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Advantages and disadvantages of the use of the CSF Amyloid β (Aβ) 42/40 ratio in the diagnosis of Alzheimer's Disease

Oskar Hansson[1,2], Sylvain Lehmann[3], Markus Otto[4], Henrik Zetterberg[5,6,7] and Piotr Lewczuk[8,9,10*]

Abstract

The cerebrospinal fluid (CSF) biochemical markers (biomarkers) Amyloidβ 42 ($A\beta_{42}$), total Tau (T-tau) and Tau phosphorylated at threonine 181 (P-tau_{181}) have proven diagnostic accuracy for mild cognitive impairment and dementia due to Alzheimer's Disease (AD). In an effort to improve the accuracy of an AD diagnosis, it is important to be able to distinguish between AD and other types of dementia (non-AD). The concentration ratio of $A\beta_{42}$ to $A\beta_{40}$ ($A\beta_{42/40}$ Ratio) has been suggested to be superior to the concentration of $A\beta_{42}$ alone when identifying patients with AD. This article reviews the available evidence on the use of the CSF $A\beta_{42/40}$ ratio in the diagnosis of AD. Based on the body of evidence presented herein, it is the conclusion of the current working group that the CSF $A\beta_{42/40}$ ratio, rather than the absolute value of CSF $A\beta_{42}$, should be used when analysing CSF AD biomarkers to improve the percentage of appropriately diagnosed patients.

Keywords: Alzheimer's Disease, Amyloidβ Peptides, $A\beta_{42/40}$ ratio, Biomarkers, Cerebrospinal Fluid

Introduction

Alzheimer's Disease (AD) is the most prevalent form of age-related dementia. The clinical manifestation of AD is generally preceded by a relatively symptom-free preclinical phase [1]. After the first clinical symptoms appear in the prodromal phase, patients transition clinically into mild cognitive impairment (MCI) [2], which eventually results in AD dementia (ADD) [3]. These phases are accompanied by biochemical changes in the brain that are reflected in cerebrospinal fluid (CSF) [4, 5]. Decreases in CSF concentrations of amyloid-beta 42 ($A\beta_{42}$) (a marker of amyloidosis) and elevations in tau species (markers of axonal damage and neurofibrillary tangles) are well-established as biomarkers useful for AD diagnosis [6, 7]. Importantly, analysis of these CSF biochemical markers (biomarkers) for AD has been shown to predict conversion from MCI to ADD with accuracies of > 80% [8–10].

Depending on their age, approximately 30–50% of patients with MCI will develop ADD within 5 years [11]. Therefore, early diagnosis is essential to enable appropriate counselling to take place, as well as for planning treatment and care. In addition, the possibility of making an early diagnosis, prior to the appearance of symptoms, is essential for the clinical evaluation of novel, potentially disease-modifying drugs for the treatment of AD.

In an effort to improve the accuracy of an AD diagnosis, it is important to be able to distinguish between AD and other types of dementia (non-AD) that are not characterized by amyloid pathology. Although the concentration of another amyloid peptide species, $A\beta_{40}$, has been reported to be unaltered in AD, the concentration ratio of $A\beta_{42}$ to $A\beta_{40}$ ($A\beta_{42/40}$ ratio) has been suggested to be superior to the concentration of $A\beta_{42}$ alone in discriminating patients with AD [12, 13]. To date, there has been a lack of comprehensive reviews on the applicability of the CSF $A\beta_{42/40}$ ratio in AD diagnosis.

Thus, the aim of the current article was to review the available evidence on the use of the CSF $A\beta_{42/40}$ ratio in the (i) differential diagnosis of AD dementia vs non-AD dementias and (ii) prediction of subsequent development of AD dementia in cases with MCI, and to discuss its value in comparison to other CSF biomarkers and compared to other diagnostic modalities, such as Aβ positron

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emission tomography (Aβ-PET). In addition, the effects of non-AD pathologies and pre-analytical handling on the various CSF biomarkers were also discussed. In order to achieve these goals, a working group was brought together to critically evaluate the evidence for use of the CSF $A\beta_{42/40}$ ratio in the diagnosis of AD and a consensus paper was drafted reviewing the advantages and disadvantages surrounding the use of the CSF $A\beta_{42/40}$ ratio.

This review addresses the advantages and disadvantages of the $A\beta_{42/40}$ ratio to detect Aβ pathology, which is an approach to normalize the $A\beta_{42}$ CSF concentration for the total Aβ CSF concentration (represented by the most abundant isoform, i.e. $A\beta_{40}$). In contrast, this paper does not address approaches to interpret the overall pattern of the AD CSF biomarkers that combine results derived from the two distinct AD pathologies (amyloidosis and neurodegeneration) to form any kind of 'ratios'.

Methods

A group of experts in the field of AD biomarkers were brought together, and several meetings of this working group were conducted. During the meetings, discussions took place based both on evidence gathered from scientific publications and the experience of the group members, as experts in the field. Studies were selected to be included in this paper based on an independent review by at least two (in most cases by all) co-authors of the report, with MEDLINE database as the primary source of the studies. Searches were conducted using keywords, such as '$A\beta_{42/40}$' and '$A\beta_{40}$' and excluding those, whose primary scope was not the role of the $A\beta_{42/40}$ ratio in AD diagnostics. The results of the discussions and the evidence gathered during the meetings are presented in this paper.

Results

$A\beta_{42}$ versus the $A\beta_{42/40}$ ratio

Comparison of the diagnostic accuracy in the context of use of differential diagnostics when discriminating AD from other neurodegenerative disorders

Due to similar clinical manifestations and overlapping brain pathologies, differentiation of AD from other neurodegenerative disorders may prove difficult even with the aid of biomarkers. For example, the symptoms and biomarker patterns observed in patients with dementia with Lewy bodies (DLB) or subcortical vascular dementia (VaD) sometimes closely resemble those of AD, which makes differential diagnosis difficult and decreases the diagnostic accuracy of the core CSF AD biomarkers, especially in the early stages of the disease. Therefore, evidence was gathered on whether adding the CSF $A\beta_{42/40}$ ratio to the existing panel of biomarkers could improve the accuracy of the differential diagnosis of AD from other dementia disorders. Here we provide details of 16 studies that have compared the diagnostic accuracy of CSF biomarkers to diagnose ADD versus non-ADDs. These studies demonstrate the usefulness of the CSF $A\beta_{42/40}$ ratio for the diagnosis of AD in patients with dementia. Studies with relevant data are also summarized in Table 1.

In a study of patients with ADD, normal controls, patients with non-ADD and patients with other neurological diseases, Shoji et al. [12] found that the ADD group had a significantly higher level of tau than the normal control group ($p < 0.001$), but $A\beta_{40}$ levels did not show any significant differences between the groups. The reduction of $A\beta_{42}$ levels in AD also resulted in a significant increase in the $A\beta_{40/42}$ ratio (note that the ratio reported in this study has $A\beta_{40}$ in the numerator, in contrast to most of the other studies summarized in the current paper) as an improved marker. The authors therefore concluded that the Aβ ratio is another important marker for AD.

Lewczuk et al. [13] measured concentrations of $A\beta_{42}$, $A\beta_{40}$ and total tau (T-tau) in order to compare their accuracy in discriminating patients with ADD, non-ADD and control subjects. The results showed that concentrations of $A\beta_{42}$ were decreased ($p < 0.001$) and of T-tau were increased ($p < 0.001$) in ADD patients, while $A\beta_{40}$ concentrations did not differ significantly among the groups. For all groups when the $A\beta_{42/40}$ ratio was used, more patients were classified correctly, compared to when the $A\beta_{42}$ concentration alone was used (94 vs 86.7% when comparing ADD to controls, 90 vs 85% when comparing ADD to non-ADD and 90.8 vs 87% when comparing ADD to non-ADD plus controls). The improvement of the diagnostic accuracy reported in this study was not significant, probably due to the small numbers of subjects and a clear ceiling effect (a relatively high number of patients were already correctly classified using $A\beta_{42}$ alone).

