Use of amyloid-PET to determine cutpoints for CSF markers

A multicenter study

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ABSTRACT

Objectives: To define CSF β-amyloid 1–42 ($A\beta_{42}$) cutpoints to detect cortical amyloid deposition as assessed by ^{11}C-Pittsburgh compound B ([^{11}C]PiB)-PET and to compare these calculated cutpoints with cutpoints currently used in clinical practice.

Methods: We included 433 participants (57 controls, 99 with mild cognitive impairment, 195 with Alzheimer disease [AD] dementia, and 82 with non-AD dementia) from 5 European centers. We calculated for each center and for the pooled cohort CSF $A\beta_{42}$ and $A\beta_{42}$/tau ratio cutpoints for cortical amyloid deposition based on visual interpretation of [^{11}C]PiB-PET images.

Results: Amyloid-PET–based calculated CSF $A\beta_{42}$ cutpoints ranged from 521 to 616 pg/mL, whereas existing clinical-based cutpoints ranged from 400 to 550 pg/mL. Using the calculated cutpoint from the pooled sample (557 pg/mL), concordance between CSF $A\beta_{42}$ and amyloid-PET was 84%. Similar concordance was found when using a dichotomized $A\beta_{42}$/tau ratio. Exploratory analysis showed that participants with a positive amyloid-PET and normal CSF $A\beta_{42}$ levels had higher CSF tau and phosphorylated tau levels and more often had mild cognitive impairment or AD dementia compared with participants who had negative amyloid-PET and abnormal CSF $A\beta_{42}$ levels.

Conclusions: Amyloid-PET–based CSF $A\beta_{42}$ cutpoints were higher and tended to reduce intercenter variability compared with clinical-based cutpoints. Discordant participants with normal CSF $A\beta_{42}$ and a positive amyloid-PET may be more likely to have AD-related amyloid pathology than participants with abnormal CSF $A\beta_{42}$ and a negative amyloid-PET.

Classification of evidence: This study provides Class II evidence that an amyloid-PET–based CSF $A\beta_{42}$ cutpoint identifies individuals with amyloid deposition with a sensitivity of 87% and specificity of 80%. *Neurology*® 2016;86:50–58

GLOSSARY

AD = Alzheimer disease; **Aβ** = β-amyloid; **$A\beta_{42}$** = β-amyloid 1–42; **AUC** = area under the curve; **MCI** = mild cognitive impairment; **NPV** = negative predictive value; **OR** = odds ratio; **PiB** = Pittsburgh compound B; **PPV** = positive predictive value; **p-tau** = phosphorylated tau; **SUVr** = standardized uptake value ratio.

Aggregation of β-amyloid (Aβ) is a histopathologic hallmark of Alzheimer disease (AD) and presents decades before clinical symptoms occur.[1,2] Biomarkers to detect Aβ pathology are cortical retention of an amyloid-PET tracer such as ^{11}C-Pittsburgh compound B ([^{11}C]PiB) and decreased levels of β-amyloid 1–42 ($A\beta_{42}$) in CSF. Furthermore, in clinical practice, amyloid-PET can be interpreted as amyloid-positive or -negative based on the presence or absence of cortical Aβ retention determined by visual inspection. In contrast, for CSF $A\beta_{42}$, there is no general cutpoint and the existing cutpoints show high variability among centers.[3]

Supplemental data at Neurology.org

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Variability in the existing CSF $A\beta_{42}$ cutpoints may have 2 explanations. First, differences in preanalytical and analytical procedures for measuring CSF biomarkers may lead to interlaboratory variability of up to 35%.[4,5] Second, because CSF $A\beta_{42}$ cutpoints are often derived from a comparison between participants with AD-type dementia and a control group, variability in the selection of these groups may introduce variability.[6–9]

In the present study, we hypothesized that the use of amyloid-PET as a reference for CSF cutpoint calculation will reduce between-center variability, as it is independent of clinical diagnosis. Another advantage of the use of amyloid-PET is its high agreement with histopathologic amyloid aggregates.[10,11] In contrast, clinical diagnosis may not accurately reflect cerebral amyloidosis because cognitively normal elderly may present with amyloid pathology while patients with AD-type dementia may lack evidence of amyloid pathology.[12,13]

The primary aim of the present study was to define a cutpoint for CSF $A\beta_{42}$ to detect cortical amyloid deposition as assessed by [^{11}C]PiB-PET images and to compare this cutpoint with cutpoints currently used in clinical practice.

