The Alzheimer’s Association international guidelines for handling of cerebrospinal fluid for routine clinical measurements of amyloid β and tau

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1 | BACKGROUND

Alzheimer’s disease (AD) is a devastating and progressive neurodegenerative disease with symptoms that exist along a continuum composed of three stages: a lengthy preclinical phase without symptoms but with measurable changes in the brain (through imaging techniques), cerebrospinal fluid (CSF), and blood, and two subsequent phases, mild cognitive impairment (MCI) and dementia, in which symptoms vary in magnitude. 1–6

Diagnosing AD at its earliest stages is challenging. However, biomarkers, biological markers that can be measured in the brain, CSF, and blood, and evaluated as potential indicators of the disease, 7 are promising tools that provide the potential for early detection and increased clinician confidence in AD diagnosis. 8 AD biomarkers can be obtained from brain imaging methods such as magnetic resonance imaging (MRI) or positron emission tomography (PET), as well as CSF or blood tests. Despite the utility of brain imaging methods, they can only identify the presence of a certain type of pathology once levels pass a certain threshold. In contrast, biomarkers from CSF are considered a more direct readout of protein levels and are therefore a more sensitive assay. 9

The four core AD CSF biomarkers include two isoforms of the protein amyloid beta (Aβ), Aβ1-42 and Aβ1-40, as well as total tau (t-tau) and phosphorylated tau (p-tau). 10 Aβ1-42 is the primary constituent of the amyloid plaques that are found in AD, 10–12 while hyperphosphorylated tau is a primary constituent of neurofibrillary tangles; 13–15 together, plaques and tangles form the pathological hallmarks of the disease.

A large body of literature has validated that each of these core CSF biomarkers are reflective of AD brain pathology. For example, low levels of CSF Aβ1-42 are correlated with brain Aβ levels, 13,16–20 while elevated levels of CSF p-tau are found in AD patients, 21 and correlate with the presence of cortical neurofibrillary tangles in the majority of studies. 22–24 In contrast, increased CSF levels of t-tau are not specific to AD, 21 but instead reflect neuronal injury/degeneration more generally and are thus also increased in conditions such as traumatic brain injury, 25 stroke, 26 and Creutzfeldt-Jakob disease 27 that do not show elevated p-tau levels. The 2018 National Institute on Aging (NIA)-Alzheimer’s Association (AA) Research Framework, created to provide guidance on how AD imaging and CSF biomarkers should be used in research, groups them into three categories that are collectively known as the AT(N) scheme. 28 The Aβ (A) designation includes CSF Aβ1-42 or the Aβ1-42/Aβ1-40 ratio, the tau (T) designation includes CSF p-tau, and the neurodegeneration or neuronal injury (N) designation includes CSF neurofilament light (NfL) or t-tau.

There are several commercially available assays that measure these core CSF biomarkers and many are approved for use in the diagnosis of AD in the European Union. 29–35 In the United States, however, PET imaging remains the only Food and Drug Administration-approved method for biomarker measurement in the disease. 36 Despite widespread use in Europe, studies of these core CSF biomarkers have found significant inter-laboratory and batch-to-batch variability. 7,37 There are three possible sources of variability to be considered when interpreting CSF biomarker results or when defining CSF biomarker cutoffs: (1) pre-analytical (i.e., due to differences in the collection, handling, and storage of CSF); 38 (2) analytical (i.e., due to differences...
RESEARCH IN CONTEXT

1. Systematic review: A workgroup led by the Alzheimer’s Association with experts from both academia and industry developed a simplified and standardized pre-analytical protocol for cerebrospinal fluid (CSF) collection, handling, and analysis for routine clinical use. This article presents two pre-analytical protocols, one for analysis of fresh CSF and one for frozen CSF.

2. Interpretation: The protocol is designed to be easy to use and to reduce the variability of CSF Alzheimer’s disease (AD) biomarker results.

3. Future directions: A widespread application of these protocols would help minimize variability in measurements, which would facilitate the implementation of unified cutoff levels across laboratories using the same platform, and foster the use of CSF biomarkers in AD diagnostics for the benefit of patients.

