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Matrix metalloproteinase-8 and tissue inhibitor of matrix metalloproteinase-1 predict incident cardiovascular disease events and all-cause mortality in a population-based cohort

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Abstract

Background: Extracellular matrix degrading proteases and their regulators play an important role in atherogenesis and subsequent plaque rupture leading to acute cardiovascular manifestations.

Design and methods: In this prospective cohort study, we investigated the prognostic value of circulating matrix metalloproteinase-8, tissue inhibitor of matrix metalloproteinase-1 concentrations, the ratio of matrix metalloproteinase-8/ tissue inhibitor of matrix metalloproteinase-1 and, for comparison, myeloperoxidase and C-reactive protein concentrations for incident cardiovascular disease endpoints. The population-based FINRISK97 cohort comprised 7928 persons without cardiovascular disease at baseline. The baseline survey included a clinical examination and blood sampling. During a 13-year follow-up the endpoints were ascertained through national healthcare registers. The associations of measured biomarkers with the endpoints, including cardiovascular disease event, coronary artery disease, acute myocardial infarction, stroke and all-cause death, were analysed using Cox regression models. Discrimination and reclassification models were used to evaluate the clinical implications of the biomarkers.

Results: Serum tissue inhibitor of matrix metalloproteinase-1 and C-reactive protein concentrations were associated significantly with increased risk for all studied endpoints. Additionally, matrix metalloproteinase-8 concentration was associated with the risk for a coronary artery disease event, myocardial infarction and death, and myeloperoxidase concentration with the risk for cardiovascular disease events, stroke and death. The only significant association for the matrix metalloproteinase-8/ tissue inhibitor of matrix metalloproteinase-1 ratio was observed with the risk for myocardial infarction. Adding tissue inhibitor of matrix metalloproteinase-1 to the established risk profile improved risk discrimination of myocardial infarction (p=0.039) and death (0.001). Both matrix metalloproteinase-8 (5.2%, $p < 0.001$) and tissue inhibitor of matrix metalloproteinase-1 (12.9%, $p < 0.001$) provided significant clinical net reclassification improvement for death.

Conclusions: Serum matrix metalloproteinase-8 and tissue inhibitor of matrix metalloproteinase-1 can be considered as biomarkers of incident cardiovascular disease events and death.

Keywords

Biomarker, cardiovascular outcomes, prediction, mortality

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Introduction

Systemic inflammation is a key contributing factor in atherosclerosis, which underlies cardiovascular diseases (CVD).[1] Myocardial infarction (MI) and ischaemic stroke, the acute and often fatal complications of CVD, account for one-third of the deaths worldwide.[2] These complications result from plaque rupture or thrombus formation in the arterial tree and are shaped largely in concert by various inflammatory mediators. Although risk factors have been mapped extensively, these events are difficult to predict since they often occur without prior symptoms. Therefore, finding specific biomarkers that can predict the outcomes and identify patients at risk would be highly valuable.

Matrix metalloproteinases (MMPs) play a critical role in inflammatory processes and are capable of degrading almost all extracellular proteins.[3] MMPs are secreted by inflammatory cells, in concert with endothelial and smooth muscle cells. They facilitate leukocyte navigation through the extracellular matrix (ECM) and modify immune responses and blood pressure by processing of non-matrix bioactive substrates.[3,4] In ex vivo studies, MMP-8 levels are shown to be higher in vulnerable plaques when compared to normal arteries.[5] ECM modulation by MMPs increases the vulnerability of the atherosclerotic plaque significantly.[6] MMPs have been shown to localise in the coronary atheroma shoulder regions – the areas especially prone to rupture leading to subsequent luminal thrombosis.[7] Disturbances in the regulation of MMP-8 have also been linked to plaque formation and maturation.[8–11] Previous reports showed that serum MMP-8 is associated with prevalent coronary artery disease (CAD) and acute coronary syndrome (ACS), and also with future CVD events in men and in a general population with no CVD at the baseline.[12–15]

