Pre-analytical sampling and storage conditions of Amyloid-β peptides in venous and capillary blood

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Short report

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Abstract

BACKGROUND: We analyzed the impact of pre-analytical storage and sampling conditions on plasma Amyloid-β (Aβ) peptides.

METHODS: Venous or capillary blood was collected and either stored as plasma or whole blood at room temperature or 4°C. Aβ 40 and Aβ 42 levels were measured using a chemiluminescence sandwich immunoassay.

RESULTS: Aβ 40 , Aβ 42 and Aβ 42/40 levels significantly decreased during storage at room temperature in whole blood or plasma, starting at 6 hours after sampling. While Aβ 42 was most prone to the time of storage, Aβ 42/40 was stable up to 24 hours. Storing whole blood or plasma at 4°C led to stable Aβ peptide concentrations up to 72 hours. In addition, Aβ peptides could be measured in capillary blood. While Aβ 40 and Aβ 42 concentrations were slightly lower in capillary blood and started decreasing during storage conditions at 4°C, the Aβ 42/40 ratio remained constant up to 72 hours and was comparable to venous whole blood.

CONCLUSION: These findings provide information that need to be respected in pre-analytical standard operating procedures for clinical and research practice to generate most reliable plasma Aβ measurements.

Background:

Alzheimer's disease (AD) is one of the most challenging and costly disorders with an incidence of 5.8 million people in the United States in 2019 by rising expectantly up to 13.8 million affected people in the 2050s [1]. While Amyloid-β is a key neuropathological feature of AD [2], soluble Aβ in cerebrospinal fluid (CSF) is one of the most important biomarker for AD in clinical practice [3].

Since Biogen's novel Amyloid-β targeting therapy, its monoclonal antibody Adacanumab, reduces amyloid plaques in the brain and most likely improve cognition [4], latest causal therapies might be close to clinical implementation [5]. However, due to the cost intensive therapy and potential side effects, patients need to be selected carefully to exclusively include patients with an underlying Alzheimer's pathology before receiving this kind of therapy. In particular, the recently introduced limbic-predominant age-related TDP-43 encephalopathy (LATE) is a clinically serious differential diagnosis, contributing to about 15–20% of the false-positive AD cases in elderly patients [6]. These patients will most likely not benefit from novel Aβ based therapies such as Adacanumab. Therefore, there is a high need for screening assays to select patients with possible AD neuropathological changes.

Many studies have shown the pre-analytical characteristics of CSF biomarkers. For example, Lewczuk et al. showed that CSF Aβ is stable up to seven days at room temperature (RT) [7]. However, the composition of blood defers from CSF. Several studies have suggested plasma Aβ, in particular the $Aβ_{42}$ to $Aβ_{40}$ peptide ratio ($Aβ_{42/40}$) as a potential biomarker for AD [8–10]. There is currently very limited data on pre-

analytic handling conditions of blood samples to measure peripheral Aβ species. As recommended by the Blood-Based Biomarker Interest Group [11], Rózga et al. analyzed different handling conditions, however relevant storage conditions, in particular storage of whole blood at 4 °C are missing [12].

Due to the high demand of easy to access screening tools, we also studied handling and storage of capillary blood. Independent of any medical centers, capillary blood can be easily collected by oneself with a small finger insertion, comparable to blood sugar measurements, and sampled in microvettes®.

This study provides complementary data to the recently published study by Rózga et al [12], outlining potential sampling, handling and storage condition for a broad general practice to pre-screen patients for further AD diagnostics.

Methods:

Sample collection, preparation and storage

Venous (K3-EDTA-monovettes®, Sarstedt) and capillary (200 µl K3 EDTA-microvettes®, Sarstedt) blood was collected from healthy volunteers without neurological disease. All participants gave their written informed consent (ethical vote: 3/4/18 and 9/2/16, ethics committee of the University Medical Center Goettingen, Germany). Whole blood EDTA-monovettes were stored at RT or at 4 °C for the indicated time before processing and freezing at -80 °C. Immediately processed plasma samples were stored in polypropylene low-bind Eppendorf tubes under equal storage conditions before long-termed storage at -80 °C. All samples were centrifugation at 2,000 x g for 10 minutes for plasma separation.