Gabelle et al. [14] evaluated the value of individual and combined measurements of CSF biomarkers. They found that both $A\beta_{40/42}$ and $A\beta_{38/42}$ ratios were significantly altered in AD. They also found that the $A\beta_{40/38}$ ratio was the only one that differentiated clearly control subjects from FTD subjects, while not being significant between AD and FTD. In the ROC curves, they found that for FTD versus AD diagnosis, the best AUCs for amyloid biomarkers were the $A\beta_{38/42}$ ratio and the Innogenetics Aβ/Tau index (IATI) (AUCs = 0.87). However, the $A\beta_{40/42}$ ratio or $A\beta_{42}$ alone had very close and statistically undifferentiated AUC values. The authors concluded that the $A\beta_{38/42}$, $A\beta_{40/42}$ and the IATI ratios were also better than individual biomarkers to identify AD therefore justifying their clinical relevance.

In another study carried out by Wiltfang et al. [15], the authors found that alterations of $A\beta_{42}$ concentrations might not only result from AD pathology but may also be related to total Aβ peptide concentrations. In

Table 1 CSF biomarkers to distinguish cases with ADD from cases with non-ADD

Study	Number of AD patients	Number of non-AD patients	Number of control patients	CSF biomarkers	Optimal cut-off*	Sensitivity % (95% CI)**	Specificity % (95% CI)**	AUC (95% CI)	SL (p value)#
Shoji et al. [12]	55	68	34	$A\beta_{42}$	158.6 fmol/mL	–	–	–	–
				$A\beta_{42/40}$ ratio##	0.078##	51	82	–	NP
Lewczuk et al. [13]	22	11	35	$A\beta_{42}$	550 pg/mL	100	80	0.923	–
				$A\beta_{42/40}$ ratio	9.75	95.2	88.4	0.944	NP
Spies et al. [22]	69	69	47	AD vs controls					
		16 DLB		$A\beta_{42}$	–	93	87	0.949	–
		27 FTD		$A\beta_{42/40}$ ratio	–	93	87	0.947	NP
		26 VaD		AD vs non-AD					
				$A\beta_{42}$	–	83	74	0.811	NP
				$A\beta_{42/40}$ ratio	–	85	85	0.903	
Hertze et al. [32]	94	166 (MCI) 29 (DD)	38	AD vs controls					
				$A\beta_{42\ MSD}$	< 523	73	89	0.88 (0.82–0.93)	–
				$A\beta_{42\ MSD}/_{40}$ ratio	< 0.069	93	86	0.91 (0.86–0.95)	NP
				$A\beta_{42\ MSD}/_{38}$ ratio	< 0.37	87	82	0.89 (0.83–0.93)	NP
				MCI-AD vs MCI					
				$A\beta_{42\ MSD}$	< 523	67	71	0.73 (0.66–0.80)	–
				$A\beta_{42\ MSD}/_{40}$ ratio	< 0.069	85	71	0.86 (0.79–0.91)	NP
				$A\beta_{42\ MSD}/_{38}$ ratio	< 0.37	88	71	0.85 (0.79–0.91)	NP
Gabelle et al. [14]	52	34	42	AD vs FTD					
				$A\beta_{42}$	> 464	79	62	0.75	–
				$A\beta_{42/40}$ ratio	≤11.1	79	76	0.85	n.s.
				$A\beta_{42}/_{38}$ ratio	≤2.00	88	86	0.87	n.s.
Slaets et al. [16]	80 69 (NP) 11 (AD+CVD)	75 24 DLB (15 NP) 29 FTD (12 NP) 22 VaD (11 NP)	30	$A\beta_{42}$	517 pg/mL	81	59	0.747 (0.670–0.827)	–
				$A\beta_{42/40}$ ratio	0.057	81	60	0.749 (0.673–0.826)	NP
Nutu et al. [17]	48	127 43 PD 33 PDD							
		51 DLB	107	AD vs control					
				$A\beta_{42}$	444 ng/L	94	72	0.871 (0.811–0.930)	-NP
				$A\beta_{42/40}$ ratio	0.125	92	79	0.871 (0.801–0.933)	
				AD vs PDD					
				$A\beta_{42}$	449 ng/L	94	61	0.805 (0.704–0.905)	–
				$A\beta_{42}/_{40}$ ratio	0.150	90	81	0.910 (0.844–0.976)	NP
				AD vs DLB					

Table 1 CSF biomarkers to distinguish cases with ADD from cases with non-ADD *(Continued)*

Study	Number of AD patients	Number of non-AD patients	Number of control patients	CSF biomarkers	Optimal cut-off*	Sensitivity % (95% CI)**	Specificity % (95% CI)**	AUC (95% CI)	SL (p value)#
				$A\beta_{42}$	387 ng/L	88	41	0.675 (0.570–0.780)	–
				$A\beta_{42/40}$ ratio	0.115	90	57	0.759 (0.664–0.853)	NP
Baldeiras et al. [18]				AD vs controls					
				$A\beta_{42}$	534 pg/mL	82	74	0.818	–
				$A\beta_{40/42}$ ratio	8.3	59	81	0.719	NP
				AD vs FTD					
				$A\beta_{42}$	538 pg/mL	70	82	0.791	–
				$A\beta_{40/42}$ ratio	5.4	59	87	0.778	NP
Dumurgier et al. [19]	367 (AD+ non-AD)	–	0	AD vs non-AD					
				$A\beta_{42}$	737–836 pg/mL	78	79	0.81	–
				$A\beta_{42/40}$ ratio	0.050–0.082	73	78	0.81	NP
Struyfs et al. [23]	100	50	50	AD vs controls					
	50 (AD)	17 (DLB)		$A\beta_{42}$	< 722 pg/mL	98.0	74.0	0.874	–
	50 (MCI-AD)	17 (FTD)		$A\beta_{42/40}$ ratio	< 0.1099	85.7	78.0	0.881	NP
		16 (VaD)		$A\beta_{42/38}$ ratio	< 0.269	81.6	82.0	0.858	NP
				AD vs non-AD					
				$A\beta_{42}$	< 694 pg/mL	95.9	40.0	0.686	–
				$A\beta_{42/40}$ ratio	< 0.1215	93.9	50.0	0.782	NP
				$A\beta_{42/38}$ ratio	< 0.2730	81.6	68.0	0.804	NP
Bousiges et al. [25]	70	55	15	Pro-AD vs pro-DLB					
	31 (AD-d)	20 (DLB-d)		$A\beta_{42}$	≤ 730 ng/L	84.6	71.4	0.84 (0.74–0.92)	–
	39 (pro-AD)	35 (pro-DLB)		$A\beta_{42/40}$ ratio	≤ 0.0529	88.9	100	0.95 (0.83–0.99)	NP
				AD-d vs DLB-d					
				$A\beta_{42}$	≤ 504 ng/L	67.7	80	0.77 (0.63–0.88)	–
				$A\beta_{42/40}$ ratio	≤ 0.0799	92.3	88.9	0.86 (0.64–0.97)	NP
Janelidze et al. [24]	Cohort 2	Cohort 2	Cohort 2	AD vs MCI					
	75 (AD)	62 (MCI)	53	$A\beta_{42}$	–	–	–	0.817 (0.743–0.890)	–
	35 (MCI-AD)	34 (VaD)	Cohort 3	$A\beta_{42/40}$ ratio	–	–	–	0.879 (0.823–0.936)	< 0.028
	Cohort 3	47 (DLB/PDD)	328	$A\beta_{42/38}$ ratio	–	–	–	0.856 (0.790–0.923)	< 0.222
	137	33 (FTD)		AD vs DLB/PDD					
		Cohort 3		$A\beta_{42}$	–	–	–	0.583 (0.476–0.690)	–
		35 (DLB/PDD)		$A\beta_{42/40}$ ratio	–	–	–	0.792 (0.707–0.877)	< 0.001
		128 (PD)		$A\beta_{42/38}$ ratio	–	–	–	0.796 (0.710–0.883)	< 0.001
				AD vs VaD					

