



## Added diagnostic value of CSF biomarkers in differential dementia diagnosis

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Received 9 July 2008; received in revised form 26 September 2008; accepted 30 October 2008

### Abstract

This study aimed to investigate whether cerebrospinal fluid (CSF) biomarkers could have helped the clinician in differential dementia diagnosis in case of clinically ambiguous diagnoses, as compared to autopsy-confirmed dementia diagnosis as gold standard.

Twenty-two patients of our autopsy-confirmed dementia population totalling 157 patients had an ambiguous clinical diagnosis at CSF sampling and were included in statistical analysis. CSF levels of  $\beta$ -amyloid peptide ( $A\beta_{1-42}$ ), total tau protein (T-tau) and tau phosphorylated at threonine 181 (P-tau<sub>181P</sub>) were determined. A biomarker-based model was applied to discriminate between AD and NON-AD dementias.

AD and NON-AD patients showed no significant differences in  $A\beta_{1-42}$  and T-tau concentrations, whereas P-tau<sub>181P</sub> concentrations were significantly higher in AD compared to NON-AD patients. The biomarker-based diagnostic model correctly classified 18 of 22 (82%) patients with clinically ambiguous diagnoses.

Using a biomarker-based model in patients with clinically ambiguous diagnoses, a correct diagnosis would have been established in the majority of autopsy-confirmed AD and NON-AD cases, indicating that biomarkers have an added diagnostic value in cases with ambiguous clinical diagnoses.

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**Keywords:** Alzheimer's disease; Biomarkers; Cerebrospinal fluid; Dementia

### 1. Introduction

The most common cause of dementia is Alzheimer's disease (AD). Diagnosis of probable AD is made according to clinical diagnostic criteria (NINCDS–ADRDA criteria) that are mainly based on the exclusion of other systemic or brain diseases (McKhann et al., 1984). Moreover, the

required diagnostic work-up is time-consuming and expensive (Dubois et al., 2007; McKhann et al., 1984). Average sensitivity and specificity figures of respectively 81% and 70% for a diagnosis of probable AD were obtained using neuropathology as gold standard (Knopman et al., 2001). These figures however were mostly achieved in specialized clinical centers and diagnoses are based on follow-up periods of several years. A much lower diagnostic accuracy can be expected in the earliest stages of the disease and when the diagnostic work-up is performed in non-specialized centers. Diagnosis of definite AD can therefore only be made through postmortem pathological examination of the brain.

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As the brain is in direct contact with the cerebrospinal fluid (CSF) and the flow of proteins from and to the brain is restricted by the blood–brain barrier, biochemical changes that reflect pathophysiological processes in the brain are reflected in CSF. Consequently, research on biomarkers for dementia focuses on CSF. Promising CSF biomarkers for AD are  $\beta$ -amyloid<sub>1-42</sub> protein (A $\beta$ <sub>1-42</sub>), total tau-protein (T-tau) and phosphorylated tau (P-tau) (Andreasen et al., 2003; Knopman et al., 2001; The Ronald and Nancy Reagan Research Institute of the Alzheimer’s Association and the National Institute on Aging Working Group, 1998). The value of those biomarkers in discriminating patients with AD from healthy elderly (Hulstaert et al., 1999; Sjögren et al., 2000), depressed elderly (Buerger et al., 2003) and subjects with Parkinson’s disease (Holmberg et al., 2003) has been demonstrated in several independent studies.

A clinically very relevant but yet unstudied question is whether CSF biomarkers can have an added diagnostic value in differential dementia diagnosis. Indeed, low average specificity levels of 48% for clinical diagnosis of possible AD reflect the overlap of clinical profiles between AD and other (NON-AD) types of dementia (Knopman et al., 2001). Should diagnostic errors occur, they most likely involve one of the other primary dementias, mixed pathologies that include a vascular component, or uncertainties associated with early diagnosis. Especially in the earliest dementia stages, inconclusive routine clinical diagnostic work-up often results in clinically ambiguous diagnoses that can often be reduced to an AD versus NON-AD differential diagnosis. However, an overlap in CSF levels of A $\beta$ <sub>1-42</sub> and T-tau between AD and NON-AD dementias like frontotemporal dementia (FTD), dementia with Lewy bodies (DLB), Parkinson’s disease dementia or vascular dementia (VAD) limits sensitivity, specificity and diagnostic accuracy of these individual biomarkers (Hulstaert et al., 1999; Sjögren et al., 2000). Tau phosphorylated at threonine 181 (P-tau<sub>181P</sub>) seems to be a more specific marker for AD (Hampel et al., 2004; Vanderstichele et al., 2006). The combined assessment of individual CSF biomarkers could further increase the specificity for discriminating AD from other (degener-

