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ORIGINAL PAPER

Mapping CSF biomarker profiles onto NIA–AA guidelines for Alzheimer's disease

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Abstract The National Institute on Aging–Alzheimer's Association (NIA–AA) guidelines for Alzheimer's disease (AD) propose the categorization of individuals according to their biomarker constellation. Though the NIA–AA criteria for preclinical AD and AD dementia have already been applied in conjunction with imaging AD biomarkers, the application of the criteria using comprehensive cerebrospinal fluid (CSF) biomarker information has not been thoroughly studied yet. The study included a monocentric cohort with healthy ($N = 41$) and disease ($N = 22$) controls and patients with AD dementia ($N = 119$), and a multicentric sample with healthy controls ($N = 116$) and patients with AD dementia ($N = 102$). The CSF biomarkers β-amyloid 1–42, total tau, and phosphorylated tau at threonine 181 were measured with commercially available assays. Biomarker values were trichotomized into positive for AD, negative, or borderline. In controls the presence of normal CSF profiles varied between 13.6 and 25.4 % across the studied groups, while up to 8.6 % of them had abnormal CSF biomarkers. In 40.3–52.9 % of patients with AD dementia, a typical CSF profile for AD was detected. Approximately 40 % of the potential biomarker constellations are not considered in the NIA–AA guidelines, and more than 40 % of participants could not be classified into the NIA–AA categories with distinct biomarker constellations. Here, a refined scheme covering all potential biomarker constellations is proposed. These results enrich the discussion on the NIA–AA guidelines and point to a discordance between clinical symptomatology and CSF biomarkers even in patients with full-blown AD dementia,

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who are supposed to have a clearly positive for AD neurochemical profile.

Keywords Dementia · Cognitive aging · Biomarkers · Diagnostic criteria

Introduction

Reflecting the tremendous progress done in the field of biomarkers of Alzheimer's disease (AD) in the last decades, the National Institute on Aging and Alzheimer's Association (NIA–AA) diagnostic guidelines for AD [1–3] propose algorithms for categorizing cognitively healthy individuals and patients with AD dementia into groups with distinct constellations of biomarkers [4]. AD biomarkers predict with high accuracy the presence of the core brain pathological alterations observed in the disease, mainly β-amyloid (Aβ) accumulation [e.g., decreased levels of cerebrospinal fluid (CSF) Aβ42] and neuronal injury [e.g., CSF total tau (t-Tau) and phosphorylated tau at threonine 181 (p-Tau)] [5].

The NIA–AA criteria do not use a uniform nomenclature for the groups with distinct biomarker constellations into which individuals without cognitive deficits and patients with dementia are categorized [2, 3]. According to the NIA–AA algorithm, each biomarker value can be classified as positive for AD, negative or borderline. Abnormality of biomarkers in cognitively healthy individuals justifies the presence preclinical AD. Preclinical AD is further divided into three stages. Preclinical stage 1 is characterized by asymptomatic Aβ accumulation, while preclinical stage 2 is characterized by asymptomatic Aβ accumulation in conjunction with evidence of neuronal injury. At preclinical stage 3, subtle cognitive deficits are present in addition to positive Aβ- and neuronal injury markers. On the other hand, the criteria for AD dementia establish how probable it is that the AD pathology is present and causes the dementia syndrome. The highest probability is indicated by a combination of both abnormal Aβ- and neural injury biomarkers, and the lowest probability by normality of both Aβ- and neural injury markers [2, 3]. If neuronal injury biomarkers are unavailable or indeterminate and Aβ biomarkers are positive, or vice versa, the patient is assigned an intermediate probability to suffer from AD [2]. In the NIA–AA guidelines for both preclinical AD and AD dementia, information yielded by conflicting biomarkers are classified as uninformative (e.g., positive p-Tau in combination with negative t-Tau) or are not considered at all (e.g., positive p-Tau in conjunction with negative Aβ).

Recently, efforts were undertaken to apply the NIA–AA criteria to actual patient populations with preclinical AD and full-blown AD dementia. However, most of those studies were exclusively focused on imaging biomarkers [6, 7], or combined imaging biomarkers with only a single neurochemical biomarker [8]. A recently published multicentric study which considers all established CSF biomarkers is exclusively focused on patients with mild cognitive impairment, being a predementia clinical syndrome [9], and not on preclinical AD or dementia due to AD [10]. Another report focused on CSF biomarkers, and cognitively healthy controls was based on dichotomization of biomarker values (i.e., negative vs. positive), neglecting the fact that the NIA–AA guidelines also consider borderline biomarker values, and that in many cases biomarker values are in fact neither clearly positive nor negative [2, 11]. As a consequence, there is a critical gap regarding the application of the NIA–AA algorithms for preclinical AD and AD dementia in conjunction with comprehensive fluid biomarker information.

The main aims of the present study were (1) to unravel the neurochemical profile of patients with AD dementia and of cognitively healthy elderly individuals and (2) to apply the NIA–AA recommendations for preclinical AD and AD dementia, using CSF biomarker information, in order to investigate whether the NIA–AA algorithms consider all biomarker constellations observed in controls and patients with AD dementia.

