A Decade of Cerebrospinal Fluid Biomarkers for Alzheimer's Disease in Belgium

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Abstract. During the past ten years, over 5,000 cerebrospinal fluid (CSF) samples were analyzed at the Reference Center for Biological Markers of Dementia (BIODEM), UAntwerp, for core Alzheimer's disease (AD) CSF biomarkers: amyloid- β peptide of 42 amino acids ($A\beta_{1-42}$), total tau protein (T-tau), and tau phosphorylated at threonine 181 (P-tau_{181P}). CSF biomarker analyses were performed using single-analyte ELISA kits. In-house validated cutoff values were applied: $A\beta_{1-42}$ <638.5 pg/mL, T-tau >296.5 pg/mL, P-tau_{181P} >56.5 pg/mL. A CSF biomarker profile was considered to be suggestive for AD if the CSF $A\beta_{1-42}$ concentration was below the cutoff, in combination with T-tau and/or P-tau_{181P} values above the cutoff (IWG2 criteria for AD). Biomarker analyses were requested for following clinical indications: 1) neurochemical confirmation of AD in case of clinical AD, 2) neurochemical confirmation of AD in case of doubt between AD and a non-AD dementia, 3) neurochemical diagnosis of prodromal AD in case of mild cognitive impairment, 4) neurochemical confirmation of AD in case of psychiatric symptoms (like depression, psychosis), or 5) other clinical indications. During these ten years, the number of yearly referred samples increased by 238% and clinical indications for referral showed a shift from neurochemical confirmation of AD in case of clinical AD to differential dementia diagnosis in case of doubt between AD and a non-AD dementia. Four percent of the patients also had a postmortem neuropathological examination. Together, these biomarker data were the basis for several research papers, and significantly contributed to the validation of these biomarkers in autopsy-confirmed subjects.

Keywords: Alzheimer's disease, amyloid, biomarkers, cerebrospinal fluid, dementia, diagnosis, mild cognitive impairment, neuropathology, tau

INTRODUCTION

The past two decades, much effort has been put in developing biomarkers for Alzheimer's disease (AD) [1–6]. This resulted in three core cerebrospinal fluid (CSF) biomarkers for AD diagnostics, namely the

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42 amino acid long amyloid-beta peptide ($A\beta_{1-42}$), total tau protein (T-tau), and tau phosphorylated at threonine 181 (P-tau_{181P}) [7]. $A\beta$ and both tau proteins can reliably be measured in CSF by the use of several analytical techniques including single-analyte enzyme-linked immunosorbent assays (ELISA) [8].

Biomarkers have been incorporated into research diagnostic criteria for AD [9–11] and, although the clinical examination (including full neuropsychological evaluation) is still the basis for AD diagnosis [12], these biomarkers are being introduced in daily clinical dementia practice as *in vivo* surrogate markers for the confirmation of AD neuropathology. The core CSF AD biomarkers increase the diagnostic accuracy for diagnosing AD (including cases with atypical presentations), also in its prodromal phase (mild cognitive impairment (MCI) due to AD) [1, 4], and are able to differentiate between AD and psychiatric disorders. The CSF biomarkers are useful to diagnose AD in patients with ambiguous clinical dementia diagnoses [13, 14] and in cases with mixed brain pathology like AD with cerebrovascular disease [15–17].

At the Reference Center for Biological Markers of Dementia (BIODEM), UAntwerp, CSF samples from referring clinical centers have been analyzed for the core AD CSF biomarkers since 2004. In what follows, an overview of a decade of CSF biomarker analyses at BIODEM will be given. This overview includes over 5,000 neurochemical analyses. Four percent of the patients also had a postmortem neuropathological examination. Together, these biomarker data were the basis for several research papers. This overview forms a basis for revealing limitations and providing new insights to improve the neurochemical diagnosis of AD.

