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Pre-analytical stability of novel cerebrospinal fluid biomarkers

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ABSTRACT

Stability of the cerebrospinal fluid (CSF) composition under different pre-analytical conditions is relevant for the diagnostic potential of biomarkers. Our aim was to examine the pre-analytical stability of promising CSF biomarkers that are currently evaluated for their discriminative use in various neurological diseases.

Pooled CSF was aliquoted and experimentally exposed to delayed storage: 0, 1, 2, 4, 24, 72, or 168 h at 4 °C or room temperature (RT), or 1–4 months at −20 °C; or up to 7 freeze/thaw (f/t) cycles, before final storage at −80 °C. Eleven CSF biomarkers were screened using immunoassays, liquid chromatography, or enzymatic methods.

Levels of neurogranin (truncP75), chitinase-3-like protein (YKL-40), beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), acetylcholinesterase (AChE) enzymatic activity, theobromine, secreted protein acidic and rich in cysteine-like 1 (SPARCL-1) and homovanillic acid (HVA) levels were not affected by the applied storage conditions. 3-Methoxy-4-hydroxyphenylglycol (MHPG) levels linearly and strongly decreased after 4 h at RT (−10%) or 24 h at 4 °C (−27%), and with 6% after every f/t cycle. 5-Methyltetrahydrofolate (5-MTHF) (−29% after 1 week at RT) and 5-hydroxyindoleacetic acid levels (5-HIAA) (−16% after 1 week at RT) were reduced and 3,4-dihydroxyphenylacetic acid (DOPAC) levels (+22% after 1 week at RT) increased, but only after > 24 h at RT.

Ten out of eleven potential CSF novel biomarkers showed very limited change under common storage and f/t

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-MTHF, 5-methyltetrahydrofolate; AChE, acetylcholinesterase; AD, Alzheimer's disease; BACE1, beta-site amyloid precursor protein cleaving enzyme 1; CI, confidence interval; CSF, cerebrospinal fluid; DOPAC, 3,4-dihydroxyphenylacetic acid; ELISA, enzyme-linked immunosorbent assay; F/t, freeze/thaw; HVA, homovanillic acid; JPND, Joint Programming - Neurodegenerative Disease Research; MHPG, 3-methoxy-4-hydroxyphenylglycol; MS, multiple sclerosis; RP-UHPLC-ECD, reversed phase ultra-high pressure liquid chromatography system coupled with electrochemical detection; RT, room temperature; SOP, standard operating procedure; SPARCL-1, secreted protein acidic and rich in cysteine-like 1; YKL-40, chitinase-3-like protein

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conditions, suggesting that these CSF biomarkers can be trustfully tested under the pre-analytical conditions present across different cohorts.

1. Introduction

Biomarkers in cerebrospinal fluid (CSF) have a high potential to support the diagnosis, monitoring and prognosis of neurological diseases to eventually improve patient care. Studies aimed at the identification and clinical validation of novel biomarkers often make use of samples originating from historical cohorts, collected before the establishment of clear biobanking protocols, or from multiple centres, between which the variation in pre-analytical conditions can be substantial. Pre-analytical variation can occur during collection, processing, and storage of the CSF and could result in non-biological variation of biomarker levels [1,2]. Excluding this non-biological variation of biomarker levels is of great importance, as biomarkers are used as clinical tools influencing medical decisions and patient care. As such, knowledge on the pre-analytical stability of potential CSF biomarkers is an essential prerequisite before proceeding to clinical validation of the biomarker assay. The classical CSF biomarkers that are used for diagnosis of Alzheimer's disease, amyloid-β1–42, phosphorylated Tau and total Tau, have been tested for stability [3,4] and these results were the basis for the current CSF collection and processing consensus protocol [5].

To facilitate a universal method for immunoassay validation, a standard operating procedure (SOP) was recently developed, which includes a section on storage stability to ensure that relevant pre-analytical conditions for CSF are addressed during the technical validation of a novel biomarker [6]. Upon application of this SOP, stability studies will be better aligned, resulting in better interpretable outcomes and more certainty regarding biomarker stability. The SOP was a result of the BIOMARKAPD project, a part of the EU Joint Programming - Neurodegenerative Disease Research (JPND) focussing on standardisation of biomarker assays for dementia (www.jpnd.eu), and was applied in this study.

The following CSF proteins and molecules were selected for stability testing, based on their potential as biomarker candidates for neurological diseases. Neurogranin and beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) are both synaptic proteins, and neurogranin CSF levels are currently being explored in a number of clinical trials, e.g. in the gantenerumab trial [7,8]. BACE1 protein levels have been explored in elderly healthy participants after chronic treatment with a BACE inhibitor [9]. CSF Acetylcholine esterase (AChE) has been proposed as a potential biochemical marker for cholinergic function in Alzheimer's disease (AD), decreasing modestly as dementia progresses, but also current AD therapy is mostly based on inhibitors of AChE which only have modest and transient therapeutic effects [10]. Chitinase-3-like protein (YKL-40) in CSF increases upon neuroinflammation in several neurodegenerative diseases, and YKL-40 levels were found to be decreased upon immunosuppressive treatment in multiple sclerosis (MS) [11,12]. Theobromine, a metabolite of caffeine, has been explored in CSF after traumatic brain injury for its potential neuroprotective effects [13], and might have a neuroprotective effect in AD as well [14]. Secreted Protein Acidic and Rich in Cysteine-like 1 (SPARCL-1) is a secreted protein involved in extracellular matrix organisation and has been studied in CSF as diagnostic biomarker for subtypes of MS and to discriminate AD patients from controls [15,16]. The monoamines, 3-methoxy-4-hydroxyphenylglycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA), are the principal metabolites of neurotransmitters and have been studied in CSF as biomarkers for AD or dementia with Lewy bodies [17,18]. 5-Methyltetrahydrofolate (5-MTHF) in CSF reflects the methionine–homocysteine transmethylation cycle in the brain and has been studied as a diagnostic marker for various CNS diseases, including AD [19].

Our aim was to assess the storage and freeze/thaw stability of eleven CSF proteins or molecules that are currently evaluated in our centre and in collaborative projects for their potential as novel biomarkers for neurological diseases.