METHODS

Participants. We included 433 participants from 5 memory clinic cohorts (n = 163, Amsterdam Dementia Cohort; n = 125, Turku University Hospital; n = 73, Copenhagen University Hospital; n = 44, Karolinska University Hospital; and n = 28, Hospital de Sant Pau). A portion of the participants of the Amsterdam Dementia Cohort and Karolinska University Hospital were previously included in comparison studies of [^{11}C]PiB-PET and CSF biomarkers.[14,15] Inclusion criteria were the presence of both CSF and [^{11}C]PiB-PET measures within 1 year. Of the 433 participants, 278 had dementia, of which 195 participants had probable AD.[16,17] Eighty-three had non-AD dementias including 30 participants with frontotemporal dementia,[18] 14 with vascular dementia,[19] 12 with dementia with Lewy bodies,[20] 7 with corticobasal degeneration,[21] 3 with progressive supranuclear palsy,[22] and 17 with other types of non-AD dementia. Furthermore, 98 had mild cognitive impairment (MCI) according to Petersen criteria,[23] and 57 participants were cognitively normal controls.

All participants underwent a standard diagnostic dementia workup, including clinical history, medical and neurologic examination, clinical chemistry, cognitive evaluation using the Mini-Mental State Examination, and rating scales for depression and neuropsychiatric symptoms. Patients were diagnosed without knowledge of PET or CSF results, except for participants included in Copenhagen and Stockholm. Therefore, participants from these centers were excluded from the analysis using diagnosis as predictor for biomarkers.

Classification of evidence. This study provides Class II evidence that a cutpoint for CSF $A\beta_{42}$ (as measured by INNOTEST b-amyloid 1–42 assay; Innogenetics, Ghent, Belgium) identifies individuals visiting a memory clinic with amyloid deposition based on amyloid-PET (using [^{11}C]PiB-PET and visually rated as amyloid-positive or -negative) with a sensitivity of 87% and specificity of 80%.

Standard protocol approvals, registrations, and patient consents. This study was approved by the local medical ethics review committees. Written informed consent allowing use of their clinical data for research purposes was obtained from all patients.

PET. Participants were scanned according to the routine local [^{11}C]PiB-PET protocol as described elsewhere[15,24–26] except for Barcelona, where standardized uptake value ratio (SUVr) images were generated from the interval from 60 to 90 minutes after injection. [^{11}C]PiB-PET images were rated by a local expert reader as either amyloid-positive (abnormal) or amyloid-negative (normal) blinded for CSF results.

A test set of 20 SUVr [^{11}C]PiB-PET images (comprising a mixture of amyloid-positive and amyloid-negative scans) was visually assessed by the expert reader of each center in order to assess the interrater agreement between centers. These SUVr images were generated 60 to 90 minutes after injection with cerebellar gray matter as reference tissue and rated as amyloid-positive or amyloid-negative.

CSF. [^{11}C]PiB-PET was performed within (mean ± SD) 74 ± 76 days of the lumbar puncture, with a maximum delay of 351 days. CSF was collected by lumbar puncture and levels of $A\beta_{42}$, total tau (t-tau), and phosphorylated tau (p-tau) in CSF were measured locally using commercially available sandwich ELISAs (INNOTEST b-amyloid 1–42, INNOTEST hTAU Ag; Innogenetics). CFS tau levels were not available for 19 participants. CSF $A\beta_{42}$ levels were dichotomized as normal or abnormal based on clinical-based cutpoints (Amsterdam, <550 pg/mL; Turku, <450 pg/mL; Copenhagen <400 pg/mL; Stockholm <450 pg/mL; and Barcelona, <550 pg/mL). Detailed prescriptions of approaches used to determine clinical-based cutpoints were described previously.[6–9]

***APOE* genotype.** In a subset (n = 252) of participants, *APOE* genotype was determined by PCR of genomic DNA extracted from ethylenediaminetetraacetic acid–anticoagulated blood. Participants were classified as *APOE* ε4 carriers or noncarriers. *APOE* genotyping was not performed in 29 cognitively normal controls, 27 patients with MCI, 82 patients with AD dementia, and 43 patients with non-AD dementia.

Statistics. Statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY), except for the Fleiss κ, which was calculated using SAS version 9.2 (SAS Institute, Cary, NC) for Windows. Baseline characteristics were compared using χ^2 tests and analysis of variance with post hoc Bonferroni corrections. CSF biomarkers were log-transformed for these analyses because of positively skewed values.