Methods

Study design and sample

The study procedures were approved by the institutional review boards of all participating centers, and written informed consent was obtained from all participants or authorized representatives. The analyses included a monocentric dataset (MUC), comprising individuals recruited at the hospital of Technische Universität München (TUM), and a multicentric dataset, encompassing participants of the first phase of AD Neuroimaging Initiative (ADNI), with available CSF concentrations of Aβ42, t-Tau, and p-Tau. ADNI is a collaborative project of academic institutions and private corporations across the USA and Canada. The ADNI data used in this study were obtained from the ADNI database at www.adni-info.org on July 31, 2013. ADNI general eligibility criteria are described at www.adni-info.org/Scientists/ADNIGrant/ProtocolSummary.aspx. The datasets consisted of patients with AD dementia and controls. Patients with AD dementia fulfilled the NIA–AA and the National Institute of Neurological and Communicative Disorders and Stroke/AD and Related Disorders Association (NINCDS-ADRDA) criteria for AD dementia and probable AD [2, 12, 13]. Healthy controls in both datasets were elderly individuals without neuropsychiatric disorders or subjective memory complaints

and with normal neurocognitive test results. They were independent in their activities of daily living [12, 14]. CSF samples of MUC healthy controls were obtained as part of scheduled urological or orthopedic surgery procedures under spinal anesthesia at the hospital of TUM [14]. The MUC dataset included also a convenience sample of disease controls, who were not diagnosed with a central nervous system disorder. They had no subjective memory complaints and were independent in their activities of daily living. Lumbar punctures and structural brain imaging did not reveal any abnormalities. It should be underscored that AD biomarker findings were not used for establishing clinical diagnoses.

CSF acquisition and analysis

The CSF peptide concentrations were measured in ADNI with a multiplex platform [15] and in MUC with commercially available enzyme-linked immunosorbent assays (ELISA) as previously described in detail [16–18].

APOE genotyping

APOE genotypes were determined using standard polymerase chain reaction methods [19]. No *APOE* genotype data were available for disease controls since no written informed consent for genotyping has been obtained from them.

Classification of neurochemical biomarker values

Each patient's biomarker values were categorized as either positive for AD, negative for AD or borderline. The definition of the range of borderline values was based on previously published biomarker cutoffs, being specific for each employed measurement method [9, 19–22], and the standard deviations (SD) which were calculated in the whole monocentric and multicentric dataset separately, since the methods employed for peptide measurements in the two datasets were different. The range of borderline values was specified with the aim to reach a reasonable compromise between minimizing the chance of an artificial categorization as positive or negative and at the same time classifying <25 % of the measured values of each biomarker as borderline. Values within 20 % of the SD from the respective cutoff were classified as borderline. Aβ42 concentrations lower than the defined range of Aβ42 borderline values and t-Tau and p-Tau levels higher than the respective borderline ranges were assumed to be AD positive. All other biomarker values were considered negative. In the MUC sample the following concentrations were regarded as positive for AD: Aβ42 < 579.72 ng/l, t-Tau > 331.11 ng/l and p-Tau > 68.68 ng/l. The following concentrations were regarded as negative for AD: Aβ42 > 704.28 ng/l, t-Tau < 172.89 ng/l and p-Tau < 53.32 ng/l. AD positivity in the ADNI dataset was defined as Aβ42 < 177.62 ng/l, t-Tau > 104.15 ng/l and p-Tau > 27.41 ng/l. AD negativity was defined as Aβ42 > 206.38 ng/l, t-Tau < 83.85 ng/l and p-Tau < 20.59 ng/l.

NIA–AA categorization of participants

According to the NIA–AA algorithms and their CSF biomarker values, controls were categorized into the preclinical AD stages 1 or 2 or as not harboring AD pathology, while patients with dementia due to AD were classified into groups with high, intermediate, or lowest probability for AD pathology, or as having biomarker combinations being uninformative with regards to the presence of AD. Participants with biomarker constellations being not considered by the NIA–AA guidelines could not be classified into the NIA–AA categories. Modifications of the NIA–AA algorithms are here proposed, so that all potential fluid biomarker constellations are integrated and specified in the refined schemata, and the nomenclature used for subjects with preclinical AD and patients with AD dementia is harmonized. In the modified algorithm, the relative importance of Aβ42 is greater compared to t-Tau and p-Tau. Individuals with positive Aβ42 values are classified at least as high AD likelihood, whereas subjects with negative Aβ42 values are categorized as having AD likelihood not higher than low. Individuals with negative Aβ42 and neurodegeneration markers negative or borderline are classified into the lowest likelihood category. Individuals with borderline Aβ42 in conjunction with at least one positive neuronal injury marker are classified as having intermediate AD likelihood, while in the absence of positive neurodegeneration markers individuals are categorized as having low AD likelihood.

In a number of participants ($N = 18$), only one neurochemical neuronal injury biomarker was available. Due to the previously reported high degree of correlation between p-Tau/t-Tau [23], it was hypothesized that the unavailable biomarker stood in agreement with the available neurodegeneration biomarker. This strategy embodies a compromise solution. It does not indicate that p-Tau and t-Tau yield exactly the same information and are interchangeable.

