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

Check for updates

Frederick Palm [a, *], Pirkko J. Pussinen [b], Anton Safer [c], Taina Tervahartiala [b], Timo Sorsa [b, d], Christian Urbanek [a], Heiko Becher [c, e], Armin J. Grau [a]

[a] Department of Neurology, Klinikum Ludwigshafen, Germany
[b] Oral and Maxillofacial Diseases, University of Helsinki, Finland
[c] Institute of Public Health, University of Heidelberg, Germany
[d] Department of Dental Medicine, Karolinska Institute, Huddinge, Sweden
[e] Institute of Medical Biometry and Epidemiology, University Medical Center Hamburg-Eppendorf, Germany

ARTICLE INFO

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.

© 2018 Elsevier B.V. All rights reserved.

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

* Corresponding author. Department of Neurology, Städtisches Klinikum Ludwigshafen, Bremserstr. 79, 67063 Ludwigshafen a. Rh., Germany.
E-mail address: frederickpalm@gmx.de (F. Palm).

stroke severity and stroke etiology [8]. Based on data from a pre-liminary 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 Ludwigs-hafen 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 cri-terion 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 apha-sia, insufficient understanding of the German language or any other relevant communication barrier and severe disability that pre-cluded 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 partici-pation 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 electro-cardiogram 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 man-ufacturer'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 previ-ously, and the interassay coefficient of variation (CV)% was 7.3% [11]. For the calculation of MMP-8 ∕ TIMP-1 molar ratios, the concen-trations 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 Treat-ment) 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 as-sesses 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 parame-ters 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 multi-variate 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, hyperlipid-emia, 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

Table 1
Baseline characteristics of cases and controls.

Variable	Cases (n = 470)	Controls (n = 809)	p-value
Age mean (SD)	65.9 (10.7)	67.3 (10.2)	0.03
Female sex n (%)	188 (40.0%)	338 (41.8%)	0.5
Hypertension n (%)	407 (86.6%)	536 (66.3%)	<0.001
Diabetes n (%)	145 (30.9%)	138 (17.1%)	<0.001
Hypercholesterolemia n (%)	342 (72.8%)	525 (64.9%)	0.004
Atrial fibrillation n (%)	92 (19.6%)	75 (9.3%)	<0.001
History of myocardial infarction n (%)	66 (14.9%)	14 (1.7%)	<0.001
Coronary artery disease n (%)	99 (21.1%)	89 (11.0%)	<0.001
Congestive heart failure n (%)	60 (12.8%)	48 (5.9%)	<0.001
Peripheral artery disease n (%)	46 (9.8%)	17 (2.1%)	<0.001
Physical activity n (%)[b]	150 (31.9%)	447 (55.3%)	<0.001
Current smoking n (%)	145 (30.9%)	118 (14.6%)	<0.001
High alcohol consumption n (%)[c]	32 (6.8%)	47 (5.8%)	0.48
Number of teeth (SD)	12.5 (11.0)	15.5 (11.1)	<0.001[a]
Leucocyte count (×1000/mm^3) mean (SD)	8.3 (2.1)	6.7 (1.8)	<0.001

p-value refers to X^2-test.
If [a] is shown, the t-test has been used; [b] leisure time physical activity that causes sweating ≥1/week; [c] > 50 g alcohol intake/day.

teeth was considerably lower in cases. Leucocyte count was significantly higher in cases. Table 2 presents mean serum MMP-8, MPO and TIMP-1 concentrations as well as MMP-8/TIMP-1 molar ratio in patients and controls. All parameters showed significant differences in univariate and multivariate analysis. They all were significantly higher in the case group as compared to controls. Sensitivity analysis leaving out 48 cases that had received iv t-PA treatment did not notably change the results (Table 3).

The serum levels of inflammatory markers are summarized by NIHSS on admission in Fig. 1A. Analysis of variance showed significant associations between stroke severity and serum levels of MMP-8 ($p < 0.001$), MPO ($p < 0.001$) and MMP-8/TIMP-1 molar ratio ($p < 0.001$) but not TIMP-1 ($p = 0.5$) with higher values being measured in patients with more severe stroke.

