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ORIGINAL COMMUNICATION

Phosphorylated tau 181 (p-tau$_{181}$) as an innovative, fast and robust biomarker for cerebrospinal fluid leaks

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Abstract

Background Cerebrospinal fluid (CSF) leaks can lead to serious complications if left untreated, making rapid and accurate diagnosis essential. Biomarkers such as β2-transferrin (B2TRF) and β-trace protein are used to detect CSF leaks, but their limitations warrant the exploration of alternative markers. This study investigates the potential of phosphorylated tau at threonine 181 (p-tau$_{181}$) as a biomarker for CSF leaks.

Methods Samples from 56 subjects were analyzed for B2TRF and p-tau$_{181}$ using immunoaffinity blotting and chemiluminescent enzyme immunoassay, respectively. Data analysis included Mann–Whitney test to assess the overall difference in median p-tau$_{181}$ concentrations between B2TRF positive and negative patients and a receiver operating characteristic (ROC) curve analysis to determine optimal p-tau$_{181}$ cutoff values for predicting B2TRF positivity.

Results p-tau$_{181}$ levels were significantly higher in B2TRF positive samples compared to negative samples ($p < 0.001$). ROC analysis showed high diagnostic performance for p-tau$_{181}$, with an optimal cutoff of 13.22 pg/mL providing 92.0% sensitivity and 93.1% specificity. Excluding hemolyzed samples improved further the diagnostic performances, maintaining high sensitivity (90.9%) and achieving perfect specificity (100.0%).

Conclusions This study highlights the potential of p-tau$_{181}$ as a valuable biomarker for the detection of CSF leaks due to its high diagnostic accuracy and practical advantages over the current biomarkers. The characteristics of p-tau$_{181}$ assay being both quantitative and rapid, with high diagnostic accuracy, suggest that it could be a valuable tool for the detection of CSF leaks. Further research are now needed to validate these findings.

Keywords p-tau$_{181}$ · Cerebrospinal · Leak · Biomarker · Neurology

Introduction

Cerebrospinal fluid (CSF) surrounds the brain and spinal cord, providing a cushion to protect them from injury. The meninges are composed of three layers that surround the spinal cord and brain, and when there is a puncture or tear in the outermost layer, called the dura mater, it may lead to a leak of CSF. There are two different types of CSF leak with different symptoms, causes and treatments. First, the spinal CSF leaks, which can occur anywhere along the spine and which are usually characterized by orthostatic headaches. Second, cranial CSF leaks, which occur within the skull and manifest by rhinorrhea or otorrhea [1–5].

The most common cause of CSF leakage occurs following post-traumatic craniofacial structural damage, which accounts for 80% of CSF leaks. Iatrogenic causes, particularly postoperative, account for 16% of CSF leaks, with the remaining 4% due to a variety of etiologies [6, 7]. If left untreated, CSF leak can lead to several complications, the most serious of these being bacterial meningitis [8]. Therefore, a rapid multidisciplinary diagnosis based on clinical, biological and radiological factors is required.

Two biomarkers are commonly used to detect the presence of CSF in a sample. The first one is β2-transferrin (B2TRF), a carbohydrate-free (desialylated) form of

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transferrin found only in CSF and in the perilymph. Its use in the detection of CSF leaks was first described in 1979 by Meurman et al. [9]. Since then, B2TRF has been widely used to diagnose CSF leaks and is considered the gold standard in this indication. Performed on an agarose gel, B2TRF detection is a qualitative and time-consuming analysis and requires an experienced eye for correct interpretation [10, 11]. The second one is β-trace protein (BTP) which is a major component of CSF representing approximatively 3% of total CSF proteins. This protein is present in CSF at concentrations 35 times higher than in serum, whereas it is almost absent in other body fluids [12, 13]. The BTP assay is quantitative and can be measured by nephelometry [14, 15]. However, it is still debated which cutoff value is appropriate to use and some conditions, like bacterial meningitis or reduced glomerular filtration, may impact BTP levels and diagnostic performances [16].

Over the last few decades, several neurological biomarkers have been studied for the diagnosis of neurodegenerative diseases, particularly for Alzheimer's disease (AD). Along with amyloid-β42 (Aβ42) and the total tau (T-tau) protein, the phosphorylated tau at threonine position 181 (p-tau$_{181}$) is a well-validated biomarker for AD diagnosis [17–19]. More recently, plasma biomarkers, including p-tau$_{181}$, have been developed as an alternative to current CSF markers. While still at the investigational stage, blood biomarkers pave the way for more accessible diagnosis and potential monitoring of neurodegenerative diseases. However, further work is still needed to determine the optimal assay that best combines sensitivity, specificity and cost-effectiveness [20, 21].

