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Impact of pre-analytical factors on blood-based biomarkers of Alzheimer's disease

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Introduction

- Alzheimer's disease (AD) is characterized by β-amyloid deposition and tau pathology in the brain.[1]
- Blood-based biomarkers (BBBMs) can aid in the detection of AD pathology and are urgently required to enable early identification of patients requiring further evaluation and initiation of disease-modifying therapies.[2,3]
- Reliable BBBM tests may allow for robust, cost-effective triage of patients with suspected AD before referral for detailed assessments.[3]
- Investigation into appropriate pre-analytical sample handling procedures across a wide range of BBBMs using fully automated platforms is a prerequisite for reliable analysis of these biomarkers to support detection of AD pathology in routine laboratory practice and clinical trials.

Objective

- **To outline appropriate pre-analytical sample handling recommendations for BBBMs of AD pathology.**

Methods

- This prospective, non-interventional study (Dec 2020–Oct 2021) evaluated the effects of:
  - Storage time and temperature in stressed whole blood (WB) and plasma;
  - Freeze/thaw (F/T) cycles and plastic tube type in fresh, never frozen, and/or previously frozen plasma; and
  - Anticoagulants and tube transfer in previously frozen plasma only.
- Blood samples were collected from eligible participants with clinically suspected AD attending routine clinical visits at University Hospital, LMU Munich (Munich, Germany); collection was approved by the ethics committee of LMU Munich, and all participants provided written informed consent
  - To assess the effects of storage time and temperature, WB samples were collected in tripotassium ethylenediaminetetraacetic acid (K3 EDTA) tubes and stored at room temperature (RT) or 4°C for ≤24 hrs as WB or plasma
  - To assess the effects of tube transfer and F/T cycles, separated plasma was transferred into polypropylene (PP) tubes and underwent ≤4 F/T cycles
  - To assess the effect of tube type, separated plasma was transferred into low-density polyethylene (PE-LD) tubes
  - To assess the effect of anticoagulants, K3 EDTA, lithium heparin (LiHep) and sodium citrate (NaCit) tubes were used.
- BBBM measurement was performed at Roche Diagnostics GmbH (Penzberg, Germany).
- Most BBBMs were measured in single determination; where duplicate measurements were available, mean values were calculated.
- BBBM plasma levels (**Table 1**) were measured using the automated Elecsys® prototype immunoassays on a cobas e 601 analyzer (all Roche Diagnostics International Ltd, Rotkreuz, Switzerland).

Table 1. BBBM plasma levels measured.

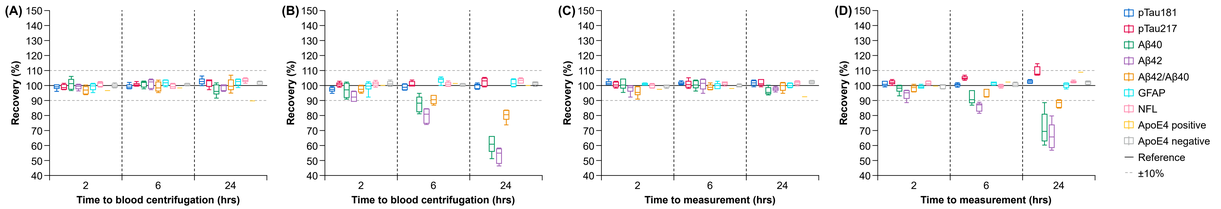
BBBMs measured	Abbreviation
β-amyloid 1–40	Aβ40
β-amyloid 1–42	Aβ42
Apolipoprotein E4	ApoE4
Glial fibrillary acidic protein	GFAP
Neurofilament light chain	NFL
Phosphorylated-tau 181	pTau181
Phosphorylated-tau 217	pTau217

ApoE4 analyte results were separated into ApoE4 positive and negative.

Results

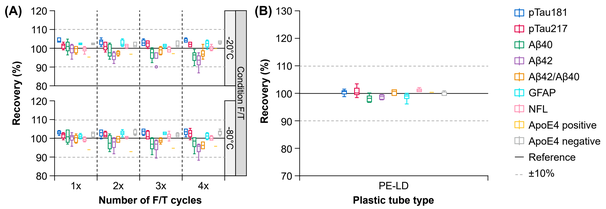
- WB samples were collected from a total of 16 patients with clinically suspected AD.

Figure 1. Effect of storage time and temperature on BBBM levels in WB stored at (A) 4°C and (B) RT, and plasma stored at (C) 4°C and (D) RT (fresh, never frozen samples).



