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Amyloid Beta Adsorption Problem with Transfer Plates in Amyloid Beta 1–42 IVD Kits

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Abstract
Adsorption of CSF Aβ1–42 during pre-analytical processing is suggested as an important confounder in testing. The aim of the present study was to assess the effect of polypropylene transfer plates (PTP) in the INNOTEST Aβ1–42 IVD-ELISA assay on Aβ1–42 levels. CSF samples from 26 individuals with subjective cognitive impairment (SCI) and 25 patients with suspected neurodegenerative disorders were tested using four different lots of kits. Aβ1–42 levels in all samples that were loaded onto the PTP were significantly lower than the levels in the same samples that were analyzed without prior loading onto the PTP. We found that the PTP may adsorb Aβ1–42 in the range 7 to 69%. The diagnosis in 20% of patients and amyloid burden assessment in 23% of SCI patients had to be modified post hoc due to initial erroneously low amyloid levels. Using a PTP prior to loading the samples onto the INNOTEST Aβ1–42 test plate may result in erroneously low Aβ1–42 levels.

Keywords Alzheimer's disease · Aβ 1–42 · CSF · Diagnosis · Biomarker · ELISA

Introduction

The amount of Aβ1–42, total tau, and hyperphosphorylated tau in the cerebrospinal fluid (CSF) are widely-accepted biomarkers of Alzheimer's disease (AD) (Tapiola et al. 2009; *Alzheimers Dement* 2012; Gezen-Ak et al. 2014). Although the most widely used method for detection of the target proteins is ELISA, there are also other semi- or fully automated methods. Recently, the need to re-define cutoff levels for these biomarkers has been suggested (Illan-Gala et al. 2017). An upward shift in CSF Aβ1–42 cutoff levels has been proposed (Schindler et al. 2018), and attention has been drawn to the protein binding capacity of the sample collection tubes (Vanderstichele et al. 2017). Initial studies have indicated lower CSF Aβ1–42 levels when samples are preloaded onto the polypropylene transfer plates (PTP) that are supplied with the kit (del Campo et al. 2012; Fourier et al. 2015). A recent study has confirmed that adsorption of CSF Aβ1–42 during pre-analytical processing is an important confounder (Willemse et al. 2017). Although polypropylene tubes and pipet tips are thought to present a low binding capacity to Aβ1–42, the aforementioned study reported that, depending on the number and the volume of the transfers, results may vary enough to alter the diagnosis (Willemse et al. 2017). Besides, studies have shown that polypropylene material does not guarantee efficient pre-analytical behavior (Perret-Liaudet et al. 2012). The peptide binding capacity of hydrophobic and hydrophilic surfaces has also been investigated and the results suggest that hydrophilic surfaces have a relatively lower peptide binding capacity (Vrlinic et al. 2012). Our study indicated the particular peptide adsorption problem that originates from the PTP supplied with the Aβ1–42 in vitro diagnostic (IVD) kit and which has been confirmed by previous studies (del Campo et al. 2012; Fourier et al. 2015).

We initially recognized a problem with the INNOTEST Aβ1–42 kit, ref no. 81576, lot no. 403813, during a routine sampling which was performed on a relatively high number of patients, requiring more than six strips. The manual with the kit recommends using a 96-well polypropylene transfer plate (PTP) before loading the samples onto the antibody-coated plate when more than six strips need to be used. In that

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particular assay, all samples and calibrators were loaded onto the PTP supplied within the kit. All samples and calibrators were tested in duplicate, and inter-run coefficients of variation (CV) were less than 10%. Although all standards were accurate and run validation controls (RVC) were within the range, we had low levels of Aβ1–42 varying between approximately 120 and 300 pg/ml for all patients. As the levels were low for all patients, this cast doubt on their accuracy. We then repeated the test for some of these patients with the same lot but without using the PTP. We found that Aβ1–42 levels in the repeated tests were higher. The only difference between the two measurements was that the samples in the first experiment were loaded onto the PTP before being transferred to the antibody-coated plate as recommended by the manufacturer's protocol. The initial studies of del Campo et al. (del Campo et al. 2012) reported a similar problem with ten patient samples and Fourier et al. (Fourier et al. 2015) with eight patients, without indicating any variation between lots. We therefore tested kits with different lot numbers to explore whether the peptide adsorption problem with the PTP supplied with the Aβ1–42 (IVD) kit was specific to a particular lot or is a more general problem.