2. Methods

2.1. Samples

CSF samples from the Alzheimer Center Biobank at the Amsterdam University Medical Center (Amsterdam UMC, Amsterdam, the Netherlands) [20] were used to prepare pools for storage and freeze/thaw stability testing. These samples had been collected according the workup for the Amsterdam Dementia Cohort and were stored at −80 °C, but were surplus due to e.g. inadequate clinical information for use in targeted biomarker studies and therefore selected for this study. Multiple CSF samples (0.5 ml per sample) from 3 to 6 different individuals were merged into one pool (50 ml PP tube), resulting in several CSF pools with different compositions. Aliquots of pooled CSF were typically 500 µl and were stored in 1.5 ml polypropylene tubes with screw caps (Sarstedt, Nümbrecht, Germany). The aliquoted CSF pools were exposed to storage conditions as described in the SOP for sample stability [6], i.e. storage up to 1 week at either 4 °C or room temperature (RT); storage at −20 °C up to 1 or 4 months, before final storage at −80 °C; or exposure up to 7 freeze/thaw cycles. A minimum of three different CSF pools were measured per marker, only for SPARCL1 the sample size was extended to 5 in view of the variation of the assay. For delayed storage of 5-MTHF, fresh CSF was centrifuged at 2000*g* for 10 min and then stored for 30 min, 1 h, 1 day, or 1 week at 4 °C or RT; other aliquots underwent 1 or 4 f/t cycles before storage at −80 °C. Reference samples for all markers were stored at −80 °C directly at time point zero. All samples were blinded and donors gave informed consent. This study was in line with the institutional research code and the biobank was approved by the local ethical committee. Samples were stored at −80 °C for maximum one year before measurement of the biomarker of interest.

2.2. Assays

2.2.1. Neurogranin

Neurogranin levels were measured using an enzyme-linked immunosorbent assay (ELISA) that specifically detects the neurogranin epitope that is C-terminally truncated at P75 (ADx Neurosciences, Ghent, Belgium) according to the manufacturer's instructions [21]. Intra- and inter-assay variabilities reported by the manufacturer were < 5% and < 7%, respectively.

2.2.2. YKL-40

YKL-40 levels were measured at the University of Gothenburg using ELISA according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). Intra- and inter-assay variabilities were established as < 10% and 6%, respectively [22].

2.2.3. Theobromine

Theobromine levels were quantified using liquid chromatography coupled to tandem mass spectrometry and compared with an internal standard at the Center for Neurosciences and Cell Biology, University of Coimbra (modified from [14] as described in the supplementary

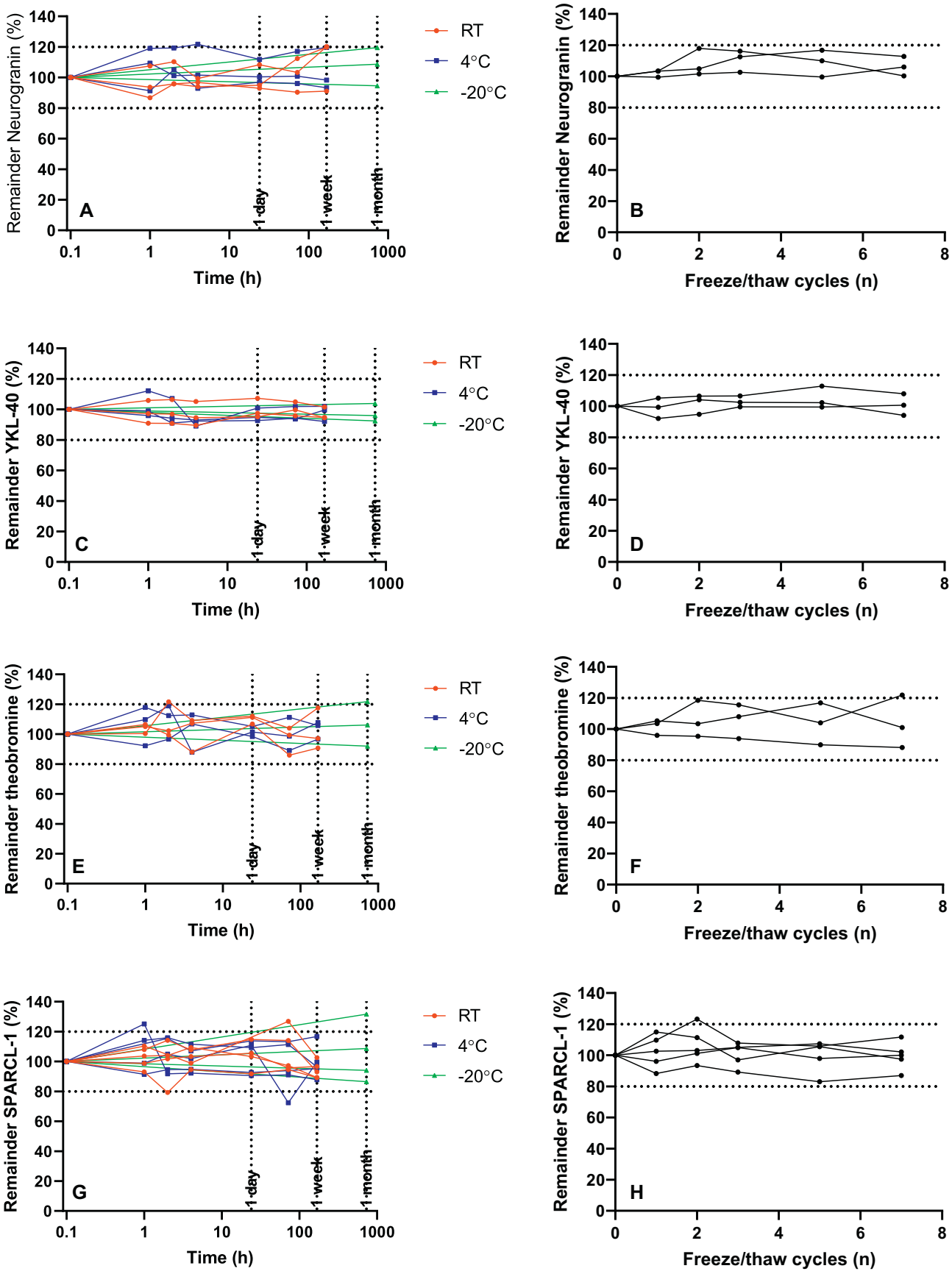

Fig. 1. Storage (left) and freeze/thaw stability (right) of potential novel biomarkers: neurogranin (A, B), YKL-40 (C, D), theobromine (E, F), SPARCL-1 (G, H), BACE1 (I, J), AchE activity (K, L), HVA (M, N), 5-HIAA (O, P), MHPG (Q, R), DOPAC (S, T), 5-MTHF (U,V). Circles show RT results, squares show 4 °C results, and triangles show −20 °C results. Dotted lines represent reference lines at 80 and 120%. Symbols represent individual data points, normalised to the reference value. RT = room temperature.

