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Serum matrix metalloproteinase-8, tissue inhibitor of metalloproteinase and myeloperoxidase in ischemic stroke

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ABSTRACT

Background and aims: Matrix metalloproteinase (MMP)-8 and myeloperoxidase (MPO) may contribute to cerebral damage in acute ischemic stroke. We tested the hypothesis that levels of MPO, MMP-8 and the ratio between MMP-8 and its regulator, tissue inhibitor of metalloproteinase (TIMP-1), are increased in acute ischemic stroke and its etiologic subgroups and they correlate with stroke severity.
Methods: In a cross-sectional case–control study, serum concentrations of MMP-8, MPO and TIMP-1 were assessed within 24 h after admission in 470 first-ever ischemic stroke patients and 809 age- and sex-matched controls, randomly selected from the population. Odds ratios (OR) per decade of log transformed dependent variables were calculated and adjusted for age, sex and vascular risk factors.
Results: Levels of MMP-8 (OR 4.9; 95% CI 3.4–7.2), MMP-8/TIMP-1 ratio (3.0; 2.2–4.1) and MPO (6.6; 4.0–11.0) were independently associated with ischemic stroke. MMP-8 levels differed between etiologic stroke subgroups ($p = 0.019$, ANOVA), with higher levels in cardioembolic stroke and stroke due to large vessel disease, and lower levels in microangiopathic stroke. MMP-8, MMP-8/TIMP-1 ratio and MPO ($p < 0.001$) concentrations showed positive associations with stroke severity independent of stroke etiology.
Conclusions: Concentrations of serum neutrophil markers are increased after ischemic stroke and associate with stroke severity and etiology. The value of these biomarkers in diagnostics and prognostics is worth being evaluated.

1. Introduction

Stroke is one of the major causes of death and disability worldwide [1]. Demographic changes will lead to increasing numbers of stroke patients [2]. Optimizing stroke prevention and acute stroke care is mandatory. Systemic inflammation plays a key role in both stroke formation and immediate tissue damage post stroke [3]. Identification of specific proinflammatory biomarkers might give an opportunity to lower stroke risk and enhance acute stroke care.

We recently described higher serum concentrations of matrix metalloproteinase (MMP)-8 and myeloperoxidase (MPO) in patients with acute ischemic stroke compared to stroke-free controls [4]. MMP-8 or collagenase-2 is a catalytically competent endoprotease that can decisively process extracellular matrix components and non-matrix bioactive substrates causing tissue destruction and modulation of immunoresponses [5]. By producing hypochlorite (HOCl), MPO can not only oxidatively activate latent MMP-8 but also inactivate tissue inhibitor of matrix metalloproteinase (TIMP)-1 [6]. TIMP-1 is an important endogenous inhibitor of MMP-8 capable of binding to the active site of MMP-8 in an equimolar ratio to maintain the physiological conditions. Although some studies of serum MMP-8, MPO, and TIMP-1 in stroke patients exist, such observations are limited. In a population-based cohort, circulating MPO and TIMP-1 concentrations, but not MMP-8, were associated with incident stroke in a 13-year follow-up [7]. However, little is known on the association between these serum factors and

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stroke severity and stroke etiology [8]. Based on data from a preliminary case-control study, we tested the hypotheses that serum levels of MMP-8 along with its activator MPO and TIMP-1 inhibitor are elevated in patients with ischemic stroke (IS), and especially that they are associated with stroke severity.

2. Patients and methods

"GENESIS" is a cross-sectional case-control study comprising 470 first-ever ischemic stroke (FEIS) cases (40% women, mean age 66.5 ± 10.8 years; 60% men, age 65.5 ± 10.7 years) and 809 age- and sex-matched controls (41.8% women, 66.4 ± 11.1; 58.2% men, age 67.9 ± 9.5 years), randomly selected from the general population. This study was established within the framework of the Ludwigshafen Stroke Study (LuSST), a population-based stroke registry that started on January 1st, 2006. Detailed descriptions of the LuSSt registry and the "GENESIS" study have been published recently [9,10]. The studies were approved by the ethics committee of the Landesärztekammer Rheinland-Pfalz (837.333.05(4991)).

2.1. Inclusion and exclusion criteria

Inclusion criteria of both cases and controls covered males and females between 20 and 80 years of age, permanent residency in the study area of the LuSSt registry, Caucasian ethnicity and written informed consent to study participation. Additional inclusion criterion for cases was the diagnosis of a FEIS based on an acute neurological deficit lasting >24 h with no other cause than cerebral ischemia. All cases received a cerebral CT or MRI.