Levels of inflammatory markers on admission are shown by stroke etiology in Fig. 1B. Analysis of variance showed significant differences between stroke subgroups for MMP-8 ($p = 0.019$) and MMP8/TIMP-1 ratio ($p = 0.047$), but not for MPO ($p = 0.113$) and TIMP-1 ($p = 0.071$). In patients with stroke due to small vessel disease (SVD), MMP-8 serum levels were significantly lower compared to patients with stroke due to large-vessel disease (LVD) or cardioembolism (CE). When analysing stroke etiology and categorized stroke severity in the ANOVA model simultaneously, only stroke severity remained significant ($p = 0.009$) while stroke etiology turned non-significant ($p = 0.241$).

4. Discussion

In this large, carefully designed case-control study of ischemic stroke, we found that serum MMP-8, MPO, and TIMP-1 concentrations were strongly associated with acute ischemic stroke. While MMP-8, MPO, and MMP-8/TIMP-1 molar ratio were positively associated with stroke severity, serum levels of MMP-8 differed significantly between stroke subtypes.

MMPs play an important role in the cascade leading to brain injury after an ischemic stroke. MMP-1, -2, -3, -8, -9, -10, -13, and TIMP-1 are overexpressed in the infarcted brain tissue of stroke patients compared to non-ischemic areas [16]. Their sources are activated or infiltrating inflammatory cells such as microglia, macrophages, and neutrophils, and they contribute to the neurovascular perturbations comprising blood-brain barrier leakage and inflammatory reactions. Therefore, MMPs have been suggested as therapeutic targets for stroke. However, since MMPs are needed in the angiogenesis during the recovery, therapeutic inhibition of their activity needs to be carefully timed. The most studied MMP in CVD is MMP-9, but considering the potential of MMPs in treatments, the research should be broadened to other MMP family members.

MMP-8 is seldom studied specifically in stroke patients [4,17]. In our study, MMP-8 was directly associated with more severe stroke as measured by NIHSS. We did not analyze infarct volume, but stroke severity measured by NIHSS is closely related to it [18]. The

Table 2
MMP-8, TIMP-1, MPO and MMP-8/TIMP-1-molar ratio in cases and controls.

Variable	Univariate[b]				Multivariate per sero-parameter[c]		Multivariate all 3 sero-parameters[c]	
	Cases	Controls	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
MMP-8 [ng/ml] mean[a](95% CI)	58.3 (53.2–63.8) N = 466	24.2 (22.7–25.8) N = 803	8.2 (6.0–11.3)	<0.0001	4.9 (3.4–7.2)	<.0001	3.4 (2.1–5.3)	<0.0001
TIMP-1 [ng/ml] mean[a] (95% CI)	127 (123–132) N = 465	122 (119–124) N = 803	3.5 (1.6–8.0)	0.025	2.0 (0.7–5.5)	0.186	7.5 (2.5–22.6)	<0.0001
MMP-8/TIMP-1 mean[a](95% CI)	0.19 (0.17–0.21) N = 465	0.08 (0.08–0.09) N = 803	4.5 (3.4–5.9)	<0.0001	3,0 (2.2–4.1)	<.0001	NA[d]	
MPO [ng/ml] mean[a] (95% CI)	214 (201–227) N = 466	130 (124–137] N = 802	10,1 (6.6–15.4)	<0.0001	6.6 (4.0–11.0)	<.0001	3.7 (2.0–6.9)	<0.0001

[a] Means and confidence limits have been calculated under log-transformation, and have been back-transformed to original scale; OR was calculated per decade of the dependent variable, as each unit on the log10-scale means a factor of 10 on metric scale.
[b] Results stratified for age and sex.
[c] Results stratified for age and sex and adjusted for diabetes, hyperlipidemia, atrial fibrillation, myocardial infarct, coronary artery disease, chronic heart failure, peripheral artery disease, smoking, physical activity, number of teeth and leucocyte count.
[d] NA (not available): this parameter would cause collinearity and cannot be calculated.

Table 3
Sensitivity analysis of MMP-8, TIMP-1, MPO and MMP-8/TIMP-1-molar ratio in cases and controls excluding rt-PA treated cases.

