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Added Diagnostic Value of Cerebrospinal Fluid Biomarkers for Differential Dementia Diagnosis in an Autopsy-Confirmed Cohort

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Abstract.
Background: Differential dementia diagnosis remains a challenge due to overlap of clinical profiles, which often results in diagnostic doubt.
Objective: Determine the added diagnostic value of cerebrospinal fluid (CSF) biomarkers for differential dementia diagnosis as compared to autopsy-confirmed diagnosis.
Methods: Seventy-one dementia patients with autopsy-confirmed diagnoses were included in this study. All neuropathological diagnoses were established according to standard neuropathological criteria and consisted of Alzheimer's disease (AD) or other dementias (NONAD). CSF levels of $A\beta_{1\text{-}42}$, T-tau, and $P\text{-}tau_{181}$ were determined and interpreted based on the IWG-2 and NIA-AA criteria, separately. A panel of three neurologists experienced with dementia made clinical consensus dementia diagnoses. Clinical and CSF biomarker diagnoses were compared to the autopsy-confirmed diagnoses.
Results: Forty-two patients (59%) had autopsy-confirmed AD, whereas 29 patients (41%) had autopsy-confirmed NONAD. Of the 24 patients with an ambiguous clinical dementia diagnosis, a correct diagnosis would have been established in 67% of the cases applying CSF biomarkers in the context of the IWG-2 or the NIA-AA criteria respectively.
Conclusion: AD CSF biomarkers have an added diagnostic value in differential dementia diagnosis and can help establishing a correct dementia diagnosis in case of ambiguous clinical dementia diagnoses.

Keywords: Ambiguous diagnosis, Alzheimer's disease, biomarkers, cerebrospinal fluid, dementia, differential dementia diagnosis, neuropathology

INTRODUCTION

Alzheimer's disease (AD) and other types of dementia (non-AD) overlap in clinical profiles, and therefore the differential dementia diagnosis remains challenging and diagnostic errors continue to appear. These errors generally concern uncertainties in early diagnosis, or could involve one of the other primary dementias, co-pathologies, or pathologies including a vascular component. Moreover, misdiagnoses of patients with vascular lesions confirmed by structural brain imaging as possible or probable vascular

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dementia (VaD) often occur [1]. Diagnostic accuracy should improve by minimizing errors in clinical diagnosis, and a possible approach is the use of cerebrospinal fluid (CSF) biomarkers. The standard AD CSF biomarker panel that consists of amyloid-β of 42 amino acids ($A\beta_{1-42}$), total tau protein (T-tau), and hyperphosphorylated tau (P-tau), increases the diagnostic accuracy for AD [2], also in its prodromal phase [3], and is used in daily clinical practice [4]. However for differential dementia diagnosis, the use of this AD CSF biomarker panel is limited due to an overlap in CSF levels of $A\beta_{1-42}$ and T-tau between AD and non-AD dementias, especially in case of AD co-pathology in the brain of non-AD dementias, like dementia with Lewy bodies (DLB) [2, 5]. P-tau_{181} is an essential component of the AD CSF biomarker panel and has the highest diagnostic power to discriminate between AD and non-AD dementias [6]. Although the added diagnostic value of the AD CSF biomarker panel was demonstrated in case of ambiguous clinical dementia diagnoses (when a clinical diagnostic work-up was not able to discriminate between AD and a non-AD dementia) [7], the added diagnostic value when applying the IWG-2 [8] and NIA-AA criteria [9] has not been taken into account for differential dementia diagnosis. We aimed to investigate a cohort of clinically assessed patients who also underwent lumbar puncture (LP) and were followed-up by autopsy. This enables us to compare the clinical (non-biomarker-based) and CSF biomarker diagnoses (IWG-2 or NIA-AA criteria based diagnoses), with the autopsy-confirmed neuropathological diagnoses as the reference.