METHODOLOGY

CSF samples from referring clinical centers

CSF samples from patients with presumed cognitive deterioration were referred to the BIODEM lab. Since 2004, clinical centers from all over Belgium as well as some international centers have been referring samples (Fig. 1). CSF biomarker analyses were requested by the physicians in the context of a diagnostic workup. The physicians were asked to fill out a request form consisting of the patient's age, gender, Mini-Mental State Examination (MMSE) score [18], sampling date, and the clinical indication for referral,

that consisted of one of the following options (tick boxes):

- 1) Neurochemical confirmation of AD in case of clinical AD. CSF biomarkers were used to increase the diagnostic accuracy.
- 2) Neurochemical confirmation of AD in case of doubt between AD dementia and a non-AD dementia, including atypical clinical presentations of AD, possible dementia with Lewy bodies (DLB), possible frontotemporal lobar degeneration (FTLD), possible vascular dementia (VaD), Creutzfeldt-Jakob's disease (CJD), etc.
- 3) Neurochemical confirmation of prodromal AD in case of MCI.
- 4) Neurochemical confirmation of AD in case of psychiatric symptoms (like depression, psychosis).
- 5) Other clinical indication: the referring clinician was asked to provide additional clinical information.

In case of CJD confirmation or AD versus CJD differential diagnosis, additional analysis of 14-3-3 protein could be requested [19–21].

CSF sampling and handling

All CSF sampling and handling steps are represented in Fig. 2. In short, CSF was obtained by lumbar puncture (LP) at the L3/L4 or L4/L5 interspace (Engelborghs et al., unpublished). Referring centers were asked to perform CSF sample handling and processing according to the standard procedure as described in the BIODEM lab guidelines (<http://www.bornbunge.be>). This includes collection of minimal 2 ml CSF in a labeled polypropylene tube to avoid adsorption of $A\beta$ to the wall of the vial. In case of a hemorrhagic LP detected by macroscopic inspection, CSF should be centrifuged for 10 min at 3000 rpm within 4 h after LP. The supernatant should thereafter be transferred to an unused polypropylene vial. CSF samples should be shipped to the BIODEM lab, unfrozen if sent within 24 h after LP (shipment at room temperature) or frozen if sent later (shipment on dry ice). Samples were stored at -80°C until analysis.

CSF analysis

CSF concentrations of $A\beta_{1-42}$, T-tau, and P-tau_{181P} were determined with commercially available single-analyte ELISA kits (INNOTEST® β -Amyloid₍₁₋₄₂₎, INNOTEST® hTau-Ag, and INNOTEST® Phospho-

Fig. 1. National and international locations of the centers referring samples between 2004 and 2014 (A). Locations of the referring centers subdivided in a time range of 2004–2006 (B) and 2012–2014 (C).

Tau_(181P), respectively; Fujirebio Europe, Ghent, Belgium). With each assay the clinical samples, together with a blank (sample diluent), calibrator solutions, and appropriate quality controls, were analyzed according to the manufacturer's instructions as described previously [2]. The 14-3-3 protein analyses were performed using western blot [22].

As described in the IWG2 criteria for AD, a CSF biomarker profile was considered pathological and suggestive for AD if the analysis resulted in a low CSF A β ₁₋₄₂ value in combination with increased levels of T-tau and/or P-tau_{181P} [10]. Cutoffs used for the

determination of normal values are A β ₁₋₄₂ >638.5 pg/ml, T-tau <296.5 pg/ml, and P-tau_{181P} <56.5 pg/ml as determined in a cohort of autopsy-confirmed AD patients and cognitively healthy elderly [23].

As a part of the Alzheimer's Association Quality Control (QC) Program for CSF biomarkers, the performance of the assays was monitored with CSF QC samples provided by the program. Two different longitudinal samples were included in the routine ELISA analyses since 2010. Sample characteristics and procedures have been described previously by Mattsson et al. [24].