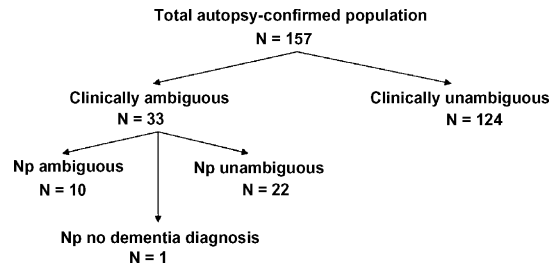


Fig. 1. Classification tree. Abbreviation: Np, neuropathology.

ative) dementias (Andreasen and Blennow, 2005; Blennow and Vanmechelen, 2003). Combined assessment of (CSF) biomarkers can be achieved by applying biomarker-based diagnostic models using different combinations of biomarkers (Engelborghs et al., 2008). In a population (N=200) of autopsy-confirmed dementia patients and healthy controls, we recently developed biomarker-based models that allow discriminating (1) AD from NON-AD dementias, (2) AD from pooled healthy controls and NON-AD dementia patients and (3) dementia from healthy controls, achieving sensitivity, specificity, and diagnostic accuracy levels, consistently exceeding 80% (Engelborghs et al., 2008).

The aim of this study was to investigate whether CSF biomarkers could have helped the clinician in differential dementia diagnosis in clinically ambiguous cases (when a clinical diagnostic work-up was not able to discriminate between AD and a NON-AD dementia), using an AD versus NON-AD biomarker-based model (Engelborghs et al., 2008) compared to autopsy-confirmed dementia diagnosis as gold standard.

**2. Methods**

*2.1. Study population*

We expanded our original neuropathologically confirmed study population (Engelborghs et al., 2007; Engelborghs et al., 2008) with 57 dementia subjects (N=157). From this

Table 1  
Summary of the clinical, pathological and biomarker-based diagnoses of the excluded subjects.

Excluded subjects	Clinical diagnosis			Pathological diagnosis	Biomarker model
	AD	or	NON-AD		
1	AD	or	DLB	AD + DLB	NON-AD
2	AD	or	CJD	AD + DLB	AD
3	AD	or	FTD	AD + FTD	AD
4	AD	or	DLB	AD + DLB	AD
5	AD	or	DLB	AD + DLB	AD
6	AD	or	FTD	AD + DLB	NON-AD
7	AD	or	FTD	AD + FTD	AD
8	AD	or	VAD	AD + DLB	AD
9	AD	or	CJD	AD + CJD	AD
10	AD	or	CJD	AD + CJD	AD
11	AD	or	CJD	Hippocampal necrosis	AD

Table 2  
Summary of the symptoms at clinical work-up of the included subjects.

Included subjects	Clinical diagnosis
1	AD or DLB Memory problems Extrapyramidal signs Visual hallucinations
2	AD or DLB Memory problems Extrapyramidal signs Neuropsychiatric symptoms (sleep disorder, aggression)
3	AD or Korsakoff syndrome Memory problems History of alcohol abuse Neuropsychiatric symptoms (depression, aggression, self neglect, delusions, sleep disorder, apathy) Extrapyramidal signs
4	AD or metachromatic leucodystrophy <sup>a</sup> Apraxia, agnosia, aphasia Neuropsychiatric symptoms (aggression, stereotyping, utilization behavior, restlessness) Dysarthria
5	AD or FTD Memory problems Neuropsychiatric symptoms (loss of insight and judgment, disinhibition, dietary hyperactivity, impaired control of emotions, echolalia, logorrhea, restlessness, utilization behavior)
6	AD or CJD <sup>b/c</sup> Memory problems Myoclonus
7	AD or CJD <sup>c</sup> Memory problems Myoclonus Cerebellar signs (ataxia, gait disturbances)
8	AD or VAD Neuropsychiatric symptoms Aphasia Pyramidal signs Extrapyramidal signs (parkinsonism)
9	AD or CJD <sup>c</sup> Memory problems Myoclonus Cerebellar signs (gait disturbances, tendency to fall) Apraxia
10	AD or CJD Memory problems Extrapyramidal signs (tremors) Neuropsychiatric symptoms Apraxia, aphasia
11	AD or CJD <sup>c</sup> Neuropsychiatric symptoms Cerebellar signs (gait disturbances, ataxia, tendency to fall) Extrapyramidal signs (parkinsonism)

Table 2 (Continued)

Included subjects	Clinical diagnosis
12	AD or CJD <sup>c</sup> Memory problems Apraxia Speech disturbances (akinetic mutism) Epilepsy
13	AD or CJD <sup>b</sup> Memory problems Behavioral disturbances (decreased initiative) Myoclonus
14	AD or DLB Memory problems Myoclonus Extrapyramidal signs Visual hallucinations Fluctuation in cognition Agnosia, aphasia, apraxia
15	AD or CJD <sup>b</sup> Memory problems Myoclonus
16	MXD or DLB Memory problems Neuropsychiatric symptoms (aggression, confusion, wandering, decreased interest) Visual and auditory hallucinations Extrapyramidal signs Apraxia, anomia
17	AD or FTD Behavioral disturbances (disinhibition, perseverations) Memory problems
18	MXD or VAD Memory problems Visual problems (hemianopsia after cerebrovascular accident) Neuropsychiatric symptoms
19	AD or FTD Neuropsychiatric symptoms (apathy, decreased interest, aggression, agitation, restlessness) Memory problems Decreased executive functions
20	AD or CJD <sup>b/c</sup> Neuropsychiatric symptoms Memory problems
21	AD or FTD Neuropsychiatric symptoms (aggression) Memory problems Dysarthria Decreased executive functions
22	AD or DLB Memory problems Neuropsychiatric symptoms (aggression) Extrapyramidal signs Hallucinations Fluctuation in cognition

<sup>a</sup> Based on brain MRI scan.