MATERIALS AND METHODS

Study population

The study population consisted of 71 demented patients with autopsy-confirmed dementia diagnoses. Patients were recruited through the Memory Clinic of Hospital Network Antwerp (ZNA) Middelheim and Hoge Beuken ($n = 64$) and through centers referring to the Biobank of the Institute Born-Bunge ($n = 7$) [1]. Based on the information collected during the clinical diagnostic work-up at enrollment in the study, a panel of three neurologists experienced with neurodegenerative diseases and dementia (JG, PPDD, SE) made a consensus clinical dementia diagnosis (not biomarker-based). The panel was blinded for the initial clinical and neuropathological diagnoses. The presented clinical information (by BF) consisted of age at inclusion/death, gender, history of and concomitant illnesses, familial and social history, onset and history of presented complaint(s), medication, physical and clinical neurological examination, a complete neuropsychological examination including (among others) Mini-Mental State Examination (MMSE) scores [1], brain magnetic resonance imaging (MRI), and/or computed tomography (CT) scan.

This study was approved by the ethics committee of UAntwerp, Antwerp, Belgium (UA A07-24). All included subjects and/or caregiver signed an informed consent.

Clinical diagnostic criteria

The clinical panel established consensus diagnoses based on standard clinical diagnostic criteria, allowing to label each clinical diagnosis as probable or possible depending on the likelihood of it being the cause of dementia. The diagnostic procedure did not include CSF biomarkers.

The diagnosis of possible/probable AD was made according to the NINCDS-ADRDA criteria [10]. In case patients fulfilled the criteria of probable AD 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 [11], patients were diagnosed with a combination of AD and CVD. For the diagnosis of VaD the NINDS-AIREN criteria were applied [11]. Criteria described by Neary [12] were applied for the diagnosis of probable frontotemporal dementia (FTD). DLB was diagnosed according to the clinical diagnostic criteria of McKeith [13]. Parkinson's disease dementia (PDD) was diagnosed when patients with idiopathic Parkinson's disease (PD) developed dementia following a dementia-free interval of at least two years. Idiopathic PD was diagnosed based on the presence of at least two out of four motor manifestations that characterize the disease and an insidious onset [14]. Diagnosing Creutzfeldt-Jakob disease (CJD) was established according to the diagnostic criteria of Weber [15].

Neuropathological criteria

All pathological diagnoses were made using standard neuropathological criteria by the same neuropathologist (JJM). The neuropathologist was blinded to the consensus diagnoses, but had access

to all neuroimaging data. AD, VaD, and DLB were neuropathologically diagnosed based on the criteria of Montine [16]. For the diagnosis of FTD the neuropathological criteria of Cairns [17] and Mackenzie [18, 19] were applied. CJD was diagnosed according to Markesbery [20]. Neuropathology was executed on the right hemisphere of the brain.

CSF sampling and storage

CSF was obtained by LP at the L3/L4 or L4/L5 interspace. CSF samples were immediately frozen in liquid nitrogen and stored at –75°C until analysis, as previous described [4]. The samples were collected at the Memory Clinic of ZNA Middelheim and other centers referring to the Biobank of the Institute Born-Bunge according to a standard protocol [4]. In here, non-blood contaminated samples did not undergo centrifugation, whereas in case of a hemorrhagic LP detected by macroscopic inspection, CSF was centrifuged for 10 min at 3000 rpm within 4 hours after LP. The supernatant was thereafter transferred to an unused polypropylene vial.

CSF analysis and interpretation

CSF analysis was performed at the BIODEM lab. The laboratory technician was blinded for the clinical and pathological diagnoses when performing and interpreting the tests. CSF levels of $A\beta_{1\text{-}42}$, T-tau, and P-tau_{181} were determined with commercially available singe-analyte ELISA kits (INNOTEST®, β-AMYLOID$_{(1\text{-}42)}$, INNOTEST® hTAU-Ag, and INNOTEST® PHOSPHOTAU$_{(181P)}$, Fujirebio Europe, Ghent, Belgium). All samples were run in duplicate. The concentration ranges of the test kits are described in the package inserts ($A\beta_{1\text{-}42}$: 125–2000 pg/mL, T-tau: 75–1200 pg/mL, P-tau_{181}: 15.6–500 pg/mL). If CSF concentrations were out of range, the concentrations were set to the upper/lower limit of the kit inserts. For the statistical analyses all patients were included as the out-of-range concentrations did not affect the CSF biomarker profiles.

