CHAPTER 3

Diagnostic impact of CSF biomarkers for Alzheimer’s disease in a tertiary memory clinic

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ABSTRACT

Background: we aimed to assess the impact of CSF biomarkers for Alzheimer’s disease on decision-making and patient management in a tertiary memory clinic.

Methods: we included all patients visiting the VUmc Alzheimer center for cognitive screening during one year. Neurologists completed questionnaires before and after CSF disclosure. We assessed change of diagnosis, diagnostic confidence, and impact on patient management.

Results: 438 patients (age 63±8 years, 39% women) were included, 351(80%) underwent lumbar puncture. After disclosure of CSF 23/351 diagnoses (7%) were changed. Diagnostic confidence increased from 84% to 89% (p<0.001). There were consequences for management in 44/351 patients (13%) with CSF, and 13/87 patients (15%) because of unavailable CSF. There was no effect of age on these results.

Conclusions: CSF biomarkers aid clinicians with decision-making during diagnostic work-up of cognitive disorders. This study may be useful for developing guidelines for implementation of CSF biomarkers in daily practice.
1. Introduction

Over the past two decades much effort has been put in developing biomarkers for Alzheimer’s disease (AD).1,5 Fairly accurate distinction of AD patients from healthy subjects is now possible using CSF biomarkers amyloid β$_{1-42}$ (Aβ$_{42}$), total tau and phosphorylated tau (p-tau).6 As such, these biomarkers have been incorporated in the recent diagnostic criteria for AD.7-9 Most studies assessing diagnostic accuracy of CSF biomarkers, however, have been performed in selected research populations,1-4 while the population of memory clinics is typically more diverse regarding clinical presentation, co-morbidity and other complicating factors. This makes the diagnostic process in routine practice of a memory clinic more challenging, even more so since diagnoses tend to be made in an earlier stage. In addition, the diagnostic criteria do not give explicit recommendations on when and how biomarkers should be used in daily practice. Recently, an effort has been made to develop criteria for appropriate use of amyloid imaging in clinical practice,10 but such recommendations are not yet available for CSF biomarkers.

Previously, we showed that knowledge of CSF biomarkers increased diagnostic confidence of clinicians in a local hospital.11 This study was performed in a selected population however, as lumbar puncture was performed only when diagnostic confidence was low. The aim of the current study was to assess the impact of CSF biomarkers in routine clinical practice in a large memory clinic. To prevent selection bias we included all patients visiting our memory clinic during one year. As part of the standardized work-up, all patients were offered to undergo lumbar puncture after informed consent. We evaluated the considerations of clinicians regarding CSF biomarkers, to assess qualitative as well as quantitative aspects of the use of these biomarkers.

2. Methods

2.1 Patients

492 patients visited our memory clinic between June 2011 and May 2012.12 All patients underwent extensive dementia screening, including physical, neurological and neuropsychological examination, EEG, brain MRI, lumbar puncture, and routine laboratory tests. Diagnoses were made by consensus without knowledge of CSF biomarker results, at weekly multidisciplinary meetings.

The core clinical NIA-AA criteria were used for AD (n=127),8 the International FTD consortium criteria for the behavioural variant of frontotemporal dementia (FTD; n=16),13 McKeith criteria for dementia with Lewy bodies (DLB; n=21),14 NINDS-AIREN criteria for vascular dementia (VaD; n=10),15 criteria of Boeve for corticobasal degeneration (CBD; n=5),16 NINDS-Society for Progressive Supranuclear Palsy criteria for PSP (n=4),17 and the core clinical
NIA-AA criteria for MCI \( (n=74) \).\(^{18}\) Seven patients presented with another type of dementia (one with Parkinson’s dementia, two with semantic dementia, one with normal pressure hydrocephalus, and three with a clinical dementia of unknown etiology), 14 with another neurological disorder (cerebrovascular disease \( n=4 \), normal pressure hydrocephalus \( n=3 \), intracranial tumor \( n=2 \), epileptic syndrome \( n=2 \), neurological symptoms of systemic disease \( n=3 \)), and 28 patients with a psychiatric disorder. The label of subjective cognitive complaints \( n=105 \) was used when results of all clinical examinations were normal, i.e. when criteria for MCI or dementia were not fulfilled, and there was no psychiatric disorder. Patients with subjective complaints, another neurological disorder or a psychiatric disorder were pooled in a ‘no dementia’ group. In 27 patients there was a diagnostic dilemma after the first visit (i.e. ‘unclear diagnosis’). Patients gave written informed consent for the use of their data for research purposes, and for storage of CSF in our biobank. Informed consent was given in accompaniment of the physician and a close relative. The local ethical review board approved the study.

