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Sample-dependent diagnostic accuracy of prostaglandin D synthase in cerebrospinal fluid leak

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ABSTRACT

Background: Prostaglandin D2 synthase, commonly known as β-trace protein (βTP), is an excellent biomarker for the assessment of cerebrospinal fluid (CSF) leaks. Despite being widely used, the limits for the diagnostic values of βTP are not well established to date, and currently suggested cut-off values in literature range from 0.25 to 6.0 mg/L. Sample-specific and more accurate thresholds are a current need.

Methods: A retrospective observational study, performed in a tertiary-care hospital, between January 2006 and January 2014. A total of 74 patients were included, with a definitive diagnosis after initial leak suspicion and at least one determination of βTP using a nephelometry-based assay. A total of 46 CSF samples were included in the control group. Samples were obtained from nasal secretions, ear secretions or spinal surgical injury, directly using sterile Eppendorf tubes. The analysis of 3 different cut-off values was performed and the receiver operating curve (ROC) analyses were calculated.

Results: Initial diagnostic suspicion was confirmed in 51% of cases, most of which were of postoperative origin (51%) and traumatic (26%). The βTP median concentration in different samples was significantly higher in the presence of cerebrospinal fluid fistula, regardless of sample type (22.0 mg/L vs. 0.24 mg/L, 95% confidence interval: 19.0–30.8 vs. 0.08–0.40; $p < 0.001$). Data from contingency tables show 100% sensitivity and specificity, depending on sample type and the cut-off value used: for rhinorrhea and otorrhea samples, the most appropriate it was 0.7 mg/L, while values >2.0 mg/L could be used for spine postoperative fluid leakage samples.

Conclusions: The cut off value for βTP in the diagnosis and follow-up of cerebrospinal fluid leaks should be modified depending on the type of secretion (sample type), for a better diagnostic accuracy.

1. Introduction

Cerebrospinal fluid (CSF) is a colorless liquid produced in the ventricular choroid plexus in the brain and provides mechanical protection, nutrient supply, metabolite transportation and waste product removal [1]. Due to constant interactions, its biomolecule content may reflect most of the processes occurring at the central nervous system [2]. CSF shows a complex composition, with about 80% of the protein content coming from plasma, while the remaining 20% of the proteome is synthesized intrathecally [3,4].

Cerebrospinal fluid leak results when there is a pathological connection (fistula) between the subarachnoid space and the nasal cavity, the paranasal sinuses or the middle ear. As a result, the biofluid drains from the ear or the nose, depending on the location of the skull bone damage. The main causes of CSF leaks are traumatisms (traumatic brain injury) or postoperative (after brain surgical intervention), although 4% of cases result from inflammatory, infectious or neoplastic processes [5–7].

Life-threatening situations may arise from undetected and untreated leaks. The risk of developing meningitis or intracranial abscess was defined as 10% when the diagnosis was delayed more than one week [8], with an associated mortality of 50% [9]. Therefore, the accurate diagnosis of CSF leak is essential for its precise location by imaging techniques, and the subsequent early surgical reparation [8].

In this study, we aimed to improve the current clinical diagnostic strategies in the assessment of CSF leaks by defining and specifying the cut-off values for prostaglandin D2 synthase, also known as β-trace protein (βTP), with highest diagnostic accuracy in different specimens, as measured by a nephelometry-based assay.

2. Methods

2.1. Data collection

This is a retrospective observational study, performed in a tertiary-care hospital, and was approved by the Ethics Board of our institution.

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Between January 2006 and January 2014, patients with an established definitive diagnosis and at least one βTP determination were included.

Inclusion criteria comprised cranial traumatism with aqueous rhinorrhea or otorrhea, spontaneous unilateral rhinorrhea or otorrhea without any objective improvement after medical treatment, recurrent meningitis or a past surgical intervention of the nose, ear, transphenoid, base of the skull or rachidic. Patients with kidney disease, defined by levels of serum creatinine above 2.5 mg/dL (221 μmol/L) or the need of dialysis treatment, were excluded as well as excessively viscous samples after 2 centrifugation-filtration cycles.