Fig. 2. Workflow of the pre-analytical, analytical and post-analytical steps of the BIODiEM lab for CSF biomarker analyses at the BIODiEM lab.

Informed consent for research purposes

Whenever possible, patients were asked to give written informed consent to use CSF leftovers for scientific research as well as to undergo brain autopsy after death [2, 13]. In case consented patients died, a brain autopsy was performed according to a standard protocol [2, 13]. Neuropathological examination was performed on the right hemisphere of the brain at the Biobank of the Institute Born-Bunge (Antwerp, Belgium). Patients were neuropathologically diagnosed as definite AD, DLB and VaD using the criteria of Montine [25]. Definite FTLTD was diagnosed through the criteria of Cairns [26] and Mackenzie [27, 28], while definite CJD was diagnosed in agreement with the criteria of Markesbery [29].

Statistical analyses

The variables were assessed for normal distribution using the Kolmogorov-Smirnov test. Since variables were not normally distributed, non-parametric tests were performed. For comparisons across groups, Kruskal-Wallis and Mann-Whitney tests were applied. Chi-Square tests were performed to explore gender distributions. Spearman's ρ was calculated to determine correlations. Confidence intervals (CI) were established at 95%. The level of significance was set at $p < 0.05$. The diagnostic

accuracies of the autopsy-confirmed patients was obtained by receiver operating characteristics (ROC) curve analysis for the determination of the area under the curve (AUC), sensitivities, specificities and the optimal cutoff for the differentiation of AD and non-AD dementia diagnosis. The optimal cutoff values were determined by calculation of the maximal sum of the sensitivity and the specificity. All statistical analyses were done using SPSS statistical package of IBM Statistics, version 22.

RESULTS

Description of the population

Table 1 shows the demographic and clinical characteristics of the patients. The database contains samples of a total of 5,022 patients with an equal distribution of males and females. The mean age at time of CSF sampling was 71 years. A proportion of the population (4%) received an autopsy-confirmed diagnosis. For a subgroup of 1,710 patients, the MMSE scores at time of CSF biomarker analysis request were available. Patients who were referred for neurochemical confirmation of AD or for differential AD versus non-AD dementia diagnosis showed significantly lower MMSE scores as compared to the prodromal AD group, and the patients who were referred for a neurochemical diagnosis of AD in case of psychiatric

symptoms and other indications ($p < 0.001$). During the past ten years, the mean MMSE scores remained unchanged (results not shown). Indeed, the relative number of samples from patients referred for neurochemical confirmation of prodromal AD did not increase significantly.

Referral of CSF samples from 2004–2014

Between 2004 and 2014, the analyses of unique samples per year have increased from 238 in 2004 to 804 in 2014 (Fig. 3A). Where initially only Antwerp centers referred samples in 2004, increasing to 381 in 2014, more and more centers from other regions in Belgium started referring samples throughout the years, leading to a total of 423 in 2014 (Fig. 3B). The relative amount of patients per indication (Fig. 3C) demonstrates a switch at the timepoint of the second quarter of 2011 from the majority of requests concerning neurochemical confirmation of AD, to most referrals being for differential dementia diagnosis. Samples referred for neurochemical diagnosis of AD in case of psychiatric symptoms also become slightly more frequent over time. The number of samples referred for other indications, such as CJD, shows a decrease over time. Over the time course of ten years, the number of samples referred for diagnosis of prodromal AD in case of MCI was stable.

