Prediction of Alzheimer's Disease Using Midregional Proadrenomedullin and Midregional Proatrial Natriuretic Peptide: A Retrospective Analysis of 134 Patients With Mild Cognitive Impairment

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Objective: Development of biomarkers for early detection of Alzheimer's disease (AD) is a major clinical research goal. On the basis of the hypothesis that cardiovascular risk factors contribute to the pathogenesis of AD, we investigated whether the cardiovascular risk markers midregional proadrenomedullin (MR-proADM) and midregional proatrial natriuretic peptide (MR-proANP) predict a major clinical milestone, ie, conversion from predementia mild cognitive impairment (MCI) to manifest AD.

Method: A group of 134 MCI patients, among 137 originally prospectively recruited at the memory disorder clinic at Malmö University Hospital, Malmö, Sweden, between July 1998 and June 2001, was clinically followed for 4–6 years. We determined whether plasma concentrations of MR-proADM and MR-proANP at baseline predicted time to conversion from MCI to clinically diagnosed AD (*DSM-III-R*). MCI was diagnosed according to Petersen criteria.

Results: During follow-up, 41.8% of MCI patients remained cognitively stable, 42.5% converted to possible and probable AD, and 15.7% converted to other forms of dementia (MCI-other). MCI converters and MCI-other patients showed increased concentrations of MR-proANP and MR-proADM compared to the stable MCI patients ($P = .0001$). At a cutoff of 87 pmol/L, MR-proANP yielded a sensitivity of 73.7% and a specificity of 64.3% for predicting conversion to AD. The survival analysis showed that higher values of MR-proANP and MR-proADM were associated with progression to AD. In a multivariate Cox regression model including known risk factors, MR-proANP and MR-proADM remained independent risk factors for conversion to AD for patients below the age of 72 years.

Conclusions: Our study shows that plasma concentrations of MR-proANP and MR-proADM have predictive value in the progression from predementia MCI to clinical AD. Sensitivity was particularly high, which may recommend this test for first-stage screening in patients at risk for AD.

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A major biologic basis for progressive cognitive decline in the elderly is characteristic neuropathology with underlying molecular mechanisms of Alzheimer's disease (AD).[1] In AD, the dementia syndrome results from a slowly progressive cognitive decline preceded by a predementia stage, termed mild cognitive impairment (MCI). In order to improve early and differential diagnosis of AD during the MCI transition stage of the disease, major efforts are ongoing during the last decade to develop diagnostic, predictive, and, ideally, surrogate biologic markers.[2,3] They are also urgently needed for clinical trials in patients with MCI and AD.[4,5] The clinical syndrome of MCI has been shown to predict conversion to AD with a rate of 10% to 15% per year compared to 2% in the general population aged 65 years and older. The risk of conversion is further substantially increased in MCI patients with overt hippocampus atrophy[6] or pathologically altered cerebrospinal fluid (CSF) concentrations of tau protein (tau), phosphorylated tau protein (p-tau), β-amyloid 42 (Aβ42) peptide, and β-site APP-cleaving enzyme 1 (BACE1; both concentration and activity).[7–10] Therefore, these core feasible biomarker candidates, which have been rigorously tested in clinical diagnostic studies for more than 10 years and which are currently at the end stage of world-wide, controlled, multicenter validation, have finally become part of recently proposed revised research criteria to define the predementia symptomatic stage of clinically probable AD.[11]

There is growing evidence that vascular factors and cardiovascular dysfunction may substantially contribute to the specific pathogenesis of AD. Therefore, vascular factors and cardiovascular dysfunction may be central and potentially specific mechanistic events and not simply unrelated converging cofactors that contribute to progression and severity of general brain pathology and present as a mere comorbidity of vascular disease in some AD patients.[12–14]

Biologic risk markers of cardiovascular disease may be helpful to predict AD-specific mechanisms underlying conversion of predementia MCI into clinical AD.

