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Oligomeric Aβ in the cerebrospinal fluid of patients with early Alzheimer's Disease: A pilot study

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Abstract

Background: Oligomerization of amyloid β (Aβ) peptides occurs decades before the onset of clinical symptoms of Alzheimer's disease (AD). It is considered, according to the amyloid hypothesis, one of the earliest pathologic events in the disease, perhaps even triggering the cascade of pathophysiologic reactions leading to neurodegeneration and dementia. One of the hypotheses, why Aβ1-42 is decreased in the cerebrospinal fluid (CSF) of AD patients, proposes formation of oligomeric Aβ as an explanation for monomers' epitope masking. Furthermore, Aβ oligomers are considered highly neurotoxic.
Methods: With a novel Amyloid-beta Toxic Oligomers ELISA (#27709, Immuno Biological Laboratories Ltd., provided by IBL International, Hamburg, Germany) designed to specifically quantify CSF concentrations of oligomeric Aβ, CSF samples were analyzed from carefully selected patients with mild cognitive impairment with AD pathology (MCI-AD, n=14), dementia due to AD (ADD, n=16), and non-demented controls (n=25), whose clinical characterization was confirmed with the results of the neurochemical dementia diagnostics (NDD) biomarkers in all cases (Aβ1-42, Aβ42/40 ratio (IBL International), Tau, and pTau181 (Fujirebio Europe, Ghent, Belgium)).
Results: Intra-assay coefficients of variation (CVs) were 1.2% and 0.8% for two CSF control samples (eight repetitions of each, sample dilution 1:2). Inter-assay CV was 6.4% (four repetitions). After adjusting for sex and age of the individuals, MCI-AD subjects, but not ADD patients, had significantly higher concentrations of the CSF toxic Aβ oligomers (87.7 ± 4.7 pg/mL, p=0.038, and 85.7 ± 11.1 pg/mL, p=0.55, respectively) compared to the controls (82.4 ± 8.3 pg/mL). Interestingly, we observed significant correlation between the concentrations of toxic Aβ oligomers and Aβ1-40. Neither age nor sex (adjusted for the concentrations of oligomeric Aβ) significantly influenced the concentrations of oligomeric Aβ.
Conclusions: The results of this pilot study suggest increased concentrations of CSF oligomeric Aβ in the early stage of AD, which then seems to normalize. Further studies are necessary to confirm our findings.

Materials and Methods

1. The assay; intra- and inter-assay imprecision

The analyses were performed with a previously described ELISA, generated with antibodies specifically recognizing toxic Aβ oligomers in the human CSF (Murakami et al, *Sci Rep*, 2016). Briefly, 100µL of a sample (pre-diluted CSF/calibrators/controls) were pipetted into a well of a microtiter plate pre-coated with 82E1 antibody. After overnight incubation and washing, HRP-labeled anti-AβE22P 24B3 monoclonal antibody was applied, followed by incubation and washing. The signal from the substrate (TMB) was read at 620 nm with a correction at 450 nm.

To study intra-assay imprecision, two quality control (QC) CSF samples were prepared and diluted 1:2 and 1:4 each, and assayed eight times on one plate. To study inter-assay imprecision, one CSF sample (diluted 1:2) was assayed on four ELISA plates on four days. Imprecision results are expressed as coefficients of variation (CVs). All analyses were performed in duplicates.

2. Patients

CSF samples were analyzed from carefully selected 55 individuals with mild cognitive impairment with high probability of Alzheimer's Disease (MCI-AD, n=14, age 71.5 ± 9.6 yrs, 5 males), AD in the dementia stage (ADD, n=16, age 67.7 ± 8.7 yrs, 8 males), and non-demented controls (n=25 age 56.1 ± 10.8 yrs, 16 males). The clinical diagnoses of all individuals were confirmed by the results of the four core neurochemical dementia diagnostics (NDD) CSF biomarkers: Aβ1-42, Aβ42/40 ratio, Tau, and pTau181.
The CSF samples were collected by lumbar puncture into polypropylene test tubes, centrifuged, portioned into aliquots of ca. 150µL, frozen, and stored at -80°C until analyses.