Results of CSF biomarker analyses

The mean biomarker concentrations are described in Table 1. All three CSF biomarkers were analyzed in 4983 of the 5025 samples, while the remaining samples were tested only for T-tau ($n = 16$), $A\beta_{1-42}$ ($n = 4$), or P-tau_{181P} ($n = 2$) or two of the three biomarkers ($n = 20$). All samples were analyzed in duplicates. These missing data are due to insufficient sample volume to analyze all three biomarkers and/or reanalyze biomarkers in case the coefficient of variation ($100 \times \text{standard deviation} / \text{mean}$) exceeded 20%. Of the entire study population, 39% ($n = 1,956$) had a neurochemical profile indicative for AD pathology, based on the IWG2 criteria [10]. Samples with a normal CSF biomarker profile accounted for 22% ($n = 1,123$). This leaves another 39% ($n = 1,943$) of the samples unclassifiable by the IWG2 criteria, including samples presenting with normal $A\beta_{1-42}$ but increased T-tau and/or P-tau_{181P} concentrations ($n = 724$) and samples presenting with low $A\beta_{1-42}$ ($< 638.5 \text{ pg/ml}$) but normal T-tau and P-tau_{181P} concentrations ($n = 1,181$). Thirty-eight samples could

not be categorized due to missing analyses of one or two of the CSF biomarkers. Of the 261 samples with very high T-tau values ($> 1,200 \text{ pg/ml}$; value higher than the upper limit of quantification of the test kits used for analysis, as described in the package inserts), 95 samples were analyzed for 14-3-3 protein of which 69 samples were positive.

In total, 170 patients had autopsy-confirmed diagnoses of which 108 with AD pathology and 62 with non-AD pathology (Table 2). Six out of 108 subjects (6%) were autopsy-confirmed AD patients who presented with normal CSF biomarker levels of $A\beta_{1-42}$, T-tau, and P-tau_{181P}, three subjects (3%) with normal $A\beta_{1-42}$ but increased T-tau and/or P-tau_{181P}, and 15 subjects (14%) with low $A\beta_{1-42}$ ($< 638.5 \text{ pg/ml}$) but normal T-tau and P-tau_{181P} levels. The biomarker profiles of these neuropathologically confirmed AD patients therefore do not comply with the IWG2 criteria for AD biomarker diagnosis. Of the autopsy-confirmed non-AD patients, 27 subjects (44%) had a CSF neurochemical profile that was in compliance with the IWG2 criteria for AD biomarker diagnosis. In 16 subjects (9% of the total number of autopsy-confirmed patients) a neurochemical diagnosis could not be established due to analysis of only $A\beta_{1-42}$ or T-tau levels. Thirteen patients with very high T-tau levels ($> 1,200 \text{ pg/ml}$) were autopsy-confirmed CJD. Four of these patients scored negative for 14-3-3 protein. Of the 70 samples with positive 14-3-3 protein analysis, one sample showed to be autopsy-confirmed hippocampal sclerosis. Inter-biomarker correlations and correlations of the biomarkers with age at LP and MMSE score are presented in Table 3. The diagnostic accuracies and optimal cutoff values determined for the individual markers as well as the $A\beta_{1-42}$ /T-tau and $A\beta_{1-42}$ /P-tau_{181P} ratios are shown in Table 4.

Alzheimer's Association QC program for CSF biomarkers

The results of the Alzheimer's Association QC Program first and second longitudinal QC (QC-L) samples are shown in Fig. 4. A second longitudinal QC sample was used in succession of the first due to a supply shortage. All BIODiEM results were within the 2SD range calculated from the mean value of all participating laboratories. The inter-run CV was 14%, 16% and 8% for $A\beta_{1-42}$, T-tau and P-tau_{181P}, respectively, for the first QC-L and 12%, 16%, and 6% for the second QC-L. No shift occurred for the three biomarkers (i.e., a systematic error, where results of six consecutive measurements lie on the same side of

Table 1

Demographic and clinical data. Biomarker concentrations that were found to be abnormal can be indicative for AD pathology, following the AD profile cutoff points: $A\beta_{1-42} < 638.5$ pg/ml, T-tau > 296.5 pg/ml and P-tau_{181P} > 56.5 pg/ml. Other autopsy-confirmed diagnosis involves hippocampal sclerosis, progressive supranuclear palsy and anoxic necrosis

