Differences and Similarities between Two Frequently Used Assays for Amyloid β42 in Cerebrospinal Fluid, Niki S.M. Schoonenboom,1,2* Coes Mulder,2 Hugo Vanderstichele,3 Yolande A.L. Pijnenburg,1 Gerard J. Van Kamp,2 Phillip Scheltens,1 Pankaj D. Mehta,4 and Marinus A. Blankenstein1 (1) Alzheimer Center and Department of Neurology, and 2 Department of Clinical Chemistry, VU University Medical Center, Amsterdam, The Netherlands; 3 Innogenetics NV, Ghent, Belgium; 4 Institute for Basic Research in Developmental Disabilities, Department of Developmental Neurobiology, Division of Immunology, Staten Island, NY; * these authors equally contributed to the work; † these authors equally contributed to the author at: Departments of Neurology and Clinical Chemistry, VU University Medical Center, PO Box 7057, 1081 HV Amsterdam, The Netherlands; fax 31-(0)204440715, e-mail niki.schoonenboom@vumc.nl

Amyloid β 42 (Aβ42) concentrations in cerebrospinal fluid (CSF) are used to identify Alzheimer disease (AD) (1), but reported concentrations differ among studies, as does diagnostic accuracy (2). These differences may relate to the patient and control groups studied (3), processing and storage methods (4), intra- and interassay variation of the assays, or to the reagent antibodies used. A recent metaanalysis (2) stressed the importance of standardizing assays for Aβ42 in CSF. In most studies, CSF Aβ42 was reported to be decreased, but in 2 studies CSF Aβ42 was not significantly changed in AD (2), and in 1 study (5), it even increased in the early stages of disease. These dissimilarities might reflect the specificities of the antibodies incorporated in the assays.

The first aim of our study was to compare Aβ42 concentrations measured by 2 different assays in the same CSF samples. The first assay, widely used in Europe (6), uses 2 monoclonal antibodies (mAbs) and detects the full-length Aβ42 peptide, Aβ(1–42) (7). The second assay [Aβ(N-42)], used mainly in the United States (8), detects both full-length Aβ42 and Aβ peptides truncated at the NH2 terminus (9).

The second aim of our study was to compare diagnostic accuracies of the assays for patients with AD compared with controls without dementia and patients with frontotemporal lobar degeneration (FTLD).

Finally, we investigated the relationship between CSF Aβ42 [Aβ(1–42) and Aβ(N-42)] concentrations and albumin ratio, age, disease duration, and disease severity.

Between October 2000 and December 2002, we recruited 39 AD patients, 24 FTLD patients, and 30 non-dementia controls at the Alzheimer Center of the VU University Medical Center (VUMC). All patients underwent a standardized investigative battery (3). A diagnosis of “probable” AD was made according to the NINCDS-ADRDA criteria (10); the clinical picture of FTLD (including frontotemporal dementia, semantic dementia, and progressive aphasia) was based on international clinical diagnostic criteria (11). Disease duration was defined as the time in years between the first symptoms by history and the lumbar puncture.

The control group (n = 30) consisted of 20 persons with subjective memory complaints, who had undergone the same battery of examinations as the patients; 5 spouses of patients; 3 individuals with a positive family history for AD, all without memory complaints; 1 patient with a suspicion of intracranial hypertension; and 1 patient with a possible neuritis vestibularis. No controls developed dementia within 1 year. The Mini Mental State Examination (MMSE) score (12) was used as a measure of global cognitive impairment. The study was approved by the ethics review board of the VUMC. All patients and controls gave written informed consent.

CSF was collected and stored as described previously (4). The albumin ratio (serum albumin/CSF albumin) was used as a measurement of the intactness of the blood–brain barrier. Except for 1 FTLD patient and 2 controls, the blood–brain barriers of the patients were intact (Table 1).

The INNOTESTTM β-AMYLOID(1–42) (Innogenetics) uses mAb 21F12, which binds the COOH terminus of the Aβ42 peptide (amino acids 36–42), as capture antibody and biotinylated mAb 3D6, which binds the NH2 terminus (amino acids 1–6), as detection antibody (6). Aβ(1–42) peptides from Bachem were used for calibration. This test was performed at the Department of Clinical Chemistry, VUMC, Amsterdam.