Aβ-measurements

$A\beta_{40}$ and $A\beta_{42}$ peptide concentrations in plasma were measured as described previously [13] using a chemiluminescence immuno-assay (VPLEX Aβ [6E10], (Mesoscale Discovery). Briefly, the assay plate was blocked using 150 µl blocking puffer for 60 min at RT followed by three washing steps. A pre-diluted plasma solution (one part plasma and one part dilution buffer) was centrifuged at 10,000 x g for 10 min. Supernatant was transferred and diluted once more equivoluminar with dilution buffer to a final one to four dilution. Following this, 25 µl of detection antibody solution, containing 2% of 50 X sulfo-tag labeled anti-Aβ antibody (6E10) and 1% $A\beta_{40}$ blocker, and 25 µl diluted plasma samples were applied to the plate. Samples were incubated for 120 min at RT while shaking at 300 rpm, followed by three consecutive washing steps. 150 µl read buffer was added immediately before measuring with MESO QuickPlex SQ 120 reader, Mesoscale Discovery.

Statistics

%-recovery of different storage conditions was compared to immediate processing and storing at -80 °C (0.5 h) using a one-sample Wilcoxon Test with Bonferroni correction for multiple testing. Significance was assumed at $p < 0.05$. Outliers were identified by ROUT with Q = 1%.

Results:

Stability of Aβ peptides in venous blood

Aβ peptide concentrations start to decrease in whole blood stored at RT within the first 6 hours. In particular, $Aβ_{42}$ is significantly lower after 6 hours compared to an immediate processing (reduction by 13.75%, 11.05%, 29.03%, 51.66%, and 73.17% at 6, 8, 24, 48, and 72 hours, respectively). $Aβ_{40}$ significantly decreases after 48 hours (30.13% and 49.81% at 48 and 72 hours), resulting in a significant lower $Aβ_{42/40}$ ratio at 24 hours (14.55%, 34,65%, and 48.40% at 24, 48, and 72 hours, respectively) (Fig. 1a, e, e).

In whole blood stored at 4 °C neither the $Aβ_{40}$ nor the $Aβ_{42}$ peptide concentration changed over the indicated time of up to 72 hours resulting in a stable $Aβ_{42/40}$ ratio (Fig. 1b, f, j).

Aβ stability in separated plasma at RT and 4 °C

Similar to storage of whole blood at RT, Aβ peptide concentrations are reduced in plasma after 6 hours storage. $Aβ_{42}$ is significantly reduced by 9.33%, 15.77%, 33.18%, and 42.60% at 6, 24, 48, and 72 hours, respectively. $Aβ_{40}$ concentrations in plasma are stable up to 8 hours at RT (reduction by 12.20%, 19.51%, and 34.38% at 24, 48, and 72 hours, respectively). Comparable reductions of $Aβ_{42}$ and $Aβ_{40}$ in plasma at RT resulting in an only slightly reduced $Aβ_{42/40}$ ratio by 9.73% and 14.46% at 48 and 72 hours (Fig. 1c, g, k).

Related to whole blood, plasma stored at 4 °C shows very stable Aβ peptides up to 72 hours. No reduction of $Aβ_{42}$, $Aβ_{40}$, or $Aβ_{42/40}$ is observed (Fig. 1d, h, l).

Stability of Aβ peptides in capillary blood

Comparing the Aβ peptide concentrations in venous and capillary blood, Aβ peptides are significantly lower in capillary blood at baseline (0.5 h). The concentration of $Aβ_{40}$ shows a slightly higher reduction (13.24%) than $Aβ_{42}$ (12.81%) negatively affecting the $Aβ_{42/40}$ ratio by rising significantly to 2.4% compared to $Aβ_{42/40}$ ratio in venous blood (Fig. 2a).

Regarding the temporal stability of capillary blood stored at 4 °C both Aβ peptide concentrations significantly decrease after 48 hours (13.95% for $Aβ_{40}$ and 14.95% for $Aβ_{42}$, respectively) and 72 hours (23.65% and 23.38% for $Aβ_{40}$ and $Aβ_{42}$, respectively). Due to the equal decrease of $Aβ_{40}$ and $Aβ_{42}$, the $Aβ_{42/40}$ ratio is stable up to 72 hours in capillary blood stored at 4 °C (Fig. 2b).