Table 1 CSF biomarkers to distinguish cases with ADD from cases with non-ADD *(Continued)*

Study	Number of AD patients	Number of non-AD patients	Number of control patients	CSF biomarkers	Optimal cut-off*	Sensitivity % (95% CI)**	Specificity % (95% CI)**	AUC (95% CI)	SL (p value)[#]
				$A\beta_{42}$	–	–	–	0.698 (0.580–0.816)	–
				$A\beta_{42/40}$ ratio	–	–	–	0.880 (0.814–0.946)	< 0.001
				$A\beta_{42/38}$ ratio	–	–	–	0.860 (0.786–0.935)	< 0.001
				AD vs non-AD					
				$A\beta_{42}$	–	–	–	0.720 (0.651–0.788)	–
				$A\beta_{42/40}$ ratio	–	–	–	0.863 (0.813–0.912)	< 0.001
				$A\beta_{42/38}$ ratio	–	–	–	0.863 (0.813–0.913)	< 0.001
Lehmann et al. [26]	342	562	0	AD vs non-AD					
	Cohort 1	Cohort 1		Cohort 1					
	124	276		$A\beta_{42}$	500 pg/mL	–	–	0.78 (0.734–0.818)	–
	Cohort 2	Cohort 2		$A\beta_{42/40}$ ratio	0.1	–	–	0.90 (0.865–0.926)	< 0.0001
	218	286		Cohort 2					
				$A\beta_{42}$	700 pg/mL	–	–	0.60 (0.553–0.641)	–
				$A\beta_{42/40}$ ratio	0.05	–	–	0.77 (0.728–0.803)	< 0.0001

*Optimal cut-offs were created using different statistical approaches—please see original articles for details. **Sensitivity and specificity are a function of the cut-off, and the cut-offs were calculated in different ways; therefore, they are not clearly comparable across different articles. [#]Significance levels (p values) of the AUC values are comparisons of the Aβ isoform ratios vs $A\beta_{42}$ alone. [##]Note that the ratio in the original article is inversed, but for consistency, the $A\beta_{42/40}$ ratio has been calculated for this table. *Aβ* Amyloid beta, *AD* Alzheimer's Disease, *ADD* AD dementia, *Ad-d* demented AD patients, *AUC* area under curve, *CJD* Creutzfeldt-Jakob Disease, *CSF* cerebrospinal fluid, *DLB* dementia with Lewy bodies, *DLB-d* demented DLB patients, *FTD* frontotemporal dementia, *MCI* mild cognitive impairment, *MCI-AD* MCI that subsequently developed ADD or MCI due to AD, *NP* neuropathologically confirmed, *NP* not provided, *NPH* normal pressure hydrocephalus, *n.s* not significant, *PD* Parkinson's Disease, *PDD* Parkinson's Disease dementia, *pro-AD* prodromal-AD patients, *pro-DLB* prodromal-DLB patients, *PsD* psychiatric disorders, *SL* significance level, *VaD* vascular dementia

such cases, healthy individuals with relatively low total Aβ might be misdiagnosed as having 'pathologically low' $A\beta_{42}$ concentrations, and vice versa, AD subjects with high total Aβ might be misinterpreted as normal $A\beta_{42}$ carriers. It was therefore concluded that the analysis of CSF $A\beta_{42}$ alone (i.e. without $A\beta_{40}$) might lead to misinterpretation of the neurochemical dementia diagnostics outcome in subjects with constitutively high or low concentrations of total Aβ peptides. Consequently, the authors conclude that the CSF $A\beta_{42/40}$ ratio can possibly improve the reliability of the neurochemical dementia diagnosis.

A study by Slaets et al. [16] compared the use of different biomarkers for the diagnosis of AD. Addition of the CSF $A\beta_{42/40}$ ratio to the existing panel of biomarkers, $A\beta_{42}$, $A\beta_{40}$ and tau phosphorylated at threonine 181 ($P\text{-}tau_{181}$) was compared to the panel without the addition of the ratio. The results showed that the CSF $A\beta_{42/40}$ ratio was significantly decreased in AD patients (0.043 ± 0.021) compared to non-AD patients (0.064 ± 0.027; $p < 0.001$) and controls (0.053 ± 0.023; $p < 0.001$). Following receiver operating characteristic (ROC) analysis, the optimal cut-offs discriminating the groups were defined as the values maximising the sum of the sensitivity and the specificity. Although the difference between the areas under the ROC curves (AUC) for $A\beta_{42}$ and $A\beta_{42/40}$ turned out to be insignificant, the diagnostic accuracy of the decision tree that contained $A\beta_{42}$, $A\beta_{40}$, $P\text{-}tau_{181}$ and the $A\beta_{42/40}$ ratio was significantly better than the diagnostic accuracy of the decision tree without $A\beta_{40}$ and the $A\beta_{42/40}$ ratio (80% vs 74%; $p < 0.001$). The authors concluded that there was no difference in CSF $A\beta_{40}$ levels found between AD and non-AD patients, but that adding CSF $A\beta_{40}$ and the CSF $A\beta_{42/40}$ ratio to a biomarker-based decision tree, might have an added value for discriminating AD from non-AD patients in cases with intermediate CSF $P\text{-}tau_{181}$ values.

Nutu et al. [17] evaluated whether the CSF $A\beta_{42/40}$ ratio could be used for differentiating AD from DLB and Parkinson's Disease Dementia (PDD). The primary finding of this study was that the CSF $A\beta_{42/40}$ ratio increased

discrimination of AD from PDD and DLB compared with either of the two Aβ biomarkers individually. Of note, in this study, $A\beta_{40}$ was significantly higher in AD. Furthermore, the authors concluded that use of the $A\beta_{42/40}$ ratio could improve the differentiation of AD from PDD and DLB.

In a study by Baldeiras et al. [18], the added value of another CSF Aβ-peptide ($A\beta_{40}$), along with the core CSF markers T-tau, P-tau_{181} and $A\beta_{42}$, in the discrimination between two large dementia groups of FTD ($n = 107$), AD ($n = 107$) and non-demented subjects ($n = 33$) was evaluated. The authors found that their data 'taken together' indicated that 'although CSF $A\beta_{40}$ has no added value in the distinction between AD and FTD patients, it might be useful in the discrimination between AD and FTD patients from non-demented controls, and therefore could be considered in patients diagnostic work-up'.

In a prospective study of subjects with cognitive disorders at three French memory centres (Paris-North, Lille and Montpellier; the PLM study), Dumurgier et al. [19] assessed whether the use of the $A\beta_{42/40}$ ratio would reduce the frequency of indeterminate CSF profiles. They found that, on the basis of local optimum cut-offs for $A\beta_{42}$ and P-tau_{181}, 22% of patients had indeterminate CSF profiles. The systematic use of the $A\beta_{42/40}$ ratio instead of $A\beta_{42}$ levels alone decreased the number of indeterminate profiles (17%; $p = 0.03$), but it failed to improve the classification of subjects (NRI = − 2.1%; $p = 0.64$). In contrast, use of the $A\beta_{42/40}$ ratio instead of $A\beta_{42}$ levels alone in patients with a discrepancy between P-tau_{181} and $A\beta_{42}$ led to a reduction by half of the number of indeterminate profiles (10%; $p < 0.001$) and was also in agreement with clinician diagnosis (NRI = 10.5%; $p = 0.003$). The authors therefore concluded that in patients with a discrepancy between CSF P-tau_{181} and CSF $A\beta_{42}$, the assessment of the $A\beta_{42/40}$ ratio led to a reliable biological conclusion in over 50% of cases that agreed with a clinician's diagnosis.