Adrenomedullin (ADM) and atrial natriuretic peptide (ANP) have been found to be associated with microvascular dysfunction, such as heart failure, myocardial infarction, respiratory tract infections, and sepsis.[15–17]

Adrenomedullin, a peptide with 52 amino acids, has immune-modulating, metabolic, and vascular properties. Due to its potent vasodilating activity, the tissue-wide production of ADM assures blood supply to the individual organs.[18,19] The concentration of ADM significantly increases in a number of diseases, including congestive heart failure, sepsis, essential hypertension, and renal impairment.[20]

Atrial natriuretic peptide consists of 28 amino acids, and it promotes natriuresis and diuresis, inhibits the renin-angiotensin-aldosterone axis, and acts as a vasodilator.[21–23] Circulating ANP concentrations were shown to be increased in patients with heart failure.[24]

As ADM and ANP are rapidly cleared from the circulation, with a half-life of 22 minutes for ADM[25,26] and 2 to 5 minutes for ANP,[27] the measurement of these 2 peptides represents a technical challenge. Recently, we have developed reliable assay systems to detect precursor fragments of these bioactive peptides in plasma that exhibit prolonged half-lives. These assays measure midregional proadrenomedullin (MR-proADM)[28] and midregional proatrial natriuretic peptide (MR-proANP)[29] as functionally inactive surrogates of the active substances.

We have recently shown that blood-based markers of microcirculation are altered in AD patients compared to controls.[30] In the present study, we investigated whether the plasma-based measurement of MR-proADM and MR-proANP concentrations contributes to the prediction of progression from predementia MCI to clinical AD. We studied 134 MCI patients, who were followed-up for 4 to 6 years. Concentrations of plasma markers were compared between MCI groups showing different clinical outcomes (stable MCI versus MCI converted to AD versus MCI converted to other forms of dementia).

METHOD

Patients

In this retrospective analysis, 137 MCI patients were included who were originally prospectively recruited in the memory disorder clinic at Malmö University Hospital, Malmö, Sweden, between July 1998 and June 2001 for the CSF biomarker study as reported by Hansson and colleagues.[31] The original study also included 39 healthy controls who had been cognitively stable for 3 years; however, this article focuses on the MCI patients only. Data of the established CSF markers Aβ42 and tau (total tau and tau protein phosphorylated at threonin 181 [p-tau181]), as given in that publication, were available to us for correlation purposes. Three patients were excluded, as they died before 4 years of follow-up. The patients were aged between 49 and 89 years, and 56% were women; 75% of patients were referred to the clinic by family practitioners. All patients underwent general physical, neurologic, and psychiatric examination performed by experienced physicians who specialized in cognitive disorders, as well as routine blood tests (C-reactive protein, hemoglobin, leukocytes, trombocytes; sodium, potassium, glycosylated hemoglobin, creatinine, albumin, thyroid-stimulating hormone, and homocysteine), analysis of apolipoprotein E (APOE) genotype; blood pressure; a computed tomography scan of the brain; and cognitive tests, including the Mini-Mental State Examination (MMSE),[32] the Alzheimer's Disease Assessment Scale-cognitive subscale,[33] and the Clock Drawing Test.[34] Mild cognitive impairment was diagnosed according to the criteria of Petersen and colleagues.[35,36] In terms of their cognitive profiles, patients exhibited what we now call the amnestic subtype of MCI.[37] No patients fulfilled *DSM-III-R*[38] criteria for dementia. The patients were allowed to exhibit white matter changes or silent brain infarcts, because these changes are common in elderly people with and without cognitive deficits.[39] Patients with low plasma concentrations of vitamin B12 or folate were treated at baseline; patients with other causes of cognitive impairment, like brain tumor, subdural hematoma, central nervous system infection, or current alcohol abuse, were excluded from the study.

Patients and controls were followed up by Hansson and colleagues until they developed dementia or until they had been cognitively stable for more than 4 years. Fifty-six (41.8%) remained cognitively stable (MCI-stable), 57 (42.5%) converted to probable AD (MCI-AD) according to National Institute of Neurological Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria, and 21 (15.7%) converted to other forms of dementia (MCI-other). The study sample was described earlier in detail by Hansson et al.[31]

Procedures

Blood was taken at the Malmö University Hospital, Malmö, Sweden, between 8 AM and noon; patients were not fasting. Blood was collected in tubes containing EDTA as anticoagulant and centrifuged at 2.000 × g for 10 minutes at +4°C. The supernatant was pipetted off, and samples were frozen at −80°C and sent on dry ice to Germany (BRAHMS AG, Hennigsdorf, Germany) for further analyses.