Discussion:

Implementing standard operating procedures for plasma Aβ handling, as extensively studied in CSF, is more than necessary due to rising number of studies analyzing blood Aβ peptides as biomarkers in AD

diagnostic. This study complements the result by Rózga et al. [12] showing a significant reduction of $A\beta_{42}$ levels starting at 6 hours at RT in plasma and whole blood. An important storage condition that was not shown by the study of Rózga et al. is the impact of temperature on the stability of whole blood plasma Aβ peptides separately [12]. In our study we clearly demonstrate a significant effect of lower storage temperature of 4 °C on Aβ peptide plasma levels both in whole blood and separated plasma being stable for up to three days. Improving pre-analytical storage condition by easily to implement factors like temperature is important to raise the reliability and quality of blood-based Aβ biomarkers in diagnosing AD patients. In particular, establishing and improving a pre-analytical protocol at 4 °C leading to highly stable biomarkers, AD diagnostics can be done by family doctors independent from major medical centers.

Another important fact in clinical diagnosing AD that we can confirm in our study is the significantly longer stability and easier storage of $A\beta_{42/40}$ levels compared to the single Aβ peptides [14, 15].

Moreover, in this study the capability and stability with the less invasive capillary blood collection is researched for the first time. Plasma Aβ peptide levels are detectable in capillary blood, even though showing a slightly reduced concentration compared to venous blood. Also, the $A\beta_{42/40}$ ratio shows high stability up to three days under improved storage condition at 4 °C. The evidence of AD biomarkers in even capillary blood leads to a potential future tool in early AD diagnostics that needs to be more investigated to increase the reliability and reproducibility. Using capillary blood collection enables a novel field of potential study populations by addressing patients at home sampling capillary blood in microvettes® without professional medical support.

We cannot distinguish between degradation of Aβ-peptides, ether protease or non-protease based, or lower Aβ levels due to aggregation. Interestingly, the reduction of plasma Aβ levels follows it aggregation potential. $A\beta_{42}$, which is known to have a high aggregation potential [16], is reduced after 6 hours at RT in plasma and whole blood, whereas $A\beta_{40}$ is stable up to 24 or 48 hours supporting the hypothesis of Aβ aggregation. However, to address and carefully analyze the Aβ stability of AD patients further studies need to be done for implementing pre-analytical blood handling SOPs for clinical Aβ measurement in diagnosis AD.

Conclusion:

This study shows that storage of blood samples is a crucial step in plasma Aβ measurements to avoid artificially decreased plasma Aβ levels. While whole blood as well as plasma can be stored at 4 °C up to 72 hours without significant Aβ reduction, blood samples should be processes within a short time if storing at RT. In addition, this study points out that capillary blood might be a potential alternative source for plasma Aβ measurements with comparable $A\beta_{42/40}$ levels.

Abbreviations:

AD: Alzheimer's disease; Aβ: Amyloid-β; CSF: cerebrospinal fluid; LATE: limbic-predominant age-related TDP-43 encephalopathy; RT: room temperature; $A\beta_{42/40}$: $A\beta_{42}$ to $A\beta_{40}$ peptide ratio

Declarations:

Ethics approval and consent to participate

This study was approved by the ethics committee of the the University Medical Center Goettingen, Germany (ethical vote: 3/4/18 and 9/2/16). All participants gave their informed consent prior to inclusion to the study.

Consent for publication

Not applicable.

Availability of data and materials

Primary data and spare material are stored at the Department of Psychiatry and Psychotherapy at the University Medical Center Goettingen, Germany. Data and material are available from the corresponding author.

Competing interests

The authors state that there are no conflict of interests to declare.

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Authors' contribution

MW collected blood samples, performed plasma Abeta measurements and wrote the manuscript. JW supervised and designed the study. JV supervised and designed the study, performed statistical analysis and wrote the manuscript. All authors read and approved the final manuscript.