The three CSF biomarkers results were interpreted based on in-house validated cut-off values (in autopsy-confirmed AD versus cognitively healthy elderly; $A\beta_{1\text{-}42}$ <638.50 pg/mL, T-tau >296.50 pg/mL, P-tau_{181} >56.50 pg/mL) [21].

CSF biomarkers were analyzed by applying the IWG-2 criteria [8] for AD and the NIA-AA criteria [9] separately (Supplementary Table 1). CSF $A\beta_{1\text{-}42}$ is indicative of AD if the concentration is below the cut-off and CSF tau biomarkers if the concentrations are above the cut-off. By applying the IWG-2 criteria a CSF biomarker profile was considered to be suggestive for AD if CSF $A\beta_{1\text{-}42}$, in combination with T-tau and/or P-tau_{181} values were altered. In all other cases, the CSF biomarker profile was not suggestive for AD. In addition, the NIA-AA criteria were applied, with a high likelihood of AD if both amyloid and neuronal injury markers were altered, whereas the low likelihood was if both markers were unaltered. Intermediate likelihood was if only one of both was altered.

Categorization of diagnoses

Subjects with neuropathological diagnoses of AD or AD with CVD were pooled in the AD group whereas we refer to non-AD in case of other dementias. The latter, was subdivided into patients without a co-pathology (non-AD) and patients with a primary non-AD pathology and AD co-pathology. Subjects with a single clinical diagnosis were categorized as "unique" (e.g., probable AD). In case of doubt between two clinical diagnoses following the clinical diagnostic work-up, subjects were categorized as "ambiguous" (there was doubt between two clinical diagnoses belonging to both AD and non-AD categories, e.g., probable AD/possible FTD).

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 23 and GraphPad Prism 6. To describe and analyze our entire cohort, categorical variables were analyzed with a Chi-Square test, and percentages, sensitivity, specificity, and positive/negative predictive values were reported. Independent pairwise comparisons were performed with Mann Whitney U test, and demographic variables were reported as mean values with SD and biomarkers variables by median with interquartile range (IQR). For all analyses, *p*-values below 0.05 were considered significant.

RESULTS

Population (Table 1)

In this cohort ($n = 71$), there was no significant difference in the proportion of gender between the three patient groups. Forty-two patients in the

Table 1
Description of the population

	Pathological diagnosis			*p*-value
	AD ($n=42$)	non-AD ($n=22$)	Primary non-AD with AD co-pathology ($n=7$)	
Gender (% male/female)	52/48	59/41	43/57	0.735
Age at inclusion (y)	80.1 [± 9.0]	69.5 [± 12.1]	75.9 [± 8.5]	0.001^
Age at death (y)	82.0 [± 8.3]	70.8 [± 11.8]	76.9 [± 8.7]	0.001^
MMSE (score out of 30) (n)	13.5 [± 6.3] (36)	16.0 [± 7.2] (16)	16.0 [± 6.8] (7)	NS
Interval inclusion - autopsy (y)	1.7 [± 2.6]	1.4 [± 1.7]	0.9 [± 1.3]	NS
Interval last clinical evaluation - autopsy (y)	0.8 [± 1.7]	0.6 [± 1.0]	0.4 [± 0.9]	NS
CSF $A\beta_{1\text{-}42}$ (pg/mL)	394.5 [280.3–506.3]	463.3 [378.0–573.8]	450.1 [438.0–537.0]	NS
CSF T-tau (pg/mL)	723.7 [358.0–1200.08]	544.7 [211.8–976.0]	645.4 [375.0–1200.0]	NS
CSF P-tau_{181} (pg/mL)	103.3 [58.3–137.3]	73.4 [25.4–69.6]	67.2 [46.3–82.5] (7)	0.001^,$

