Fibrinolysis and von Willebrand factor in Alzheimer's disease and vascular dementia – a case-referent study

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Introduction: The importance of vascular risk factors for Alzheimer's disease (AD) is not settled. Our aim was to compare patients with AD or vascular dementia (VaD) with non-demented subjects with regard to endothelial derived fibrinolytic and hemostatic factors.

Materials and methods: In a cross-sectional mono-center case-referent study in Örebro, Sweden, we consecutively included 95 patients with AD and 55 with VaD and 154 non-demented active seniors (AS). Plasma biomarkers including the endothelial derived fibrinolytic factors: mass concentrations of tissue plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1), tPA/PAI-1 complex and von Willebrand factor (vWF), as well as clinical data were analyzed.

Results: None of the endothelial derived fibrinolytic markers or vWF differed between AD vs. VaD. In comparison with

the AS group, tPA was higher in AD ($p=0.001$) and VaD ($p=0.023$) but its inhibitor, PAI-1 mass concentration did not differ significantly; tPA/PAI-1 complex was higher in both VaD ($p=0.038$) and AD ($p=0.005$). vWF concentration was lower in the AS group ($p<0.001$) than in both dementia groups.

Conclusion: Thus, endothelial derived fibrinolytic factors, tPA/PAI-1 complex and vWF, discriminated between the reference group of non-demented elderly and the AD and VaD groups, but not between AD and VaD. This suggests similar disturbances for endothelial derived fibrinolytic and hemostatic factors among AD and VaD patients and may reflect shared vascular pathophysiological mechanisms in the dementias.

Keywords: vascular dementia, Alzheimer's disease, fibrinolysis, hemostasis, von Willebrand factor

Introduction

Hemostasis has been proposed as a possible pathogenetic factor in dementia, especially vascular dementia [1-3]. To our knowledge there is only one study by Mari et al [1] in which endothelial derived fibrinolytic markers and vWF has been evaluated in AD vs. VaD patients.

In the fibrinolytic system plasmin is regulated by tissue plasminogen activator (tPA), urokinase-type plasminogen activator, and plasminogen activator inhibitor-1 (PAI-1). In the presence of fibrin, tPA converts the proenzyme plasminogen into active plasmin within the thrombus. PAI-1 regulates plasminogen activation by inhibiting free tPA and forming an enzymatically inactive tPA/PAI-1 complex, which results in a loss of plasminogen activation potential and thereby a decreased level of proteolytic and fibrinolytic activity. A common finding in patients with myocardial infarction is an impaired function in the fibrinolytic system, mainly due to increased plasma levels of mass concentration of tPA and PAI-1 [4] which are considered as primary risk factors of cardiovascular disease [5].

vWF is an essential factor in the primary hemostasis. Increased level of vWF was found to be a predictor of cardiovascular events in survivors of myocardial infarction, suggesting its relation to atherosclerosis and/or increased risk of thromboembolic complications [6].

Disturbances of fibrinolysis and vWF may reflect endothelial dysfunction and or damage, an important feature of

atherosclerotic pathophysiology. Increased mass concentrations of tissue plasminogen activator (tPA) in plasma is an example of such a disturbance which predisposes to both a first myocardial infarction [5] and to reinfarction [7-10].

The metabolic syndrome, also characterized by an atherosclerotic tendency, is associated with increased plasma levels of the fibrinolytic key components PAI-1 and tPA, as are various inflammatory conditions [11, 12]. Fibrinolytic markers and vWF are thus closely related to atherosclerotic disease while there is very sparse data for the association between fibrinolytic markers and vWF and AD.

We here bring new data as we also compared tPA and tPA/PAI-1 complex in the two great dementia diagnose groups, AD and VaD, and also compared with a cognitively intact and healthy reference group in the same age range. Further we adjusted for, in addition to age, parameters known to influence the fibrinolytic and hemostatic system, e. g. BMI.