Patient data were obtained with permission from the hospital medical records, and the diagnostic process was followed up until a definitive conclusion was reached (either presence or absence of CSF leak). Data regarding age, gender, imaging technique results, βTP determinations, definitive diagnosis and treatment were registered.

2.2. Control group

A total of 46 CSF samples from the Emergency and Neurology departments were included, obtained from both pediatric and adult populations. Samples from patients with a clinical suspicion of meningitis or CSF fistula were excluded as control group.

2.3. Biochemical analysis

Samples were obtained from nasal secretions, ear secretions or spinal surgical injury, directly using sterile Eppendorf tubes, and were analyzed within 24 h after extraction.

Preanalytical sample treatment included a 10 min centrifugation at 1000*g*. Samples with high viscosity were previously filtered using filter centrifugation tubes (Amicon Ultra, Merck Millipore, US) and centrifuged 20 min at 13,500 g. If viscosity persisted, samples were diluted 1:2 or 1:5 with a phosphate buffer and a further 13,500 g centrifugation was carried out.

The quantification of βTP was performed using an end-point immunonephelometric assay, N Latex betaTP (Dade Behring, Marburg, Germany) on the analyzer BN™ II (Siemens Healthcare, Malvern, US). The reagent uses polystyrene particles covered with rabbit polyclonal antibodies against human βTP and avoids interferences due to rheumatoid factor.

With a minimum sample volume of 200 μL, the dynamic range covers from 0.25 to 15.8 mg/L. Analytical imprecision is <6.5% [17], in-house imprecision was determined to 15 series of two controls of known concentrations of βTP (1.7 mg/L and 0.85 mg/L) in triplicate, obtaining a 4.84% of imprecision and standard deviation of a 0.1%.

Table 1
Epidemiological descriptive data.[a]

	Rhinorrhea	Otorrhea	Spinal fluid leakage	Total
N	44	17	13	74
Age, years	52 (21)	36 (27)	52 (20)	49 (22)
Male, %	22 (50)	12 (71)	4 (31)	38 (51)
Diagnostic techniques				
CT	12 (27)	10 (59)	3 (23)	25 (34)
MRI	6 (14)	0	6 (46)	12 (16)
CT + MRI	19 (43)	7 (41)	4 (31)	30 (41)
Endoscopy	11 (25)	0	0	11 (15)
CSF fistula				
Yes	18 (41)	11 (65)	9 (69)	38 (51)
Surgical	8 (44)	3 (27)	9 (100)	20 (52)
Traumatic	4 (22)	6 (55)	0	10 (26)
Tumoral	3 (17)	1 (9)	0	4 (11)
Spontaneous	3 (17)	1 (9)	0	4 (11)
βTP, mg/L	**22.2 (6.2–45.3)**	**25.2 (4.4–79.3)**	**20.6 (11.3–29)**	
No	26 (59)	6 (35)	4 (31)	36 (49)
βTP, mg/L	**0.10 (0.01–0.52)**	**0.25 (0.02–0.63)**	**0.96 (0.51–1.89)**	

CT: computerized tomography, MRI: magnetic resonance imaging, βTP: beta-trace protein.
[a] Data are expressed as mean(SE), n(%), or median(percentile5–percentile95).

Table 2
Contingency data according to sample type.

