The impact of pre-analytical variables on measuring CSF biomarkers for Alzheimer's disease diagnosis: A review

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Abstract

INTRODUCTION:

Cerebrospinal fluid (CSF) biomarkers have the potential to improve the diagnostic accuracy of Alzheimer's disease. A lack of harmonized pre-analytical CSF-handling protocols accounts for a large proportion of observed variation and limits between-study comparison of CSF biomarker concentrations.

METHODS:

This systematic review summarizes the current literature on the influence of different pre-analytical variables on CSF biomarker concentration. We evaluated the evidence for three core CSF biomarkers: β-Amyloid(1–42), total tau and phosphorylated tau.

RESULTS:

This review highlights where previous literature agrees on the influence of certain variables on CSF biomarkers, and where there is a lack of consensus, or little evidence available.

DISCUSSION:

A unified CSF handling protocol is recommended to reduce pre-analytical variability and facilitate comparison of CSF biomarkers across studies and laboratories.

Keywords: Cerebrospinal fluid; Biomarkers; Pre-analytical variables; Alzheimer's disease diagnosis; β-amyloid 42; Total tau; Phosphorylated tau

Research in context

Systematic review: The PubMed and Cochrane databases were searched between 1995 and March 2017 with specific search terms to identify studies that analyzed the effect of pre-analytical variables on cerebrospinal fluid (CSF) biomarkers of Alzheimer's disease (AD). Relevant publications were identified, screened, and assessed. A total of 49 publications were analysed for the influence of pre-analytical variables and the results are discussed. We also summarize the variations between currently used CSF handling protocols.

Interpretation: This review highlights the consensus in the field on the influence of different pre-analytical variables. It also indicates where there is a lack of consensus or a need for further research.

Future directions: We recommend developing a unified CSF handling protocol; this could reduce the impact of pre-analytical variables on biomarker measurement. This in turn could improve the diagnostic accuracy for AD and enhance clinical trial recruitment.

Highlights

- CSF biomarkers of Alzheimer's disease are influenced by pre-analytical variables.
- Inconsistencies in published evidence on each variable's effect.
- A unified protocol may reduce CSF biomarker variability and improve diagnosis.

1. Introduction

Alzheimer's disease (AD) is thought to be a continuum with three main stages: preclinical (cognitively unimpaired), prodromal (with mild cognitive symptoms), and dementia (more advanced clinical symptoms) [1]. Disease-modifying treatments in development targeted at AD will likely have greatest clinical benefit early in the AD continuum, before neuronal damage is widespread [2,3]. It is challenging to definitively diagnose early AD using clinical criteria alone [1]; however, biomarkers can detect changes in underlying neuropathology when only mild cognitive symptoms are present [4–7], or even at preclinical stages [8–12].

1.1 The potential impact of biomarkers in AD diagnosis and research

A biomarker is an objective measure of a biological or pathological process that can be used to monitor normal physiological processes, or evaluate disease risk or prognosis, to guide clinical diagnosis or to monitor therapeutic interventions [13]. Several physiological changes related to the pathogenesis of AD (such as neuritic plaques, tangles, and synapse loss) have been well documented. These are accompanied by changes in the levels of some molecules, both in the brain and cerebrospinal fluid (CSF), several of which have been suggested as potential biomarkers in the field of AD for specific applications (e.g., diagnosis, treatment follow-up) [14].

Patients with AD have a characteristic profile of altered concentrations of three CSF protein biomarkers: β-Amyloid (Aβ) (1–42), total tau (tTau), and phosphorylated tau (pTau) [15–17]. While these biomarkers may be individually affected by non-AD-related pathologies, for example Aβ(1–42) in subcortical vascular dementia [18], the combination of the three core biomarker changes is known as the AD 'signature' or 'profile' [4,5,19–22].

Aβ(1–42) is the main component of amyloid plaques associated with AD [23–25]. Currently, the only FDA-approved method to detect Aβ deposits within the brain is β-amyloid positron emission tomography (PET) [26]. However, several commercially available assays for measuring Aβ(1–42) (and other core AD biomarkers) in CSF are approved for diagnostic use in the European Union [27–29]. In addition, ~10% of cognitively normal elderly individuals have biomarker evidence of amyloidosis but no cognitive symptoms [30]. Low CSF Aβ(1–42) could be an early indicator of preclinical AD before amyloid deposition rises to levels visible by PET imaging [31].

Brain amyloid pathology is correlated with abnormally low levels of Aβ(1–42) in the CSF [2,32–36]. There is high concordance of CSF Aβ(1–42) with β-amyloid PET status in both AD dementia and prodromal AD [4]. Concordance is further improved by using the ratio of CSF Aβ(1–42)/(1–40) , CSF pTau/Aβ(1–42) or CSF tTau/Aβ(1–42) [16,18,37–40]. Altered levels in the concentration ratio of CSF Aβ(1–42) and Aβ(1–40) are observed in subjects influenced by the apolipoprotein E *(APOE)* ε4 allele [41], a strong genetic risk factor for AD [42].

CSF tTau is increased following neuronal injury or degeneration and is associated with cognitive decline [43,44]. An increase in CSF tTau concentration is characteristic of neurodegenerative disorders such as AD [45], but is also found in acute neuronal injury, for example, ischaemic stroke [46], other tauopathies (e.g., corticobasal syndrome [47]), and very high concentrations are found in disorders with rapid neuronal degeneration, for example Creutzfeldt-Jakob disease (CJD) [48].

Hyperphosphorylated tau is an important component of neurofibrillary tangles, which are a pathologic hallmark of AD [2,14,49]. High CSF pTau correlated with cortical tangle pathology in some studies [50,51], with the exception of Engelborghs, et al.

(2007) [52], while high levels of CSF pTau were found in AD patients (up to 3.4-fold higher than healthy controls) [53]. Studies have found that CSF pTau$_{181P}$ correlated with the amount of cortical amyloid measured via PET imaging [16] or CSF Aβ(1–42)/(1–40) ratio [41]. The inclusion of pTau as a biomarker for AD together with Aβ(1–42) and tTau can help differentiate AD from normal ageing and difficult diagnoses (e.g., Parkinson's disease, CJD, some forms of non-AD dementia), and improve diagnostic performance [54,55]. CSF pTau$_{181P}$ concentration was found to be the most statistically significant single variable of the three for discrimination between AD and dementia with Lewy bodies in one study [54]. While pTau$_{181P}$ is the most studied form, different phosphorylated epitopes, detected using different antibody combinations, may also result in a better separation of AD from non-AD dementia [56,57].

Research guidelines from the National Institute on Aging and the Alzheimer's Association (NIA-AA; [58,59]) and International Working Group (IWG-2; [60]) recommend including core biomarkers in AD diagnostic assessment, while the European Academy of Neurology recommends CSF biomarker assessment to aid AD differentiation [61]. As well as having diagnostic potential, changes in the core AD biomarkers precede cognitive changes and predict clinical progression in patients with mild cognitive impairment (MCI) [5,7,17,62,63], and effectively stratify patients for their risk of developing AD dementia [5,7,20,21,39,64]. Promisingly, these biomarkers also detect pathological changes associated with preclinical AD in cognitively healthy elderly individuals [12,40,65,66], and can enhance both differential diagnosis and prognostic stratification within AD populations.

Accurate, consistent, and reliable biomarker measurement remains a goal for researchers and clinicians alike, but requires consensus to establish universal cut-off

values. However, the significant variability documented in CSF biomarker measurements across research and clinical studies [2,67–69] has hampered these efforts. The translation of cut-off values between clinical sites and studies has been investigated [64,70] but can lead to patient misclassification, which in turn could influence clinical decision-making or clinical trial eligibility.