3. Statistical analysis

Linear regression was used to model oligomeric Aβ CSF concentration as a function of the diagnostic categories, age, and sex, followed by estimation of marginal predictors and 95% confidence intervals (95%CI). The correlation between the CSF concentrations of oligomeric Aβ and Aβ1-40 was tested with Spearman's rank correlation coefficient. A p<0.05 was considered significant. All analyses were performed with Stata 14.2 (StataCorp, College Station, TX, USA).

Results

Intra-assay CVs of the four QC samples were between 0.9% - 2.1%, with slightly lower CVs in the two samples diluted 1:2, and hence this dilution factor was used in the following analyses of the patients' CSF samples. Inter-assay CV was 6.4%.
The results of the patients' samples are presented in figure 1 and table 1. After adjusting for sex and age of the individuals, MCI-AD subjects, but not ADD patients, had significantly higher concentrations of the CSF toxic Aβ oligomers (87.7 ± 4.7 pg/mL, p=0.038, and 85.7 ± 11.1 pg/mL, p=0.55, respectively) compared to the non-demented controls (82.4 ± 8.3 pg/mL). Neither age nor sex (adjusted for the concentrations of oligomeric Aβ) significantly influenced the concentrations of oligomeric Aβ.
Interestingly, we observed significant, apparently non-linear correlation between the concentrations of the Aβ oligomers and Aβ1-40 (r=0.73, p<0.001, figure 2).

Discussion and conclusions

Oligomerization of Aβ is, according to the amyloid hypothesis, the first pathophysiologic event in AD. Therefore, it is reasonable to assume that development of a method specifically quantifying oligomeric forms of Aβ would significantly improve early NDD. Unfortunately, the task seems to be uneasy, and the results published so far are conflicting and difficult to reproduce.
In this study, we applied a currently developed method to quantify CSF concentrations of oligomeric Aβ (Murakami et al, *Sci Rep*, 2016), and aimed at its validation on a larger and better characterized cohort. For example, in contrast to the original report, the diagnoses of the patients of this study were confirmed by the results of the four core CSF NDD biomarkers in all cases.
The results of the current study suggest a transient increase in the concentrations of the CSF oligomeric Aβ at the earlier stages of the disease (MCI-AD group), which then seems to normalize (ADD group). This would correspond to the assumption that the Aβ oligomerization occurs early in the AD process, or perhaps even triggers it.
Somehow unexpectedly, we observed statistically highly significant correlation between the CSF concentrations of oligomeric Aβ and Aβ1-40; this finding might be explained by a cross-reactivity of the assay (which, however, was claimed to be excluded in the study describing the development of the method) or by the presence of Aβ40 oligomers in the human CSF, as suggested f. e. by Hölttä et al (*PLoS ONE*, 2013). In any case, further studies are necessary to confirm if the current method could be included into the panel of the routine NDD biomarkers.