Our hypothesis was that the referent group and AD patients could have lower concentrations of endothelial derived hemostatic factors and vWF compared with VaD. Our primary aim was therefore to explore the levels of endothelial derived hemostatic factors and vWF in a cross-sectional mono-center study in consecutive patients with either VaD or AD diagnosed according to uniform criteria. Secondly we investigated if patients with VaD or AD differed from a reference group of non-demented elderly in these variables.

Materials and Methods

Study population

The present study comprises two different groups. **A:** Patients at Örebro University Hospital, Örebro, Sweden, the Dementia, Genetic and Milieu (DGM) study group. This study population consists of 300 consecutive patients (157 women and 143 men), see Figure 1. They were all referred to the Memory Care Unit at the Department of Geriatrics for diagnostic assessment and treatment of suspected cognitive problems. The cognitive problems had to be mild or at worst moderate, defined as MMSE score ≥ 10 . At the time of inclusion no one of the subjects were living in sheltered housing. All subjects were examined according to a structured protocol including thorough clinical investigation (medical and family history, socio-economic data, physical as well as neurological and psychiatric examination and CT scan). Ongoing pharmacological use was registered according to the Anatomical Therapeutic Chemical (ATC) Classification System. For differential diagnostic purposes lumbar puncture were accomplished in 243 subjects at L3/L4 or L4/L5 level. Analyses of the Csf biomarkers for AD: total tau protein, phosphorylated tau protein and β -amyloid protein₁₋₄₂ were performed according to our clinical routine at the Department of Psychiatry and Neurochemistry, Institute of Clinical Neuroscience, Sahlgrenska University Hospital, Mölndal, Sweden. Fasting blood

and Csf samples were taken on subjects in sitting position after a minimum of 15 minutes rest in order to diminish effects of acute physical activity on the biomarkers, and always between 08 and 09 AM in order to reduce the effect of circadian rhythm variation (see below).

ICD-10 [13] criteria was used to divide dementia patients into different diagnostic categories. Probable AD was also diagnosed in accordance with the NINCDS-ADRDA criteria [14]. Csf biomarkers for AD (i.e. elevated concentration of Csf-total-tau and Csf-phospho-tau and low concentration of Csf- β -amyloid₁₋₄₂) were used to sharpen the differential diagnostic process.

Probable VaD was diagnosed in accordance with ICD-10 criteria (F01) and the NINDS-AIREN criteria [15]. In addition, in the VaD group there were no Csf signs of AD and albumin index [16] was significantly higher in the VaD group compared with AD group. There were no post-mortem data.

B: Subjects in the referent group were recruited by a multi-phase sampling procedure aimed at an elderly population, living in various communities in central Sweden. The locations for the recruitment were selected to represent a broad range of socioeconomic levels and included rural as well as urban and suburban areas. The sample consisted of 154 senior citizens (100 female and 54 men with mean age 73.4 years) taking part in various study circles. Being retired (usual age ≥ 65 yr in Sweden) and living independently in their

own homes in addition to the ability to participate in the study circles, were the sole inclusion criteria, not preset health criteria. They were all assessed with Mini Mental State Examination, MMSE [17] and Clock Drawing Test, CDT [18, 19]. According to these tests they were all cognitively intact with MMSE ≥ 28 and CDT ≥ 4 . Since the subjects were drawn from an accessible population and do not represent a random population sample of elderly Swedes, we designated this referent group as ‘Active Seniors’ (AS-study group). Blood samples were taken on subjects in sitting position after a minimum of 15 minutes rest in order to diminish effects of acute physical activity on the biomarkers. There are no medical history data and no physical examination data besides blood pressure and blood sample analyses. Our study thus comprised three groups: AS ($n=154$), AD ($n=95$) and VaD ($n= 55$). The reason for this categorization is to explore the train of thought in the literature that alteration in fibrinolysis could promote neurodegeneration through a vascular mechanism.