Of the 492 patients, 54 could not be included due to biomarkers known before presentation at our clinic, incomplete or incorrectly filled questionnaires, or absence of informed consent (shown in figure 1). As a result, 438 patients were included in the study.

### 2.2 CSF biochemical analysis

CSF was obtained by lumbar puncture, and collected in 10 mL polypropylene tubes (Sarstedt, Nümbrecht, Germany). Part of the CSF was used for routine analysis including cell count, glucose and total protein concentration. Within two hours, the remaining CSF was centrifuged at 1800 g for 10 minutes at 4°C, transferred to 0.5 mL polypropylene tubes, and stored at -20°C until biomarker analysis, or immediately at -80°C for future research purposes. Aβ42, total tau and p-tau were measured on a routine basis within two months, after thawing once. Commercially available ELISAs (Innotest β-amyloid \(^{1-42} \), Innotesth TAU-Ag and Innotest Phosphotau \(^{181P} \); Innogenetics, Ghent, Belgium) were used, as described before.\(^{19}\) The intra-assay coefficient of variation (CV; mean±SD) was 2.0±0.5% for Aβ42, 3.2±1.3% for tau and 2.9±0.8% for p-tau as calculated from averaging CVs of duplicates from 5 runs randomly selected over 2 years. The inter-assay CV (mean±SD) was 10.9±1.8% for Aβ42, 9.9±2.1% for tau and 9.1±1.8% for p-tau. Cut-offs for abnormal biomarker values were used as previously described; for Aβ42 <550 pg/mL, for tau >375 pg/mL, and for p-tau >52 pg/mL.\(^4\)

### 2.3 Outcome measures

After clinical assessment and establishment of consensus diagnosis, but prior to analysis of CSF AD biomarkers, neurologists (PS, NP, AL, YP or FB) were asked to complete a questionnaire in which they had to estimate their level of diagnostic confidence on a visual analogue scale from 0 to 100%. In addition, the questionnaire included questions on whether they wished to
use biomarker results, and if so, for which reasons they wished to use the results (more than one answer allowed). Neurologists completed this questionnaire irrespective of whether lumbar puncture was actually performed.

After the CSF biomarkers were disclosed, the neurologists completed a second questionnaire, again including estimation of diagnostic confidence, re-evaluation of the diagnosis and questions on consequences of biomarker results for the patient. All clinicians were aware of our in-house established cut-off levels for individual biomarkers. Nevertheless, as we wished to assess how clinicians dealt with sometimes conflicting or ambiguous biomarker results, we invited them to judge the profile as ‘certainly not/maybe/probably/definitely’ an AD profile. The neurologists completed this second questionnaire irrespective of their initial wish to use biomarkers. When no lumbar puncture had been performed, they had to indicate whether they would have wished to use biomarkers, and if yes, whether the fact that there was no CSF had consequences for further management. When completing the second questionnaire, neurologists were blinded to answers they had given on the first questionnaire, to avoid overestimation of change in diagnostic confidence. Mean interval between the first and second questionnaires was 1.4±0.6 months. Main outcome measures were change in diagnosis, diagnostic confidence level and patient management after disclosure of CSF AD biomarker results. In addition, we assessed which proportion of the cohort would hypothetically meet the ‘Appropriate use criteria’ for amyloid imaging (i.e. subjects with unexplained/persisting MCI, patients satisfying the core clinical criteria for possible AD, and patients presenting with a cognitive disorder possibly caused by AD, with atypical age of onset), and assessed differences between these patients and patients not meeting the criteria. Finally, one week after lumbar puncture patients were asked about complaints after the procedure.