We calculated the area under the curve (AUC) of the CSF $A\beta_{42}$ and the CSF $A\beta_{42}$/tau ratio for amyloid-PET positivity using receiver operating characteristic analysis. We defined cutpoints that maximized the Youden index (sensitivity + specificity − 1) for predicting amyloid-PET positivity and cutpoints that predicted amyloid-PET positivity with a sensitivity of 85%. Cutpoints were calculated in each cohort separately and in the whole sample. Using the dichotomized CSF biomarkers, we calculated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and odds ratio (OR). Statistical difference was detected by nonoverlapping confidence intervals (95% CIs). Variance between clinical-based cutpoints and calculated cutpoints was compared using 1-tailed *F* tests.

Concordance of CSF biomarkers and [^{11}C]PiB-PET was defined as the proportion of individuals with an identical classification of both biomarkers, e.g., normal CSF biomarkers (not Alzheimer-like) (either $A\beta_{42}$ alone or $A\beta_{42}$/tau) and abnormal (positive) amyloid-PET or abnormal (Alzheimer-like) CSF biomarkers (either $A\beta_{42}$ alone or $A\beta_{42}$/tau) and normal (negative) amyloid-PET. Discordance of CSF biomarkers and [^{11}C]PiB-PET was defined as the proportion of individuals with only one abnormal or "AD-positive" biomarker, while the other one was normal or "AD-negative."

RESULTS

Participant characteristics. Participant characteristics according to diagnostic group are presented in table 1. Groups did not differ in age or sex. As expected, both the patients with AD and those with non-AD dementia had lower Mini-Mental State Examination scores than participants with MCI and controls. In addition, participants with AD dementia showed higher CSF tau and lower CSF $A\beta_{42}$ levels compared with the other groups. Controls had higher CSF $A\beta_{42}$ levels than participants with MCI and non-AD dementia, while tau levels did not differ between the groups. Participants with AD dementia and MCI were more often *APOE* ε4 carriers than participants with non-AD dementia and healthy controls. Participants with AD dementia and MCI more often had an abnormal amyloid-PET scan compared with participants who had non-AD dementia and controls. Diagnoses of included participants according to center are shown in table e-1 on the *Neurology*® Web site at Neurology.org.

Interreader agreement of PET images. The intercenter agreement of the rating of the test set of 20 SUVr [^{11}C]PiB-PET images was 100% (Fleiss $\kappa = 1.0$).

Accuracy of CSF $A\beta_{42}$ for detection of amyloid-PET positivity. CSF $A\beta_{42}$ levels were significantly lower in the amyloid-PET–positive participants (average CSF $A\beta_{42}$ levels of centers ranged from 336 to 475 pg/mL) compared with the amyloid-PET–negative participants (average CSF $A\beta_{42}$ levels ranged from 634 to 844 pg/mL; $p < 0.01$) in each center. The AUC for detection of amyloid-PET positivity was 0.89 (95% CI = 0.83–0.96) for Amsterdam, 0.86 (95% CI = 0.78–0.93) for Turku, 0.80 (95% CI = 0.69–0.90) for Copenhagen, 0.84 (95% CI = 0.65–1.00) for Stockholm, 0.97 (95% CI = 0.91–1.00) for Barcelona, and 0.86 (95% CI = 0.82–0.90) in the whole sample.

Clinical-based cutpoints. When using center-specific clinical-based cutpoints, ORs for amyloid-PET positivity varied between 3.6 and 75.0, sensitivity between 47% and 88%, specificity between 70% and 91%, PPV between 74% and 94%, and NPV between 27% and 83% (table 2). In the whole sample using the average of the center-specific cutpoints (480 pg/mL), the OR was 10.4, with a sensitivity of 66%, specificity of 84%, PPV of 87%, and NPV of 60%.

Amyloid-PET–based cutpoints. All center-specific $A\beta_{42}$ cutpoints that maximized the Youden index for predicting amyloid-PET positivity (521–616 pg/mL) were higher than the clinical-based cutpoints in each center (400–550 pg/mL). In addition, when maximizing the Youden index for predicting amyloid-PET positivity, there was a trend for lower variability between centers in cutoff points compared with clinical-based cutpoints (521–616 vs 400–550 pg/mL, $p = 0.11$).