Statistical analysis

The statistical analyses were performed in SPSS v19.0 for Windows (IBM corp., Somers, NY, USA) and in MATLAB R2012a version (MathWorks, Natick, MA). Normality of data distribution was checked using the Kolmogorov–Smirnov test. Differences between diagnostic groups with regards to demographic and biomarker data and *APOE* ε4 allele distribution were assessed with analysis of variance, Bonferroni post hoc analysis, Kruskal–Wallis test, Mann–Whitney test and Chi-square test as appropriate and in each dataset separately. The raw biomarker data of both datasets

Table 1 Description of the study sample

Group	Monocentric dataset (MUC)			*P* value	Multicentric dataset (ADNI)		*P* value
	HC	DC	AD		HC	AD	
N	41	22	119		116	102	
Age (years)*	67.44 (10.62)	62.82 (9.65)	68.50 (8.90)	0.04	75.61 (5.16)	75.13 (7.87)	0.60
Gender (female %)	29.3	40.9	55.5	0.01	50.0	42.2	0.28
APOE ε4 carriers (%)	($N = 40$), 36.6	NA	($N = 87$), 49.6	<0.01	24.1	69.6	<0.01
MMSE*	29.20 (1.01)	NA	21.81 (4.90)	<0.01	28.09 (1.02)	23.56 (1.90)	<0.01
CSF Aβ42 (ng/l)*	998.46 (325.20)	772.82 (282.31)	565,61 (220.76)	<0.01	206.36 (54.68)	142.98 (40.79)	<0.01
CSF Aβ42 values positive/borderline/ negative for AD (%)	9.8/7.3/82.9	27.3/22.7/50.0	62.2/19.3/18.5	<0.01	21.9/11.2/56.9	87.3/4.9/7.8	<0.01
CSF p-Tau (ng/l)*	49.95 (16.77)	43.68 (13.82)	($N = 102$) 81.46 (42.40)	<0.01	25.85 (16.51)	41.60 (19.74)	<0.01
CSF p-Tau values positive/borderline/ negative for AD (%)	19.5/14.6/65.9	4.5/13.6/81.8	($N = 102$) 55.9/23.5/20.6	<0.01	13.8/12.1/74.1	57.8/12.7/29.4	<0.01
CSF t-Tau (ng/l)*	259.20 (106.62)	($N = 21$) 219.90 (77.83)	654.94 (419.31)	<0.01	70.13 (30.29)	120.27 (57.80)	<0.01
CSF t-Tau values positive/borderline/ negative for AD (%)	26.8/46.3/26.8	($N = 21$) 9.5/61.9/28.6	83.2/15.1/1.7	<0.01	13.8/4.5/74.1	52.0/12.7/29.4	<0.01

MUC: sample recruited at the Hospital of Technische Universität München; ADNI: sample recruited with the framework of the Alzheimer's Disease Neuroimaging Initiative; HC: Healthy controls; DC: Disease controls; AD: Dementia due to Alzheimer's disease; APOE: Apolipoprotein E; MMSE: Mini mental state examination; CSF Aβ42 positive/negative for AD: β-amyloid 1–42 levels in cerebrospinal fluid (CSF) < 579.72 or 177.62 ng/l/>704.28 or 206.38 ng/l for the monocentric and multicentric dataset, respectively; CSF p-Tau values positive/negative for AD: tau phosphorylated at threonine 181 levels in CSF > 68.68 or 27.41 ng/l/<53.32 or 20.59 ng/l l for the monocentric and multicentric dataset, respectively; CSF t-Tau values positive/negative for AD: total tau levels in CSF > 331.11 or 104.15 ng/l/<172.89 or 83.85 ng/l for the monocentric and multicentric dataset, respectively

* Data presented as mean (SD)

were graphically presented by means of nonnegative matrix factorization (NNMF) [24], a data-learning technique that is particularly suited for analyzing positive valued data, in order to condense the available information in a low-dimensional (2D) space. The overall set of measurements $X_i = \{$Aβ42, t-Tau, p-Tau$\}$unlikely $_i$, $i = 1, 2, \ldots, N$, where N is the total number of participants, was approximated as $X_{[N\times 3]} \approx W_{[N\times 2]} B_{[2\times 3]}$ so as to minimize the reconstruction error induced by the Frobenius norm: $\|X - WB\|^2$. In this way, the vector of measurements X_i associated with the ith participant took the form of $X_i = w_{i1} B_1 + w_{i2} B_2$, where B_1, B_2 were the unit length vectors for a parsimonious 2D representation and w_{i1}, w_{i2} were the corresponding components. A two-sided level of significance of 0.05 was used.

Results

Sample characteristics

The characteristics of the datasets are presented in Table 1. Age and gender distribution significantly differed across the MUC groups; disease controls were significantly younger in comparison with patients with AD dementia ($P = 0.03$), while women in the healthy control group were significantly less frequent than in the AD dementia group ($P < 0.01$). As expected, in both datasets the presence of *APOE* ε4 was significantly higher in patients with AD dementia compared with healthy controls. In the MUC dataset, CSF levels of Aβ42, t-Tau, and p-Tau were [mean (SD)] 688.17 (311.38), 514.82 (395.56), and 68.59 (28.39) ng/l, respectively. In the ADNI dataset, Aβ42, t-Tau, and p-Tau concentrations were 176.70 (57.99), 93.59 (51.67), and 33.22 (19.72) ng/l, respectively. Figure 1, being a graphical presentation of participants' Aβ42, t-Tau, and p-Tau CSF levels using NNMF points to discordance between CSF profiles and diagnostic status. It highlights that in both datasets despite the clearly distinct diagnostic status of the participants (controls vs. patients with AD dementia), their biomarker profiles are distributed over a continuous spectrum between the two opposite edges, in which controls with normal biomarkers (lower right quadrant of the graphs) and patients with AD dementia and positive neurochemical biomarkers (upper left quadrant of the graphs) represent the extreme ends.