The balance between pro- and anti-degradative factors determine the integrity of the atherosclerotic plaque ECM. The key regulators of MMP activity are tissue inhibitors of matrix metalloproteinases (TIMPs), TIMP-1 being the most potent and well-studied of the four major endogenous inhibitors.[16] TIMP-1 has also been reported as an independent predictor of CVD events and cardiac death.[17,18] The imbalance between MMP-8 and TIMP-1 may indicate vulnerability of the atherosclerotic plaque to rupture.[13]

The aim of our study was to evaluate the prognostic value of serum MMP-8 and TIMP-1 concentrations, and their ratio for various cardiac endpoints and death. Myeloperoxidase (MPO) and the classical inflammatory marker C-reactive protein (CRP) were both included as reference values. We hypothesise that both MMP-8 and TIMP-1 are significantly associated with the risk of CVD events and that both can improve the discrimination and reclassification of the current general cardiovascular risk profile.

Methods

Study population

This study is based on the FINRISK97 Study, a national population-based cohort ($n = 8446$) with 25–74 year-old subjects.[19] The study was approved by the Ethics Committee of the National Public Health Institute and carried out according to the recommendations of the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Data collection and study design

The subjects filled out a mailed questionnaire and participated in a clinical examination at which blood samples were drawn. Body mass index (BMI), systolic and diastolic blood pressure, and waist/hip ratio were measured in the clinical examination, and years of education and smoking habits were recorded using the self-administered questionnaires.[19]

Prevalent diabetes and CVD events were defined as a doctor-diagnosed disease using the questionnaire, and in the register data either as an intake of related drugs or as hospitalisations with the disease. CAD events included the history of MI, revascularisations, or percutaneous transluminal coronary angioplasty. Additionally, history of stroke (excluding subarachnoid haemorrhage) was included in the prevalent CVD.

The study design was a prospective cohort study with several endpoints (Figure 1). Some participants with incident CVD suffered from multiple events classified as stroke, MI or CAD, thus the numbers of subjects do not sum up to the number of incident CVD events. During the 13-year follow-up (median follow-up time 13.83, years 1997–2012; interquartile range (IQR) 0.115), the endpoints were ascertained through record linkage of the FINRISK data with the National Causes of Death Register and the National Hospital Discharge register, and they included: (a) CVD, (b) CAD, (c) MI, (d) stroke, and (e) all-cause death. The register derived endpoints have been shown to be valid indicators for cardiovascular and stroke events.[20,21]

Serum and plasma determinations

The blood sample collection was made in public health centres and other survey sites. In the invitation letter, the participants were asked to fast at least for four hours and to avoid heavy meals before the sampling. The baseline laboratory measurements included serum total and high-density lipoprotein (HDL) cholesterol,

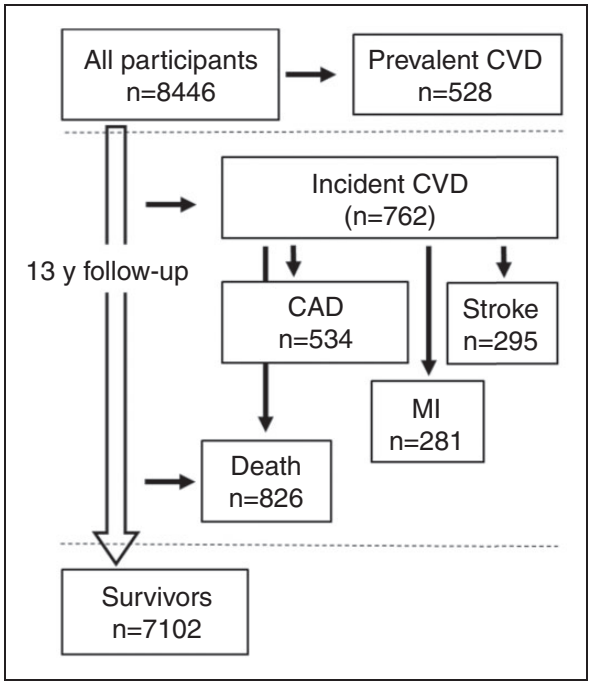

Figure 1. Study design. CAD: coronary artery disease; CVD: cardiovascular disease; MI: myocardial infarction.

triglyceride (TG), high-sensitivity CRP and γ-glutamyltransferase (γ-GT) concentrations.[22] Serum samples were taken without coagulation activators.