Analysis of Biomarkers

Midregional proadrenomedullin was detected using a novel commercial assay in the chemiluminescence/coated tube-format (MR-proADM luminescence immunoassay, BRAHMS AG) as described.[28] The immunoassay was performed by incubating 10 µL of samples/standards and 200 µL tracer in coated tubes for 2 hours at room temperature. Tubes were washed 4 times with 1 mL of LIA wash solution (BRAHMS AG), and bound chemiluminescence

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was measured using a LB952T luminometer (Berthold Technologies, Bad Wildbad, Germany). Median concentration of MR-proADM in healthy individuals (n = 264) was 0.33 nmol/L (range, 0.1–0.64 nmol/L). The analytic assay sensitivity was 0.08 nmol/L, and the interlaboratory coefficient of variation was < 20% for values > 0.12 nmol/L.[28]

Midregional proatrial natriuretic peptide was assessed using a novel sandwich immunoassay (MR-proANP LIA; BRAHMS AG) as described elsewhere.[29] As a modification of the published assay, control and patient samples (1:40 dilution of 5 µL EDTA-plasma in incubation buffer) or standards were added to antibody-coated tubes and incubated for 30 minutes at room temperature. After 5 washings with 1 mL washing buffer, 200 µL tracer was added, followed by 30 minutes incubation at room temperature. Tubes were washed 3 times with 1 mL washing solution, and bound chemiluminescence was measured using a LB952T luminometer (Berthold Technologies). The functional assay sensitivity (interassay coefficient of variance < 20%) was 20 pmol/L, median MR-proANP in 325 healthy individuals in previous investigations was 45 pmol/L (95% CI, 43–49 pmol/L).[29]

Statistical Analysis

Due to their log-normal distribution, biomarker measurements were log-transformed for statistical analysis (base 10). Median and interquartile ranges (IQRs) were used to describe continuous variables. Demographic characteristics and clinical and biomarker data were compared between groups using the nonparametric Kruskal-Wallis 1-way analysis of variance by ranks test, followed, if significant, by Tukey-type post hoc tests between any 2 groups.[40] Categorical variables were tested using the Pearson χ^2 test for classification tables. The Spearman correlation coefficient was used for comparing continuous variables. The clinical milestone and primary end point, time to conversion to AD, was analyzed using Cox proportional hazard models. Conversions to other forms of dementia were treated as censoring events. Hazard ratios for continuous variables are reported as standardized hazard ratios, ie, the hazard ratio per IQR increase.[41] Kaplan-Meier plots were used to illustrate the association between biomarkers and progression to AD. Area under the curve (AUC) values at 6 years were determined from censored survival data using the nearest neighbor estimation method.[42]

RESULTS

Group Characteristics

A total of 134 MCI patients were followed-up for 4 to 6 years. Group characteristics are presented in Table 1. The group of MCI-stable patients was significantly younger compared to the MCI-AD and MCI-other groups ($P < .0001$). The MCI-AD group comprised significantly more APOE ε4 allele carriers than the MCI-stable and MCI-other groups ($P = .001$).

Table 1. Demographic and Clinical Characteristics and Concentration of Parameters in MCI-Stable, MCI-AD, and MCI-Other Groups

Characteristic	MCI-Stable (n = 56)	MCI-AD (n = 57)	MCI-Other (n = 21)
Age, y[a]			
Mean ± SD	64.3 ± 9.0	74.1 ± 5.9	73.1 ± 9.4
Range	49–81	59–85	54–89
Sex, male, n (%)[b]	30 (54)	16 (28)	13 (62)
APOE ε4 carrier status, n (%)[c]	28 (50)	43 (75)	6 (29)
MMSE score			
Mean ± SD	27.3 ± 1.8	26.8 ± 1.4	26.8 ± 1.5
Range	24–30	24–30	25–30
MR-proANP, pmol/L[d]			
Median	73.4	113.0	118.0
Interquartile range	58.9–102.0	86.9–137.0	68.9–148.0
MR-proADM, nmol/L[d]			
Median	0.715	0.828	0.926
Interquartile range	0.567–0.833	0.703–0.944	0.825–1.060