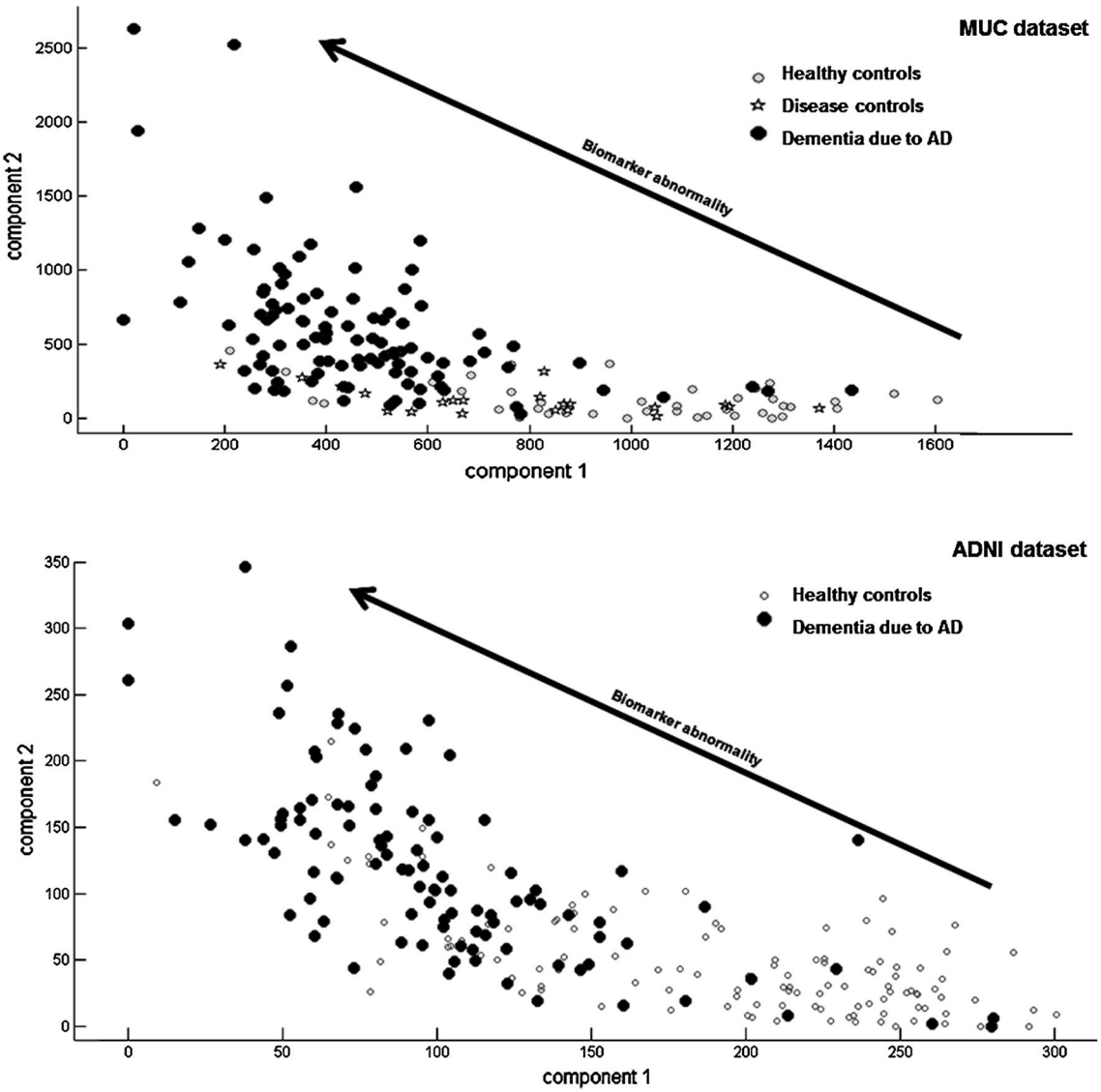
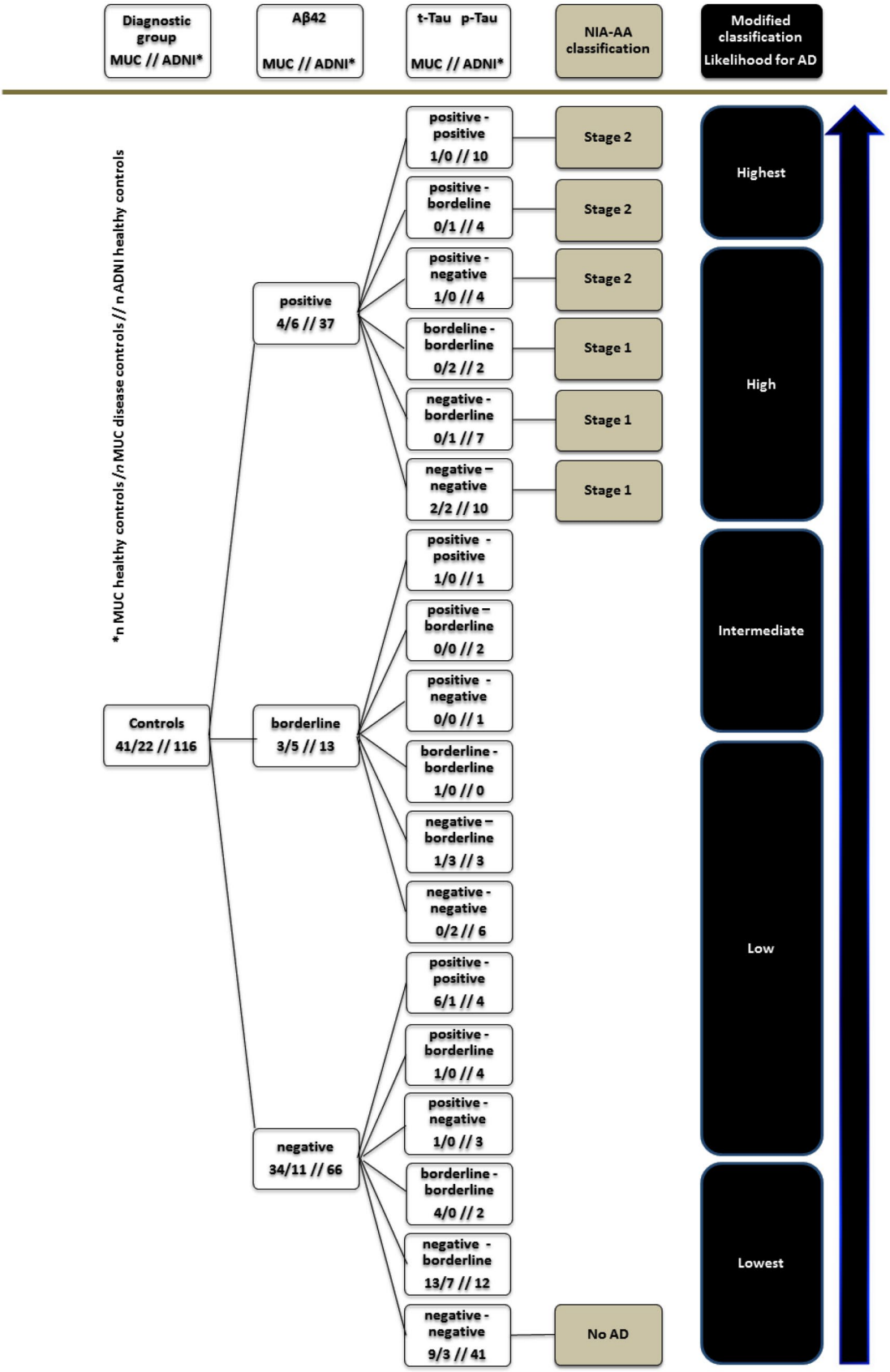


