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Plasma Aβ peptides and apolipoprotein E in sporadic Alzheimer's disease and mild cognitive impairment

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Summary

Introduction: Plasma Aβ levels have been examined in sporadic Alzheimer's disease (AD) patients yielding conflicting results. Some studies showed no difference between plasma concentrations of $A\beta_{1-42}$ and $A\beta_{1-40}$ in sporadic cases of AD as compared to controls, others found increased levels of $A\beta_{1-42}$ in at least some AD patients. The results of several recent studies suggest that elevated plasma $A\beta_{1-42}$ levels may be detected several years before the onset of symptoms, though the value of that effect in predicting progression to dementia in mildly cognitively impaired (MCI) subjects is not known. Finally, it's been proposed that plasma Aβ levels increase merely with age and are neither sensitive nor specific for AD or MCI.

Material and method: Levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ were measured in plasma from 54 patients with AD, 39 subjects with MCI and 35 controls using a commercially available ELISA.

Results: Mean plasma $A\beta_{1-42}$ levels were significantly higher in MCI as compared to both AD ($p<0.001$) and control subjects ($p<0.001$) while levels of $A\beta_{1-40}$ did not differ between the groups. In contrast to some earlier reports no correlations were observed between Aβ species levels and age or MMSE scores. However, $A\beta_{1-42}$ were significantly lower in subjects carrying at least one apoliprotein ε4 allele. Employing ROC curve analysis we found that the maximum accuracy in discriminating MCI versus both controls and AD subjects has been achieved using a cut-off value of 3.8.

Conclusions: Mean plasma levels of Aβ peptides differ between AD, MCI and control subjects though their usefulness in the differential diagnosis of AD is doubtful. Further studies are needed to establish the value of Aβ peptides levels in identifying patients with MCI and (possibly) in prediction of their progression to clinically overt AD.

amyloid / **plasma** / **apoliprotein E** / **Alzheimer's disease** / **mild cognitive impairment**

INTRODUCTION

Dementia is nowadays considered one of the leading causes of death worldwide [1, 2] and Alzheimer's disease (AD) is the most common form of dementia in the Western countries. Population studies show that 5–10% of persons older than 65 suffer from dementia while in the subgroup older than 80 this percentage amounts to almost 50% [3, 4, 5]. Limited Polish data allows the suggestion of dementia prevalence as similar to Westernized countries, however, vascular and mixed forms of dementia might be marginally more frequent than AD [6].

Routine clinical assessments, even if enriched with neuropsychological and neuroimaging procedures, while costly and time-consuming, can at

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best result in diagnosis of "probable" AD as defined by DSM-IV or ICD–10 sets of criteria; only in specialized centres does the overall diagnostic accuracy reach 65–90% [7], being considerably lower in the earlier stages of the disorder.

An ideal biomarker, according to the Nancy & Ronald Reagan Institute recommendations, should not only permit the reliable diagnosis but also predict the course of the disorder, monitor its progression and serve as surrogate marker in clinical studies evaluating treatment procedures. Preferably, it should also reflect pathogenetic cascades and major neuropathological changes of the disease, in case of AD the neurodegeneration of neuronal cells and synapses as well as the development of amyloid plaques and neurofibrillary tangles (NFT). It is also suggested that such an ideal biomarker should claim at least 80% of both sensitivity and specificity in pathologically confirmed cases and, last but not least, should be easily determined in body fluids, such as urine, blood or cerebrospinal fluid (see also http://www/alzforum.org/res/enab/workshops/biomarkers.asp). It is needless to say that to date the ideal biomarker of AD has not yet been established.

Mild cognitive impairment (MCI) is a clinical construct of uncertain validity, still by many authors considered an early, preclinical phase of AD [8, 9]. Data from longitudinal studies show that a significant proportion of MCI cases (particularly its amnestic form) evolve to AD (so-called conversion rate is estimated at 5–10% a year). On the other hand, however, predictors of conversion are not clearly established and in some cohorts up to 40% of MCI subjects return to normal or remain stable even after long-term surveillance [10, 11]. It is then a matter of debate whether MCI is indeed an early phase of AD and the lack of biomarkers precludes an internal verification of a hypothesis claiming pathogenetical equality between MCI and AD [12, 13].