Data are presented as mean [± SD] values, percentage (%), or number (n). CSF biomarker data are presented as median values [IQR]. ^Significantly different between AD and non-AD patient group. $Significantly different between AD and non-AD with AD co-pathology. AD, Alzheimer's disease; $A\beta_{1\text{-}42}$, amyloid-β of 42 amino acids; CSF, cerebrospinal fluid; IQR, interquartile range; MMSE, Mini-Mental State Examination; non-AD, other type of dementia (than Alzheimer's disease); NS, not significant; P-tau_{181}, phosphorylated tau at Threonine 181; SD, standard deviation; T-tau, total tau protein.

population (59%) had neuropathologically confirmed AD, whereas 29 (41%) patients showed non-AD neuropathology findings. Seven out of the 29 non-AD patients (24%) had primary non-AD neuropathology with an AD co-pathology (DLB $n=4$, VaD $n=1$, FTD $n=1$, CJD $n=1$). The non-AD group (FTD $n=8$, DLB $n=6$, VaD $n=5$, CJD $n=3$) was significantly younger at inclusion and at death than the AD group. MMSE scores were not significantly different comparing the three groups. The interval between inclusion (time of LP) and autopsy was in most cases short (1.5 ± 2.3 years, of which 70.4% of the patients died within one year following inclusion), likewise the interval between last clinical evaluation and autopsy (0.7 ± 1.5 years). No significant differences were observed comparing intervals between inclusion/last clinical evaluation and autopsy between AD and non-AD groups. CSF P-tau_{181} concentrations were significantly different, comparing the AD and non-AD patient group ($p=0.001$), whereas no significant differences were detected for CSF $A\beta_{1\text{-}42}$ and T-tau. No significant differences were observed between AD or non-AD and the non-AD with AD co-pathology. The CSF $A\beta_{1\text{-}42}$ concentration was lower, whereas T-tau and P-tau_{181} concentrations were higher in the AD group as compared to the non-AD group. The non-AD with AD co-pathology group had intermediate CSF biomarker concentrations.

Diagnostic value of CSF biomarkers (Table 2)

By comparing the clinical consensus and CSF biomarker diagnoses in subjects with unique clinical diagnoses ($n=47$, Tables 2a-b and 3, Supplementary Table 2), the diagnostic accuracy of CSF biomarker diagnoses (based on IWG-2 criteria or NIA-AA criteria) was not significantly different from the clinical diagnostic accuracy ($p=0.162$ and $p=0.473$, respectively) using the autopsy-confirmed diagnosis as a reference. Therefore, no added value of the CSF biomarkers was detected as compared to the clinical diagnosis in patients with a unique clinical diagnosis. Nevertheless, 60% of the clinically incorrect diagnosed patients would have been correctly diagnosed with CSF biomarkers following the IWG-2 criteria, and 75% following the NIA-AA criteria. Those patients had a biomarker profile suggestive for AD and were neuropathologically diagnosed as AD ($n=5$) or DLB with AD co-pathology ($n=1$).

Four patients had incorrect CSF biomarker diagnoses (both IWG-2 and NIA-AA criteria) with correct clinical diagnosis and were neuropathologically confirmed as CJD ($n=2$), FTD ($n=1$), or DLB ($n=1$). In addition, three subjects were incorrect CSF biomarker-based diagnosed as non-AD following the IWG-2 criteria with correct clinical diagnoses and neuropathologically confirmed as AD (abnormal CSF $A\beta_{1\text{-}42}$ with normal tau values).