2.4 Statistical analysis

For statistical analysis, SPSS version 21.0 (IBM for Windows) was used. Descriptive statistics were performed using chi-squared tests, independent samples T-tests, or ANOVA with post-hoc Bonferroni corrections when appropriate. Differences between patients with and without CSF, and between patients in which neurologists did and did not express the wish to use CSF were assessed with univariate GLM (for continuous variables) or with logistic regression analysis (for categorical and dichotomous variables). Differences between pre- and post-CSF diagnostic confidence levels were assessed with ANOVA for repeated measures, in the total population as well as stratified according to age (<65 vs. ≥65 years). Cases in which the diagnosis changed after disclosure of biomarkers were not included in this last analysis. All analyses were adjusted for clinician (entered as dummy variables). The level of significance was set at p<0.05.
3. Results

Figure 1 shows the study flow chart; table 1 shows baseline characteristics of the study population. Age of the patients was (mean±SD) 63±8 years, 169 (39%) were female, MMSE was 24±5. Of the 438 patients included in the study, 351 patients (80%) underwent lumbar puncture. In the other 20% there was a contraindication such as use of anti-coagulants (n=20), an unsuccessful procedure (n=30), or refusal of the patient to undergo the procedure (n=37). Patients in whom no CSF was obtained had a slightly lower MMSE (23 vs. 25, p<0.05), there were no other differences between these groups.

Table 1. Baseline characteristics of study population

<table>
<thead>
<tr>
<th></th>
<th>Total population (n=438)</th>
<th>LP performed</th>
<th>CSF wished by clinician</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n=351)</td>
<td>No (n=87)</td>
<td>Yes (n=326)</td>
</tr>
<tr>
<td>Age, mean±SD</td>
<td>63 ± 8</td>
<td>63 ± 8</td>
<td>63 ± 10</td>
</tr>
<tr>
<td>Sex, n (%) female</td>
<td>169 (39)</td>
<td>133 (38)</td>
<td>36 (41)</td>
</tr>
<tr>
<td>MMSE, mean±SD</td>
<td>24 ± 5</td>
<td>25 ± 5</td>
<td>23 ± 5 *</td>
</tr>
<tr>
<td>LP performed, n (%)</td>
<td>351 (80)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Wished CSF, n (%)</td>
<td>326 (74)</td>
<td>262 (75)</td>
<td>64 (74)</td>
</tr>
<tr>
<td>Pre-CSF diagnosis, n (%):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No dementia</td>
<td>147 (34)</td>
<td>122 (83) a</td>
<td>25 (17)</td>
</tr>
<tr>
<td>MCI</td>
<td>74 (17)</td>
<td>61 (82) a</td>
<td>13 (18)</td>
</tr>
<tr>
<td>AD</td>
<td>127 (29)</td>
<td>100 (79) a</td>
<td>27 (21)</td>
</tr>
<tr>
<td>Other dementia</td>
<td>63 (14)</td>
<td>48 (76) a</td>
<td>15 (24)</td>
</tr>
<tr>
<td>Unclear diagnosis</td>
<td>27 (6)</td>
<td>20 (74) a</td>
<td>7 (26)</td>
</tr>
<tr>
<td>Meets PET-AUC, n (%)</td>
<td>189 (43)</td>
<td>153 (44)</td>
<td>36 (41)</td>
</tr>
</tbody>
</table>

Displayed are patient characteristics of the total study population, as well as stratified according to performance of lumbar puncture, and whether neurologists expressed the wish to use the CSF results. Analyses were adjusted for clinician (entered as dummy variable) using logistic regression analysis (for categorical variables) or univariate GLM (for continuous variables). Crude data are displayed in the table; presented p-values are adjusted for clinician.

a Percentage of total number of patients in each diagnostic group.
* p < 0.05 vs patients in whom lumbar puncture was performed.
* p < 0.01 vs cases in which neurologists wished CSF results.
† p < 0.01 vs patients with no dementia.
Chapter 3

3.1 Considerations of clinicians

Irrespective of actual availability of CSF biomarkers, neurologists expressed the wish to use biomarkers in 74% (n=326) of patients. On average these patients were older, and had a lower MMSE than patients from whom neurologists indicated biomarker results were not needed (table 1). They more often wished CSF in patients with MCI, AD, other dementia or an unclear diagnosis compared to the ‘no dementia’ group (p<0.01). Figure 2 displays reasons neurologists indicated for wishing to use biomarkers beforehand and for using them after disclosure. Reasons for wishing to use biomarkers were most often to confirm or exclude AD pathology (46% and 36% respectively) or for assessment of prognosis (24%, especially in MCI patients). After biomarker disclosure, neurologists indicated to have used the results in 179/351 cases (51%). Reasons to use CSF were largely similar compared to what they had indicated before disclosure. In 76 patients neurologists indicated after disclosure to find the CSF important for their understanding, yet had not used the results. These were mostly AD patients or non-demented subjects with high pre-CSF diagnostic confidence. In 96 patients (27%) neurologists indicated that CSF had no additional value at all.