1.2 Variables affecting CSF biomarker concentration

CSF biomarker concentration can be affected by clinical, analytical, and pre-analytical variables [71–73]. Clinical variables (besides AD and non-AD pathology) include age and *APOE* genotype [30]. Analytical procedures are related to the assay itself, for example, differences in technician skills and training, operating procedures, assay manufacturing or batch-to-batch variations in kits [74]. Considerable work has been done to standardize CSF biomarker measurement across different assays and laboratories. Notable efforts include: 1) the development of certified reference materials for the CSF biomarkers, currently underway within the International Federation of Clinical Chemistry Working Group for CSF proteins [75], 2) the introduction of reference measurement procedures (RMPs) based on liquid chromatography tandem mass spectrometry for CSF Aβ(1–42) quantification, which were recently formally certified by the Joint Committee for Traceability in Laboratory Medicine (C11RMP9 and C12RMP1, respectively) [76,77], and 3) the establishment of quality control programs for monitoring between-laboratory and between-batch variability of commercially available immunoassays [28,72,78–82].

Pre-analytical variables include CSF sampling materials and methodology, CSF handling, and storage procedures (see [71,80] for reviews). Following notable improvements in analytical methods, recent studies have revealed that pre-analytical factors account for a considerable proportion of the total variability observed in

biomarker concentrations [71,83]. Thus, there is a growing view that standardization of selected pre-analytical factors might well significantly reduce this variability, improve biomarker diagnostic accuracy and encourage greater inclusion of CSF biomarker testing in both clinical research and routine clinical practice.

1.3 Rationale for this systematic review

Standardization of key analytical issues is increasingly being embraced by the AD community. In contrast, clinics and diagnostic laboratories have commonly developed their own site-specific protocols for the pre-analytical handling of CSF [14,80]. While some centres have published their protocols, the majority follow a small number of published protocols or recommendations, while a variety of adjustments to these protocols also have been published [35,71,80,84–87]. In addition, many unpublished adjustments to pre-analytical protocols have been adopted (Fagan A, personal observation). Where differences in pre-analytical procedures exist, direct comparison of CSF biomarker data between groups is difficult or biased, albeit possible [88]. As new studies are published, there is a need to update protocols and work towards their subsequent global implementation.

2. Aim

This review has two aims: 1) to compare current pre-analytical recommendations and protocols for handling CSF samples prior to measurements of the core AD biomarkers of Aβ(1–42), pTau and tTau and 2) to summarize the effects of pre-analytical variables on core AD CSF biomarker concentration based on a systematic literature review.

3. Methods

In accordance with PRISMA guidelines, we conducted a systematic review of the PubMed (comprising citations from MEDLINE, life science journals and online books) and Cochrane Library databases from 1995 to March 2017 with five sets of search terms to identify studies that analyzed the effect of pre-analytical variables on AD CSF biomarkers. In addition, we examined reference lists of reviews and selected original research articles for relevant studies; the authors also suggested additional relevant publications. The PubMed search strategy was designed to select studies with pre-analytical variables of Aβ(1–42), pTau, or tTau concentration in CSF (Appendix A). Duplicates within searches were removed by a reviewer and abstracts were screened using predefined criteria:

- eligible studies described AD CSF biomarker measurements when one or more pre-analytical variable(s) were investigated
- studies were excluded if 1) they did not collect and analyze CSF, 2) did not specifically include or mention Aβ(1–42), pTau or tTau, 3) steps investigated referred to analytical rather than pre-analytical variables, 4) were not in English, 5) were not based on humans, 6) were conference abstracts only, or 7) were review articles.

Studies examining the effect of pre-analytical variables were evaluated and evidence that a variable had no effect, or altered concentration, was extracted and summarized.

4. Results

4.1. Comparison of pre-analytical protocols for CSF measurements of core AD biomarkers

We identified six pre-analytical protocols used in multicentre, international studies including AD biomarker measurements (Table 1). Several steps were different and these steps were compared in Table 1. Only the recommended location of sampling (LP) and storage temperature was the same in all protocols examined. In contrast, recommendations for LP needle size, collection volume, and centrifugation steps varied.

4.2. Summary of pre-analytical variable effects on CSF measurements of core AD biomarkers

The systematic review identified 593 articles; 211 duplicates were removed and 49 studies were assessed (Fig. 1). The evidence for the influence of CSF sampling procedure (Table 2), CSF storage conditions (Table 3) and CSF treatment conditions (Table 4) on core AD biomarker concentration (see Fig. 2) are discussed in more detail below.

4.2.1. The influence of sampling procedure on CSF biomarker concentration

Several variables within the process of taking CSF samples from individuals could affect biomarker concentration. These include the conditions immediately prior and during the sampling procedure, for example, the timing, method (e.g., needle type) and the technique used.

4.2.2 Timing of CSF sampling

Most current protocols recommend a time window to draw CSF for Aβ(1–42) measurements (Table 1). Despite this recommendation, not all studies assessed describe significant differences in CSF biomarker concentration across a 24-hour period [89,90]. There were several reports of significant changes in CSF Aβ(1–42) concentration over a number of hours (Table 2). CSF samples collected hourly in 15 healthy individuals revealed a significant (up to 4-fold) change in Aβ(1–42) levels, with increasing concentrations over 36 hours [91]. Huang, et al. (2012) also described a longitudinal linear rise in CSF Aβ(1–42) concentration over 36 hours in young (18–60 years old) control individuals, in addition to a circadian-like sinusoidal pattern. The amplitude of diurnal change in CSF Aβ(1–42) concentration decreased with age, while hourly dynamics and linear rise were attenuated in individuals with known amyloid deposition [92]. A recent report using pooled data from several sites also found significant diurnal variation in Aβ(1–42) levels [93], with a gradual increase in concentration in the first 12 hours (up to 200% higher from initial baseline draw), which did not return to baseline levels after 24 hours. Samples from indwelling catheters used in this study derived from individuals who participated in clinical research studies and were given placebo treatment [93]. This study comprised the highest sample number examining this variable to date (n = 178). All other studies examined had relatively small sample sizes (n = 10–15).

It is notable that, in these studies, CSF was repeatedly sampled via indwelling lumbar sac catheterization. Therefore, the frequency of CSF sampling and aspects of the procedural technique may have influenced the interpretation of diurnal variation. All groups employing this approach noted a steady increase in CSF Aβ levels over the first few hours. Materials in the catheter (e.g., the presence of a

bacterial filter), sampling frequency and the volume of CSF being withdrawn (especially nearer the first part of the sampling period) may have influenced the results of studies investigating CSF concentration changes over short periods of time [93–96]. Moreover, the initial sampling may leave a hole in the dura mater allowing CSF to leak in to the surrounding tissues; this could change the CSF circulation dynamics, thereby altering CSF Aβ(1–42) concentration.

The limited data available on diurnal variability of CSF tTau or pTau did not generally reveal any significant diurnal change for tTau [89,90], with a linear increase that was reported by Slats, et al. (2012) attributed to a methodological artefact [95]. This same study was the only one to report a steady increase in pTau and a circadian-like fluctuation (over 24 hours using a cosinor fit, a fluctuation of 2.5 pg/mL for individuals with AD and 1.4 pg/mL for the control group was seen, n = 6, $P < .005$) [95].

