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***FAST*TRACK CLINICAL RESEARCH**
ESC Hot Line: Late Breaking Registry Results 2

Prevention and epidemiology

Fibrosis as measured by the biomarker, tissue inhibitor metalloproteinase-1, predicts mortality in Age Gene Environment Susceptibility-Reykjavik (AGES-Reykjavik) Study

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Background Fibrosis is a key pathological process in many chronic inflammatory disease states.

Aims We hypothesized that tissue inhibitor metalloproteinase-1 and matrix metalloproteinase-9 (TIMP-1 and MMP-9), biomarkers of fibrosis, would predict all-cause mortality and we assessed the incremental value of these biomarkers when adjusting for clinical and other biomarkers.

Methods The cohort included 5511 community-dwelling participants in the AGES-Reykjavik Study. The baseline Cox proportional hazards regression model was based on the Framingham Risk Score variables; we added TIMP-1, MMP-9, serum high-sensitivity C-reactive protein (hsCRP), and estimated glomerular filtration rate (eGFR). The primary outcome was all-cause 10-year mortality. Cause of death was categorized as cardiovascular death (CVD), cancer death, and other causes.

Results Participants averaged 76 years and 43% were male. Ten-year mortality was 41% (2263 deaths). Of these, 915 (16.6%) died of cardiovascular disease (CVD), 543 (9.9%) with cancer, and 805 (14.6%) from other causes. For 10-year mortality, age was the strongest predictor (log likelihood $\chi^2 = 798.7$, $P < 0.0001$), followed by TIMP-1 ($\chi^2 = 125.2$, $P < 0.0001$), female gender, current smoker, diabetes mellitus, total cholesterol, eGFR (χ^2 16.7, $P < 0.0001$), body mass index, and hsCRP (χ^2 11.3, $P = 0.0008$) in that order. TIMP-1 and hsCRP had the highest continuous net reclassification improvement over the baseline model for 5-year survival [net reclassification index (NRI) 0.28 and 0.19, respectively, both $P < 0.0001$] and for 10-year survival (NRI 0.19 and 0.11, respectively, both statistically significant).

Conclusion TIMP-1 is the strongest predictor of all-cause mortality after age. The metabolic pathways regulating extracellular matrix homeostasis and fibrogenic processes appear pathologically relevant and are prognostically important.

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Introduction

Fibrosis is a key pathological outcome in many chronic inflammatory disease states.[1] The extracellular matrix (ECM) is a highly dynamic structure, constantly undergoing remodelling. Abnormal ECM dynamics play a role in deregulated cell proliferation and in excessive tissue fibrosis.[2] Ultimately, fibrosis can contribute to permanent scarring, organ malfunction, heart failure, and death.

Under normal conditions, tissue inhibitor metalloproteinases (TIMPs) and matrix metalloproteinases (MMPs) are actively involved in regulation and remodelling of the ECM.[3] Imbalances or dysregulation of TIMPs and MMPs activate fibrotic pathways. Since TIMPs and MMPs are measurable in the serum, they may be able to detect subclinical disease and may aid in risk stratification.[3,4]

The current study is part of the Age, Gene/Environment Susceptibility-Reykjavik (AGES-Reykjavik) Study which examined 5764 survivors of the original Reykjavik Study cohort from 2002–2006.[5,6] As a study focused on older, community-dwelling subjects, AGES-Reykjavik is an interesting population for addressing questions related to fibrosis and how they relate to mortality.

The specific aim of this study is to understand the prognostic significance of fibrotic processes in the AGES-Reykjavik cohort of older community-dwelling subjects. We hypothesize that the novel biomarkers TIMP-1 and MMP-9 provide prognostic information over risk factors and established biomarkers [high sensitivity C-reactive protein (hsCRP)[7–12] and estimated glomerular filtration rate (eGFR)].[13] The second aim is to assess the incremental value of the biomarkers for predicting mortality.