<sup>b</sup> Based on EEG.

<sup>c</sup> Rapidly progressive dementia.

study population, we selected all subjects ( $N=22$ ) who (1) at time point of CSF sampling, had an ambiguous clinical diagnosis, i.e. where the clinician was not able to categorize the patient in the AD group or the NON-AD group based on strictly applied clinical diagnostic criteria and (2) had an unambiguous neuropathological dementia diagnosis, i.e. where the neuropathological examination showed exclusively or predominantly features of a single type of dementia (Fig. 1). The biomarker profile and pathological spectrum of the excluded neuropathologically ambiguous cases are presented in Table 1. Clinical symptoms of the included patients are summarized in Table 2.

All subjects' samples (CSF and brain) originated from the Biobank facilities of the Institute Born-Bunge (Antwerp, Belgium). Required minimal information consisted of patient's date of birth, date of death, CSF sampling date, gender, clinical and neuropathological diagnosis. The study was approved by the local medical ethics committee.

## 2.2. Clinical and neuropathological diagnostic criteria

All dementia patients were diagnosed by a neurologist experienced in dementia diagnosis at the time point of lumbar puncture (LP) according to strictly applied clinical diagnostic criteria (without knowledge of CSF biomarker data). The diagnosis of probable AD was established using NINCDS–ADRDA criteria (McKhann et al., 1984). All patients also fulfilled the Diagnostic and Statistical Manual of Mental Diseases (DSM-IV-TR) criteria (American Psychiatric Association, 2000). MXD was diagnosed when patients fulfilled the criteria of probable AD according to NINCDS–ADRDA criteria and, in addition, displayed cerebrovascular disease (CVD) on brain CT and/or MRI that, however, did not meet the criteria of relevant CVD according to NINDS–AIREN criteria of VAD (Roman et al., 1993), thus excluding multiple large-vessel infarcts, strategically placed infarcts, multiple basal ganglia and white matter lacunes or extensive white matter lesions. VAD was diagnosed according to the NINDS–AIREN criteria (Roman et al., 1993). For the diagnosis of probable FTD, the criteria described by Neary et al. (1998) were applied. DLB was diagnosed applying the clinical diagnostic criteria of McKeith et al. (1996, 2005). Creutzfeldt–Jakob disease (CJD) was diagnosed according to the diagnostic criteria of Weber (2000). There were two patients with a NON-AD clinical differential diagnosis not belonging to one of the NON-AD diagnoses mentioned above, in particular metachromatic leucodystrophy and Korsakoff syndrome, which were diagnosed according to the DSM-IV-TR criteria (American Psychiatric Association, 2000). CSF sampling was performed during the clinical diagnostic work-up.

All pathological diagnoses were established by the same neuropathologist (JJM) who was blinded for the CSF results. For AD patients, the neuropathological criteria of Braak and Braak (1991), Jellinger and Bancher (1998) and Braak et al. (2006) were applied, whereas FTD patients were neuropatho-

logically diagnosed according to Jackson and Lowe (1996) and Markesbery (1998). The pathological criteria of Kosaka et al. (1988) were applied for diagnosing DLB. CJD, MXD and VAD were diagnosed according to Markesbery (1998). All patients died between April 1995 and September 2007.

To summarize, the stains routinely applied in our laboratory on paraffin blocks representative of the whole central nervous system (but not for the spinal cord) are haematoxylin–eosin, cresyl violet and Bodian's technique. The routinely applied immunostains are 4G8 (amyloid), AT8 (P-tau<sub>181P</sub>) and an anti-ubiquitin antibody (ubiquitin). When the presence of Lewy bodies is suspected on haematoxylin–eosin and ubiquitin immunoreactivity, an anti-alpha-synuclein staining is applied. In non-tau fronto-temporal dementias or when the ubiquitin staining shows immunoreactive dystrophic neurites in the upper cortical layers and “cat's eyes” intranuclear inclusions, TDP-43 immunostaining is performed. When the routine histology suggests a spongiform encephalopathy of the CJD type, a 3F4 antibody is applied.