The primary aim of the present study was to investigate the use of a plasma p-tau$_{181}$ chemiluminescent enzyme immunoassay (CLEIA) to detect CSF leak in fluids of various origins. Indeed, this biomarker has several advantages over B2TRF and BTP, in particular its ease of use in the laboratory. We hypothesized that high levels of p-tau$_{181}$ would be associated with the presence of CSF in the sample.

Methods

Patients and study design

Samples analyzed for B2TRF between September 2022 and February 2024 at the Cliniques Universitaires Saint-Luc (Belgium), were stored at − 20 °C for later inclusion in our study. Samples with insufficient residual volume (< 25 µL) to perform p-tau$_{181}$ analysis were excluded. A total of 56 subjects (26 females and 30 males) with a median (± IQR) age of 61.5 ± 25.5 years (range 3–84) were included in the study. The samples include 34 nasal discharges, 3 ear discharges, 12 deep collection fluids and 7 from other sites. These samples were divided into two groups: those with a negative B2TRF (29/54–53.7%) and those with a positive B2TRF (25/54–46.3%) result. There was no statistical difference in age or gender between these two groups ($p = 0.52$ and $p = 0.29$, respectively). Two samples had an indeterminate B2TRF result and were not included in the method comparison.

Analytical methods

β2-Transferrin assay

B2TRF detection was performed with an immunoaffinity mediated blotting technique, according to a previously described protocol [22]. Briefly, samples were applied to a nitrocellulose membrane that had been coated overnight with anti-human transferrin IgG antibodies. After application, the membrane was washed and electrophoresed on an agarose gel. The gel was incubated with anti-transferrin serum after another washing step. At the end of the incubation period and after a further wash, the gel was stained for analysis before reading by an experienced person (VvP or JLB).

p-tau$_{181}$ assay

Samples were tested using a CLEIA (Lumipulse® G pTau 181 Plasma, reference 81288) on a Lumipulse® G600II analyzer (Fujirebio®, Tokyo, Japan) after appropriate calibration and successful internal control measurement (Lumipulse® pTau181 Plasma Controls, reference 81297). Each assay uses 130 µL of sample with a minimum dead volume of 100 µL when using the recommended sample beakers. Therefore, the total sample volume required per assay is at least 230 µL. In case of insufficient volume, samples were diluted up to tenfold with the company's specific diluant (Lumipulse® G Specimen Diluant 1). Measuring range of the assay ranges from 0.05 to 60.00 pg/mL.

BTP assay

BTP quantification was performed using an immuno-nephelometric technique (Siemens Healthineers®, Atellica® NEPH 630 System/BN II System/BN ProSpec® System). The principle of the method involves the use of polystyrene particles coated with anti-human BTP antibodies, which aggregate when mixed with BTP-containing samples. The intensity of the light scattered by the aggregates is proportional to the concentration of BTP in the sample.

Data analysis

The results obtained with the p-tau$_{181}$ assay was compared to the B2TRF, which was used as a gold standard.

Mann–Whitney test was used to assess the overall difference in median p-tau$_{181}$ concentrations between B2TRF positive and negative patients. Differences were considered statistically significant at $p < 0.05$. Receiver operating characteristic (ROC) analysis was performed to assess area under the curve (AUC) and the Youden Index was used to define the optimal p-tau$_{181}$ cutoff value to predict B2TRF positivity. All statistical analyses were performed using GraphPad Prism 9 software.

Results

The median concentration of p-tau$_{181}$ [IQR] was statistically higher in B2TRF positive samples compared to B2TRF negative samples: 29.83 pg/mL [22.83–55.62] vs 2.86 pg/mL [1.38 pg/mL–7.08 pg/ml] respectively; $p < 0.001$ (Fig. 1). This difference was even higher when hemolyzed samples were excluded, with a median p-tau$_{181}$ concentration of 30.57 pg/mL [21.30–55.24] in B2TRF positive patients and 2.46 pg/mL [1.22–5.64] in the negative group; $p < 0.001$ (Fig. 1).

ROC curve was plotted to determine whether p-tau$_{181}$ could be useful as a biomarker for the diagnosis of CSF leaks and to calculate an optimal cutoff. The resulting AUC (95% confidence interval (IC_{95})) was 0.954 (IC_{95} 0.902–1.000; $p < 0.001$) and, based on the Youden index, the statistically optimal cutoff was determined to be 13.22 pg/mL, giving a sensitivity of 92.0% (IC_{95} 74.0–99.0) and a specificity of 93.1% (IC_{95} 77.2–99.2). Without hemolyzed samples, the AUC was 0.974 (IC_{95} 0.934–1.000; $p < 0.001$) and the optimal calculated cutoff was 9.39 pg/mL. This gave a sensitivity of 90.9% (IC_{95} 70.8–98.9) and a specificity of 100.0% (IC_{95} 86.8–100.0) (Table 1 and Fig. 2).