Variable	Univariate[b]				Multivariate per sero-parameter[c]		Multivariate all 3 sero-parameters[c]	
	Patients	Controls	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
MMP-8 [ng/ml] mean[a] (95% CI)	56.5 (51.4–62.1) N = 426	24.2 (22.7–25.8) N = 803	7.7 (5.6–10.6)	<0.0001	4.4 (3.0–6.5)	<.0001	3.9 (1.9–4.9)	<0.0001
TIMP-1 [ng/ml] mean[a] (95% CI)	128 (123–133) N = 425	122 (119–124) N = 803	4.1 (1.7–9.7)	0.001	2.4 (0.8–6.9)	0.11	9.0 (2.9–28.5)	<0.0001
MMP-8/TIMP-1 mean[a] (95% CI)	0.18 (0.16–0.20) N = 425	0.08 (0.08–0.09) N = 803	4.2 3.1–5.5)	<0.0001	2.7 (1.9–3.8)	<.0001	NA[d]	
MPO [ng/ml] mean[a] (95% CI)	210 (197–224) N = 426	130 (124–137] N = 802	9.1 (5.9–14.0)	<0.0001	6.0 (3.6–10.0)	<.0001	3.8 (2.0–7.0)	<0.0001

[a] Means and confidence limits have been calculated under log-transformation, and have been back-transformed to original scale; OR was calculated per decade of the dependent variable, as each unit on the log10-scale means a factor of 10 on metric scale.
[b] Results stratified for age and sex.
[c] Results stratified for age and sex and adjusted for diabetes, hyperlipidemia, atrial fibrillation, myocardial infarct, coronary artery disease, chronic heart failure, peripheral artery disease, smoking physical activity, number of teeth and leucocyte count.
[d] NA (not available): this parameter would cause collinearity and cannot be calculated.

role of MMP-8 both in local plaque pathology and adverse cardio-vascular events is obvious [7,11,12,19–21]. Although we could demonstrate significant differences between stroke subtypes with higher serum values of MMP-8 in stroke due to CE or LVD, these differences are largely explained by CE and LVD contributing to higher stroke severity [22]. However, further research might focus on MMP-8 predicting stroke volume. Using longitudinal study-design, impact of MMP-8 predicting ischemic stroke in certain stroke etiologies (e.g. asymptomatic carotid stenosis) as well as the impact on stroke prognosis might be evaluated. This might facilitate decision-making process with regards to therapy adjudication (e.g. carotid endarterectomy), as usage in certain clinical situation is questionable. In addition, utilizing of MMP-8 determination in clinical practice might also potentially help to identify stroke mimics in future, avoiding application of risky therapies such as iv-treatment with t-PA.

Circulating TIMP-1 concentrations have been consistently associated with CVD development and prognosis including stroke [7,23,24] and its associations especially with fatal events have been repeatedly shown [7,25–27]. In our recent study including several biomarkers, TIMP-1 concentrations were directly associated with the highest hazards for stroke and death, and including the biomarker to the established risk profile improved the risk discriminations [7]. In addition of being an inhibitor of several MMPs, TIMP-1 plays an important regulatory role in numerous biological pathways, such as proliferation, apoptosis, and angiogenesis [28]. In the present study TIMP-1 did not independently associate with stroke after adjusting for confounders. However, when all measured biomarkers were entered simultaneously into the final model, TIMP was independently associated with stroke and its OR was the highest (9.5/10 units, $p < 0.001$), describing the importance of the whole proteolytic and oxidative tissue destructive cascade.

In the present study, MPO had a strong association with both stroke and its severity, which is in agreement with earlier studies [4,8]. It has also been reported to predict poor outcome in patients with significant stenosis [29,30] and in CVD-free populations [7,31]. It is a well-known inflammatory marker produced mainly by neutrophils as a response to microbial insult. In addition, MPO enhances the proteolytic activity of MMP-8 and inactivates TIMP-1 and has, therefore, synergetic effect for MMP-8 function [32].

