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Association of matrix metalloproteinases with MRI indices of brain ischemia and aging

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Abstract

Magnetic resonance imaging (MRI) findings of large white matter hyperintensities (LWMH), decreased brain volume and silent cerebral infarcts (SCI) are subclinical indices of brain ischemia and aging. Although the pathophysiology of these findings remains uncertain, extracellular matrix (ECM) remodeling, a process regulated by matrix metalloproteinases (MMPs) and their inhibitors (TIMPs), may be implicated.

We evaluated the cross-sectional relations of circulating MMP-9 and TIMP-1 to these MRI indices in 583 stroke and dementia-free, Framingham Offspring participants (mean age 57 years, 58% women). Using multivariable regression MMP-9 (detectable versus non-detectable) and TIMP-1 (modeled as sex-specific quartiles) were related to LWMH (>1 S.D. above age-specific mean; yes/no), SCI (yes/no) and total brain volume (ratio of parenchymal to intracranial volume, TCBVr).

Mean TCBVr was 0.78 (S.D. 0.03), 13% of subjects had LWMH and 12% had SCI. Detectable MMP-9 was associated with higher prevalence of LWMH (OR 2.09, 95% confidence interval (CI) 1.00–4.37), but not with TCBVr. TIMP-1 was associated with a high prevalence of LWMH (OR for Q4 versus Q1–3: 1.83, 95%CI 1.06–3.18) and with lower mean TCBVr (Q4 associated with 0.17 S.D. units lower value relative to Q1–3; $p = 0.04$). Neither biomarker was associated with SCI.

Our findings are preliminary but if confirmed in further studies, suggest a pathophysiological role for the MMP/TIMP pathway in processes of brain ischemia and aging.

Keywords

MMPs; Brain MRI; Framingham; Brain aging

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Conflicts of interest

Reports no actual or potential conflicts of interest.

1. Introduction

Several findings on magnetic resonance imaging (MRI) of the brain including decreased brain volume, increased white matter hyperintensities (LWMH) and silent cerebral infarcts (SCI) have been associated with increased risk of mild cognitive impairment, dementia, and stroke (O'Brien and Ames, 1996; Pantoni and Garcia, 1997; Yamauchi et al., 2002; Vermeer et al., 2003). However, the pathophysiological basis for these MRI findings remains uncertain.

More recently, experimental and clinical observations have suggested that extracellular matrix (ECM) remodeling may contribute to alterations in brain volume, and to the presence of white matter hyperintensities or SCI (Sykova et al., 1998; Ellison et al., 1999; Asahi et al., 2000; Uspenskaia et al., 2004). ECM remodeling is a process mediated by the matrix metalloproteinases (MMPs), a family of zinc endopeptidases that are kept under control by their physiological antagonists, the tissue inhibitors of MMPs (TIMPs) (Matrisian, 1990; Messerli, 2004). Post mortem studies of brain tissue have demonstrated an increased expression of MMPs and TIMPs in the brain of patients with diseases where LWMH are frequent, such as multiple sclerosis (Anthony et al., 1997), conditions with decline in brain volumes such as Alzheimer disease (AD) (Asahina et al., 2001), and those associated with SCI such as stroke (Peress et al., 1995). On a parallel note, clinical investigators have noted elevation of circulating MMP-9 concentrations in patients with AD (Lorenz et al., 2003b), and in acute stroke (Horstmann et al., 2003), and elevated CSF concentrations of MMP-9 in vascular dementia and AD (Lorenz et al., 2003a; Adair et al., 2004). There is paucity of data evaluating the relation of ECM changes and brain MRI findings of ischemia and aging.

In the present study we evaluated the relation of select circulating markers of ECM remodeling (MMP-9 and TIMP-1) to MRI markers of subclinical cerebral disease (decreased cerebral volume, increased white matter hyperintensities and SCI) assessed via volumetric brain MRI.

2. Methods

2.1. Study sample

Participants were eligible for the present investigation if they had available biomarker measurements (either MMP-9 or TIMP-1) and brain MRI. Biomarker measurements were done as part of a prior study that assessed the biochemical correlates of left ventricular remodeling (Sundstrom et al., 2004a), therefore the sampling scheme is dependent on the LV remodeling study design. Participants were sampled according to LV echocardiographic dimensions and categorized in three groups: subjects with LV internal dimension (LVEDD) and wall thickness (LVWT) below the sex-specific median ("referent group" $n = 724$), and subjects with values equal to or exceeding the sex-specific 90th percentile of LVEDD ($n = 276$) or LVWT ($n = 276$) (Sundstrom et al., 2004a,b).