Fig. 1 Condensed representation, in the form of a 2D scatterplot, of the monocentric (MUC) and multicentric (ADNI) dataset (*upper* and *lower panel*, respectively). The ensemble of trivariate measurements of CSF β-amyloid 1–42, hyperphosphorylated tau at threonine 181, and total tau for all participants has been analyzed via nonnegative matrix factorization (NNMF) and approximated by means of a bivariate data swarm that conveniently represents the total variation in the original data. In the derived map, the *labels* indicate the different groups and lend semantics to the plot

Neurochemical profiles

In the MUC dataset, only 22 % of healthy controls and 13.6 % of disease controls had a clearly normal neurochemical profile, while in ADNI the proportion of controls with a clearly normal CSF profile tended to be significantly higher (35.4 %) ($P = 0.06$) (Fig. 2) The respective proportions of healthy controls with all biomarkers abnormal (typical AD neurochemical profile) were 2.5 % in MUC and 8.6 % in ADNI. No disease controls had all CSF biomarkers positive for AD. The distribution of controls in whom all biomarkers were abnormal did not differ across the datasets ($P = 0.16$). Interestingly, 19.5 % of MUC and 18.9 % of ADNI healthy controls and 4.5 % of MUC disease controls had positive p-Tau and/or t-Tau values in conjunction with negative or borderline Aβ42 values.

Regarding the neurochemical profile of patients with clinically diagnosed AD dementia, 40.3 % in MUC and 52.9 % in ADNI had a typical fluid biomarker profile for AD. The distribution of the typical AD neurochemical profile did not differ between patients with AD dementia in MUC and ADNI ($P = 0.08$) (Fig. 3). All available CSF markers were negative in only 0.8 % of patients with AD dementia in MUC and 2.9 % in ADNI ($P = 0.34$).


Diagnostic group
MUC // ADNI*
Aβ42
MUC // ADNI*
t-Tau p-Tau
MUC // ADNI*
NIA-AA classification
Modified classification
Likelihood for AD
*n MUC healthy controls /n MUC disease controls // n ADNI healthy controls
Controls
41/22 // 116
positive
4/6 // 37
borderline
3/5 // 13
negative
34/11 // 66
positive - positive
1/0 // 10
positive - bordeline
0/1 // 4
positive - negative
1/0 // 4
bordeline - borderline
0/2 // 2
negative - borderline
0/1 // 7
negative – negative
2/2 // 10
positive - positive
1/0 // 1
positive – borderline
0/0 // 2
positive - negative
0/0 // 1
borderline - borderline
1/0 // 0
negative – borderline
1/3 // 3
negative - negative
0/2 // 6
positive - positive
6/1 // 4
positive - borderline
1/0 // 4
positive - negative
1/0 // 3
borderline - borderline
4/0 // 2
negative - borderline
13/7 // 12
negative - negative
9/3 // 41
Stage 2
Stage 2
Stage 2
Stage 1
Stage 1
Stage 1
No AD
Highest
High
Intermediate
Low
Lowest

◀**Fig. 2** Biomarker profiles of healthy and disease controls of the monocentric (MUC) and multicentric (ADNI) dataset, National Institute on Aging–Alzheimer's Association (NIA–AA) assignments and a refined classification scheme based on CSF β-amyloid 1–42 (Aβ42), hyperphosphorylated tau at threonine 181 (p-Tau) and total tau (t-Tau)

In 30.3 % of MUC and 6.9 % of ADNI patients with AD dementia, positive p-Tau and/or t-Tau values with negative or borderline Aβ42 concentrations were observed.