	Rhinorrhea	Otorrhea	Spinal fluid leakage
Cut off *βTP*	**3.0 mg/L**	**3.0 mg/L**	**3.0 mg/L**
Sensitivity	100 (83.2–100)	100 (71.5–100)	100 (66.4–100)
Specificity	100 (86.8–100)	100 (54.1–100)	100 (54.1–100)
PPV	100 (83.2–100)	100 (71.5–100)	100 (66.4–100)
NPV	100 (86.8–100)	100 (54.1–100)	100 (54.1–100)
Cut off *βTP*	**1.1 mg/L**	**1.1 mg/L**	**1.1 mg/L**
Sensitivity	100 (83.2–100)	100 (71.5–100)	100 (66.4–100)
Specificity	100 (86.8–100)	100 (54.1–100)	66.7 (22.3–95.7)
PPV	100 (83.2–100)	100 (71.5–100)	81.8 (48.2–97.7)
NPV	100 (86.8–100)	100 (54.1–100)	100 (39.8–100)
Cut off *βTP*	**0.7 mg/L**	**0.7 mg/L**	**0.7 mg/L**
Sensitivity	100 (83.2–100)	100 (71.5–100)	100 (66.4–100)
Specificity	100 (86.8–100)	100 (54.1–100)	33.3 (4.3–77.7)
PPV	100 (83.2–100)	100 (71.5–100)	69.2 (38.6–90.9)
NPV	100 (86.8–100)	100 (54.1–100)	100 (15.8–100)
Cut off *βTP*	**0.35 mg/L**	**0.35 mg/L**	**0.35 mg/L**
Sensitivity	100 (83.2–100)	100 (71.5–100)	100 (66.4–100)
Specificity	88.5 (69.9–97.6)	66.7 (22.3–95.7)	0 (0–45.9)
PPV	87 (66.4–97.2)	84.6 (54.6–98.1)	60 (32.3–83.7)
NPV	100 (85.2–100)	100 (39.8–100)	0
AUC	**1.0 (1)**	**0.9 (0.7–1.0)**	**1.0 (1)**

Data are shown as % (CI 95%).
PPV: positive predictive value, NPV: negative predictive value, AUC: area under curve. *βTP:* beta-trace protein.

2.4. Statistical analysis

Categorical variables were shown as absolute numbers and percentages. Continuous data following a normal distribution were shown as mean and standard deviation. βTP concentrations were shown as median, 5th–95th percentiles, due to a non-Gaussian distribution (checked using the Kolmogorov-Smirnov test).

Medians were compared using the Mann Whitney's *U* test. Statistical analyses were performed using SPSS v 17 (SPSS, Chicago), and the probability of type I error as $\alpha = 0.05$.

Contingency tables for the analysis of 3 different cut-off values were performed (rhinorrhea, otorrhea and spinal fluid leakage). The cut-off values to be analyzed were based on previous studies: 0.35 mg/L [18],

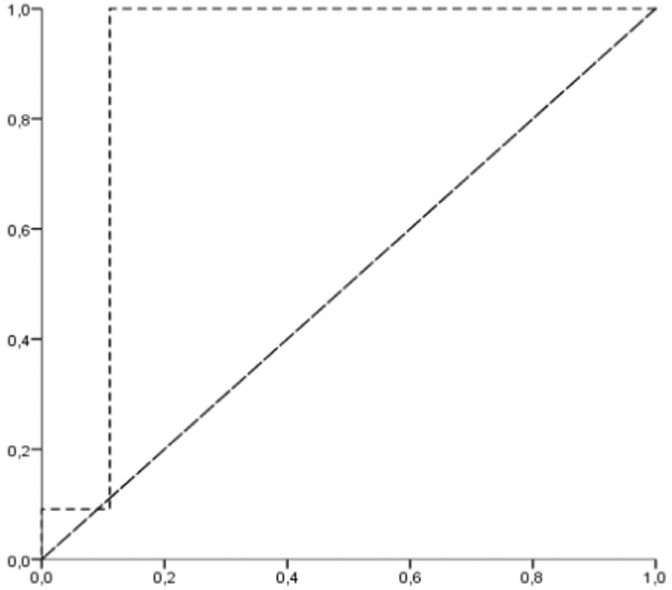

Fig. 1. ROC curve for otorrhea samples ($n = 17$), AUC = 0.899 (CI 95%: 0.711–1.00). Optimal cut-off: 0.6 mg/L (y-axis: sensitivity; x-axis: 1-specificity).

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