[a]Significant differences in age distribution between the MCI-stable group and the MCI-AD and MCI-other groups ($P < .0001$).
[b]The MCI-AD group comprised more female patients than the MCI-stable and MCI-other groups ($P = .006$).
[c]The MCI-AD group comprised more APOE ε4 allele carriers compared to the MCI-stable and MCI-other groups ($P = .001$).
[d]The MCI-AD and MCI-other groups had significantly higher concentrations of MR-proANP and MR-proADM compared to the MCI-stable group ($P = .0001$).
Abbreviations: AD = Alzheimer's disease, APOE = apolipoprotein E, MCI = mild cognitive impairment, MMSE = Mini-Mental State Examination, MR-proADM = midregional proadrenomedullin, MR-proANP = midregional proatrial natriuretic peptide.

The MCI-AD group comprised more female patients than the MCI-stable and MCI-other groups ($P = .006$). The groups did not differ in MMSE baseline scores ($P = .219$).

Association Between MR-ProANP, MR-ProADM, and Covariates and Cardiovascular Risk Factors

The values of MR-proANP and MR-proADM correlated with age (Spearman correlation $r = 0.6$ and $r = 0.57$, respectively; $P < .001$, 95% CI, 0.47–0.69 and 0.47–0.67, respectively) but not with sex, APOE ε4 status, or MMSE baseline scores (data not shown).

There was no correlation between MR-proADM and MR-proANP and blood pressure (systolic/diastolic: ANP $r < 0.1/r < 0.1$; ADM $r < 0.1/r < 0.2$).

On the basis of our data set, we cannot exclude a correlation between history of hypertension (given as "medicated hypertension") and MR-proANP ($P = .09$); MR-proADM was correlated ($P = .02$, median = 0.83 in patients with versus 0.77 in patients without treatment of hypertension).

Only 6 patients had diabetes mellitus. Therefore we could not calculate the correlation between diabetes mellitus and the peptides studied here.

Correlation Between MR-ProANP, MR-ProADM, and Established CSF Markers

We found no correlation between established biologic markers in the CSF (Aβ42, total tau, p-tau181) and MR-proANP ($r = -0.18$, 0.32, and 0.22, respectively) or MR-proADM ($r = -0.12$, 0.13, and 0.02, respectively).

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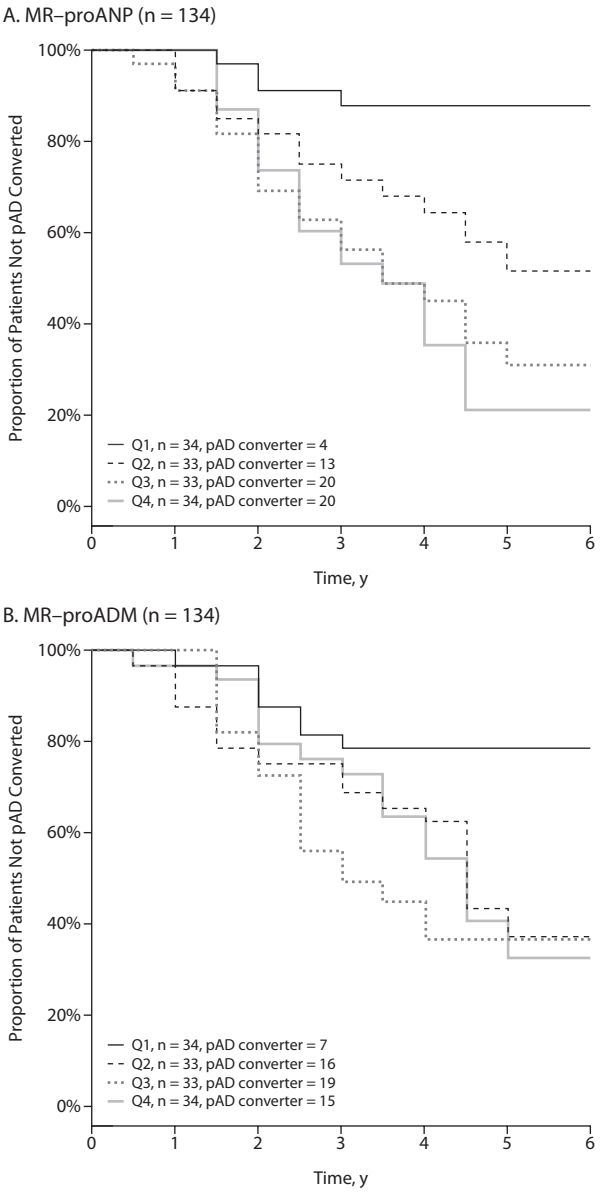
Table 2. Association Between MR-proANP, MR-proADM, and Progression to AD: Results of a Univariate Cox Regression (unadjusted),[a] Standardized HRs, and AUCs at 6 Years