Material and Methods

Patients and Patient Consent

Fifty-one individuals (26 with a clinical diagnosis of subjective cognitive impairment (SCI) with a mean age of 56.4 [6.4] and 25 patients with suspected neurodegenerative disorders including AD or MCI with a mean age of 65.9 [12.7]) were included in this study. The sample selection was designed to give a large distribution of Aβ1–42 values. We included samples randomly to exclude any possible bias due to positive selection of any patient group. CSF data assessment of the individuals was done according to Mulder and Hulstaert formulas and tau/Aβ1–42 ratios (Duits et al. 2014) rather than local cutoff values. Signed informed consent was obtained from all study participants.

CSF Collection, Processing, and Storage

As part of the JPND-BIOMARKAPD project, we adhered to the standard operating procedures for CSF collection, processing, and storage that were defined by del Campo et al. (del Campo et al. 2012). Briefly, a lumber puncture (LP) was performed at the L3/4 level with 20G atraumatic needles and 10 ml of CSF was obtained. Samples were collected into 12-ml sterile polypropylene tubes (187261, Greiner-bio-one) and transferred to the laboratory within no more than 2 h within a cold chain. Samples were centrifuged at 2000 *g* for 10 min at 4 °C and aliquoted into 1.2-ml polypropylene cryogenic vials (430658, Corning); each aliquot received a volume of 900 μl. The aliquots were stored at − 80 °C. In all assays, freshly thawed aliquots of CSF that had been frozen only once were used. Different aliquots from a given patient were used in each experiment.