Applying the criteria for appropriate use of amyloid imaging, amyloid measurement would be indicated in 189 patients (43%) of the total cohort. Overall concordance between the wish

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**Table 2. Interpretation of CSF biomarker profile**

<table>
<thead>
<tr>
<th>AD profile according to clinician</th>
<th>AD profile according to cut-off values</th>
<th>All normal</th>
<th>Only Aβ42 abnormal</th>
<th>Only tau and/or p-tau abnormal</th>
<th>Aβ42 + tau and/or p-tau</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Certainly not, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>179</td>
</tr>
<tr>
<td>Aβ42, mean±SD</td>
<td></td>
<td>154 (98)</td>
<td>4 (14)</td>
<td>20 (27)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Tau, mean±SD</td>
<td></td>
<td>1001±188</td>
<td>518±35</td>
<td>1079±286</td>
<td>526</td>
<td></td>
</tr>
<tr>
<td>P-tau, mean±SD</td>
<td></td>
<td>221±67</td>
<td>240±96</td>
<td>425±131</td>
<td>372</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>34±9</td>
<td>33±18</td>
<td>10±2</td>
<td></td>
<td>61</td>
</tr>
<tr>
<td>Maybe, n (%)</td>
<td></td>
<td>4 (2)</td>
<td>15 (52)</td>
<td>35 (47)</td>
<td>3 (3)</td>
<td>57</td>
</tr>
<tr>
<td>Aβ42, mean±SD</td>
<td></td>
<td>897±391</td>
<td>448±78</td>
<td>923±354</td>
<td>467±71</td>
<td></td>
</tr>
<tr>
<td>Tau, mean±SD</td>
<td></td>
<td>241±104</td>
<td>265±79</td>
<td>565±216</td>
<td>458±117</td>
<td></td>
</tr>
<tr>
<td>P-tau, mean±SD</td>
<td></td>
<td>36±13</td>
<td>38±11</td>
<td>69±15</td>
<td>796±308</td>
<td></td>
</tr>
<tr>
<td>Probably/Definitely, n (%)*</td>
<td></td>
<td>0 (0)</td>
<td>10 (34)</td>
<td>19 (26)</td>
<td>86 (96)</td>
<td>115</td>
</tr>
<tr>
<td>Aβ42, mean±SD</td>
<td></td>
<td>391±78</td>
<td>615±62</td>
<td>447±71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tau, mean±SD</td>
<td></td>
<td>260±80</td>
<td>826±280</td>
<td>796±308</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-tau, mean±SD</td>
<td></td>
<td>38±10</td>
<td>89±20</td>
<td>86±26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>158</td>
<td>29</td>
<td>74</td>
<td>90</td>
<td>351</td>
</tr>
</tbody>
</table>

Shown are opinions of the clinician compared with the strict cut-off levels of the biomarkers (grouped into all possible combinations of normal and abnormal biomarkers according to the cut-off levels). Cut-off levels were defined as previously described: Aβ42 < 550 pg/mL, tau > 375 pg/mL, p-tau >52 pg/mL. Percentages shown are percentage per column of biomarker combination. Shown in italics are the mean±SD of the biomarker levels within each group.

* Categories ‘probably’ and ‘definitely’ AD profile were combined because proportions of biomarker combinations were not different between these two categories.
492 patients with cognitive complaints presented in our memory clinic in one year. Several patients were excluded from the study: three patients did not give informed consent for use of their clinical data for research purposes, in 22 patients CSF biomarkers were recently analyzed at their first visit at our memory clinic, in 9 patients final clinical assessment by the neurologist – and therefore completion of the questionnaires – was not possible (i.e. when the results of the screening were only communicated by phone, with the patient’s partner or the referring clinician), and in 19 cases questionnaires were incorrectly or not completed. Finally, 438 patients were included in the study.