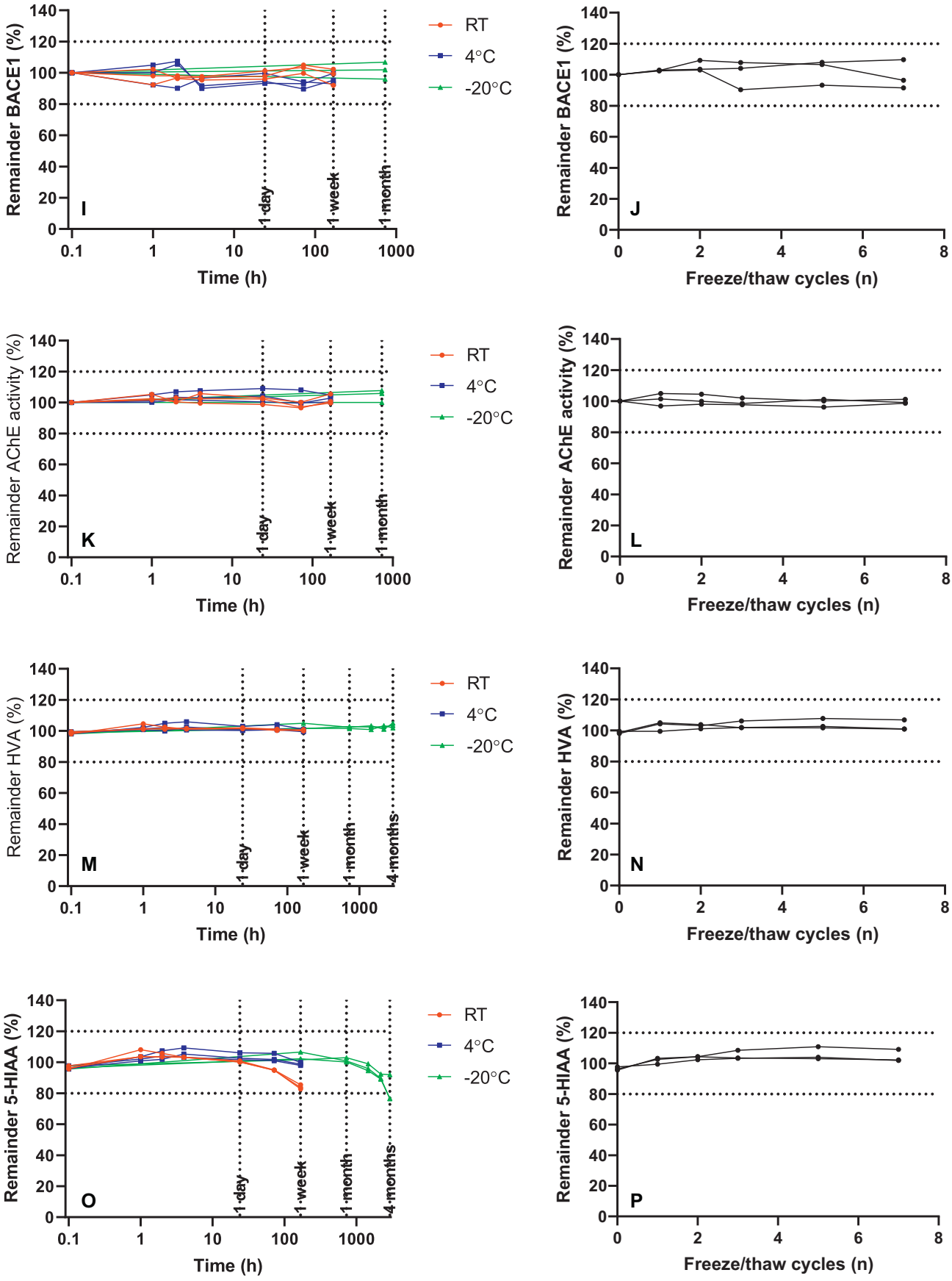

Fig. 1. (*continued*)

material). The coefficient of variation reported for this assay was 6% and 17% for intra and inter-assay, respectively.

2.2.4. SPARCL-1

SPARCL-1 levels were measured at Amsterdam UMC using a DuoSet ELISA Development kit according to the manufacturer's instructions (R &D systems, Abingdon, United Kingdom) [15]. Intra- and inter-assay coefficients of variation reported in that study were 6% and 12% respectively.

2.2.5. BACE1

BACE1 levels were measured using ELISA according to the manufacturer's instructions (ADx Neurosciences, Ghent, Belgium) [23]. Intra- and inter-assay variabilities reported by the manufacturer were 3,8% and 8,5%, respectively.

2.2.6. AChE enzymatic activity

AChE activity was determined by a modified microassay version of the colorimetric Ellman's method [24] at the University Miguel Hernández. AChE was assayed with 1 mM acetylthiocholine and 50 µM tetraisopropyl pyrophosphoramide (Iso OMPA), a specific inhibitor of butyrylcholinesterase, a second cholinesterase that co-exists with AChE in brain and CSF. The coefficient of variation reported for this assay was 3%.