Variable	Model χ^2_1	LR *P* value	HR (95% CI)[b]	$AUC_{72\ months}$[c]
Age	33.67	< .00001	4.10 (2.43–6.93)	0.79
Sex	9.34	.00224	2.36 (1.32–4.21)	0.63
Systolic blood pressure	0.02	.88784	0.98 (0.68–1.39)	0.61
Diastolic blood pressure	7.10	.0077	0.65 (0.47–0.90)	0.67
APOE ε4 carrier	13.11	.00029	2.85 (1.55–5.23)	0.65
MMSE at baseline	2.82	.09313	0.77 (0.56–1.05)	0.57
MR-proADM	5.82	.01586	1.52 (1.08–2.13)	0.71
MR-proANP	11.97	.00054	1.81 (1.30–2.53)	0.77

[a]Conversion to other forms of dementia is treated as censoring event; MR-proANP, MR-proADM $\log_{10}$ transformed.
[b]Hazard ratios (HRs) are standardized to a change of one interquartile range.
[c]AUC values at 6 years are determined from censored survival data using the nearest neighbor estimation method as described by Heagerty et al.[42]
Abbreviations: AD = Alzheimer's disease, APOE = apolipoprotein E, AUC = area under the curve, LR = likelihood ratio, MMSE = Mini-Mental State Examination, MR-proADM = midregional proadrenomedullin, MR-proANP = midregional proatrial natriuretic peptide.

Figure 1. Associations Between (A) MR-proANP and Progression to AD and (B) MR-proADM and Progression to AD: Kaplan-Meier Plots by Quartiles[a]



[a]For quartile cutoffs, see Table 1.
Abbreviations: AD = Alzheimer's disease, MR-proADM = midregional proadrenomedullin, MR-proANP = midregional proatrial natriuretic peptide, pAD = probable Alzheimer's disease, Q = quartile.

Association Between MR-ProANP, MR-ProADM and Progression to AD (univariate analysis)

Patients with MCI who converted to AD and other forms of dementia had significantly higher concentrations of MR-proANP and MR-proADM compared to the MCI-stable group ($\chi^2 = 21.1$, $P < .0001$ and $\chi^2 = 18$, $P = .0001$ [Kruskal-Wallis], post hoc $P < .05$ for all comparisons with MCI-stable group).

The results of the univariate Cox regression are shown in Table 2. MR-proANP (hazard ratio = 1.8; 95% CI, 1.3–2.5; $P = .00054$) and MR-proADM (hazard ratio = 1.5; 95% CI, 1.1–2.1; $P = .0159$) are predictors for progression to AD. In the Kaplan-Meier analysis (Figure 1), the higher values of MR-proANP and MR-proADM were associated with progression to AD.

In a time-dependent receiver operating characteristic analysis, which took into account censoring, time to conversion, and available follow up time, the $AUC_{72\ months}$ was 0.77 and 0.71 for MR-proANP and MR-proADM, respectively. At a cutoff of 87 pmol/L, MR-proANP yielded a sensitivity of 74.7% and specificity of 63.1%. Sensitivity and specificity values for MR-proADM at a cutoff of 0.72 nmol/L were 76.3% and 47.3%, respectively. Both cutoffs were previously identified in an independent sample,[30] separating healthy controls from patients with AD.