Methods

This study was approved by the National Bioethics Committee of Iceland, the institutional review board of the Intramural Research Program of the National Institute on Aging, and the Data Protection Authority in Iceland.

All subjects enrolled in the AGES-Reykjavik Study ($N = 5764$) were eligible for this study. We excluded subjects missing measurements of the biomarkers TIMP-1, MMP-9, hsCRP, and eGFR. We also excluded subjects missing any variables of the baseline model that define the variable within the Framingham Risk Score which included: gender, age, type 2 diabetes, smoking status (current and previous smoker), treated and untreated systolic blood pressure, total cholesterol, HDL cholesterol as well as statin use and body mass index (BMI). The final study size was 5511 participants (analysis cohort).

Blood was drawn at the initial AGES-Reykjavik characterization and citrate plasma samples were frozen and stored at -80° C. MMP-9 and TIMP-1 were measured using ELISA assay kits. C-reactive protein concentration was measured in serum with a high-sensitivity assay (Roche Diagnostics) as an established biomarker. Serum creatinine was measured and estimated eGFR was calculated.[13]

The primary study outcome was all-cause mortality at 10 years based on the Icelandic National Roster. Complete mortality follow-up for this analysis went through to December 2006. Fact and cause of death were obtained from Statistics Iceland, which classified cause of death based on a nosologist review of medical and death records. An additional endpoint in this study was 5-year all-cause mortality. We also studied cardiovascular death (CVD), cancer-related deaths and 'other' causes of death. CV-related death included International Classification of Disease-10th revision codes I10-I25, I42-I52, I61, I63-I74 (hypertensive disease, myocardial infarction, coronary artery disease, ischaemic and haemorrhagic stroke, and aneurysms), Non-CV deaths included International Classification of Disease-10th revised codes for all neoplasms and 'other' encompassed airway disease, diseases of the nervous system other than cerebrovascular diseases, disease of the digestive system, disease of the genitourinary system, mental and behavioural disorders, and other causes.[14]

Statistics

Statistical analysis was performed using R within R-Studio and IBM SPSS Statistics® statistical software. The follow-up was the time from entering the study until death or 1 January 2016, whichever came first. The Cox proportional hazards regression model was used in survival analysis to estimate the association between all the predictors in the baseline model (Framingham Risk Score variables, BMI, and statin use) and the four biomarkers with mortality, and with CVD and cancer as causes of death. Time since entering the study was used as the time scale after also considering age as a time scale and age-group stratification in the survival models. The biomarkers TIMP-1 and MMP-9 were used on the original scale and hsCRP[11] and eGFR (mL/min/1.73 m^2) were used on the log-scale. Time dependency of the biomarker estimates was inspected using Schoenfeld residual plots and testing for interaction with time. The hazard ratios (HRs) associated with each biomarker were calculated for percentile ranges and for 1 standard deviation increments in each biomarker assuming log-linear relationship with the hazard for each end point. The log-linear relationships were inspected using cubic spline functions. All cause and cause specific HRs were estimated. The likelihood ratio statistic, as a χ^2 value, from a test of significance of each biomarker in a multiple variable model, containing all the biomarkers, was used to compare the strength of the biomarkers as predictor variables. Continuous net reclassification index (NRI) and the integrated discrimination improvement (IDI) was calculated using the method of Pencina.[15] Receiver operator characteristic (ROC) analysis and comparison of the area under ROC curves was performed using the method of Blanche for time dependent area under the curve (AUC).[16] Internal validation of the AUC estimates was made using bootstrap methods.

Results

The baseline characteristics of our analysis cohort ($N = 5511$) can be seen in *Table 1*. The mean age was 76.8 years (range 68–98 years) and approximately 57% were female. Type 2 diabetes mellitus was present in 13% of the population. The mean systolic blood pressure in treated patients was 144 mmHg and 12% were current smokers. The mean body mass index was 27 kg/m^2. The all-cause mortality was 16% (886 deaths) at 5-year follow-up and mortality was 41% (2263 deaths) at 10-year follow-up.

