How to handle adsorption of cerebrospinal fluid amyloid-β (1–42) in laboratory practice? Identifying problematic handlings and resolving the issue by use of the Aβ42/Aβ40 ratio

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Chapter 5

Abstract
We aimed to investigate factors defining amyloid-β (1–42) (Aβ1–42) adsorption during preanalytical workup of cerebrospinal fluid (CSF). CSF was transferred to new tubes 4 times. Variables tested were different polypropylene tube brands, volumes, CSF Aβ1–42 concentrations, incubation times, pipettes, vortex intensities, and other CSF proteins, including hyperphosphorylated tau and Interleukin 1 Receptor Accessory Protein (IL-1RAcP). An enquiry assessed the number of transfers in current practice. In diagnostic practice, the number of transfers varied between 1 and 3. Every tube transfer resulted in 5% loss of Aβ1–42 concentration, even 10% in small volumes. Adsorption was observed after 30 seconds and after contact with the pipette tip. Tube brand, vortexing, or continuous tube movement did not influence adsorption. Adsorption for Aβ1–40 was similar, resulting in stable Aβ1–42/Aβ1–40 ratios over multiple tube transfers. We confirmed that adsorption of CSF Aβ1–42 during preanalytical processing is an important confounder. However, use of the Aβ1–42/Aβ1–40 ratio overcomes this effect and can therefore contribute to increased diagnostic accuracy.
Introduction

Amyloid-β (1–42) (Aβ1–42) in cerebrospinal fluid (CSF), together with total tau (tTau) and hyperphosphorylated tau (pTau), is used as a diagnostic biomarker for Alzheimer’s disease (AD) diagnosis in research settings. The optimal and universal use of Aβ1–42 has been hampered in part because of technical problems related to hydrophobicity of Aβ1–42, which leads to aggregation and adherence to surfaces. In 1998, it was observed that Aβ1–42 concentrations were significantly lower after storing in glass and polystyrene tubes, compared with tubes composed of polypropylene (PP), whereas no effects for tTau and pTau181P were found. This important finding was replicated and captured in a standardization protocol for biobanking recommending the use of laboratory plastics composed of PP. Other studies focused on the degree of Aβ1–42 adsorbance in different types of collection tubes and different types of biobanking vials, showing recovery of Aβ1–42 when Tween-20 was added to the samples. A multicenter study showed that harmonization of the CSF collection tube between test centers led to a 12% increase in clinical sensitivity for Aβ1–42 as a biomarker for AD.

Other than the type of tube, several studies have described a larger adsorption effect when proportionally little tube volume was used. In addition, Aβ1–42 adsorption was found to be a repetitive process, occurring again when exposed to a novel surface such as a second tube. To identify the critically important preanalytical variation factors in laboratory routine, these factors need to be studied in parallel, mimicking the clinical route of CSF as good as possible. The aim of the present study was to comprehensively map the factors defining Aβ1–42 adsorption in laboratory practice to finally optimize the current biobanking protocols. More specifically, we studied the effects of the number of CSF transfers from tube to tube, the type of biobanking vial, the aliquot volume, the Aβ1–42 concentration, the exposure time of the CSF to the tube, and the type of pipette used during tube transfers. In addition, we measured Aβ1–42 adsorption during other common laboratory steps, such as continuous movement of the CSF collection tube, using a PP instead of polyethylene screw cap of the collection tube, and excessive vortexing. Subsequently, we verified adsorption specificity for Aβ1–42 and assessed the frequency of tube transfers in current laboratory practice.

Methods

Number of tube transfers in clinical practice—Small-scale questionnaire

A small questionnaire was sent out to identify the variation in the number of transfers in diagnostic routine. Four academic research centers were compared: BIODEM at the University of Antwerp (Belgium); Sahlgrenska University Hospital, Gothenburg (Sweden); University Clinic Erlangen (Germany); and VU University Medical Center, Amsterdam (Netherlands). Participants were asked to comment on the number of transfers, the transfer method they applied, whether

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they worked according to the standard operating procedures, and if they applied additional transfers to external samples that were sent for AD biomarker determination.