In summary, the evidence for diurnal variability of CSF Aβ(1–42) and pTau concentration is inconclusive. However, differences in observed diurnal variability of CSF Aβ(1–42) between studies may be dependent on CSF sampling procedure (e.g., catheterization), age and/or, the presence of amyloid pathology. More accurate assays with increased sensitivity in future may aid the resolution of this open question. In addition, it would be useful to compare CSF collected at different time points in the same individuals over several weeks.

4.2.3. Location and volume of CSF sampling

CSF can be sampled from the lumbar region of the spine or the cerebral ventricles/cisternae via a shunt. AD biomarker protocols universally recommended sampling to be performed by LP at the level of L3/4 or L4/L5, i.e., a level safely below the spinal cord (supported by recent consensus guidelines [97]) (Table 1).

Collection volumes in these protocols vary from ≥ 1.5 mL to 20 mL (Table 1). CSF biomarkers for other neurodegenerative disease (e.g., α-synuclein as a biomarker in Parkinson's disease) may vary along the rostro-caudal gradient [98,99]. If large volumes of CSF are obtained via the lumbar sac, CSF will be in effect drawn from more rostral reaches of the spinal canal and so any rostro-caudal gradient in biomarker concentration would have implications for interpreting results. However, we found limited evidence in the literature that CSF AD biomarker concentrations are gradient dependent. No significant change in the concentration of CSF Aβ(1–42) was observed along the rostro-caudal gradient, when analyzing concentrations in different portions of large CSF volume samples [73,100]. This included a comparison of CSF biomarker concentration between four successive 10 mL volumes [73]. Volumes larger than 50 mL may lead to a slight increase in Aβ(1–42) concentration (though this effect is below 5–10% [Hansson O, unpublished observation]); however, such excessive volumes are not relevant in most clinical settings.

The majority of studies examined in this review also found no significant difference between CSF Aβ(1–42) concentration from lumbar or ventricular locations [90,101,102]. However, a study of 15 patients with normal pressure hydrocephalus (NPH) reported higher concentration of CSF Aβ(1–42) from lumbar versus ventricular catheter samples [103]. Conversely, higher pTau concentrations were noted in ventricular versus lumbar CSF from patients with NPH, in agreement with Djukic, et al. [102]. Similar findings were observed for tTau [90,103].

Available evidence indicates that there is no significant rostro-caudal effect on CSF Aβ(1–42) concentrations with the volumes of CSF collected routinely for biomarker samples. Further evidence may be required to determine rostro-caudal gradient

changes in tTau and pTau in control populations. The rostro-caudal gradient of the AD biomarkers may also depend on the patient's underlying medical conditions.

4.2.4. Type of puncture needle

In this review, we did not identify any studies directly comparing the effect of needle type on CSF AD biomarkers, probably due to the technical difficulties in varying this factor objectively. However, current protocols all recommend an atraumatic (pen-point) needle, although with different sized gauges (22, 24 or 25 G; Table 1). A study found that conventional needles (compared with atraumatic ones) led to minor serum protein contamination of CSF [104], though this may not affect AD CSF biomarker concentrations (see section 4.2.6).

As we have found no evidence of an effect of puncture needle on core AD CSF biomarker concentration, the current protocol recommendations of needle type may relate more to prevention of patient side effects. For example, in multicentre studies, atraumatic needles prevented post-LP complaints such as back pain and headache. A larger needle diameter was associated with severe headache [105], whereas smaller 24 gauge Sprotte needles were associated with a low incidence of post-LP headache [106].

4.2.5. Collection method

Most current protocols do not specify a drainage method, though the Alzheimer's Disease Neuroimaging Initiative (ADNI) and BioFINDER protocols recommend gravity drip to allow CSF to flow freely (Table 1). It is well recognized that different tube materials can significantly affect CSF AD biomarker concentration (see below), which led to a suggestion that aspirating CSF in plastic syringes could cause a significant decrease in Aβ(1–42) concentration compared with gravity drip.

Conversely, use of a syringe could enable faster collections and direct aliquoting, which could help minimize CSF Aβ(1–42) loss through adsorption. A direct comparison of the two methods in a cohort of 54 study participants (38 healthy controls, eight with MCI and eight with AD) found no significant differences in CSF Aβ(1–42), pTau, or tTau concentration [107]. In this study, aspiration samples were taken following sequential gravity drip samples, and so could retain possible gradient effects on concentration.

CSF may also be collected through a catheter (first used to measure the CSF pressure), and a comparison of two catheter types found no difference in CSF Aβ(1–42) concentration [73]. Another study found that manometer use significantly reduced (by 4–6%) CSF Aβ(1–38), Aβ(1–40) and Aβ(1–42) concentrations [108]. As mentioned, this may be a result of tube or pipette material adsorption (see section 4.2.1). Overall, there are few studies that have directly compared drainage methods and no conclusive evidence of an effect of collection method on core CSF AD biomarker concentration has been reported. It would be very challenging to directly compare this parameter in more detail without potential confounders.

4.2.6. Blood contamination

Blood contamination of CSF from tissue trauma has been reported to occur in ~15% of LPs [109], but is most often minor (Blennow K, personal observation). Proteins, including albumin and proteases present in blood, may bind or degrade Aβ(1–42), [110,111], while blood cells themselves contain significant amounts of Aβ(1–42) [112]. Many of these proteins are naturally occurring in the CSF and may be even more abundant if the blood-CSF barrier is impaired (e.g., with acute meningitis) or when the CSF samples are contaminated with blood during the collection process. Accordingly, CSF collection protocols consistently recommend discarding the initial

1–2 mL of CSF if blood contamination is noted based on visual assessment, or if the CSF sample contains > 50 erythrocytes per µL (Table 1). However, there is conflicting evidence on whether plasma proteins can affect biomarker concentration. For example, two studies found no significant effect of the addition of albumin at the time of sample incubation [113] or minimal blood contamination [73] on Aβ(1–42) concentration. Conversely, CSF Aβ(1–42) concentration in samples spiked with plasma (corresponding to a CSF/serum albumin ratio of 55; Table 2) was significantly reduced up to 49% [73]. It should be noted that CSF/serum albumin ratios in this range are not found in AD cases or other chronic neurodegenerative disorders. The opposite effect was seen by Leitao, et al. (2015), who spiked samples with 5000 erythrocytes/µL and found a small but significant increase in both CSF Aβ(1–42) and pTau concentration (6 and 11%, respectively) [114]. Experimental design may therefore explain some of these conflicting results. Red blood cell contamination may be more critical for the concentration of biomarkers for other neurological conditions, such as Parkinson's disease, than the core AD biomarkers [115,116].

Taken together, these studies suggest that blood contamination may affect core AD biomarker concentration and that grossly contaminated samples should be discarded. However, further research may be needed to determine acceptable contamination levels.

4.3. The influence of CSF storage on biomarker concentration

The steps between performing a LP and measuring CSF biomarker concentration may introduce variability. These include the types and size of pipettes and tubes for transferring and processing samples, the CSF volume-to-surface ratio, and the temperature at which samples are held.

4.3.1. Tube material

Many published studies examined tube material and the majority showed that different tubes adsorb CSF biomarkers to varying degrees. CSF samples are often exposed to many tubes, not only collection and storage tubes, but also those used for pooling CSF for certain purposes. Of the three CSF AD biomarkers examined in this review, Aβ(1–42) concentration was affected to the greatest extent, likely due to the hydrophobic nature of the peptide.