The baseline concentrations of MMP-8, MPO and TIMP-1 were measured according to manufacturer's instructions by following assays: serum TIMP-1 and plasma MPO, chemiluminescent micro particle immunoassay (CMIA), Abbott, Architect *i*2000 and serum MMP-8, time-resolved IFMA, Medix Biochemica, Espoo, Finland. The interassay coefficients of variation (CV%) of TIMP-1, MPO and MMP-8 assays were 3.1, 4.1 and 7.3%. For calculation of MMP-8/TIMP-1 molecular ratio, the concentrations were converted into moles using MWs 65000 and 28000 g/mol for MMP-8 and TIMP-1, respectively.

Statistical analysis

The statistical analyses were performed with SPSS 23.0 for PC, and R (http://www.R-project.org). Before the analysis CRP, MMP-8, MPO and TIMP-1 concentrations were transformed with natural logarithm due to the skewed distributions. The subjects with prevalent CVD were excluded from the analyses. The missing values were excluded from the statistical analysis. In participants with incident CVD the number of missing values for MMP-8, TIMP-1, MMP-8/TIMP-1, MPO and CRP were 12, 74, 76, 68 and 78, respectively. The significance of differences in baseline characteristics between the individuals without and with incident disease during the follow-up was tested with Mann-Whitney U-test. The differences between categorical variables were tested with Chi-square test. In all analyses, a two-sided *p*-value below 0.05 was considered statistically significant.

The associations of MMP-8, TIMP-1, MMP-8/TIMP-1, MPO and CRP concentrations with different endpoints were estimated by Cox proportional hazards model using age as the time-scale. The model was further stratified by sex and adjusted with covariates, which were selected based on current general cardiovascular risk profile presented in the Framingham Heart Study.[23] They included logarithmic total and HDL cholesterol concentration, systolic blood pressure, use of antihypertensive medication, smoking and prevalent diabetes. In our population-based sample, the geographic area has been previously recognised as an important confounder and was thus added among the covariates.[24] The socioeconomic status was monitored by years of education, which is considered an optimal parameter of this status in Finnish studies.[25] The education variable with three categories is a modified version of the standard three-level education variable, which takes into account the subjects' age and sex, thus eliminating the bias caused by changes in the education system. The assumption of proportional hazards was assessed by extending the Cox model with a time by biomarker interaction factor.

Finally, the potential performance improvement added by MMP-8 and TIMP-1 in the Framingham prediction model regarding MI and death was evaluated using the C-index, net reclassification improvement (NRI), and integrated discrimination improvement (IDI) statistics.[26] In the analyses for categorical and clinical NRI, the subjects were assigned according to the predicted risk to four groups (0–5%, 5–10%, 10–20%, > 20%) and three groups (0–5%, 5–20%, > 20%), respectively.[27] For the 13-year absolute risk predictions, we used 10 × cross validation to reduce overoptimism. The cross validation was stratified by sex. Cross-validated model calibration was tested using the Hosmer-Lemeshow goodness-of-fit test.

Results

As presented in Figure 1, 7928 subjects were free from CVD at baseline. Of them, 89.6% survived without registered endpoints, while 9.6, 6.7, 3.7 and 3.5% developed a CVD event, CAD event, stroke or MI, respectively, and 10.4% died. The baseline characteristics of individuals with and without registered events in the follow-up are presented in Table 1. Subjects with a CVD event were significantly older, and had higher BMI, systolic blood pressure and total cholesterol concentration, as well as lower serum HDL cholesterol

Table 1. Baseline characteristics of the participants with and without incident cardiovascular disease (CVD) in a 13-year follow-up.