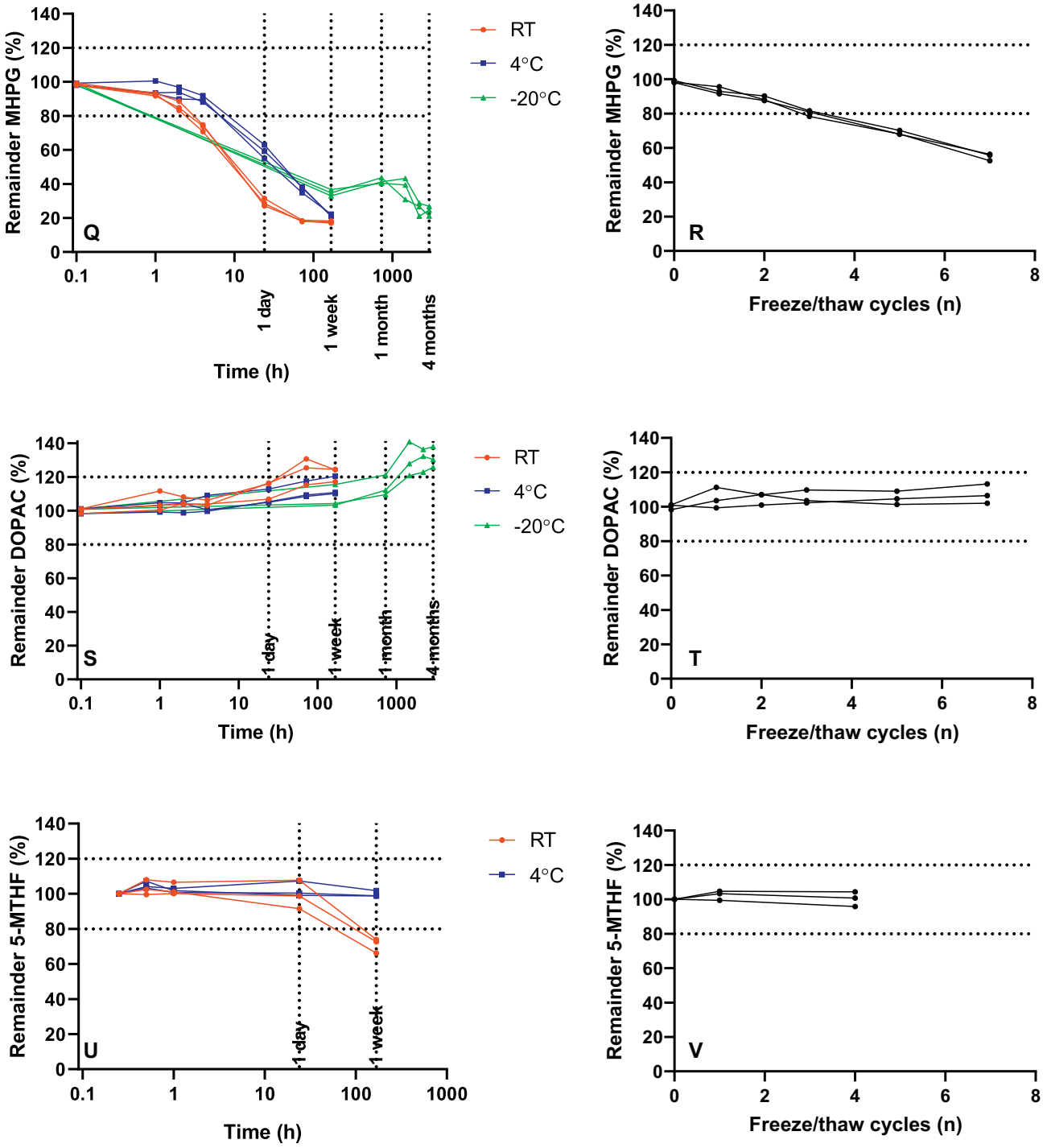

Fig. 1. (*continued*)

2.2.7. Monoamine metabolites

The principal metabolites of noradrenaline, dopamine and serotonin, i.e. MHPG, DOPAC and HVA, and, 5-HIAA, respectively, were determined using a reversed phase ultra-high pressure liquid chromatography system coupled with electrochemical detection (RP-UHPLC-ECD) (Alexys™ Neurotransmitter Analyzer, Antec Leyden, Zoeterwoude, Netherlands). The previously validated and optimized method was applied [25] with modifications regarding the installed column (BEH C18 Waters column, 150 mm × 1 mm, 1.7 µm particle size), pump preference (LC110S pump, 487 bar; isocratic flow rate of 75 µL/min) and mobile phase composition (11% MeOH, 100 mM citric and phosphoric acid, 2.8 mM octane-1-sulfonic acid sodium salt; pH 3.0). To remove excess proteins, pre-column purification of CSF samples was conducted using Amicon® Ultra 0.5 Centrifugal Filters (cut-off 3000 Da, Millipore, Ireland; 14,000 x g, 40 min, 4 °C) [17], after which samples were injected. Intra- and inter-assay precisions were reported < 3% [25].

2.2.8. 5-MTHF

5-MTHF, a metabolite of folic acid, was quantified through a compound-specific redox reaction after separation of chemical compounds in CSF using RP-HPLC-ECD [26]. This method was established with an intra-assay variation of 4.2% and an inter-assay variation of 5.7% (Metabolic Laboratory, Dept, of Clinical Chemistry, VUmc).

2.3. Validation of the stability SOP

The laboratories at the University of Antwerp and at the University Miguel Hernández prepared and tested their own CSF stability sets next to the Amsterdam UMC-prepared samples according to the standardised guidelines [27] and stability SOP [6]. This was to validate the sample preparation, exposures, and part of the findings.

2.4. Data analyses

Biomarker concentrations were normalised to the value of the reference sample ($t = 0$), and 95% confidence intervals (CIs) of the mean per time point, per temperature were reported. Markers for which the CI includes 100% were considered stable for that time point; markers for which the CI did not include 100% were considered unstable.

3. Results

The absolute concentrations of the reference samples were comparable to previously reported concentration ranges for all biomarkers as summarized in Table S1 [14,15,17,22–24,26,28,29].

3.1. Storage stability of potential biomarkers

When we analysed storage stability over 1 week storage at 4 °C or RT, or 1 month at −20 °C compared to the reference sample stored at −80 °C, levels of neurogranin, YKL-40, theobromine, SPARCL-1, BACE1, AChE and HVA did not change (Fig. 1A, C, E, G, I, K, M). Similarly, levels of these seven biomarkers were not changed after up to 7 freeze/thaw cycles (Fig. 1B, D, F, H, J, L, N).

5-HIAA levels did not change after 1 week at 4 °C, 3 days at RT, or 2 months at −20 °C, but started decreasing after longer periods at RT or − 20 °C storage (Fig. 1O). After 1 week storage at RT levels decreased with −16%, after 4 months storage at −20 °C levels decreased with −18%. 5-HIAA levels did not change after 7 freeze/thaw cycles (Fig. 1P).