Table 1 Demographic and clinical characteristics according to diagnostic groups

	Controls	MCI	AD dementia	Non-AD dementia
No.	57	98	195	83
Age, y	65 ± 9	65 ± 9	65 ± 8	63 ± 10
Sex, female	24 (42)	40 (41)	84 (43)	29 (35)
MMSE score	29 ± 1	27 ± 2	23 ± 4[a,b,c]	25 ± 4[a,b]
APOE ε4 carrier[d]	9 (32)	36 (59)	66 (58)	11 (28)
CSF $A\beta_{42}$, pg/mL	763 ± 247	608 ± 266[a]	414 ± 166[a,b,c]	633 ± 314[a]
CSF tau, pg/mL	286 ± 172	380 ± 219	593 ± 344[a,b,c]	278 ± 158
CSF p-tau, pg/mL	49 ± 22	65 ± 28[a,c]	87 ± 38[a,b,c]	45 ± 20
Amyloid-PET–positive	10 (18)	58 (59)[a,c]	183 (94)[a,b,c]	18 (22)

Abbreviations: $A\beta_{42}$ = β-amyloid 1–42; AD = Alzheimer disease; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; p-tau = phosphorylated tau.
Data are presented as mean ± SD or n (%). Differences between diagnostic groups were assessed using analysis of variance with post hoc Bonferroni correction (age, log-transformed CSF $A\beta_{42}$, tau, and p-tau [untransformed CSF levels are shown], and MMSE) and χ^2 (sex, *APOE* ε4 carrier, and amyloid-PET–positive).
[a] Significantly different from controls ($p < 0.05$).
[b] Significantly different from patients with MCI ($p < 0.05$).
[c] Significantly different from patients with non-AD dementia ($p < 0.05$).
[d] *APOE* genotype was determined in a subset of the sample.

Table 2 Performances of CSF $A\beta_{42}$ cutpoints for detection of amyloid-PET positivity

$A\beta_{42}$, center	Cutpoint	SE, %	SP, %	PPV, %	NPV, %	OR
Clinical						
All	480	66 (60–72)	84 (78–89)	87 (82–92)	60 (54–67)	10.4
AMS	550	80 (71–88)	89 (79–95)	90 (82–96)	78 (67–86)	32.4
TUR	450	72 (61–81)	84 (70–93)	89 (79–96)	62 (48–74)	13.3
COP	400	61 (44–75)	70 (51–85)	74 (57–87)	55 (38–71)	3.6
STO	450	47 (30–65)	88 (47–97)	94 (72–99)	27 (12–48)	6.3
BAR	550	88 (64–98)	91 (59–98)	94 (70–99)	83 (52–97)	75.0
Youden-PET						
All	557	87 (82–91)	80 (73–86)	88 (83–91)	79 (72–85)	26.5
AMS	616	95 (88–98)	82 (71–90)	87 (79–93)	92 (82–97)	77.6
TUR	521	84 (74–91)	84 (70–93)	91 (82–96)	74 (60–85)	27.7
COP	562	93 (81–98)	60 (41–77)	77 (63–87)	86 (64–97)	20.0
STO	570	89 (74–97)	75 (35–96)	94 (80–99)	60 (26–88)	24.0
BAR	571	94 (71–99)	91 (59–98)	94 (71–99)	91 (59–98)	160.0
85% SE-PET						
All	551	86 (82–90)	80 (74–86)	88 (83–92)	78 (71–84)	25.9
AMS	557	85 (76–91)	89 (79–95)	91 (82–96)	82 (71–90)	43.9
TUR	551	85 (76–92)	80 (65–90)	88 (79–95)	74 (60–86)	22.4
COP	526	86 (72–95)	60 (41–77)	76 (61–87)	75 (53–90)	9.3
STO	555	85 (67–94)	75 (35–96)	94 (79–99)	50 (21–79)	15.0
BAR	533	88 (64–98)	91 (59–98)	94 (70–99)	83 (52–97)	75.0

Abbreviations: $A\beta_{42}$ = β-amyloid 1–42; All = all centers; AMS = Amsterdam; BAR = Barcelona; COP = Copenhagen; NPV = negative predictive value; OR = odds ratio; PPV = positive predictive value; SE = sensitivity; SP = specificity; STO = Stockholm; TUR = Turku.
Cutpoint is presented as CSF $A\beta_{42}$ level (pg/mL).

Center-specific ORs for amyloid-PET positivity using the Youden-based $A\beta_{42}$ cutpoints varied between 20.0 and 160.0. Sensitivity ranged between 84% and 95%, specificity between 60% and 91%, PPV between 77% and 94%, and NPV between 60% and 92% (table 2). In the pooled sample, the optimal $A\beta_{42}$ cutpoint based on the Youden index was 557 pg/mL and had an OR of 26.5. Compared with the average clinical-based cutpoint, the overall Youden-based cutpoint increased sensitivity (66%–87%, $p < 0.05$) and NPV (60%–79%, $p < 0.05$), while specificity and PPV remained similar. Within centers, Youden-based $A\beta_{42}$ cutpoints increased sensitivity for amyloid-PET in Amsterdam, Copenhagen, and Stockholm.