The measurements of plasma MMP-9 and TIMP-1 were acquired at the sixth examination of the Framingham Offspring Study from 1995 to 1998. Volumetric brain MRI was acquired between 1998 and 2002 in 1965 participants. Participants with claustrophobia or contraindications to MRI were excluded as well as those with a clinical diagnosis of stroke, dementia, multiple sclerosis, brain tumor, head injury or other neurologic conditions that affect brain MRI measurements. Of the remaining 1882 participants with available brain MRI, 583 participants also had available measurements of biomarkers and constituted our study sample. Both biomarkers (TIMP-1 and MMP-9) were available in 377 persons, only TIMP-1 in 200, and only MMP-9 in 6 persons. The Institutional Review Board of Boston University approved the study protocol and informed consent was obtained from all subjects.

2.2. Plasma MMP-9 and TIMP-1 measurements

Blood samples were drawn in fasting state in a supine position, centrifuged, and the plasma frozen at -70°C until assayed. Plasma total MMP-9 was measured in duplicate with the use of a two-site sandwich ELISA assay (Amersham Pharmacia Biotech; assay range of 4–128 ng/mL), which measures MMP-9, ProMMP-9, and the ProMMP-9/TIMP-1 complex (Sundstrom et al., 2004a). Plasma total TIMP-1 was measured similarly with a two-site sandwich ELISA assay (Amersham Pharmacia Biotech), which measures free TIMP-1 and TIMP-1 complexed with various MMPs (Sundstrom et al., 2004b). The intra-assay coefficient of variation was $<18\%$ for MMP-9 and $<5\%$ for TIMP-1 measurements (Sundstrom et al., 2004a).

2.3. Brain MRI measurements

Detailed information about acquisition and data processing of MRI scans, is available elsewhere (DeCarli et al., 1992; Seshadri et al., 2004). The scans were processed and analyzed by a neuroradiologist (CD) who was blind to the subjects' MMP and TIMP concentrations, demographic, vascular risk factor data and clinical information. All analyses were performed using a custom-designed image analysis package, QUANTA 6.2, operating on a Sun Microsystems (Santa Clara, CA) Ultra 5 workstation.

Brain volume was determined in coronal sections by manually outlining the intracranial vault above the tentorium to determine the total cranial volume (TCV). Next, the skull and other non-brain tissues were removed from the image, followed by mathematical modeling to determine total brain volume (TBV). TBV includes the supratentorial gray and white matter and excludes the CSF. We used the ratio of TBV to TCV as a measure of brain volume to correct for differences in head size (TCBv_r).

The volume of abnormal white matter hyperintensities was determined according to previously published methods of documented high reliability. The inter-rater reliabilities range between 0.90 and 0.94 for TCV, TBV, and white matter hyperintensities, and intra-rater reliabilities average 0.98 across all measures (Jeerakathil et al., 2004; DeCarli et al., 2005). Large WMH on brain MRI (LWMH) were defined as white matter hyperintensity volumes >1 S.D. above age-specific mean (Jeerakathil et al., 2004; DeCarli et al., 2005). The presence or absence of SCI was determined manually by the operator based on the size, location and imaging characteristics using previously described methods (DeCarli et al., 2005). Lesions were superimposed on T2-weighted and proton density images and SCI were identified in the subtraction image (PD-T2) as lesions ≥ 3 mm, having cerebrospinal fluid signal intensity and distinct from the circle of Willis for basal ganglia infarcts (Das et al., 2008). Inter-rater reliabilities for SCI ranged between 0.73 and 0.90 (Das et al., 2008).

2.4. Stroke risk factors data

The following risk factors were included as covariates in multivariable analyses: systolic blood pressure; use of anti-hypertensive medication; current smoking; diabetes mellitus (fasting blood glucose of at least 126 mg/dl, current use of hypoglycemic medication or insulin, or previous diagnosis of diabetes); prior cardiovascular disease (coronary artery disease, congestive heart failure and intermittent claudication); history of atrial fibrillation; and electrocardiographic left ventricular hypertrophy.