In contrast, MHPG levels steadily decreased with −10% after 4 h at 4 °C, and, −27% after 4 h at RT, whereas at −20 °C storage for 1 month, a larger reduction of −58% was observed (Fig. 1Q). Similarly, after 7 freeze/thaw cycles, MHPG steadily decreased with an average of −6.4% per freeze/thaw cycle (Fig. 1R).

In addition, DOPAC levels slightly increased with + 8% after 24 h storage at 4 °C, and with + 13% after 24 h at RT, accumulating to + 14% after 1 week storage at 4 °C, and, + 22% after one week storage at RT (Fig. 1S). At −20 °C storage, DOPAC levels similarly increased after one week, starting from + 8% up to + 30% after 2 months, where DOPAC levels reached a plateau (+ 31% after 3 and 4 months storage at −20 °C). No change in DOPAC levels after 7 freeze/thaw cycles was observed (Fig. 1T).

5-MTHF acid levels remained unaltered after 1 week of storage at 4 °C, but showed a steep decline so that, after 1 week at RT, only 70% of the initial 5-MTHF concentration was detected (Fig. 1U). 5-MTHF levels were not changed after 4 freeze/thaw cycles (Fig. 1V).

3.2. Validation of the stability SOP

To validate the sample preparation and exposures according to the SOP at Amsterdam UMC and to validate part of the findings, the laboratories at the University of Antwerp and the University Miguel Hernández prepared and tested their own CSF stability sets next to the VUmc-prepared samples. In these sets, again, no changes were observed in neurogranin, BACE1, or AChE activity levels when CSF was exposed to up to 1 week of storage at 4 °C or RT, or, 1 month at −20 °C (supplementary material).

4. Discussion

We here gained insight into the storage stability of several CSF proteins and metabolites that are currently relevant and evaluated as potential novel biomarkers for various neurological diseases. Under the pre-analytical conditions tested in the present study, which are extreme compared to common biomarker research practice, the CSF proteins and compounds neurogranin, YKL-40, BACE1, AChE activity, theobromine, SPARCL-1 and HVA were stable to all subjected storage and freeze/thaw conditions. However, a few compounds did not show complete stability: MHPG levels showed a moderate decrease at 4 °C, RT and − 20 °C, as well as upon multiple freeze/thaw cycles. As for 5-MTHF and 5-HIAA, we found decreased, and for DOPAC increased, CSF levels after 1 week of delayed storage time at RT. The standardised duration of laboratory handlings with CSF is recommended to be maximum 2 h at 4 °C between collection and centrifugation, maximum 2 h between centrifugation and freezer storage at −80 °C, and maximum 2 freeze/thaw cycles [27]. Apart from MHPG, none of the markers tested showed any alterations under these recommended pre-analytical conditions.

Some biomarkers that we analysed here have been previously evaluated for their stability. A previous study showed that full length CSF neurogranin levels were stable at 4 °C and − 20 °C for one week, while after 2 days of storage at RT a decrease of 20% was observed [30], which contrasts with our results. Our finding, i.e. no decline in truncated neurogranin levels at any temperature, could be explained by the use of a different assay that targets the P75 truncation of neurogranin specifically. Surprisingly, others reported slightly reduced levels of truncated P75 neurogranin (on average − 3.2%) already after one freeze/thaw cycle [31], although this small change remains within the intra-assay variation. YKL-40 levels were previously reported to be stable in CSF after short-term (< 2 h) storage at RT [32], which is in agreement with the stable YLK-40 levels we observed. AChE activity levels were previously found to be unchanged during repeated freeze/thaw cycles [33], similarly to results from the current study. In addition, 5-HIAA and HVA levels were previously published to be stable under delayed storage conditions up to 3 days [34,35]. Again, this is in line with our results, which extended the findings for delayed storage from 3 days to one week at RT, thereby showing stable levels of HVA, but a 16% decrease in 5-HIAA levels. Overall, this study expands the current knowledge on CSF stability, as we examined many storage time intervals at regular intervals, and a high number of freeze/thaw cycles.

Moreover, these conditions were measured within the same CSF pools, thus examining all conditions in parallel.

Remarkably, MHPG levels have previously been found to be stable for up to 72 h at RT using HPLC-ECD [34], while we detected a substantial decrease of MHPG levels after 4 h at RT already. No other studies so far investigated the stability of MHPG in CSF, however, its overall low (patho)physiological concentration in CSF and very early elution of the column (ca. 2 mins; polar compound) make MHPG a rather difficult metabolite to detect [36] (own observations (YV)). On the contrary, the chromatographic method used in the current study has been thoroughly optimized for specific MHPG detection, amongst others by sample pre-purification to remove the MHPG bound to the sulphate conjugate, in combination with optimal column length, mobile phase and detector settings ($E_{1/2}$ detection potential of 670 mV). These methodological differences could underlie the conflicting results. Also, the study of Langlais et al. [34] analysed a mere 6 CSF samples. Studies on CSF stability of theobromine, SPARCL-1, BACE1, DOPAC, and 5-MTHF have not been previously reported.

The proteins tested comprised of various chemical structures. In this study, we found no evidence for instability of specific chemical structures resulting from specific pre-analytical conditions. Apart from the metabolite MHPG, no effective instability was observed for any of the proteins or metabolites tested. Previous studies support our findings, as no effect of long-term (2–14 years) biobank storage on several CSF biomarker concentrations was shown [37], neither did evaporation occur after > 4 years of biobank storage [41]. Adsorption to lab plastics, however, significantly decreased levels of CSF Aβ42 [38–40], emphasising that the effect of pre-analytical conditions is crucial to consider during biomarker development. We encourage the use of the currently presented standardised approach for testing the effect of pre-analytical processing conditions on levels of novel biomarkers, to serve as a solid basis for future biomarker validation tests.

The major strength of this study is the standardised approach of sample preparation that was used. To validate the sample preparation according to the SOP [6] at Amsterdam UMC, the laboratories at the University of Antwerp and the University Miguel Hernández also prepared and tested their own CSF stability samples ($n = 3$ at each centre). These samples gave similar results as the sets prepared at the Amsterdam UMC, indicating that the SOP can be successfully applied in other laboratories.

