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Sysmex Corporation

【Overview presentation】

15th Clinical Trials on Alzheimer's Disease (CTAD)

Effects of pre-analytical parameters on plasma β-amyloid level

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Overview presentation

Backgrounds

Blood-based biomarkers of Alzheimer's disease (AD) are expected to be promising tools for assisting diagnosis in combination with conventional biomarkers such as neuroimaging and cerebrospinal fluid (CSF) biomarkers. In particular, the ratio of plasma β-amyloid$_{1-42}$ (Aβ42) to β-amyloid $_{1-40}$ (Aβ40) is known to be associated with brain Aβ pathology. Therefore, it could assist in the selection of the candidates for clinical trials of disease-modifying therapies targeting Aβ. We have developed assays that can specifically measure Aβ40 and Aβ42 in plasma using a fully automated immunoassay platform, the HISCL™ series. The assay showed high performance for predicting brain Aβ pathology defined by amyloid positron emission tomography (PET).

However, it is widely recognized that plasma Aβ peptides are unstable molecules, and thus the measured values are affected by pre-analytical parameter such as external factors, measurement equipment and sample handling. Especially, blood collection and storage conditions in sample handling affect the quality of plasma Aβ levels, leading to false selection.

Therefore, clarification of the influence of pre-analytical parameters on plasma Aβ levels measured by our assay is required to obtain reliable data in clinical trials.

Objectives

The purpose of this study was to clarify the effect of pre-analytical parameters on plasma Aβ levels measured by HISCL series.

Methods

	Whole blood samples were collected in K_2 EDTA tubes from healthy volunteers. After centrifugation, plasma Aβ40 and Aβ42 levels were immediately measured using the HISCL series and plasma Aβ42/Aβ40 was calculated. We compared the impacts of the 11 pre-analytical parameters such as duration between blood collection and centrifugation at room temperature (RT) or 4°C, the interval between plasma storage at RT or 4°C before measurement, the number of freeze/thaw (F/T) cycles, the conditions of thawing, the length of time that the plasma was thawed before measurement, the types of tubes and manufacturers for storing plasma, the tip type using plasma separation, and the number of tube transfers on plasma Aβ42/Aβ40. **Results** Plasma Aβ42/Aβ40 levels remained unchanged after 2 hours at RT and 6 hours at 4°C after blood collection. After the plasma separation, it is unaltered even after 18 hours of storage at 4°C. Longer sample storage time and higher storage temperature after plasma separation tended to affect the reduction of the plasma Aβ42/Aβ40 ratio, but did not have an effect beyond our established criteria. The time for holding the thawed plasma at RT was also investigated, and no difference was detected until 4 hours. For the freezing samples, up to three F/T cycles did not significantly change the Aβ42/Aβ40 ratio compared to the reference value. Thawing at RT and 37°C were also used to assess the thawing state. The 37°C conditions were verified using a water bath, a dry block incubator, and an air incubator. Furthermore, neither of the thawing conditions affects the Aβ42/Aβ40 values. The Aβ42/Aβ40 values are unaffected by tube manufacturer, tip type, or tube transfer up to one time. **Conclusion** In this study, we evaluated the influences of 11 pre-analytical parameters on plasma Aβ42/Aβ40 ratio measured by HISCL series. In our evaluation, the sample storage time and temperature after plasma separation were the most influential factors in plasma Aβ42/Aβ40 ratio. These results were consistent with the previous reports of pre-analytical handling using another assay platform. This study will contribute to the determination of pre-analytical handling for plasma Aβ measurement using HISCL. By establishing pre-analytical handling based on the findings of this study, the high amyloid PET predictive performance we have shown so far could be achieved in clinical trials.
Session	Poster, Clinical Trials: Biomarkers including plasma（LP74）