Exclusion criteria for cases were acute transient ischemic attack, intracerebral, subdural or subarachnoid hemorrhage and ischemic stroke due to cerebral trauma or brain malignancy. Exclusion criteria for both cases and controls included any previous stroke, myocardial infarction within past 90 days, dementia, severe aphasia, insufficient understanding of the German language or any other relevant communication barrier and severe disability that precluded interview participation.

2.2. Recruitment

For recruitment of controls, a random sample of Ludwigshafen residents was drawn from the population registry including name, age, sex and address. Subsamples were consecutively taken to match the age and sex distribution of cases. Those selected received invitation letters with detailed information on the study and request for their participation. The participation rate for controls was 46.6%. The cases included incident stroke cases from the LuSSt registry. According to the study protocol only in-patients at the Klinikum Ludwigshafen were asked for participation in "GENESIS". This group represents about 89% of all cases in LuSSt. The participation rate for cases was 73.7%.

2.3. Data collection and laboratory tests

Cases and controls were interviewed by trained personnel using a standardized questionnaire. We collected data on age, sex, anthropometric measures, previous diseases, frequency of previous dentist visits as markers of health behavior, number of teeth, smoking habits, alcohol intake, physical activity, dietary patterns, and medication and social history. In both, cases and controls, blood pressure was measured after 5 min of resting, a 12-lead electrocardiogram and a Duplex-sonography of brain supplying arteries were performed. Venous blood samples were collected in cases and controls, immediately frozen and stored at −70 °C until processing. In all patients, venipuncture was performed within first 24 h after hospital admission. Serum inflammation marker concentrations were determined by commercial ELISA kits according to the manufacturer's instructions. The precision of the analysis are expressed as coefficient of variation (CV) in percent. Precision for TIMP-1 (Amersham Biotrak, GE Healthcare, Buckinghamshire, UK) was 3.1%, for MPO (Immundiagnostik AG, Bensheim, Germany) 4.8%. The serum MMP-8 concentrations were determined by a time-resolved immunofluorometric assay (IFMA) as described previously, and the interassay coefficient of variation (CV)% was 7.3% [11]. For the calculation of MMP-8 / TIMP-1 molar ratios, the concentrations were converted to molarity using MWs of 65 kDa and 28 kDa as described previously [12]. Leucocyte count (XE analyserXE-2100; Sysmex) was determined shortly after admission.

2.4. Definition of variables

Cardiovascular risk factors were defined according to current national and international guidelines and have been described in detail [13]. Etiological subtypes of IS were ascertained using modified TOAST criteria (Trial of ORG 10172 in Acute Stroke Treatment) as described recently [13]. Stroke severity was measured by the National Institutes of Health Stroke Scale (NIHSS) on admission, as well as by the modified Ranking Scale (mRS) [14,15].

2.5. Statistical analysis

The Chi-squared (X^2) test was used to compare categorical data. The *t*-test was applied to analyse normally distributed continuous variables. The Box-Cox method was used to check the compliance of each laboratory parameter with the normal distribution, taking into account the group influence (case-control). This method assesses the optimal transformation for the parameters to achieve normal distribution. If necessary, parameters were log transformed prior to multivariate analysis. All biomarkers of inflammation were additionally adjusted for cardiovascular risk-factors and parameters significantly different between cases and controls by univariate analysis using conditional multiple logistic regression analysis, stratified for the matching parameters sex and age. In the multivariate logistic model it was impossible to include the quotient MMP-8/TIMP-1 molar ratio. This quotient is a direct derivative of two predictors already included in the model, and would result in collinearity. We displayed the univariate statistics just for purpose of comparability with literature. Logistic regression analysis was used to calculate odds ratios (OR) for stroke with 95% confidence intervals (CI). To assess influence of iv-treatment with t-PA, we additionally performed sensitivity analysis excluding t-PA treated cases. Investigation of the influence of stroke etiology and NIHSS on admission on biomarkers of inflammation was performed by analysis of variance (ANOVA) complemented by Dunn's procedure for multiple comparisons, which corrects for potential bias by multiple testing. NIHSS was separated into 4 classes: 0–2; 3–4; 5–9; >9. Influence of stroke etiology and severity was investigated in restriction to stroke cases. All tests were two-sided and level of significance was set to 5%. Data were analyzed using the software package SAS 9.4, and SAS JMP 1.2.

3. Results

Distribution of baseline characteristics is shown in Table 1, as previously published [10]. Patients were significantly more often diagnosed as having hypertension, diabetes mellitus, hyperlipidemia, coronary artery disease (CAD), myocardial infarct, chronic heart failure, peripheral artery disease (PAD), and atrial fibrillation (AF). They were more often physically inactive and more frequently current smokers. Compared to the control group, the number of