Sauvee et al. [20] investigated whether the CSF $A\beta_{42/40}$ ratio could be used to improve the accuracy of diagnostically relevant conclusions in patients with ambiguous CSF $A\beta_{42}$ or tau results. They found that one third of the biomarker profiles of patients with atypical dementia were ambiguous. The addition of the CSF $A\beta_{42/40}$ ratio increased the proportion of interpretable profiles from 69 to 87%, without changing the conclusion when the usual biomarkers ($A\beta_{42}$ and P-tau_{181}) were concordant. The authors therefore concluded that their results support the use of the $A\beta_{42/40}$ ratio in addition to the usual CSF AD biomarkers for patients with ambiguous profiles. They added that this method could be specifically directed to this population (i.e. patients with ambiguous results) in order to improve the level of certainty for clinical routine practice.

Lewczuk et al. [21] also compared the diagnostic accuracies of the CSF $A\beta_{42/40}$ ratio and CSF $A\beta_{42}$ alone. Analysis of $A\beta_{42}$ gave a sensitivity and specificity of 69.3% and 88.9%, respectively, whereas the $A\beta_{42/40}$ ratio showed significantly improved performance with sensitivity and specificity of 93.3% and 100%, respectively. Thus, the authors concluded that the CSF $A\beta_{42/40}$ ratio concentration shows significantly better diagnostic performance compared to the CSF $A\beta_{42}$ concentration alone. It should be noted, however, that this study must not be interpreted as providing absolute values of the diagnostic accuracies, but their relative comparison.

In another study including various CSF biomarkers, Spies et al. [22] investigated the CSF $A\beta_{42/40}$ ratio under the assumption that $A\beta_{40}$ closely represents the total cerebral Aβ load. They found that the $A\beta_{42/40}$ ratio improves differentiation of AD patients from VaD, DLB and non-ADD patients when compared to $A\beta_{42}$ alone. Furthermore, they found that the $A\beta_{42/40}$ ratio is a more easily interpretable alternative to the combination of $A\beta_{42}$, P-tau_{181} and T-tau when differentiating AD from either frontotemporal dementia (FTD) or other non-ADDs. Since they found different $A\beta_{40}$ concentrations in the various dementia groups, the authors also added that it can be debated if the $A\beta_{42/40}$ ratio is a good representation of the $A\beta_{42}$ fraction of the total Aβ load and thus eliminates inter-individual differences in total Aβ concentrations.

A study of patients with AAD, non-ADDs (DLB, FTD, VaD), MCI due to AD and non-demented controls found that the CSF $A\beta_{42/40}$ ratio improved the diagnostic performance of $A\beta_{42}$ in most differential diagnostic situations. Struyfs et al. [23] also found that the $A\beta_{42/40}$ ratio was the best biomarker to distinguish between AD-MCI and FTD.

Similarly, Janelidze et al. [24] also found that the CSF $A\beta_{42/40}$ ratio, as well as the CSF $A\beta_{42/38}$ ratio, was 'superior biomarkers of AD pathology compared with $A\beta_{42}$ alone'. Using three commercially available CSF biomarker immunoassays, this study found that the CSF $A\beta_{42/40}$ and $A\beta_{42/38}$ ratios improved differentiation of AD from non-ADs, especially when separating AD from DLB/PDD and VaD.

The authors point to several potential explanations for the improved accuracy when using the CSF $A\beta_{42/40}$ and $A\beta_{42/38}$ ratios instead of $A\beta_{42}$. They suggest that it might be that subcortical pathologies not specific to AD, such as WMLs and alpha-synuclein pathology, cause reduced levels of all CSF Aβ species, including $A\beta_{42}$. A second explanation for the improved diagnostic accuracy of the $A\beta_{42/40}$ and $A\beta_{42/38}$ ratios could be that differences in the overall production and clearance of Aβ probably contribute to inter-individual variability in total CSF Aβ levels. This is supported by the present finding that in CSF $A\beta_{42}$ correlates with $A\beta_{38}$ and $A\beta_{40}$ even in healthy controls. Consequently, when detecting $A\beta_{42}$ brain pathology with CSF $A\beta_{42}$, using ratios to $A\beta_{40}$ or $A\beta_{38}$ might correct for inter-individual differences in total Aβ levels.

In another study, which evaluated the differential diagnosis of DLB and AD, once again, the CSF $A\beta_{42/40}$ ratio was found to aid diagnosis. The study by Bousiges et al. [25] found that the $A\beta_{42/40}$ ratio remained unchanged in DLB patients between the prodromal and demented stages, contrary to what was observed in AD. The $A\beta_{42/40}$ ratio therefore makes it possible to distinguish between the two pathologies 'at a time when the differential diagnosis is difficult'.

Finally, in a study by Lehmann et al. [26], the $A\beta_{42/40}$ ratio was added to a previously described PLM scale (Paris-Lille-Montpellier study), which combines $A\beta_{42}$, T-tau and P-tau_{181} biomarkers, in order to evaluate an optimized PLM_R scale (PLM ratio scale). Nine hundred and four participants (342 AD and 562 non-AD) were studied, in two chronologically different cohorts (400 Mtp-1 and 504 Mtp-2). For AD patients, the mean CSF $A\beta_{42}$ and CSF $A\beta_{42/40}$ ratio was 553 ± 216 pg/mL and 0.069 ± 0.022 pg/mL in Mtp-1 and 702 ± 335 pg/mL and 0.045 ± 0.020 pg/mL in Mtp-2. The distribution of AD and non-AD differed between the PLM and the PLM_R scales ($p < 0.0001$). The percentage AD well-classified (class 3) increased with PLM_R from 38 to 83% in Mpt-1 and from 33 to 53% in Mpt-2. A sharp reduction of the discordant profiles going from 34 to 16.3% and from 37.5 to 19.8%, for Mtp-1 and Mtp-2 respectively, was also observed. The authors concluded that the integration of the $A\beta_{42/40}$ ratio in the PLM_R scale resulted in an easy-to-use tool which reduced the discrepancies in biologically doubtful cases and increased the confidence in the diagnosis.

In order to try to assess what is the overall impact on diagnosis, an estimation was made of what the actual percentage of patients that are misdiagnosed by $A\beta_{42}$ alone and that become correctly classified with the $A\beta_{42/40}$ ratio. Assuming normal distribution of $A\beta_{40}$ across the population of interest [15], a very conservative estimation is that neglecting $A\beta_{40}$ (which is equivalent to applying $A\beta_{42}$ as the sole CSF biomarker of amyloidosis) leads to misdiagnoses of ca. 5–10% of cases. This is further confirmed by Baldeiras et al. [27], who found an increase in the proportion of interpretable CSF profiles from 61 to 75% (i.e. ca. 20% of the baseline value) of the MCI patients. Also, Dorey et al. [28] report that determining CSF $A\beta_{40}$ concentrations corrected diagnosis in AD patients with non-pathological CSF $A\beta_{42}$ levels in 76.2% of cases using the CSF $A\beta_{42/40}$ ratio.

In summary, the accumulation of evidence clearly points to the usefulness of the CSF $A\beta_{42/40}$ ratio for the diagnosis of AD in patients with dementia. The CSF $A\beta_{42/40}$ ratio is also better than CSF $A\beta_{42}$ alone at distinguishing AD dementia from non-AD dementias, not only from controls. The evidence therefore strongly suggests that the CSF $A\beta_{42/40}$ ratio, rather than CSF $A\beta_{42}$ alone, should be used in the clinical work-up of AD, as a way to improve the diagnostic accuracy for distinguishing ADD from other dementia disorders.

Comparison of the diagnostic accuracy for predicting the development of ADD in patients with MCI

With disease-modifying therapies on the horizon, there is a need to be able to predict the risk of developing ADD before the onset of dementia, i.e. during the MCI and subjective cognitive decline (SCD) stages. The increased percentage of MCI subjects with pathologic CSF who convert to ADD, compared to those having normal CSF, is considered a very strong argument in favour of the use of CSF biomarkers as predictors of MCI to ADD conversion. Here we provide details of six studies that have compared the diagnostic accuracy of CSF biomarkers when predicting the development of ADD in patients with MCI. All of the studies show the added value of the CSF $A\beta_{42/40}$ ratio in accurately predicting progression to ADD. Studies with relevant data are also summarized in Table 2.