	No CVD	Incident CVD				Death
	All ($n = 7166$)	All ($n = 762$)	CAD ($n = 528$)	MI ($n = 290$)	Stroke ($n = 375$)	All ($n = 826$)
	Mean (SD)					
Age, years	46.7 (12.9)	61.1 (9.1)	61.1 (8.7)	61.8 (8.5)	61.9 (9.4)	61.5 (10.4)
BMI, kg/m^2	26.4 (4.5)	28.5 (4.3)	28.5 (4.0)	28.8 (4.1)	28.6 (4.6)	27.7 (4.8)
Systolic bp, mm Hg	134.2 (19.1)	149.2 (21.8)	148.2 (20.2)	149.6 (20.9)	150.7 (23.6)	147.5 (22.3)
Total chol, mmol/l	5.5 (1.1)	5.9 (1.1)	5.9 (1.0)	5.9 (1.1)	5.9 (1.1)	5.7 (1.1)
HDL chol, mmol/l	1.41 (0.36)	1.25 (0.33)	1.23 (0.32)	1.23 (0.34)	1.26 (0.32)	1.32 (0.37)
Education, years	11.7 (3.9)	8.9 (3.7)	8.8 (3.6)	8.5 (3.2)	8.9 (3.8)	8.9 (3.7)
	n (%)					
Gender, male	3345 (46.7)	536 (70.3)	387 (73.3)	205 (73.0)	198 (67.1)	554 (67.1)
Current smoker	1666 (23.2)[a]	183 (24.0)	125 (23.7)[a]	73 (27.0)[a]	72 (24.4)[a]	257 (31.1)
Prevalent DM	305 (4.3)	115 (15.1)	81 (15.3)	41 (14.6)	46 (15.6)	111 (13.4)
HTN medication	840 (11.7)	109 (14.3)	78 (14.8)	52 (18.5)	41 (13.9)	110 (13.3)

bp: blood pressure; CAD: coronary artery disease; chol: cholesterol; DM: diabetes mellitus; HDL: high-density lipoprotein; HTN: hypertension; MI: myocardial infarction; SD: standard deviation.
All cases with prevalent CVD were excluded.
[a]Only non-significant (NS) differences (no CVD versus incident CVD or death) are marked (NS: $p > 0.05$).

concentration. Further, they were more often men, less educated and suffered from diabetes. A smoking habit was significantly more frequent only in those who died during the follow-up.

Baseline MMP-8, TIMP-1, MPO and CRP concentrations as well as MMP-8/TIMP-1 ratios of individuals with and without different incident outcomes were compared (Table 2). MMP-8 concentrations were significantly lower in individuals with future CVD event ($p = 0.005$) or stroke ($p = 0.003$). On the contrary, TIMP-1 levels were higher in the individuals with a future CVD outcome or death from any cause ($p < 0.001$ for all). As expected, based on these concentrations, the MMP-8/TIMP-1 ratios were significantly lower in individuals with incident CVD, CAD, stroke or death ($p < 0.001$ for all). MPO and CRP levels were significantly higher in individuals with any of the studied outcomes ($0.015 < p < 0.001$).

Table 3 presents multivariate associations of continuous values of each marker (per standard deviation (SD)) with incident CVD events and death during 13-year follow-up. Serum TIMP-1 and CRP concentrations associated significantly with increased risk for all studied endpoints. Additionally, MMP-8 concentration was associated with the risk for a CAD event and MI, and MPO concentration with the risk for CVD and stroke. The only significant association for the MMP-8/TIMP-1 ratio was observed with the risk for MI.

The largest magnitude of risk was generally associated with the CRP concentration; TIMP-1 concentration displayed higher hazard ratios (HRs) only for stroke and death. After CRP, TIMP-1 provided the largest HRs also for CVD and CAD events. Risk for MI was mainly associated with MMP-8 concentration and its ratio with TIMP-1 with hazards of the same magnitude.