2.5. Statistical analysis

We used multivariable linear regression to examine the associations of plasma MMP-9 and TIMP-1 to TCBv_r and multivariable logistic regression to relate MMP-9 and TIMP-1 to SCI (presence versus absence), and LWMH (presence versus absence). We conducted separate analyses for each MRI phenotype and for each biomarker.

TIMP-1 was modeled as a continuous variable (logarithmically transformed to normalize the right-skewed distribution) and using sex-specific quartiles. Plasma MMP-9 was modeled as a binary variable (detectable versus undetectable) because it was detectable in only 19% of individuals.

We examined differences across quartiles in prevalence of MRI findings of interest (LWMH and SCI) or their mean values (TCBVr). We also evaluated models with biomarker quartiles (for TIMP-1): comparing each of quartiles 2–4 to the first quartile that served as referent (LWMH, SCI, wherein higher values indicate increasing abnormality) and comparing quartile 4 to quartiles 1–3 (TCBVr, for which lower values indicate increasing abnormality) to evaluate for non-linear relations with a threshold effect at the upper end of the distribution of the markers. The covariates were incorporated in the multivariable models hierarchically:

- a. Adjusted for age, sex and echocardiographic LV mass measures (LVEDD and LVWT) (since biomarker sampling was based on echocardiographic measures), and for the time interval between the sixth examination cycle (when biomarkers were measured) and the acquisition of brain MRI at the seventh examination cycle.
- b. Additionally adjusted for stroke risk factors, previously reported to be related to the brain MRI indices in our cohort (Jeerakathil et al., 2004; Seshadri et al., 2004).

We used multivariable regression to examine linear trends in covariate-adjusted mean TCBVr across sex-specific quartiles of plasma TIMP-1 concentrations and for the two MMP-9 groups (detectable versus non-detectable). All analyses were determined a priori and performed using Statistical Analyses System software Version 9.1 (SAS Institute, Cary, NC). A two-sided p -value <0.05 was considered statistically significant.

3. Results

The baseline characteristics of the participants are presented in Table 1. We observed LWMH in 74 participants (12%) and SCI in 69 participants (12%). The mean TCBVr, ratio of brain volume to head size, was 0.78 (S.D. 0.03, range 67–85) and was not significantly different in men (mean 77.9 ± 3.3 S.D.) compared to women (mean 78.6 ± 2.9 S.D.). TIMP-1 was detected in 577 participants (range 447–1796 ng/ml) and MMP-9 was detectable in 73 participants (19%) (range 20–248 ng/ml). Table 2 presents the relation of baseline characteristics, and MMP-9 and TIMP-1.

3.1. Relation of biochemical and MRI markers

3.1.1. TIMP-1—We found a significant association of higher TIMP-1 concentrations with a higher prevalence of LWMH (odds ratio (OR) 3.20 for Q2–4 versus Q1, 95% CI 1.41–7.25, $p = 0.005$). However, the association was not linear and the main effect was a lower prevalence of LWMH in the lowest quartile of TIMP-1. The association remained largely the same after adjusting for stroke risk factors (OR 3.27, 95% CI 1.44–7.46, $p = 0.005$). Plasma TIMP-1 concentration was inversely related to TCBVr after adjusting for age, sex, LV mass, and time interval ($\beta -0.21 \pm 0.08$ Q4 versus Q1–3, $p = 0.012$). This association was maintained after additional adjustment for stroke risk factors ($\beta -0.17 \pm 0.08$ Q4 versus Q1–3, $p = 0.044$). There was no significant association of plasma TIMP-1 concentrations with SCI (Table 3).

We also evaluated the effect of circulating CRP level, a marker of inflammation, and use of lipid lowering treatment on the associations reported above (Table 4). Adjustment for these confounders did not change the results.

3.1.2. MMP-9—Detectable plasma MMP-9 was associated with a higher prevalence of LWMH after adjusting for age, gender, LV mass measures, the time interval between biomarker

collection and MRI acquisition, and stroke risk factors. The odds of having LWMH were twice as high in the group with detectable MMP-9 (OR 1.89, 95%CI 0.95–3.73, $p = 0.068$) and were significant in the multivariable adjusted model (OR 2.09, 95%CI 1.00–4.37, $p = 0.049$). There was no significant association of detectable MMP-9 with TCBVr or SCI, however the sample size was small ($n = 71$) and we cannot exclude a modest association. Analysis of MMP-9 as a continuous variable showed no significant associations, but given the small sample size we deemed it more appropriate to use MMP-9 as a dichotomous variable, with results as reported above. Similar to the case of TIMP-1, adjustment for CRP levels and lipid lowering therapy did not change the results (Table 4).