In a study validating the previously introduced Erlangen Score interpretation algorithm [29], Lewczuk et al. compared three- and four-biomarker-based approaches in the German Competence Network Dementias cohort. They found that the score based on four biomarkers (i.e. including the $A\beta_{42/40}$ ratio in addition to $A\beta_{42}$) correlated better with the ratio of pre-dementia subjects having progressed to ADD than the score based on three biomarkers [30].

In a study by Hansson et al. [31], baseline levels of $A\beta_{40}$ and $A\beta_{42}$ in CSF were measured in a cohort of patients with MCI ($n = 137$) in relation to the final diagnosis after 4–6 years of follow-up. The $A\beta_{42}$ concentration at baseline and the $A\beta_{42/40}$ ratio were significantly decreased in MCI patients who developed ADD compared to cognitively stable MCI patients and MCI patients who developed other forms of dementia ($p < 0.001$). The baseline levels of $A\beta_{40}$ were similar in all MCI groups but correlated with change in Mini-Mental State Examination scores in converters to ADD. The $A\beta_{42/40}$ ratio was superior to $A\beta_{42}$ concentration with regard to identifying incipient AD in MCI ($p < 0.05$). The authors concluded that the data provides support for the view that amyloid precursor protein metabolism is disturbed in early sporadic AD and points to the usefulness of the $A\beta_{42/40}$ ratio as a predictive biomarker for AD.

A study by Hertze et al. [32] investigated the diagnostic accuracy of CSF biomarkers to predict the development of ADD within 5 years in patients with MCI. The results showed that the predictive values of the $A\beta_{42/40}$ and $A\beta_{42/38}$ ratios were higher compared to that of $A\beta_{42}$ alone ($p < 0.01$) when using the electrochemiluminescence technology of the Meso Scale Discovery (MSD) platform to quantify amyloid in MCI patients. However, $A\beta_{42}$ quantified with a

Table 2 CSF biomarkers for predicting the development of AD in patients with MCI

Study	Number of AD patients	Number of MCI/ non-AD patients	Number of control patients	CSF biomarkers	Optimal cut-off*	Sensitivity % (95% CI)**	Specificity % (95% CI)**	AUC (95% CI)	SL (p value)#
Hansson et al. [31]	0	137 (MCI)	0	$A\beta_{42}$	0.64 ng/mL	93 (82–98)	53 (41–64)	0.77 (0.69–0.84)	–
				$A\beta_{42/40}$ ratio	0.95	87 (76–95)	78 (67–86)	0.87 (0.80–0.92)	< 0.05
Hertze et al. [32]	94	166 (MCI) 29	38	MCI-AD vs MCI					
		(DD)		$A\beta_{42\ MSD}$	< 523	67	71	0.73 (0.66–0.80)	–
				$A\beta_{42\ MSD}/_{40}$ ratio	< 0.069	85	71	0.86 (0.79–0.91)	NP
				$A\beta_{42\ MSD}/_{38}$ ratio	< 0.37	88	71	0.85 (0.79–0.91)	NP
Parnetti et al. [33]	28 (AD) 32	58 (MCI-MCI)	0	MCI-AD vs MCI-MCI					
	(MCI-AD)	28 (OND)		$A\beta_{42}$	420.5 pg/mL	56 (38–74)	96 (88–99)	0.85	–
				$A\beta_{42/40}$ ratio	5.3	71 (48–89)	92 (79–98)	0.82	NP
				AD vs non-AD (OND)					
				$A\beta_{42}$	500.0 pg/mL	63 (42–81)	79 (59–92)	0.70	–
				$A\beta_{42/40}$ ratio	5.0	74 (54–89)	79 (59–92)	0.78	NP
Lewczuk et al. [21]	75 (AD-MCI)	0	45	$A\beta_{42}$	691 pg/mL	69.3	88.9	0.895 (0.819–0.946)	–
				$A\beta_{42/40}$ ratio	0.06	93.3	100	0.970 (0.916–0.993)	< 0.0001
Baldeiras et al. [27]	168	197 (MCI)	66	$A\beta_{42}$	585 pg/mL	82	83	0.882 (0.837–0.927)	–
				$A\beta_{42/40}$ ratio	0.068	79	86	0.874 (0.827–0.921)	n.s.
Frölich et al. [34]	0	115 (MCI)	0	$A\beta_{42}$	< 600 pg/mL	74	64	0.68	–
				$A\beta_{42/40}$ ratio	–	59	75	0.66	NP

*Optimal cut-offs were created using different statistical approaches—please see original articles for details.**Sensitivity and specificity are a function of the cut-off, and the cut-offs were calculated in different ways, therefore they are not clearly comparable across different articles. #Significance levels (p values) of the AUC values are comparisons of the Aβ isoform ratios vs $A\beta_{42}$ alone. *Aβ* Amyloid beta, *AD* Alzheimer's Disease, *AD-MCI* early AD and MCI, *AUC* area under curve, *DD* depressive disorder, *MCI* mild cognitive impairment, *MCI-AD* mild cognitive impairment patients progressing to AD, *MCI-MCI* stable MCI patients, *MSD* Meso Scale Discovery assay, *NP* not provided, *n.s* not significant, *OND* other neurological diseases, *SL* significance level

bead-based multiplexing (xMAP) technology performed as well as the MSD $A\beta_{42/40}$ ratio.

A 4-year follow-up study carried out by Parnetti et al. [33] measured CSF $A\beta_{40}$, $A\beta_{42}$, T-tau and P-tau_{181} in patients with AD, stable MCI (MCI-MCI) and MCI evolving into ADD (MCI-AD) in order to evaluate the power of each biomarker and/or their combination in predicting AD progression. Although they found that inclusion of the $A\beta_{42/40}$ ratio instead of $A\beta_{42}$ alone did not improve the prediction power of the model in the multivariate analysis, when univariate statistics were employed, they found that the $A\beta_{42/40}$ ratio had an increased sensitivity with respect to $A\beta_{42}$.

In a recently published paper, Baldeiras et al. [27] showed that replacing $A\beta_{42}$ by the $A\beta_{42/40}$ ratio resulted in a significant increase in the proportion of interpretable biological profiles (from 61 to 75%, $p = 0.001$) of MCI patients, due to a reduction by half in the number of suspected non-Alzheimer pathophysiology cases and an increase in the proportion of the high-AD-likelihood subgroup. In their study, the risk of progression to ADD was highest in the 'high-likelihood' group and increased when the $A\beta_{42/40}$ ratio, instead of $A\beta_{42}$, combined with T-tau and P-tau_{181} was used for biomarker-based categorization.

Frölich et al. [34] investigated whether the progression of MCI to AD dementia can be predicted by cognitive, neuroimaging and CSF markers. They studied 115 complete datasets from MCI patients of the 'Dementia Competence Network', a German multi-centre cohort study with annual follow-up up to 3 years. They hypothesized that since most biomarkers reveal complementary information, a combination of biomarkers may increase the predictive power. Their results showed that two- to four-parameter

combinations of the eight predictor/biomarker indices (MMSE, CDR-sb, CERAD-DR, HCV, $A\beta_{42}$, $A\beta_{42/40}$, T-tau and P-tau_{181}) were numerically superior over the performance of a single biomarker index in predicting MCI subjects who progressed to AD. In this dataset, however, the Aβ42/Aβ40 ratio was not consistently superior to $A\beta_{42}$ alone for predicting AD dementia in MCI patients.

Together these reports provide evidence that clearly demonstrates the added value of the CSF $A\beta_{42/40}$ ratio in accurately predicting progression to ADD.

In the last years, studies have also been published applying the CSF $A\beta_{38}$ concentration as the reference isomer for normalization of the $A\beta_{42}$ concentration (in the form of $A\beta_{42/38}$ ratio). However, it needs to be stressed that neither the results of these studies, nor the physiological rationale behind using $A\beta_{38}$ instead of $A\beta_{40}$ is convincing enough to replace $A\beta_{40}$.

