Influence of delayed CSF storage on concentrations of phospho-tau protein (181), total tau protein and beta-amyloid (1–42)

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Abstract

It is generally accepted that cerebrospinal fluid (CSF) biomarkers such as tau protein, phosphorylated tau protein (threonine 181) and beta-amyloid (1–42) can facilitate early and differential diagnosis of Alzheimer’s disease (AD). Since the respective concentrations can only be measured in a number of specialized centers, time to CSF specimen work-up has been considered as crucial for the stability of the respective biomarkers. When shipping of CSF samples is needed for biomarker measurement and immediate freezing of samples is not available, an overnight delay of up to 24 h frequently occurs. Therefore, we investigated the potential impact of a 24 h delayed freezing on CSF biomarker concentrations and compared it to 2 h storage (room temperature, 20 °C) and an immediate freezing. First, storage at room temperature for 2 h had only marginal, non-significant effects on the concentrations of CSF total tau protein and phospho-tau protein (181) compared to immediate freezing. Second, storage at room temperature for 24 h did not significantly affect total tau protein or phospho-tau protein but beta-amyloid (1–42) concentrations which increased significantly compared to the samples frozen immediately. These results indicate that CSF samples for the evaluation of total tau and phospho-tau protein may be kept at room temperature for up to 24 h whereas CSF samples for beta-amyloid (1–42) need to be frozen immediately.

Keywords: Alzheimer’s disease; Tau proteins; Beta-amyloid; CSF samples; Storage conditions

In the present study, we investigated CSF concentrations of total tau protein, phospho-tau protein (181) and beta-amyloid (1–42) under the condition of 24 h delayed freezing and compared it to a 2 h delayed and immediate sample freezing at −80 °C. In a first study, CSF samples (250 μl in polypropylene tubes) were taken from 12 patients (8 male, 4 female, age: 67.2 ± 10.1 years) undergoing diagnostic lumbar puncture (LP). Patients’ diagnoses were mild cognitive impairment (MCI) (n = 4), Alzheimer’s disease (AD) (n = 6), vascular dementia (n = 1) and major depressive disorder (n = 1). Samples were frozen in liquid nitrogen directly (t = 0 h) and 2 h (t = 2 h) after LP without centrifugation, and stored at −80 °C.

In a second study, CSF samples (250 μl in polypropylene tubes) were taken from 20 patients (10 male, 10 female, age 65.4 ± 13.2 years). Patients’ diagnoses were MCI (n = 6), AD (n = 5), major depressive disorder (n = 5), bipolar disorder (n = 2), Lewy body dementia (n = 1) and vascular dementia (n = 1). Samples were frozen in liquid nitrogen directly after LP (t = 0 h) and 24 h later (t = 24 h) without centrifugation, and stored at −80 °C. Concentrations of phospho-tau protein (181), total tau protein and beta-amyloid (1–42) were measured by...
ELISA (INNOTEST Phospho-Tau (181)-Ag, INNOTEST hTau-Ag, and INNOTEST Beta-Amyloid (1–42) Ag-kits; coefficient of variation (CV) for intra-assay variation of INNOTEST hTau-Ag (1.2–5.9%), CV for inter-assay variability (1.7–6.0%); INNOTEST Phospho-Tau (181)-Ag interlot variability <10%; CV for intra-assay variation of INNOTEST Beta-Amyloid (1–42) 5.6%, and CV for inter-assay variability 7.7%) [1]. For the discrimination of AD from controls sensitivity (specificity) of CSF tau protein, phospho-tau protein and beta-amyloid (1–42) were reported as follows: 81% (90%), 80% (92%), and 86% (90%), respectively [2]). Examiners were blinded to patients’ characteristics and diagnosis during measurement. Informed consent was obtained from all patients. Statistical analysis was performed by using t-tests (significance level at \( p < 0.05 \)).

The concentrations of tau protein showed only marginal, non-significant differences between the groups of \( t = 0 \) h and \( t = 2 \) h (mean 304.3 ± 178.0 pg/nl; Coefficient of Variation (CV) = 58.5% versus 308.0 ± 169.2 pg/nl; CV = 54.9%; \( p = \text{not significant} \)) as well as between \( t = 0 \) h and \( t = 24 \) h (mean 350.6 ± 249.6 pg/nl; CV = 71.2% vs. 366.1 ± 260.3 pg/nl; CV = 71.1%; \( p = \text{not significant} \)) (Figs. 1 and 2). Similarly, concentrations of phospho-tau protein (181) showed only minor changes between the groups of \( t = 0 \) h and \( t = 2 \) h (mean 59.4 ± 29.9 pg/nl; CV = 50.3% versus 60.0 ± 27.4 pg/nl; CV = 45.6%; \( p = \text{not significant} \)) as well as between \( t = 0 \) h and \( t = 24 \) h (mean 61.5 ± 32.8 pg/nl CV = 53.3% versus 58.4 ± 31.4 pg/nl; CV = 53.8%; \( p = \text{not significant} \)) (Figs. 3 and 4). However, we found a statistically significant increase of beta-amyloid (1–42) \( t = 0 \) h versus \( t = 24 \) h (mean 790.8 ± 329.2 pg/nl; CV = 41.6% versus 848.5 ± 287.1 pg/nl; CV = 33.8%; \( p < 0.05 \)) (Fig. 5).

Our study revealed two major findings: according to our data total tau protein and phospho-tau protein (181) remained stable 2 h up to 24 h after LP (storage at room temperature). In contrast, beta-amyloid (1–42) showed a significant increase after 24 h under the same storing conditions. These results confirm and extend previous findings from Schoonenboom et al. [4] who demonstrated the stability of total tau protein at room temperature for up to 12 days followed by a consecutive decrease, probably due to proteolytic processes and degradation of the protein. Even repeated freeze/thaw cycles did not cause a statistically significant difference in the concentrations measured. According to our findings, phospho-tau protein (181) concentrations are of a similar stability and show only marginally,
non-significant changes after a period of 2 h and 24 h at room temperature. On the other hand beta-amyloid (1–42) concentrations showed a slight but significant increase after 24 h, whereas the data of Schoonenboom et al. demonstrated only an initial decrease within the first 2 days. However, a very small sample size \((n=4)\) may have contributed to divergent results in the cited study [4]. These findings demonstrate that studies focussing on CSF storage conditions and sample treatment can contribute to a better understanding of sample work-up procedures and thereby enabling researchers and clinicians to attain comparable and standardized CSF measurements. Accurate CSF protein measurements are important for the establishment of reliable reference values [5].

In conclusion, our findings emphasize that CSF samples for the evaluation of total and phospho-tau protein concentrations do not need to be immediately frozen but may be kept at room temperature for at least 24 h. This does not apply for CSF beta-amyloid (1–42) concentrations since an increase occurred after 24 h. Further studies are warranted to investigate stability of CSF biomarker concentrations in larger samples with respect to common shipping conditions.

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References