4. Discussion

4.1. Principal findings

The present study evaluates, for the first time in a community-based sample, the cross-sectional relations between circulating biomarkers of ECM remodeling and volumetric brain MRI indices. Our principal findings are twofold: a significant association of higher blood concentrations of TIMP-1 and detectable MMP-9 with the presence of LWMH, and a significant association of higher TIMP-1 concentrations with lower TCBVr. These associations were significant after adjusting for standard vascular risk factors (age, hypertension, smoking, diabetes, prior cardiovascular disease, and atrial fibrillation), echocardiographic markers of ventricular remodeling, circulating CRP level, and use of lipid lowering therapy.

4.2. Relations of ECM biomarkers to LWMH

Although our findings are preliminary and need further investigation, the association of TIMP-1 and MMP-9 with LWMH raises the possibility that this pathway may have a role in the pathophysiology of white matter disease seen on brain MRI. Several cells in the central nervous system produce MMPs and TIMPs (neurons, astrocytes, microglia and endothelial cells) (Rosenberg et al., 2001; Lorenl et al., 2003b). At least two processes have been involved in the development of LWMH, ischemia and inflammation, and MMPs and TIMPs have been implicated in both of these processes (Cunningham et al., 2005). The pathological changes underlying white matter lesions include microvascular occlusion, disturbed vascular permeability and altered vascular autoregulation (Pantoni and Garcia, 1997). MMP-9 and TIMP-1 play a key role in maintaining the integrity of the microvascular basal lamina in the brain and disturbances in their levels could result in these pathological changes. Concentrations of these markers may be increased by cerebral ischemia (Hamann et al., 1999), and higher concentrations of these markers may in turn exacerbate ischemic complications by altering the blood-brain barrier.

Chronic hypoperfusion also has been related to increased WMH and brain atrophy (Kawamura et al., 1993). Chronic cerebral hypoperfusion activates microglia and astrocytes (Wakita et al., 1994), which are capable of producing MMPs and TIMPs, thereby contributing to tissue damage.

In addition, cerebral ischemia may activate inflammatory pathways and inflammation itself may contribute to the development of WMH. For instance, elevated CRP blood level, a marker of inflammation, has been associated with WMH lesions (mean age 71 years) (van Dijk et al., 2005). Inflammatory mediators have been demonstrated to regulate MMP-9 activity in experimental studies (Gottschall and Yu, 1995) and MMP-9 has been related to blood brain barrier disruption during inflammation (Mun-Bryce and Rosenberg, 1998), which might contribute to the development of WMH. Inflammation has been related to the pathogenesis of AD, a disease process associated with increased WMH (Aktas et al., 2007). Elevated MMPs also have been found in patients with multiple sclerosis, a white matter autoimmune disease

characterized by a prominent inflammatory response (Rosenberg, 2002), and prior studies have shown that MMPs are capable of degrading myelin basic protein (Chandler et al., 1995), one of the components of myelin in white matter tracts. Although CRP levels have been associated with WMH as noted, adjustment for CRP levels in our study did not change the results; however, our cohort is younger than in previous studies (mean age 57 years at biomarker measurement) and it is possible that age has an effect on this relation. Our data suggests an association of MMP-9 and TIMP-1 with the brain MRI markers independent of circulating CRP levels and use of lipid lowering therapy.

4.3. Relations of ECM biomarkers to brain volume (TCBv)

Brain atrophy is known to increase with aging (Heijer et al., 2003; DeCarli et al., 2005) and prior studies have implicated vascular risk factors, in particular hypertension, in this process (Heijer et al., 2003). Our study provides preliminary evidence suggesting that brain tissue remodeling mediated by TIMP/MMP may play a role in determining brain atrophy, after accounting for the vascular risk factors examined.

Although definite explanations are speculative at present, it is likely that ischemia and chronic hypoperfusion, inflammation and neuronal loss are all synergistic contributing factors to this process.