Samples
Surplus CSF from the routine diagnostic laboratory was used, according to the "Research Code for Proper Secondary Use of Human Material" of the VU University Medical Center (VUmc), Amsterdam. Pools were prepared from CSF samples with both high (>1000 pg/mL) and low (400–500 pg/mL) Aβ1–42 concentrations as determined with the INNOTEST β-Amyloid (1–42) (Fujirebio, Gent, Belgium) or from CSF samples with unknown Aβ1–42 concentrations, if not mentioned otherwise. The total protein concentration in the CSF pools ranged from 378 to 479 mg/L.

Adsorption during tube transfer experiments
CSF was transferred 0, 1, 2, or 4 times into a new tube with an incubation time of 5 minutes, at room temperature (Figure 1, Supplementary Material). Different conditions were tested during the transfers (n = 3 per experiment): Aβ1–42 concentrations; starting volumes; different PP tubes (Sarstedt 72.694.007; FluidX 65-7532; Nalgene 5000-1020), which were selected based on pilot experiments comparing adsorption between 18 tubes (n) (Supplementary Material 4). The final tubes were kept at −80°C until Aβ1–42 and Aβ1–40 measurement. The transfer series with volumes 500 and 1000 mL were refrozen at −80°C and later used for pTau and tTau measurement.

Effect of CSF incubation time on Aβ1–42 adsorption to tube wall
To determine the influence of incubation time on Aβ1–42 adsorption, we tested the effects of 5 minutes, 15 minutes (suggested as minimum 1), 30 seconds as extreme minimum, and 2 hours as extreme maximum. For this experiment, 3 new, high and low Aβ1–42 CSF pools were used, starting volume was 150 µL, 2.0 mL Sarstedt tubes (72.690.001) were used, and 0 versus 4 tube transfers were compared in one series. Samples were kept at −80°C until Aβ1–42 and Aβ1–40 measurements. Samples were refrozen at −80°C for later use with the INNOTEST β-Amyloid (1–42).

Adsorption because of pipetting
To distinguish Aβ1–42 adsorption to the tube wall from Aβ1–42 adsorption in the pipette tip, we extended the original transfer experiment: 4 CSF transfers with a new pipette into a new tube; 4 CSF transfers with a new pipette into the same tube; and CSF remained in the tube without transfers (n = 6) (Supplementary Material 1). Sarstedt tubes (72.690.007; 2.0 mL) were used with 150 or 1000 µL CSF. Samples were kept at −80°C until Aβ1–42 and Aβ1–40 measurement.

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Aβ₁₋₄₂ and Aβ₁₋₄₀ enzyme-linked immunosorbent assays

Aβ₁₋₄₂ and Aβ₁₋₄₀ enzyme-linked immunosorbent assays (ELISAs) (EUROIMMUN AG, Lübeck, Germany) were performed according to the manufacturer’s instructions. More technical details on the assay are described by Sutphen et al.¹° Samples were run in duplicate and all conditions for each pool within one plate.

To exclude assay-related effects from interfering with the adsorbance results, Aβ₁₋₄₂ measurements were repeated with the INNOTEST β-Amyloid (1–42) in a subset of samples, n = 47. Aβ₁₋₄₂ levels were correlated using Pearson correlation statistics.

Statistical analyses

All concentrations were normalized to the reference value and expressed as percentages. A P value < 0.05 was considered significant. Statistical analyses were performed using IBM SPSS Statistics 22, and graphs were prepared in GraphPad Prism 6.

Linear regression analysis was performed to assess the adsorption effect for Aβ₁₋₄₂ and Aβ₁₋₄₀ over tube transfers, using the relative Aβ₁₋₄₂ or Aβ₁₋₄₀ concentration as the dependent variable and the number of tube transfers, starting volume, starting concentration, ratio contact surface area to volume, and tube brand as independent variables. Multiple group comparisons were statistically tested using analysis of variance (ANOVA) with Bonferroni post hoc correction; two-group comparisons with t tests; and correlations with Pearson or Spearman, if data were not normally distributed. Normal distribution was checked using frequency histograms, Q–Q plots, skewness and kurtosis values, and the Shapiro-Wilk test for normality.

Results

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We verified the number of transfers in four academic AD diagnostic reference centers as confirmation that standardization of this parameter is relevant to the field. The survey indicated that the number of CSF transfers occurring in diagnostic practice can range from 0 to 2 and that various transfer methods are applied (Table 1) (Engelborghs, Zetterberg and Andreasson, Lewczuk, Van Uffelen, personal communication). According to our adsorption results (Section 3.3), this would already account for an artificial difference of 10% in Aβ₁₋₄₂ concentration between these centers.

