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Cerebrospinal Fluid Biomarkers

Effect of long-term storage in biobanks on cerebrospinal fluid biomarker $A\beta_{1-42}$, T-tau, and P-tau values

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Abstract

Introduction: We studied the effect of long-term storage at −80°C on cerebrospinal fluid (CSF) biomarker levels. Our approach assumed consistency of mean biomarker levels in a homogenous Alzheimer's disease patient cohort over time.

Methods: We selected 148 Alzheimer's disease samples that had inclusion dates equally distributed over the years 2001 to 2013 from our biobank. The concentrations of CSF biomarkers, amyloid β_{1-42} ($A\beta_{1-42}$), total tau (T-tau), and phosphorylated tau_{181} (P-tau), were measured with one enzyme-linked immunosorbent assay lot. Results were compared with historical results obtained at biobank inclusion.

Results: Linear regression analyses showed that the levels of CSF biomarkers, $A\beta_{1-42}$, T-tau, and P-tau, were not related to storage time at −80°C ($\beta = 0.015$, 0.048, and 0.0016 pg/mL per day, not significant). However, the differences between remeasured concentrations of $A\beta_{1-42}$ and concentrations at biobank inclusion measured for more than 30 assay batches increased with increasing time difference.

Discussion: The levels of CSF biomarkers, $A\beta_{1-42}$, T-tau, and P-tau, did not significantly change during the maximum period of 12 years of storage at −80°C. Batch variation for $A\beta_{1-42}$ is a factor that should be controlled for when using historical cohorts.

Keywords: Alzheimer's disease; Cerebrospinal fluid; Biomarkers; Amyloid β_{1-42}; Total tau; Phosphorylated tau_{181}; Long-term storage; Preanalytical variation; Batch variation; ELISA

1. Introduction

Cerebrospinal fluid (CSF) biomarkers, amyloid β_{1-42} ($A\beta_{1-42}$), total tau (T-tau), and phosphorylated tau_{181} (P-tau), support the diagnosis of Alzheimer's disease (AD) [1]. Unfortunately, susceptibility to preanalytical/analytical variation has hampered clinical implementation [2–9]. The effect of long-term storage on CSF biomarkers is relevant because historical cohorts are needed to establish universal cutoff values. Evaporation during biobank storage is not an issue [10,11], but the long-term stability of biomarkers has been poorly studied. The first study on this topic monitored $A\beta_{1-42}$ and T-tau for 22 days and extrapolated these results using Arrhenius equations showing long-term stability at −80°C [12]. Next, a study reported on a repetitive measurement of a quality control CSF sample for more than 2 years, resulting in stable $A\beta_{1-42}$ concentrations [13]. Another study compared aliquots of clinical samples for more than 0 to 6 years and reported stable $A\beta_{1-42}$ and T-tau concentrations [14]. In both the latter studies, however, analysis was done in different assay batches, which could have interfered with the potential effects of long-term storage.

We studied the levels of CSF biomarkers, $A\beta_{1-42}$, T-tau, and P-tau, in relation to storage duration, starting from the

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assumption that mean biomarker levels of the average AD patient will not change over time. Similar collection procedures and assay batches thus precluded any preanalytical bias other than the length of biobank storage.

2. Methods

2.1. Sample collection

Patients underwent a standard clinical assessment and were diagnosed as probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [15]. We assumed that the average biomarker concentrations in AD patients within a narrow age range and equal apolipoprotein E (*APOE*) genotype and gender distribution on a group level will remain similar, regardless of the inclusion year. CSF was collected by lumbar puncture in 10 mL polypropylene tubes (Sarstedt, Nümbrecht, Germany). CSF samples were centrifuged at 1800 to 2100*g* for 10 minutes at 4°C within 2 hours and a small amount, 0.35 to 2.5 mL, was transferred to 5 mL polypropylene tubes (Sarstedt) and used for routine analysis and biomarker measurement (Innotest β-AMYLOID (1–42), hTAU-Ag, and PHOSPHO-TAU (181p); Innogenetics, Belgium). The remaining CSF was divided into polypropylene tubes (1.5 or 2.0 mL; Sarstedt) in 0.5 mL volumes and stored in the biobank at −80°C [16]. The protocol was approved by the institutional review board and subjects gave written consent.