Comparison of the diagnostic accuracy of the CSF biomarkers and Aβ-PET

CSF $A\beta_{42}$ and Aβ-PET have both been found to correlate highly with brain biopsy findings [35, 36]. Decreased concentrations of CSF $A\beta_{42}$ and increased retention of amyloid tracers in the brain on PET are considered the earliest biomarkers of AD, although some evidence suggests that the alterations measurable in the CSF occur earlier [43]. A potential advantage of Aβ-PET over CSF $A\beta_{42}$ as an early diagnostic marker is the possibility to detect regional Aβ depositions that might occur before the global neocortical signal becomes pathologic. On the other hand, analysis of CSF offers a quantitative result of the net effect of the biomarkers. Running costs of CSF analysis are 10- to 15-fold lower than those for Aβ-PET (total costs for lumbar puncture and analysis of four CSF biomarkers in Germany do not exceed €200). CSF analysis is also more accessible to patients, it does not require the patient and caregiver to travel to a distant centre equipped with PET facilities and it enables simultaneous analysis of dozens of biomarkers in one sample volume, including biomarkers of neurodegeneration, neuroinflammation and others [37–39]. In contrast to neuroimaging modalities, a CSF-based approach enables aliquoting and storage of a sample's volume for further analyses in the future, also in other laboratories.

Finally, although lumbar puncture is frequently regarded as an invasive procedure, serious complications are extremely rare, with the most common, headache, only being observed in 2% of the elderly population and being easily treated with simple analgesia [40]. On the other hand, it is questionable to consider injection of radioactive tracers, designed to target brain tissue, as a 'non-invasive' procedure. Here we provide details of five studies that have compared the diagnostic accuracy of CSF biomarkers and Aβ-PET, all showing that both methods can identify early AD with high accuracy. Studies with relevant data are also summarized in Table 3.

In Cohort 1 of a study carried out by Janelidze et al. [24], which included 215 MCI patients, a discrepancy between CSF $A\beta_{42}$ and Aβ-PET status was observed. In fact, they found that 10–20% of healthy individuals and MCI patients showed a mismatch in CSF $A\beta_{42}$ and Aβ-PET status. In their study, they found that the CSF $A\beta_{42/40}$ and $A\beta_{42/38}$ ratios better predict abnormal cortical amyloid deposition (visualized with PET) compared with $A\beta_{42}$. The ratios increased the classification performance both for patients who were falsely classified as positive (by low CSF $A\beta_{42}$) and for patients who were falsely classified as negative (by high CSF $A\beta_{42}$).

One possible explanation proposed by the authors for the improved concordance between Aβ-PET and CSF Aβ, when using the $A\beta_{42/40}$ and $A\beta_{42/38}$ ratios instead of $A\beta_{42}$, was that subcortical pathologies not specific to AD could cause reduced levels of all CSF Aβ species, including $A\beta_{42}$, but not the ratios. For example, in patients with MCI, low CSF $A\beta_{42}$, $A\beta_{40}$ and $A\beta_{38}$ were all linked to subcortical injury, including increased white matter lesions (WML) and enlarged lateral ventricles. The mechanisms underlying these associations are likely related to dysregulation in β amyloid precursor protein pathways with a general decline in the production of Aβ.

A study by Lewczuk et al. [41] reported an association between amyloid alterations reflected in the CSF with those in Aβ-PET in a cohort of 199 patients. The results showed that the CSF $A\beta_{42/40}$ ratio corresponded better than $A\beta_{42}$ with PET results, with a larger proportion of concordant cases (89.4% vs 74.9%, respectively, $p < 0.001$) and a larger area under the ROC curve (AUC 0.936 vs 0.814, respectively, $p < 0.001$) associated with the ratio. For both CSF biomarkers, the percentage of CSF-abnormal/PET-normal cases was larger than that of CSF-normal/PET abnormal cases. The authors concluded that the CSF $A\beta_{42/40}$ ratio is superior to $A\beta_{42}$ alone as a marker of amyloid-positivity by PET, which may be explained by the fact that the ratio compensates for general between-individual variations in CSF total Aβ concentrations. Furthermore, they speculated, that the fact that there was a higher proportion of subjects with pathologic CSF (as reflected by $A\beta_{42}$ or $A\beta_{42/40}$) and normal PET compared to those with pathologic PET and normal CSF might suggest that CSF reflects amyloidosis earlier than PET does. Similar conclusions based on earlier findings in CSF compared to PET were also made by Mattsson et al. and Palmqvist et al. [42, 43].

Janelidze et al. [44] studied the concordance between CSF $A\beta_{42}$ levels measured using five different immunoassays [Innotest, Modified Innotest (increased antibody concentration and incubation time, and lower CSF volume), fully automated Lumipulse, Euroimmun and MSD

Table 3 Studies comparing CSF biomarkers and Aβ-PET

Study	Number of AD patients	Number of MCI/ non-AD patients	Number of control patients	CSF biomarkers/ PET	Optimal cut-off*	Sensitivity% (95% CI)**	Specificity% (95% CI)**	AUC (95% CI)	SL (p value)#
Mattsson et al. [42]	121	68 (SCD)	161	$Aβ_{42}$	< 192 ng/L	–	–	–	–
		419 (MCI)		$Aβ_{42/40}$ ratio		–	–		NP
				Florbetapir PET	> 1.11 SUV	–	–	–	NP
Janelidze et al. [24]	Cohort 1	Cohort 1	Cohort 1	Euroimmun					
	0	215 (MCI)	0	$Aβ_{42}$	< 507.5 pg/mL	83.2	83.3	0.894 (0.850–0.937)	–
				$Aβ_{42/40}$ ratio	< 0.10	97.2	88.0	0.954 (0.923–0.986)	0.008
				$Aβ_{42/38}$ ratio	< 0.29	92.5	88.9	0.943 (0.911–0.975)	0.007
				MSD					
				$Aβ_{42}$	< 495.9 pg/mL	85.0	88.9	0.916 (0.876–0.956)	–
				$Aβ_{42/40}$ ratio	< 0.09	95.3	95.4	0.975 (0.952–0.998)	< 0.001
				$Aβ_{42/38}$ ratio	< 0.17	97.2	91.7	0.964 (0.935–0.992)	0.007
				Quanterix					
				$Aβ_{42}$	< 1742 pg/mL	73.3	77.5	0.810 (0.707–0.913)	–
				$Aβ_{42/40}$ ratio	< 0.16	90.0	90.0	0.912 (0.834–0.991)	0.002
Lewczuk et al. [41]	0	199 CN &	0	$Aβ_{42}$	735 pg/mL	81.6 (68.0–91.2)	72.7 (64.8–79.6)	0.814 (0.752–0.865)	–
		abnormal (150 PET−/ 49 PET+)		$Aβ_{42/40}$ ratio	0.05	95.9 (86.0–99.5)	88.0 (81.7–92.7)	0.936 (0.892–0.966)	< 0.001
Janelidze et al. [44]	0	262 (MCI/SCD)	0	Innotest					
				$Aβ_{42}$	≤ 548 pg/mL	96	82	0.92 (0.89–0.95)	–
				$Aβ_{42/40}$ ratio	≤ 0.06	91	82	0.92 (0.88–0.95)	NP
				Modified Innotest					
				$Aβ_{42}$	≤ 1091 pg/mL	92	74	0.87 (0.83–0.91)	–
				$Aβ_{42/40}$ ratio	≤ 0.12	92	87	0.93 (0.90–0.96)	≤ 0.01
				Euroimmun					
				$Aβ_{42}$	≤ 449 pg/	82	80	0.88	–
				$Aβ_{42/40}$ ratio	mL ≤ 0.10	93	88	(0.84–0.92) 0.93 (0.90–0.96)	≤ 0.01
				MSD					
				$Aβ_{42}$	≤ 506 pg/mL	94	76	0.89 (0.85–0.93)	–
				$Aβ_{42/40}$ ratio	≤ 0.08	96	89	0.95 (0.93–0.98)	≤ 0.001