An incorrect diagnosis for both the clinical and CSF-biomarker diagnosis (both IWG-2 and NIA-AA criteria) was detected in two subjects, namely neuropathologically diagnosed as CJD or DLB. In addition, two subjects were incorrect CSF biomarker-based diagnosed as non-AD following the IWG-2 criteria incorrect clinical diagnoses and neuropathologically confirmed as AD (abnormal CSF $A\beta_{1\text{-}42}$ with normal tau values).

Table 2

a. Clinical diagnosis versus CSF biomarker-based diagnosis according to the IWG-2 criteria, using the autopsy-confirmed diagnosis as a reference ($p = 0.162$)

	Biomarker diagnosis Correct	Biomarker diagnosis Incorrect	Total
Clinical diagnosis Correct	30	7	37
Clinical diagnosis Incorrect	6	4	10
Total	36	11	47

b. Clinical diagnosis versus CSF biomarker-based diagnosis according to the NIA-AA criteria, using the autopsy-confirmed diagnosis as a reference ($p = 0.473$)

	Biomarker diagnosis Correct	Biomarker diagnosis Incorrect	Total
Clinical diagnosis Correct	24	4	28
Clinical diagnosis Incorrect	6	2	8
Total	30	6	36

In total, 24 subjects had an ambiguous clinical diagnosis and were excluded from this analysis. The other 47 individuals were subdivided into concordance/discordance between the clinical diagnoses and CSF biomarker diagnosis, both for the IWG-2 criteria (a) and NIA-AA criteria (b), compared to neuropathological diagnosis. Eleven subjects with an intermediate clinical diagnosis based on the NIA-AA criteria were not included in Table 2b as we could not decide if they were correctly or incorrectly CSF biomarker-based diagnosed compared to the neuropathological diagnosis. Table 2a ($n = 47$), the sensitivity, specificity, diagnostic accuracy, and positive/negative predicted values were calculated for both the clinical and CSF biomarker diagnosis compared to the neuropathological diagnosis (respectively, clinically 78%, 80%, 79%, and 89%/63%; and for CSF biomarker-based diagnosis 84%, 40%, 70%, and 75%/55%). Table 2b ($n = 36$), the sensitivity, specificity, diagnostic accuracy, and positive/negative predicted values were calculated for both the clinical and CSF biomarker diagnosis compared to the neuropathological diagnosis (respectively, clinically 82%, 67%, 78%, and 88%/55%; and for CSF biomarker-based diagnosis 100%, 67%, 92%, and 90%/100%). CSF, cerebrospinal fluid; IWG-2, International Working Group; NIA-AA, National Institute on Aging/Alzheimer's Association.

Diagnoses of ambiguous clinical cases (Table 3)

In 24 out of the 71 subjects an ambiguous clinical diagnosis was detected, i.e., where the panel was not able to categorize the patient in the AD group or the non-AD patient group. The outcome was either a success (when diagnostic categories of pathology and of CSF biomarkers according to the IWG-2 or NIA-AA criteria for individual patients matched) or a failure (when diagnostic categories did not match).

A correct CSF biomarker diagnosis based on the IWG-2 criteria was established in 16 (AD $n = 9$, non-AD $n = 3$, or non-AD with AD co-pathology (DLB $n = 2$, VaD $n = 1$, FTD $n = 1$)) out of 24 (67%) patients as compared to the autopsy-confirmed diagnosis. Of the eight patients who were incorrectly diagnosed based on CSF biomarkers, the confirmed diagnosis of three patients was not one of the ambiguous clinical diagnosed probabilities (e.g., the clinical differential diagnosis consisted of AD versus VaD, whereas the autopsy-confirmed diagnosis was FTD). The other five patients were neuropathologically confirmed as one of the ambiguous clinical diagnosed probabilities (e.g., the clinical differential diagnosis consisted of AD versus DLB, and the autopsy-confirmed diagnosis was DLB).