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Table 1. Variance in number of tube transfers in four academic AD referral centers.

<table>
<thead>
<tr>
<th>Center</th>
<th>Number of transfers</th>
<th>Transfer method</th>
<th>Procedure according to SOP</th>
<th>Extra transfer for external CSF samples</th>
</tr>
</thead>
<tbody>
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<td>Decanting</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Center 2</td>
<td>0/1</td>
<td>PE Pasteur pipette</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Center 3</td>
<td>1*2</td>
<td>PE Pasteur pipette*</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Center 4</td>
<td>1</td>
<td>Pipette with tip (PP)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Indicates the procedure for a minority of the CSF samples (Engelborghs, Zetterberg and Andreasson, Lewczuk, Van Uffelen, personal communication). Abbreviations: AD, Alzheimer’s disease; CSF, cerebrospinal fluid; PE, polyethylene; PP, polypropylene; SOP, standard operating procedure.

NOTE: Samples were CSF that was screened for AD biomarkers. Results are from a small-scale questionnaire.

ELISAs

The present study was done using the Aβ_{1–40} and Aβ_{1–42} assays from EUROIMMUN. To exclude a potential bias by using EUROIMMUN ELISAs linked to a difference in assay design, Aβ_{1–42} was remeasured using the INNOTEST β-Amyloid (1–42) (Fujirebio) in a subset of the tube transfer samples. The correlation factor for the CSF samples (n = 47) amounted to r = 0.97, P < 0.001 (Pearson correlation).

Aβ_{1–42} concentration decreases per tube transfer

Absolute CSF Aβ_{1–42} values for the CSF pools varied between 257 and 340 pg/mL for the low Aβ_{1–42} pools and between 795 and 978 pg/mL for the high Aβ_{1–42} pools before exposure to tube transfers. We observed a loss of approximately 5% Aβ_{1–42} with every tube transfer (Figure 1). In addition to the number of transfers (standardized β = −0.75, P < 0.001), we observed in a linear regression model that the extent of concentration loss was dependent on the starting volume (standardized β = −0.29, P < 0.001) and the starting concentration (standardized β = −0.21, P < 0.001).

Different tested tube brands lacked an impact on Aβ_{1–42} adsorption. The ratio of the contact surface area of the CSF and tube to the CSF-volume was specific per tube brand because of the shape of the tubes (Figure 2, x-axis). Higher contact surface area to volume ratios led to more adsorption after 4 tube transfers (Spearman’s r = −0.71, P < 0.01). In a linear regression model, including “starting volume” changed the standardized β of “ratio of contact surface area to CSF volume” from −0.27 (P < 0.001) to −0.03 (not significant), indicating that starting volume and surface area to volume ratio are closely related.

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Adsorption of CSF Aβ1-42 in laboratory practice

Figure 1. Effect of volume on relative decrease in Aβ1–42 (A and B) and Aβ1–40 (C and D) and stability of the Aβ1–42/Aβ1–40 ratio (E and F) over CSF tube transfers. Starting CSF Aβ1–42 concentration in (A, C, and E) was >1000 pg/mL and in (B, D, and F) was between 400 and 500 pg/mL. Percentages in red indicate the remaining Aβ1–42 (A and B) or Aβ1–40 (C and D) concentration. Error bars represent the standard error of the mean of n between 3 and 9.

Abbreviations: Aβ1–40, amyloid-β (1–40); Aβ1–42, amyloid-β (1–42); CSF, cerebrospinal fluid.

Aβ1–40 decreases per tube transfer whereas the Aβ1–42/Aβ1–40 ratio is stable

Absolute values before transfers for Aβ1–40 were 5667 pg/mL for the low-concentration Aβ1–42 pools and were between 5470 and 5974 pg/mL for the high concentration Aβ1–42 pools (from 4 pools described in Section 2.2). We observed a loss of Aβ1–40 over tube transfers (Figure 3), which depended on the number of transfers and the starting volume. This decrease followed the same pattern as the Aβ1–42 adsorption pattern, with no difference between tube brands and a final mean decrease after 4 transfers of 83% for both Aβ1–42 and Aβ1–40. Interestingly, when calculating the Aβ1–42/Aβ1–40 ratio, it remained constant over 4 transfers (Figure 1).