## 2.3. CSF sampling, storage and analysis

CSF was obtained in clinical centers during clinical work-up of the patient by LP at the L3/L4 or L4/L5 interspace. Samples from 12 patients were collected in the Memory Clinic at Middelheim General Hospital (Antwerp, Belgium) between June 1995 and August 2007, whereas samples from 10 patients were collected in referring centers between March 1999 and April 2005. In Middelheim General Hospital, CSF sampling was performed according to a standard protocol. CSF samples were collected in polypropylene vials and immediately frozen in liquid nitrogen. In case of macroscopically hemorrhagic CSF, samples were immediately centrifuged (10 min at  $4000 \times g$ ); the supernatant was distributed in a different polypropylene vial and frozen in liquid nitrogen. Routine investigation of CSF was performed and included cell count, total protein and glucose analysis. Whenever CSF cell count revealed more than 10 white blood cells or more than 500 red blood cells per  $\text{mm}^3$ , samples were not included in the study (which did not apply to any of the samples). Referring centers were asked to sample at least 1 ml of CSF in polypropylene vials. Referring centers could either ship fresh, unfrozen samples (within 24 h after sampling, shipment at room temperature) or frozen samples (within 1 week after sampling, shipment on dry ice). Despite these instructions, some CSF samples ( $N=6$ ) from referring centers were not collected in polypropylene vials.

CSF samples were stored at the Biobank facilities of the Institute Born-Bunge (Antwerp, Belgium) at  $-75^\circ\text{C}$  until analysis.

CSF analysis was performed at R&D facilities of Innogenetics NV (Ghent, Belgium) or at the Reference Centre for Biological Markers of Memory Disorders (Antwerp, Belgium). CSF biomarker analysis was performed without knowledge of the clinical or pathological diagnosis.

Table 3

Summary of the clinical and pathological diagnoses of the included subjects and categorization of patients in the AD or NON-AD group according to pathology and biomarker model.

Included subjects	Clinical diagnosis			Pathological diagnosis	Pathological category	Biomarker model
	AD	or	NON-AD			
1	AD	or	DLB	AD	AD	AD
2	AD	or	DLB	DLB	NON-AD	NON-AD
3	AD	or	Korsakoff syndrome	AD	AD	AD
4	AD	or	Metachromatic leucodystrophy	MXD	AD	AD
5	AD	or	FTD	AD	AD	AD
6	AD	or	CJD	AD	AD	(AD)
7	AD	or	CJD	AD	AD	AD
8	AD	or	VAD	VAD	NON-AD	AD
9	AD	or	CJD	AD	AD	AD
10	AD	or	CJD	AD	AD	NON-AD
11	AD	or	CJD	AD	AD	(AD)
12	AD	or	CJD	AD	AD	AD
13	AD	or	CJD	AD	AD	AD
14	AD	or	DLB	AD	AD	AD
15	AD	or	CJD	AD	AD	AD
16	MXD	or	DLB	DLB	NON-AD	NON-AD
17	AD	or	FTD	AD	AD	NON-AD
18	MXD	or	VAD	VAD	NON-AD	NON-AD
19	AD	or	FTD	FTD	NON-AD	NON-AD
20	AD	or	CJD	CJD	NON-AD	AD
21	AD	or	FTD	AD	AD	AD
22	AD	or	DLB	AD	AD	AD

Diagnostic categories between brackets are patients with out-of-range values set equal to the lowest/highest calibrator concentrations for A $\beta$ <sub>1-42</sub> and/or P-tau<sub>181P</sub> or both and who are not included in the analysis in Table 3b. Abbreviations: AD, Alzheimer's disease; CJD, Creutzfeldt–Jakob disease; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; MXD, mixed dementia; NON-AD, non-Alzheimer's disease; VAD, vascular dementia.

CSF levels of A $\beta$ <sub>1-42</sub>, T-tau and P-tau<sub>181P</sub> were determined with commercially available single-parameter ELISA kits (respectively, INNOTEST<sup>®</sup>  $\beta$ -AMYLOID<sub>(1-42)</sub>, INNOTEST<sup>®</sup> hTAUAg, INNOTEST<sup>®</sup> PHOSPHO-TAU<sub>(181P)</sub>; Innogenetics, Ghent, Belgium). With each assay, the clinical samples, together with a blank (sample diluent), the (prepared) calibrator solutions and the appropriate controls, were tested strictly following the test

instructions provided in the kit inserts. All samples were run in duplicate. If the intra-assay coefficient of variation (CV) was >20% (calculated as range  $\times$  100/average) or if concentrations obtained were out-of-range (OD values not between mean OD values of highest and lowest calibrator concentration), samples were retested (by extension of the calibrator concentration range in case of out-of-range concentrations). The concentration ranges of the test kits are

Table 4

Demographic and biomarker data.