Figure 1. Design of Dementia, Genetic and Milieu Study in Örebro, the DGM study.

Biochemical assays

Venous samples were drawn with a minimum of stasis in siliconized evacuated Stabilyte™ tubes (Biopool®, Umeå, Sweden) as earlier described [20]. The blood samples from both cohorts were stored in a biobank freezer at -70°C . All plasma samples were thawed and analyzed simultaneously at the same occasion. The tPA-PAI-1 complex, tPA, PAI-1 and vWF concentrations in plasma were determined using enzyme-linked immunosorbent assays [21]. The reagent kits for the tPA/PAI-1 complex, the tPA and the PAI-1 assays were purchased from (TintElize

tPA/PAI-1 complex) from Biopool® AB (Umeå, Sweden). vWF was measured with an ELISA [22] using reagents purchased from DAKO (Copenhagen Denmark). High-sensitivity CRP (hs-CRP) was determined with an automated chemiluminiscent immunoassay, using an IMMULITE® analyzer from DPC (Los Angeles, CA). All samples were thawed and analyzed at the same time.

All coefficients of variation were under 7.5%. Serum samples were analysed on a Hitachi 911 multianalyser (Roche, Mannheim, FRG): Total cholesterol, triglycerides, and creatinine (Crea plus, enzymatic method) were analysed using kits from Roche/Boehringer (Mannheim, FRG). Albumin was analysed with immunoturbidimetry with reagents from DAKO, Copenhagen, Denmark. HDL and LDL cholesterol were measured by direct, homogeneous assays based on detergent treatment of the serum or plasma (N-geneous™ HDL-c and N-geneous™ LDL reagents, respectively, from Genzyme Corporation, Cambridge, MA, USA).

Statistical analyses

Statistical analyses were performed using SPSS for Windows, version 15.0 (Chicago, Illinois, USA). The results are presented as means $\pm$ SD and for the ANCOVA analyses as mean $\pm$ SEM. Statistical significance were considered with a probability value <0.05 . ANOVA with post hoc analyses (Tukey) were used in group comparisons with continuous variables in Table 1. In group comparisons

with categorical variables, ² tests were used. Relations between the hemostatic variables and selected cardiovascular risk factors (see Table 1) were assessed by Pearson's correlations, and variables showing a bivariate correlation with significance levels of $p<0.10$ were entered as independent variables into four separate ANCOVA analyses, with each of the four hemostatic variables as outcome variables. The diagnostic group variable was used as a fixed factor in these models. Post hoc analyses according to Bonferroni were used in the ANOVA and also in the ANCOVA analyses. The following independent variables were used in the multivariate model: age, BMI, cystatin C, HDL-cholesterol, hs-CRP, sex, systolic blood pressure in sitting position. The calculations were re-done after adding statins, Angiotensin Converting Enzyme (ACE)-inhibitors, and Angiotensin II Receptor Blocker (ARB) to the model.

Variables that did not follow the normal distribution according to Kolmogorov-Smirnovs test were logarithmically transformed (BMI, systolic blood pressure, hs-CRP, cystatin-C, HDL-cholesterol, LDL-cholesterol and HbA1c, tPA, PAI-1, tPA/PAI-1 complex and vWF). Due to missing data points, the number of subjects in the multivariate calculations is lower than in the univariate calculations. Numbers are given in the tables.

Ethics

The studies were performed in accordance with the Helsinki declaration and the

Ethical Committee of the Örebro County Council approved the study. Permission to store personal data on files and in digital form was obtained from the Swedish Data Inspection Board. All subjects gave a specific informed consent to participate in the study, including genotyping and biobanking of the donated samples, and signed an informed consent form.