AMYLOID β PEPTIDES AS BIOMARKERS OF AD AND MCI

Amyloid cascade hypothesis is, at present, a commonly accepted pathogenetic concept of AD. In its abridged form, it suggests that an active process of amyloid deposition in the brain is central to AD pathogenesis. Fibrillar (and intermediate) forms of amyloid are considered to be not only directly toxic to neurons but also able to initiate and maintain several processes (oxidative stress, inflammation, formation of ion channels, dysregulation of cholinergic transmission, vascular changes, to name a few) that ultimately lead to neuronal loss and clinically overt dementia [14]. The role of amyloid is quite obvious in familial cases of AD (familial AD, FAD) with the majority of known mutations leading to either overproduction of the amyloid precursor protein (βAPP), which also operates in Down's syndrome with dementia cases, or a change in its metabolism in favour of release of a longer, more prone to fibrillogenesis forms of amyloid β peptides (Aβ). Pathogenesis of the most common, sporadic cases of AD is less well known. It is assumed that an interplay of various genetic (e.g. polymorphisms of apolipoprotein E or CYP46), metabolic (hormonal dysfunction, cholesterol mismatebolism), vascular (microcirculation changes) and environmental (education, mental activity, general fitness) factors, acting synergistically, lead to neuropathological changes and clinical picture similar to FAD [15].

Aβ is synthesized during normal cellular metabolism of its membrane-anchored precursor protein and released to extracellular space which allows its detection in body fluids, including cerebrospinal fluid (CSF) and plasma [16]. Studies of the CSF levels of total Aβ in AD produced inconsistent results; however, most of the authors were able to show a statistically significant decrease in the $A\beta_{1-42}$ isoform concentration over the course of the disorder, the finding which is usually explained by the precipitation of $A\beta_{1-42}$ in the brain along with plaques formation and consequently its reduced clearance [17, 18]. Sensitivity and specificity of the decrease in $A\beta_{1-42}$ isoform levels in CSF as a biomarker of AD have been estimated at up to 100% and 65%, respectively [18]. Interestingly, a similar finding has been reported in MCI cases as well [19]. However, the clinical usefulness of the CSF $A\beta_{1-42}$ isoform levels has been questionable. Firstly, the specificity is low and the decrease might also be detected in other than AD forms of dementia [7]. Secondly, a procedure of lumbar puncture required for CSF assessments is considered risky in many countries (except for Scandinavia and Japan) and relative-

ly rarely performed, mostly due to patients' reluctance.

Since blood samples are easily acquired and no additional risk (as in the case of CSF sampling) is recognized, plasma levels of Aβ isoforms possibly represent an attractive candidate for a clinically useful method supporting AD diagnosis and serving as a biomarker. Studies in FAD and Down's syndrome with dementia have consistently shown an increase in Aβ levels in plasma [20, 21]. However, unequivocal results have so far been published in sporadic AD cases, with both an increase [22, 23] and no change [24, 25] as compared to normal controls having been reported. The results of one study also pointed to age but not diagnosis as a main predictor of Aβ plasma levels [26]. Studies in MCI are limited. It has been shown however, that Aβ plasma levels might be increased years before clinical diagnosis of AD is possible [27], while an increase has been reported in one study but only in women [28].

MATERIAL AND METHODS

We aimed to compare the plasma levels of Aβ peptides in sporadic AD patients, MCI subjects and cognitively healthy controls matched for age, gender and education. An estimation of the sensitivity and specificity of plasma levels of Aβ peptides as biomarkers of AD and MCI has also been undertaken.