NIA–AA categorization

The attempt to apply the NIA–AA algorithm for preclinical AD to healthy and disease controls revealed that only seven of 18 observed biomarker constellations could be categorized according to the NIA–AA algorithm (Fig. 2). Moreover, 4.9 % of MUC and 15.5 % of ADNI healthy controls and 4.5 % of MUC disease controls met the criteria for preclinical stage 2 (at least one amyloid and one neural injury marker positive), while 4.8 % of healthy controls in MUC and 6.4 % in ADNI and 22.7 % of MUC disease controls fulfilled the criteria for preclinical stage 1 (Aβ positivity only). 68.3 % of healthy controls of the MUC dataset and 32.8 % of ADNI had combinations that are not specified in the NIA–AA guidelines. The respective proportion in MUC disease controls was 59.1 %.

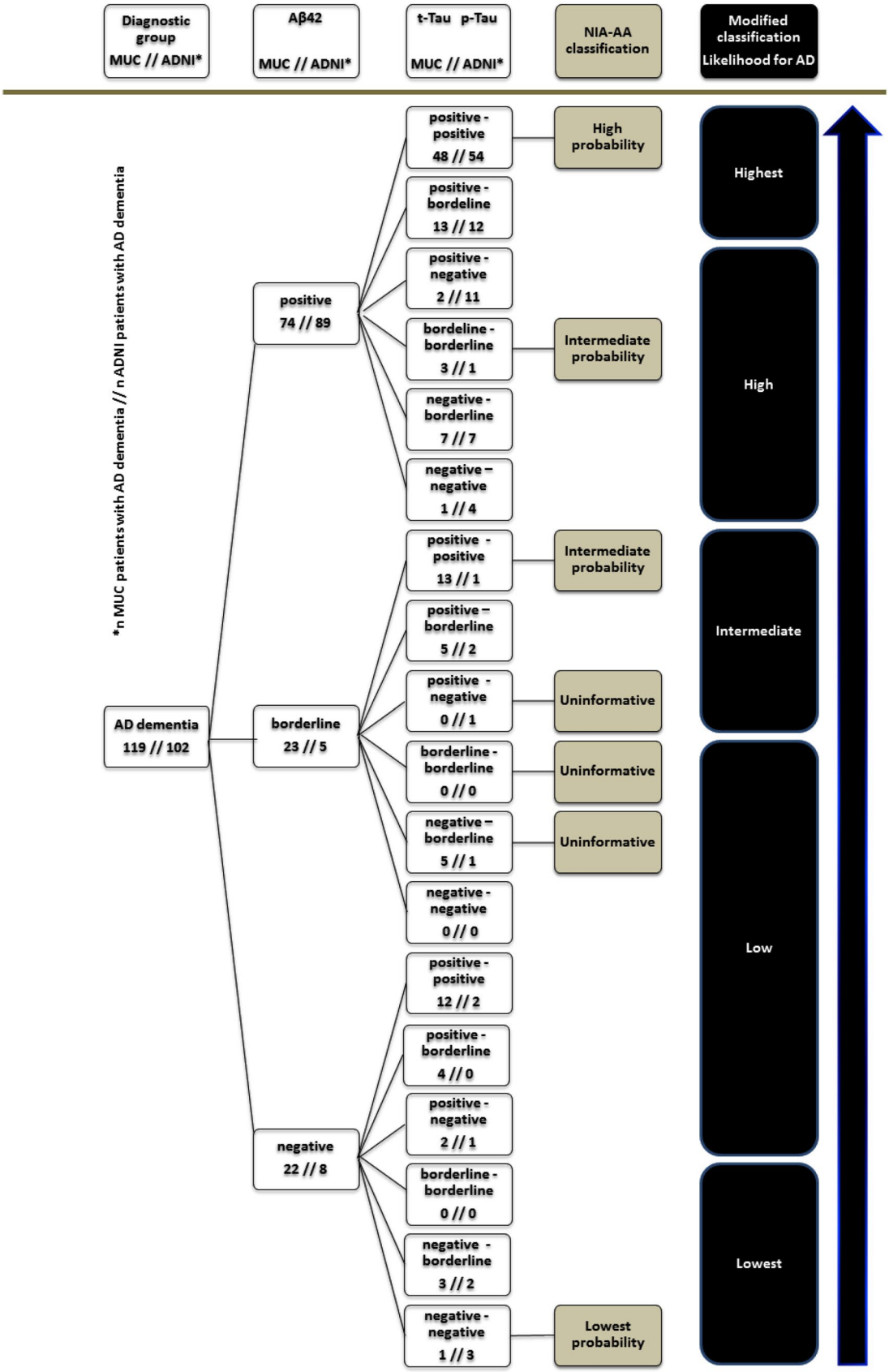
Only four of the 18 potential biomarker constellations can be classified into categories with different probability for the presence of AD according to the NIA–AA algorithm for AD dementia. In MUC 40.3 and 13.4 % of patients with AD dementia were categorized into the groups with high (all biomarkers positive) and intermediate (positive Aβ42 and both pTau and tTau borderline or vice versa) AD probability, respectively. With the exception of a single patient with the lowest probability of AD (all available biomarkers negative), all other MUC patients' biomarker combinations (45.4 %) either would be classified as uninformative by the NIA–AA guidelines or will not specified by them at all. In ADNI 52.9, 2 and 3 % of patients could be classified as having high, intermediate, and lowest probability of AD, respectively. All other ADNI patients (42.1 %) had combinations of biomarker findings that are either uninformative or are left undefined by the NIA–AA criteria (Fig. 3).