Brain atrophy and gliosis have been related to chronic hypoperfusion (Kobari et al., 1990; Kawamura et al., 1993) and chronic hypoperfusion may activate astrocytes, which have been shown to respond by producing TIMP-1 (Rivera et al., 1997). Activated astrocytes may participate in the development of gliosis, eventually leading to brain atrophy.

The MMPs (especially MMP-9) have been implicated in neuronal cell death (Gu et al., 2002). Administrations of neutralizing antibodies to MMP-9 (Romanic et al., 1998) or lack of the MMP-9 gene (Asahi et al., 2000) have been reported to attenuate neuronal damage in experimental animal stroke models. It may be argued that if higher MMP-9 concentrations are associated with greater ischemic damage, an opposite relation should be observed for TIMP-1. However, we measured total TIMP-1 that includes activity of TIMPs complexed to MMPs. Thus, total TIMP-1 concentrations may indicate greater MMP-9 activity.

Although TIMP-1 has been shown to inhibit excitotoxic death in neurons (Tan et al., 2003), as previously mentioned the assay used to measure TIMP-1 concentrations in our study measures total TIMP-1 complexed with MMPs, and therefore the relation observed between elevated concentrations of TIMP-1 and brain atrophy, may actually reflect an increase in concentrations of MMPs in addition to a compensatory elevation of TIMP-1, though insufficient to prevent brain atrophy. Adjustment for circulating CRP levels and for use of lipid lowering therapy did not alter the results, suggesting that pathways besides inflammation may explain the relation of these ECM biomarkers with brain MRI measures.

4.4. Relations of ECM biomarkers to SCI

The lack of association observed in our study between SCI and biomarkers despite their association to LWMH is intriguing. Although it has been postulated that both MRI markers may be the result of small vessel disease, they may represent different processes with different pathophysiology but sharing risk factors. Prior studies have shown that macrovascular disease is also implicated in the etiology of SCI (Roquer et al., 2004). It is possible that macrovascular disease contributed significantly to the SCI observed in our study sample. The lower prevalence of hypertension in our study (20% compared to 49% in some prior studies of older population samples with SCI) (Bryan et al., 1999), is another factor that may explain the lack of association of TIMP-1 with SCI, because hypertension is the strongest vascular risk factor for the

development of SCI and hypertension has also been shown to increase circulating concentrations of TIMP-1 (Tayebjee et al., 2004). Overall, this study cannot exclude a modest effect of TIMP/MMPs on SCI.

4.5. Strengths and limitations

Our study has several strengths including the middle-aged community-based sample, that included men and women, the use of reproducible quantitative MRI techniques, and the interpretation of biomarkers and imaging data independent of each other and blinded to clinical data. We also evaluated the effect of several possible confounders including CRP levels, a marker of inflammation, and use of lipid lowering therapy.

Limitations of our study include the predominantly European descent of our participants; further studies are required to confirm our results in other ethnic and racial groups. A potential limitation is the acquisition of MRI and biomarkers at separate times; however adjustment for the time interval between the two measures did not alter the results. The sampling design for the ECM biomarkers was based on the distribution of LV remodeling phenotypes. Nevertheless, adjustment for LV mass in our analyses did not alter the association between elevated biomarkers and extensive WMH.

As discussed earlier lack of association of MMP-9 with TCBVr and both of the biomarkers with SCI, could be due to limited power in our study. We had a 90% power to detect a threefold increase in the risk of SCI for MMP-9 assuming $\alpha < 0.05$, but the power to detect a relative risk of twofold was lower, only 55%. As previously noted, the sample size of subjects with detectable MMP-9 was a limitation. The power for detecting a threefold increase in the risk of SCI for TIMP-1 was 95%, assuming $\alpha < 0.05$, but it was only 60% to detect a relative risk of 2. Thus modest associations of the biomarkers with SCI cannot be excluded.

Evaluation of the TIMP/MMP pathway was limited in this study, and future studies could include measurements of enzyme activity and other MMPs. We did not evaluate for other factors, which could potentially affect the results, such as acute silent cerebral infarcts at the time of biomarker blood sample collection, or acute infectious processes. However, all participants were free of cancer for the 6 months prior to biomarker collection, and those with overt cardiovascular disease were excluded. In addition, the cohort was ambulatory, able to participate in a 3–4 h examination, and had the option of rescheduling to a more convenient date during an acute event, or other minor or major illness, making it less likely that an acute inflammatory process accounted for our observations.