In most previous studies established clinical criteria (e.g. NINCSD-ADRDA or DSM-IV) for diagnosing AD were used; as the specificity of those is insufficient, both mixed cases and some patients with other than AD dementias might be included using the above criteria. We have therefore used NINCSD-ADRDA criteria as a first step and then excluded all subjects with clinically and/or radiologically significant vascular pathology as well as those who fulfilled criteria for other than AD primary dementias, including dementia with Lewy bodies (DLB) and frontotemporal dementias (FTD). In case of MCI only predominantly amnestic subjects were included. MCI patients also needed to be free of any unstable somatic disorder, uncontrolled vascular risk factors or other identifiable cognitive decline predictors, like drug or alcohol abuse.

Our initial AD sample included 132 subjects fulfilling NINCSD-ADRDA criteria for AD. Of those, we have excluded 36 fulfilling ICD–10 criteria for mixed dementia, 11 with probable or possible DLB, 5 with FTD as well as 8 with a history or current drug or alcohol abuse or dependence. Eighteen subjects with significant family history were also excluded.

From the initial MCI sample of 70 subjects, we have excluded 31 due to not fulfilling criteria for a predominantly amnesic MCI (either isolated non-memory deficit or multiple domains deficits, n=11), the presence of any uncontrolled somatic (including vascular) or neuropsychiatric (including depressive episode) disorders that might directly influence cognition (n=11), a history of drugs or alcohol abuse or dependence (n=3) and, finally, familial history of AD (n=7).

The protocol of the study has been accepted by the Medical University of Łódź Ethics Committee as conforming to the declaration of Helsinki and all the subjects as well as their informal caregivers have signed an informed consent.

Whole blood was collected from fasting subjects in EDTA-containing recipients and cellular material was pelleted by centrifugation. Platelets have been regarded a primary source of circulating βAPP and Aβ. However, no sampling technique modification preventing the activation of platelets was applied, based on data indicating lack of any associations between platelet activation and plasma Aβ levels measured with a similar method [29]. As an increasing number of reports fail to observe a correlation between statins treatment and plasma Aβ levels, we did not introduce any additional procedures in cases of hypercholesterolemia treated with statins [30]. Plasma was stored at –4°C for a maximum of 8 hours and then frozen in 1 ml aliquots and stored at –70°C until measurements. The concentrations of Aβ peptides (Aβ40 and Aβ42) in plasma were measured using commercially available sandwich ELISA colorimetric assay (BioSource Intl, Inc) which has been shown to be sensitive enough (range 15.6–1000 pg/ml) to ensure an accurate result in plasma. Apolipoproetin E was genotyped using standardized protocol in a subset of 44 AD subjects who consented independently.

Table 1. Demographic variables of the study cohort

Study group	N	Age (mean ± standard deviation)	Gender (fraction of women)	Years of formal education (mean ± standard deviation)	Age at symptomatic onset (AD cases only)	MMSE score (mean ± standard deviation)	ADAS-cog score (mean ± standard deviation)
AD	54	77.5±4.4	0.69	7.3±3.3	73.5±4.2	17.5±3.4	30.5±10.6
MCI	39	74.0±3.4	0.66	10.0±3.2	N/A	27.3±0.9	10.4±2.7
Controls	35	75.0±2.9	0.68	8.6±2.9	N/A	29.5±0.6	3.0±1.9
Total	128	75.6±4.1	0.68	8.5±3.3	N/A	23.8±5.9	16.9±13.9

RESULTS

One hundred and twenty eight subjects were included, of those 54 with sporadic AD (17 men, mean age 77.5 ± 4.4 years, mean MMSE 17.5 ± 3.4), 39 with predominantly amnestic MCI (13 men, mean age 74 ± 3.4, mean MMSE 27 ± 0.9) and 35 cognitively intact controls (11 men, mean age 75 ± 2.9, mean MMSE 29.5 ± 0.6) (Table 1).