The application of the refined NIA–AA algorithm is presented in Fig. 2 for controls and in Fig. 3 for patients with AD dementia. The refined algorithm considers all potential CSF biomarker constellations.

Discussion

The findings of the present study indicate a continuum of neurochemical biomarker profiles from cognitively healthy aging to AD dementia despite the clearly distinct diagnostic status of the study participants. They are in line with reports from large clinical trials of disease-modifying drug candidates which showed that 10–35 % of patients with clinically diagnosed AD dementia have negative Aβ positron emission tomography scans, i.e., no measureable Aβ pathology [25]. The detected atypical for AD biomarker profiles in patients suffering from AD dementia can be attributed to the relatively low, in the absence of biomarker data, accuracy of current clinical AD diagnostic methods in predicting histopathologic diagnoses (sensitivity 71–88 %, specificity 44–71 %) validated by the standard pathologic diagnosis at autopsy [26]. Clinical symptoms in AD dementia are not a straightforward consequence of the presence of AD pathology, being reflected in biomarker abnormality. As autopsy reports underscore, a plethora of pathologies accompany AD pathological alterations in the aging brain (for instance, cerebrovascular alterations or Lewy body pathology) [27, 28]. Such concurrent pathologies can synergistically lower the threshold for the development of clinical symptoms, making it more likely that an individual will develop cognitive deficits, which will then justify the diagnosis of AD dementia. Such co-pathologies potentiate the clinical expression of AD-associated brain alterations which would have remained clinically silent in the absence of co-pathologies, because they are still not sufficiently advanced to become clinically recognizable [29, 30]. Thus, clinical symptoms in AD dementia are not a straightforward consequence of AD pathology, but the consequence of a complex interplay [30].

CSF biomarker abnormalities were detected in both the multicentric and monocentric groups of controls. Our observations are in line with previous reports which showed that more than approximately 50 % of cognitively healthy elderly individuals have at least one positive imaging AD biomarker [6, 7]. As already underscored, the presence of positive biomarkers does not straightforwardly lead to clinical symptoms. Biomarker abnormality in the absence of clinical symptoms is compatible with the recently defined concept of preclinical AD [3]. According to it, AD pathological hallmarks begin to develop many years prior the onset of clinical symptoms. As a consequence, AD-type brain changes are often found in individuals without any cognitive symptoms. The brain is in fact able to tolerate, mask, or even respond to structural changes [29]. For instance, the concept of neural or cognitive reserve provides an explanation why pathological alterations can accumulate for a long time without any clinical signs or symptoms [31, 32]. The discordance between biomarker profiles and clinical symptoms warrants thorough investigation, since it embodies a crucial parameter not only for clinical trials and for defining surrogate endpoints within


Diagnostic group MUC // ADNI*
Aβ42 MUC // ADNI*
t-Tau p-Tau MUC // ADNI*
NIA-AA classification
Modified classification Likelihood for AD
*n MUC patients with AD dementia // n ADNI patients with AD dementia
AD dementia 119 // 102
positive 74 // 89
borderline 23 // 5
negative 22 // 8
positive - positive 48 // 54
positive - bordeline 13 // 12
positive - negative 2 // 11
bordeline - borderline 3 // 1
negative - borderline 7 // 7
negative – negative 1 // 4
positive - positive 13 // 1
positive – borderline 5 // 2
positive - negative 0 // 1
borderline - borderline 0 // 0
negative – borderline 5 // 1
negative - negative 0 // 0
positive - positive 12 // 2
positive - borderline 4 // 0
positive - negative 2 // 1
borderline - borderline 0 // 0
negative - borderline 3 // 2
negative - negative 1 // 3
High probability
Intermediate probability
Intermediate probability
Uninformative
Uninformative
Uninformative
Lowest probability
Highest
High
Intermediate
Low
Lowest

◀**Fig. 3** Biomarker profiles of patients with dementia due to Alzheimer's disease (AD) of the monocentric (MUC) and multicentric (ADNI) dataset, National Institute on Aging–Alzheimer's Association (NIA–AA) assignments and a refined classification scheme based on CSF β-amyloid 1–42 (Aβ42), hyperphosphorylated tau at threonine 181 (p-Tau) and total tau (t-Tau)

their framework, but also for developing effective prevention strategies not pertaining to AD pathomechanism.