	Total	Clinical indications				
		Neurochemical confirmation of AD	Differential AD versus non-AD dementia diagnosis	Diagnosis of prodromal AD in case of MCI	Differential AD versus depression or other psychiatric disorder diagnosis	Other indication
	(n = 5022)	(n = 1596)	(n = 2136)	(n = 651)	(n = 307)	(n = 332)
Age: mean (CI) (y)	70 (69.4–70.0)	72 (71.1–72.2)	70 (69.3–70.2)	70 (69.5–71.0)	65 (64.1–66.6)	63 (61.3–64.7)
Gender: female / male	2493 / 2529	890 / 706	988 / 1148	317 / 334	166 / 141	132 / 200
MMSE score: mean (CI) (n)	22 (21.4–22) (1710)	20 (19.9–21.0) (486)	21 (20.1–21.0) (776)	25 (25.0–25.8) (310)	24 (22.5–24.9) (87)	24 (22.5–25.6) (51)
Abnormal concentrations: n (%)						
$A\beta_{1-42}$	3140 (62)	1097 (69)	1361 (64)	374 (57)	172 (56)	135 (41)
T-tau	2630 (52)	939 (59)	1111 (52)	331 (51)	109 (36)	137 (41)
P-tau _{181P}	1851 (37)	692 (43)	738 (35)	261 (40)	70 (23)	88 (26)
Biomarker results (pg/ml)						
$A\beta_{1-42}$: mean $\pm$ SD	594 $\pm$ 292	557 $\pm$ 266	603 $\pm$ 306	619 $\pm$ 300	611 $\pm$ 273	645 $\pm$ 301
T-tau: mean $\pm$ SD	409 $\pm$ 294	420 $\pm$ 294	418 $\pm$ 303	399 $\pm$ 292	357 $\pm$ 266	355 $\pm$ 255
P-tau _{181P} : mean $\pm$ SD	58.2 $\pm$ 36.1	63.8 $\pm$ 40.0	56.9 $\pm$ 34.7	53.7 $\pm$ 32.6	51.5 $\pm$ 34.4	54.9 $\pm$ 31.6
Autopsy-confirmed diagnosis: n	170	82	71	5	1	11
AD	108	72	29	4	1	2
Non-AD	62	10	42	1	0	9
FTLD	16	3	13	0	0	0
DLB	12	1	11	0	0	0
VaD	14	5	9	0	0	0
CJD	13	0	5	0	0	8
Other	9	2	5	1	0	1

the mean) nor was there a visible trend for all three biomarkers (i.e., a systematic error, were the measurements move in one direction, heading towards an 'out-of-control' value).

DISCUSSION

During ten years of AD CSF biomarker analyses, the amount of samples referred to the BIODM lab at UAntwerp from clinical centers has increased with 238%. Due to the revisions of diagnostic criteria for AD diagnosis and the herein described use of CSF biomarkers [9–11], confidence in the importance of biomarkers has grown. Not only are they used more often by clinicians, they are also useful in clinical trials as enrichment strategy or outcome measures due to their *in vivo* pathophysiological characteristics [30]. The observed increase of referrals could also have been influenced by different milestones, e.g. the publications of biomarker-based diagnostic

criteria for AD. These specific milestones did not show clear associations with the time course of the number of samples referred. However, there is a general shift from samples referred for neurochemical confirmation of AD diagnosis to referrals for differential AD versus non-AD dementia diagnosis. This may be due to the growing scientific support for biomarker-based differential diagnosis between AD and non-AD dementias [13, 14, 31–33].