The sources of elevated serum MMP-8 and MPO in CVD has remained partially unclear. MMP-8 and MPO are stored in different neutrophil granules and released by degranulation in response to inflammatory stimuli [33]. Recent studies demonstrated that t-PA treatment in ischemic stroke patients led to neutrophil degranulation with consecutive peaks of MMP-8 and MPO followed by increased production of TIMP-1 [34]. As indicated by sensitivity analyses, tPA treatment did not significantly contribute to higher biomarker concentrations in our study. Overall, the serum concentrations of these biomarkers are higher than those of plasma probably due to the sample preparation, where the anticoagulants inhibit the clotting cascade and complement activation [35]. Therefore, the deviation of serum MMP-8 and MPO concentrations, but not plasma, are partially explained by the genetic variation of the complement factor H [36,37]. However, these SNPs did not significantly associate with the risk of prevalent or incident stroke in a large cohort with a relatively low number of cases [37]. Further research is needed to investigate the usefulness of serum or plasma biomarkers in stroke and the genetic variation of the complement cascade predisposing to stroke. Our study has several limitations. Lower response rate in controls might potentially bias study results. We did not assess infarct volume in stroke patients and there are no data available on C-reactive protein or renal function in controls. Longitudinal study design might have given more information on the course of MMP-8, TIMP-1 and MPO in ischemic stroke. Strengths of our study include the ample information on stroke risk factors, stroke etiology and stroke severity.

4.1. Conclusion

Our data demonstrate significantly higher serum concentrations of inflammatory biomarkers MMP-8, MPO and TIMP-1 in acute ischemic stroke cases compared to controls, even after adjustment for signs of inflammation (leucocyte count). Serum levels of MMP-8 and MPO were strongly associated with stroke severity and stroke etiology.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Financial support
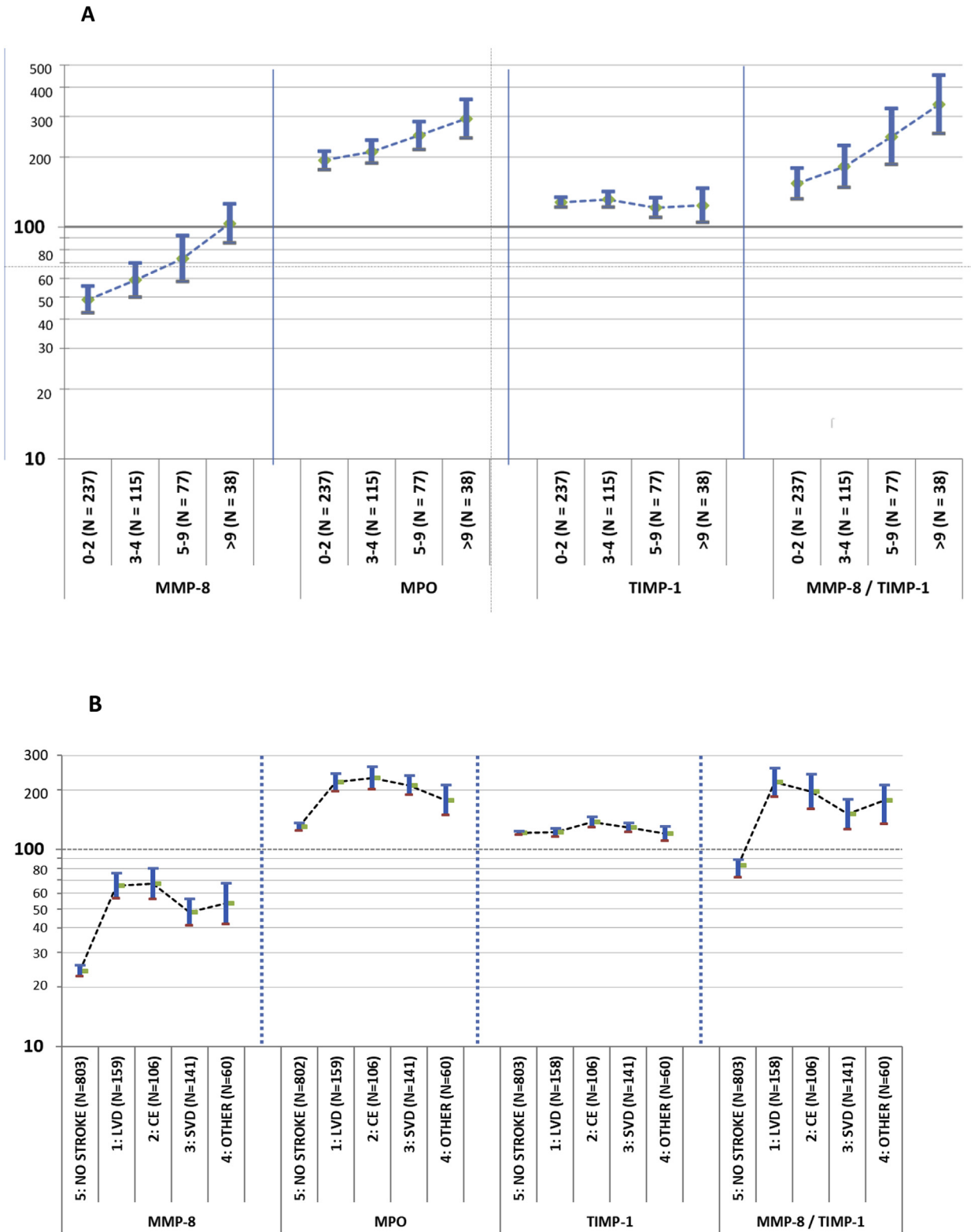
Fig. 1. Distribution of biomarkers by stroke severity and stroke etiology.
(A) Distribution of biomarkers by NIHSS on admission (means and 95% CIs). In this graph, MMP-8/TIMP-1 molar ratio has been multiplied by a factor 1000 for scaling reasons. (B) Distribution of biomarkers by TOAST classification (means and 95% CIs). In this graph, MMP-8/TIMP-1 molar ratio has been multiplied by a factor 1000 for scaling reasons.