Center-specific $A\beta_{42}$ cutpoints that predicted amyloid-PET positivity with 85% sensitivity were somewhat lower compared with the Youden-based cutpoints except for one center (Turku) but were all higher than the clinical-based cutpoints. Center-specific ORs for amyloid-PET positivity varied between 9.3 and 75.0, specificity between 60% and 91%, PPV between 76% and 94%, and the NPV between 50% and 83% (table 2). In the pooled sample, the optimal $A\beta_{42}$ cutpoint based on 85% sensitivity was 551 pg/mL and had an OR of 25.9, with a specificity of 80%, PPV of 88%, and NPV of 78%. Compared with clinical-based cutpoints, and similar to the Youden cutpoint, the 85% sensitivity–based overall cutpoint increased sensitivity (66%–86%, $p < 0.05$) and NPV (60%–78%, $p < 0.05$), while specificity and PPV remained similar. Within centers, this cutpoint yielded a higher sensitivity in one center (Stockholm) ($p < 0.05$) compared with the clinical-based cutpoint, while no differences in accuracy measures were found when compared with the Youden cutpoints overall and within centers.

Accuracy of CSF $A\beta_{42}$/tau ratio for detection of amyloid-PET positivity. The AUC of CSF $A\beta_{42}$/tau ratio for amyloid-PET positivity was 0.92 (95% CI = 0.86–0.96) for Amsterdam, 0.88 (95% CI = 0.78–0.93) for Turku, 0.94 (95% CI = 0.89–0.99) for Copenhagen, 0.86 (95% CI = 0.68–1.00) for Stockholm, 1.00 (95% CI = 1.00–1.00) for Barcelona, and 0.91 (95% CI = 0.88–0.94) in the whole sample, which did not differ from those based on CSF $A\beta_{42}$ only for detection of amyloid-PET positivity. None of the

centers used a clinical-based cutpoint for a CSF $A\beta_{42}$/tau ratio.

Amyloid-PET–based cutpoints. Center-specific cutpoints varied between 1.16 and 2.38 and had an OR for amyloid-PET positivity between 47.2 and infinity, sensitivity between 85% and 100%, specificity between 75% and 100%, PPV between 91% and 100%, and NPV between 78% and 100% (table 3). In the pooled sample, the optimal $A\beta_{42}$/tau cutpoint based on the Youden index was 1.58 and had an OR of 46.0, sensitivity of 85%, specificity of 89%, PPV of 93%, and NPV of 78%. Furthermore, center-specific cutpoints for 85% sensitivity varied between 1.16 and 2.21 and had ORs between 18.00 and infinity, specificity between 75% and 100%, PPV between 89% and 100%, and NPV between 55% and 100% (table 3). In the pooled sample, the optimal $A\beta_{42}$/tau cutpoint based on 85% sensitivity was 1.61 and had an OR of 38.3, specificity of 87%, PPV of 92%, and NPV of 78%.

Concordance between CSF $A\beta_{42}$ and amyloid-PET. Clinical-based cutpoints showed an overall agreement with amyloid-PET of 76%, which was highly variable between centers (55%–89%). When CSF $A\beta_{42}$ levels were dichotomized according to the cutpoint that maximized the Youden index in the whole sample, 365 participants (84%) had concordant markers (n = 131 [30%] both normal; n = 234 [54%] both abnormal) and 68 (16%) had discordant markers (n = 35 [8%] positive amyloid-PET and normal CSF $A\beta_{42}$; n = 33 [8%] negative amyloid-PET and abnormal CSF $A\beta_{42}$), as shown in table 4.

Of the 35 participants with a positive amyloid-PET but normal CSF $A\beta_{42}$, 62% had an abnormal CSF tau and 83% had abnormal p-tau. Of these participants, 23 were genotyped and 11 (48%) of them carried at least one *APOE* ε4 allele. Of the 33 participants with a negative amyloid-PET but abnormal CSF $A\beta_{42}$, 16% showed an abnormal CSF tau and 40% abnormal p-tau. Furthermore, 11 were genotyped and 7 (64%) of them carried at least one *APOE* ε4 allele. In addition, clinical diagnosis differed between groups, as participants with a positive amyloid-PET but normal CSF $A\beta_{42}$ were more often classified as MCI or AD dementia, while participants with a negative amyloid-PET but abnormal CSF $A\beta_{42}$ were more often controls or diagnosed with non-AD dementia ($p < 0.05$).