For samples that did not match between the B2TRF research and p-tau$_{181}$ measurement, we performed verification by testing for BTP. We did the same with 2 other samples whose B2TRF electrophoresis gels were uninterpretable. Of these samples, 5 out of 6 were consistent with our p-tau$_{181}$ assay (Table 2).

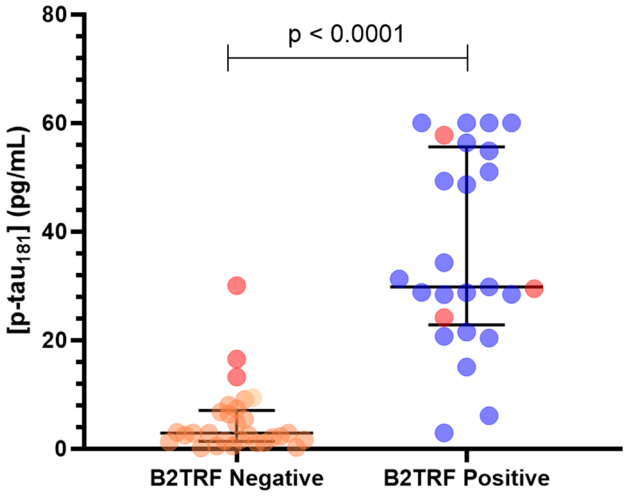

Fig. 1 p-tau$_{181}$ concentrations (median with IQR) in samples negative or positive for B2TRF. Red dots indicate hemolyzed samples

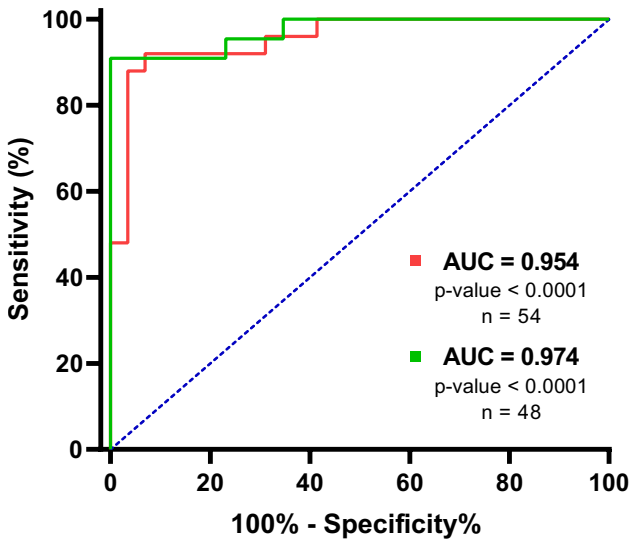

Fig. 2 Receiver operating characteristic (ROC) curves for determining the optimal cutoff of p-tau$_{181}$ to predict B2TRF positivity. Red curve: with hemolyzed samples; green curve: without hemolyzed samples

Table 1 Sensitivity and specificity of p-tau$_{181}$ level to predict B2TRF positivity

	n	Cutoff (pg/mL)	Sensitivity (%)	Specificity (%)
All CSF leaks	54	> 2.88	100.0	58.6
		> 13.22*	92.0	93.1
		> 30.0	48.0	100.0
Without hemolyzed samples	48	> 2.88	100.0	65.38
		> 9.39*	90.9	100.0

*Optimal cutoffs were calculated according to the Youden index

Table 2 Comparative table of B2TRF, p-tau$_{181}$ and BTP results for discordant samples (patient 1–4) and samples for which the B2TRF gel could not be interpreted (patient 5–6)

	B2TRF	p-tau$_{181}$ (pg/mL)	BTP (mg/L)	Hemolyzed ?	Agreement BTP/p-tau$_{181}$
Patient 1	Positive	2.92	0.06	No	Yes
Patient 2	Positive	6.08	29.0	No	No
Patient 3	Negative	30.04	0.846	Yes	Yes
Patient 4	Negative	16.53	2.74	Yes	Yes
Patient 5	Undetermined	24.32	1.16	Yes	Yes
Patient 6	Undetermined	12.7	0.804	No	Yes

The positive cutoff is 13.22 pg/mL for p-tau181 and 0.7 mg/mL for BTP

Discussion

The primary objective of this study was to evaluate the potential of p-tau$_{181}$ as a biomarker for CSF leaks compared to the current gold standard. Our results show that p-tau$_{181}$ is significantly higher in B2TRF-positive samples than in B2TRF-negative samples, suggesting that p-tau$_{181}$ may serve as a reliable biomarker for the detection of CSF leaks.