This study has nonetheless a few limitations. First, to prepare the stability sets according to the SOP, CSF pools were formed from biobanked CSF. This required one freeze/thaw cycle before actual exposure to the experimental storage conditions. Therefore, protein changes that might have occurred during long-term storage at −80 °C or during the first freeze/thawing of the CSF could not be assessed. Second, the protein stabilities described in this study are limited to the specific (bio)chemistry of the used assays and cannot be extrapolated to other measurement platforms. Stability testing may thus still be required for novel methods, even if the protein had already shown stability with other techniques. Third, stability testing results depend on the characteristics of the assay, i.e., low detection levels in CSF and high variation coefficients decrease the power of stability testing. Some stability results had to be excluded from this study for that reason.

In conclusion, levels of potential novel CSF biomarkers appeared resistant to common experimental storage and freeze/thaw conditions, except for persistent – but slight – decreases of MHPG. It is, therefore, recommended to always store CSF samples for MHPG analyses almost instantaneously following lumbar puncture at −80 °C or in liquid nitrogen, and reduce the number of pre-analytical sample preparation steps (at 4 °C) before analysis. The influence of the tested different pre-analytical storage procedures in biomarker studies that use historical cohorts or cohorts in multicentre studies is thus likely trivial, but should be confirmed by systematic stability testing for each novel biomarker or novel test platform.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.07.024.

References

[1] M. Plebani, Errors in clinical laboratories or errors in laboratory medicine? Clin. Chem. Lab. Med. 44 (2006) 750–759, https://doi.org/10.1515/CCLM.2006.123.
[2] S.J.B. Vos, P.J. Visser, F. Verhey, P. Aalten, D. Knol, I. Ramakers, P. Scheltens, M.G.M.O. Rikkert, M.M. Verbeek, C.E. Teunissen, Variability of CSF Alzheimer's disease biomarkers: implications for clinical practice, PLoS One 9 (2014) e100784, , https://doi.org/10.1371/journal.pone.0100784.
[3] N. Le Bastard, P.P. De Deyn, S. Engelborghs, Importance and impact of Preanalytical variables on Alzheimer disease biomarker concentrations in cerebrospinal fluid, Clin. Chem. 61 (2015) 734–743, https://doi.org/10.1373/clinchem.2014.236679.
[4] M. Bjerke, E. Portelius, L. Minthon, A. Wallin, H. Anckarsäter, R. Anckarsäter, N. Andreasen, H. Zetterberg, U. Andreasson, K. Blennow, Confounding factors influencing amyloid Beta concentration in cerebrospinal fluid, Int. J. Alzheimers Dis. 2010 (2010) 1–12, https://doi.org/10.4061/2010/986310.
[5] M. del Campo, B. Mollenhauer, A. Bertolotto, S. Engelborghs, H. Hampel, A.H. Simonsen, E. Kapaki, N. Kruse, N. Le Bastard, S. Lehmann, J.L. Molinuevo, L. Parnetti, A. Perret-Liaudet, J. Sáez-Valero, E. Saka, A. Urbani, E. Vanmechelen, M. Verbeek, P.J. Visser, C. Teunissen, Recommendations to standardize pre-analytical confounding factors in Alzheimer's and Parkinson's disease cerebrospinal fluid biomarkers: an update, Biomark. Med 6 (2012) 419–430, https://doi.org/10.2217/bmm.12.46.
[6] U. Andreasson, A. Perret-Liaudet, L.J.C. van Waalwijk van Doorn, K. Blennow, D. Chiasserini, S. Engelborghs, T. Fladby, S. Genc, N. Kruse, H.B. Kuiperij, L. Kulic, P. Lewczuk, B. Mollenhauer, B. Mroczko, L. Parnetti, E. Vanmechelen, M.M. Verbeek, B. Winblad, H. Zetterberg, M. Koel-Simmelink, C.E. Teunissen, A practical guide to immunoassay method validation, Front. Neurol. 6 (2015) 179, https://doi.org/10.3389/fneur.2015.00179.
[7] S. Ostrowitzki, R.A. Lasser, E. Dorflinger, P. Scheltens, F. Barkhof, T. Nikolcheva, E. Ashford, S. Retout, C. Hofmann, P. Delmar, G. Klein, M. Andjelkovic, B. Dubois, M. Boada, K. Blennow, L. Santarelli, P. Fontoura, SCarlet RoAD investigators, a phase III randomized trial of gantenerumab in prodromal Alzheimer's disease, Alzheimers Res. Ther. 9 (2017) 95, https://doi.org/10.1186/s13195-017-0318-y.
[8] H. Zetterberg, K. Blennow, Neurogranin levels in cerebrospinal fluid: a new addition to the Alzheimer disease diagnostic toolbox, JAMA Neurol. 72 (2015) 1237–1238, https://doi.org/10.1001/jamaneurol.2015.2075.
[9] M. Timmers, S. Barão, B. Van Broeck, I. Tesseur, J. Slemmon, K. De Waepenaert, J. Bogert, L.M. Shaw, S. Engelborghs, D. Moechars, M. Mercken, L. Van Nueten, L. Tritsmans, B. de Strooper, J.R. Streffer, BACE1 dynamics upon inhibition with a BACE inhibitor and correlation to downstream Alzheimer's disease markers in elderly healthy participants, J. Alzheimers Dis. 56 (2017) 1437–1449, https://doi.org/10.3233/JAD-160829.