Aβ adsorption to tube wall occurs within 30 seconds

With varying incubation times of CSF in the tubes, the tube transfer experiments were repeated using 3 high Aβ1–42 pools (>1000 pg/mL) and 3 low Aβ1–42 pools (400–500 pg/mL). As depicted in Figure 3, the relative Aβ1–42 remainder after 4 transfers was 70% with 30 seconds of exposure, 70% with 5 minutes of exposure, 72% with 15 minutes of exposure, 62% with 2 hours of exposure (repeated measures ANOVA, P = 0.001); the relative Aβ1–40 remainder was 68% with 30 seconds of exposure, 71% with 5 minutes of exposure, 70% with 15 minutes of exposure, 64% with 2 hours of exposure (repeated measures ANOVA, P = 0.001), the relative Aβ1–42 remainder was 68% with 30 seconds of exposure, 71% with 5 minutes of exposure, 70% with 15 minutes of exposure, 64% with 2 hours of exposure (repeated measures ANOVA, P = 0.001); and the ratio of Aβ1–42/Aβ1–40 remained constant over 4 transfers (Figure 1).
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of exposure (repeated measures ANOVA, P < .05). Thus, adsorption occurs within 30 seconds.

The ratio $A_1{\beta}_{1–42}/A_1{\beta}_{1–40}$ remained stable at all incubation times (repeated measures ANOVA, n.s.).

**Figure 2.** Relation between surface area to volume ratio and $A_1{\beta}_{1–42}$ adsorption after four tube transfers. The ratio was calculated by taking the tube surface area that is in contact with CSF (tube sizes supplied by the manufacturer) divided by the CSF volume. The surface area to volume ratio for the Sarstedt tube was 0.58 with 1 mL volume, 0.68 with 0.5 mL, and 0.83 with 150 µL. For FluidX, the ratio was 0.49 with 1 mL CSF, 0.54 with 0.5 mL, and 1.29 with 150 µL. For Nalgene, the ratio was 0.58 with 1 mL CSF, 0.61 with 0.5 mL, and 1.07 with 150 µL. Spearman correlation between the adsorption after four transfers and the ratio surface area to volume was $p = – 0.71$, $P < 0.01$. Every symbol indicates one sample (Section 3.2). Abbreviations: $A_1{\beta}$, amyloid-$b$ (1–42); CSF, cerebrospinal fluid.

**Adsorption effect is partially explained by adsorption in pipette tips**

We next verified whether the adsorption problems observed during tube transfers might be also linked to adsorption in the pipette tip as described in Section 2.4. We here assumed that pipetting into the same tube does not lead to extra adsorption. Taking up CSF with a pipette 4 times and releasing the CSF into the same tube led to losses of 13% and 7% of $A_1{\beta}_{1–42}$ concentration in small and large volumes, respectively. Taking up CSF with a pipette 4 times and releasing it into a new tube led to 30% and 13% of losses in $A_1{\beta}_{1–42}$ concentration in small and large volumes, respectively (Figure 4). Adsorption thus occurred in the pipette tip and is doubled when transferred into a new tube instead of into the same tube. ANOVA showed that this effect was significant for $A_1{\beta}_{1–40}$ and $A_1{\beta}_{1–40}$, both $P < 0.001$, and that the $A_1{\beta}_{1–42}/A_1{\beta}_{1–40}$ ratio was not affected.

**Discussion**

Our study shows that $A_1{\beta}_{1–42}$ adsorption is a relevant preanalytical factor in laboratory practice, and we aimed to present a comprehensive overview of potential confounding factors regarding adsorption. On average 5% of $A_1{\beta}_{1–42}$ in CSF was lost because of adsorption with every tube transfer.
transfer. In smaller aliquot volumes, the adsorption increased up to 10% and approximately half of the Aβ1–40 loss during a transfer occurred in the pipette tip. Other laboratory handlings, including excessive vortexing, rolling transport of CSF collection tubes, material of the screw cap, and material of the transfer pipette did not influence Aβ1–40 concentrations (Supplementary Material 2). Incubation times of 30 seconds, 5 minutes, and 30 minutes caused similar adsorption, whereas 2 hours of contact in the tube led to slightly more adsorption. Use of the ratio Aβ1–40/Aβ1–40 completely eliminates the adsorption effect as was suggested before. Adsorption in biobanking tubes was specific for amyloid peptides, since tTau, pTau, and IL-1RACp values were not decreased because of tube transfers (Supplementary Material 3).