Using the CSF biomarkers following the NIA-AA criteria, a correct diagnosis was established in 14/21 (67%) individuals as compared to the autopsy-confirmed diagnoses. One patient was diagnosed with the low likelihood of AD and CSF biomarkers in the context of the IWG-2 criteria resulted in a 'non AD' CSF biomarker profile. This patient was neuropathologically diagnosed with non-AD (FTD). Thirteen patients had a high likelihood of AD and were neuropathologically confirmed as AD ($n = 9$) or non-AD with AD co-pathology ($n = 4$). The other seven patients were incorrectly diagnosed using the CSF biomarkers in the context of the NIA-AA criteria, all with a high likelihood of AD, and were neuropathologically confirmed as VaD ($n = 4$), FTD ($n = 2$), or DLB ($n = 1$). Three patients who had an intermediate likelihood of AD based on the NIA-AA criteria were not included in this analysis.

Intermediate NIA-AA CSF biomarker-based diagnosis

In total 14 subjects had an intermediate NIA-AA diagnosis (Table 3) and were not included in the clinical versus CSF biomarker-based diagnosis comparison (Table 2b) as we could not decide if they were

Table 3
Patients with ambiguous clinical diagnoses as compared to CSF biomarker-based diagnoses using the IWG-2 criteria

Clinical diagnosis (n)	Suggestive for AD	Correct biomarker diagnosis (%)	Not suggestive for AD	Correct biomarker diagnosis (%)
Probable AD/Probable non-AD (5)	4	75	1	100
Probable AD/Possible non-AD (15)	13	62	2	50
Probable non-AD/Possible AD (3)	2	50	1	100
Possible AD/Possible non-AD (1)	1	100	0	0

Data are number of patients (n) and correct relative number of diagnoses (%). The category 'suggestive for AD' consists of patients with an AD CSF biomarker profile according to the IWG-2 criteria, whereas 'not suggestive for AD' were patients with CSF biomarkers not suggestive for AD according to the IWG-2 criteria. A correct diagnosis was based if the diagnostic categories of pathology and of CSF biomarkers according to the IWG-2 for individual patients matched, for instance if definite AD and cases with AD co-pathology had a positive biomarker profile. The sensitivity, specificity, diagnostic accuracy, and positive/negative predicted values were calculated for the CSF biomarker diagnoses based on the IWG-2 criteria compared to the neuropathological diagnosis (respectively, 90%, 50%, 67%, and 56%/88%). AD, Alzheimer's disease; CSF, cerebrospinal fluid; IWG-2, International Working Group; NIA-AA, National Institute on Aging/Alzheimer's Association; non-AD, other type of dementia (than Alzheimer's disease).

correctly or incorrectly diagnosed according to the NIA-AA criteria in comparison to neuropathology as the reference.

In total six patients were neuropathologically confirmed as AD with incorrect CSF biomarker diagnosis based on the IWG-2 criteria (abnormal CSF $A\beta_{1\text{-}42}$ with normal tau values, $n=5$; or normal $A\beta_{1\text{-}42}$ with abnormal T-Tau values, $n=1$). The other eight patients with an intermediate likelihood based on the NIA-AA criteria (abnormal $A\beta_{1\text{-}42}$ with normal tau values, $n=6$ (FTD, $n=3$; DLB, $n=3$); or normal $A\beta_{1\text{-}42}$ with abnormal tau values, $n=2$ (VaD and FTD)) were correctly diagnosed with the CSF biomarkers based on the IWG-2 criteria, and were neuropathologically diagnosed as FTD, DLB, or VaD.