**Figure 1.** Flow chart of study population

- 492 patients for dementia screening
- 3 no informed consent to use clinical data for research
- 22 in which biomarkers were already known
- 9 without final clinical assessment by neurologist
- 19 with incorrectly/not completed questionnaires

**Diagnosis**
- No dementia
- MCI
- AD
- Other dementia
- Diagnosis unclear

**Wish to use CSF results:** 326 patients
- With LP: 262 patients (80%)
- Without LP: 64 patients (20%)

**Do not wish to use CSF results:** 112 patients
- With LP: 89 patients (80%)
- Without LP: 23 patients (20%)

**438 patients included in study**

**Changed diagnoses (n=23)**
- 8 No dementia
- 7 MCI
- 6 AD
- 4 Other dementia
- 2 Diagnosis unclear

**Other consequences (n=57)**
- 6 No dementia
- 9 MCI
- 7 AD
- 16 Other dementia
- 19 Diagnosis unclear
to use CSF and these criteria was 57%, in 163 patients biomarkers were indicated according to the criteria as well as the clinician, while in 86 patients biomarkers were not indicated according to both criteria and clinician (table 1). The largest discordant group consisted of cases where neurologists wished CSF, but the criteria were not met \((n=163)\). A substantial proportion were AD patients \((n=64)\), where neurologists wished to confirm AD pathology.

**Figure 2.** Reasons for wishing to use CSF (beforehand) and actually using CSF (after disclosure of CSF)

In light grey: frequencies of reasons for wishing to use CSF results, as indicated by neurologists before disclosure of the results. Displayed are percentages of all cases in which neurologists indicated they wished to use CSF results \((n=326; 74\% \text{ of all cases})\). In dark grey: frequencies of reasons for using CSF results, as indicated by neurologists after disclosure of the results. Displayed are percentages of all cases in which neurologists indicated to have used CSF results \((n=179; 51\% \text{ of all cases with CSF})\). Note that clinicians could select more than one reason, therefore percentages count to more than 100%.

* Trial selection was in some cases a reason to know CSF results, as the possibility of inclusion in a trial could be dependent on values of CSF biomarkers.
There were also 74 ‘no dementia’ patients in this group, in which neurologists wished to exclude AD or neurodegeneration in general.

Table 2 shows the interpretation of the clinicians regarding biomarker profile according to strict cut-off levels. The ratings of ‘probably’ and ‘definitely’ AD profile are combined because they showed similar distribution of biomarker combinations. Fifty-seven (16%) patients showed ambiguous biomarkers, defined as ‘maybe’ an AD profile. In patients with only abnormal $A\beta$42 levels, the clinicians deemed the CSF more likely an AD profile with a more evident decrease of $A\beta$42. In addition, in patients with only abnormal tau and/or p-tau, cases with ‘probably’ an AD profile according to the clinician had lower $A\beta$42 values as well as more clearly abnormal tau levels.

### 3.2 Change of diagnosis

In 23 cases (7% of patients with CSF) the pre-CSF diagnosis changed due to biomarker results, as indicated in figure 1. Detailed descriptions of these cases are shown in table 3. Eight patients had an unclear pre-CSF diagnosis and received a diagnosis after disclosure of CSF. In six AD patients (of which one had mixed AD/VAD) the diagnosis changed to another type of dementia because biomarkers were discordant with AD pathology. Stratification for age (<65 vs ≥65) showed comparable proportions for younger and older patients (14 [7%] vs 9 [6%], p=0.67). Diagnosis changed in 19 (12%) of patients meeting the criteria for amyloid imaging, versus 4 (2%) of those not meeting these criteria (p<0.001). Ambiguous biomarker profiles never led to a change in the patient’s diagnosis.

In addition to the above mentioned 23 cases, in 10 patients the diagnosis was changed for other reasons than biomarkers at the time the neurologist completed the second questionnaire: clinical follow-up (n=5), consultation of other specialists (n=2) or other investigations (n=3). In four patients with CSF and an unclear diagnosis at baseline the diagnosis was still unclear when the neurologist completed the second questionnaire. All four patients were admitted in hospital for clinical observation and further investigations.