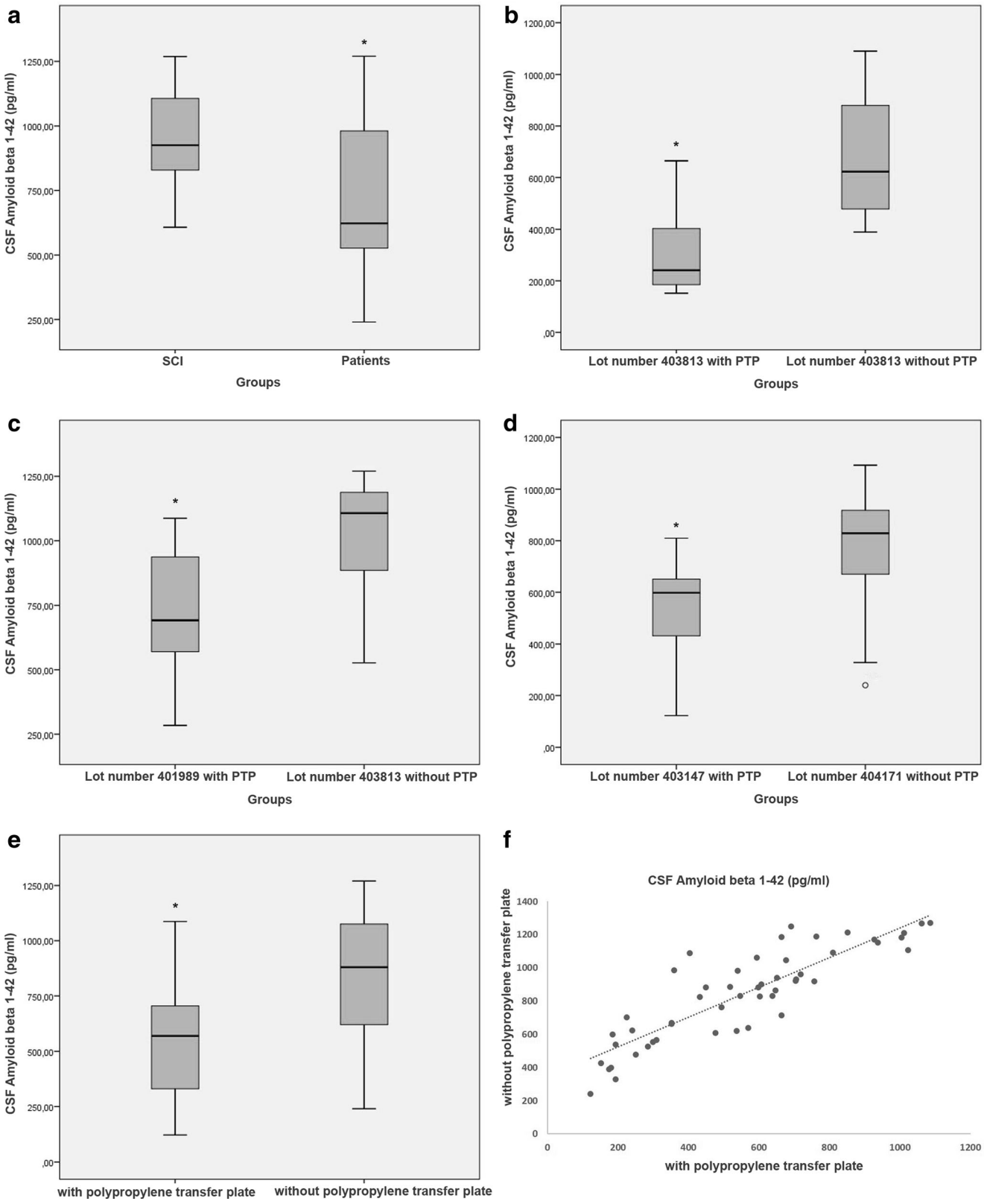
CSF Aβ 1–42 Assay

Prior to the assay, each sample was brought to room temperature for 1 h and then vortexed as described in the manufacturer's protocol. Aβ1–42 levels were determined by an enzyme-linked immunosorbent assay using INNOTEST Aβ1–42 IVD assays (Fujirebio [formerly Innogenetics], Ghent, Belgium). We used manual testing with a Thermo MultiScan EX ELISA reader. To eliminate lot-to-lot variations, we used four different lots of INNOTEST Aβ1–42 IVD assays (lots 403147, 401989, 404171, 403813). Altogether, 13 samples were tested with lot number 403813 using a PTP. In a subsequent analysis, samples from these patients were tested again with the same lot number, but without using the PTP. A further 21 samples were tested with lot number 401989 with and without using a PTP. These same samples were then analyzed with lot number 403813 without using a PTP. In another sample of 17 patients, we used lot number 403147 using a PTP, and these same samples were then analyzed with lot number 404171 without using the PTP.

Statistics

Each assay included a blank and eight standards. All samples and standards were tested in duplicate. The inter-run

Fig. 1 Comparison of CSF Aβ 1–42 levels. **a** Distribution of CSF Aβ1–42 levels in patients and SCI groups: the CSF Aβ1–42 levels of patient samples ($n = 25$) were significantly lower than those of the SCI samples ($p = 0.0018$). SCIs ($n = 26$, 957.2 (185.8), 95% CI 882.2–1032.3); patients ($n = 25$, 720.1 (302.6), 95% CI, 595.2–845.0). Comparison of CSF Aβ 1–42 levels of the samples that were assayed with and without a PTP; **b**–**d**) Lot-to-lot comparison: CSF amyloid beta 1–42 levels of the samples that were loaded onto a PTP were significantly lower than those of the same samples that were not loaded onto a PTP; **b** *n* 13, mean difference 368, 95% CI 203–534; $p = 0.0012$; **c** *n* 21, mean difference 277, 95% CI 116–438; $p = 0.0012$; **d** *n* 17, mean difference 238, 95% CI 94–382; $p = 0.002$); **e** total analysis of four different lots: CSF amyloid beta 1–42 levels of all the samples that were loaded onto a PTP were significantly lower than those of the same samples that were not loaded onto a PTP (*n* 51, mean difference 287.20, 95% CI 180.7–393.7; $p < 0.0001$). Data are given as the mean (SD); **f** correlation analysis of the CSF Aβ 1–42 levels of all samples that were assayed with and without a PTP. The data of the samples loaded onto a PTP were correlated with the data of the same samples that were not loaded onto a PTP ($n = 51$; correlation coefficient (r) = 0.8690; 95% CI 0.78–0.92; coefficient of determination (r^2) = 0.76). PTP polypropylene transfer plate