The change in CSF biomarker concentration caused by the tube material, or 'tube effect', was shown to happen quickly (< 5 minutes) and was not further affected by long incubation time (up to 48 hours, at 2–8°C [117]). The 'tube effect' on CSF Aβ(1–42) concentration loss was greater when the initial CSF Aβ(1–42) concentration was higher (>1000 pg/mL compared with 400–500 pg/mL [83]).

Standard protocols recommend polypropylene (PP) as a tube material (Table 1); indeed, polystyrene and glass tubes significantly reduced CSF Aβ(1–42) concentration [73,118,119]. However, recent groups have shown that the type of PP - as homo or copolymers - or with additives such as polyethylene copolymers, also significantly altered CSF Aβ(1–42) concentration [117,120–122]. For example, variation of up to 48% was observed among 11 tubes all listed by their manufacturer as PP [117].

Tubes treated to be 'low binding' (LoB) were found to yield significantly greater CSF Aβ(1–42) concentration compared with untreated PP tubes [123,124]. Moreover, the chemical composition of the tube could influence the effects of freezing and tube volume on CSF Aβ(1–42) concentration; for example, an additional freeze/thaw cycle

reduced CSF Aβ(1–42) concentration by > 25% in a PP tube, but had almost no effect when LoB storage tubes were used [124].

CSF Aβ(1–42) adsorption onto tube surfaces will occur with each transfer to a new tube [83,124,125]. Reduced recovery of measurable CSF Aβ(1–42) can result from adsorption to transfer pipettes, in addition to collection or storage tubes [83]. In one study, transfer of CSF samples from LoB tubes to PP tubes decreased Aβ(1–42) concentration by 42.5%. This is significant since a recent survey across four academic AD diagnostic reference centres found that 0–2 transfers of CSF occur on average (occasionally more) [83].

Different tube types have been shown to influence CSF pTau and tTau concentration, but within the range of acceptable assay variation (± 8%, [117]). Although one study found that CSF tau concentration was not affected by adsorption when transferred between different brands of tubes [83] another recent study did find transfer to a PP tube caused a significant decrease in CSF tTau [126], while a reduction was also reported for transfers to polystyrene tubes [119].

In summary, the evidence indicates that CSF Aβ(1–42) concentration is significantly affected by tube type, while CSF pTau and tTau concentration is not notably affected. Peptide ratios can be more consistent than single peptide concentrations as they are not altered as much by interactions of single peptides with tube material [119]. Interestingly, it has been shown that using Aβ(1–42)/ (1–40) ratios [83,119], but not Aβ(1–42)/tau [126], can reliably compensate for tube-based variation in Aβ(1–42) concentration.

4.3.2. Aliquot tube volume

Current protocol recommendations for both tube volume (between 0.1–0.75 mL) and the CSF fill volume (either no specification, or between 50 and > 75% full) vary considerably (Table 1).

The CSF surface to tube volume ratio can affect CSF Aβ(1–42) concentration; there is a consensus in the literature that a larger ratio results in lower CSF Aβ(1–42) concentration (Table 3; [83,114,124,127]). For example, one study observed that a loss in CSF Aβ(1–42) concentration following tube transfer depended on starting volume, with higher surface area to volume ratios increasing Aβ(1–42) adsorption [83]. In contrast, there is limited evidence of an effect of tube volume on CSF tTau concentration, although a decrease in concentration with decreased volume, dependent on tube material, was reported for tTau [124] and a weak association also noted [127]. We found no reports of an effect of tube volume on the concentration of pTau. Current evidence supports limiting the surface area to volume ratio when using samples for CSF Aβ(1–42) concentration, but specifics vary, warranting further investigation.

4.3.3. Temperature between collection and analysis/storage

There are several differences in the recommended time that CSF samples can be left at room temperature (RT) or cooled (Table 1). Many studies have investigated the stability of CSF biomarkers at different temperatures, with emphasis on the length of time samples were left at RT immediately following LP as a practical consideration in the clinical setting (Table 3).

Several studies that started with fresh samples agreed that storing CSF at RT for up to 24 hours had no significant effect on CSF Aβ(1–42) concentration [73,100,128].

Measurements on samples left at RT for longer than 24 hours have been more variable, with studies reporting increasing [100,129] or decreasing [130] CSF Aβ(1–42) concentration, or no effect [131–133]. Hypotheses for the increase or decrease include Aβ(1–42) release from amyloid-binding proteins [100], increased Aβ(1–42) adsorption to tube material [83], or proteolytic degradation [129].

In comparison with CSF Aβ(1–42), studies report no changes in CSF pTau or tTau concentration over short timeframes when kept at RT [128,129,131,134]. A decrease in tTau protein at 37°C or RT has been reported after ~12 days, though this was not seen in samples kept at 4°C [130,132].

A few studies have assessed the influence of different cooling methods or freezer temperatures, with limited evidence that colder temperature may limit biomarker concentration loss. For example, snap freezing samples in liquid N_2 increased CSF Aβ(1–42) concentration compared with freezing in a -80°C freezer ($P = .048$), but may not be practical in the clinic setting. In addition, freezing at -80°C yielded a higher CSF pTau concentration than freezing at -20°C ($P = .001$) [100].

Most evidence for CSF Aβ(1–42) concentration supports limiting the time at RT to less than 24 hours, followed by analysis or freezing. However, a comparison using newer fully automated assays with fresh CSF stored for various times compared with samples frozen at -20°C or -80°C for the same period is warranted. Although no evidence of significant temperature effect was found for tTau and pTau, freezing method and temperature effects on tau concentrations also merits further study.

4.3.4. Freeze/thaw cycles

Evidence on the effects of freeze/thaw cycles on biomarker concentration is inconsistent; however, up to three cycles was the standard recommendation in

current protocols (Table 1). Following many reports on freezing effects on CSF biomarker concentration, most studies have shown that multiple freeze/thaw cycles decrease CSF biomarker concentration. The majority of studies found no significant effect of one freeze/thaw cycle on the CSF concentration of Aβ(1–42), pTau or tTau, compared with fresh samples (Table 3), or on mass spectra intensities [135]. One notable exception was a multicentre analysis that suggested frozen samples had higher diagnostic accuracy than fresh samples for CSF tTau and Aβ(1–42) concentration [29], although this has not been confirmed.

Significant reductions or unsystematic changes in CSF Aβ(1–42) concentration have been described after strict application of two [113,120], three [130,131,136], four [100] or five [114] cycles of freeze/thawing. A freeze/thaw-dependent decrease in CSF Aβ (1–42) concentration may be more pronounced at lower initial concentrations of CSF Aβ (1–42) [113].

For CSF pTau and tTau, similar disagreement exists regarding the number of freeze/thaw cycles that are possible before concentration is significantly reduced. For example, no group has described a significant change in concentration from one freeze/thaw cycle, though a significant reduction was seen in CSF tTau concentration after two [100] or three [131,132] cycles. Conversely, Leitao, et al. (2015) found up to at least five freeze/thaw cycles had no significant effect on CSF tTau or pTau concentration.

In summary, studies agreed that increasing the number of freeze/thaw cycles decreases CSF concentration of Aβ(1–42), tTau and pTau, but evidence on the exact number of cycles is inconclusive. A note of caution is recommended when assessing this variable, however; the influence of freeze/thaw cycles reported here

may not be purely a result of the temperature change, but rather due to the pipetting and tube transfer steps or a change in surface area/volume ratio increasing adsorption to these materials.