Several research papers found their origin in the BIODM database of AD CSF biomarker analyses and an important contribution is ascribed to the subjects that gave informed consent for brain autopsy. Our lab was the first to demonstrate the diagnostic value of the CSF biomarkers $A\beta_{1-42}$, T-tau, and P-tau_{181P} in clinical AD, using the neuropathological diagnosis as a reference [2, 34]. The data demonstrated that all three biomarkers provide useful information, showing promising sensitivity and specificity values that systematically exceed the 80% threshold. The use of a biomarker-based model

Table 2

AD CSF biomarker profile and biomarker levels of autopsy-confirmed AD and non-AD dementia patients. n.s., no significant differences

	AD (n = 108)	Non-AD (n = 62)	Significance
Normal			
n	6	7	
T-tau mean $\pm$ SD (pg/ml)	212 $\pm$ 49	244 $\pm$ 33	n.s.
A β ₁₋₄₂ mean $\pm$ SD (pg/ml)	914 $\pm$ 209	962 $\pm$ 188	n.s.
P-tau _{181P} mean $\pm$ SD (pg/ml)	41.4 $\pm$ 9.3	40.0 $\pm$ 5.2	n.s.
A β ₁₋₄₂ /T-tau mean $\pm$ SD	4.38 $\pm$ 0.64	3.99 $\pm$ 0.84	n.s.
A β ₁₋₄₂ /P-tau _{181P} mean $\pm$ SD	22.62 $\pm$ 5.59	24.16 $\pm$ 4.30	n.s.
Age at LP	78 $\pm$ 6	71 $\pm$ 6	n.s.
MMSE score	24 $\pm$ 2	17 $\pm$ 15	n.s.
Suggestive for AD			
n	72	27	
T-tau mean $\pm$ SD (pg/ml)	585 $\pm$ 216	446 $\pm$ 155	n.s.
A β ₁₋₄₂ mean $\pm$ SD (pg/ml)	402 $\pm$ 131	435 $\pm$ 134	n.s.
P-tau _{181P} mean $\pm$ SD (pg/ml)	84.0 $\pm$ 42.9	61.6 $\pm$ 34.8	$p = 0.001$
A β ₁₋₄₂ /T-tau mean $\pm$ SD	0.72 $\pm$ 0.42	0.88 $\pm$ 0.58	n.s.
A β ₁₋₄₂ /P-tau _{181P} mean $\pm$ SD	5.61 $\pm$ 2.91	8.62 $\pm$ 4.08	$p < 0.001$
Age at LP	76 $\pm$ 13	74 $\pm$ 10	n.s.
MMSE score	15 $\pm$ 7	19 $\pm$ 7	n.s. ($p = 0.58$)
Normal Aβ₁₋₄₂ but increased T-tau and/or P-tau_{181P}			
n	3	13	
T-tau mean $\pm$ SD (pg/ml)	552 $\pm$ 134	481 $\pm$ 204	n.s.
A β ₁₋₄₂ mean $\pm$ SD (pg/ml)	702 $\pm$ 57	832 $\pm$ 116	n.s. ($p = 0.057$)
P-tau _{181P} mean $\pm$ SD (pg/ml)	78.9 $\pm$ 24.9	59.9 $\pm$ 23.3	n.s.
A β ₁₋₄₂ /T-tau mean $\pm$ SD	1.17 $\pm$ 0.67	1.56 $\pm$ 0.77	n.s.
A β ₁₋₄₂ /P-tau _{181P} mean $\pm$ SD	20.53 $\pm$ 6.38	15.83 $\pm$ 6.01	n.s.
Age at LP	81 $\pm$ 5	67 $\pm$ 9	$p = 0.014$
MMSE score	18 $\pm$ 6	19 $\pm$ 7	n.s.
Low Aβ₁₋₄₂ but normal T-tau and P-tau_{181P}			
n	15	11	
T-tau mean $\pm$ SD (pg/ml)	117 $\pm$ 54	166 $\pm$ 65	$p = 0.033$
A β ₁₋₄₂ mean $\pm$ SD (pg/ml)	324 $\pm$ 117	408 $\pm$ 154	n.s.
P-tau _{181P} mean $\pm$ SD (pg/ml)	30.9 $\pm$ 7.6	25.7 $\pm$ 8.9	n.s. ($p = 0.066$)
A β ₁₋₄₂ /T-tau mean $\pm$ SD	1.95 $\pm$ 0.90	3.00 $\pm$ 2.07	n.s.
A β ₁₋₄₂ /P-tau _{181P} mean $\pm$ SD	11.84 $\pm$ 6.10	14.44 $\pm$ 9.04	n.s.
Age at LP	72 $\pm$ 14	69 $\pm$ 10	n.s.
MMSE score	16 $\pm$ 7	19 $\pm$ 3	n.s.