The median and interquartile ranges for biomarkers levels are summarized in *Table 1*. Of note, the median hsCRP is close to a level used to distinguish lower risk from higher risk. Similarly, the median eGFR is close to the level used to define normal renal clearance.

Kaplan–Meier analysis of the biomarkers when stratified by percentiles showed that the highest TIMP-1 percentile category (>95th percentile) had the worst survival for all biomarkers at both 5 and 10 years (*Figure 1*) with a HR 2.32 at 10 years (*Table 2*). The percent survival for subjects with TIMP-1 in the highest percentile category was 54% at

5 years and 19% at 10 years. For hsCRP, the percent survival was 72% and 47% at 5 and 10 years for the highest percentile category and the HR was 1.29 (*Table 2*). For eGFR, there was little separation in survival for the percentiles categories corresponding to most normal renal clearance (>median, 5–25%, 25–50%); only subjects with the renal function in the worst category had significantly increased HR. For MMP-9, no percentiles showed significant differences in survival.

The multivariable regression analysis (*Table 3*) was composed of the Framingham Risk Score variables, BMI and statin use, plus the four biomarkers for predicting 5- and 10-year follow-up. The HRs for 1 standard deviation difference in the biomarkers were based on assuming log-linear relationship with the hazard of death. This assumption held well for TIMP-1 and MMP-9 but there was a hint of a J-shape both for CRP and eGFR at extreme values upper values. However, over most of the range the relationship was log-linear. The χ^2 values in *Table 3* are likelihood ratio tests and are differences in likelihood ratio statistics between a model with and without the corresponding variable. For 10-year mortality, age was the strongest multivariable predictor (log likelihood $\chi^2 = 798.7$, $P < 0.0001$), followed by TIMP-1 ($\chi^2 = 125.2$, $P < 0.0001$), current smoker, female gender, diabetes mellitus, BMI, eGFR ($\chi^2 = 16.7$, $P < 0.0001$), total cholesterol and hsCRP ($\chi^2 = 11.3$, $P = 0.0008$) in that order. However, for 5-year mortality, the top three predictors were age, TIMP-1 ($\chi^2 = 83.14$, $P < 0.0001$) and current smoker (*Table 3*).

Table 1 Demographics of the analysis cohort

Demographics	Analysis cohort ($N = 5511$) number (proportion %) or mean (SD)
Age (years)	76.81 (5.75)
Gender (females)	3166 (57.4%)
Type 2 diabetes mellitus	694 (12.6%)
Current smoker	675 (12.2%)
Previous smoker	2403 (43.6%)
Treated systolic blood pressure	143.89 [21.15]
Untreated systolic blood pressure	142.38 [20.54]
Total cholesterol	217.75 [44.87]
HDL cholesterol	61.31 [17.32]
Statin use	1221 (22.2%)
Body mass index (kg/m^2)	27.03 [4.43]
Biomarkers	Median [interquartile]
MMP-9 (ng/mL)	18.66 [15.05, 23.84]
TIMP-1 (ng/mL)	224.6 [195.14, 261.90]
hsCRP (mg/L)	1.9 [1.00, 3.90]
eGFR (mL/min/1.73 m^2)	63.92 [53.6, 74.96]

Q1, quartile 1; Q3, quartile 3; SD, standard deviation; TIMP-1, tissue inhibitor metalloproteinase-1; matrix metalloproteinase-9, MMP-9; hsCRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate.

Cause of death analysis

For the overall cohort of 5511 individuals, 3248 (58.9%) were alive at 10 years or latest follow-up while 915 (16.6%) had died of cardiovascular disease (CVD), 543 (9.9%) had died from cancer, and 805 (14.6%) had died from other causes. Thus, 2263 (41.1%) had died by the 10-year follow-up.