References

[1] D. Summers, A. Leonard, D. Wentworth, J.L. Saver, J. Simpson, J.A. Spilker, et al., Comprehensive overview of nursing and interdisciplinary care of the acute ischemic stroke patient: a scientific statement from the American Heart Association, Stroke 40 (2009) 2911—2944.

[2] V.L. Feigin, B. Norrving, G.A. Mensah, Global burden of stroke, Circ. Res. 120 (2017) 439—448.

[3] H.C. Emsley, C.J. Smith, C.M. Gavin, R.F. Georgiou, A. Vail, E.M. Barberan, et al., An early and sustained peripheral inflammatory response in acute ischaemic stroke: relationships with infection and atherosclerosis, J. Neuroimmunol. 139 (2013) 93—101.

[4] F. Palm, L. Lahdentausta, T. Sorsa, T. Tervahartiala, P. Gokel, F. Buggle, et al., Biomarkers of periodontitis and inflammation in ischemic stroke: a case-control study, Innate Immun. 20 (2014) 511—518.

[5] C.S. Miller, C.P. King Jr., M.C. Langub, R.J. Kryscio, M.V. Thomas, Salivary biomarkers of existing periodontal disease: a cross-sectional study, J. Am. Dent. Assoc. 137 (2006) 322—329.

[6] H. Saari, K. Suomalainen, O. Lindy, Y.T. Konttinen, T. Sorsa, Activation of latent human neutrophil collagenase by reactive oxygen species and serine proteases, Biochem. Biophys. Res. Commun. 171 (1990) 979—987.

[7] I. Kormi, M.T. Nieminen, A.S. Havulinna, T. Zeller, S. Blankenberg, T. Tervahartiala, et al., Matrix metalloproteinase-8 and tissue inhibitor of matrix metalloproteinase-1 predict incident cardiovascular disease events and all-cause mortality in a population-based cohort, Eur. J. Prev. Cardiol. 24 (2017) 1136—1144.

[8] A. Tay, Y. Tamam, B. Yokus, M. Ustundag, M. Orak, Serum myeloperoxidase levels in predicting the severity of stroke and mortality in acute ischemic stroke patients, Eur. Rev. Med. Pharmacol. Sci. 19 (2015) 1983—1988.