	Total	AD	NON-AD	Statistical analysis: Rank Sum Test
Age at CSF sampling (years)	75 (70–83) N=22	77 (71–86) N=16	71 (62–78) N=6	<i>p</i> = 0.140, <i>T</i> = 49.0
Age at death (years)	77 (71–83) N=19	79 (72–87) N=14	71 (68–80) N=5	<i>p</i> = 0.195, <i>T</i> = 36.0
Interval CSF sampling–death (months)	1.6 (1.0–5.7) N=19	1.8 (0.8–7.6) N=14	1.3 (0.9–22.4) N=5	<i>p</i> = 0.926, <i>T</i> = 49.0
Disease duration (months)	33 (14–60) N=22	27 (13–57) N=16	36 (18–60) N=6	<i>p</i> = 0.911, <i>T</i> = 70.5
MMSE score (/30)	17 (6–22) N=16	9 (6–20) N=11	18 (15–25) N=5	<i>p</i> = 0.212, <i>T</i> = 53.5
CSF A $\beta$ <sub>1-42</sub> levels (pg/ml) <sup>a</sup>	375 (131–548) N=22	236 (127–517) N=16	519 (327–581) N=6	<i>p</i> = 0.302, <i>T</i> = 83.0
CSF A $\beta$ <sub>1-42</sub> levels (pg/ml) <sup>b</sup>	399 (139–549) N=20	304 (137–557) N=14	519 (327–581) N=6	<i>p</i> = 0.409, <i>T</i> = 73.0
CSF T-tau levels (pg/ml) <sup>a</sup>	532 (215–1070) N=22	532 (219–1094) N=16	489 (198–1071) N=6	<i>p</i> = 0.941, <i>T</i> = 68.0
CSF T-tau levels (pg/ml) <sup>b</sup>	362 (193–701) N=19	436 (193–724) N=14	352 (174–827) N=5	<i>p</i> = 0.853, <i>T</i> = 48.0
CSF P-tau <sub>181P</sub> levels (pg/ml) <sup>a</sup>	49.0 (39.7–92.8) N=22	71.1 (41.7–112.0) N=16	36.9 (25.4–49.2) N=6	<b><i>p</i> = 0.022, <i>T</i> = 38.0</b>
CSF P-tau <sub>181P</sub> levels (pg/ml) <sup>b</sup>	45.4 (39.4–88.5) N=21	66.2 (40.7–102.5) N=15	36.9 (25.4–49.2) N=6	<b><i>p</i> = 0.029, <i>T</i> = 38.0</b>

Significant differences are indicated in bold. Data are presented as median with interquartile ranges between brackets. Abbreviations: AD, Alzheimer's disease; A $\beta$ <sub>1-42</sub>,  $\beta$ -amyloid<sub>1-42</sub> protein; CSF, cerebrospinal fluid; MMSE, Mini Mental State Examination; NON-AD, NON-Alzheimer's disease; P-tau<sub>181P</sub>, tau hyperphosphorylated at threonine 181; T-tau, total protein tau.

<sup>a</sup> Biomarker concentrations with out-of-range data set equal to the lowest/highest calibrator concentration in cases still displaying out-of-range data following retesting or when insufficient CSF was available to allow retesting.

<sup>b</sup> Biomarker concentrations with out-of-range data considered as missing data.

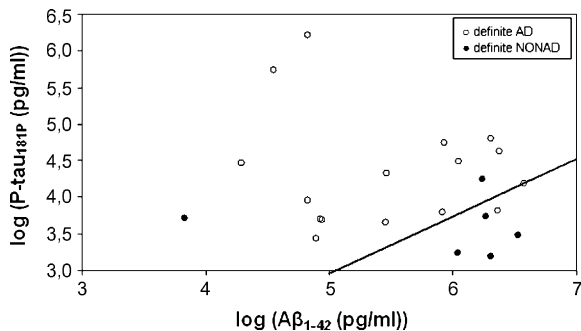


Fig. 2. Biomarker-based diagnostic model: AD versus NON-AD dementia using CSF Aβ<sub>1-42</sub> and P-tau<sub>181P</sub> levels. Discrimination between AD and NON-AD:  $\log (P\text{-tau}_{181P}) = -0.9450 + 0.7813 \times \log (A\beta_{1-42})$ . Natural log-transformed biomarker concentrations in the area above the discrimination line are indicative for AD, natural log-transformed biomarker concentrations in the area under the discrimination line are indicative for NON-AD.

described in the package inserts (Aβ<sub>1-42</sub>: 125–2000 pg/ml, T-tau: 75–1200 pg/ml, P-tau<sub>181P</sub>: 15.6–500 pg/ml).

2.4. Biomarker-based diagnostic model

The AD versus NON-AD biomarker-based model discriminates AD from NON-AD patients by means of a discrimination line that is based on two biomarker concentrations and that is defined by the following equation:  $\log (P\text{-tau}_{181P}) = -0.9450 + 0.7813 \times \log (A\beta_{1-42})$  (Engelborghs et al., 2008). According to this model, the probability of AD significantly increases with an increasing concentration of P-tau<sub>181P</sub> and significantly decreases with increasing concentration of Aβ<sub>1-42</sub>. CSF biomarker concentrations in this model are natural log-transformed concentrations.

2.5. Statistical analyses

Data between AD and NON-AD patients were compared using Mann–Whitney Rank Sum Test. A binomial test (one sample test of a proportion) was used to investigate the performance of the CSF biomarkers in the biomarker-based model using the pathological diagnosis as the gold standard. Sensitivity and specificity figures and diagnostic accuracy were calculated. A probability level of 0.05 was considered significant. Statistical analyses were performed using SPSS® version 13.0 and SAS® version 9.1.