The relative strength of the Framingham variables and the biomarkers was examined in a multivariable Cox regression model of cause-specific mortality (*Table 4*). After age, TIMP-1 was the second strongest predictor of CVD and the third strongest predictor of cancer death and other causes of death. Current smoking was the second strongest predictor of cancer death. Diabetes was the second strongest predictor of non-cardiovascular and non-cancer related deaths.

Hazard ratios for the biomarkers for 10-year end point showed some variation depending on cause of death (*Table 5*). Based on HRs, TIMP-1 had the same strength of association with CVD death and cancer death at 10 years. hsCRP was slightly more strongly associated with cancer deaths than CVD but both were significant. Renal function, as measured by eGFR, was a moderately strong predictor of CVD and a weak but significant predictor of non-cancer/non-CVDs. MMP-9 was not significantly associated with any of the specific causes of death or all-cause mortality.

Net reclassification index and receiver operator characteristic analysis

TIMP-1 and hsCRP had the highest continuous net reclassification improvement indices when added to the baseline model for 5-year survival (NRI 0.28 and 0.19, respectively, both $P < 0.0001$, *Table 6*) and for 10-year survival (NRI 0.19 and 0.11, respectively, TIMP-1 $P < 0.0001$ and hsCRP $P = 0.013$, *Table 6*).

For TIMP-1, the NRI was dominated by an improved classification of participants who did not die within 5 years, and had almost no change in classification of participants that died (*Table 6*). There was a similar pattern for the TIMP-1 NRI regarding 10-year mortality in subjects with and without events (*Table 6*). The NRI for hsCRP improved as a result of reclassification of both participants that died or did not die by 5-year follow-up. For 10-year follow-up, the hsCRP NRI was mostly limited to survivors that were reclassified towards lower risk. For eGFR, the NRI was negatively affected among subjects that died by 5-year follow-up and was weakly significant for 10-year mortality. The overall continuous NRI for MMP-9 was not statistically significant and neither improved nor impaired re-classification.

The IDI index was used as a tool to evaluate the relative strength of variables to predict an outcome of interest. TIMP-1 had the highest IDI indices when added to the baseline model for 5-year survival (IDI 0.027, $P < 0.0001$, *Table 6*) and for 10-year survival (IDI 0.017, $P < 0.0001$) but these were relatively weak changes. For hsCRP at 5-year and 10-year survival, the IDI was significant ($P < 0.0001$ and $P = 0.0008$, *Table 3*) but modest strength.

Starting with the area under the receiver operator characteristic (ROC) curve for the baseline model (0.7349 and 0.7694 for 5-year mortality and 10-year mortality), TIMP-1 increased the AUC for both 5-year and 10-year mortality prediction over the baseline model ($P < 0.0001$ for both end points, see Supplementary material online, *Addendum Table S2*) although the magnitude of change was small. MMP-9 did not significantly change the AUC relative to the