Association Between MR-ProANP, MR-ProADM, and Progression to AD (multivariate analysis)

In the multivariate Cox regression model including age, sex, systolic and diastolic blood pressure, as well as APOE ε4 carrier status (base model), the greatest influence on the progression to AD was age (likelihood ratio $\chi^2 = 19.8$; hazard ratio = 3.5; 95% CI, 2.01–6.07; $P < .0001$). Introduction of MR-proANP value and its interaction term with age added predictive value to the base model (likelihood ratio $\chi^2_2 = 6.6$, $P = .037$). Introduction of MR-proADM value and its interaction term with age also added predictive value to the base model (likelihood ratio $\chi^2_2 = 7.7$, $P = .021$). To illustrate the biomarker-by-age interaction, the estimated standardized hazard ratio for MR-proANP-by-age is plotted in Figure 2. While low (high) values of the biomarkers are associated with a low (high) risk for progression in patients below the age of 72 years (Figure 3A), the risk to progression is independent of MR-proANP concentration in patients older than 72 years

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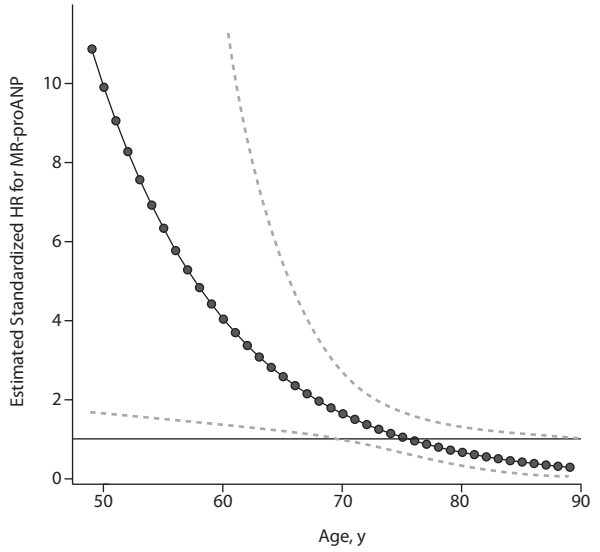
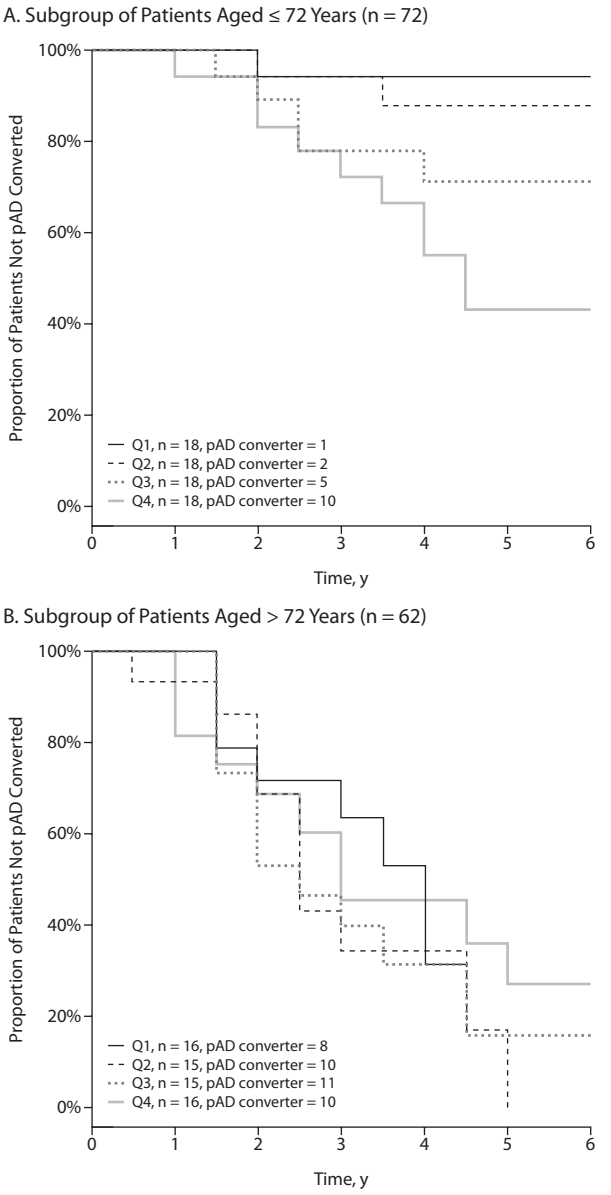
Figure 2. Interaction Between Age and MR-proANP: Estimated Standardized Hazard Ratio (HR) for MR-proANP by Age[a]



[a]Solid line indicates estimated standardized HR for MR-proANP by age. Dotted lines show 95% CI.
Abbreviation: MR-proANP = midregional proatrial natriuretic peptide.

