REVIEW

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Diagnostic values of beta-2 transferrin and beta-trace protein as markers for cerebrospinal fluid fistula*

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SUMMARY

Introduction: *During recent decades, β_2-transferrin and β-trace protein (prostaglandin D synthase) have been used as immunological markers for the diagnosis of CSF fistula. A method for detecting CSF traces should be non invasive, reliable and cheap.*

Methods: *The characteristics of the two immunological markers are described based on own experience and a literature review. PubMed (1966 – 2007) was searched and 39 articles were retrieved from the period 1987 – 2007.*

Results: *The β_2-transferrin marker showed a high reliability during the last decades using immunofixation or immunoblotting. The performance of β_2-transferrin assay requires between two and four hours hands-on time in the laboratory depending on the assay. The β-trace protein protein marker showed a high reliability when assayed using immunoelectrophoresis or laser-nephelometry. Laser-nephelomety is automated, non- time consuming, provides quantitative results and last but not least, is cheap. A cut-off point at 1.11 mg/l for β-trace protein gave the best trade-off between high sensitivity and high specificity when including the secretion/serum ratio.*

Conclusion: *Both β_2-transferrin and β-trace protein are reliable immunological markers for the detection of CSF traces. High diagnostic accuracy values were found for both β_2-transferrin and β-trace protein protein.*

Key words: cerebrospinal fluid, fistula, liquorrhea, β-trace protein, Prostaglandin H_2-D isomerase, Protein isoforms, β2-transferrin, surgery, complication, skull base

INTRODUCTION

Bacterial meningitis is a live-threatening disease. The mortality of bacterial meningitis was estimated to be 25 – 50%, and about 20% of cases of bacterial meningitis are due to a CSF leak [(1)]. CSF fistulas can be a sequel of head trauma, surgical procedures at the skull base, increased intracranial pressure, arachnoid granulations, malignancy or congenital malformation. The estimated cumulative risk for patients with an acute traumatic CSF leak without dural repair to develop bacterial meningitis is more than 85% at 10 years [(2)]. Sometimes, patients with a CSF leak remain undiagnosed and manifest CSF rhinorrhea with meningitis that can occur decades after a head injury [(3-7)].

The barrier between the external environment and the brain

In healthy individuals, the intracranial cavity, the middle ear and the paranasal sinuses are sterile. The nasal cavity and the epipharynx contain bacteria. During episodes of sinusitis or otitis, which are common diseases, the paranasal sinuses and the middle ear also contain infective agents. These two compartments, the intracranial cavity and the pneumatized sinuses of the skull base have to be separated by a reliable barrier. During evolution, nature has developed several different layers, in order to provide a reliable, durable and water-tight barrier. Under normal conditions it is impossible for bacteria to pass through the mucosa, the periosteum, the bone, the dura mater and the arachnoid membrane, which are the main structures of this barrier [(8)]. However, trauma or other conditions may cause a defect of this barrier creating thus an opening between the intracranial space and the pneumatized space of the skull base, which is by definition a CSF fistula. In three areas, these two compartments are narrowly separated so that the brain is more exposed to the external environment or to iatrogenic damage during surgical procedures: these are the labyrinth within the temporal bone, the orbit and even more so, the olfactory region.

The surgical treatment of CSF fistula using endoscopic approaches showed low morbidity and is highly effective.

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A reliable diagnostic method to detect CSF or to exclude the presence of CSF is therefore of importance for the rhinologist, otologist and skull base surgeon.

MATERIAL AND METHODS

PubMed (1966-2007) was searched for the terms "(cerebrospinal fluid AND fistula) AND (beta-trace protein OR beta-2 transferrin)" yielding 39 articles from the period 1987 – 2007. Eleven of the articles focused on the diagnosis of perilymphatic fluid fistula and were therefore excluded. In addition, secondary references collected during the last decade were reviewed. The main focus was on the diagnostic values of β_2-transferrin and beta-trace protein (β-TP) and on the cut-off of β-TP as a marker for CSF fistula.