[10] M.-S. García-Ayllón, D.H. Small, J. Avila, J. Sáez-Valero, Revisiting the role of acetylcholinesterase in Alzheimer's disease: cross-talk with P-tau and β-amyloid, Front. Mol. Neurosci. 4 (2011) 22, https://doi.org/10.3389/fnmol.2011.00022.
[11] F. Llorens, K. Thüne, W. Tahir, E. Kanata, D. Diaz-Lucena, K. Xanthopoulos, E. Kovatsi, C. Pleschka, P. Garcia-Esparcia, M. Schmitz, D. Ozbay, S. Correia, Â. Correia, I. Milosevic, O. Andréoletti, N. Fernández-Borges, I.M. Vorberg, M. Glatzel, T. Sklaviadis, J.M. Torres, S. Krasemann, R. Sánchez-Valle, I. Ferrer, I. Zerr, YKL-40 in the brain and cerebrospinal fluid of neurodegenerative dementias, Mol. Neurodegener. 12 (2017) 83, https://doi.org/10.1186/s13024-017-0226-4.
[12] C. Malmeström, M. Axelsson, J. Lycke, H. Zetterberg, K. Blennow, B. Olsson, CSF levels of YKL-40 are increased in MS and replaces with immunosuppressive treatment, J. Neuroimmunol. 269 (2014) 87–89, https://doi.org/10.1016/j.jneuroim.2014.02.004.
[13] K.T. Sachse, E.K. Jackson, S.R. Wisniewski, D.G. Gillespie, A.M. Puccio, R.S. Clark, C.E. Dixon, P.M. Kochanek, Increases in cerebrospinal fluid caffeine concentration are associated with Favorable outcome after severe traumatic brain injury in humans, J. Cereb. Blood Flow Metab. 28 (2008) 395–401, https://doi.org/10.1038/sj.jcbfm.9600539.
[14] M. Travassos, I. Santana, I. Baldeiras, M. Tsolaki, O. Gkatzima, G. Sermin, G.G. Yener, A. Simonsen, S.G. Hasselbalch, E. Kapaki, B. Mara, R.A. Cunha, P. Agostinho, K. Blennow, H. Zetterberg, V.M. Mendes, B. Manadas, A. de Mendon, Does caffeine consumption modify cerebrospinal fluid amyloid-β levels in patients with Alzheimer's disease? J. Alzheimers Dis. 47 (2015) 1069–1078, https://doi.org/10.3233/JAD-150374.
[15] C. Bridel, M.J.A. Koel-Simmelink, L. Peferoen, C. Derada Troletti, S. Durieux, R. Gorter, E. Nutma, P. Gami, E. Iacobeus, L. Brundin, J. Kuhle, H. Vrenken, J. Killestein, S.R. Piersma, T.V. Pham, H.E. De Vries, S. Amor, C.R. Jimenez, C.E. Teunissen, Brain endothelial cell expression of SPARCL-1 is specific to chronic multiple sclerosis lesions and is regulated by inflammatory mediators in vitro, Neuropathol. Appl. Neurobiol. 44 (2018) 404–416, https://doi.org/10.1111/nan.12412.
[16] B. Vafadar-Isfahani, G. Ball, C. Coveney, C. Lemetre, D. Boocock, L. Minthon, O. Hansson, A.K. Miles, S.M. Janciauskiene, D. Warden, A.D. Smith, G. Wilcock, N. Kalsheker, R. Rees, B. Matharoo-Ball, K. Morgan, Identification of SPARC-like 1 protein as part of a biomarker panel for Alzheimer's disease in cerebrospinal fluid, J. Alzheimers Dis. 28 (2012) 625–636, https://doi.org/10.3233/JAD-2011-111505.
[17] J. Janssens, Y. Vermeiren, E. Fransen, T. Aerts, D. Van Dam, S. Engelborghs, P.P. De Deyn, CSF and serum MHPG improve Alzheimer's disease versus dementia with Lewy bodies differential diagnosis, Alzheimers Dement. 10 (2018) 172–181, https://doi.org/10.1016/j.dadm.2018.01.002.
[18] M.K. Herbert, M.B. Aerts, H.B. Kuiperij, J.A.H.R. Claassen, P.E. Spies, R.A.J. Esselink, B.R. Bloem, M.M. Verbeek, Addition of MHPG to Alzheimer's disease biomarkers improves differentiation of dementia with Lewy bodies from Alzheimer's disease but not other dementias, Alzheimers Dement. 10 (2014) 448–455, https://doi.org/10.1016/j.jalz.2013.05.1775.
[19] C. Mulder, N.S.M. Schoonenboom, E.E.W. Jansen, N.M. Verhoeven, G.J. van Kamp, C. Jakobs, P. Scheltens, The transmethylation cycle in the brain of Alzheimer patients, Neurosci. Lett. 386 (2005) 69–71, https://doi.org/10.1016/j.neulet.2005.03.073.
[20] W.M. van der Flier, Y.A.L. Pijnenburg, N. Prins, A.W. Lemstra, F.H. Bouwman, C.E. Teunissen, B.N.M. van Berckel, C.J. Stam, F. Barkhof, P.J. Visser, E. van Egmond, P. Scheltens, Optimizing patient care and research: the Amsterdam dementia cohort, J. Alzheimers Dis. 41 (2014) 313–327, https://doi.org/10.3233/JAD-132306.
[21] A. De Vos, D. Jacobs, H. Struyfs, E. Fransen, K. Andersson, E. Portelius, U. Andreasson, D. De Surgeloose, D. Hernalsteen, K. Sleegers, C. Robberecht, C. Van Broeckhoven, H. Zetterberg, K. Blennow, S. Engelborghs, E. Vanmechelen, C-terminal neurogranin is increased in cerebrospinal fluid but unchanged in plasma in Alzheimer's disease, Alzheimers Dement. 11 (2015) 1461–1469, https://doi.org/10.1016/j.jalz.2015.05.012.
[22] K. Hellwig, H. Kvartsberg, E. Portelius, U. Andreasson, T.J. Oberstein, P. Lewczuk, K. Blennow, J. Kornhuber, J.M. Maler, H. Zetterberg, P. Spitzer, Neurogranin and YKL-40: independent markers of synaptic degeneration and neuroinflammation in Alzheimer's disease, Alzheimers Res. Ther. 7 (2015) 74, https://doi.org/10.1186/s13195-015-0161-y.
[23] A. De Vos, H. Struyfs, D. Jacobs, E. Fransen, T. Klewansky, E. De Roeck, C. Robberecht, C. Van Broeckhoven, C. Duyckaerts, S. Engelborghs, E. Vanmechelen, The cerebrospinal fluid Neurogranin/BACE1 ratio is a potential correlate of cognitive decline in Alzheimer's disease, J. Alzheimers Dis. 53 (2016) 1523–1538, https://doi.org/10.3233/JAD-160227.
[24] M.-S. García-Ayllón, M.-X. Silveyra, N. Andreasen, S. Brimijoin, K. Blennow, J. Sáez-Valero, Cerebrospinal fluid acetylcholinesterase changes after treatment with donepezil in patients with Alzheimer?S disease, J. Neurochem. 101 (2007) 1701–1711, https://doi.org/10.1111/j.1471-4159.2007.04461.x.