The high AUC value (0.955) of the ROC curve indicates excellent diagnostic performance, with an optimal cutoff of 13.22 pg/mL, providing high sensitivity (92.1%) and specificity (93.1%); therefore further supporting the utility of p-tau$_{181}$ in this indication. When hemolyzed samples were excluded, the AUC (0.973) improved further and the optimal cutoff was adjusted to 9.39 pg/mL, maintaining high sensitivity (90.9%) and achieving perfect specificity (100.0%). Furthermore, the high concordance between p-tau$_{181}$ and BTP results for discordant samples underlines the reliability of p-tau$_{181}$ in detecting CSF leaks and highlights the difficulty in interpreting B2TRF electrophoresis. However, this concordance was not perfect, as shown for the case of patient number 2. In this case, the BTP and B2TRF were clearly positive but the p-tau$_{181}$ was negative.

While the phosphorylation process of the axonal protein tau is a physiological process [23], higher p-tau concentrations is a pathological hallmark of AD. However, it is also well-known that this process is not specific to AD and that higher concentrations are observed in other neurological conditions. For example, higher blood p-tau concentrations have been described following traumatic brain injury (TBI), with acute elevations of various p-tau isoforms being detectable within hours post-injury [24–26]. Whether p-tau$_{181}$ performs better to highlight CSF leak following traumatic injury, in comparison to postoperative or iatrogenic causes, should require larger and specific studies.

Our results are particularly promising given the advantages of the p-tau$_{181}$ assay over the B2TRF. The p-tau$_{181}$ assay is quantitative and can be performed rapidly, within 35 min per sample, on the fully automated Lumipulse® G600II analyzer, making it a practical tool for clinical laboratories. In contrast, the B2TRF assay is qualitative and labor-intensive, requiring manual steps that can introduce variability and delay diagnosis. As a matter of fact, only one laboratory performs this analysis in Belgium, with a turnaround time of 10 days. With the advent of blood-based biomarkers of AD, it is expected that these markers, like p-tau$_{181}$, p-tau$_{217}$ and the Aβ42/Aβ40 ratio, will play a key role in the large-scale diagnosis of Alzheimer's disease, making these assays potentially available in many laboratories.

We noticed that most of our discordant cases were hemolytic. In fact, the manufacturer's insert advises to exclude hemolyzed samples without giving a clear interference threshold, as this assay is still for research use only (RUO). However, as shown by Musso et al. [27], there is a significant correlation between the concentration of p-tau$_{181}$ and the hemolysis index. This must be taken into account as it may lead to falsely elevated results and, therefore, wrong diagnosis. Further studies on the effect of hemolysis on the measurement of this biomarker are needed.

It is important to note that although p-tau$_{181}$ is promising in this indication, further validation studies are needed. Our study was limited by sample size and the retrospective nature of the analysis. In addition, the results obtained were only compared with the results of B2TRF, which was used as a reference method, but as discussed above, the B2TRF assay has a number of limitations. Larger and prospective studies, eventually with related radiological data, are needed to confirm these findings and to establish standardized protocols for the use of p-tau$_{181}$ in clinical practice.

Conclusion

This study demonstrates that p-tau$_{181}$ is a promising biomarker for the detection of CSF leaks, with high diagnostic accuracy and practical advantages over the current gold standard, B2TRF. The automated processing and accessibility of the p-tau$_{181}$ assay make it a potential and viable option for rapid and reliable diagnosis in clinical settings. However, further research are needed to validate these findings and

establish standardized protocols for its use. If confirmed, p-tau$_{181}$ could significantly improve the diagnostic process for CSF leaks, ultimately improving patient care and outcomes.

Author contributions MB and JLB contributed to the conception and design of the study. FB and JLB participated in sample collection. All authors contributed to the acquisition of data. MB and JLB carried out all statistical analyses, contributed to drafting the manuscript and preparing the figures. All authors take responsibility for the integrity of the data and the accuracy of the data analysis and have critically reviewed interim and final versions of the manuscript.

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Data availability The data sets that were used and/or analyzed in the current study are available on reasonable request from the corresponding author.

Declarations

Conflicts of interest The authors do not report any competing financial interests or personal relationships likely to influence the work reported in this paper.

Ethical approval Patients admitted to the Cliniques Universitaires Saint-Luc sign an internal regulatory document, stating that leftovers from biological samples used for routine diagnostic procedures can be used for retrospective academic studies, without an additional informed consent (ethics committee approval 2007/10SEP/233).

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