RESULTS AND DISCUSSION

β_2-transferrin

The β_2-transferrin protein was introduced as a marker for CSF by Meurman et al. in 1979 [9]. Further clinical experience was published by several authors [10-18]. Three different electrophoretic assays including immunofixation or immunoblotting were described.

Advantages: The minimum sample volume of a pure CSF sample to be detected by β_2-transferrin gel electrophoresis is 2 µl.

Disadvantages: It takes between two and four hours hands-on time in the laboratory to perform the assay. Recently, an automated immunofixation electrophoresis system was introduced [19]. A reliable β_2-transferrin analysis is not possible in samples which are contaminated by blood [20]. The normal values of β_2-transferrin in CSF have been quantified between 12 – 78 mg/l [21]. Quantitative values of β_2-transferrin in serum have not yet been published. The CSF/serum ratio of β_2-transferrin is not known. Increased β_2-transferrin values have been found in serum from subjects with liver failure [22-23].

Beta-trace protein

The protein was first described by Clausen in 1961 [24]. Initially, an immunoelectrophoresis was used. Later, a nephelometric research assay was introduced for the quantification of β-TP in 2001 (N latex βTP, Dade Behring) [25]. In 1993, after amino acid sequence determination, it was shown that β-TP is identical to prostaglanding D synthase (EC 5.3.99.2) [26].

Advantages: Beta-trace protein showed a CSF/serum ratio of 33, which is the highest of CSF specific proteins known today [27]. The minimum sample volume to detect CSF traces was 5 µl. Samples with or without blood contamination could be measured because of the 1:100 default dilution. In three prospective studies, both CSF markers were compared. The diagnostic values favoured β-TP (Table 1). The serum values of β-TP were stable. Beta-trace protein was used as a marker for standardised quality control after endoscopic CSF fistula repair [28-30].

Disadvantages: The protein is contained in urine and increased β-TP values have been found in serum from subjects with renal failure [31-32].

The cut-off: A first cut-off point for β-TP was proposed at 6 mg/l and resulted in high specificity and relatively low sensitivity (between 0.91 and 0.93). Later, lower cut-offs were proposed (Table 2). Using a cut-off point at 1.31 mg/l, Arrer et al. demonstrated sensitivity and specificity of 100 % (95% confidence interval, 88 – 100% and 98 – 100% respectively). Reiber et al. proposed a cut-off at 0.35 mg/l [33]. It astonishes that Reiber and co-workers did not find false positive results. A possible explanation is that they only used samples which did not contain blood or serum. As the mean β-TP value in blood is about 30 times higher compared to its mean value in nasal secretions, a very low cut-off value would seem to be justified in samples that are devoid of blood or serum. A cut-off at 0.35 mg/l is only justified in samples not containing blood or serum. Such a low cut-off should not be used in samples containing blood or serum. The range of β-TP in serum is between

Table 1. Diagnostic values of immunological markers for CSF fistula.

Author	N	β_2-transferrin		β-TP	
		Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Arrer E et al. 2002	30	93 (78-99)	97 (94-99)	100 (88-100)	100 (98-100)
Schnabel C et al. 2003	25	73	Not stated*	100 (78-100)	100 (86-100)
Risch L et al. 2005	105	84	100	99	100

The diagnostic values for β_2-transferrin or β-TP. *In 37% of all investigated patients the interpretation of the immunofixation was not possible.

Table 2. The cut-off of β-TP and the corresponding sensitivity and specificity.

Author	cut-off [mg/l]	Subjects	sensitivity	specificity
Bachmann G et al. 2000	6	98	0.91	1
Petereit HF et al. 2001	6	100	0.93	1
Arrer E et al. 2002	1.31	160	1	0.97
Reiber H et al. 2003	0.35	49 + 132	Not stated	not stated
Schnabel C et al. 2004	1.0	26 + 7	1	1
Risch L et al. 2005	1.11*	105	0.99	1

The study from Risch et al. was the only study applying receiver-operating characteristics. *In addition to a β-TP cut-off at 1.11 mg/l, a secretion/serum ratio cut-off at 4.9 was proposed.

Table 3. Beta-trace protein values in serum.