We measured CSF Aβ1–40 concentrations over up to four tube transfers to be able to detect subtle effects on tube adsorption, because of potential variation factors such as different tube brands, volumes, and Aβ1–40 concentrations. Initially, we considered four transfers as an extreme, merely experimental, condition. Surprisingly, a small-scale questionnaire revealed that up to three transfers can occur in clinical settings. On top of that, an additional aliquoting step is sometimes required for research, suggesting that a total of four CSF transfers is not a merely experimental condition in biobanking practice.

The finding that small aliquot volumes result in higher adsorption rates is explained by relatively more tube wall surface area that is in contact with the CSF in smaller aliquot volumes than in larger aliquot volumes. As such, we found a correlation between the degree of adsorption after four tube transfers and the ratio of contact surface area to volume, and thereby we confirm previous findings. Because our pilot studies and other published studies indicated a difference in adsorption between different types of CSF vials, we were surprised not to retrieve this result in the current experiments. A limitation of our study is that we did not include commercial tubes having a surface coating designed to avoid adsorption in our comparison. The contact surface area of the tube to the aliquot volume ratio varies between tube brands because the shapes of the biobanking vials are slightly different. We think that the effect of aliquot volume and shape of the tube may have contributed the observed differences in adsorption between tubes with different material compositions in previous studies and was at least not included as a biasing factor in those studies.

We found an increased adsorption of CSF with high Aβ1–40 concentrations (>1000 pg/mL) compared with low Aβ1–40 concentrations (400–500 pg/mL), which could be explained by accumulation of Aβ1–40 protein aggregations at the tube walls enhanced by the abundant presence of this hydrophobic aggregation-prone protein (for review see ). We showed that the adsorption effect was specific for the Aβ1–40 and Aβ1–40 proteins, because tTau, pTau, Interleukin 1 Receptor Accessory Protein (IL-1RACp), and total protein did not decrease in concentration after tube transfers, which is confirmed in other studies for the tau proteins.
Aβ₁₋₄₂ adsorption had already occurred after 30 seconds of CSF incubation. CSF tube incubation of 5 or 15 minutes showed similar Aβ₁₋₄₂ concentrations, indicating that adsorption occurs immediately. This implies that adsorption is also relevant for the pipette tip and that it cannot be avoided by quick handling, although we did not study contact of less than 30 seconds, which was practically impossible. Remarkably, after similar adsorption for 30 seconds to 15 minutes of incubation time, we found a significant difference between 15 minutes and 2 hours of incubation, indicating that a longer incubation allows more protein to adhere to the tube wall, although a previous study showed no additional adsorption with incubation increasing from 1 to 24 hours. Our results emphasize the susceptibility of the diagnostic AD marker Aβ₁₋₄₂ to preanalytical variation. Adsorption leads to underestimation of the Aβ₁₋₄₂ concentration, which in the case of an Aβ₁₋₄₂ value close to the cutoff level could falsely classify a healthy individual as AD. The hypothesis that the Aβ₁₋₄₂/Aβ₁₋₄₀ ratio would improve diagnostic accuracy in AD was first proposed to be patient related as it would control for high and low amyloid producers. In a prospective clinical study, the Aβ₁₋₄₂/Aβ₁₋₄₀ ratio was found to better predict conversion to AD in a mild cognitive impairment (MCI) group than CSF Aβ₁₋₄₀ alone. Still, the added value of using the Aβ₁₋₄₂/Aβ₁₋₄₀ ratio is debated. Our findings advocate for addition of Aβ₁₋₄₀ to the diagnostic CSF biomarker panel for AD, because it would reduce preanalytical variation for the Aβ₁₋₄₀ concentration and thereby increase diagnostic accuracy. The ratio of total extracted Aβ₁₋₄₂/Aβ₁₋₄₀ also showed good discriminative power for AD versus control subjects in a study where matrix interference was precluded. Recent studies also found increased diagnostic accuracy with additional use of the Aβ₁₋₄₂/Aβ₁₋₄₀ ratio, especially in case of inconclusive CSF AD profiles.
Several strategies have been proposed to influence Aβ₄₂ adsorption. The most commonly studied treatment preventing Aβ₄₂ adsorption is the addition of Tween-20, which completely prevents Aβ₄₂ from adsorbing. We also added Tween-20 to a subset of our samples, retrospectively, and found a slightly improved recovery of Aβ₄₀ and Aβ₃₈ (data not shown). We did not find complete recovery, as is expected considering our study design, because Aβ₄₂ was lost in prior tubes during transfer and in pipette tips, so only the Aβ₄₀ adsorbed by the wall of the final tube could be recovered. Addition of Tween-20 should be done at the time of CSF withdrawal, which is impractical. Moreover, addition of Tween-20 did not improve the diagnostic accuracy of discriminating AD patients from control subjects. Taking these two points into consideration, using the Aβ₄₀/Aβ₄₂ ratio seems to be the preferred solution for variation owing to adsorption as was proposed for Aβ₁₋₄₀ and Aβ₁₋₃₈. Notwithstanding, measuring Aβ₄₀ requires additional analytical work, and the use of two assays instead of one will in theory slightly increase the analytical variance. Importantly, manufacturers will need to examine the variability on the ratio of both proteins instead of the variability of each protein separately. Moreover, a reference method and a certified reference material for Aβ₄₀ should be developed. Because both were recently developed for Aβ₁₋₄₀, extending these to Aβ₄₀ could be relatively straightforward.