[25] D. Van Dam, Y. Vermeiren, T. Aerts, P.P. De Deyn, Novel and sensitive reversed-phase high-pressure liquid chromatography method with electrochemical detection for the simultaneous and fast determination of eight biogenic amines and metabolites in human brain tissue, J. Chromatogr. A 1353 (2014) 28–39, https://doi.org/10.1016/j.chroma.2014.05.004.
[26] K. Hyland, R. Surtees, Measurement of 5-methyltetrahydrofolate in cerebrospinal fluid using HPLC with coulorimetric electrochemical detection, Pteridines. 3 (1991) 149, https://doi.org/10.1515/pteridines.1991.3.12.149.
[27] C.E. Teunissen, A. Petzold, J.L. Bennett, F.S. Berven, L. Brundin, M. Comabella, D. Franciotta, J.L. Frederiksen, J.O. Fleming, R. Furlan, R.Q. Hintzen, S.G. Hughes MD, M.H. Johnson, E. Krasulova, J. Kuhle, M.C. Magnone, C. Rajda, K. Rejdak, H.K. Schmidt, V. van Pesch, E. Waubant, C. Wolf, G. Giovannoni, B. Hemmer, H. Tumani, F. Deisenhammer, A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking, Neurology. 73 (2009) 1914–1922, https://doi.org/10.1212/WNL.0b013e3181c47cc2.
[28] E.A.J. Willemse, A. De Vos, E.M. Herries, U. Andreasson, S. Engelborghs, W.M. van der Flier, P. Scheltens, D. Crimmins, J.H. Ladenson, E. Vanmechelen, H. Zetterberg, A.M. Fagan, K. Blennow, M. Bjerke, C.E. Teunissen, Neurogranin as cerebrospinal fluid biomarker for Alzheimer disease: an assay comparison study, Clin. Chem. (2018), https://doi.org/10.1373/clinchem.2017.283028 clinchem.2017.283028.
[29] C.G. Schipke, A. De Vos, M. Fuentes, D. Jacobs, E. Vanmechelen, O. Peters, Neurogranin and BACE1 in CSF as potential biomarkers differentiating depression with cognitive deficits from early Alzheimer's disease: a pilot study, Dement. Geriatr. Cogn. Dis. Extra. 8 (2018) 277–289, https://doi.org/10.1159/000489847.
[30] H. Kvartsberg, E. Portelius, U. Andreasson, G. Brinkmalm, K. Hellwig, N. Lelental, J. Kornhuber, O. Hansson, L. Minthon, P. Spitzer, J.M. Maler, H. Zetterberg, K. Blennow, P. Lewczuk, Characterization of the postsynaptic protein neurogranin in paired cerebrospinal fluid and plasma samples from Alzheimer's disease patients and healthy controls, Alzheimers Res. Ther. 7 (2015) 40, https://doi.org/10.1186/s13195-015-0124-3.
[31] H. Vanderstichele, L. Demeyer, S. Janelidze, E. Coart, E. Stoops, K. Mauroo, V. Herbst, C. François, O. Hansson, Recommendations for cerebrospinal fluid collection for the analysis by ELISA of neurogranin trunc P75, α-synuclein, and total tau in combination with Aβ(1–42)/Aβ(1–40), Alzheimers Res. Ther. 9 (2017) 40, https://doi.org/10.1186/s13195-017-0265-7.
[32] C. Østergaard, J.S. Johansen, T. Benfield, P.A. Price, J.D. Lundgren, YKL-40 is elevated in cerebrospinal fluid from patients with purulent meningitis, Clin. Diagn. Lab. Immunol. 9 (2002) 598–604, https://doi.org/10.1128/cdli.9.3.598-604.2002.
[33] S.I. Deutsch, R.C. Mohs, M.I. Levy, A.B. Rothpearl, D. Stockton, T. Horvath, A. Coco, K.L. Davis, Acetylcholinesterase activity in CSF in schizophrenia, depression, Alzheimer's disease and normals, Biol. Psychiatry 18 (1983) 1363–1373 http://www.ncbi.nlm.nih.gov/pubmed/6661467 (accessed September 1, 2017).
[34] P.J. Langlais, E.D. Bird, W.J. McEntee, Stability of monoamine metabolites in human cerebrospinal fluid, Ann. Neurol. 12 (1982) 48–51, https://doi.org/10.1002/ana.410120109.
[35] J.R. Strawn, N.N. Ekhator, T.D. Geracioti, In-use stability of monoamine metabolites in human cerebrospinal fluid, J. Chromatogr. B Biomed. Sci. Appl. 760 (2001) 301–306 http://www.ncbi.nlm.nih.gov/pubmed/11530989 accessed August 31, 2017.
[36] K. Hyland, Clinical utility of monoamine neurotransmitter metabolite analysis in cerebrospinal fluid, Clin. Chem. 54 (2008) 633–641 http://clinchem.aaccjnls.org/content/54/4/633.long (accessed September 1, 2017).
[37] E.A.J. Willemse, K.W.J. van Uffelen, W.M. van der Flier, C.E. Teunissen, Effect of long-term storage in biobanks on cerebrospinal fluid biomarker Aβ 1-42, T-tau, and P-tau values, Alzheimer's dement, Diagnosis, Assess. Dis. Monit. 8 (2017) 45–50, https://doi.org/10.1016/j.dadm.2017.03.005.
[38] E. Willemse, K. van Uffelen, B. Brix, S. Engelborghs, H. Vanderstichele, C. Teunissen, 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 Aβ 42 /Aβ 40 ratio, Alzheimers Dement. 13 (2017) 885–892, https://doi.org/10.1016/j.jalz.2017.01.010.
[39] J. Toombs, R.W. Paterson, J.M. Schott, H. Zetterberg, Amyloid-beta 42 adsorption following serial tube transfer, Alzheimers Res. Ther. 6 (2014) 5, https://doi.org/10.1186/alzrt236.
[40] A. Perret-Liaudet, M. Pelpel, Y. Tholance, B. Dumont, H. Vanderstichele, W. Zorzi, B. ElMoualij, S. Schraen, O. Moreaud, A. Gabelle, E. Thouvenot, J. Thomas-Anterion, P. Catherine Touchon, G.G. Krolak-Salmon, A. Kovacs, I. Coudreuse, S. Lehmann Quadrio, Cerebrospinal fluid collection tubes: a critical issue for Alzheimer disease diagnosis, Clin. Chem. 795 (2012) 787–789.
[41] E.A.J. Willemse, M.J.A. Koel-Simmelink, S. Durieux-Lu, W.M. van der Flier, C.E. Teunissen, Standard biobanking conditions prevent evaporation of body fluid samples, Clin. Chim. Acta 447 (2015) 119–120.