DISCUSSION

This study investigated whether CSF biomarkers levels could help the physician in differential dementia diagnoses using a cohort of 71 autopsy-confirmed patients, whereof 24 patients had an ambiguous clinical diagnosis. This study showed that by applying CSF biomarkers using the IWG-2 or NIA-AA criteria in patients with an ambiguous clinical diagnosis a correct diagnosis would have been established in 67% of patients using autopsy-confirmed dementia diagnosis as the reference. Moreover, IWG-2 and NIA-AA criteria performed equally well. We thus confirm that CSF biomarkers have an added diagnostic value in cases with ambiguous clinical diagnoses [7]. Therefore, biomarkers should be included in the diagnostic work-up in case of doubt between AD versus non-AD. CSF biomarkers contribute to a high accuracy for identifying AD, also in prodromal AD [22–25], but several other brain diseases can lead to pathological values of these AD CSF biomarkers, which was also observed in this study. For instance, an increase in T-tau is also detected in disorders with extensive and/or rapid neuronal degeneration, such as CJD [26]. Moreover, both $A\beta_{1\text{-}42}$ and T-tau are detected at intermediate levels in non-AD patients, in between normal control and abnormal AD values [2, 27–29], especially in DLB but also in FTD, VaD, and CJD. In order to improve the discriminatory power for the differential diagnosis of dementia, additional markers, more specific to the non-AD dementia can be valuable.

Even though, no added diagnostic value of CSF biomarkers was detected in case of unique clinical diagnosis, a correct CSF biomarker-based diagnosis following the IWG-2 or NIA-AA criteria with incorrect clinical diagnosis would have been established in 60% and 75% of the cases, respectively. When CSF biomarkers showed incorrect diagnosis compared to correct clinical diagnosis, the CSF biomarkers were in concordance with the neuropathology. Of those patients, three were neuropathologically confirmed with AD and had abnormal CSF $A\beta_{1\text{-}42}$ concentrations, nevertheless the CSF tau concentrations were normal and neuropathologically neurofibrillary tangles were found. The neuronal loss may not have been severe enough for tau to be released into the interstitial fluid, resulting in normal CSF tau values. In addition, four patients were neuropathologically diagnosed with non-AD (CJD; $n=2$, FTD; $n=1$, and DLB; $n=1$) and all had abnormal CSF $A\beta_{1\text{-}42}$ values and amyloid plaques were found at autopsy, thus the biomarker was in concordance with the pathology. Nevertheless, the CJD patients had very high T-tau values (abnormal), indicating a severe neuronal

loss, typical of CJD, which is in line with the current literature [30, 31]. The FTD and DLB patients also had abnormal T-tau values and neuronal loss was neuropathologically confirmed.

Additionally, when patients had an intermediate likelihood CSF biomarker-based diagnosis of AD (according to the NIA-AA criteria), the IWG-2 criteria could correctly diagnose eight of the 14 individuals in this study. The other six individuals with an intermediate likelihood CSF biomarker-based diagnosis of AD (according to the NIA-AA criteria) had an incorrect CSF biomarker-based diagnosis (according to the IWG-2 criteria: abnormal $A\beta_{1\text{-}42}$ and normal tau values). For those six patients the CSF biomarkers actually correctly reflected the pathology. This agreement was also found in case patients had both incorrect clinical and incorrect CSF biomarker-based diagnosis ($n = 4$). A possible solution to correct for the discordancy between CSF and clinical diagnosis could be the introduction of the CSF $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio [32]. These findings further underbuilt the recently published consensus recommendations that stress that AD CSF biomarkers should be applied as an add-on to the clinical evaluation in selected clinical indications [33, 34].

A limitation of this study might be the rather low cut-offs compared to other studies. However, our lab participates in the Alzheimer's Association external quality control program (AA QC) for CSF. Within this program our longitudinal samples were always lower in comparison to mean of all participating laboratories. Since we switched to kits with ready-to-use calibrators our longitudinal samples are in agreement with the mean of the other participating labs. Nevertheless, the individuals in this cohort were analyzed before the transition to ready-to-use calibrator kits. In addition, the in-house established cut-offs (based on autopsy-confirmed dementia subjects and cognitively healthy elderly) were also calculated before this transition, and therefore, we could rely on the applied cut-offs.

In conclusion, these findings show that the AD CSF biomarkers have an added diagnostic value in differential dementia diagnosis and can help establishing a correct dementia diagnosis in case of ambiguous clinical dementia diagnoses.

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SUPPLEMENTARY MATERIAL

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