EFFECT OF PRE-ANALYTICAL FACTORS ON AD BIOMARKER LEVELS IN CSF

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Introduction: CSF biomarker analyses (β-amyloid (Aβ₁₋₄₂), total tau (T-tau) and hyperphosphorylated tau (P-tau₁₈₁P)) are becoming routine in dementia diagnosis. However, lack of standardized pre-analytical sample procedures hampers between-laboratory comparisons.

Aim: To explore the influence of fractionated sampling, centrifugation, freezing conditions, and freeze-thaw cycles on CSF biomarker levels.

Methods: Fractionated sampling and centrifugation was studied by taking sequentially 8 aliquots of 1.5 mL and 1 aliquot of 3 mL which was redistributed in two vials of 1.5 mL, one of which was centrifuged before freezing. In addition, centrifuged CSF from each patient underwent different freezing protocols (liquid N₂; -80°C; -20°C; 24h at 2-8°C; 24h, 48h and 72h at room temperature). To study the influence of freeze-thaw cycles, centrifuged samples from each patient were thawed up to four times and (re)frozen at -80°C. CSF was collected in polypropylene tubes. CSF biomarker levels were determined with commercially available single-analyte INNOTEST assays.

Results: No significant effect of fractionated sampling and centrifugation was detected with maximal differences of about 5% and 7%, respectively. Pair-wise comparisons of the freezing conditions showed biomarker-dependent differences. The fourth freeze-thaw cycle caused a significant decrease in Aβ₁₋₄₂ (maximally 16%).

Conclusion: CSF biomarker concentrations from non-hemorrhagic samples are not significantly influenced by centrifugation or fractionated sampling. Freezing conditions can affect biomarker concentrations. Consecutive freezing and thawing of CSF samples for up to three times demonstrated little effect on biomarker concentrations. The present results could contribute to evidence-based guidelines for standardization of CSF sampling as part of biomarker analysis.