[9] F. Palm, C. Urbanek, S. Rose, F. Buggle, B. Bode, M.G. Hennerici, et al., Stroke incidence and survival in Ludwigshafen am Rhein, Germany: the Ludwigshafen Stroke Study (LuSSt), Stroke 41 (2010) 1865—1870.

[10] H. Becher, F. Palm, A. Aigner, A. Safer, C. Urbanek, F. Buggle, et al., Socioeconomic conditions in childhood, adolescence, and adulthood and the risk of ischemic stroke, Stroke 47 (2016) 173—179.

[11] A.M. Tuomainen, K. Nyyssonen, J.A. Laukkanen, T. Tervahartiala, T.P. Tuomainen, J.T. Salonen, et al., Serum matrix metalloproteinase-8 concentrations are associated with cardiovascular outcome in men, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 2722—2728.

[12] A.M. Tuomainen, I. Kormi, A.S. Havulinna, T. Tervahartiala, V. Salomaa, T. Sorsa, et al., Serum tissue-degrading proteinases and incident cardiovascular disease events, Eur. J. Prev. Cardiol. 21 (2014) 806—812.

[13] F. Palm, C. Urbanek, J. Wolf, F. Buggle, T. Kleemann, M.G. Hennerici, et al., Etiology, risk factors and sex differences in ischemic stroke in the Ludwigshafen Stroke Study, a population-based stroke registry, Cerebrovasc. Dis. 33 (2012) 69—75.

[14] G. Teasdale, B. Jennett, Assessment of coma and impaired consciousness. A practical scale, Lancet 2 (1974) 81—84.

[15] L.B. Goldstein, C. Bertels, J.N. Davis, Interrater reliability of the NIH stroke scale, Arch. Neurol. 46 (1989) 660—662.

[16] E. Cuadrado, A. Rosell, A. Penalba, M. Slevin, J. Alvarez-Sabin, A. Ortega-Aznar, et al., Vascular MMP-9/TIMP-2 and neuronal MMP-10 up-regulation in human brain after stroke: a combined laser microdissection and protein array study, J. Proteome Res. 8 (2009) 3191—3197.

[17] S. Lorenzl, G. De Pasquale, A.Z. Segal, M.F. Beal, Dysregulation of the levels of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases in the early phase of cerebral ischemia, Stroke 34 (2003) e37—38.

[18] J.N. Fink, M.H. Selim, S. Kumar, B. Silver, I. Linfante, L.R. Caplan, et al., Is the association of National Institutes of Health Stroke Scale scores and acute magnetic resonance imaging stroke volume equal for patients with right- and left-hemisphere ischemic stroke? Stroke 33 (2002) 954—958.

[19] M.M. Turu, J. Krupinski, E. Catena, A. Rosell, J. Montaner, F. Rubio, et al., Intraplaque MMP-8 levels are increased in asymptomatic patients with carotid plaque progression on ultrasound, Atherosclerosis 187 (2006) 161—169.

[20] P.J. Pussinen, S. Sarna, M. Puolakkainen, H. Ohlin, T. Sorsa, E. Pesonen, The balance of serum matrix metalloproteinase-8 and its tissue inhibitor in acute coronary syndrome and its recurrence, Int. J. Cardiol. 167 (2013) 362—368.

[21] W. Peeters, F.L. Moll, A. Vink, P.J. van der Spek, D.P. de Kleijn, J.P. de Vries, et al., Collagenase matrix metalloproteinase-8 expressed in atherosclerotic carotid plaques is associated with systemic cardiovascular outcome, Eur. Heart J. 32 (2011) 2314—2325.

[22] D.A. Dulli, H. Stanko, R.L. Levine, Atrial fibrillation is associated with severe acute ischemic stroke, Neuroepidemiology 22 (2003) 118—123.

[23] M.J. West, P.J. Nestel, A.C. Kirby, R. Schnabel, D. Sullivan, R.J. Simes, et al., The value of N-terminal fragment of brain natriuretic peptide and tissue inhibitor of metalloproteinase-1 levels as predictors of cardiovascular outcome in the LIPID study, Eur. Heart J. 29 (2003) 923—931.