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Figures

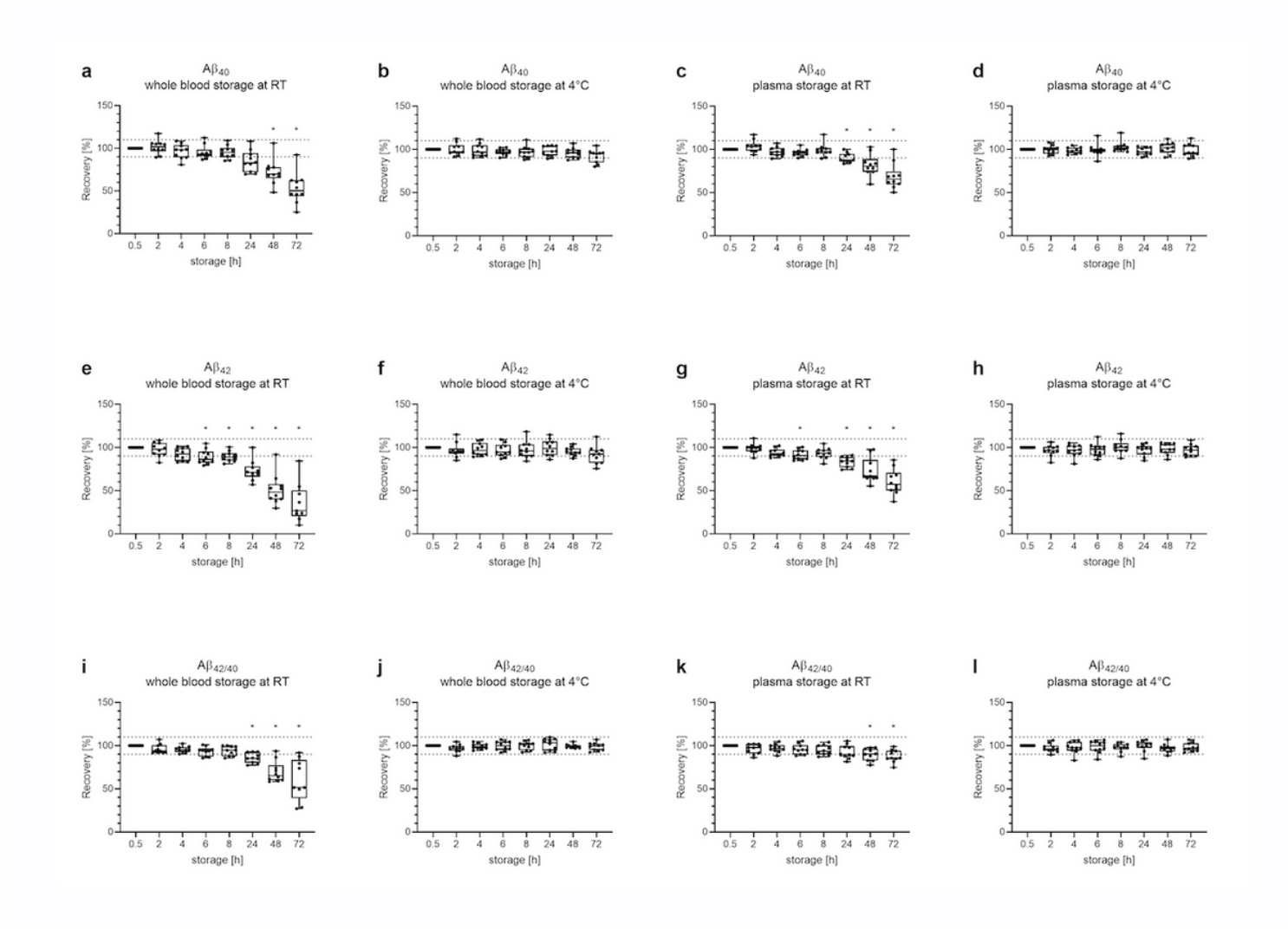

Figure 1

Effect of storage temperature of venous whole blood and separated plasma on the stability of plasma Aβ peptides and Aβ42/40 ratio. Samples are processed 0.5, 2, 4, 6, 8, 24, 48 and 72h after blood collection and stored at RT or at 4°C before freezing at -80°C. Plasma levels of Aβ40, Aβ42 and Aβ42/40 ratio are measured. All values are normalized to baseline (0.5h). Each storage condition includes a number of ten samples (n=10). Time to centrifugation of whole blood stored at RT (a, e, i) and of whole blood stored at 4°C (b, f, j). Time to freezing freshly separated plasma stored at RT (c, g, k) and at 4°C (d, h, l). Bars represent minimum and maximum Aβ levels, the dashed lines represent ± 10% recovery. p-value is adjusted according to Bonferroni correction. *: $p < 0.05$.