Results

We studied 304 subjects of whom 95 were diagnosed as AD and 55 as VaD and 154 subjects comprised the referent group of AS. The basic characteristics of the subjects are shown in Table 1. The group with VaD was significantly older than the AD and the AS group and had significantly higher plasma concentrations of cystatin C and high sensitive C-reactive protein (hs-CRP), and diastolic blood pressure was also significantly higher in the VaD group. Their HDL-cholesterol levels were significantly lower than in the reference group. LDL-cholesterol concentration was significantly higher in the AD group than in the AS group. When comparing VaD vs. AD there were no significant differences with regard to the endothelial derived fibrinolytic variables or vWF. Table 2 shows the fibrinolytic variables. Values adjusted for age, sex, hs-CRP, cystatin C, HDL cholesterol, systolic blood pressure and HbA1c are shown (ANCOVA statistics with Bonferroni post hoc analyses). Adding statins, Angiotensin Converting Enzyme (ACE)-inhibitors, and Angiotensin II Receptor Blocker (ARB) to the model did not change the differences shown.

When comparing AS vs. AD we found that tPA mass concentration ($p=0.001$), tPA/PAI-1 complex ($p=0.005$) and vWF ($p<0.001$) differed significantly.

AS compared with VaD showed the following significant differences: tPA mass concentration ($p=0.023$), tPA/PAI-1 complex ($p=0.038$) and vWF ($p<0.001$).

Discussion

Our main finding was that AD patients did not differ significantly from VaD patients with regard to hemostatic and fibrinolytic factors. When compared to non-demented active seniors tPA mass concentration, tPA/PAI-1 complex and vWF were significantly higher in AD compared to AS. Both tPA mass concentration, tPA/PAI-1 complex, and vWF were significantly higher in VaD patients than in the AS group. These results did not change when also adjusting for the pharmaceutical groups: statins, angiotensin converting enzyme inhibitors and angiotensin II (A II) receptor blockers.

PAI-1 concentrations and fibrinolytic activity are strongly influenced by anthropometric and life style factors such as BMI and glucose metabolism including the metabolic syndrome and diabetes mellitus type 2 [23], but not diabetes mellitus type 1 [24]. The dementia state usually implies a weight loss [25] and this could be the explanation why PAI-1 did not differ significantly between the reference group and the dementia groups, neither in the study by Mari *et al* [1], nor in our study (Table 2). The current study

implicated that endothelial fibrinolytic and hemostatic factors may share similar pathophysiological pathways for AD and VaD patients.

In accordance with epidemiological papers concerning other vascular risk factors and diseases associated with dementia we also found similarities between VaD and AD.

The review by Skoog et al 1999 [26] documented several vascular risk factors and diseases e.g. hypertension (both systolic and diastolic), atrial fibrillation, diabetes mellitus, cholesterol and homocysteine as risk factors for late-onset

Basic characteristics of the study population. ANOVA statistics.