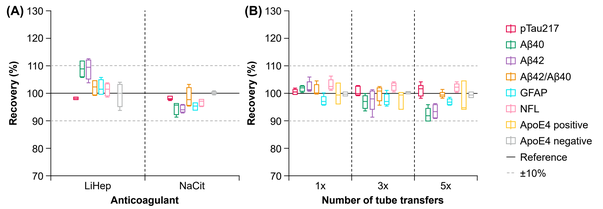
For all BBBMs measured n=6, except for ApoE4 positive and negative, where n=5 and n=1, respectively. Recovery signals compared with the reference sample (black solid line) were reported, with acceptance criteria of ±10% (grey dotted line). For panels A–D, the reference sample underwent centrifugation 30–60 mins after blood draw, and was then measured immediately (i.e. the optimal pre-analytical technique). Boxes represent median and IQR; lower whisker represents the higher of the minimum values and the 25th quartile to 1.5*IQR; higher whisker represents the lower of the maximum values and the 75th quartile to 1.5*IQR. IQR, inter-quartile range.

Figure 2. Effect of (A) F/T cycles and (B) plastic tube type on BBBM levels in fresh, never frozen plasma.



For all BBBMs measured n=6, except for ApoE4 positive and negative, where n=1 and n=5, respectively. Recovery signals compared with the reference sample (black solid line) were reported, with acceptance criteria of ±10% (grey dotted line). For panel A, the reference sample underwent zero F/T cycles. For panel B, the reference sample was kept in a PP tube. Boxes represent median and IQR; lower whisker represents the higher of the minimum values and the 25th quartile to 1.5*IQR; higher whisker represents the lower of the maximum values and the 75th quartile to 1.5*IQR. Values above or below the whiskers are plotted as dots.

Figure 3. Effect of (A) anticoagulants and (B) tube transfer on BBBM levels in previously frozen plasma.



For panel A, for all BBBMs measured n=4, except for ApoE4 positive and negative, where n=0 and n=4, respectively. For panel B, for all BBBMs measured n=5, except for ApoE4 positive and negative, where n=3 and n=2, respectively. pTau181 was not measured in this experiment. Recovery signals compared with the reference sample (black solid line) were reported, with acceptance criteria of ±10% (grey dotted line). For panel A, the reference sample was mixed with K3 EDTA. For panel B, the reference sample underwent zero tube transfers. No data was available on the effect of anticoagulants on plasma samples from ApoE4 positive individuals. Boxes represent median and IQR; lower whisker represents the higher of the minimum values and the 25th quartile to 1.5*IQR; higher whisker represents the lower of the maximum values and the 75th quartile to 1.5*IQR.

Figure 4. Summary of recommendations for blood collection and pre-analytical sample handling for the analysis of BBBMs of AD pathology.

Effect of storage time and temperature on WB and plasma

- Using fresh, never frozen samples, all BBBMs were stable for ≤24 hrs at 4°C in WB and K3 EDTA plasma (**Figure 1**)
  - Aβ42 and Aβ40 were unstable if stored for >2 hrs at RT
    - This could only be partially compensated by the Aβ42/Aβ40 ratio
  - The median recovery signal for pTau217 increased by ~10% in K3 EDTA plasma when kept at RT for 24 hrs, compared with the reference
    - This effect was not observed in WB samples kept at RT
    - This was the only difference in stability observed between BBBMs measured in WB versus plasma.
- There were no differences in the median recovery signal between fresh, never frozen and previously frozen plasma samples (data not shown).

Effect of F/T cycles on fresh, never frozen plasma

- Up to two F/T cycles were acceptable for all BBBMs (**Figure 2A**).
- Median recovery signals for all BBBMs were comparable between samples frozen at -20°C and samples frozen at -80°C.

Effect of plastic tube type

- There was no change in the median recovery signal between PP and PE-LD tubes for any BBBMs measured in fresh, never frozen plasma (**Figure 2B**).
- Median recovery signals for all BBBMs were comparable between fresh, never frozen and previously frozen plasma samples (data not shown).

Impact of anticoagulant type

- For all BBBMs, except Aβ42 and Aβ40, analyte levels were comparable between K3 EDTA, LiHep, and NaCit tubes (**Figure 3A**).

Effect of tube transfer

- All BBBMs assessed were stable for up to five tube transfers in K3 EDTA plasma (**Figure 3B**).
- The median recovery signals for Aβ42 and Aβ40 decreased progressively between one, three and five tube transfers; however, this decrease was within pre-defined acceptance criteria.

Conclusions

- **Aβ42 and Aβ40 were most sensitive to pre-analytical sample handling compared with the other BBBMs measured, and the effects could only be partially compensated by the Aβ42/Aβ40 ratio.**
- **For all BBBMs and pre-analytical effects measured, there was no marked difference in the median recovery signal between fresh, never frozen and previously frozen plasma samples.**
- **Our recommendations for optimal handling of fresh blood samples for analysis of BBBMs of AD pathology are shown in Figure 4:**
  - **WB and K3 EDTA plasma should be stored at 4°C for ≤24 hrs, while storage at RT should be avoided or limited to 2 hrs.**
- **This protocol would be appropriate for use in routine practice.**

References

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