The discrimination and reclassification of individuals after addition of MMP-8 and TIMP-1 to the Framingham risk score for incident MI and all-cause death is shown in Table 4. The baseline model provided C-indexes of 0.848 and 0.820 for MI and all-cause death, respectively. Calibration for all endpoints was found successful (Hosmer-Lemeshow test; $0.60 > p > 0.11$).

Neither MMP-8 nor TIMP-1 concentration significantly improved the C-index for MI. However, addition of TIMP-1 to the baseline model for MI resulted in a significant IDI (0.004; $p = 0.039$) and the inclusion of correctly reclassified MI cases in the intermediate risk category of the clinical NRI (4.6%; $p = 0.043$). MMP-8 did not produce significant NRI results for incident MI.

Inclusion of MMP-8 concentration in the model of all-cause death did not significantly affect the C-index, but inclusion of TIMP-1 improved it (difference 0.006; $p = 0.004$). Both MMP-8 (5.2%, $p < 0.001$) and TIMP-1 (12.9%, $p < 0.001$) increased the number of cases classified correctly into a higher clinical risk category.

Table 2. Baseline matrix metalloproteinase (MMP)-8, tissue inhibitor of matrix metalloproteinase (TIMP)-1, myeloperoxidase (MPO) and C-reactive protein (CRP) concentrations and MMP-8/TIMP-1 ratios in cases with and without incident cardiovascular diseases (CVDs) or death.

		MMP-8 (ng/ml)	TIMP-1 (ng/ml)	MMP-8/TIMP-1	MPO (ng/ml)	CRP (mg/l)
		Geometric mean (IQR)				
CVD	No CVD	30.8 (15.3–58.4)	86.5 (74.8–97.1)	0.35 (0.18–0.69)	14.7 (10.4–19.3)	1.14 (0.52–2.27)
	CVD	28.0 (14.0–49.1)	97.7 (83.9–110.2)	0.28 (0.14–0.53)	16.2 (11.2–21.4)	1.96 (0.91–3.88)
	p	**0.005**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
CAD	No CAD	30.6 (15.2–57.8)	86.8 (75.0–97.8)	0.35 (0.17–0.68)	14.8 (10.4–19.5)	1.16 (0.52–2.32)
	CAD	29.3 (14.2–51.5)	97.5 (84.0–109.5)	0.30 (0.14–0.55)	15.9 (11.3–20.3)	1.99 (0.92–3.96)
	p	0.201	**<0.001**	**0.001**	**0.007**	**p<0.001**
MI	No MI	30.5 (15.2–57.6)	87.1 (75.0–98.0)	0.35 (0.17–0.68)	14.8 (10.4–19.5)	1.17 (0.53–2.34)
	MI	31.4 (15.5–56.1)	99.6 (85.6–112.0)	0.31 (0.16–0.59)	16.3 (11.2–21.6)	2.20 (0.97–4.38)
	p	0.723	**<0.001**	0.251	**0.015**	**<0.001**
Stroke	No stroke	30.7 (15.2–57.7)	87.1 (75.1–98.1)	0.35 (0.17–0.68)	14.8 (10.4–19.4)	1.17 (0.53–2.35)
	Stroke	26.4 (13.9–46.1)	99.6 (84.1–113.0)	0.27 (0.14–0.50)	16.9 (11.1–22.9)	2.00 (0.88–3.87)
	p	**0.003**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Death	No death	30.6 (15.3–57.5)	86.2 (74.8–96.7)	0.35 (0.17–0.68)	14.6 (10.4–19.2)	1.13 (0.52–2.26)
	Death	30.1 (14.4–58.5)	100.2 (84.6–112.0)	0.29 (0.14–0.59)	17.0 (11.4–22.1)	2.03 (0.93–4.14)
	p	0.592	**<0.001**	**<0.001**	**<0.001**	**<0.001**

CAD: coronary artery disease; IQR: interquartile range; MI: myocardial infarction.
Mann-Whitney U-test was used for analyses. Statistically significant values ($p < 0.05$) highlighted (bold). The levels of biomarkers were converted with natural logarithm for the statistical analyses.