In conclusion, it must be emphasized that clinical laboratories should try to reduce the number of transfers of CSF and keep the workflow of CSF processing as consistent as possible, including types of collection and biobanking tubes and aliquot volumes. The Aβ₄₀/Aβ₄₂ ratio can eliminate adsorption as a preanalytical factor as we demonstrated here. Therefore, adding Aβ₄₀ to the AD diagnostic biomarker panel increases the diagnostic accuracy as has been shown in recent

![Figure 4. Effect of adsorption in pipettes on relative Aβ₄₀ concentration and the Aβ₄₀/Aβ₄₂ ratio. CSF that was 4 times pipetted into the same tube showed about half of the adsorption of Aβ₄₀ observed for CSF that was pipetted into a new tube (ANOVA, P < 0.001). The ratio Aβ₄₀/Aβ₄₂ remained constant under all circumstances (ANOVA, n.s.). Means with SEM of n = 6 are shown. Abbreviations: ANDOA, analysis of variance; Aβ₄₀, amyloid-β (1–40); Aβ₄₂, amyloid-β (1–42); CSF, cerebrospinal fluid; SEM, standard error of the mean.](image)
studies by others. A reference method and a certified reference material should be developed for $\text{A}\beta_{1-40}$ to realize implementation. Diagnostic studies using the $\text{A}\beta_{1-42}/\text{A}\beta_{1-40}$ ratio show promising results with respect to defining a universal cutoff to discriminate patients and control subjects, which would stimulate multicentre approaches for biomarker studies and clinical trials. Moreover, implementation of the CSF biomarkers in the AD diagnostic criteria would be a feasible purpose in the near future.

Acknowledgments
The authors acknowledge EUROMMUN for supplying with $\text{A}\beta_{1-42}$ and $\text{A}\beta_{1-40}$ enzyme-linked immunosorbent assays for this project. The authors thank Prof. Dr Engelborghs from the University of Antwerp and Bjorn Fetlaar from Sopachem B.V. for donation of part of the tubes that were tested. The authors also thank Prof. Dr Zetterberg and Dr Andreasson from the University of Gothenburg, Prof. Dr Lewczuk from the University of Erlangen, and Prof. Dr Engelborghs from the University of Antwerp for filling out the questionnaire on tube transfers in practice. This research was financially supported by Biobanking and BioMolecular resources Research Infrastructure The Netherlands (BBMRI-NL), a research infrastructure financed by the Dutch government (NWO 184.021.007) under project CP2013-68.