3. Results

3.1. Description of the study population

The definite AD group consisted of six males and ten females, whereas the definite NON-AD group consisted of three males and three females. All patients had a clinical differential diagnosis of probable AD (N=22, among whom 2 patients with MXD) and a diagnosis of a probable NON-AD dementia, such as VAD (N=2), FTD (N=4), DLB (N=5), CJD (N=9), metachromatic leucodystrophy (N=1) or Korsakoff syndrome (N=1) (Table 3). Pathological diagnoses consisted of AD (N=15), MXD (N=1), VAD (N=2), FTD (N=1), DLB (N=2) and CJD (N=1) (Table 3).

Demographic data are summarized in Table 4. The exact date of death was missing for three patients, although the year of death was available. The majority (17/22) of CSF samples were taken within 1 year preceding death. There were no significant differences between AD and NON-AD patients for age at CSF sampling, age at death, interval between CSF sampling and death and Mini Mental State Examination score (Table 4). The study population consisted of moderate to severe dementia patients.

3.2. CSF analyses and CSF biomarker levels

Biomarker data are summarized in Table 4. Seven CSF samples showed out-of-range concentrations for at least one of the biomarkers. Five out-of-range samples originated from AD patients and two from NON-AD patients. In total, four T-tau concentrations and one P-tau<sub>181P</sub> concentration exceeded the highest calibrator concentrations, whereas five Aβ<sub>1-42</sub> concentrations were below the lowest calibrator concentration. These samples were retested with extended calibrator curves; two T-tau concentrations still exceeded the highest calibrator concentration and for two samples insufficient CSF was available for retesting (2 Aβ<sub>1-42</sub>, 1 T-tau and 1 P-tau<sub>181P</sub> concentration).

Therefore, data analyses concerning CSF biomarker levels were performed twice: (1) with out-of-range data set equal to the highest (T-tau: 1200 pg/ml; P-tau<sub>181P</sub>: 500 pg/ml) or lowest (Aβ<sub>1-42</sub>: 125 pg/ml) calibrator concentrations in cases still displaying out-of-range data following retesting or when insufficient CSF was available to allow retesting and (2) with these out-of-range data considered as missing data (but

Table 5 Summary of binomial tests for the biomarker-based AD versus NON-AD model.

	Number of patients	AD according to biomarker model		NON-AD according to biomarker model		Statistical analysis: Binomial test
		Correctly identified AD	Total AD	Correctly identified NON-AD	Total NON-AD	
(a) With out-of-range data set equal to highest/lowest calibrator concentration	22	14	16	4	6	p=0.003
(b) With out-of-range data considered as missing data	20	12	14	4	6	p=0.007

including the out-of-range samples for which a valid result was obtained through extension of the calibrator curve).

Comparing AD and NON-AD patients, no significant differences in  $A\beta_{1-42}$  and T-tau levels were found, whereas P-tau<sub>181P</sub> levels were significantly higher in AD patients (Table 4). None of the CSF biomarkers correlated with the MMSE scores in the total group ( $A\beta_{1-42}$ :  $r=0.248$   $p=0.355$ ; T-tau:  $r=-0.450$ ,  $p=0.081$ ; P-tau<sub>181P</sub>:  $r=-0.393$ ,  $p=0.132$ ) or in the AD ( $A\beta_{1-42}$ :  $r=0.027$ ,  $p=0.936$ ; T-tau:  $r=-0.451$ ,  $p=0.164$ ; P-tau<sub>181P</sub>:  $r=-0.214$ ,  $p=0.527$ ) and NON-AD ( $A\beta_{1-42}$ :  $r=0.027$ ,  $p=0.936$ ; T-tau:  $r=-0.451$ ,  $p=0.164$ ; P-tau<sub>181P</sub>:  $r=-0.214$ ,  $p=0.527$ ) subgroups.

### 3.3. Recategorization of pathological diagnoses and added diagnostic value of CSF biomarkers

Subjects pathologically diagnosed as AD or MXD were pooled in the AD category whereas other dementias (VAD, FTD, DLB and CJD) were pooled in the NON-AD category (Table 3). CSF biomarker levels of  $A\beta_{1-42}$  and P-tau<sub>181P</sub> were modeled in the AD versus NON-AD biomarker model (Fig. 2). A comparison of the diagnostic categories was made for each patient (Table 3) and the data were analysed by means of a binomial test. The outcome was either a success (when diagnostic categories of pathology and of biomarkers for individual patients matched) or a failure (when diagnostic categories did not match).

In patients with clinically ambiguous diagnoses, a correct biomarker diagnosis was established in 18/22 (82%) patients (Table 5) as compared to pathological diagnosis ( $p=0.003$ ). Comparable results were achieved excluding patients with out-of-range concentrations for  $A\beta_{1-42}$  and/or P-tau<sub>181P</sub> ( $p=0.007$ ) (Table 5).