The observations of the present study with regard to combinations of abnormal p-Tau and/or t-Tau values with negative or borderline Aβ42 concentrations further support the presence of individuals with normal or borderline amyloid biomarkers and abnormal biomarkers of neuronal injury. The term "suspected non-AD pathophysiology (SNAP)" has been recently proposed to designate subjects without evidence of amyloid accumulation but with abnormal biomarkers of neuronal injury [6, 8, 33]. In accordance with prior reports, proportions of individuals with SNAP in our study did not exceed 30 % in each diagnostic group [6, 8, 33]. Interestingly, the monocentric AD dataset encompassed clearly more patients with SNAP than the ADNI AD sample (30.3 vs. 6.9 %). This difference could be explained by the different characteristics of the two samples. The ADNI cohort was mainly recruited for research purposes at specialized research centers, while the MUC samples were recruited in a more naturalistic clinical setting and not exclusively within the framework of research activity. As a consequence, the latter are less contingent on over-selection and research center enrollment biases.

Our results underscore that the NIA–AA algorithms do not consider all possible biomarker constellations. Approximately 40 % of biomarker combinations observed could not be classified according to the NIA–AA algorithms. Conflicting biomarker results within the same biomarker category (e.g., neuronal injury) or between different biomarker categories as well as biomarker constellations indicating SNAP are not considered or are classified as uninformative. However, in the era of personalized medicine [34, 35], it is important that all potential biomarker combinations are considered and incorporated into the algorithm for defining groups with different AD likelihoods. In the modified assignment scheme, which is here presented, a harmonization of the nomenclature used for the groups with distinct biomarker constellations regardless clinical symptoms is proposed, since the classification into groups is based on the same biomarkers and refers to the same pathological changes [2, 3]. Group assignment does not indicate a specific pattern of clinical prognosis or symptomatology, since prognosis and clinical symptoms are in fact not exclusively contingent on AD pathology [30]. In the proposed modified algorithm, amyloid biomarkers are prioritized compared with biomarkers of neuronal injury, since Aβ42 is more specific to AD than t-Tau, and Aβ42 levels become abnormal earlier than p-Tau and t-Tau according to the model of temporal evolution of AD biomarkers [36]. The NIA–AA algorithm for preclinical AD also prioritizes amyloid information [3]. Previously proposed refinements of the NIA–AA algorithm for AD dementia, which were based on different biomarker modalities, prioritized amyloid biomarker information too [8]. Nonetheless, the proposed modified assignment schema has to be justified through empirical evidence, for instance through pathological diagnoses established in close temporal relation to the acquisition of CSF.

The present study should be viewed in the light of some limitations. The diagnostic workup of the disease control group did not include a neuropsychological assessment. As a result, it cannot be excluded that some of the disease controls suffered from very mild cognitive deficits, which, however, did not cause any subjective memory complaints or impairment of their activities of daily living. In addition, the size of the disease control group is relatively small. Moreover, the healthy controls' neuropsychological assessment was restricted to established cognitive measures that cannot detect very subtle cognitive impairment [3]. As a consequence, it cannot be ruled out that some of the cognitively healthy individuals with a typical neurochemical AD profile could have been assigned to stage 3 of preclinical AD, if they had been tested for subtle cognitive deficits Furthermore, no histopathologic (definite) diagnoses were available, and we did not consider imaging biomarker data. However, it should be underscored that while combining imaging with neurochemical biomarker data may be relevant for research settings, it is rarely applicable to clinical settings because of limitations related to scanner equipment and sophisticated image analyses expertise. Moreover, the NIA–AA guidelines do not necessitate the availability of imaging biomarker data [2, 3]. Despite the previously reported significant influences of age and sex on the development of AD pathology [37, 38], it seems unlikely that the detected significant differences in age and sex distribution between the diagnostic groups of the MUC cohort have biased our observations, since our study aimed to describe naturalistically the biomarker profiles of controls and patients with AD dementia and to apply the NIA–AA criteria. In line with such an assumption, the proportion of controls with normal biomarkers was significantly higher in ADNI compared with MUC, though ADNI controls were older than MUC controls. In addition the distribution of controls with abnormal biomarkers did not differ across the datasets. Furthermore, it can be reckoned that the observed high proportion of participants who could not be classified by the NIA–AA algorithms is attributable to the defined range of borderline values. In the light of the lack of empirical data with regard to definitions of the range of borderline values, our findings should be treated

with caution. Nonetheless, the NIA–AA guidelines clearly specify the presence of borderline biomarker values and do not take into account approximately 40 % of the potential biomarker constellations. As a result, further studies considering borderline biomarker values are warranted.

To conclude, the findings of the present study illustrate the polymorphy of the neurochemical profiles of patients with AD dementia and elderly cognitively healthy individuals. They point to discordance between CSF biomarker profile and diagnostic status. This discordance is a consequence of the complexity of the genesis of clinical symptoms in AD. Our observations enrich the discussion on the NIA–AA guidelines and possibly contribute to paving the way toward refining the guidelines, so that they address all potential biomarker constellations.

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Compliance with ethical standards

Conflict of interest Dr. Alexopoulos serves on the editorial board of the Journal of Alzheimer's Disease and has received speaker honoraria from IBL International. Dr. Buck has received compensation for activities with Bayer HealthCare, BiogenIdec, MerckSerono, and Novartis. She was supported by the Commission for Clinical Research of the Faculty of Medicine, Technische Universität München, Abirisk and the PML Consortium. All other authors report no disclosures.

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