Research in context
1. Systematic review: The authors reviewed the literature using PubMed, meeting abstracts, and presentations. It has been previously described that cerebrospinal fluid (CSF) amyloid-$\beta$ (1–42) ($\text{A}\beta_{1-42}$) adsors to polypropylene tubes; however, the adsorption issue had never been addressed as thoroughly, extensive, and translatable to clinical practice as described in the present study.
2. Interpretation: Our study proves that using the CSF $\text{A}\beta_{1-42}/\text{A}\beta_{1-40}$ ratio instead of CSF $\text{A}\beta_{1-42}$ alone strongly reduces the confounding effect of adsorption during preanalytical processing of CSF.
3. Future directions: Replication of this study using the novel automated platforms that are currently under development would be relevant, because these platforms show less analytical variation and can thus better detect changes caused by preanalytical variation. Our findings advocate for additional routine measurement of CSF $\text{A}\beta_{1-42}$ and $\text{A}\beta_{1-40}$ next to $\text{A}\beta_{1-42}$ Tau and pTau, to universally implement the use of the CSF $\text{A}\beta_{1-42}/\text{A}\beta_{1-40}$ ratio in clinical practice and hereby increase the diagnostic accuracy for Alzheimer’s disease.

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Research in context
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Adsorption of CSF Aβ1-42 in laboratory practice

References


Supplementary data
Supplementary material 1. Schematic representation of tube transfer methods

Supplementary figure 1. Experimental set-up for the tube transfer experiments. CSF was incubated for 5 minutes at room temperature before the next transfer. A) This set-up was repeatedly executed with varying sample volumes (150 µl, 500 µl, 1000 µl), in different PP biobanking tubes (Sarstedt, FluidX, Nalgene), for 3 high-amyloid pools and 3 low-amyloid pools as explained in text sections 2.3, 2.4 and 2.5. tf=transfer. B) This set-up was used to verify whether Aβ1-42 adsorption during transfers was partly explained by adsorption in the pipette tip as explained in text sections 2.5 and 3.6. For every pipetting step clean pipette tips were used. This set-up was repeatedly executed with varying sample volumes (150 µl and 1000 µl), n = 6.

Supplementary material 2. Adsorption during other laboratory handlings
For the following handlings and the following materials (PP vs PE) applied during the laboratory processing of CSF, no effects on Aβ1-42 adsorption were observed.

Aβ1-42 adsorption is not enhanced by vortexing
Next, Aβ1-42 adsorption induced by vortexing was assessed in 5 ml PP Sarstedt tubes (55.525) filled with 3.5 ml pooled CSF. No vortexing (n = 5) was compared to 3 or 10 times vortexing (both n = 5) for 3 seconds. Aliquots of 300 µl CSF were stored in 2 ml Sarstedt tubes (72.694.007) and stored at -80°C until Aβ1-42 measurement.

The Aβ1-42 concentration in 5 CSF pools was 100.3% compared to samples that were not vortexed after 3 times vortexing, and 99.3% after 10 times vortexing the tubes (Supplementary figure 2A), indicating that Aβ1-42 adsorption is not influenced by vortex intensity.
Aβ1-42 adsorption in collection tubes is not influenced by continuous movement during transport

10 ml Sarstedt collection tubes (62.610.018, PP) were filled with 6.0 ml CSF and were put either on the roller bench, to mimic transport of CSF collection tubes, or in upright position, for 48 hours. At start and after 1, 4, 24, and 48 hours, 250 µl was sampled from the tubes and stored in 2 ml Sarstedt tubes (72.694.007) at -80°C until Aβ1-42 measurement. Stable Aβ1-42 concentrations indicated that adsorption was not influenced by the type of transport, nor the duration of transport (Supplementary figure 2B).

Aβ1-42 adsorption in collection tubes not influenced by PE or PP screw cap

Polypropylene (PP) CSF collection tubes are the standard since discovery of the adsorption to polystyrene, however, standard tube caps are composed of polyethylene (PE). We compared Aβ1-42 adsorption in PP caps versus PE caps, both placed on PP tubes (Sarstedt, 60.540.016, 13 ml, screw cap PP; Sarstedt, 62.9924.284, 10 ml, screw cap PE) filled for 50% with pooled CSF. At start, and after 1 hour and 24 hours on a roller bench, 300 µl was sampled from CSF tubes (n = 5) and stored in 2 ml Sarstedt tubes (72.694.007) at -80°C until Aβ1-42 measurement. Aβ1-42 concentrations were not decreased after 1 nor after 24 hours, indicating that there is no influence of the type of screw cap (Supplementary figure 3B).
Polypropylene (PP) and polyethylene (PE) transfer pipettes led to similar adsorption levels