Regardless of an isoform, plasma levels of Aβ peptides did not differ between AD and controls (Mann-Whitney U test, adjusted for age, MMSE and education). However, in the MCI group plasma levels of $A\beta_{1-42}$ were significantly higher and the $A\beta_{1-40}/A\beta_{1-42}$ ration lower than both in AD and controls (table 2 and figure 1). Contrary to one previous report [26] ANOVA linear regression failed to reveal any correlation between age and Aβ levels; between-gender differences were also not observed.

Surprisingly and contrary to most of the previous reports, plasma levels of $A\beta_{1-42}$ were significantly lower in carriers of at least one apolipoprotein E ε4 allele as compared to non-carriers (33.2±7.7 *versus* 41.8±12.8 pg/ml; Mann-Whitney U test, p=0.01) (figure 2).

As a next step, we have performed area under the curve ROC analyses to estimate the cut-off

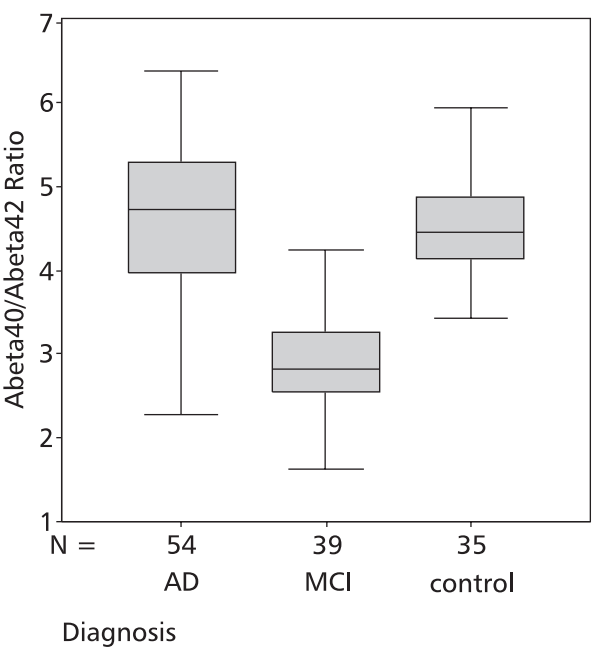

Fig. 1. Mean values (bold line in boxplots) and 95% confidence intervals (grey zones of boxplots) of $A\beta_{1-40}/A\beta_{1-42}$ ratio in AD, MCI and controls.

Table 2. Mean plasma levels of Aβ peptides (pg/ml ± standard deviation)

Study group	N	$A\beta_{1-40}$	$A\beta_{1-42}$*	$A\beta_{1-40}/A\beta_{1-42}$*
AD	54	168.7 ± 32.2	37.8 ± 10.3	4.6 ± 0.9
MCI	39	160.1 ± 20.2	56.8 ± 9.3	2.9 ± 0.6
Controls	35	160.1 ± 15.2	36.3 ± 6.3	4.5 ± 0.6

* differences significant between MCI and both AD and controls at p<0.001(U Mann-Whitney test)

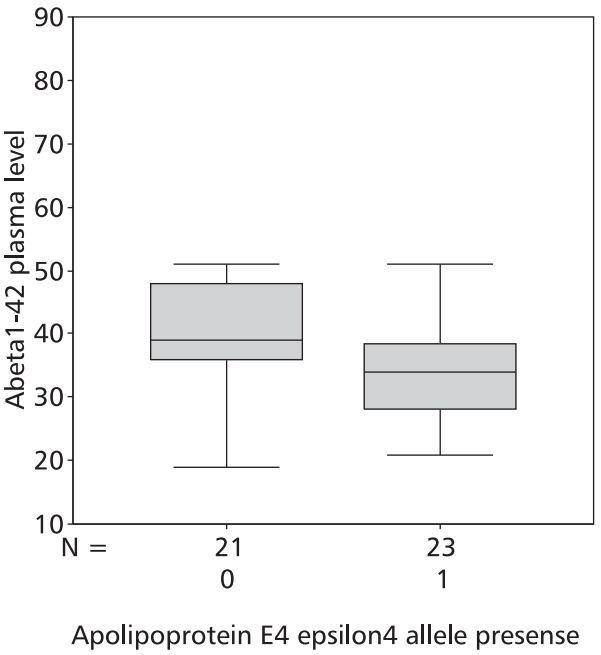

Fig. 2. Mean values and 95% confidence intervals of $A\beta_{1-42}$ plasma levels in AD subjects in regard of the presence of at least one apoliprotein E $\varepsilon 4$ allele (1 = present, 0 = absent)