5. Conclusion

Higher concentrations of ECM turnover markers, TIMP-1 and MMP-9, are associated with MRI markers of aging and brain ischemia after adjusting for standard vascular risk factors in a community-based sample of middle-aged adults, free of clinical stroke and dementia. Our findings are preliminary, and require further investigation; if replicated in further studies, these data suggest a role for the MMP/TIMP pathway in the pathophysiology of structural brain changes associated with aging and ischemia.

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Table 1

Baseline characteristics of study sample.

Stroke risk factors	Men (n = 247)	Women (n = 336)
Clinical characteristics		
Age (years), mean $\pm$ S.D.	57 $\pm$ 9	57 $\pm$ 9
Systolic blood pressure (mm Hg), mean $\pm$ S.D.	127 $\pm$ 17	123 $\pm$ 21
Anti-hypertensive medication (%)	22	19
Current smokers (%)	9	17
Diabetes mellitus (%)	9.8	4.2
Prevalent cardiovascular disease (%)	8.5	5.1
Atrial fibrillation (%)	4.1	0.3
Electrocardiographic left ventricular hypertrophy (%)	3.7	1.2

Table 2

Relation of baseline characteristics of study sample and biomarkers.

	TIMP-1			MMP-9		
	Q1	Q2	Q3	Q4	Not detectable (n = 313)	Detectable (n = 71)
Clinical characteristics						
Male (%)	42	42	42	42	43	51
Age (years), mean ± S.D.	53 ± 8	55 ± 9	58 ± 9	61 ± 9	57 ± 10	59 ± 9
Systolic blood pressure (mm Hg), mean ± S.D.	120 ± 19	123 ± 17	125 ± 17	130 ± 23	126 ± 21	130 ± 21
Anti-hypertensive medication (%)	11	12	23	35	22	39
Current smokers (%)	14	11	12	18	10	25
Diabetes mellitus (%)	2.1	2.8	1.7	14.6	5.8	18.3
Prevalent cardiovascular disease (%)	0.7	3.5	6.2	15.3	6.7	15.5
Atrial fibrillation (%)	0.7	2.1	1.4	3.5	2.2	1.4
Electrocardiographic left ventricular hypertrophy (%)	0.7	1.4	4.1	2.8	2.3	7.4

Table 3

Relation of biomarkers and brain MRI indices.

Model	TCBvT ($\beta \pm$ S.D.)	P	LWMH (OR [95% CI])	P	SCI (OR [95% CI])	P
(I) TIMP-1						
(A) Age-, sex-, time to MRI- and LV mass-adjusted						
1 S.D. increase in ln(TIMP-1)	-0.12 $\pm$ 0.04	0.002	1.31 [1.02–1.68]	0.037	1.06 [0.81–1.38]	0.672
Q1	Referent		Referent		Referent	
Q2	0.09 $\pm$ 0.10	0.374	3.10 [1.26–7.63]	0.014	1.80 [0.83–3.90]	0.134
Q3	0.02 $\pm$ 0.10	0.873	2.59 [1.04–6.49]	0.042	1.29 [0.58–2.88]	0.539
Q4	-0.17 $\pm$ 0.10	0.091	4.10 [1.67–10.04]	0.002	1.76 [0.80–3.88]	0.161
Trend across TIMP-1 quartiles ^a	-0.06 $\pm$ 0.03	0.074	1.41 [1.11–1.80]	0.006	1.13 [0.89–1.44]	0.315
Q4 versus Q1–3	-0.21 $\pm$ 0.08	0.012				
Q2–4 versus Q1			3.20 [1.41–7.25]	0.005	1.60 [0.82–3.15]	0.171
(B) Multivariable-adjusted (age, sex, time to MRI, LV mass, stroke risk factors)						
1 S.D. increase in ln(TIMP-1)	-0.09 $\pm$ 0.04	0.017	1.45 [1.09–1.94]	0.012	1.01 [0.77–1.34]	0.919
Q1	Referent		Referent		Referent	
Q2	0.09 $\pm$ 0.10	0.362	3.23 [1.30–7.99]	0.011	1.80 [0.82–3.92]	0.141
Q3	0.02 $\pm$ 0.10	0.840	2.52 [1.00–6.38]	0.051	1.28 [0.57–2.90]	0.552
Q4	-0.13 $\pm$ 0.10	0.203	4.35 [1.75–10.78]	0.002	1.70 [0.76–3.82]	0.200
Trend across TIMP-1 Quartiles ^a	-0.04 $\pm$ 0.03	0.172	1.44 [1.12–1.84]	0.005	1.12 [0.87–1.43]	0.376
Q4 versus Q1–3	-0.17 $\pm$ 0.08	0.044				
Q2–4 versus Q1			3.27 [1.44–7.46]	0.005	1.58 [0.80–3.14]	0.190
(II) MMP-9 (Detectable versus non-detectable)						
(A) Age-, sex-, time to MRI- and LV mass-adjusted						
	-0.07 $\pm$ 0.11	0.525	1.89 [0.95–3.73]	0.068	1.37 [0.66–2.83]	0.396
(B) Multivariable-adjusted (age, sex, time to MRI, LV mass, and stroke risk factors)						
	0.03 $\pm$ 0.12	0.769	2.09 [1.00–4.37]	0.049	1.33 [0.62–2.85]	0.469