Concordance between CSF $A\beta_{42}$/tau ratio and amyloid-PET. With use of the $A\beta_{42}$/tau ratio, 353 participants (85%) showed concordant markers (n = 138 [33%] both normal; n = 215 [52%] both abnormal), and 61 participants (15%) showed discordant markers (n = 42 [10%] positive amyloid-PET and normal CSF $A\beta_{42}$/tau; n = 19 [5%] negative amyloid-PET and abnormal CSF $A\beta_{42}$/tau). Participant characteristics of each group are shown in table 5.

DISCUSSION Calculated CSF $A\beta_{42}$ cutpoints based on amyloid-PET for detection of cortical amyloid pathology were higher than the existing clinical-

Table 3 Performances of CSF $A\beta_{42}$/tau ratio cutpoints for detection of amyloid-PET positivity

$A\beta_{42}$/tau, center	Cutpoint	SE, %	SP, %	PPV, %	NPV, %	OR
Youden-PET						
All	1.58	85 (80-89)	89 (83-94)	93 (89-96)	78 (71-84)	46.0
AMS	1.57	85 (76-91)	90 (81-96)	92 (84-97)	82 (72-90)	50.3
TUR	2.38	90 (81-96)	84 (68-94)	91 (82-97)	82 (66-92)	47.2
COP	1.16	86 (72-95)	93 (78-99)	95 (83-99)	82 (65-93)	86.3
STO	1.74	97 (85-100)	75 (35-96)	94 (81-99)	86 (42-98)	102.0
BAR	1.61	100 (71-100)	100 (80-100)	100 (71-100)	100 (80-100)	∞
85% SE-PET						
All	1.61	85 (80-89)	86 (80-91)	92 (87-94)	78 (71-84)	38.3
AMS	1.64	86 (77-92)	86 (76-93)	89 (80-94)	82 (72-90)	36.6
TUR	2.21	86 (76-93)	84 (68-94)	91 (82-97)	76 (60-88)	31.5
COP	1.16	86 (72-95)	93 (78-99)	95 (83-99)	82 (65-93)	86.3
STO	1.42	86 (70-95)	75 (35-96)	94 (79-99)	55 (24-83)	18.0
BAR	1.61	100 (71-100)	100 (80-100)	100 (71-100)	100 (80-100)	∞

Abbreviations: $A\beta_{42}$ = β-amyloid 1–42; All = all centers; AMS = Amsterdam; BAR = Barcelona; COP = Copenhagen; NPV = negative predictive value; OR = odds ratio; PPV = positive predictive value; SE = sensitivity; SP = specificity; STO = Stockholm; TUR = Turku.
Cutpoint is presented as CSF $A\beta_{42}$/tau ratio. ∞ = infinite.

Table 4 Characteristics of concordant and discordant participants based on CSF $A\beta_{42}$ levels and amyloid-PET positivity

	Concordant		Discordant	
	CSF−/PET−	CSF+/PET+	CSF−/PET+	CSF+/PET−
No.	131	234	35	33
Age, y	65 ± 10	65 ± 9	66 ± 7	61 ± 10
Sex, female	41 (31)	112 (48)[a,b,c]	12 (35)	12 (36)
MMSE score	26 ± 3	24 ± 4[a,b]	25 ± 4	26 ± 3
CSF $A\beta_{42}$, pg/mL	854 ± 211[b,c,d]	377 ± 120	683 ± 126[b,d]	375 ± 101
CSF tau, pg/mL	288 ± 141	540 ± 307[a,b]	559 ± 448[a,b]	269 ± 230
CSF p-tau, pg/mL	51 ± 22	81 ± 37[a,b]	82 ± 36[a,b]	42 ± 28
APOE ε4 carrier	22 (28)[b,c,d]	82 (65)	11 (48)	7 (64)
Diagnosis[e]				
Controls (n = 46)	30 (65)	7 (15)	2 (5)	7 (15)
MCI (n = 74)	28 (38)	32 (43)	11 (15)	3 (4)
AD dementia (n = 133)	6 (4)	110 (83)	14 (11)	3 (2)
Non-AD dementia (n = 63)	43 (68)	14 (22)	0 (0)	6 (10)

Abbreviations: $A\beta_{42}$ = β-amyloid 1–42; AD = Alzheimer disease; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; p-tau = phosphorylated tau.
Data are presented as mean ± SD or n (%). CSF−/PET+ = normal CSF $A\beta_{42}$ and positive amyloid-PET; CSF+/PET− = abnormal CSF $A\beta_{42}$ and negative amyloid-PET. CSF $A\beta_{42}$ levels were dichotomized according to overall Youden-based cutpoint (557 pg/mL). Differences between diagnostic groups were assessed using analysis of variance with post hoc Bonferroni correction (age, log-transformed CSF $A\beta_{42}$, tau and p-tau [untransformed CSF levels are shown], and MMSE) and χ^2 (sex and *APOE* ε4 carrier).
[a] Significantly different from CSF−/PET− ($p < 0.05$).
[b] Significantly different from CSF+/PET− ($p < 0.05$).
[c] Significantly different from CSF−/PET+ ($p < 0.05$).
[d] Significantly different from CSF+/PET+ ($p < 0.05$).
[e] Data from Copenhagen and Stockholm were excluded from this analysis because PET and/or CSF results were used to establish a diagnosis.