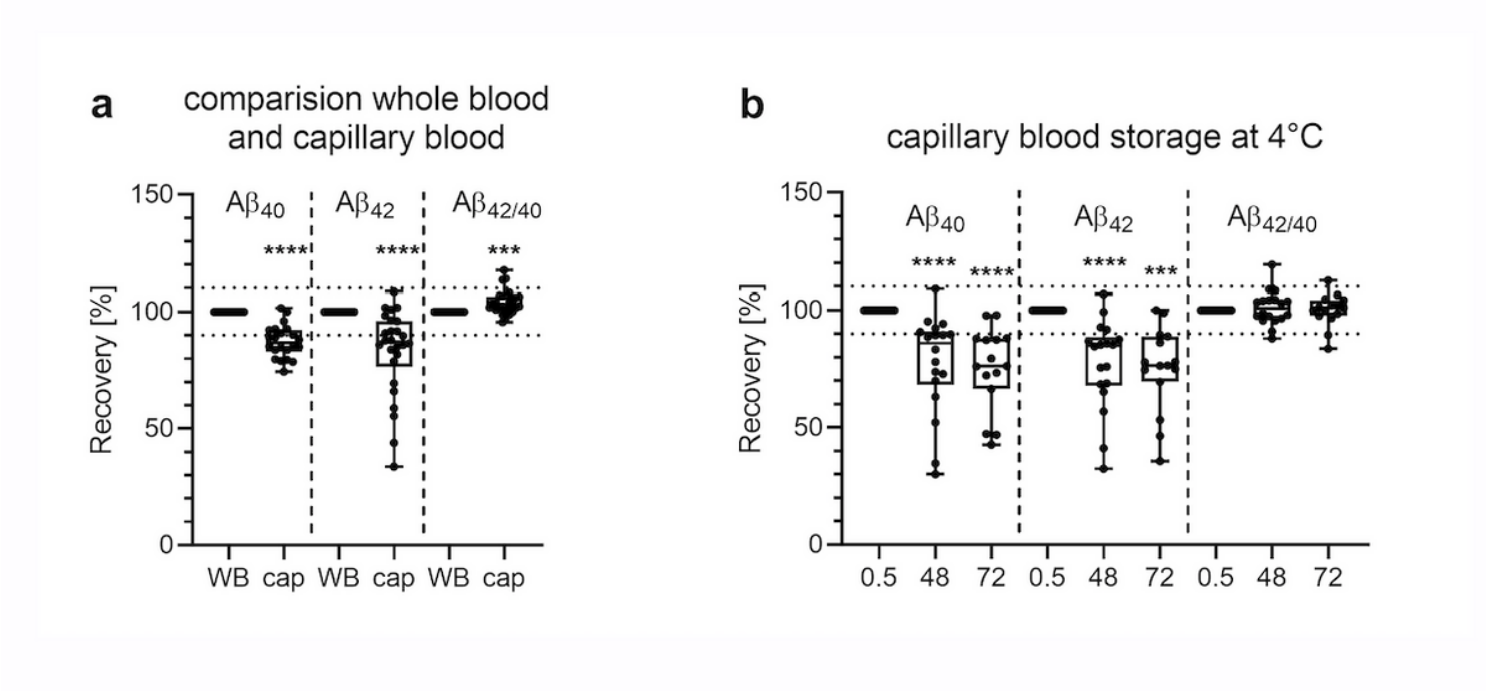

Figure 2

Aβ peptides in capillary blood. Capillary blood (cap) is compared to venous whole blood (WB) at baseline after processing within 0.5h (n=22) (a). Temporal stability of Aβ peptides is shown in capillary blood stored at 4°C for 0.5, 48 (n=18) and 72h (n=15) after blood collection. Capillary blood is collected and stored in microvettes® (Sarstedt) until processing and freezing at 80°C (b). All values are normalized to the baseline at 0.5h after blood extraction. Bars represent minimum and maximum Aβ levels, the dashed lines represent ± 10% recovery. p-values are adjusted according to Bonferroni correction. ***: $p < 0.001$; ****: $p < 0.0001$.