Table 3 Studies comparing CSF biomarkers and Aβ-PET *(Continued)*

Study	Number of AD patients	Number of MCI/ non-AD patients	Number of control patients	CSF biomarkers/ PET	Optimal cut-off*	Sensitivity% (95% CI)**	Specificity% (95% CI)**	AUC (95% CI)	SL (p value)[#]
Schindler et al. [46]	0	22 (MCI)	176 (CN)	$A\beta_{42}$	< 1.098 pg/mL	–	–	0.85 (0.80–0.90)	–
		(~ 25% PiB+)	(~ 25% PiB+)	$A\beta_{42/40}$ ratio	< 0.075	–	–	0.93 (0.89–0.96)	NP

*Optimal cut-offs were created using different statistical approaches—please see original articles for details. **Sensitivity and specificity are a function of the cut-off, and the cut-offs were calculated in different ways, therefore they are not clearly comparable across different articles. [#]Significance levels (p values) of the AUC values are comparisons of the Aβ isoform ratios vs $A\beta_{42}$ alone. *Aβ* amyloid beta, *AD* Alzheimer's Disease, *ADAS-cog* Alzheimer's Disease Assessment Scale-cognitive subscale, *AUC* area under curve, *cen* centiloid, *CN* cognitively normal, *CU* centiloid units, *DLB* dementia with Lewy bodies, *FTD* frontotemporal dementia, *MCI* mild cognitive impairment, *MCI-AD* MCI that subsequently developed AD dementia, *MSD* Meso Scale Discovery, *NP* not provided, *n.s* not significant, *PD* Parkinson's Disease, *PDD* Parkinson's Disease with dementia, PET, positron emission tomography, *MS-RMP* mass spectrometry-based candidate reference measurement procedure, *PiB* carbon-11 labelled thioflavin-T derivative, Pittsburgh compound B, *SCD* subjective cognitive decline, *SL* significance level, *SUV* standardized uptake value, *VaD* subcortical vascular dementia, *vis* visual

assays) and visual read of Aβ-PET images in 262 patients with MCI or SCD. They found that although the accuracy to correlate with visual 18F-flutemetamol PET was decreased when using newer $A\beta_{42}$ assays, this limitation is overcome by using the CSF $A\beta_{42/40}$ ratio. They also found that the CSF $A\beta_{42/40}$ ratio from the newer assays showed improved accuracy for detection of cortical Aβ fibrils as measured by PET. Moreover, the sensitivities and specificities of these newer assays were less influenced by moderate changes in the cut-offs when the CSF $A\beta_{42/40}$ ratio was used, a finding that is important when samples will be analysed consecutively over time.

In another study analysing the agreement between data on cerebral amyloidosis, derived using Pittsburgh compound B (PiB) PET and immunosorbent assay of CSF $A\beta_{42}$, Leuzy et al. [45] examined 243 subjects with normal cognition and patients with MCI. They found that agreement between PiB classification and MSD immunosorbent assay/mass spectrometry reference measurement procedure findings was further improved when using the CSF $A\beta_{42/40}$ ratio.

A study by Schindler et al. [46] studied the relationship between CSF biomarkers, as measured by a novel automated immunoassay platform and Aβ-PET. They found that CSF biomarker ratios (T-tau/$A\beta_{42}$, P-tau_{181}/$A\beta_{42}$ and $A\beta_{42/40}$) best discriminated PiB-positive from PiB-negative individuals.

These reports show that both Aβ-PET and CSF biomarkers can identify early AD with high accuracy. However, several studies strongly suggest that Aβ alterations in the CSF occur earlier [42, 43]. Similarly, there is no convincing evidence that the spatial distribution of Aβ deposits in the brain tissue observed in PET deliver clinically relevant information [47]. In addition, the CSF $A\beta_{42/40}$ ratio can better predict abnormal cortical amyloid deposition (visualized with PET) compared with $A\beta_{42}$. This then leads to fewer patients being diagnosed as false positive (low CSF $A\beta_{42}$) or false negative (high CSF $A\beta_{42}$).

Effects of non-AD pathologies on $A\beta_{42}$, $A\beta_{40}$ and the $A\beta_{42/40}$ ratio, such as WMLs, PDD and DLB

In clinical practice, CSF biomarker analyses are often carried out in patients with atypical or mixed presentation of dementia. This makes the diagnosis complex and highlights the importance of being able to discriminate AD from other neurodegenerative processes such as FTD, WMLs, VaD, DLB, PDD and cerebral amyloid angiopathy [5].

Selnes et al. [48] studied the effects of cerebrovascular disease on amyloid precursor protein metabolites in CSF in 37 patients with SCD or MCI without stroke, and 26 after acute stroke. They found that CSF levels of $A\beta_{38}$, $A\beta_{40}$ and $A\beta_{42}$ were inversely correlated with chronic WML volume ($p \leq 0.01$; $p \leq 0.05$; $p \leq 0.05$, respectively), but not with acute WML or infarct volumes.

Similarly, van Westen et al. [49] studied the association of cerebral WML with Aβ isoforms and Aβ-PET in 831 subjects with cognitive performance ranging from normal to ADD. The results showed that all Aβ isoforms were consistently inversely correlated with WML, but the CSF $A\beta_{42/40}$ ratio and Aβ-PET were not. These results indicate that the presence of WMLs affects the levels of CSF $A\beta_{38}$, $A\beta_{40}$ and $A\beta_{42}$, but not the CSF $A\beta_{42/40}$ ratio or Aβ-PET.

Finally, Gabelle et al. [14] observed that $A\beta_{40}$ levels were decreased in FTD suggesting that it could represent a diagnostic biomarker in this pathology.

It can therefore be concluded that $A\beta_{42}$, as well as $A\beta_{38}$ and $A\beta_{40}$, might be reduced in CSF due to non-AD related pathologies, but usually, the $A\beta_{42/40}$ ratio, as well as other Aβ ratios, including $A\beta_{42}$, are not affected. These studies therefore point to the usefulness of the CSF $A\beta_{42/40}$ ratio in improving the accuracy of the differential diagnosis in patients with ambiguous biological profiles or with other conditions that might affect the concentrations of the Aβ peptides.

Effects of pre-analytical handling on $A\beta_{42}$ and the $A\beta_{42/40}$ ratio

Various papers have pointed out pre-analytical and analytical variability between laboratories for the concentration of the $A\beta_{42}$ peptide in CSF [50–54]. Tubes for collection, sample handling and sample storage conditions, in particular, have been noted as critical factors [55, 56]. These factors have also been highlighted as a possible reason for problems linked to inter-centre variability when it comes to analysing CSF biomarkers [57].

The type of sampling and storage tubes used is an important source of variability because of the tendency of Aβ peptides to adsorb on plastic surfaces [21, 54, 56]. It has been proposed that there is parallel adsorption of CSF $A\beta_{42}$ and $A\beta_{40}$ onto the sampling tube surface, regardless of the type of plastic, however with significant differences across different type of plastics [58]. They also suggest that systematic use of the CSF $A\beta_{42/40}$ ratio would provide a complete interpretation of CSF amyloid biomarker results, integrating the impact of plastic tube type.

Lewczuk et al. [58] measured biomarkers of AD (T-tau, P-tau_{181}, $A\beta_{42}$, $A\beta_{40}$) in CSF samples in collection tubes made of different materials. The results suggest that the CSF $A\beta_{42/40}$ ratio and CSF P-tau_{181} are much less prone to methodologic error introduced by interactions of the biomarkers' molecules with the test tube surfaces compared with $A\beta_{40}$ and $A\beta_{42}$ concentrations, similarly to the concentrations of T-tau. The authors concluded that the CSF $A\beta_{42/40}$ ratio is a more reliable biomarker than pure Aβ concentrations, as the $A\beta_{42/40}$ ratio is less altered by interaction with the surface of the collection tubes.