4.3.5. Storage duration

Current protocols do not specify a time limit on long-term storage at -80°C, except the < 2 years recommended in the BIOMARKAPD protocol [80] (Table 1). Investigations have consistently shown that CSF biomarkers (Aβ(1–42), pTau and tTau) are reasonably stable over time, when stored at -80°C (Table 3; [73,123,130,131,133,134,137–140]); a recent study found that, if using a single batch of assays following varying sample storage times, storage for up to 12 years had no significant effect [140]. Only one report was found in this review that deviated from this, describing an increase in concentration of some samples following 7 months [138]. However, it should also be noted that lot-to-lot variability or assay storage may have an influence when comparing CSF concentration changes analyzed on different occasions over time [140].

In general, the literature is in agreement that storage at -80°C for up to 6 months has little or no effect on CSF Aβ(1–42), $pTau_{181P}$, and tTau concentration.

4.4 The influence of CSF treatment on biomarker concentration

The treatment of CSF prior to biomarker assessment can affect biomarker concentration. This includes additives, heat treatment, centrifugation, and/or mixing of samples.

4.4.1 Additives

Additives are not recommended by current diagnostic protocols (Table 1). Additives such as mild detergents (Triton-X 100 or Tween-20) can alter protein binding, and

several studies have shown that CSF samples that have been treated with~0.05%

Tween-20 have higher CSF Aβ(1–42) concentration (Table 4). For example, 0.1%

Tween-20 or 0.05% TritonX-100 both increased CSF Aβ(1–42) concentration by

~75% in individuals with AD [73], although Willemse, et al. (2017) found that the

addition of Tween-20 did not improve the discrimination of AD patients from control

subjects [83].

The detergent possibly interferes with binding of CSF Aβ(1–42) to tube material

[122,127], and therefore could be a way of mitigating adsorption [123].

Subsequently, the standard strong correlation between tube surface area and

increased CSF Aβ(1–42) concentration, probably reflecting Aβ(1–42) adsorption, is

not present in samples containing 0.05% Tween-20 [127]. Even a pre-wash step of

tubes with detergent-containing buffers was enough to considerably reduce CSF

Aβ(1–42) tube adsorption (optimal dose recovery was 0.01% Tween-20) [124]. To

prevent Aβ(1–42) loss in earlier steps in pipette tips or tubes prior to transfer,

detergent could be added at the time of CSF withdrawal [83].

Detergents or other additives could affect the concentration and detection of other

analytes used as biomarkers and modify the equilibrium between protein-bound and

free analytes in CSF, or the ratio of Aβ isoforms, and so should be used with caution

[124]. In addition, detergents could affect reagent antibodies in immunoassays.

Despite this, 0.05% detergent (Triton-X 100 or Tween-20) did not seem to affect

sensitivity, specificity, diagnostic accuracy [141] or intra-assay variability for CSF

Aβ(1–42) concentration [124]. Some authors do explicitly recommend detergent use

to mitigate CSF Aβ(1–42) adsorption [123,127], for example, for older samples

stored in high absorption tubes.

In summary, there is evidence that 0.05% Tween-20 may help mitigate CSF Aβ(1–42) adsorption onto tubes and boost concentration [124,127,141,142]. Though there is limited evidence that 0.05% detergent has any effect on CSF tTau or pTau [141,142], unknown effects on potential interactions of additives with other proteins and assay reagents understandably restricts any recommendation for their use. It should also be noted that the certified reference material for Aβ(1–42) is composed of neat CSF without additive, calibrated against mass spectrometry methods [75]. It would be interesting to directly compare CSF Aβ(1–42) concentration between the lowest binding tubes on the market with and without the addition of 0.05% Tween-20 to quantify potential loss in more detail.

4.4.2 Heat denaturation

Current protocols do not recommend heat denaturation as a protocol step prior to CSF biomarker measurement (Table 1). Nevertheless, the possible effect of this step on CSF Aβ(1–42) concentration has been sporadically investigated (Table 4). In 2000, Vanderstichele et al demonstrated that boiling CSF samples had no effect on CSF Aβ(1–42) concentration [113]. In contrast, heat denaturation was recently reported to increase CSF Aβ(1–42) concentration (15 mins at 100°C increased levels by 42–71%), and this increase was hypothesized to be due to detection of Aβ(1–42) that was previously bound in protein complexes or masked by epitopes [73]. The evidence is inconclusive about the effect of heat treatment on CSF biomarkers; however, a heating step is unlikely to occur in or be recommended for routine clinical use.

4.4.3 Centrifugation

Centrifugation has been investigated often and is recommended in several standard protocols, particularly with haemorrhagic CSF samples (Table 1). However, results

remain inconsistent (Table 4). Significant reductions in CSF Aβ(1–42) concentration following centrifugation have been reported [73,141,143], possibly reflecting increased adhesion of Aβ(1–42) to the cell walls, or precipitation of lysed cells during the process.

Leitao, et al. (2015) found no effect of different centrifugation speeds on CSF Aβ(1–42), tTau or pTau concentration except when total protein levels were high [114]. No significant differences were reported in CSF tTau or pTau concentration from including or omitting a centrifugation step [100,114,130,141]. Centrifugation may be required if samples contain some blood contamination (4.1.5 Blood contamination), and if so the step may reduce CSF Aβ(1–42) but not tTau or pTau concentration (Table 4). The potential reduction in Aβ(1–42) must be taken into consideration, and we conclude centrifugation effects need further quantification if it is to be used sporadically to mitigate blood contamination.

4.4.4 Shaking

The effect of shaking on CSF biomarker concentration has not been extensively examined and is not listed in current protocols (Table 1). Shaking CSF samples for 48 hours at RT led to a small but significant decrease in median Aβ(1–42) concentration of 7% (n = 20, $P < .05$) and tTau of 30% (n = 20, $P < .001$; [144]). Conversely, excessive vortexing did not have any effect on CSF Aβ(1–42) concentration in a recent report [83]. Sample mixing may be more important if samples have been frozen and then thawed (Hansson O., personal observation). The benefits of creating more homogeneous samples by shaking or mixing, especially following freezing steps, requires further study.

4.5 Additional variables

During this review process, a small number of potential pre-analytical variables were uncovered that have not received much consideration to date. This includes steps contained in current protocols without extensive investigation (for example, whether fasting before the CSF collection procedure is necessary), and also steps not included in current protocols for which some evidence indicates an influence on CSF biomarker concentration. The latter includes bacterial growth conditions, where bacterial contamination significantly reduced levels of Aβ(1–42) and diminished tau concentration [144]. However, this could be alleviated by preventing bacterial growth with 0.1% sodium azide – which did not alter starting concentrations even after 5 days at RT [133]. In addition, tube sterilization methods can significantly increase their adsorbance properties (e.g., irradiation significantly increased the adsorbance properties of homopolymer tubes [121]). Further analysis into these potential variables may be required.

5. Discussion

5.1. Conclusions and recommendations

The range of pre-analytical protocols used has introduced the potential for significant variability in CSF AD biomarker measurements. In this review, we summarized the current body of evidence and highlighted the most important key pre-analytical variable steps to consider in the future, such as tube material, the length of time samples are kept at RT before storage or analysis, and the relationship of tube surface area to sample volume (Fig. 2). In particular, CSF Aβ(1–42) concentration is significantly affected by tube type, with potentially important consequences for clinical and research evaluation. More consistent sample processing and a consensus on LoBind tube choice would help the field. In the future, it is also recommended that a better tube, capable of inhibiting CSF biomarker adsorption, be developed and adopted.