based cutpoints,[17–20] while intercenter variability in cutpoints was reduced. Using CSF $A\beta_{42}$ cutpoints based on amyloid-PET positivity, concordance was present in 84% of the participants. Cutpoints based on a CSF $A\beta_{42}$/tau ratio showed similar concordance.

The increase in $A\beta_{42}$ cutpoint based on amyloid-PET compared with the clinical-based cutpoints can probably be explained by the fact that clinical-based cutpoints are often selected to maximize the discriminability between cognitively normal participants and participants diagnosed with AD dementia. However, since 20%–30% of the cognitively normal controls had abnormal CSF $A\beta_{42}$ levels,[13] this cutpoint calculation method may result in a more conservative and lower CSF $A\beta_{42}$ cutpoint. The use of amyloid-PET positivity as a standard of truth overcomes this problem. A previous study[27] also found a higher CSF $A\beta_{42}$ cutpoint based on amyloid-PET compared with routinely used cutpoints, although our cutpoint of 557 pg/mL was lower than in the previous study. It should be noted that the present study addressed data derived from 5 different laboratories, while in particular $A\beta_{42}$ is sensitive to preanalytical and analytical factors with interlaboratory variability up to 28%.[4,5] Furthermore, our study included a heterogeneous cohort of participants visiting the memory clinics, including both participants with and without dementia, while the previous study was restricted to only participants without dementia. However, post hoc analysis showed that calculated cutpoints did not differ between diagnostic groups (data not shown), which renders this explanation less likely.

The high concordance between CSF $A\beta_{42}$ and amyloid-PET is in line with our previous report[14] and other studies comparing CSF biomarkers and [^{11}C]-PiB[28–32] or [^{18}F]-labeled amyloid-PET tracers.[27,33] Discordant participants with a positive amyloid-PET but normal CSF $A\beta_{42}$ had elevated CSF tau and p-tau and were typically diagnosed with MCI or AD dementia. Furthermore, these participants often had CSF $A\beta_{42}$ close to the cutpoint, which may suggest that in these participants amyloid pathology is related to AD dementia. Discordant interpretation of biomarkers may be attributable to a false-negative classification of CSF $A\beta_{42}$, possibly because of the arbitrary nature of a cutpoint, as

Table 5 Characteristics of concordant and discordant participants based on CSF $A\beta_{42}$/tau ratio and amyloid-PET positivity

	Concordant		Discordant	
	CSF−/PET−	CSF+/PET+	CSF−/PET+	CSF+/PET−
No.	140	218	39	17
Age, y	64 ± 10	65 ± 8	65 ± 8	67 ± 8
Sex, female	45 (32)	104 (48)[a]	14 (36)	6 (35)
MMSE score	26 ± 4	24 ± 4[b]	26 ± 4	26 ± 3
CSF $A\beta_{42}$, pg/mL	791 ± 258[b,c,d]	396 ± 142[c]	550 ± 188	466 ± 146
CSF tau, pg/mL	250 ± 112[b,d]	599 ± 324[c]	230 ± 72[d]	567 ± 227
CSF p-tau, pg/mL	45 ± 19[b,d]	87 ± 36[c]	47 ± 14[d]	79 ± 34
APOE ε4 carrier	21 (27)[b,c,d]	78 (62)	12 (63)	8 (80)[b,c]
Diagnosis[e]				
Controls (n = 46)	35 (76)	6 (13)	3 (7)	2 (4)
MCI (n = 70)	27 (38)	25 (36)	14 (20)	4 (6)
AD dementia (n = 126)	5 (4)	109 (87)	8 (6)	4 (3)
non-AD dementia (n = 56)	41 (72)	7 (13)	7 (13)	1 (2)