In a similar study by Gervaise-Henry et al. [59], the impact of collection tubes and repetitive freeze/thaw cycles on CSF $A\beta_{42}$ and $A\beta_{40}$ concentrations was investigated. CSF from 35 patients was collected in different polypropylene (PP) tubes and stored at − 80 °C. Samples were also subjected to three successive freeze-thaw cycles. The results showed that CSF $A\beta_{42}$ and $A\beta_{40}$ values were significantly different depending on the type of collection tube and the number of freeze/thaw cycles. Although the calculation of the CSF $A\beta_{42/40}$ ratio eliminated the effect of PP tube-dependent variation, this was not the case for freeze-thaw cycle-associated variation. The authors concluded that the use of $A\beta_{42/40}$ ratio rather than $A\beta_{42}$ alone could contribute toward pre-analytical standardization, thus allowing for the general use of CSF AD biomarkers in routine practice.

Willemse et al. [60] tested several variables as potential confounders influencing adsorption of Aβ peptides on the surfaces of test tubes, including different polypropylene tube brands, volumes, CSF $A\beta_{42}$ concentrations, incubation times, pipettes, vortex intensities and other CSF proteins. They found that every sample transfer from one tube to another resulted in 5% loss of $A\beta_{42}$ concentration, which reached as high as 10% when small volumes were used. This decrease in concentration was, however, similar for $A\beta_{40}$, resulting in stable $A\beta_{42/40}$ ratios over multiple tube transfers. Correspondingly, they conclude that use of the $A\beta_{42/40}$ ratio overcomes the effect of adsorption-derived Aβ concentration loss and can therefore contribute to increased diagnostic accuracy.

Furthermore, Vanderstichele et al. [61] determined that using the CSF $A\beta_{42/40}$ ratio mitigates many of the effects of additional freeze/thaw cycles, tube type and CSF volumes for PP storage tubes. In fact, they concluded that the CSF $A\beta_{42/40}$ ratio is clearly a more robust biomarker than $A\beta_{42}$ toward (pre-) analytical interfering factors. They also observed that the rate of adsorption to PP recipients is higher for $A\beta_{42}$ than for the other amyloid isoforms, such as $A\beta_{40}$, and therefore using the $A\beta_{42/40}$ ratio does not completely eliminate the effects of binding of Aβ to the tube walls of PP tubes. However, they found that 'low binding recipients' are able to reduce the binding of Aβ species to the tube walls. They concluded that integration of the $A\beta_{42/40}$ ratio and 'low-binding tubes' into guidance criteria may speed up worldwide standardization of CSF biomarker analysis.

In summary, evidence from studies on the effect of pre-analytical handling on biomarkers of AD suggest that use of the CSF $A\beta_{42/40}$ ratio would improve the interpretation of CSF amyloid biomarker results, by reducing the impact of these factors on the final outcome. The use of the CSF $A\beta_{42/40}$ ratio could therefore contribute toward pre-analytical standardization, allowing for the use of CSF AD biomarkers in routine clinical practice.

Disadvantages of the use of the CSF $A\beta_{42/40}$ ratio

The main disadvantage of the use of the CSF $A\beta_{42/40}$ ratio is economical and not interpretational in nature. Considering the laboratory costs of the AD biomarkers, the inclusion of $A\beta_{40}$ increases the costs by ca. €40–50, which is negligible when considering the total costs of the diagnostic work-up and treatment of patients with suspected AD assessed at specialized memory clinics. Furthermore, the amount of CSF sample needed to perform this additional test is also negligible (5–10 μL, depending on the method). In addition, interpretation of the results when four biomarkers (instead of three) are used is not more complex, when a solid interpretational algorithm is validated and consequently used.

Conclusion

There is a growing body of evidence that suggests the better diagnostic performance of the CSF $A\beta_{42/40}$ ratio compared to CSF $A\beta_{42}$ alone. In addition, it also appears to be clear that including the CSF $A\beta_{42/40}$ ratio in the clinical workup of MCI patients improves the accuracy of predicting progression to AD.

It has also been shown that both Aβ-PET and CSF biomarkers can identify early amyloid pathology with high accuracy. The CSF $A\beta_{42/40}$ ratio can also better predict abnormal cortical amyloid deposition (visualized with PET) compared with CSF $A\beta_{42}$. This then leads to fewer patients being diagnosed as false positive (low CSF $A\beta_{42}$) or false negative (high CSF $A\beta_{42}$). In addition, there is evidence to suggest that Aβ alterations in CSF occur earlier than they are detectable in Aβ-PET.

Addition of the CSF $A\beta_{42/40}$ ratio to the usual panel of CSF AD biomarkers for patients with ambiguous biological profiles also increases the number of interpretable results.

It has also been found that the use of the CSF $A\beta_{42/40}$ ratio rather than CSF $A\beta_{42}$ alone contributes toward pre-analytical standardization, removing the effects of (pre-)analytical interfering factors, such as tube type, freeze/thaw cycles and CSF volumes.

The working group therefore makes the following recommendations:

1. The $A\beta_{42/40}$ ratio should always be analysed, irrespective of the results of other AD biomarkers, and without paying attention to whether $A\beta_{42}$ is normal or pathologic. This is driven by the fact that the $A\beta_{42/40}$ ratio can equally change the CSF interpretation from 'normal to pathologic' as from 'pathologic to normal'. Analysing the $A\beta_{42/40}$ ratio only in cases with abnormal $A\beta_{42}$ (leaving cases with normal $A\beta_{42}$ without further consideration, i.e. as truly not having amyloidosis) would neglect the former scenario. On the other hand, performing $A\beta_{42/40}$ ratio analysis only in cases with normal $A\beta_{42}$ (with subjects with abnormal $A\beta_{42}$ considered as truly having amyloidosis) would neglect the latter case.
2. It should be mandatory for every laboratory to establish their own specific reference values (cut-offs) for the $A\beta_{42/40}$ ratio and to participate in an internal quality control programme to ensure longitudinal stability in the measurements of $A\beta_{42}$ and $A\beta_{40}$, just as it is mandatory to establish reference values and control quality of all other biomarkers.
3. Following on from (2), it is possible to combine laboratory results obtained on different platforms and from different vendors. The point is only that the reference value for the $A\beta_{42/40}$ ratio must be carefully validated. It is beyond the scope of this paper to make suggestions on statistical methods to calculate reference values and/or their transfer from other laboratories or analytical platforms.

Based on the body of evidence collected here it is the conclusion of the current working group that the CSF $A\beta_{42/40}$ ratio, rather than the absolute value of CSF $A\beta_{42}$, should be used when analysing CSF AD biomarkers to improve the percentage of appropriately diagnosed patients. It is also suggested that the CSF $A\beta_{42/40}$ ratio could therefore be used as a proxy for amyloid status in AD clinical trials and eventually in clinical care settings.

Abbreviations

AD: Alzheimer's Disease; ADD: AD dementia; AUC: Area under the ROC curve; $A\beta_{42}$: Amyloid β 42; $A\beta_{42/40}$ Ratio: Concentration ratio of $A\beta_{42}$ to $A\beta_{40}$; Aβ-PET: Aβ positron emission tomography; CSF: Cerebrospinal Fluid; DLB: Dementia with Lewy Bodies; FTD: Frontotemporal Dementia; MCI: Mild cognitive impairment; MCI-AD: MCI evolving into ADD; MCI-MCI: Stable MCI; non-AD: Other types of dementia; PDD: Parkinson's Disease Dementia; PiB: Pittsburgh compound B; PLM_R-scale: PLM ratio scale; PLM-scale: Paris-Lille-Montpellier scale; P-tau_{181}: Tau phosphorylated at threonine 181; ROC: Receiver Operating Characteristic; SCD: Subjective cognitive decline; T-tau: Total Tau; VaD: Subcortical vascular dementia; WML: White matter lesions

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This review does not contain any analyzable data. All sources cited in this paper are publicly available.

Authors' contributions

OH and PL prepared the initial draft of the paper. All authors read and approved the final paper.

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