Although there are differences in the variables that specifically affect CSF Aβ(1–42), tTau, and pTau, we found that generalizations could be made across these three biomarkers. In particular, our results showed that the variables (in addition to tube material) with the largest amount of conflicting data were: temperature between collection and analysis/storage, the number of freeze/thaw cycles advised, centrifugation and additives. In contrast, steps for which either little variability exists or where there is some consensus regarding parameters that should be followed were: CSF collection steps and storage time (although the effect of storage temperature and duration should be studied using new, high-precision assays). A number of pre-analytical variables may require further research, for example tube volume and shaking (Fig. 2).

One limitation of this review may be that some unstudied variables or interactions of variables may yet have a significant influence on biomarker concentration. Further comprehensive research with more robust and precise assays will hopefully shed light on this. The wide variety in methodology in the research studies included in this review, where most studies did not combine several variables or measure several biomarkers in the same experiments, may make some comparisons weaker. In addition, this review was limited to analyzing evidence for pre-analytical variables affecting the concentration of CSF Aβ(1–42), tTau, and pTau. Other biomarkers for AD have been suggested, alone or in combination, for example Aβ(1–38), Aβ(1–40), sAPPα, sAPPβ, ApoE, neurofilament light, neurogranin, YKL-40 and VILIP-1 [39,145-147], and there is an emerging body of evidence for potential CSF biomarkers of other neurodegenerative disorders [148,149].

We recommend that a universal pre-analytical protocol for CSF handling be developed and incorporated into future clinical trials, registries, and routine AD diagnosis. This protocol should attempt to control for the variables identified in this review as having the most influence on biomarker concentration. Aβ(1–42) appeared to be the most affected by the pre-analytical variables evaluated; however, a universal protocol would reduce variability of all CSF biomarkers. This protocol could reflect the latest evidence on potentially important pre-analytical variables, while ideally being easy to implement and practical for clinical settings. Although clinical and analytical variables will still have an influence, a universal pre-analytical protocol will help enable accurate comparison of results between studies and further limit potential diagnostic variability.

Appendix A

Search terms used in PubMed literature search:

Search

1   ((pre-analytic* OR pre-analytic*) AND (CSF OR cerebrospinal fluid) AND

         ("1995"[Date - Publication] : "3000"[Date - Publication])

2   (variab* AND (CSF OR cerebrospinal fluid) AND (amyloid beta 42 OR

         amyloid-β 42 OR Aβ42 OR Abeta42) AND ("1995"[Date - Publication] :

         "3000"[Date - Publication])

3   variab* AND (CSF OR cerebrospinal fluid) AND (phosphorylated tau OR

         ptau OR p-tau OR phospho tau OR phospho-tau) AND ("1995"[PDAT] :

         "3000"[PDAT])

4   (variab* AND (CSF OR cerebrospinal fluid) AND (total tau OR ttau OR t-

         tau OR totaltau OR total-tau) AND ("1995"[Date - Publication] :

         "3000"[Date - Publication])

5   validation AND (CSF OR cerebrospinal fluid) AND biomarker* AND

         (alzheimer's OR alzheimer OR AD) AND (amyloid beta 42 OR amyloid-β

         42 OR Aβ42 OR Abeta42 OR phosphorylated tau OR ptau OR p-tau OR

         phospho tau OR phospho-tau OR total tau OR ttau OR t-tau OR totaltau

         OR total-tau) AND ("1995"[Date - Publication] : "3000"[Date -

         Publication])

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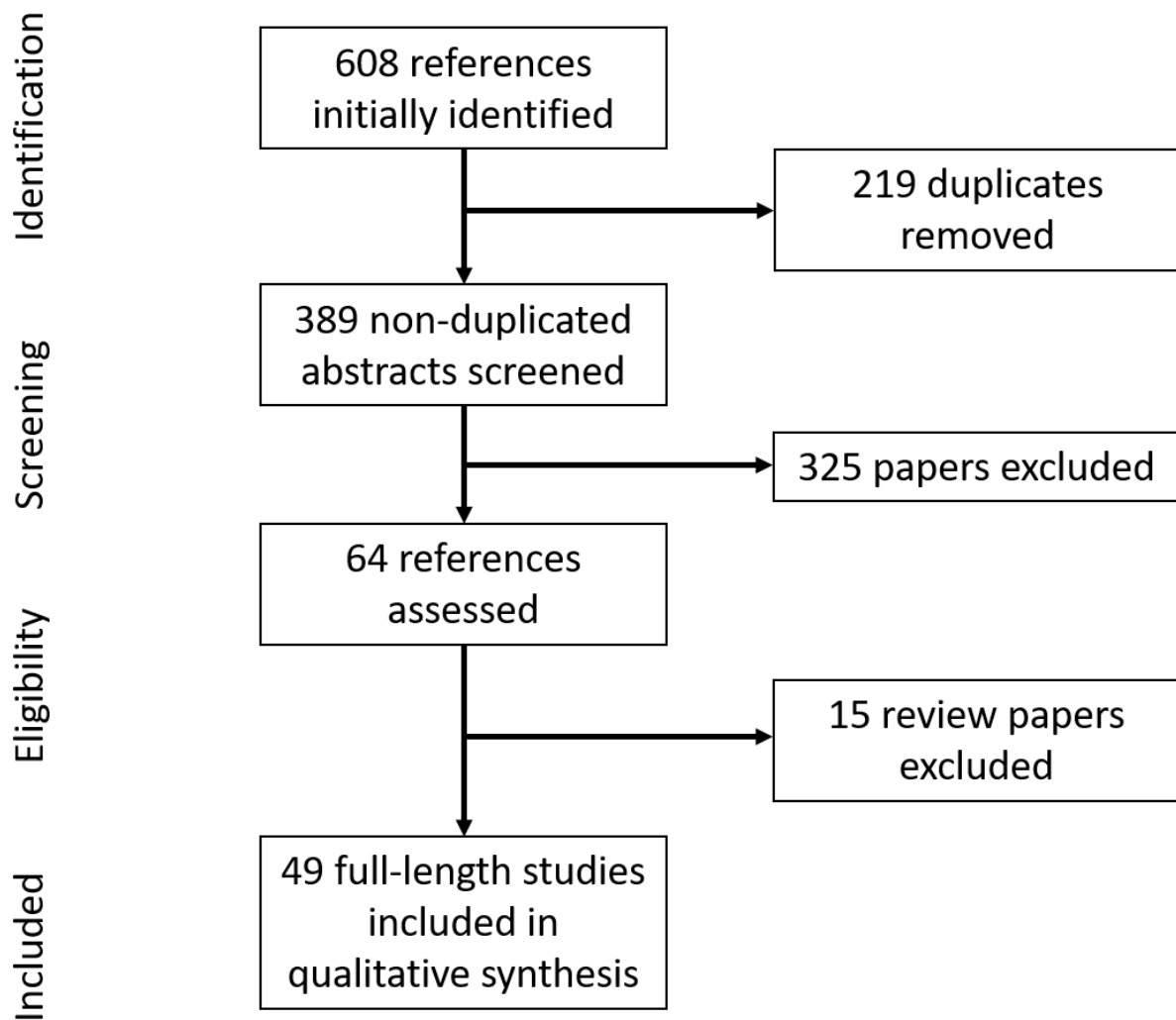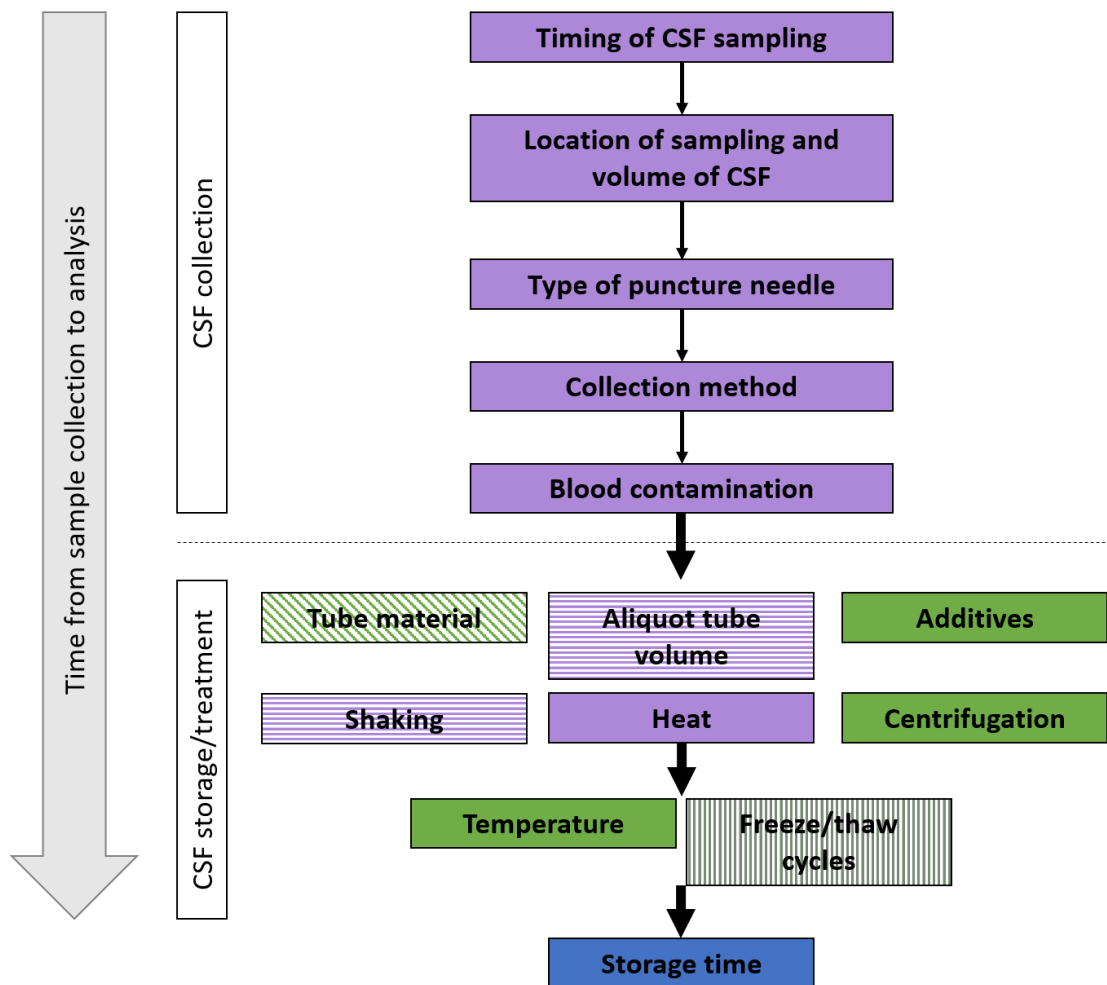
Fig. 1. PRISMA flow diagram of literature search and study selection flow for systematic reviews.

