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Invited critical review

Pre-analytical and analytical factors influencing Alzheimer's disease cerebrospinal fluid biomarker variability

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ABSTRACT

A panel of cerebrospinal fluid (CSF) biomarkers including total Tau (t-Tau), phosphorylated Tau protein at residue 181 (p-Tau) and β-amyloid peptides ($A\beta_{42}$ and $A\beta_{40}$), is frequently used as an aid in Alzheimer's disease (AD) diagnosis for young patients with cognitive impairment, for predicting prodromal AD in mild cognitive impairment (MCI) subjects, for AD discrimination in atypical clinical phenotypes and for inclusion/exclusion and stratification of patients in clinical trials. Due to variability in absolute levels between laboratories, there is no consensus on medical cut-off value for the CSF AD signature. Thus, for full implementation of this core AD biomarker panel in clinical routine, this issue has to be solved. Variability can be explained both by pre-analytical and analytical factors. For example, the plastic tubes used for CSF collection and storage, the lack of reference material and the variability of the analytical protocols were identified as important sources of variability. The aim of this review is to highlight these pre-analytical and analytical factors and describe efforts done to counteract them in order to establish cut-off values for core CSF AD biomarkers. This review will give the current state of recommendations.

Contents

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1. Introduction

Alzheimer's disease (AD) is the most common type of dementia and is characterized by progressive neuronal degeneration, aggregation of β-amyloid and hyper phosphorylated Tau proteins into plaques and tangles, leading to progressive loss of cognitive functions [1]. A diagnosis of AD made on pure clinical criteria is uncertain even in the clinical stage of mild dementia; this uncertain diagnosis has caused problems in clinical trials, where 10–30% of enrolled patients did not have AD pathology [2]. In the prodromal stage of the disease (mild cognitive impairment due to AD), the diagnostic criteria, including cerebrospinal fluid (CSF) biomarkers, still remain in the research field [3,4]. It is well accepted that the use of biomarkers (imaging or CSF biomarkers) in specialized centers can improve the diagnostic certainty for AD [5]. The core CSF biomarker panel for AD diagnosis includes a decrease in the concentration of the 42 amino acid long amyloid-β peptide ($A\beta_{42}$) reflecting plaque pathology, together with an increase of total Tau (t-Tau) and phosphorylated Tau 181 (p-Tau) proteins, which reflect axonal degeneration and Tau pathology [6,7]. More recently, a decrease of the $A\beta_{42}/A\beta_{40}$ ratio has also been implemented in several specialized centers [8–10], [Dorey et al. submitted]. The use of AD biomarkers for routine diagnostic purposes is at the present time only proposed to be optional for demented patients when deemed appropriate by the clinician, especially in patients with early-onset dementia, or with atypical AD [11].

The significant variability in measured biomarker levels found in various studies, resulting in a high variability of both the diagnostic accuracy [12] and the clinical cut-off for the diagnostic of AD, with two to threefold differences between the highest and the lowest reported cut-off values in Europe [13], is a hindrance to the general implementation of these markers and their integration in the diagnostic criteria [3]. Recently, a consensus report established the main pre-analytical factors contributing to the variation of the laboratory results before the analysis of the sample and concluded that pre-analytical phase should be standardized for CSF AD biomarker analysis [14]. However, the importance of some pre-analytical confounding factors highlighted in that report remains to be elucidated. Concerning the analytical phase, the introduction of an external quality control program revealed a great dispersion of results among participants [15]. This variability could be partly explained by the lack of reference material and relatively unstandardized operating procedures. The aim of this report is to discuss and focus on main critical points in the pre-analytical and analytical steps likely to be responsible for the variability of data.

2. Influence of confounding factors in the pre-analytical phase

The confounding factors in pre-analytical phases of biochemical analysis may have a great impact on the reliability of the results. Several experimental studies support this assessment for the core CSF AD biomarkers [16–18]. Confounding factors are classically listed in a "catalog" dichotomized in two different groups: "in vivo" or biological factors directly linked to the patient and "in vitro" factors linked to the procedure of sample handling and processing. However, we chose to present them based on the effect size of their potential influence: main factors requiring standardization and minor factors for which no specific recommendation is needed.

2.1. Confounding factors with major effects

Here we present factors causing major modifications of CSF biomarkers concentrations in a logical order, from sampling to freezing/thawing of samples before analysis.

2.1.1. The kind of needle used for CSF collection

The type and the internal diameter of needle may be a factor contributing both to the side effects observed in some patients and to the presence of blood contamination. Comparative studies gave a consensus that decreasing the inner diameter of the needle and using preferentially atraumatic than traumatic needles could decrease the percentage of hemorrhagic CSF samples and the percentage of post-lumbar puncture headaches [19–22]. However, the exact inner diameter to be used remains debating and seems to depend partially on the age of patients [23].

2.1.2. The nature of sampling tubes

Several reports have shown that polypropylene (PP) tubes should be preferred to glass or polystyrene (PS) tubes for collection of CSF since Aβ peptides, but also t-Tau and p-Tau, may bind in a non-specific manner to the two last ones [16,18,24]. Yet, these studies generalized the results to generic PP tubes whereas they did not test a large panel of different PP tubes leading to the conclusion about the apparent superiority of PP tubes against PS or glass tubes. It should be noted that the guidelines used today are based on these reports. Within the PP family, there is a high heterogeneity of plastic polymer composition as we have reported by calorimetry and spectroscopy analysis [17]. Moreover, surface treatments (as plasma gas treatment of tetra fluorine carbon, of anionic or cationic detergents…) at the late stages of their manufacturing are also a source of variability, modifying the hydrophobic/hydrophilic properties of their surface. For example, two independent studies reported significant differences on $A\beta_{42}$ levels when CSF was collected in PP tubes from different suppliers [17,25]. This adsorption occurs quickly (15 min) and is highly dependent on the total amount of proteins present in CSF [17]. The main message learnt from these studies is that pure untreated PP tubes were the worst, probably due to their hydrophobic nature which enables hydrophobic interactions with Aβ peptides. Finally, the best tubes regarding Aβ recovery were found to have been treated onto the walls, independently of the nature of plastic. The exact nature of this treatment is unfortunately not available, the information being protected by companies [17,26]. It has been shown that the adsorption of Aβ peptides was significantly reduced when Tween-20 was mixed with CSF in the tube for example [25,27]. In line with this, we reported similar results using various plasma treatments of the tube surface, which modified the adsorption of different proteins such as prion protein, Tau and alpha synuclein [26]. Indeed, tubes that performed better for $A\beta_{42}$ gave on the other side a slight decrease of p-Tau levels (only a trend with a mean decrease of 10%, in the analytical coefficient of variation of the assay) while t-Tau levels remained unmodified, suggesting once again that hydrophobic/hydrophilic balance is an important aspect in protein adsorption [17,26]. In addition to the previous observation, it is worth noting that adsorption was most pronounced if the sample volume was low, i.e., if there was a low volume to surface ratio [28].