to surgical intervention if indicated, and thereby reduce the risk of developing life-threatening bacterial meningitis and confirm the success of such surgery.

References:

1. Bachmann G, Petereit H, Djenabi U, et al. Predictive values of β-trace protein (prostaglandin D synthase) by use of laser-nephelometry assay for the identification of cerebrospinal fluid. Neurosurgery. 2002;50:571-7.
2. Meco C, Oberascher G, Arrer E, et al. β-Trace protein test: new guidelines for the reliable diagnosis of cerebrospinal fluid fistula. Otolaryngol Head Neck Surg. 2003;129:508-17.
3. Durand ML, Calderwood SB, Weber DJ, et al. Acute bacterial meningitis in adults. A review of 493 episodes. N Engl J Med. 1993;328:21-8.
4. Eljamel MS, Foy PM. Acute traumatic CSF fistulae: the risk of intracranial infection. Br J Neurosurg. 1990;4:381-5.
5. Schoentgen C, Henaux PL, Godey B, et al. Management of post-traumatic cerebrospinal fluid (CSF) leak of anterior skull base: 10 years' experience. Acta Otolaryngol. 2013;133:944-50.
6. Warnecke A, Averbeck T, Wurster U, et al. Diagnostic relevance of beta2-transferrin for the detection of cerebrospinal fluid fistulas. Arch Otolaryngol Head Neck Surg. 2004;130:1178-84.
7. Meurman OH, Irjala K, Suonpaa J, et al. A new method for the identification of cerebrospinal fluid leakage. Acta Otolaryngol. 1979;87:366-9.
8. Kelly RH, Kelly CM, Busis SN. Factitious hearing loss and otorrhea in an adolescent boy. Clin Chim Acta. 2000;299:205-9.
9. Schnabel C, Di Martino E, Gilsbach JM, et al. Comparison of β2-transferrin and β-trace protein for the detection of cerebrospinal fluid in nasal and ear fluids. Clin Chem. 2004;50:661-3.
10. Arrer E, Meco C, Oberascher G, et al. β-Trace protein as a marker for cerebrospinal fluid rhinorrhea. Clin Chem. 2002;48:939-41.
11. Reiber H, Walther K, Althaus H. Beta-trace protein as a sensitive marker for CSF rhinorrhea and CSF otorrhea. Acat Neurol Scand. 2003;108:359-62.
12. Bachmann G, Nekic M, Michel O. Clinical experience with beta-trace protein as a marker for cerebrospinal fluid. Ann Otol Rhinol Laryngol. 2000;109(12 Pt 1):1099-1102.
13. Kleine TO, Damm T, Althaus H. Quantification of ß-trace protein and detection of transferrin isoforms in mixtures of cerebral fluid and blood serum as models of rhinorrhea and otorrhea diagnosis. Fresenius J Anal Chem. 2000;366:382-6.
14. Petereit HF, Bachmann G, Nekic H, et al. A new nephelometric assay for β-trace protein (prostaglandin D synthase) as an indicator of liquorrhea. J Neurol Neurosurg Psychiatry. 2001;71:347-51.
15. Risch L, Lisec I, Jutzi M, et al. Rapid, accurate and non-invasive detection of cerebrospinal fluid leakage using combined determination of β-trace protein in secretion and serum. Clin Chimica Acta. 2005;351:169-76.
16. McCudden CR, Senior BA, Hainsworth S, et al. Evaluation of high resolution gel β(2)-transferrin for detection of cerebrospinal fluid leak. Clin Chem Lab Med. 2013;51:311-15.
17. Mase M, Yamada K, Iwata A, et al. Acute and transient increase of lipocalin-type prostaglandin D synthase (beta-trace) level in the cerebrospinal fluid of patients with aneurysmal subarachnoid hemorrhage. Neurosci Lett. 1999;270:188-90.
18. Mase M, Yamada K, Shimazu N, et al. Lipocalin-type prostaglandin D synthase (beta-trace) in the cerebrospinal fluid: a useful marker for the diagnosis of normal pressure hydrocephalus. Neurosci Res. 2003;47:455-9.
19. Bernasconi L, Pötzl T, Steuer C, et al. Retrospective validation of a ß-trace protein interpretation algorithm for diagnosis of cerebral fluid leakage. Clin Chem Lab Med. 2017;55:554-560.
20. Mantur M, Lukaszewicz-Zając M, Mroczko B, et al. Cerebrospinal fluid leakage--reliable diagnostic methods. Clin Chim Acta. 2011;412(11-12):837-40.
21. Deisenhammer F, Egg R, Giovannoni G, et al. EFNS guidelines on disease-specific CSF investigations. Eur J Neurol. 2009;16:760-70.

Product availability may vary from country to country and is subject to varying regulatory requirements. Please contact your local representative for availability.

Local Contact Information

Siemens Healthcare Diagnostics Inc.
Laboratory Diagnostics
511 Benedict Avenue
Tarrytown, NY 10591-5005
USA
Phone: +1 914-631-8000
siemens.com/healthcare

Siemens Healthineers Headquarters
Siemens Healthcare GmbH
Henkestr. 127
91052 Erlangen
Germany
Phone: +49 9131 84-0
siemens.com/healthcare

Order No. A91DX-HHS-151609-XC1-4A00 | 06-2017 | © Siemens Healthcare Diagnostics Inc., 2017

siemens.com/healthcare