### 3.3 Diagnostic confidence

After biomarker disclosure, confidence level increased from 84% to 89% (p<0.001; figure 3). When change in confidence level was assessed according to the neurologist’s wish to use CSF results, pre-CSF confidence level was lower in cases in which neurologists beforehand had expressed the wish to use biomarkers (82% vs 90%, p<0.001), and there was an increase in confidence in these patients only (figure 3). When we stratified the analysis according to age, the increase tended to be larger in older patients, but this did not reach significance (6% [82% to 88%] vs 4% [86% to 90%], p=0.09). Increase in confidence was similar in patients meeting versus not meeting criteria for amyloid imaging (5% [83% to 88%] vs 4% [86% to 90%], p=0.37).
### Table 3. Description of patients with changed diagnoses after disclosure of CSF results (n=23)

<table>
<thead>
<tr>
<th>Primary diagnosis</th>
<th>Diagnosis after CSF</th>
<th>CSF biomarkers (pg/mL)</th>
<th>Description *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aβ42</td>
<td>tau</td>
</tr>
<tr>
<td>subjective complaints (n=1)</td>
<td>Preclinical AD (MCI at follow-up)</td>
<td>439</td>
<td>1020</td>
</tr>
<tr>
<td>MCI (n=3)</td>
<td>1x due to AD (AD at follow-up)</td>
<td>341</td>
<td>566</td>
</tr>
<tr>
<td></td>
<td>2x not due to AD (FTD at follow-up)</td>
<td>1003</td>
<td>314</td>
</tr>
<tr>
<td>AD (n=5)</td>
<td>2x FTD</td>
<td>872</td>
<td>229</td>
</tr>
<tr>
<td></td>
<td>2x other dementia</td>
<td>1011</td>
<td>273</td>
</tr>
<tr>
<td></td>
<td>1x DLB</td>
<td>894</td>
<td>164</td>
</tr>
<tr>
<td>Mixed AD/VaD (n=1)</td>
<td>VaD</td>
<td>1053</td>
<td>411</td>
</tr>
<tr>
<td>VaD (n=3)</td>
<td>Mixed AD/VaD</td>
<td>336</td>
<td>526</td>
</tr>
<tr>
<td></td>
<td></td>
<td>433</td>
<td>776</td>
</tr>
<tr>
<td></td>
<td></td>
<td>560</td>
<td>307</td>
</tr>
<tr>
<td>CBD (n=1)</td>
<td>AD</td>
<td>538</td>
<td>872</td>
</tr>
<tr>
<td>Alcohol abuse AD (n=1)</td>
<td>AD</td>
<td>349</td>
<td>699</td>
</tr>
<tr>
<td>Diagnosis postponed (n=8)</td>
<td>3x AD</td>
<td>322</td>
<td>852</td>
</tr>
<tr>
<td></td>
<td></td>
<td>342</td>
<td>615</td>
</tr>
<tr>
<td></td>
<td></td>
<td>325</td>
<td>342</td>
</tr>
<tr>
<td></td>
<td>3x FTD</td>
<td>891</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td></td>
<td>942</td>
<td>182</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1124</td>
<td>187</td>
</tr>
<tr>
<td></td>
<td>1x psychiatric disorder</td>
<td>791</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>1x other dementia</td>
<td>671</td>
<td>230</td>
</tr>
</tbody>
</table>

* CSF biomarkers were defined by clinicians as ‘AD profile’ according to their own interpretation with the use of cut-off values as previously described.4
Displayed are confidence levels before disclosure of CSF results (in light grey), and confidence levels after disclosure of CSF results (in dark grey) of the total study population (on the left), as well as stratified according to whether neurologists indicated beforehand they wished or did not wish to use the CSF results (on the right). The number of patients is indicated beneath the graph. Only patients with CSF and unchanged diagnosis after disclosure of CSF were included in this analysis. Analyses were performed using univariate GLM and GLM for repeated measures, and were adjusted for clinician (entered as dummy variables in the model). Stars indicate significant differences.