coefficients of variation were less than 10%. Aβ1–42 concentrations were calculated using standard curves ($R^2 = 0.998$). Raw data for each group were analyzed with an unpaired t test, given that the data were normally distributed, and $p < 0.05$ was considered statistically significant. Data were given as the mean (SD).

Data Availability All data analyzed during this study are included in this published article.

Results

The distribution of CSF Aβ1–42 levels in patients and SCI groups are shown in Fig. 1a. The CSF Aβ1–42 levels of patients were significantly lower than the SCIs ($p = 0.0018$) (Fig. 1a). Each of the four different PTP lots of INNOTEST Aβ1–42 IVD assays had significant peptide adsorption in initial loading ($p < 0.002$) (Fig. 1b–d). CSF Aβ1–42 levels in all samples that were loaded onto a PTP were significantly lower than the levels for the same samples that were analyzed without loading onto the PTP (Fig. 1e). The mean reduction in the CSF Aβ1–42 levels, i.e., peptide adsorption when loaded onto the PTP, was 287 (139) pg/ml (median 258, min 50, max 687; 95% CI 248.2–326.4). Mean percentage reduction was 37% (17) (median 35, min 7, max 69; 95% CI 31.8–41.4). Results of samples loaded onto a PTP correlated well with those of the same samples that were not loaded onto a PTP (Fig. 1f). The diagnosis in 20% of the patients was modified post hoc, due to initial erroneously low amyloid beta 1–42 levels (Fig. 2a). Likewise, the status of amyloid positivity was revised in 23% of subjects with SCI (Fig. 2b).

Discussion

Our study design included individuals with a clinical diagnosis of SCI and patients with suspected neurodegenerative disorders including AD or MCI. The sample selection was designed to give a large distribution of Aβ1–42 values. We included samples randomly to exclude any possible bias due to positive selection of any patient group. The significantly-low levels of CSF Aβ1–42 in patient samples compared to the SCI group (Fig. 1a) confirmed the discriminative value of the CSF Aβ1–42 levels as previously stated in other articles.

Recent studies have indicated that adsorption of CSF Aβ1–42 during pre-analytical processing can be an important confounder (Willemse et al. 2017). Conventionally, polypropylene tubes and pipet tips are thought to have a low binding capacity to Aβ 1–42. Yet, Willemse et al. reported that, depending on the number and the volume of transfers, results may vary enough to alter the diagnosis. This study indicated particularly high adsorption rates in relatively smaller volumes (Willemse et al. 2017). This may be relevant, as the transfer plates in the IVD kits are designed to be loaded with quite a small volume, i.e., 50 µl. Accordingly, two previous studies have indicated lower CSF Aβ1–42 levels resulting from the preloading of samples onto the PTP supplied with the kits (del Campo et al. 2012; Fourier et al. 2015). Fourier et al. also reported that CSF Aβ1–42 levels were significantly reduced when the samples were incubated on the PTPs for 5 min (reduction of 14.3%) and 15 min (reduction of 24.8%). In addition, in the absence of a commercially available pre-analysis 96-well plate with minimal adsorption of amyloids to plastic, they investigated if there was any significant within-plate variability from the first to the last rows of wells, and they found a significant alteration for data in the same sample. They suggested that, for Aβ1–42, no more than half a plate should be tested at the same time when not using a PTP (Fourier et al. 2015).