Table 3

Correlations between the three biomarkers and correlations between the biomarkers and the clinical data of the autopsy-confirmed AD and non-AD dementia patients. Correlations are shown by 'Spearman's ρ (p -value)'; n.s., not significant

		T-tau (pg/ml)	A β ₁₋₄₂ (pg/ml)	Age at LP	MMSE score
AD	T-tau (pg/ml)	–	–	n.s.	–0.320 ($p = 0.004$)
	A β ₁₋₄₂ (pg/ml)	n.s.	–	n.s.	n.s.
	P-tau _{181P} (pg/ml)	0.726 ($p < 0.001$)	n.s.	n.s.	n.s.
non-AD	T-tau (pg/ml)	–	–	n.s.	n.s.
	A β ₁₋₄₂ (pg/ml)	n.s.	–	n.s.	n.s.
	P-tau _{181P} (pg/ml)	0.618 ($p < 0.001$)	0.307 ($p = 0.016$)	0.257 ($p = 0.045$)	n.s.

in patients with a clinically ambiguous diagnosis, resulted in a correct diagnosis in the majority of autopsy-confirmed AD and non-AD cases, indicating that biomarkers have an added diagnostic value in these cases [12]. Moreover, the diagnostic value of the AD biomarkers A β ₁₋₄₂, T-tau, and P-tau_{181P} is independent of the platform (single-analyte ELISA or multiplex) on which they are tested, despite the

differences in absolute values, provided that appropriate cutoff points are being used [8].

Investigating autopsy-confirmed AD and non-AD dementia patients has also improved our knowledge and insights with regard to the differential diagnostic value of the existing AD CSF biomarkers. The ratio of A β ₁₋₄₂/P-tau_{181P} was shown to have a higher diagnostic accuracy (sensitivity of 73.5%

Fig. 3. A) Number of unique samples analyzed per year. B) Number of unique samples per year, grouped by location of referring centers. C) Representation of the number of patients for each indication relative to the total amount of patients per quartile.

Table 4

Diagnostic accuracies for the differentiation of AD and non-AD dementia. The optimal cutoff values were determined by calculation of the maximal sum of the sensitivity and the specificity. AUC, area under the curve; CI, confidence intervals; sens, sensitivity; spec, specificity

AD versus non-AD	AUC	95% CI	Cutoff (pg/ml)	Sens (%)	Spec (%)
T-tau	0.670	0.474–0.660	414	65.5	56.2
A β ₁₋₄₂	0.647	0.555–0.739	615	90.0	38.7
P-tau _{181P}	0.676	0.591–0.760	54.5	65.7	63.9
A β ₁₋₄₂ /T-tau	0.635	0.544–0.726	1.55	80.8	45.9
A β ₁₋₄₂ /P-tau _{181P}	0.734	0.655–0.813	9.05	73.5	63.9

Fig. 4. CSF biomarker results of the first and second QC-L samples for Aβ₁₋₄₂, T-tau, and P-tau_{181P}. Our results are plotted (filled squares batch 1, filled circles batch 2) against the mean value (dashed line) with their 2SD ranges (dotted lines) of all participating laboratories.