## 4. Discussion

To test whether CSF biomarkers could have helped the clinician in differential dementia diagnosis in clinically ambiguous cases, we analysed CSF biomarker levels of 22 patients with clinically ambiguous diagnoses and compared their pathological diagnoses with the diagnoses obtained by modeling the CSF biomarkers in an AD versus NON-AD biomarker-based model.

The current clinical diagnostic accuracy of AD using neuropathology as reference standard shows an 81% sensitivity and a 70% specificity (Knopman et al., 2001). Consequently, when clinical diagnosis is used as standard, terms of sensitivity, specificity and accuracy should preferentially be replaced by terms such as “negative, positive, or overall percent agreement” with clinical diagnosis (Food and Drug Administration, 2007). Such an approach, combined with corroborating studies as the one described here, will pave the way forward to improve current clinical dementia diagnosis, especially in early phases of the disease.

### 4.1. Study population

Selection criteria were (1) having an ambiguous clinical diagnosis of AD versus NON-AD dementia and (2) an unambiguous neuropathological diagnosis. The autopsy-confirmed dementia population ( $N=157$ ) patients consisted of 33 patients with ambiguous clinical diagnoses. However, 10 patients also had ambiguous neuropathological diagnoses as discrimination between AD or NON-AD dementia could not be made according to neuropathological diagnostic criteria (Fig. 1). Indeed, the relative extent to which AD lesions and other coexisting pathological lesions contribute to clinical symptoms cannot always be determined with certainty (The National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease, 1997). Moreover, these data show that neuropathology also has its limitations as gold standard for dementia diagnosis. Following exclusion of these 10 subjects, the final study population consisted of 22 subjects as one patient with a clinically ambiguous dementia diagnosis appeared to have no neuropathology of dementia. Most neuropathologically ambiguous patients would have been classified as AD patients according to the model that is based on CSF  $A\beta_{1-42}$  and P-tau<sub>181P</sub> levels.

Based on neuropathological diagnoses, the study population was re-categorized and consisted of 16 (73%) definite AD and 6 (27%) definite NON-AD patients. MXD patients were categorized in the AD group, as diagnosis of MXD mainly implies AD brain lesions with an additional but minor vascular component (Markesbery, 1998). Since most (17/22, 77%) of the CSF samples were taken within 1 year preceding death, we can assume that CSF biomarker profiles reliably reflected pathological diagnoses. Moreover, dementia pathology starts many years before onset of clinical symptoms (Shaw et al., 2007).

### 4.2. CSF biomarker levels

We were not able to detect significant differences in CSF  $A\beta_{1-42}$  and T-tau concentrations between AD and NON-AD patients, which is in accordance with previous studies that demonstrated a substantial overlap between AD and NON-AD dementias with regard to CSF  $A\beta_{1-42}$  and T-tau levels (Andreasen et al., 2003; Blennow and Vanmechelen, 2003). Despite a relatively small study population, significantly higher CSF P-tau<sub>181P</sub> levels were found in AD patients compared to NON-AD patients. This supports the assumption that P-tau<sub>181P</sub> is a more specific marker for AD (Hampel et al., 2004; Vanderstichele et al., 2006).

### 4.3. Diagnostic performance of CSF biomarkers in differential dementia diagnosis

Diagnostic accuracy and sensitivity of the AD versus NON-AD biomarker model systematically exceeded 80%.

Lower specificity levels could be due to the relatively small NON-AD population. The patients with clinically ambiguous diagnoses included in the statistical analyses ( $N=22$ ) were selected from a large group ( $N=157$ ) of neuropathologically confirmed patients. The focus on clinically ambiguous cases, which pose the greatest challenge in daily clinical practice, accounts for the relatively small number of patients, especially in the NON-AD group, as prevalence of NON-AD dementias is lower than the prevalence of AD. A larger test group would have strengthened the conclusions drawn here but is not easy to accomplish given the highly specific selection criteria for the study population.

This study showed that by applying CSF biomarkers, a correct diagnosis would have been established in 18 out of 22 (82%) patients with ambiguous clinical diagnoses (AD or NON-AD) using autopsy-confirmed diagnosis as gold standard, achieving a high level of statistical significance (binomial test:  $p=0.003$ ). Thus, CSF biomarkers could have helped the clinician to establish a correct dementia diagnosis in case of clinically ambiguous diagnosis. Especially in the earliest dementia stages, clinical differential diagnosis can be ambiguous given the substantial overlap in clinical symptoms between AD and NON-AD dementias and given the limited specificity of the clinical diagnostic dementia criteria. As an early and accurate dementia diagnosis will be of growing importance when disease-modifying drugs will become available, the use of CSF biomarkers for dementia diagnosis might prove to be a very valuable and clinically relevant tool when clinical diagnostic criteria fail to discriminate between AD and NON-AD dementias. Also, AD is a multifactorial and heterogeneous disorder. CSF biomarker concentrations may not only be useful in the differential diagnosis of dementia, but also in identifying various subgroups of AD. CSF levels of  $A\beta_{1-42}$ , T-tau and ubiquitin have been proven successful in identifying subgroups of AD patients, also reflecting their histopathological lesions. One group with low  $A\beta_{1-42}$  and without a marked increase in T-tau was found to have a high incidence of Lewy bodies (Iqbal et al., 2005). To diagnose (different subgroups of) NON-AD patients, other protein markers than the ones presented in this paper, such as  $\alpha$ -synuclein, may also be useful.