Table 3. Associations of baseline proteinase and inflammatory marker levels with incident cardiovascular disease (CVD) events and death.

	Multivariable adjusted HR (95% CI) per SD				
	CVD	CAD	MI	Stroke	Death
MMP-8	1.07 (0.99–1.16) $p = 0.099$	1.12 (1.01–1.23) $\mathbf{p = 0.025}$	1.22 (1.07–1.39) $\mathbf{p = 0.003}$	1.02 (0.90–1.16) $p = 0.789$	1.10 (1.01–1.19) $\mathbf{p = 0.022}$
TIMP-1	1.18 (1.09–1.28) $\mathbf{p < 0.001}$	1.15 (1.05–1.26) $\mathbf{p = 0.004}$	1.26 (1.11–1.41) $\mathbf{p < 0.001}$	1.28 (1.13–1.45) $\mathbf{p < 0.001}$	1.37 (1.27–1.49) $\mathbf{p < 0.001}$
MMP-8/TIMP-1	1.05 (0.97–1.14) $p = 0.259$	1.10 (0.99–1.21) $p = 0.072$	1.19 (1.03–1.36) $\mathbf{p = 0.014}$	1.00 (0.87–1.13) $p = 0.852$	1.06 (0.97–1.15) $p = 0.191$
MPO	1.11 (1.02–1.20) $\mathbf{p = 0.017}$	1.06 (0.96–1.16) $p = 0.278$	1.10 (0.97–1.25) $p = 0.147$	1.19 (1.04–1.36) $\mathbf{p = 0.010}$	1.19 (1.10–1.29) $\mathbf{p < 0.001}$
CRP	1.26 (1.15–1.37) $\mathbf{p < 0.001}$	1.25 (1.14–1.38) $\mathbf{p < 0.001}$	1.35 (1.18–1.56) $\mathbf{p < 0.001}$	1.25 (1.09–1.43) $\mathbf{p = 0.001}$	1.30 (1.19–1.41) $\mathbf{p < 0.001}$

CAD: coronary artery disease; CI: confidence interval; CRP: C-reactive protein; HDL: high-density lipoprotein; MI: myocardial infarction; MMP: matrix metalloproteinase; MPO: myeloperoxidase; SD: standard deviation; TIMP: tissue inhibitor of matrix metalloproteinase.
Estimated with Cox proportional hazards model using age as the time scale, stratified with sex, and adjusted with systolic blood pressure, use of antihypertensive medication, prevalent diabetes, current smoking, log (total cholesterol), log (HDL cholesterol), education (three categories) and geographical area (east/west). Statistically significant values are highlighted (bold).
The levels of biomarkers were transformed with natural logarithm for the analyses. SDs for each biomarker are as follows: lnCRP 1.11825, lnMPO 0.61527, lnMMP-8 0.97403, lnMMP-8/TIMP-1 0.22460, lnTIMP-1 0.22306.

Discussion

The present study shows that serum levels of the proteolytic enzyme MMP-8 and its inhibitor TIMP-1 are associated with increased risk for future CVD events and death in a 13-year follow-up based on a large population-based cohort. Inclusion of these biomarkers in the current general cardiovascular risk profile, the

Table 4. Improvement of discrimination and reclassification of future myocardial infarction and death by matrix metalloproteinase (MMP)-8 and tissue inhibitor of matrix metalloproteinase (TIMP)-1.