The CSF Pre-Analytics Consortium was formed with the goal of developing a simplified and standardized pre-analytical protocol intended for routine clinical test use. The consensus protocol is critical to establishing universal cut-off values for the measurement of CSF AD biomarker levels, establishment of general biomarker cut-off values and the adoption of a standard CSF biomarker protocol, makes proper diagnosis and treatment more difficult, and presents challenges for clinical trial enrollment.

There have been a number of efforts to reduce analytic variability and harmonize results obtained from different vendors. The International Federation of Clinical Chemistry and Laboratory Medicine Working Group for CSF proteins (WG-CSF), working closely with the Alzheimer’s Association Global Biomarker Standardization Consortium (GBSC), developed certified reference materials (CRMs). For use as value assignment of CSF Aβ1-42 in the CRMs, they developed four liquid chromatography mass spectrometry (LC-MS) methods for absolute quantification of Aβ1-42, of which two were fully validated analytically and approved by the Joint Committee for Traceability in Laboratory Medicine as Reference Measurement Procedures (C11RMP9 and C12RMP1, respectively). Initiatives to obtain standardization of Aβ1-40 and t-tau are ongoing.

Variability surrounding patient selection has also been sufficiently addressed due to the recently published appropriate use criteria (AUC) for lumbar puncture (LP) and CSF testing for AD diagnosis, established by a multidisciplinary workgroup convened by the Alzheimer’s Association.

However, a large source of variability remains with respect to pre-analytics. After the integration of CRMs for Aβ1-42 in commercial assays, pre-analytics are considered the last piece of the variability puzzle that remains to be solved. This pre-analytic variability still precludes the establishment of general biomarker cut-off values and the adoption of a standard CSF biomarker protocol, makes proper diagnosis and treatment more difficult, and presents challenges for clinical trial enrollment.

Although some groups have led development initiatives for clearer pre-analytical protocols, these protocols have not been adopted, nor have they been widely implemented (e.g., across both academic and industry settings), despite their proven success. In the present communication, we report a unified, impartial approach by a workgroup, led by the Alzheimer’s Association and composed of key opinion leaders in academia and industry, to develop a simplified and standardized pre-analytical protocol intended for routine clinical testing involving Aβ1-42. The workgroup examined the impact of a number of pre-analytical factors on measured CSF biomarker concentrations: sample collection methodology, blood contamination, tube type and filling volume, tube transfer steps and pipetting, mixing, transport, and sample stability. The primary goal of this effort is to ensure that stakeholders in industry and academia will adopt and implement the recommended protocol in clinical routine. The adoption of a global standard pre-analytic protocol is critical to establishing universal cut-off values for the measurement of CSF AD biomarker levels, especially Aβ1-42. Without such standardization, variability will continue to weaken the confidence of clinicians when evaluating measured levels of biomarkers as diagnostic criteria for AD.

2 | RECOMMENDATIONS

The CSF Pre-Analytics Consortium was formed with the goal of developing a simple, easy to implement, data-driven, and consensus-based pre-analytical protocol for routine clinical use. The consensus protocol was based on the available literature focusing on Aβ1-42 as this marker is particularly susceptible to variations in pre-analytical handling.
TABLE 1  Main findings of recent studies on the effects of pre-analytical factors on cerebrospinal fluid (CSF) Alzheimer’s disease (AD) biomarkers