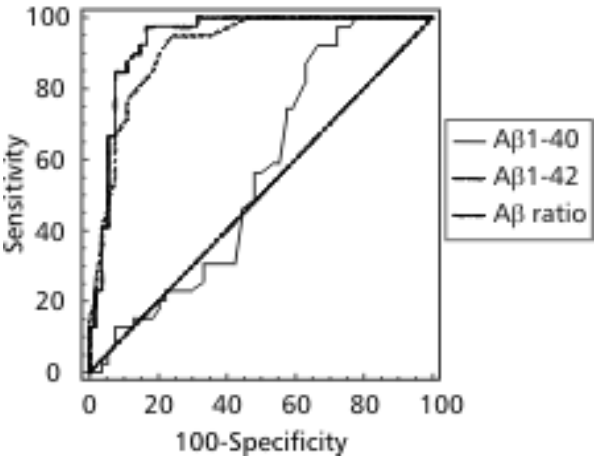

Fig. 4. A comparison of ROC curves (see legend) for evaluated parameters in MCI and AD. Areas under the curve for $A\beta_{1-42}$ (dashed line) and $A\beta_{1-42}/A\beta_{1-42}$ ratio (dotted line) differ significantly from 0.5 (0.91±0.03; 95% CI=0.84–0.96 and 0.94±0.02; 95% CI=0.87–0.98. respectively) while no difference exists for $A\beta_{1-40}$ (solid line; 0.56±0.036; 95% CI=0.46–0.67).

values of $A\beta_{1-42}$ and $A\beta_{1-40}/A\beta_{1-42}$ ratio that yield a highest accuracy in discriminating the samples. The value of $A\beta_{1-40}/A\beta_{1-42}$ ratio lower than 3.8 discriminated MCI from AD with a sensitivity of 97.4% (95%CI=86.5–99.6) and specificity of 83.3% (70.7–92.1), and MCI from controls with a sensitivity of 97.4% (86.5–99.6) and specificity of 88.6% (73.2–96.7). The level of $A\beta_{1-42}$ higher than 45 pg/ml discriminated MCI from AD with a sensitivity of 94.9% (82.6–99.2) and specificity of 75.9% (62.4–86.5), while MCI from controls with a sensitivity of 94.4% (82.6–99.2) and specificity of 91.4% (76.9–98.1) (figures 3 and 4).

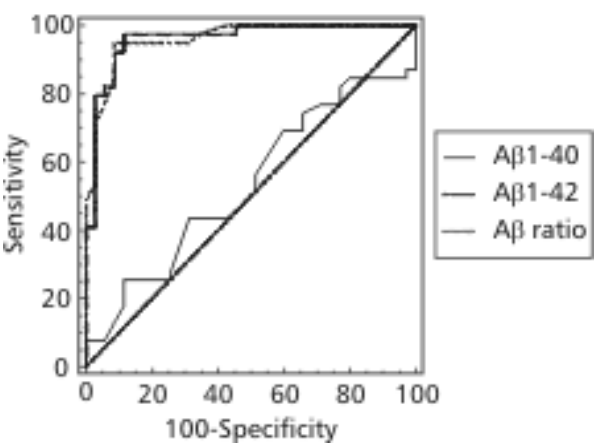

Fig. 3. A comparison of ROC curves (see legend) for evaluated parameters in MCI and controls. Areas under the curve for $A\beta_{1-42}$ (dashed line) and $A\beta_{1-42}/A\beta_{1-42}$ ratio (dotted line) differ significantly from 0.5 (0.96±0.02; 95% CI=0.88–0.99 and 0.96±0.02; 95% CI=0.89–0.99. respectively) while no difference exists for $A\beta_{1-40}$ (solid line; 0.53±0.07; 95% CI=0.41–0.65).