TCBvT, total cranial to brain volume ratio; LWMH, large white matter hyperintensities; SCI, silent cerebral infarcts; β , correlation coefficient; S.D., standard deviation; OR, odds ratio; CI, confidence interval.

^aEvaluated using multivariable regression. Stroke risk factors= systolic blood pressure; use of anti-hypertensive medication; current smoking; diabetes mellitus; prior cardiovascular disease; atrial fibrillation; electrocardiographic left ventricular hypertrophy.

Table 4

Relation of biomarkers, brain MRI markers, CRP levels and lipid lowering therapy.

Model	(I) TIMP-1		(A) Age-, sex-, time to MRI- and LV mass-adjusted		(B) Multivariable-adjusted (age, sex, time to MRI, LV mass, stroke risk factors)			
	CRP level adjusted	Lipid lowering therapy adjusted	CRP level adjusted	Lipid lowering therapy adjusted	CRP level adjusted	Lipid lowering therapy adjusted	CRP level adjusted	Lipid lowering therapy adjusted
	Q4 versus Q1-3	Q2-4 versus Q1	Q4 versus Q1-3	Q2-4 versus Q1	Q4 versus Q1-3	Q2-4 versus Q1	Q4 versus Q1-3	Q2-4 versus Q1
TCBvT	0.18 ± 0.08 ^a	0.21 ± 0.08 ^b	0.15 ± 0.08	0.18 ± 0.08 ^a	0.18 ± 0.08 ^a	0.18 ± 0.08 ^a	0.18 ± 0.08 ^a	0.18 ± 0.08 ^a
LWMH	3.12 [1.38–7.09] ^b	3.21 [1.41–7.29] ^b	3.19 [1.40–7.27] ^b	3.33 [1.46–7.62] ^b	3.19 [1.40–7.27] ^b	3.33 [1.46–7.62] ^b	3.19 [1.40–7.27] ^b	3.33 [1.46–7.62] ^b
Model	(II) MMP-9 (Detectable versus non-detectable)		(A) Age-, sex-, time to MRI- and LV mass-adjusted		(B) Multivariable-adjusted (age, sex, time to MRI, LV mass, stroke risk factors)			
	CRP level adjusted	Lipid lowering therapy adjusted	CRP level adjusted	Lipid lowering therapy adjusted	CRP level adjusted	Lipid lowering therapy adjusted	CRP level adjusted	Lipid lowering therapy adjusted
TCBvT	-0.05 ± 0.11	-0.07 ± 0.11	0.06 ± 0.12	0.04 ± 0.12	0.06 ± 0.12	0.04 ± 0.12	0.06 ± 0.12	0.04 ± 0.12
LWMH	1.96 [0.99–3.92]	1.94 [0.98–3.85]	2.14 [1.02–4.49] ^a	2.17 [1.04–4.56] ^a	2.14 [1.02–4.49] ^a	2.17 [1.04–4.56] ^a	2.14 [1.02–4.49] ^a	2.17 [1.04–4.56] ^a

TCBvT, total cranial to brain volume ratio; LWMH, large white matter hyperintensities. Effect measures on TCBvT are correlation coefficient ± standard deviation. Effect measures on LWMH are odds ratio [95%confidence interval].

^a $P < 0.05$.

^b $P < 0.01$.