Department of Psychiatry and Psychotherapy
Chairman: Prof. Dr. med. Johannes Kornhuber

Universitätsklinikum Erlangen

Early diagnosis of Alzheimer's disease with the Amyloid β 42/40 CSF concentration ratio: Analytical and clinical validation of two novel assays

Piotr Lewczuk*, Natalia Lelental, Juan Manuel Maler, Philipp Spitzer, Johannes Kornhuber

Department of Psychiatry and Psychotherapy, Universitätsklinikum Erlangen, and Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany

Conclusions

(1) In this study, the analytical validation of the novel Aß1-40 and Aß1-42 ELISA assays (IBL International GmbH, Hamburg, Germany) taking into consideration their specificities, linearity, precision, repeatability of the standard curves, recovery, etc., showed that the novel IBL International Aß1-40 and Aß1-42 ELISA assays characterize with excellent analytical performance;
(2) Moreover, the simultaneous measurement of the two Aß isoforms by splitting one diluted CSF sample and using the same protocol for both assays means a huge improvement over other currently available assays for establishing the Aß42/40 ratio; and
(3) A clinical study in order to test the hypothesis of better CSF Aß42/40 diagnostic performance compared to the CSF Aß42 concentration alone reconfirmed that the CSF Aß42/40 concentration ratio shows significantly better diagnostic performance compared to measuring the CSF Aß42 concentration alone.

Background

Cerebrospinal fluid (CSF) biomarkers for Neurochemical Dementia Diagnostics (NDD) constantly gain attention for the early diagnosis of Alzheimer's disease (AD), which is reflected by their increasing role in different diagnostic and/or research criteria. The growing body of evidence resulting from our studies as well as the research of other laboratories suggests better diagnostic performance of the Amyloid ß (Aß) 42/40 concentration ratio compared to measuring the CSF Aß42 concentration alone.

Materials and Methods

1. Preparation of the assays; generation of the antibodies; standards The Amyloid-ß (1-40) CSF ELISA (Catalog-No: RE59651) and the Amyloid-ß (1-42) CSF ELISA (Catalog-No: RE59661) were provided by IBL International GmbH (Hamburg, Germany). Both assays employ the sandwich ELISA principle. The assays use a monoclonal antibody either directed against the C-terminus of the Aß1-40 peptide or against the C-terminus of the Aß1-42 peptide, which are coated onto the surface area of the microtiter plate. The presence of the captured peptides (Aß1-40 or Aß1-42) is detected by the concomitant binding by the N-terminus specific monoclonal antibody (clone 82E1) conjugated with a horseradish peroxidase (HRP). Tetramethylbenzidine (TMB) is used as a chromogenic substrate.
2. Imprecision of the assays The intra- and inter-assay, as well as the inter-lot imprecision were tested by repeated measurements of the quality control (QC) samples with different concentrations of the two commercial peptides. These QC samples were derived from stabilized pooled human CSF, into which various amounts of Aß1-40 or Aß1-42 were spiked.
3. Patients; CSF samples handling, assays comparisons The study in the human samples was approved by the ethical committee of the University of Erlangen-Nürnberg. Patients with early AD and MCI (the AD-MCI group, n=75) were diagnosed according to the recently proposed research criteria, taking into consideration not only clinical and neuropsychological testing but also a broad spectrum of neurochemical and neuroimaging biomarkers. The Control group (n=45) consisted of patients without memory impairments.

Results

1. Cross-reactivity Only marginal cross-reactivity (Aß42 vs. Aß40) was observed; recoveries were in the range of 85 - 100% for the samples diluted 1:20 - 1:640 (Aß1-40), and 92 - 104% for the samples diluted 1:20 - 1:320 (Aß1-42). The goodness of the fit of the average standard curves was > 0.99 for both assays, and the imprecision of the optical densities in ten repetitions of the standard curves was ≤ 5% for all standards.
2. Direct method comparison Figure 1 shows the data of the direct method comparison between the two novel Aß assays (IBL International, Hamburg, Germany) and the 'reference assays' (Aß1-40 from IBL Japan, and Aß1-42 from Fujirebio Europe):

- For Aß 1-40 (left), the observed correlation coefficient (R) was 0.93 (95% CI: 0.90-0.95);
- For Aß 1-42 (right), the correlation coefficient was 0.92 (95% CI: 0.88-0.94).

3. Intra-assay, inter-assay, and inter-lot imprecision Table 1 shows intra- and inter-assay, and inter-lot, imprecision of the two novel Aß assays.