We compared Aβ1-42 adsorption during pipetting with a Pasteur pipette (Sarstedt, low-density PE, 86.1172) versus a pipette with a blue tip (Eppendorf, PP). 4 mL CSF was transferred in 4 times from a 10 mL Sarstedt tube (62.610.018, PP) to a 5 ml Sarstedt tube (55.525, PP), left for 10 minutes and in the same way transferred to a new 5 ml Sarstedt tube (n = 5). The final tubes were stored at -80°C until measurement of Aβ1-42. The Aβ1-42 values were comparable when either the 1 ml polyethylene (PE) Pasteur pipette (locally used for clinical routine CSF work-up), or the 1 ml Eppendorf pipette with a blue PP pipette tip was used (figure 6).

Supplementary figure 3. Impact of transfer pipettes and screw caps composed of PP and PE on Aβ1-42 adsorbance. A) After 4 transfers using the Pasteur PE pipette the remainder of Aβ1-42 was 89.0%, and using the blue tip PP pipette tip this was 88.5%, n.s. Volume of CSF transferred was 4 mL. B) The Aβ1-42 concentration in PP CSF collection tubes is not influenced by material of the screw cap, PE or PP. Means with SEM of n = 5 are shown.

Supplementary material 3. Adsorption is specific for Aβ1-42 and Aβ1-40: no adsorption of tTau, pTau, total protein, nor IL-1 RAcP

To verify specificity of Aβ1-42 adsorption to tube walls, total Tau (Innotest hTAU-Ag, Fujirebio, Belgium) and phosphorylated Tau (Innotest PhosphoTAU-181p, Fujirebio, Belgium) concentrations were measured according to manufacturer’s instructions in the CSF samples that underwent 0, 1, 2, or 4 transfers (500 and 1000 µl volumes) and that had been used for the Aβ1-42 and Aβ1-40 experiments. For both tTau and pTau, no decrease in concentration over tube transfers was observed.

All conditions from one high Aβ1-42 pool of the tube transfer series were measured for total protein levels using spectrophotometer NanoDrop ND-1000 (NanoDrop Technologies). Method ‘Protein A280’ was used according to the manufacturer’s instructions. The total protein levels measured by Nanodrop similarly remained constant.
IL-1 RAcP was measured in fresh aliquots of all conditions from one low pool of the tube transfer series. A sandwich ELISA was used according to the manufacturer’s instructions (Human IL-1 RAcP/IL-1 R3 DuoSet ELISA, R&D Systems, MN). Also, there was no decrease in IL-1 RAcP concentration after tube transfers (Supplementary figure 4).

Supplementary figure 4. Effect of tube transfers on relative IL-1 RAcP concentration. No decrease in CSF IL-1 RAcP concentrations was observed after CSF tube transfers. Error bars represent the SEM of n = 3.

Supplementary material 4. Pilot selection of biobanking tubes

Pilot experiments were performed using 18 tubes of varying volumes and from different manufacturers, in which the Aβ1-42 concentrations of CSF samples with a high, medium, and low Aβ1-42 concentration were compared (n = 1 per concentration) (supplemental figure 5). Three tubes were selected for further experiments: 1) Sarstedt 2.0 ml with screw-cap with o-ring (catalogue number 72.694.007) since it showed high Aβ1-42 levels compared to other tubes and it was the tube type used in the VUmc biobank, 2) FluidX 1.8 ml with screw cap (product number 65-7532) since this showed high Aβ1-42 levels too and is barcoded, and 3) Nalgene 1.5 ml with screw cap (product number 5000-1020) since this tube performed poorly showing low Aβ1-42 concentrations compared to other tubes. Aβ1-42 concentrations were measured with Innotest ELISA (Fujirebio, Belgium).
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Supplementary figure 5. Adsorption of Aβ1-42 in 18 different biobanking tubes. 400 µl of high, medium and low CSF pools was transferred into 18 different biobanking tubes using CSF to screen for adsorption characteristics. Note: in the 0.2 ml Thermo Scientific Micronic tube 100 µl CSF was transferred. N = 1.