[24] J. Hansson, R.S. Vasan, J. Arnlov, E. Ingelsson, L. Lind, A. Larsson, et al., Biomarkers of extracellular matrix metabolism (MMP-9 and TIMP-1) and risk of stroke, myocardial infarction, and cause-specific mortality: cohort study, PLoS One 6 (2011), e16185.

[25] R.S. Velagaleti, P. Gona, J. Sundstrom, M.G. Larson, D. Siwik, W.S. Colucci, et al., Relations of biomarkers of extracellular matrix remodeling to incident cardiovascular events and mortality, Arterioscler. Thromb. Vasc. Biol. (30) (2010) 2283—2288.

[26] E. Lubos, R. Schnabel, H.J. Rupprecht, C. Bickel, C.M. Messow, S. Prigge, et al., Prognostic value of tissue inhibitor of metalloproteinase-1 for cardiovascular death among patients with cardiovascular disease: results from the AtheroGene study, Eur. Heart J. 27 (2006) 150—156.

[27] E. Cavusoglu, C. Ruwende, V. Chopra, S. Yanamadala, C. Eng, L.T. Clark, et al., Tissue inhibitor of metalloproteinase-1 (TIMP-1) is an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction, Am. Heart J. 151 (2006) 1101—1108.

[28] M.L. Lindsey, A. Yabluchanskiy, Y. Ma, Tissue inhibitor of metalloproteinase-1: actions beyond matrix metalloproteinase inhibition, Cardiology 132 (3) (2015) 147—150.

[29] N. Vuilleumier, F. Montecucco, G. Spinella, S. Pagano, M. Bertolotto, B. Pane, et al., Serum levels of anti-apolipoprotein A-1 auto-antibodies and myeloperoxidase as predictors of major adverse cardiovascular events after carotid endarterectomy, Thromb. Haemost. 109 (2013) 706—715.

[30] C.L. Heslop, J.S. Frohlich, J.S. Hill, Myeloperoxidase and C-reactive protein have combined utility for long-term prediction of cardiovascular mortality after coronary angiography, J. Am. Coll. Cardiol. 55 (2010) 1102—1109.

[31] M. Karakas, W. Koenig, A. Zierer, C. Herder, W. Rottbauer, J. Baumert, et al., Myeloperoxidase is associated with incident coronary heart disease independently of traditional risk factors: results from the MONICA/KORA Augsburg study, J. Intern. Med. 271 (2012) 43—50.

[32] X. Fu, S.Y. Kassim, W.C. Parks, J.W. Heinecke, Hypochlorous acid generated by myeloperoxidase modifies adjacent tryptophan and glycine residues in the catalytic domain of matrix metalloproteinase-7 (matrilysin): an oxidative mechanism for restraining proteolytic activity during inflammation, J. Biol. Chem. 278 (2003) 28403—28409.

[33] N. Borregaard, O.E. Sorensen, K. Theilgaard-Monch, Neutrophil granules: a library of innate immunity proteins, Trends Immunol. 28 (2007) 340—345.

[34] F. Carbone, N. Vuilleumier, M. Bertolotto, F. Burger, K. Galan, G. Roversi, et al., Treatment with recombinant tissue plasminogen activator (r-TPA) induces neutrophil degranulation in vitro via defined pathways, Vasc. Pharmacol. 64 (2015) 16—27.

[35] T.E. Mollnes, P. Garred, G. Bergseth, Effect of time, temperature and anticoagulants on in vitro complement activation: consequences for collection and preservation of samples to be examined for complement activation, Clin. Exp. Immunol. 73 (1988) 484—488.

[36] A.P. Reiner, J. Hartiala, T. Zeller, J.C. Bis, J. Dupuis, M. Fornage, et al., Genome-wide and gene-centric analyses of circulating myeloperoxidase levels in the charge and care consortia, Hum. Mol. Genet. 22 (2013) 3381—3393.

[37] A. Salminen, E. Vlachopoulou, A.S. Havulinna, T. Tervahartiala, W. Sattler, M.S. Lokki, et al., Genetic variants contributing to circulating matrix metalloproteinase 8 levels and their association with cardiovascular diseases: a genome-wide analysis, Circ. Cardiovasc. Genet. 10 (6) (2017 Dec).