DISCUSSION

The results of the present study failed to validate the usefulness of the plasma levels of Aβ as a biomarker of sporadic AD, which is in agreement with the majority of similar reports to date [24–26]. We also failed to observe any correlation between plasma Aβ levels and age [26], gender, severity of disease or any other demographic variable. However, unexplainably lower Aβ levels in subjects with at least one apoliprotein E $\varepsilon 4$ allele as compared to non-carriers were detected. This finding is in sharp contrast to previous studies showing no such correlation [24, 25, 26, 31]. One possible explanation is a different mode of patients selection we employed (so, formally, as compared to previous studies it would be a selec-

tion bias) that attempted to exclude subjects with vascular pathology. However, due to a relatively small sample, our finding might also be completely spurious and requires replication.

Changes in MCI plasma Aβ levels suggest their capability as a possible biomarker [22, 27].

One may hypothesize that an increase in A$\beta_{1–42}$ levels in plasma of subjects with MCI might represent an attempt of its clearance from the brain *via* the still intact brain-blood barrier. Later on in the course of the disease, when the brain-blood barrier becomes dysfunctional at first and then anatomically damaged, further clearance turns out to be inefficient and the peptide itself accumulates in the brain to form plaques. Amyloid brain-to-blood clearance has been proved to be intact in the early stages of amyloidosis in the animal models of AD [32] and may be, at least in part, responsible for the efficacy of both active and passive immunization in reducing the number of amyloid deposits [33, 34]. Moreover, there is evidence from both human and animal studies that the brain-blood barrier is being gradually damaged as AD progresses from mild to more severe cognitive decline [35, 36]. Aβ itself has also been shown to influence brain-blood barrier permeability and probably contributes to its progressive damage [37, 38].

It is then possible that in the earliest stages of AD (clinically corresponding to MCI) an overproduced A$\beta_{1–42}$ is efficiently cleared to plasma *via* the intact brain-blood barrier, resulting in an increase in its plasma levels. Meanwhile, there is no increase in its shorter counterparts (e.g. A$\beta_{1–40}$) due to operative retrograde transport [37]. Indeed, the results of our study seem to confirm the above hypothesis showing increased plasma levels of A$\beta_{1–42}$ but not A$\beta_{1–40}$ in MCI. In the later stages of the neurodegenerative process (clinically manifesting itself as overt dementia) due to the progressive injury to the brain-blood barrier and further changes in amyloid production, its clearance from the brain would become inefficient. This in turn would result in its accumulation in the form of plaques [39], while retrograde blood-to-brain transport of Aβ peptides would mostly be responsible for its vascular deposition and the development of congophilic angiopathy [40].

Although, it looks as if we may, with reasonably high accuracy (sensitivity of 95% and specificity of 75%) discriminate between MCI and controls using only Aβ peptides plasma levels, it remains unknown whether these might also be used to predict "conversion" from MCI to dementia. Longitudinal studies (one has already been started in our centre) are needed to investigate this issue.

CONCLUSIONS

1. Plasma levels of Aβ peptides do not differ between sporadic AD cases and cognitively intact controls. Demographic variables (including age and gender) do not predict Aβ levels in AD but carriers of at least one apoliprotein E ε4 allele may have lower levels of A$\beta_{1–42}$, the validity of the latter finding needs to be confirmed.
2. Plasma levels of A$\beta_{1–42}$ allow discrimination of MCI and both sporadic AD and cognitively intact controls with reasonable sensitivity (>90%) and specificity. Discriminative power might be improved with the use of A$\beta_{1–40}$/A$\beta_{1–42}$ ratio.
3. It is to be verified whether the increase in plasma levels of A$\beta_{1–42}$ reflects the natural course of neurodegeneration in AD and whether it might serve as an individual marker of MCI to AD conversion.

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