#### 4.4. Limitations of the study

Although several limitations have already been discussed, the major limitations of the present study can be summarized as follows:

1. Given the longitudinal follow-up that was required to obtain autopsy-confirmed diagnoses and given the highly specific selection criteria for the study population included, only a small population was included. Moreover, the sample size of the NON-AD group was less than half the size of the AD group which might further have compromised statistical validity.
2. Out-of-range CSF biomarker values were found in 7/22 (32%) samples, which were mostly samples from AD

patients ( $N=5$ ). The low out-of-range  $A\beta_{1-42}$  concentrations are possibly due to the collection of CSF in polystyrene vials in case samples originated from referring centers. The reduction in  $A\beta_{1-42}$  concentration due to collection of CSF in polystyrene is about 20% (Lewczuk et al., 2006a). This accounts for 4 out of the 5 out-of-range  $A\beta_{1-42}$  concentrations. There is however no straightforward (methodological) explanation for the out-of-range results for T-tau and P-tau<sub>181P</sub> although we previously reported that out-of-range concentrations occurred more often in the diseased group than in a healthy control group. Consequently, it can be speculated that the observation of an “out-of-range” result can by itself be indicative of an underlying dementia (Engelborghs et al., 2008).

3. Biomarker concentrations were determined in two different laboratories. Since none of these samples were determined in both centers, we cannot provide data on the interlaboratory variance of these samples. Although we can assume that the interlaboratory variance is low because all analyses have been performed according to the manufacturer’s instructions, interlaboratory CV have been determined by means of other samples ( $N=31$ ) that were analyzed in both laboratories. For  $A\beta_{1-42}$ , T-tau and P-tau<sub>181P</sub>, the mean CV was 18.5%, 13.9% and 9.8%, respectively, which is less than the mean CV ( $A\beta_{1-42}$  CV 29%, T-tau CV 26%, P-tau<sub>181P</sub> CV 27%) from an international quality control survey of CSF biomarkers (Lewczuk et al., 2006b).
4. Eight of the 22 included samples were used to develop the AD versus NON-AD biomarker model. Although this might be considered a drawback, the objective of this study was by no means to validate the biomarker model itself.
5. The limited time interval between LP and autopsy does not reflect daily clinical practice. Indeed, most dementia patients included were at the moderate to severe stages of dementia at CSF sampling. Although it is unclear whether similar findings would have been achieved including early stage dementia subjects, the lack of any correlation between dementia severity and CSF biomarker concentrations in both AD and NON-AD groups is reassuring. Moreover, most studies have demonstrated intra-individual stability of CSF  $A\beta_{1-42}$ , T-tau and P-tau<sub>181P</sub> levels over time. One study reported an increase of CSF P-tau<sub>181P</sub> levels in MCI patients that converted to AD at follow-up (Andersson et al., 2008). Applying the biomarker-based AD versus NON-AD model, an increase of CSF P-tau<sub>181P</sub> levels would even imply that the biomarker profile moves further away from the discrimination line.

## 5. Conclusions

Using the AD versus NON-AD biomarker-based model in cases with ambiguous clinical diagnoses, a correct diagnosis would have been established in the majority (82%)

of autopsy-confirmed AD and NON-AD cases. These findings indicate that CSF biomarkers have a diagnostic value in differential dementia diagnosis and can help establishing a correct clinical dementia diagnosis.

### Disclosure statement

EV and HV are employees of Innogenetics NV.

### Acknowledgements

This research was supported by the Special Research Fund of the University of Antwerp; Stichting Alzheimer Onderzoek; the Thomas Riellaerts Research Fund; the Institute Born-Bunge; the agreement between the Institute Born-Bunge and the University of Antwerp; the central Biobank facility of the Institute Born-Bunge/University of Antwerp; Neurosearch Antwerp; the Fund for Scientific Research–Flanders (FWO–F); the Interuniversity Attraction Poles (IAP) program P6/43 of the Belgian Federal Science Policy Office, Belgium. NLB holds a National Grant of UNESCO–L'Oréal for Women in Science. NLB is a PhD fellow and SE is a postdoctoral fellow of the FWO–F. The authors acknowledge the technical assistance of E. De Leenheir, L. Aerts, G. Van de Vijver, F. Franck and T. Aerts, the administrative assistance of S. Hicketick, and W. Wittebolle, and the clinical staff involved. We are grateful to Dirk Wouters (Innogenetics) for his helpful comments and support.

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