- For Aß1-40, the median intra-assay imprecision was 2.1%, the median inter-assay imprecision was 4.4%, and the median inter-lot imprecision was 5.4 %;
- For Aß1-42, the values were 3.1%, 6.2%, and 6.9%, respectively;
- Median imprecision of the duplicate determinations of the human CSF samples, expressed as the range-to-averages, was 3.2% for Aß1-40, with 2 values out of 119 higher than 15%, and 2.6% for Aß1-42 with all 120 values below 15%.

4. Clinical validation; comparison of sensitivities and specificities Figure 2 shows the CSF Aß40 and Aß42 concentrations and the Aß42/40 concentrations ratio.

- At the cut off value 691 pg/mL, Aß1-42 showed sensitivity of 69.3% and specificity of 88.9%;
- At the cut off value 0.06, Aß42/40 ratio showed sensitivity of 93.3%, and specificity of 100 %.

Figure 3 shows that the area under the ROC curve (AUC) for Aß42/40 (0.974) was highly significantly larger compared to the AUC of the Aß1-42 concentration ROC curve (0.827, p<0.0001).

Discussion

Interestingly, Aß1-40 concentrations in the AD-MCI group turned out to be significantly higher compared to the Controls. Aß1-42 concentrations were highly significantly lower in the AD-MCI group compared to the Controls, and similarly Aß42/40 ratio was highly significantly lower in AD-MCI compared to the Controls. To avoid a potential bias, resulting from the fact that in our AD-MCI group Aß1-40 concentrations were higher compared to the Controls, we repeated the ROC curves comparisons after the adjustment of the groups to equalize the Aß40 concentrations. Even after this adjustment, the area under the ROC curve of the Aß42/40 ratio remained highly significantly larger compared to the AUC of the Aß1-42 ROC curve (p=0.0018; not shown).

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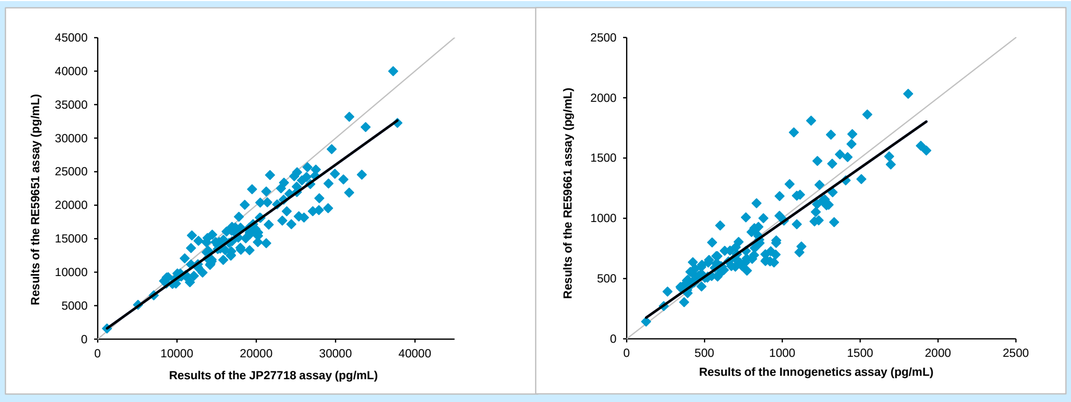

Fig. 1. The results of the method comparisons; presented are the individual results, the Passing-Bablok regression lines (solid lines), and the lines of identity.
Left panel, Aβ1-40 (R=0.93); right panel, Aβ1-42 (R=0.92).

Assay	Variable	Sample				
		1	2	3	4	5
Aβ1-40	Intra-assay	1.8 % (4,732)	2.1 % (9,937)	4.5 % (3,080)	1.9 % (10,497)	2.8 % (13,506)
	Inter-assay	4.4 % (4,967)	4.0% (19,875)	4.4 % (10,913)	6.4 % (2,257)	4.8 % (7,764)
	Inter-lot	5.4 % (2,418)	6.3 % (3,830)	4.1 % (19,407)	5.8 % (13,164)	2.7 % (9,405)
Aβ1-42	Intra-assay	3.4 % (548)	3.0 % (1,023)	3.0 % (849)	3.1 % (951)	3.1 % (1,034)
	Inter-assay	6.3 % (566)	5.7% (418)	6.2 % (770)	7.1 % (1,010)	5.6 % (1,088)
	Inter-lot	4.5% (193)	7.6 % (476)	6.9 % (661)	4.7 % (728)	7.4 % (873)

Tab. 1. Imprecision of the assays. Presented are the coefficients of variation (CV) and, in brackets, the average concentrations obtained in the experiments (in pg/mL).