Fig. 2. Pre-analytical variables in CSF biomarker analysis.

Abbreviations: Aβ, β amyloid; CSF, cerebrospinal fluid

Table 1

Comparison of existing protocols used for pre-analytical handling of CSF samples before measuring concentrations of core AD biomarkers

	ADNI [150][151] [29]	BioFINDER [14,35][*]	BIOMARKAPD [80]	AA [152]	ABSI [71]	Standard Biobanking Protocol [85]
Fasting before LP	Minimum 6 h	Not required	Not required	Not required	Not required	Not mentioned
Timing of CSF sampling	Morning	Standardized (0800–1200 h)	Day time	0800–1200 h	Any time	Standardized within each centre
Location of sampling (LP)	L3–L5	L3–L5	L3–L5	L3–L5	L3–L5	L3–L5
Type of LP needle	22G and 24G atraumatic	22G atraumatic	25G atraumatic	22G, atraumatic	22G, atraumatic	Atraumatic

Collection method	Gravity drip (22G, recommended) or suction (24G)	Gravity drip	Not specified	Not specified	Not specified	Not specified
Tube type	PP (Sarstedt)	PP	PP	PP	PP – standardized small volume	PP, screw cap
Collection volume (mL)	15–20	10 x 2, gentle mix	12	10–12, gentle mix	≥1.5	12
Temperature between collection and analysis/storage	One transfer (for shipping), dry ice	RT < 30 mins, freeze and ship on dry ice if longer	< 5 days, 4°C	RT < 2 days	RT < 5 days	RT, 30–60 mins (max 2 hours), ship on dry ice
Blood contamination	Should be clear CSF only; discard initial 1–	Discard initial 1–2 mL if bloody	< 50 erythrocytes/µL CSF (for	Should be clear CSF only; discard initial	< 50 erythrocytes/µL CSF. Discard	< 50 erythrocytes/µL CSF

	2 mL (or more if needed)		biomarkers highly abundant in peripheral blood)	0.5–1 mL (or more if needed)	initial 1–2mL if bloody	
Centrifugation	No [151]; yes 2000 x *g* 10 mins 4°C [29]	Yes– 2000 x *g* 10 mins RT	Yes – 2000 x *g* 10 mins RT	Yes	No (unless visually haemorrhagic; 2000 x *g* 10 mins RT)	Yes – 2000 x *g* 10 mins RT
Freezing temperature (°C)	-80°C (following dry ice)	-80°C	-80°C	-80°C	-80°C	-80°C
Aliquots Volume (mL)	0.5 [29]	≤ 1	0.25–0.5 tubes	1	small	0.1–0.75 aliquots in 1–2mL tubes

Fill level (%)	Not specified	≥ 50	> 75	Not specified	≥ 50	75
Freeze/thaw cycles (n)	≤ 2	1	≤ 2	1	≤ 2	1
Storage time (years)	Not specified	Not specified	≤ 2	Not specified	≤ 2	Not specified

[*]Hansson O, personal communication.

Abbreviations: AA, Alzheimer's Association; ABSI, Alzheimer's Biomarkers Standardization Initiative; ADNI, Alzheimer's Disease Neuroimaging Initiative; CSF, cerebrospinal fluid; LP, lumbar puncture; PP, polypropylene; RT, room temperature.