	MI		Death	
	MMP-8	TIMP-1	MMP-8	TIMP-1
C-statistics				
Reference risk score[a]	0.848	0.848	0.820	0.820
Extended risk score[b]	0.849	0.850	0.821	0.825
Difference	0.001	0.002	0.001	0.006
p-Value	0.496	0.374	0.209	**0.004**
Improvement				
IDI	0.003 (–0.0003–0.006)	0.004 (–0.0002–0.008)	0.00008 (–0.002–0.002)	0.008 (0.003–0.013)
p-Value	0.072	**0.039**	0.928	**0.001**
Categorical NRI	0.002 (–0.06–0.06)	0.003 (–0.052–0.057)	0.010 (–0.007–0.026)	0.013 (–0.016–0.042)
p-Value	0.939	0.927	0.252	0.394
Clinical NRI	0.039 (–0.01–0.09)	0.046 (0.001–0.09)	0.052 (0.025–0.079)	0.129 (0.078–0.18)
p-Value	0.127	**0.043**	<**0.001**	<**0.001**

IDI: integrated discrimination improvement; MI: myocardial infarction; NRI: net reclassification improvement.
All cases with prevalent cardiovascular disease (CVD) were excluded. Statistically significant *p*-values are highlighted (bold).
[a]Baseline models, Framingham risk factors only.
[b]Biomarker added to the baseline model. Categorical NRI includes four risk categories (0–5%, 5–10%, 10–20%, > 20%) and clinical NRI considers only the 5–20% risk category. For the 13-year absolute risk predictions 10 × cross validation stratified by sex was used.

Framingham risk score,[23] significantly improved the discrimination and reclassification of the baseline model for MI and all-cause death. By including TIMP-1 concentration in the risk prediction, more individuals previously classified to be at intermediate risk (5–20%) could be correctly reclassified: non-cases to a low-risk category (<5% risk) and cases to a higher risk category (>20% risk). The clinical NRI produced by TIMP-1 in the models for MI and mortality was moderate 4.6% ($p = 0.043$) and remarkably high 12.9% ($p < 0.001$), respectively. Despite improved prevention and treatment modalities, CVD still remains the leading cause of death and its prediction is problematic. Therefore, novel biomarkers with predictive potential which are easily obtained from blood samples would help to screen patients at risk and focus treatment strategies.

Although TIMP-1 is a potent inhibitor of several MMPs, many of its functions are independent of this action. TIMP-1 is a regulator of a broad range of biological functions, including cell growth, proliferation, apoptosis, migration and angiogenesis.[28,29] Within the heart and arterial tree, TIMP-1 is expressed by various cells, i.e. cardiac myocytes, fibroblasts, endothelial cells, smooth muscle cells and monocytes/macrophages. These properties provide the basis for, and may explain, the present results that circulating TIMP-1 concentrations are associated with all the CVD endpoints including mortality. The results we show further strengthen the findings by others. In the Framingham offspring cohort, higher plasma TIMP-1 concentrations were associated with mortality during a 10-year follow up.[30] Cavusoglu et al. described the marked association and prognostic ability of TIMP-1 levels for mortality and MI in a two-year follow-up.[17] In 1979 patients with suspected baseline CAD, TIMP-1 was shown to predict cardiovascular death.[18] TIMP-1 was also found to be an independent predictor of MI, stroke and cardiovascular death in patients with stable CAD in a case-control setting.[31] In conclusion, our study completes these findings by revealing the association of TIMP-1 with incident CAD in a population-based cohort of persons free of CAD at baseline. Amongst all biomarkers, TIMP-1 concentrations were directly associated with the highest risk for stroke and death (HR 1.28 and HR 1.37, per SD increase respectively). Elevated TIMP-1 concentrations have been reported in patients with hypertension, poor left ventricular systolic function and left ventricular hypertrophy, which are risk factors of stroke and therefore may explain the significant association.[32–34] To support the role in CAD, MI and stroke, upregulation of TIMP-1 has been shown at tissue level in coronary thrombi and ruptured plaques.[35,36]