	Serum range (mg/l)	Mean (mg/l)
Bachmann et al. 2002	0.2 – 1.8	0.69 ± 0.33
Arrer et al. 2002	0.117 - 1.44	0.59 ± 0.23
Reiber et al. 2003	0.38 - 0.86	0.59 ± 0.11

Normal values of β-TP in serum.

0.38 and 0.86 or between 0.117 and 1.44 mg/l (Table 3). Accordingly, for blood-contaminated samples, the cut-off value for CSF contamination should be above the average serum values. The correct β-TP value in nasal secretions is only measurable when the samples are processed undiluted 1:1 (or diluted 1:2, 1:4 or 1:10 in order to avoid highly viscous samples). However, in clinical situations, samples from the nose or the ear often contain blood or serum. Therefore, a cut-off point, which is lower than the mean value of β-TP in serum, cannot be universally recommended. In clinical situations, it may be difficult to know whether a sample contains serum or not. The inclusion of serum values allows the calculation of the secretion/serum ratio. Using a cut-off at 1 mg/l, Schnabel et al. found a sensitivity and specificity of 100%. However, a study population of 26 subjects in this study might not be representative of all patients with CSF leaks. Risch et al. investigated β-TP and β_2-transferrin both in nasal secretions and serum samples from 105 patients [34]. Using receiver-operating-characteristics, a cut-off at 1.11 mg/l was recommended. As proposed by Kleine, the secretion/serum-ratio was investigated as an additional diagnostic tool. A β-TP secretion/ serum-ratio cut-off at 4.9 was recommended for secretion values in the range between 0.68 and 1.11 mg/l. In this study, a specificity of 100% and a sensitivity of 99% were pertained.

Diagnostic management of CSF fistula

The diagnosis of CSF fistula might be a challenge because fistulas producing little CSF do not give any clinical sign. Patients with vasomotor rhinitis or autonomic dysfunction after trauma may present with clear watery rhinorrhea mimicking CSF fistula. To exclude the presence of CSF by a laboratory method will prevent these patients from further expensive and potentially hazardous diagnostic work up. A sensitive and specific laboratory method for the detection of CSF traces is clinically important. A secretion sample for β-TP or β_2-transferrin should always be obtained first (Figure 1). A secretion sample (and in some cases a serum sample for β-TP) can be repeated several times at low costs and without side effects. Only when the presence of CSF is confirmed, more invasive methods are indicated to localise the site of the defect. High resolution CT and flow sensitive MRI are the methods of choice. When the presence of CSF is proven but the site of the fistula can not be demonstrated by imaging techniques, pre-operatively intrathecally administered fluorescein dye is indicated to identify the defect during surgical exploration [35].

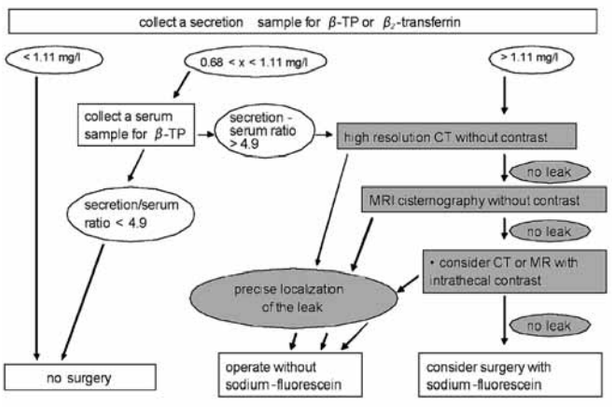

Figure 1. Diagnostic approach to CSF fistula using β-TP or β_2-transferrin. The diagnostic approach consists of two parts: The detection of CSF traces (white fields) and the localisation of the CSF fistula (grey fields). For part one β_2-transferrin or β-TP can be assayed.

CONCLUDING REMARKS

Both, β-TP and β_2-transferrin are reliable markers of traces of CSF. Comparably high diagnostic values were found for both of these markers. Beta$_2$-transferrin can be measured in the lowest sample volumes when analysing pure CSF samples. From a laboratory point of view, β-TP is easier to handle and the assay is faster. In 2003, Meco et al. stated the costs for β_2-transferrin to be $ 50 per sample and for β-TP to be $ 20 per sample [36].

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