The sandwich ELISA for Aβ(N-42) uses the commercially available mAb 6E10 (Signet Labs), specific to an epitope covering N-terminal amino acid residues 1–17 of Aβ42, as capture antibody and the polyclonal antibody R165 as detector antibody. R165 was made by immunizing rabbits with conjugated Aβ35–42 peptides (Ana Spec). Aβ(1–42) from Bachem was used for calibration, although production procedures for the calibrators were slightly different between the 2 laboratories. This test was performed at the New York site according to an in-house protocol.

For statistical analysis, we used SPSS (Ver. 11.0). Passing and Bablok regression analyses (13) were performed with Medcalc, Ver. 4.30 (Medcalc Software), and we also prepared a Bland–Altman plot (14). For group differences, we applied the Kruskal–Wallis test, followed by the Mann–Whitney U-test applying the Bonferroni correction. The χ2 test with continuity correction was used to test group differences within genders.

The sensitivities and specificities for CSF Aβ(1–42) and Aβ(N-42) were calculated. Cut points corresponded with a sensitivity ≥85% (15), but if a higher sensitivity was obtained for a reasonable specificity, it was used. ROC curves were constructed, and the areas under the curves (AUCs) were calculated and compared (16). Spearman correlations were calculated. A test was considered significant at P <0.05. All reported tests are 2-tailed unless stated otherwise.

The CSF Aβ(1–42) and Aβ(N-42) concentrations were not statistically significantly different (Table 1 and Figs. 1 and 2 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol51/issue6/). Concentrations of both CSF Aβ(1–42) and Aβ(N-42) were significantly lower in AD...
patients than in patients with FTLD and in controls (Table 1). CSF Aβ (1–42) concentrations differed significantly between FTLD patients and controls, whereas CSF Aβ (N-42) concentrations did not differ significantly between the 2 groups (Table 1). The ratio of Aβ (1–42) to Aβ (N-42) differed significantly only between the AD and FTLD patient groups.

ROC curves for CSF Aβ (1–42) and Aβ (N-42) are shown in Fig. 1. In AD patients vs controls, the sensitivity and specificity for CSF Aβ (1–42) were 90% and 93%, respectively at 473 ng/L and for CSF Aβ (N-42), they were 90% and 87%, respectively, at 383 ng/L. The AUCs were not different (Fig. 1A) for Aβ (1–42) and Aβ (N-42) [0.94 (95% confidence interval, 0.86–0.99) and 0.92 (0.83–0.97), respectively; P = 0.47].

When we compared the AD and FTLD patient groups, we obtained specificity of 67% for CSF Aβ (1–42) at a sensitivity of 85% (448 ng/L). For CSF Aβ (N-42), the specificity was 75% at a sensitivity of 87% (373 ng/L). The AUCs for CSF Aβ (N-42) and CSF Aβ (1–42) tended to be different [Fig. 1B; 0.87 (0.76–0.97) and 0.77 (0.64–0.90); P = 0.045].

The AUCs for CSF Aβ (1–42) and CSF Aβ (N-42) in distinguishing FTLD patients from controls were significantly different [Fig. 1C; 0.69 (0.55–0.81) and 0.54 (0.39–0.67); P = 0.007], but the discriminatory value was small for Aβ (1–42) and negligible for Aβ (N-42) (with the confidence interval for the AUC including 0.5).

We found no significant correlation of either CSF Aβ (1–42) or Aβ (N-42) with albumin ratio, MMSE score, age, or disease duration (AD and FTLD) in either group. The absolute concentrations of CSF Aβ (1–42) and Aβ (N-42) were comparable. However, in earlier studies, concentrations of CSF Aβ (N-42) ranged from 36 to 623 ng/L in AD patients and from 111 to 629 ng/L in controls (8,17,18). The reason for the low CSF Aβ (N-42) concentrations measured in these studies could be a difference in the affinity of the Aβ (N-42) polyclonal antiserum samples or the purity and solubility of the peptides used as calibrators (8). The sensitivity of an ELISA depends largely on the binding characteristics of the antigen, which may vary with temperature and buffer solutions, or among different reagent lots (6). In addition, the affinity of the antibodies used in the assays might vary for the various Aβ 42 peptides involved in the pathogenesis of AD, including oligomers of the Aβ 42 peptide. A future study exchanging calibrators and antibodies among various ELISAs is necessary for harmonization.