<table>
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<th>Main findings</th>
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<tr>
<td>Fasting has no effect on CSF Aβ concentrations^{58}</td>
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<td>When collecting CSF using the drip method, the concentrations of Aβ are stable when collecting up to at least 20 mL of CSF (after removing the first 1 to 2 mL CSF);^{56,59}</td>
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<td>The use of LoB tubes mitigates loss of Aβ. However, even when using LoB tubes all new tube types must be evaluated to determine whether extended contact between sample and tube (including the cap of the tube) affects the Aβ42 levels;^{49,59}</td>
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<tr>
<td>A low CSF volume versus tube surface area might reduce Aβ concentrations, even when using LoB tubes;^{49,52,59}</td>
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<td>Transfer of CSF from one tube to another results in loss of Aβ;^{49,57}</td>
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<td>Aβ concentrations in fresh CSF are stable at RT for up to 2 days and at 4°C for up to 14 days;^{56,59}</td>
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<td>Mixing of fresh CSF is not necessary before analyses and can reduce Aβ levels. However, if a frozen CSF sample is used it should be mixed using a roller mixer after thawing to reduce variation;^{56,59}</td>
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<td>Centrifugation of CSF samples is not necessary (if no visible blood contamination);^{56,59}</td>
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<td>Blood contamination visible to the naked eye (&gt;0.1% to 1.0%) might reduce Aβ concentrations in fresh CSF stored at room temp, but this effect is reduced by centrifugation and subsequent storage at 4 or −20°C;^{56,58}</td>
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<td>Transportation of fresh CSF can affect Aβ concentrations, especially if (a) the tube is not transported in upright position, (b) the CSF volume versus tube volume is low (higher tube fill volume results in less variability of Aβ recovery), or (c) the temperature is &gt;2–8°C;^{57}</td>
</tr>
<tr>
<td>The CSF Aβ42/40 ratio is generally more resistant to variations in pre-analytical procedures than Aβ42 alone;^{49,53,59}</td>
</tr>
<tr>
<td>Freezing of CSF at −20 or −80°C for at least up to 2 weeks does not change Aβ concentrations.^{56}</td>
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Abbreviations: Aβ, amyloid beta; CSF, cerebrospinal fluid; LoB, low-binding; RT, room temperature.

**FIGURE 1**  Recommended pre-analytical protocol for collection, storage, sample handling, and measurement of fresh cerebrospinal fluid (CSF). Aβ, amyloid beta; LoB, low-binding

Our research identified several areas in which data was insufficient, especially in regard to experiments in which a proper standard of truth (i.e., fresh CSF obtained directly in protein low-binding [LoB] tubes, which minimize binding of Aβ to the test tube walls, and analyzed after LP without any pre-analytical steps in between collection and analyses within hours after LP) was lacking. We consequently performed new experiments that have now been published elsewhere (Janelidze et al.,^{56} Hansson et al^{60}, Darrow et al^{58}).

Several pre-analytical factors were systematically evaluated by the CSF Pre-Analytics Consortium, including tube type, storage procedures, optimal filling volume, transportation conditions, mixing procedures, the need for centrifugation, and tube type. Of these factors, tube type, tube filling volume, transfer of CSF from one tube to another, transportation conditions, and mixing procedure had the biggest impact on the measured concentrations of the core AD biomarkers (Table 1).

Based on current evidence, we recommend the following protocol when analyzing fresh CSF samples (Figure 1). Fasting is not necessary when collecting CSF. We recommend using an ordinary LP needle.

The first 1 to 2 mL of collected CSF should not be used for AD biomarker diagnostics. The next CSF should be collected using the drip method, directly into a LoB tube, and should come from the first 20 mL
Remove first 2mL CSF; directly collect CSF into a polypropylene LoB tube*

* Follow manufacturer recommendations of tube type and filling volume

† Follow tube and assay manufacturer’s instructions of use

**FIGURE 2**  Recommended pre-analytical protocol for collection, storage, sample handling, and measurement of frozen cerebrospinal fluid (CSF). Aβ, amyloid beta; LoB, low-binding
is adequate. Frozen CSF protocol is identical to the fresh CSF protocol, except that gentle mixing before analyses is recommended to reduce variability. Frozen protocol should only be used if freezing is described in the assay manufacturer’s instructions of use. For freezing at -80°C, tubes validated for this temperature must be used.

3 | SUMMARY

This report presents unified pre-analytical protocols for the handling and measurement of fresh and short-term frozen CSF samples for use in routine clinical diagnosis. The protocol is designed to be easy to use and to reduce the variability of CSF AD biomarker results.

Assay manufacturers may indicate use of a specific tube type, and it is beyond the scope of this pre-analytical protocol to establish universal cut-off values or detail individual cut-off values that may change with new validation studies. Therefore, it is important to observe individual cut-offs as determined and validated for a specific tube type. We also want to emphasize that established biomarker cut-offs for a certain method can only be used in clinical practice when the current protocols are used together with a validated tube filled with a predefined volume of CSF. It is important to note that forthcoming studies referencing this unified protocol will present pre-analytical study results from experiments conducted using additional LoB tubes consistent with the data presented herein.