Figure 3. Interaction Between Age and MR-proANP: Kaplan-Meier Plots of MR-proANP by Quartiles



Abbreviations: HR = hazard ratio, MR-proANP = midregional proatrial natriuretic peptide, pAD = probable Alzheimer's disease, Q = quartile.

(Figure 3B). For example, the standardized hazard ratio for MR-proANP for a patient of age 60 is 4.4 (95% CI, 1.5–13.3). The same is true for MR-proADM (data not shown).

In a time-dependent receiver operating characteristic analysis for patients younger than 72 years, the $AUC_{72\ months}$ was 0.82 and 0.75 for MR-proANP and MR-proADM, respectively (compared to 0.77 and 0.72, respectively, in the full population).

DISCUSSION

To our knowledge, this is the first study of the blood-based biologic markers of microcirculation—MR-proADM and MR-proANP—in patients with MCI. We investigated whether the measurement of MR-proADM and MR-proANP concentrations contribute to the prediction of MCI progression to clinically diagnosed AD as a most relevant milestone in the course of the disease. The main finding of the present study is that concentrations of MR-proANP and MR-proADM have predictive value in MCI progression to AD in patients younger than 72 years.

In light of the high prevalence of AD in the elderly and upcoming potentially disease-modifying treatments, specific biologic markers reflecting molecular mechanisms and neuropathological characteristics of the disease are urgently needed, particularly for key clinical functions such as early detection and differential diagnosis (classification). CSF biologic marker candidates of neurodegeneration, such as Aβ42, and total and phosphorylated tau-protein have been shown to be helpful diagnostic measures validated against clinical AD patients and have promise for prediction and prognosis of conversion from predementia MCI to syndromal dementia.[8,31,43] Blood-based biologic candidate markers of AD are currently under intense investigation, since they provide a more suitable diagnostic means than biomarkers derived from CSF. As there is growing evidence that vascular risk factors and cerebrovascular events may play a major role in AD and interact with neurodegenerative processes,[12,44] we investigated the blood-based biologic markers of microcirculation in MCI patients. We had recently shown that they are altered in patients with a clinical diagnosis of AD dementia compared to healthy elderly controls.[30]

It is well known that elderly persons with MCI are at higher risk of development of AD and other types of dementia than are those without MCI.[36] In the present study, we

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investigated the potential predictive value of MR-proADM and MR-proANP concentration in MCI conversion to AD. A total of 134 MCI patients were followed up for 4 to 6 years. In the survival analysis, we found that higher values of MR-proANP and MR-proADM were associated with progression to AD.

Concentrations of MR-proANP and MR-proADM were correlated with age. Therefore, we performed a multivariate Cox regression analysis. In the base model we included age, sex, systolic and diastolic blood pressure, and APOE ε4 carrier status. Age had the greatest influence on the progression to AD. The addition of MR-proANP and MR-proADM concentrations increased the predictive value of the base model, if interaction with age was accounted for and therefore was not merely an effect of age. MR-proANP and MR-proADM concentrations predicted the MCI progression to AD in persons below the age of 72 years. In patients older than 72 years, however, the measurement of MR-proANP and MR-proADM concentrations did not improve prediction of MCI conversion to AD. Therefore, we recommend further investigation of MR-proANP and MR-proADM concentrations for specific prognostic use in the population of MCI-patients below 72 years.

It is well known that the prevalence of heart failure and other vascular disorders is age-dependent. The concentrations of MR-proANP and MR-proADM increase in patients with heart disorders. Although the patients in the study population did not have clinical signs of manifest heart failure, it can be assumed that, in patients older than 72 years, subclinical heart failure or other unknown vascular comorbidity dilutes the biomarker signal. Further studies with different comparison groups (eg, healthy older controls, older patients without cognitive problems but with cardiological disorders, older patients with cognitive decline but cardiologically intact) should be tested in order to confirm our findings.