ROC curve analysis revealed no difference in the ability of the 2 assays to differentiate AD patients from controls. In addition to the C-terminal heterogeneity, various N-terminally truncated peptides are found in the Aβ pools

---

Table 1. Demographic data and CSF analyses for each diagnostic category.\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>AD (n = 39)</th>
<th>FTLD (n = 24)</th>
<th>Controls (n = 30)</th>
<th>AD vs FTLD</th>
<th>AD vs controls</th>
<th>FTLD vs controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>62 (52–79)</td>
<td>63 (49–85)</td>
<td>64 (32–79)</td>
<td>0.58</td>
<td>0.14</td>
<td>0.66</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>20/19</td>
<td>16/8</td>
<td>14/16</td>
<td>0.26</td>
<td>0.90</td>
<td>0.41</td>
</tr>
<tr>
<td>Duration of disease, years</td>
<td>4 (1–11)</td>
<td>3 (1–11)</td>
<td>30 (25–30)</td>
<td>0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MMSE score</td>
<td>24 (3–29)</td>
<td>23 (3–29)</td>
<td>30 (25–30)</td>
<td>0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aβ 1–42, ng/L</td>
<td>315 (140–626)</td>
<td>495 (202–1087)</td>
<td>651 (337–1224)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.02</td>
</tr>
<tr>
<td>Aβ N-42, ng/L</td>
<td>288 (116–674)</td>
<td>588 (150–1324)</td>
<td>620 (218–1075)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.66</td>
</tr>
<tr>
<td>Aβ 1–42/Aβ N-42</td>
<td>1.1 (0.5–1.7)</td>
<td>0.9 (0.4–1.3)</td>
<td>1.0 (0.6–2.6)</td>
<td>0.001</td>
<td>0.24</td>
<td>0.07</td>
</tr>
<tr>
<td>Albumin ratio</td>
<td>4.8 (2.0–10.6)</td>
<td>5.3 (1.5–17.3)</td>
<td>5.2 (2.8–18.5)</td>
<td>0.6</td>
<td>0.47</td>
<td>0.99</td>
</tr>
</tbody>
</table>

\(^a\) Values are the median (minimum–maximum). P values refer to statistical difference between AD vs FTLD, AD vs controls, or FTLD vs controls.

---

Fig. 1. ROC curves comparing Aβ (1–42) (thick line) with Aβ (N-42) (thin line) in AD vs controls (A), AD vs FTLD (B), and FTLD vs controls (C).
of AD brains (19, 20). These peptides are considered to play a role in the increased Aβ 42 production in developing AD. We speculate that Aβ (1–42) and Aβ (N-42) concentrations go hand in hand at a certain stage of disease, in mild to moderate AD as well as in controls. Because N-terminally truncated Aβ 42 peptides can be demonstrated early in the disease process (9), they might be promising markers for the preclinical diagnosis of AD, when used simultaneously with Aβ (1–42) (21).

Several authors found decreased Aβ (1–42) in CSF from a subset of FTLD patients (3, 22). Very little information is available about the CSF Aβ (N-42) concentration in FTLD (17). The reason for a decrease in CSF Aβ (1–42) in FTLD is unknown, although there might be a relationship with the presence of an ε4 allele or with age (23). Interestingly, a few studies have shown the involvement of 3 mutations in the presenilin 1 gene (PSEN1) in familial forms of FTLD (24–26). These possible “loss of function” PSEN1 mutations might act as inhibitors of the γ-secretase cleavage of APP (27), leading to a decrease of Aβ (1–42) in the brain. Although most FTLD patients included in our study had the sporadic form of FTLD, we cannot exclude the possibility of a mutation in the PSEN1 gene in some of them.

References


DOI: 10.1373/clinchem.2005.048629