In 40 patients with an unchanged diagnosis (11% of patients with CSF) diagnostic confidence decreased substantially after disclosure of biomarkers (≥ -10%). Most often these were patients with subjective complaints (n=10) or a psychiatric disorder (n=5) with abnormal biomarkers, or patients diagnosed with AD (n=8) but normal biomarkers. Additionally, confidence decreased in eight MCI patients; four had normal biomarkers while underlying AD pathology was suspected clinically, and four had abnormal biomarkers while no underlying AD was suspected. Unexpected CSF results had consequences for 16 (40%) of these patients, such as additional investigations or more intensive follow-up, as described in detail below.

3.4 Consequences for patient management

All consequences for patient management are displayed in figure 1. In several patients more than one consequence was indicated. In addition to change of diagnosis, there were consequences of CSF results in 44 patients (13% of patients with CSF), most often pre-selection for clinical trials (n=20), the wish for intensive follow-up (n=13) or imaging studies (n=18) – the last two categories especially in patients with unexpected biomarker results, as described above. In 13 of the 57 patients with ambiguous biomarkers (23%) there were consequences...
for management: extra imaging studies \((n=3)\), more follow-up \((n=4)\), trial selection \((n=5)\), or referral to another specialist \((n=3)\). Of the 87 patients in whom no lumbar puncture was performed, neurologists indicated CSF results would have been useful in 48 cases \((55\%\); figure 1). There were consequences for 13 patients \((15\%)\), most frequently performance of a PiB- and FDG-PET scan \((n=11)\). In two patients a lumbar puncture was performed at a later time point, which led to exclusion of AD pathology in one patient with a postponed diagnosis and to confirmation of AD pathology in a young \((53\) years old\) patient suspected of AD.

When neurologists had beforehand indicated not to wish biomarkers \((irrespective of actual performance of lumbar puncture)\), there were less frequent consequences for management \((for 7\% vs. 23\%, p<0.001)\), these were all \((n=6)\) driven by unexpected CSF results \(see figure 1)\). Stratification for age showed similar percentage of consequences in younger and older patients \((11\% vs 14\%, p=0.41)\). There were more often consequences for management in patients meeting criteria for amyloid imaging compared to those not meeting these criteria \((28\% vs 11\%, p<0.001)\).

### 3.4 Complications of lumbar puncture

85/351 patients \((24\%)\) who had undergone lumbar puncture reported complaints. Forty-seven \((13\%)\) experienced typical post-punctional headache; of which 23 \((7\%)\) classified it as ‘moderate to severe’ headache. Twenty-nine \((8\%)\) used pain medication after lumbar puncture. Seventy-five \((21\%)\) patients reported back pain to some extent, of which 17 \((5\%)\) classified the pain as ‘evident for several days’. There were no severe complications needing medical intervention or hospitalization.

### 4. Discussion

The main finding of our study is that knowledge of CSF biomarker results aided neurologists to increase diagnostic confidence, and to establish a diagnosis in a considerable proportion of patients with a diagnostic dilemma despite thorough clinical work-up. In a small proportion, the initial clinical diagnosis changed after disclosure of CSF biomarker results. Patient management was influenced in a substantial subset of patients, either because of biomarker results, or because CSF could not be obtained.

We assessed in a detailed, descriptive way the impact of CSF biomarkers on patient management in clinical practice. Diagnostic confidence as measured with a visual analogue scale increased, albeit modestly, as pre-CSF confidence was already high. In our previous study on impact of CSF biomarkers, performed in a local memory clinic, we found that 10% of diagnoses changed after CSF results,\(^{11}\) while in a recent multicenter study a considerably larger proportion of 27% of diagnoses changed.\(^{20}\) In both studies however, there was a much lower pre-test diagnostic confidence compared to this study. By including a large
and unselected sample, incorporating both patients with and without CSF available, we tried to avoid potential inclusion bias, and provide a more realistic view of clinical impact of biomarkers in daily practice. In addition, neurologists were blinded for their answers on the first questionnaire, to prevent overestimation of change in confidence. By doing so however, neurologists might have underestimated their change in confidence, but the modest increase of confidence might also be due to the unselected sample. Together with the reasons neurologists indicated for using CSF and the relative low percentage of changed diagnoses, these findings therefore suggest a primary confirmatory role for CSF AD biomarkers. Nevertheless, there were consequences for management in a substantial proportion of patients, sometimes due unexpected biomarker results, giving rise to doubts about the primary diagnosis. In our view this reflects the need neurologists feel to make an etiological diagnosis of the dementia syndrome, in line with the tertiary nature of the center.