According to the expert consensus report criteria,[45] an "ideal" biomarker of AD should reach sensitivity of at least 85% and specificity to differentiate AD from age-matched controls and other dementias of at least 75%. In our study, the sensitivity and specificity for MR-proANP and MR-proADM in prediction of MCI conversion to AD did not reach these thresholds. It was recently shown that core biomarker candidates of neurodegeneration amyloid β (Aβ)40 and Aβ42,[46] as well as their ratio Aβ42/Aβ40 in plasma could not successfully predict the conversion of MCI patients to clinical AD. This failure is probably due to a lack of correlation between the concentration of Aβ42 in CSF and plasma.[47] Sensitivity and specificity figures obtained in our study, however, come close to figures shown for CSF biologic markers and are therefore promising as blood-based candidate biologic markers for AD that merit further investigation.

Sensitivity was particularly high, which may recommend these blood-based biomarkers as phase 1 screening tests to select predementia patients at increased risk of AD for enrichment in clinical trials and for further phase 2 diagnostic workup, ie, expensive and/or invasive procedures with high specificity, like amyloid–positron emission tomography and CSF investigation.

Our results support the hypothesis of impaired microcirculation in AD even in the predementia stage of MCI. Microvascular dysfunction and neurodegenerative process are tightly related in the pathogenesis of AD. The Aβ peptide is found in senile plaques and can also be detected in human plasma. The Aβ peptide leads to cerebral angiopathy, which is associated with increased risk of stroke. Furthermore, several experimental studies demonstrated that the Aβ peptide may induce endothelial dysfunction of both cerebral and systemic vessels.[48,49] The strength of our study is the hypothesis-driven investigation of MR-proANP and MR-proADM for prediction of MCI conversion to AD on the basis of the assumption of impaired microcirculation in AD. MR-proANP and MR-proADM should pass through a validation process in multicenter studies like the Alzheimer's Disease Neuroimaging Initiative studies of neurodegeneration biomarkers being conducted in the United States and Europe.

The question remains, however, whether levels of the peptides studied here simply reflect AD pathology or are a reflection of another potentially modifiable disease process that greatly increases risk of AD. We found no correlation between blood pressure and MR-proANP and MR-proADM concentrations, respectively. As to history of hypertension, we cannot exclude a correlation with MR-proANP, and we see a correlation with MR-proADM.

These findings are in line with results showing normal or even low blood pressure in AD but an elevated risk for AD for persons with high blood pressure, ie, a history of hypertension, in midlife.[50] Furthermore, medicated hypertension is an independent risk factor for cognitive decline in elderly individuals.[51] For our patients, we do not have the data when hypertension was diagnosed and treated. However, this finding points in the direction of an underlying (potentially modifiable) vascular pathology contributing to the development of AD. Therefore, the lack of a correlation with established CSF markers (Aβ42, total tau, and p-tau181 proteins) could be expected, since we consider the alteration of MR-proANP and MR-proADM to reflect disease processes different from those that are considered part of "pure" AD pathology.

Moreover, our results of changes of MR-proADM and MR-proANP in MCI and a correlation to progression to AD indicate potentially modifiable processes in the MCI stage of AD that have to be followed in future studies.

Another approach in biomarker research is exploratory proteome-based analyses that have yielded promising results in recent studies.[52] The overall expression pattern, however, could not be replicated as a biomarker to differentiate MCI from AD and depression.[53] The strength of our monocenter study is the hypothesis-driven approach, whereas the relevance of exploratory studies with respect to AD is uncertain.

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The population studied here and conversion rate to AD is well comparable to other published studies.[54] Approximately 30%–50% of patients with MCI in memory clinics develop AD within a 4- to 6-year period.[55] The study population was recruited consecutively, which reduced the risk of ascertainment and participation bias.[56–58]

In the future, further studies investigating the correlations between blood-based microcirculation markers and neuroimaging perfusion parameters in the brain could help to support our findings and to further highlight the contribution of microvascular dysfunction to the pathogenesis of AD.

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