Mean ($\pm$ SD)	AS <i>n</i> =154	AD <i>n</i> = 95	VaD <i>n</i> =54	<i>P</i> *
Age. yr	73.0 ($\pm$ 5.2)	74.9 ($\pm$ 8.8)	76.4 ($\pm$ 7.4)	0.004 <i>AS vs. VaD: p=0.005</i>
Sex (F/M) %	65/35	53/47	35/65	<0.001 ¥
BMI (kg·m ⁻²)	26.5 ($\pm$ 3.9)	25.1 ($\pm$ 3.3)	26.5 ($\pm$ 3.5)	0.008 <i>AS vs. AD: p= 0.009</i>
Systolic BP (sitting) (mm Hg)	151.1 ($\pm$ 27.0)	150.1 ($\pm$ 23.6)	151.2 ($\pm$ 21.3)	0.948 <i>ns</i>
Diastolic BP (sitting) (mm Hg)	77.0 ($\pm$ 11.5)	81.8 ($\pm$ 11.6)	84.2 ($\pm$ 11.6)	<0.001 <i>AS vs. AD: p=0.005</i> <i>AS vs. VaD p< 0.001</i>
hs-CRP (mg/L)	2.52 ($\pm$ 3.02)	2.13 ($\pm$ 2.62)	8.07 ($\pm$ 19.67)	<0.001 <i>AS vs. VaD: p= 0.002</i> <i>AD vs. VaD: p< 0.001</i>
Cystatin-C (mg/L)	0.93 ($\pm$ 0.26)	1.00 ($\pm$ 0.54)	1.13 ($\pm$ 0.38)	0.009 <i>AS vs. VaD: p= 0.008</i>
HDL-cholesterol (mmol/L)	1.63 ($\pm$ 0.46)	1.56 ($\pm$ 0.42)	1.42 ($\pm$ 0.38)	0.010 <i>AS vs. VaD: p=0.010</i>
LDL-cholesterol (mmol/L)	3.54 ($\pm$ 0.96)	3.92 ($\pm$ 1.03)	3.62 ($\pm$ 1.09)	0.017 <i>AS vs. AD: p=0.014</i>
B-HbA1c (%)	5.51 ($\pm$ 0.91)	4.89 ($\pm$ 0.63)	5.41 ($\pm$ 1.25)	<0.001 <i>AS vs. AD: p<0.001</i> <i>AD vs. VaD: p=0.004</i>
Statin use (Y/N)	No data	10/85	14/40	0.014 ¥
ACE-inhibitor use (Y/N)	No data	2/93	13/41	<0.001 ¥
ARB use (Y/N)	No data	3/92	5/49	0.112 ¥
Brain infarcts on CT scan (Y/N)	No data	0/95	32/22	<0.001 ¥
White matter lesions on CT scan (Y/N)	No data	33/57	22/32	<0.001 ¥
Vascular signs on CT scan (Y/N)	No data	33/57	54/0	<0.001 ¥
Albumin index	No data	6.4 ($\pm$ 1.9)	11.3 ($\pm$ 9.2)	<0.001 ¶

Table 1. ¥ = Pearson 2 test. ¶ = Student's t-test. * Variables logarithmically transformed for calculations of significance level (except diastolic BP). Significant post hoc values are given in italics. ns= not significant. Post hoc analyses according to Bonferroni, significant comparisons are shown in the table. Unadjusted data.

Fibrinolytic variables in active seniors and in dementia patients.

Mean ($\pm$ SEM)	AS <i>n</i> =109	AD <i>n</i> =78	VaD <i>n</i> =35	<i>p</i>
tPA (μ g/L)	11.6 ($\pm$ 0.4)	14.0 ($\pm$ 0.5)	14.1 ($\pm$ 0.8)	<0.001 <i>AD vs. VaD: p=1.000</i> <i>AS vs. AD: p=0.001</i> <i>AS vs. VaD: p=0.017</i>
PAI-1 (μ g/L)	26.4 ($\pm$ 1.7)	23.6 ($\pm$ 2.1)	21.6 ($\pm$ 3.1)	0.243 <i>AD vs. VaD: p=1.000</i> <i>AS vs. AD: p=0.370</i> <i>AS vs. VaD: p=0.760</i>
tPA/PAI-1 complex (μ g/L)	9.1 ($\pm$ 1.4)	13.2 ($\pm$ 1.7)	10.9 ($\pm$ 2.5)	0.001 <i>AD vs. VaD p=1.000</i> <i>AS vs. AD: p=0.002</i> <i>AS vs. VaD: p=0.029</i>
vWF (%)	195.5 ($\pm$ 10.1)	290.8 ($\pm$ 12.0)	308.7 ($\pm$ 18.0)	<0.001 <i>AD vs. VaD: p=1.000</i> <i>AS vs. AD: p<0.001</i> <i>AS vs. VaD: p<0.001</i>

Table 2. Data adjusted for age, sex, BMI, systolic blood pressure, cystatin C, hs-CRP, HDL-cholesterol and HbA1c [23, 27-29]. ANCOVA statistics.