Abbreviations: $A\beta_{42}$ = β-amyloid 1–42; AD = Alzheimer disease; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; p-tau = phosphorylated tau.
Data are presented as mean ± SD or n (%). CSF−/PET− = both markers normal; CSF+/PET+ = both markers abnormal; CSF−/PET+ = normal CSF ratio and positive amyloid-PET; CSF+/PET− = abnormal CSF ratio and negative amyloid-PET. The CSF $A\beta_{42}$/tau ratio was dichotomized according to overall Youden-based cutpoint (1.58). Differences between diagnostic groups were assessed using analysis of variance with post hoc Bonferroni correction (age, log-transformed CSF $A\beta_{42}$, tau and p-tau [untransformed CSF levels are shown], and MMSE), and χ^2 (sex and *APOE* ε4 carrier).
[a] Significantly different from CSF−/PET− ($p < 0.05$).
[b] Significantly different from CSF+/PET+ ($p < 0.05$).
[c] Significantly different from CSF−/PET+ ($p < 0.05$).
[d] Significantly different from CSF+/PET− ($p < 0.05$).
[e] Data from Copenhagen and Stockholm were excluded from this analysis because PET and/or CSF results were used to establish a diagnosis.

continuous measures close to the cutpoint suggest near-pathologic levels of CSF $A\beta_{42}$.

Discordant patients with negative amyloid-PET and abnormal CSF $A\beta_{42}$ levels, on the contrary, showed normal levels of both CSF tau and p-tau. Furthermore, these patients were more often cognitively normal controls or had a non-AD dementia diagnosis. It is possible that these participants are in a very early stage of AD in which CSF $A\beta_{42}$ is abnormal but amyloid-PET is not. Another possibility is that these participants have amyloid pathology unrelated to AD, or that the low $A\beta_{42}$ levels in CSF have resulted from technical factors as mentioned previously. Furthermore, although both CSF $A\beta_{42}$ and amyloid-PET are considered biomarkers for amyloid pathology, some studies suggest that they do not measure the same features of pathology. For example, participants with an arctic *APP* mutation and participants with HIV infection had reduced $A\beta_{42}$ levels in the absence of cortical amyloid burden on PET.[34,35] A possible explanation is that CSF reflects only the soluble pool of $A\beta_{42}$ while amyloid-PET mainly reflects the fibrillary component. Furthermore, a recent study showed that CSF $A\beta_{42}$ and amyloid-PET are partly independently and differentially related to other, nonamyloid aspects of AD pathology.[36] This finding underlines the discrepancies in amyloid biomarker information of both modalities.

The CSF $A\beta_{42}$/tau ratio did not increase concordance with amyloid-PET, as in line with a previous study.[14] However, among discordant participants, a positive amyloid-PET and normal CSF $A\beta_{42}$/tau ratio was more common than the converse. The ratio is not typically used in clinical practice and therefore cutpoints were not available from our centers. A recent multicenter study recommended a cutpoint of 1.9,[37] which was chosen to obtain a sensitivity of 93% for a clinical diagnosis of AD dementia. The present CSF $A\beta_{42}$/tau ratio cutpoint of 1.58 is more restrictive, and showed a lower sensitivity (85%) but higher specificity (89% vs 81%) while the Youden index was identical (0.74).

A limitation of this study was the lack of a gold standard for assessment of cortical amyloid burden. In the present study, we used visually rated [^{11}C]PiB-PET images as a reference for CSF biomarkers because

it consistently showed high agreement with fibrillary amyloid plaques in postmortem neuropathologic studies.[10,11] Furthermore, multiple [18F]-labeled amyloid tracers show high agreement with [11C]PiB-PET,[33,38,39] suggesting that these more widely available amyloid tracers might be suitable for CSF cutpoint calculation as well.

Our findings suggest that amyloid-PET could help define cutpoints for CSF biomarkers. While the present study showed that variability between clinical-based cutpoints could be reduced, some variability in CSF cutpoints between centers remained, which may be explained by preanalytical or analytical factors. Therefore, standardization of analytical procedures[5] and further harmonization of CSF biomarker measurements are needed.[40]

AUTHOR CONTRIBUTIONS

Dr. Rinne, Dr. Hasselbalch, Dr. Nordberg, Dr. Lleó, Dr. van Berckel, and Dr. Visser designed the study. Drs. Zwan and Dr. Visser completed the statistical analysis. Drs. Zwan, Dr. Rinne, Dr. Hasselbalch, Dr. Nordberg, Dr. Lleó, Dr. Fortea, Dr. Blesa, Dr. van Berckel, and Dr. Visser interpreted the data. Drs. Zwan, Dr. Rinne, Dr. Hasselbalch, Dr. Nordberg, and Dr. Visser wrote the manuscript. Dr. Herukka, Dr. Soininen, Dr. Law, Dr. Bahl, Dr. Carter, Dr. Fortea, Dr. Blesa, Dr. Teunissen, Dr. Bouwman, and Dr. van Berckel revised the manuscript.

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DISCLOSURE

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