and specificity 63.9%) than Aβ₁₋₄₂ (sensitivity of 90.0% and specificity 38.7%), T-tau (sensitivity of 65.5% and specificity 56.2%), P-tau_{181P} (sensitivity of 65.7% and specificity of 63.9%), or the Aβ₁₋₄₂/T-tau ratio (sensitivity of 80.8% and specificity 45.9%), while P-tau_{181P} was found to perform better than Aβ₁₋₄₂ or T-tau to discriminate between AD and non-AD dementias (Table 4). This clearly signifies the importance of P-tau_{181P} in the biomarker panel for differential dementia diagnosis [14]. Concomitant AD pathology in DLB, which occurred in 72% of the autopsy-confirmed DLB patients, is reflected by low CSF Aβ₁₋₄₂ values in these patients [35]. However, the differential diagnostic value against pure AD is limited due to the fact that only AD pathology will be detected because of a lack of established CSF DLB biomarkers. Very high T-tau concentrations in CSF (>1200 pg/ml) are indicative for CJD. In case of suspicion of CJD, analysis of CSF 14-3-3 protein can be useful.

The differential diagnostic value of the existing biomarker panel might be improved by the addition

of Aβ₁₋₄₀, as this biomarker better reflects the total pool of Aβ. As the analysis of Aβ₁₋₄₂ may lead to false negative values in case of “high Aβ producers”, normalizing the CSF Aβ₁₋₄₂ concentration to that of the most abundant and stably produced Aβ isoform (i.e. Aβ₁₋₄₀) may improve the diagnostic value for these samples [36–39]. Since formation of amyloid-plaques starts up to twenty years before the onset of the clinical symptoms, the use of low CSF Aβ₁₋₄₂ and the CSF Aβ₁₋₄₂/Aβ₁₋₄₀ ratio opens new possibilities for the identification of ‘asymptomatic at risk for AD’ subjects for clinical trials [40]. Differential diagnosis of AD versus non-AD dementia may also benefit of adding other Aβ isoforms [41] as well as other biomarkers, such as α-synuclein [42, 43] and TDP-43 [44], as they are specific for non-AD dementias.

Even with an elevated confidence in the use of CSF biomarkers for the clinical work-up of dementia diagnosis, multicenter trials have shown that there is a substantial center-to-center variation [45]. The different pre-analytical sample procedures used by the different centers contribute to variability of CSF

biomarker concentrations. This causes an important concern as direct comparisons of measurements between laboratories and across techniques are not reliable, hampering biomarker development and their utility for clinical routine diagnosis [46]. Temperature of freezing, delay until freezing and number of freeze-thaw cycles significantly influence CSF biomarker concentrations, stressing the need for standard operating procedures for pre-analytical sample handling [47]. The differences observed in the latter study were, however, relatively small. An exploratory study in MCI patients with clinical follow-up and autopsy-confirmed AD patients provided evidence that, for a specific context of use, the impact on clinical diagnostic accuracy of biomarker concentration shifts might be lower than originally expected [46]. However, standardization of (pre)analytical sample handling as well as the cutoff thresholds should be accomplished as they influence biomarker results [47, 48]. With this in mind, several programs for standardization and harmonization were set up, such as the QC program of the Alzheimer's Association, as well as the Alzheimer Biomarker Standardization Initiative (ABSI) and the JPND BIOMARKAPD consortium [49–52].

CONCLUSION

This paper provides insight in the use of CSF biomarkers for the clinical diagnostic work-up of AD and related disorders in Belgium during the past decade. Within this period, over 5,000 CSF samples have been analyzed. Several research papers found their origin in the AD CSF biomarker analyses, with a significant contribution of subjects who gave informed consent for brain autopsy. Studies on autopsy-confirmed AD patients that do not comply with the criteria for a neurochemical diagnosis of AD, which accounted for 30% of the definite diagnoses, are needed to improve our insights in the boundaries of the current AD CSF biomarkers. Improving the quality of CSF biomarker analyses for AD diagnosis can be accomplished by complying with recommendations of standardization programs.

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