Post hoc effects are compared according to Bonferroni, figures are given in italics.

AD, a picture shared by several recent authors [30-32]. From 1996-98 onwards, the hyperhomocysteinemic aspect of VaD (and AD) has attracted attention [33-36]

An increase in hs-CRP may probably *per se* cause increased plasma tPA concentrations [29]. In our study we have shown (Table 1) that hs-CRP is significantly lower in AD than in VaD and comparable to the AS group, indicating that low-grade systemic inflammatory activity is not prevalent in AD. This difference in the inflammatory response between patients with AD and VaD despite similar cardiovascular risk

factors may be an important pathophysiological mechanism that separates the two diseases. However, it does not explain why tPA mass concentration is increased in both VaD and AD as compared to non-demented active seniors, since the values in Table 2 were adjusted for hs-CRP.

Mari *et al.* [1] found that vWF and activated factor VII were significantly higher in VaD and also in AD compared with controls. PAI-1 and D-dimer were significantly higher in VaD than in both controls and AD. Bots *et al.* [2] performed a case-control study in Rotterdam and

found an OR 1.76 (CI: 1.00 – 3.10) for association with unspecified dementia vs. the control group when comparing the highest vs. the lowest quintile for D-dimer. In blood samples taken 3-10 days after acute stroke with further review at 1-3 months Stott *et al.* [3] found that fibrinogen as well as vWF and D-dimer were significantly higher in patients with acute cerebrovascular infarction and the same changes were found in VaD patients compared to controls.

To our knowledge there is no other study comparing our set of biomarkers, hemostatic and fibrinolytic markers in VaD, AD and a reference group of cognitively intact elderly. Our study is the largest on endothelial-derived hemostatic factors in AD and VaD so far. Other strengths are that the patients were consecutively collected and thus resemble a real, not preselected, sample of the incident dementia population and that diagnostics were made at a single center in accordance with current state-of-the-art diagnostic manuals and Csf-biomarkers. In spite of being the largest study so far, no differences of endothelial derived fibrinolytic variables or vWF between VaD and AD might of course still reflect a lack of power to determine a statistical effect, rather than inferring a common pathogenesis. A limitation in this study is that the diagnoses are not neuropathologically confirmed. Another limitation is that the groups were not matched with respect to age, sex or BMI, this was however handled with

adjustments in ANCOVA multivariate analysis.

In spite of their distinctive differences clinically and regarding common plasma biomarkers (Table 1), the VaD and AD groups did not differ significantly regarding hemostatic and fibrinolytic markers (Table 2). These well characterized groups of different dementias instead seem to share common risk factors indicating in some way similar pathophysiological mechanisms. In the Nun-study [37] it was suggested that cerebrovascular disease might play an important role in explaining presence and severity of clinical symptoms in AD. Our findings thus probably have interesting nosological repercussions, which should be further explored in the future. The vascular component in AD is important to recognize since it might open new fields for preventive and therapeutic strategies. The Alzheimer vascular pathway might benefit from additional ways of prevention and of treatment. For instance, in the SCOPE-study [38], comparing different anti-hypertensive drugs, the cognitive function was well maintained in both treatment arms. Since drugs against AD have limited efficacy today, general prevention through treatment of established arteriosclerotic risk factors could be a more successful pathway. But even such a strategy would have to be tested in prospective randomized trials.

In conclusion, we found that patients with AD and VaD differ from a cognitively intact elderly reference group with respect

to plasma levels of tPA, tPA-PAI-1 complex, and vWF. When adjusting for traditional cardiovascular risk factors the endothelial derived fibrinolytic factors and vWF still discriminated the reference group from the AD and VaD groups but did not

discriminate between AD and VaD. This invites to novel strategic thinking of shared pathophysiological mechanisms for endothelial derived fibrinolytic and hemostatic disturbances.

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