In an attempt to quantify the impact of CSF biomarker results, we used ‘change of diagnosis’ as an objective measure, but also the neurologist’s diagnostic confidence, change in confidence, and assessment of the importance of CSF results. As these last three are subjective measures and opinions on the importance of CSF may differ between clinicians, it could be considered a limitation. However, the clinician’s level of confidence in a diagnosis is an important factor for decision-making and patient management, and we adjusted all analyses for clinician. We deliberately asked the clinician’s own opinion on whether biomarkers were concordant with AD, allowing clinicians to have subtle differences in interpretation of borderline values in individual patients. This was done on purpose, as in our view CSF biomarkers should always be considered in the context of thorough clinical evaluation. Moreover, there is still debate on how to define a CSF ‘AD profile’. Caution is however required regarding generalization of these results to a local hospital memory clinic. The memory clinic at the VUmc Alzheimer Center acts as a tertiary referral center, with a high proportion of relatively young patients with complex clinical presentations. It is plausible that CSF biomarkers have less impact in an older population, as older patients may either have more mixed pathology and age-related amyloidosis, or a more typical clinical presentation.

We were not able to assess the effect of biomarkers on treatment. The currently available acetylcholine-esterase inhibitors are symptomatic treatments, and their use is based on a clinical presentation with typical AD-type memory disturbance, rather than on biomarker evidence. Future studies should investigate this aspect of patient management, which will become especially relevant when anti-amyloid or anti-tau treatments become available. Nevertheless, in our study biomarkers were used for inclusion of patients in trials.

Similar to the recently published criteria on appropriate use of amyloid imaging, recommendations for the appropriate use of CSF biomarkers are needed, as they too have been incorporated in the recent diagnostic criteria for AD. The criteria state that biomarkers
could be useful ‘when deemed appropriate by the clinician’, and as such do not present clear recommendations for implementation of CSF biomarkers in clinical practice. At the same time clinicians increasingly use these markers, although opinions on the use of CSF differ, even within this study. We retrospectively applied the criteria for appropriate use of amyloid imaging, and found that an amyloid-scan would have been indicated for more than 40% of our patients, which showed reasonable but not complete overlap with the clinician’s wish to use biomarkers. Most importantly, clinicians more often indicated wishing to use CSF results to rule out AD. According to recent literature, amyloid imaging and CSF biomarkers show a high rate of concordance with each other. Lumbar puncture is a safe and relatively inexpensive procedure. If a PET-scan could be avoided for the majority of patients by making use of CSF biomarkers instead, a substantial cost reduction could be gained, especially considering the low rate of complications after lumbar puncture. Moreover, CSF includes tau as a measure of general neurodegeneration, which lacks in amyloid imaging but could give important additional information.

In conclusion, our results suggest that CSF biomarkers have additional value on top of the standard diagnostic work-up of cognitive disorders, and influence patient management in a subset of patients. This study may be useful for development of guidelines for implementation of CSF biomarkers in daily practice.

Research in context

**Systematic review:** We searched PubMed for reports on impact of biomarkers (either PET imaging or CSF biomarkers) on the diagnostic process of cognitive disorders. Most identified studies were performed using PET imaging, with change in diagnosis after imaging ranging from 9% to 55%. In two studies investigating diagnostic impact of CSF biomarkers, 10% to 27% of diagnoses changed.

**Interpretation:** In the current study, including all patients presenting at our memory clinic during one year, only 7% of diagnoses changed after disclosure of CSF results. This is less than reported before, and suggests CSF biomarkers mostly have a confirmatory role in clinical practice of a tertiary memory center. However, in patients with low pre-CSF diagnostic confidence or an unclear diagnosis we show that CSF results do influence decision-making and patient management.

**Future directions:** CSF biomarkers aid clinicians in a subset of cases. This study may be used as guide for development of recommendations for the appropriate use of CSF biomarkers.
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Disclosures and conflicts of interests

None of the sponsors had any role in the design of the study; in the collection, analysis, and interpretation of the data; in the writing of the manuscript or in the decision to submit the manuscript for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Reference List