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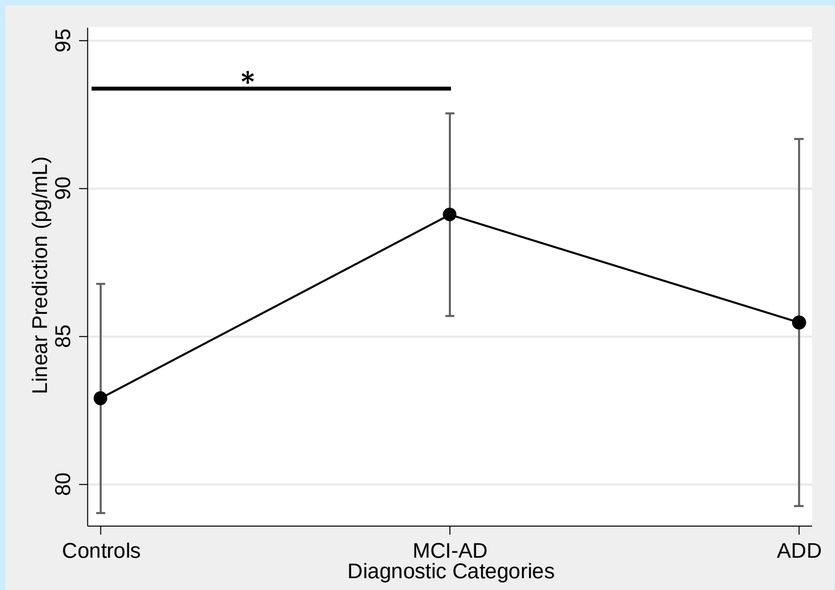

Fig. 1. Predictive margins of the CSF oligomeric Aβ concentrations, adjusted for age and sex, and the corresponding 95%CI.

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Linear regression                               Number of obs     =         55
                                                F(4, 50)          =       3.03
                                                Prob > F          =     0.0258
                                                R-squared         =     0.1170
                                                Root MSE          =      8.644

------------------------------------------------------------------------------
                             |               Robust
Toxic Aß Oligomers           |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-----------------------------+------------------------------------------------------------------
                      Groups |
(Controls (0), reference)    |
                   MCI-AD (1)|    6.21071   2.916655     2.13   0.038     .3524358    12.06898
                     ADD (2) |    2.56388   4.172919     0.61   0.542    -5.817674    10.94543
                             |
                         Age |   .0495459   .1140543     0.43   0.666     -.179539    .2786308
                Female gender|   .8899537   2.376184     0.37   0.710    -3.882752    5.66266
                    Constant |   79.34896   7.051529    11.25   0.000     65.18555    93.51237
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Tab. 1. Linear regression model with the diagnostic categories (Groups), age, and sex as explanatory variables for the CSF concentrations of oligomeric Aβ.

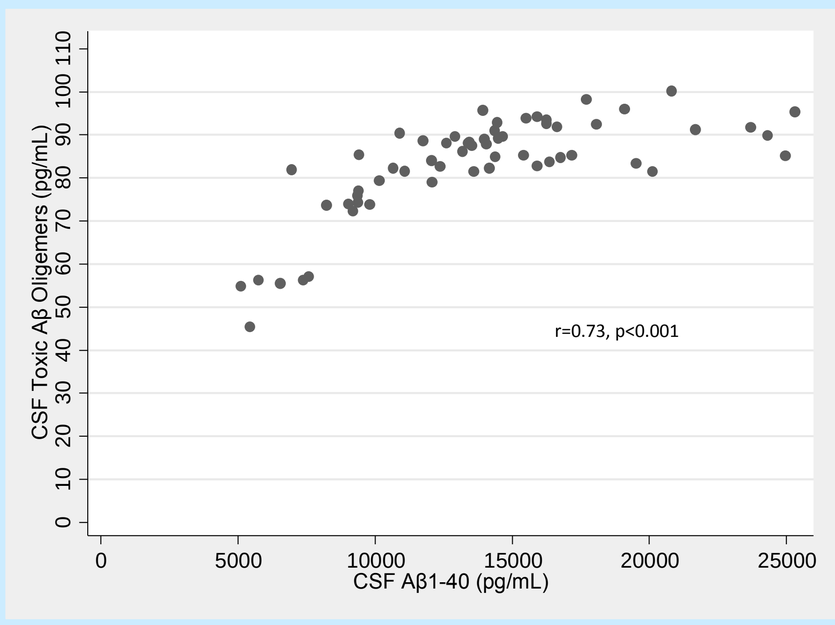

Fig. 2. Correlation between the CSF concentrations of Aβ1-40 and oligomeric Aβ.