2.2. Cohort selection

We selected 148 patients diagnosed with probable AD included between September 2001 and October 2013: aged 64 to 72 years, MMSE score from 14 to 27, *APOE* genotypes ε3/ε3 (n = 41), ε3/ε4 (n = 69), and ε4/ε4 (n = 38), and a gender distribution of 78 females and 70 males. We aimed for an equally scattered distribution of these clinical characteristics of the samples over the inclusion years. The biobank collection protocol remained unchanged during this period, apart from a small change as follows: from 2008 to 2011 biobank vials of 0.5 mL (Brand, ref. 211-3211) were used.

2.3. Enzyme-linked immunosorbent assays

We determined $A\beta_{1–42}$, T-tau, and P-tau concentrations in fresh biobank aliquots using one batch of sandwich Enzyme-linked immunosorbent assays (Innotest β-AMYLOID (1–42), Innotest hTAU-Ag, and Innotest PHOSPHO-TAU (181p); Fujirebio, Ghent, Belgium, former Innogenetics) between November 2015 and February 2016.

2.4. Statistical analysis

Linear regression analyses were performed to assess the effect of storage time in days on (1) biomarker levels (all reassessed in one batch) and (2) differences between new and old biomarker measurements. "β" expresses the slope of the linear regressions in picograms per milliliter per day ± standard error of the mean (SEM). To compare the remeasured values with the old values, Spearman correlation and Passing-Bablok regression were performed. P value $<.05$ was considered significant. Analyses were done in R version 3.3.1.

3. Results

3.1. Levels of CSF biomarkers $A\beta_{1–42}$, T-tau, and P-tau are not related to storage time at −80°C

Biomarker values (median + range) were 516 (287–1314) pg/mL for $A\beta_{1–42}$, 769 (112–2856) pg/mL for T-tau, and 83 (22–224) pg/mL for P-tau; these values were not part of the selection criteria. Linear regression analysis showed that there was no relation between storage time and biomarker value (β[slope ± SEM] = 0.015 ± 0.012 pg/mL per day for $A\beta_{1–42}$, β = 0.048 ± 0.031 pg/mL per day for T-tau, and β = 0.0016 ± 0.0025 pg/mL per day for P-tau, all $P > .05$) (Fig. 1). Confidence intervals (95%; CIs) of the linear regression model fit show that the yearly concentration changes for $A\beta_{1–42}$, T-tau, and P-tau were between −3.2 and 14.2, −4.7 and 39.7, and between −1.2 and 2.4 pg/mL, respectively.

3.2. Comparison of old and new biomarker measurements

We compared the remeasured $A\beta_{1–42}$, T-tau, and P-tau concentrations with those measured at the time of inclusion in the biobank, showing strong correlations: ρ = 0.725 for $A\beta_{1–42}$, ρ = 0.922 for T-tau, and ρ = 0.903 for P-tau (Fig. 2A–C). However, Passing-Bablok regression analyses showed systematic differences, indicated by the intercept of regression line, for $A\beta_{1–42}$ (intercept = −98.0 [95% CI = −171 to −24.0], β = 1.09 [95% CI = 0.92–1.24]), proportional differences, indicated by the slope of the regression line, for T-tau (intercept = −4.06 [95% = −28.9 to 22.5], β = 0.83 [95% CI = 0.79–0.89]) but no difference for P-tau levels (intercept = −5.91 [95% CI = −11.6 to 0.00], β = 1.09 [95% CI = 1.00–1.17]) (Fig. 2A–C).

3.3. Differences between new and old values is related to batch changes

Differences between new and old measurements of $A\beta_{1–42}$ were strongly related to storage time (β ± SEM = 0.046 ± 0.0062 pg/mL per day; $P < .001$) (Fig. 2D), where the more recent measurements were consistently higher. For T-tau, a similar trend was observed (β ± SEM = 0.031 ± 0.011 pg/mL per day; $P < .01$) (Fig. 2E), although not as strong as for $A\beta_{1–42}$. For P-tau, no trend was observed (β ± SEM = 0.00023 ± 0.0011 pg/mL per day, *n.s.*) (Fig. 2F).

From 2004 to 2015, 30 batches of $A\beta_{1–42}$ kits were sequentially used in our laboratory. Before 2004, biomarker measurements were too infrequent to consider batch shifts.