Table 2

The influence of LP on CSF core AD biomarker concentration

Pre-analytical variable	Aβ(1–42)		tTau		pTau	
	Evidence of influence of variable	Evidence of no influence of variable	Evidence of influence of variable	Evidence of no influence of variable	Evidence of influence of variable	Evidence of no influence of variable
Timing of CSF sampling	1.5–4-fold change [91], slight but significant ↓9.3% after ~5 hours, return after 24 hours [73]; low	No significant effect [89,90], CV 5.5% [89]	Linear ↑ over 36 hours in AD patients only [95]	No significant effect [89,90,95], CV 8.2% [89]	Linear ↑ over 36 hours in AD patients only and significant fluctuation pattern in both AD patients and controls [95]	No significant effect [90], not significant, CV 11.9 % [89]

	circadian-like fluctuation up to 1.7-fold change [95], up to 200% increase in indwelling catheter studies [93]; ↑ over time in younger participants [92]					
Location and volume of CSF sampling	↑ in lumbar vs ventricular in NPH patients [103]	No significant effect [73,90,100,102]	↑ concentration from ventricular vs lumbar catheter [90]; in NPH patients [103]	No significant effect [100,102]	↑ concentration in ventricular vs lumbar fraction in NPH patients [102,103]	No significant effect [100]

Type of puncture needle	None reported		None reported		None reported	
Collection method	Use of a styrene-butadiene copolymer manometer caused sig ↓4.3% (± 2.4 SE, P = .047; [108])	No difference in catheter type [73]; no difference between gravity drip or aspiration (n = 44; [107])	None reported	No difference between gravity drip or aspiration (n = 44; [107])	None reported	No difference between gravity drip or aspiration (n = 44; [107])
Blood contamination	Plasma contamination of CSF/albumin ratio of 55 ↓	No significant effect: up to 5000 per µl [73], up to 10%	None reported	No significant effect [114]	Blood spiked samples 5000 per µl	None reported

| | concentration by up to 49% [73]; blood spiked samples 5000 per µl ↑6% (*P* < .05; [114]) | contamination by haemolysed blood [123]. No effect of albumin [113] | | | ↑11% (*P* < .05; [114]) | |

Abbreviations: Aβ, β amyloid; AD, Alzheimer's Disease; CSF, cerebrospinal fluid; CV, coefficient of variation; LP, lumbar puncture;

NPH, normal pressure hydrocephalus; pTau, phosphorylated tau; SE, standard error; tTau, total tau

Table 3

The influence of CSF storage on core AD biomarker concentration

Pre-analytical variable	Aβ(1–42)		tTau		pTau	
	Evidence of influence of variable	Evidence of no influence of variable	Evidence of influence of variable	Evidence of no influence of variable	Evidence of influence of variable	Evidence of no influence of variable
Tube material	Significant ↓ polystyrene storage versus PP (mean 19%, P = .002, [73], mean 36% ↓ polystyrene, [118]) or polystyrene	No significant difference between tube brands [83]; no significant difference between PP and glass [73]; PP no	Significant differences from different types of PP tube (acceptable range; [117]); Significant ↓ in PS compared with other tubes	No significant effect [117]; No significant difference between tube brands [83]	Significant differences from different types of PP tube (acceptable range; [117]	No significant effect ; No significant difference between tube brands [83]

	versus PP, PC or PX ($P < .001$, [119]) or ↓ when stored in glass PP (mean 33%, $P < .001$ [118]); Significant differences from different types of PP tube [117,120,122]; copolymer and Sarstedt tubes significant ↓ versus homopolymer ;	significant effect [118]; LoB no significant effect when tested after one freeze/thaw [123]	($P < .001$ [119]; decrease when transferred to PP from LoB tube [126]		

	much higher concentration in Sarstedt PP [117]; Significant ↓ in PP versus LoB tubes (11.0%; [124]); Significant ↓ in Nunc PP cryotubes compared with LoB [123]				

Aliquot tube volume	↓ volume from 75 to 50% = ↓3.7% (*P* = .03; [114]); ↑ volume x 30 = ↑ of ~2-fold [127]; ↑ surface area/volume ratio = ↓ [83], in PP [124]	None reported	↓ volume PP tubes 1.5mL to 0.5mL = ↓ 4.5% (*P* = .001 [126])	No significant effect [114,127]	None reported	No significant effect [114]
Temperature between collection and analysis/storage	2 days RT or 4°C ↓ ~20% [130]; 2 days RT ↑ ~15% [100];	Up to 24 hours RT = no significant effect [73,100,128]; up to 6 hours RT or 3 days 4°C also	↓ after holding at -20°C compared with -80°C [100]; ↓after ~12 days at 37°C	No difference up to 22 days at 4°C or 18°C [130]; 24 hours RT [128,134]; 7 days RT [131];	↓ after freezing at -20°C versus -80° (*P* = .001 [100])	No effect 24 hours at RT [128,129,134]; up to 3 days [54]; 7 days RT [131]; up to 5–

	↑ after 24 hours RT [129]; significant changes in peptide patterns (mass spec) after 24 hours RT [135]; ↓ after 14 days -20°C [132]	had no effect on CSF mass spectra [135]; 7 days RT [131]; 14 days RT or 4°C [132]; up to 5–7 days routine storage conditions [133]	[130]; ↓ after 14 days RT [132]	no significant effect 14 days at -20°C or 4°C [132] up to 5–7 days routine storage conditions [133]		7 days routine storage conditions [133]; 14 days RT, 4°C or -20°C [132]
Freeze/thaw cycles	Two cycles ↓ [113]; ↓ 17.6% (P < .001; [120]); three cycles ↓~30%	One freeze/thaw = no difference [73,120,130,153] or on mass spectrometry	Significant ↓ > 2 cycles [100]; significant ↓ 17% ≥3 cycles [132]	One freeze/thaw = no difference [130,153]; up to three [131]; up	Significant ↓ 16% ≥ 3 cycles [132]	Up to three [131]; up to four [100]; up to five [114]

	[136], ↓~20% [130]; four cycles ↓16% ($P < .05$; [100]); five cycles ↓5% ($P < .05$; [114]	intensities ; up to three [100,123,131] [132][114]		to five [114]; up to six [130]		
Storage time (-80°C)	↑ after 7 months [138]	> 3 months [123]; up to ~1 year [133,137]; 2 years [73,131]; prolonged storage [130,138-140];	None reported	> 1 year -70°C no effect [134]; prolonged storage does not have an effect [130,131,139,140,154]	None reported	>1 year -70°C no effect [134]; prolonged storage does not have an effect [131,140,154]

		up to 7 months [138]				

Abbreviations: Aβ, β amyloid; AD, Alzheimer's Disease; CSF, cerebrospinal fluid; LoB, low binding; PC, polycarbonate; PP, polypropylene; PS, polystyrene; pTau, phosphorylated tau; PX, a copolymer of polystyrene and acrylonitrile; RT, room temperature; SE, standard error; tTau, total tau.

Table 4

The influence of CSF treatment on biomarker concentration

Pre-analytical variable	Aβ(1–42)		tTau		pTau	
	Evidence of influence of variable	Evidence of no influence of variable	Evidence of influence of variable	Evidence of no influence of variable	Evidence of influence of variable	Evidence of no influence of variable
Additives	0.05% Tween-20 = ↑ of ~29% (control and MCI) to 35.9% (AD), $P < .001$ [141], ↑ of 69%, $P < .001$ [142]; ↑	No significant effect on concentration of up to 0.05% Tween-20 or Triton X-100 [124]; no effect of 0.1% NaN$_3$ on	0.05% Tween-20 = ↑ of 4%, $P = .001$ [141]	Any observed changes not significant reported [142]	None reported	Any observed changes not significant [141] [142]

	[73,122,123,127,136,138]	concentration 5 days RT [133]				
Heat denaturation	↑ [73]; SDS heat prevented a ↓ otherwise seen following freezing, with SDS [155]	No significant effect [113]	None reported		None reported	
Centrifugation	↓ [73,141,143]	No significant effect [100,114,130]	↓ [143]	No significant effect [100,114,130,141]	↓ [143]	No significant effect [100,114,130]
Shaking	↓ 7% 48 hours RT ($P < .05$; [144]	No effect of vortexing [83]	↓ 30% 48 hours RT ($P < .001$; [144])	None reported	None reported	

Abbreviations: Aβ, β amyloid; AD, Alzheimer's Disease; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; NaN$_3$, sodium azide; pTau, phosphorylated tau; RT, room temperature; SDS, sodium dodecyl sulphate tTau, total tau.