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Increased Detection of Aβ Oligomers in the Cerebrospinal Fluid of Alzheimer's Disease: Fact or Artifact?

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The peptides Amyloid beta 1–40 and 1–42 ($A\beta_{40}$, $A\beta_{42}$) are physiological derivatives of the amyloid precursor protein (APP), a normal constituent of neuronal and glial membranes. $A\beta_{40}$ and $A\beta_{42}$ are released from the brain tissue into the interstitial and the cerebrospinal fluid (CSF), and are removed through the blood-brain barrier. Huge amount of studies clearly demonstrated that CSF $A\beta_{42}$ levels in Alzheimer's disease (AD) correlate inversely with the total Aβ load deposited in amyloid plaques in the brain which together with neurofibrillary tangles represent the histological hallmark of AD. Therefore, it is generally accepted that monomeric Aβ in the CSF is decreased because aggregated Aβ is deposited in plaques in the brain. Actually, a variable number of Aβ monomers can self-aggregate into soluble Aβ oligomers of different molecular sizes that in turn may constitute the precursors of insoluble large fibrils deposited in amyloid plaques in the brain. Although the decrease of $A\beta_{42}$ monomers in CSF is related to the pathologic process in the brain and represents a good diagnostic biomarker of AD *in vivo*, this constitutes in essence an epiphenomenon of the disease. Unlike Aβ monomers, Aβ oligomers display neurotoxic activity *in vitro*, and together with Aβ fibrils could play a pathogenic role in the AD process [1].

A soluble pool of $A\beta_{40}$ and $A\beta_{42}$ oligomers together with the large insoluble Aβ fibrils can be extracted by analytical ultracentrifugation of tissue homogenates from the cerebral cortex of AD affected brains, demonstrating that soluble oligomeric Aβ species are intrinsic to the brain AD pathology [2]. Therefore, interest has raised to whether Aβ oligomers could constitute a better biomarker of AD pathology than Aβ monomers: the presence of Aβ oligomers in the CSF *in vivo* could indeed represent a biomarker of the high propensity of Aβ peptides to aggregate more in AD than in normal healthy controls.

A few studies have tried to detect Aβ oligomers in the CSF using different sophisticated techniques, probably because the detection of Aβ oligomers in the CSF is limited by their low content. At variance with studies showing low levels of $A\beta_{42}$ monomers in CSF of AD patients, the level of Aβ oligomers seems to be higher in body fluids of AD than in control patients [3]. The detection of Aβ self-aggregation was first reported by Pitschke et al. [4] in the CSF of Alzheimer's patients using fluorescence correlation spectroscopy. In the study of Pitschke et al. [4] fluorescent labeled synthetic $A\beta_{42}$ monomers were added to human CSF samples and detected by fluorescence correlation spectroscopy, demonstrating the rapid aggregation of the exogenous $A\beta_{42}$ onto the endogenous multimeric $A\beta_{42}$, pre-existing in the CSF and acting as "seeds" for polymerization in patients with AD. Such process of "seed" Aβ42 polymerization appeared to be specific to patients with AD [4]. Several years later, Georganopoulou et al. [5] used monoclonal and polyclonal antibodies specific for so called amyloid-derived diffusible ligands (ADDLs): the detection of the immune-reaction product was amplified using a nanoparticle-based biobarcode assay to demonstrate that ADDLs (molecular weights between 17 and 42 kDa) are elevated significantly in AD patients compared to age-matched controls. Fukumoto et al. [6] developed a novel enzyme- linked immunosorbent assay and demonstrated elevated levels of high molecular weight (HMW) Aβ oligomers of 45- to 90-KDa in the CSF of AD patients. Such HMW oligomers account however for a very small amount (<1%) of the total oligomer mixture in the CSF of AD patients, which is instead predominantly composed of low molecular weight (LMW) oligomers [6].

Actually, the levels of Aβ oligomers in the CSF have become a controversial research topic. Gao et al. [7] developed a complex method using a synthetic ligand, the Aggregate Specific Reagent 1 (ASR1) that preferentially binds aggregated proteins over monomeric proteins. The CSF samples were incubated with ASR1 and the captured Aβ was detected by a multiplex immunoassay specific for $A\beta_{40}$ or $A\beta_{42}$. Preliminary validation of this assay with 26 clinically diagnosed AD patients and 10 age-matched controls surprisingly found only enrichment of $A\beta_{40}$ oligomers, but not of $A\beta_{42}$ oligomers in the CSF of AD patients. Recently, Santos et al. [8] used Aβ specific antibodies and subsequent detection based on a fluorescence resonance energy transfer (FRET) setup by flow cytometry: using such setup no difference was observed in the levels of oligomers between AD and control groups [8]. Finally, Klyubin et al. [9] found that Aβ dimers which in vitro disrupt synaptic plasticity were more frequently selected in CSF from people cognitively normal than from patients with AD.

Moreover, Sehlin et al. [10] cast doubt as to whether the reported increased levels of Aβ oligomers in the CSF by Georganopoulou et al. and Fukumoto et al. [5,6] are true or due to the interference of the heterophilic antibodies (HA) in the detection of Aβ oligomer with ELISAs [10]. HA, i.e. naturally occurring human antibodies to immunoglobulins of animal origin, can be source of interference in immunometric routine assays. Sehlin et al. [10] indeed studied the Aβ oligomer content with a sandwich ELISA in CSF samples from 104 individuals, and showed that the Aβ oligomer signals from the positive samples were strongly reduced when analyzed in the presence of factors blocking HA. It is worth noting that sick and hospitalized patients have higher levels of HA, so that these individuals may have an increased risk of generating a false positive response in any sandwich immunoassay [10,11].

In conclusion, increased detection of Aβ oligomers in CSF of AD patients could be due to an interference from HA generating a false positive response when using sandwich-like immunoassays. Therefore, there is an urgent need to validate a high sensitive and specific method of detection of Aβ oligomers in CSF before their reproducible assay

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could be pursued in the setting of common biomedical research institutions.

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