MMPs are highly expressed by the inflammatory cells, mainly neutrophils and macrophages, in atherosclerotic lesions ubiquitously in the vascular network and recognised as prominent regulators of ECM integrity. Our very recent genome-wide (GWAS) data from similar populations further enlightens the origin of MMP-8, as complement activation, especially the alternative pathway of the cascade, slightly but clearly

seems to contribute to serum MMP-8 levels.[37] This illustrates the inflammatory aetiology and association of MMP-8 with CVD. MMP-8 is a key player in the regulation of atherosclerotic plaque phenotype and outcome. The elevated levels have been shown to correspond particularly with unstable plaque phenotype.[9,38] Peeters et al. investigated the levels of MMP-2, -8 and -9 in a follow-up study, and showed that only MMP-8 levels were predictive for cardiovascular outcome.[12] We have previously reported that high serum MMP-8 concentrations are associated with ACS as well as with incident CVD events.[13,15,39] MMP-8 is a predictor of cardiovascular outcome and left ventricular remodelling after acute MI.[40] Increased plasma MMP-8 levels have been shown to correlate with the severity of CAD, and also to associate with vulnerability of the carotid artery plaque.[9,41] In this study, MMP-8 was associated with increased risk of CAD events, MI and mortality. To the best of our knowledge, the improvement of the baseline risk model for all-cause mortality by MMP-8 has not been shown earlier. MMP-8 improved reclassification of intermediate risk individuals for all-cause death (NRI 5.2%, $p<0.001$) and with a borderline level for MI (3.9%, $p=0.1$). The present findings support and further extend the evidence that both MMP-8 and TIMP-1 are important markers of CVD events and mortality. In terms of MMP-8/TIMP-1 ratio, the analysis showed a significant association with the risk of incident MI. Our data previously showed that the MMP-8/TIMP-1 ratio is associated increased risk for CVD death.[15] Thus, the results further signifies the potential role of this imbalance in the pathology of acute cardiac events.

MPO and CRP are well known markers of inflammation, and were thus included in the analyses. MPO is mainly produced by neutrophils in order to kill phagocytosed microbial pathogens. However, it can also act as an inflammatory mediator i.e. by regulating the activity of MMPs and TIMPs.[3,42] It can contribute to vascular injury and atherosclerosis by causing oxidative damage.[43,44] It has potential prognostic value for a future cardiac event in healthy individuals and patients with CAD and ACS.[45–47] Use of both MPO and CRP in the risk assessment has been shown to improve the prediction model.[48] In our study, MPO was associated significantly only with mortality and stroke. The predictive potential of MPO has been studied by multiple groups, but the incremental value in risk assessment remains open.[49]

Higher CRP concentrations were significantly associated with all CVD events and all-cause death, similarly as observed with TIMP-1. The added improvement by CRP has been assessed previously in a large meta-analysis, and the studies using similar NRI analyses show a moderate improvement.[50] Also, its role in the causal relationship with CAD seems unlikely.[51] CRP has been shown to be elevated in conditions, such as depression and in sick building syndrome.[52,53] This illustrates its nature as a non-specific acute phase protein.

To our knowledge there is no previous study that investigates the prognostic significance of MMP-8 and TIMP-1 in such a comprehensive and representative population-based cohort. Furthermore the long follow-up of 13 years was complete with a high number of incident events. Limitations of the study need consideration as well. The population consisted only of those with Caucasian ethnicity. There are different opinions whether the use of plasma instead of serum in the analysis of MMP-8 would be optimal, since the clotting process during the serum preparation is known to release MMPs from circulating leukocytes. Therefore, measuring the proteinase from serum also reflects the potential of the neutrophils to degranulate and release it, and this degree may depend on genetic variations.[37] Comparison between different studies may thus be challenging. Nevertheless, serum and plasma MMP-8 levels have a strong correlation with each other.[15] It is also important to note that there are multiple other MMPs and several other TIMPs that can be crucial in the circuitry behind CVD pathology.

In conclusion, this study further elucidates the significance of MMP-8 and TIMP-1 in predicting future CVD events and death. Together, these markers improve prognostic power to address the cardiac risk of apparently healthy individuals.

Author contribution

VS, ASH and SB contributed to the design of the work. PJP, IK, TT, TZ, MTK and TS contributed to the acquisition, analysis or interpretation of data for the work. IK and MTN drafted the manuscript. VS, PJP and TZ critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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