The protocol is not intended for long-term storage of samples in clinical studies (i.e., collection of multiple aliquots, and long-term storage). For clinical studies and/or long-term storage, please refer to the individual manufacturer’s recommendations. Data for long-term storage will be presented in a separate publication.

The protocol is based on the drip technique for collection. The extra time to collect this CSF volume by the drip technique is negligible. If syringe pull is standard in some centers, this technique can be applied after the protocol for additional sampling.

Note that foreseen commercially available In Vitro Diagnostic assays will explicitly prescribe a specific CSF pre-analytical protocol that corresponds to the fresh handling protocol described in this article. To achieve the best clinical performance (i.e., keep patient misclassification rate at a minimum), these recommendations have to be followed, given that handling differences may impact to the measured value of Aβ1-42, and may influence the biomarker result in relation to the cut-offs.

There are many benefits to a unified pre-analytical protocol, nevertheless a number of challenges and unanswered questions remain. The most significant challenge will be getting the standardized protocol implemented by different centers and users. While changing applied procedures across a wide range of settings may be a major hurdle, the benefits of switching to established protocols following the initial sample collection described here should facilitate broad implementation. Furthermore, this protocol contributes to the development of universal cut-offs and is an important step to optimize patient care in AD.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Alzheimer’s Association Global Biomarkers Standardization Consortium and James A. Hendrix, PhD; April Ross, PhD; and Emily A.S. Meyers, PhD for their contributions to the manuscript and bringing together this multi-disciplinary team. ELECSYS is a registered trademark of Roche. LUMIPULSE is a registered trademark of Fujirebio. OH was supported by the Swedish Research Council, the Knut and Alice Wallenberg Foundation, and the Swedish federal government under the ALF agreement. HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532); the European Research Council (#681712); Swedish State Support for Clinical Research (#ALFGBG-720931); the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862); and the UK Dementia Research Institute at UCL. KB is supported by the Swedish Research Council (#2017-00915); the Swedish Alzheimer Foundation (#AF-742881); Hjärnfonden, Sweden (#FO2017-0243); and the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986).

CONFLICTS OF INTEREST

Oskar Hansson has acquired research support (for the institution) from Roche, Pfizer, GE Healthcare, Biogen, Eli Lilly, and AVID Radiopharmaceuticals. In the past 2 years, he has received consultancy/speaker fees from Biogen and Roche. Britta Brix is a full-time employee of EUROIMMUN. Maria C. Carrilo, Rebecca M. Edelmayer, Christopher J. Weber are full-time employees of the Alzheimer’s Association. Veronika Corradin, Richard Batrla, Sandra Rutz, Simon Wahl were full-time employees and shareholders of Roche Diagnostics at the time of the study. Rianne N. Esquivel is a full-time employee of Fujirebio Diagnostics Inc., Malvern PA, USA. Christina Hall is a full-time employee of Fujirebio Diagnostics AB, Sweden. Jose Luis Molinuevo has served/serves as a consultant or on advisory boards for the following for-profit companies, or has given lectures in symposia sponsored by the following for-profit companies: Roche Diagnostics, Roche, Genentech, Novartis, Lundbeck, Oryzon, Biogen, Lilly, Janssen, Green Valley, MSD, Eisai, Alector, BioCross, GE Healthcare, ProMIS Neurosciences, NovoNordisk, Cytos, Nutricia, and Zambon. Laura K. Nisenbaum is a full-time employee of Biogen. Nathalie Le Bastard and Manu Vandjick are full-time employees of Fujirebio Europe. Christopher Traynham has a collaboration contract with ADx Neurosciences and Quanterix, and performed contract research or received grants from AxonNeurosciences, Biogen, Boehringer, and Brainstorm. Henrik Zetterberg has served on scientific advisory boards for Denali, Roche Diagnostics, Wave, Sanummed, Siemens Healthineers, Pintelon Therapeutics, Celgene, EIP Pharma, Esai, Janssen prevention center, Roche, Toyama, Vivoryon, and CogRx; has given lectures in symposia sponsored by Fujirebio, Alzecure, and Biogen; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. Kaj Blennow has served...
as a consultant, on advisory boards, or on data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. Salvatore J. Salamone is a full-time employee and shareholder of Saladax Biomedical, Inc.

REFERENCES