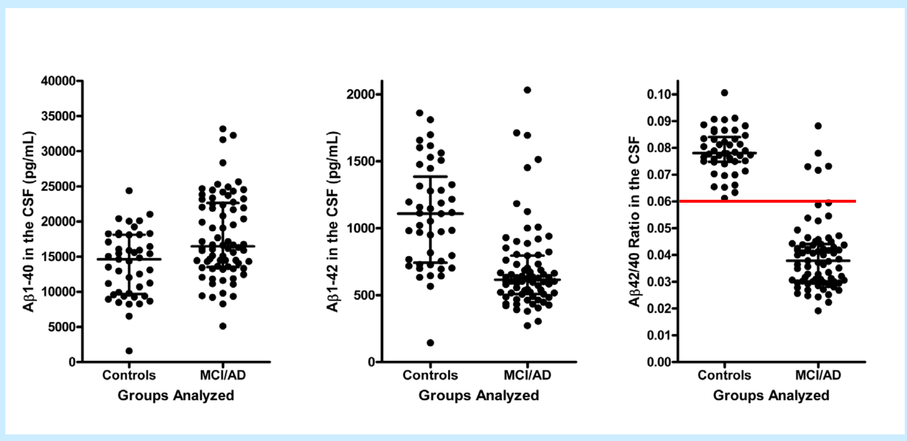

Fig. 2. Concentrations of Aβ1-40, (left) and Aβ1-42 (middle), and the Aβ42/40 ratios (right) in the patients groups; presented are the individual results, the medians and the interquartile ranges; dotted line on the right panel represents the Aβ42/40 cut off at the highest Youden index.

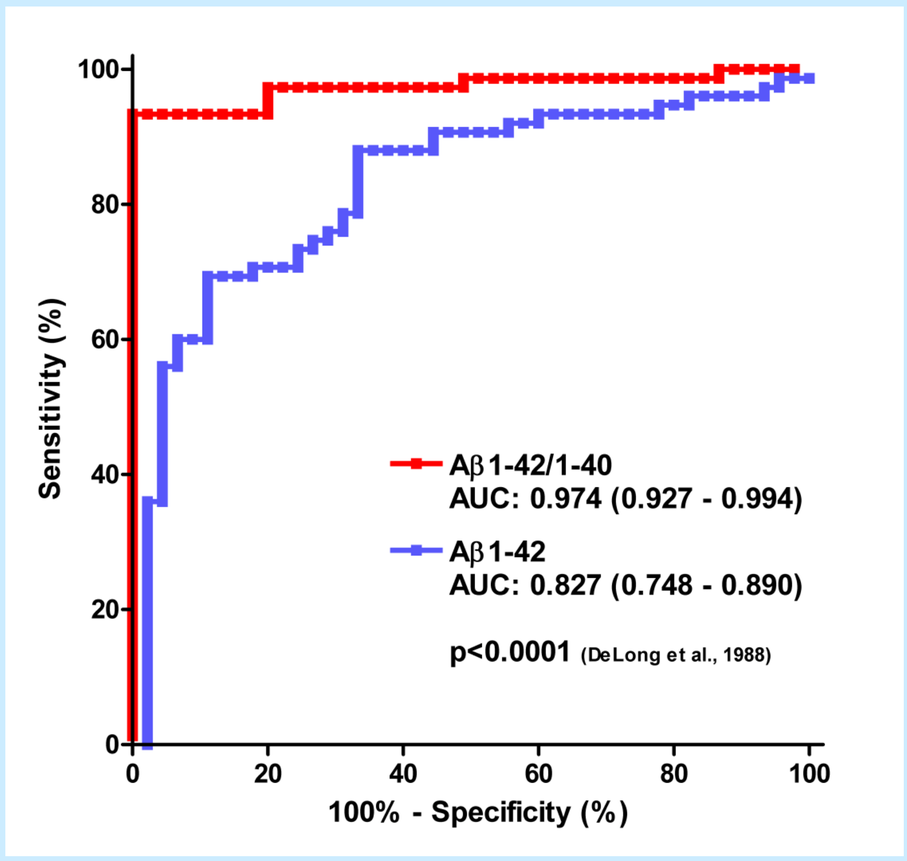

Fig. 3. Receiver operating characteristic (ROC) curves of the two biomarkers; presented are the areas under the curves (AUC) and the corresponding 95% confidence intervals; the statistical comparison of the AUC was performed with the DeLong test.

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MEDIZINISCHE FAKULTÄT

***Corresponding author:**
Prof. Dr. med. Piotr Lewczuk; Lab for Clinical Neurochemistry and Neurochemical Dementia Diagnostics,
Department of Psychiatry and Psychotherapy, Universitätsklinikum Erlangen, Schwabachanlage 6, 91054 Erlangen, GERMANY
E-mail: Piotr.Lewczuk@uk-erlangen.de