We determined that the 96-well PTPs that are supplied with the CSF Aβ1–42 IVD kit can adsorb Aβ1–42 in the range 7 to 69%. The calibrators and quality controls RCV1 or RCV2 remained in the correct ranges, unaffected in all assays. This is not surprising, as the calibrators are a set of Aβ 1–42 synthetic peptide in assay buffer (Cullen et al. 2012) and RVCs are synthetic Aβ1–42 peptide in an artificial CSF-like matrix (https://www.alz.org/research/downloads/update_innogenetics-vandijck.pdf). We also showed that synthetic Aβ1–42 peptides exhibit lower specific bioactivity and need longer incubation periods in inducing Aβ deposits than purified Aβ1–42 peptide (Stohr et al. 2012). The peptide adsorption problem with the PTP seems to be a general one, because plates from different lot numbers gave similar results. The correlation analysis indicated that similar reductions in amyloid levels could be found in all samples assayed with a PTP due to peptide adsorption.

Use of a PTP was suggested to avoid a significant decrease in the concentration of the samples while loading them onto the test plate (Fourier et al. 2015). Since sample incubation time takes 1 h, the manufacturer's protocol suggests reducing

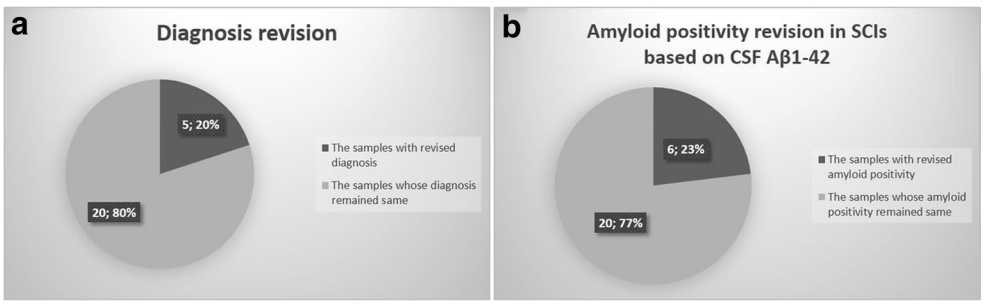


Fig. 2 Alteration in clinical diagnosis and amyloid positivity. Alterations in clinical diagnosis of patients and amyloid positivity of SCI samples were based on Mulder and Hulstaert formulas and the tau/Aβ1–42 ratio, and the clinical assessment of individuals with neuropsychological and neuroimaging data (*n*; %)

the dispensing time to a minimum. To reduce the reactivity shift, an uncoated PTP is supplied with the kit and recommended to be used as a waiting station when more than six strips are to be used for testing. When testing more than six strips, the manufacturer's protocol recommends dispensing CSF samples, calibrators and RCVs in amounts of 60 μl or higher to the PTP initially and then transferring them to the coated test plate with a multichannel pipet. Yet using the PTP with a multichannel pipet to reduce the sample distribution time does not solve the peptide adsorption problem, but adds additional steps that involve polypropylene materials including tips and the PTP, both of which have the potential to alter the results. Our study suggests testing no more than four to five strips at one time. This may result in a reduction in the number of samples tested with one kit, but may provide a way to avoid false reductions in the assay levels. Our routine protocol involves testing four strips at a time, involving duplicates of patient samples, RCVs, and standards in each run. This design allows only ten patient samples to be tested at once. This might not be cost efficient, but it ensures low preanalytical errors depending, especially, on the time consumed. It also prevents the extra adsorption of peptide from using a PTP and extra pipetting from the PTP to the test plate. Additionally, Willemse et al. found that the CSF volume/area ratio of the wall tube was also critical to the binding intensity and avoiding such a problem may require using the Aβ1–42/Aβ1–40 ratio (Willemse et al. 2017).

Conclusion

Using polypropylene plates prior to loading the samples onto the test plate can result in erroneously low amyloid levels and may lead to misdiagnosis. These errors in the measurement of Aβ1–42 levels can also affect the determination of cutoff values for CSF Aβ1–42.

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Compliance with Ethical Standards

Signed informed consent was obtained from all study participants.

Conflict of Interest The authors declare that they have no conflict of interest.

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