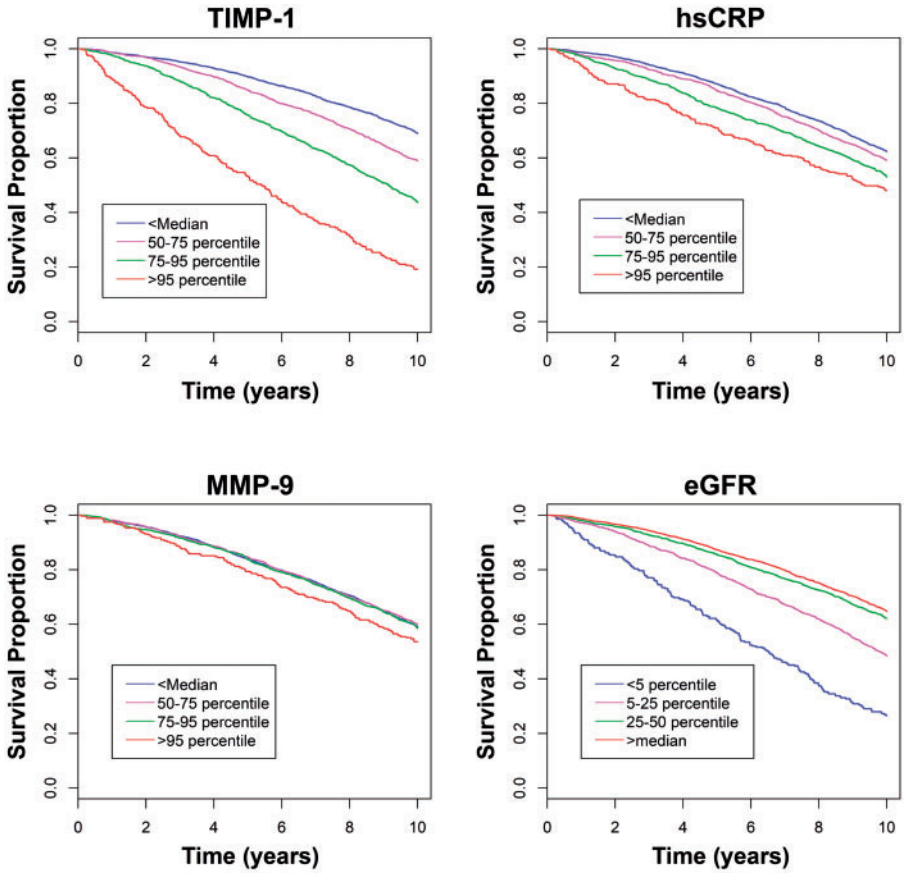

Figure 1 Kaplan–Meier curves for all-cause mortality related to biomarkers and percentiles.

Table 2 Hazard ratios from Kaplan–Meier survival analysis when categorized by percentiles

Biomarker	50–75th percentile HR (95% CI)	75–95th percentile HR (95% CI)	>95th percentile HR (95% CI)	Reference
TIMP-1	1.17 (1.05–1.30)	1.34 (1.20–1.50)	2.32 (1.97–2.74)	<median
hsCRP	1.06 (0.96–1.18)	1.27 (1.14–1.42)	1.29 (1.08–1.55)	<median
MMP-9	0.93 (0.84–1.03)	1.03 (0.92–1.15)	1.02 (0.85–1.23)	<median
Biomarker	<5 percentile HR (95% CI)	5–25th percentile HR (95% CI)	25–50th percentile HR (95% CI)	Reference
eGFR	1.42 (1.20–1.68)	1.08 (0.96–1.20)	0.92 (0.83–1.03)	>median

TIMP-1, tissue inhibitor metalloproteinase-1; matrix metalloproteinase-9, MMP-9; hsCRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate; HR, hazard ratio; CI, confidence interval.

baseline model. The change in AUC for the biomarker eGFR was borderline significant for 5-year mortality and not significant for 10-year mortality. Finally, hsCRP statistically increased the AUC for 5-year outcome but not 10-year mortality (see Supplementary material online, *Addendum Table S2*) but the magnitude of change was small. The bias in the AUC estimates was found to be less than 1%.

The calibration of the baseline model and other models were assessed (see Supplementary material online, *Addendum Figure S1*).

Addendum Figure demonstrates 2 Kaplan Meier Curves for all-cause mortality related to biomarkers and quartiles. Addendum Table 1 demonstrates the hazard ratios for the survival analysis based on quartiles.

Beyond pre-specified analyses, we added Troponin-T (TnT) and Troponin-I (TnI) to our multivariable model that included all risk factors and the other biomarkers (see Supplementary material online, *Addendum Table S3*). TIMP-1 remained a strong and significant predictor all-cause mortality and CV mortality at 10 years

Table 3 The Cox proportional hazards regression model was based on the Framingham Risk Score variables, BMI, statin use, plus the biomarkers TIMP-1, MMP-9, hsCRP, and eGFR

	Five-year multivariable analysis			Ten-year multivariable analysis		
	Wald χ^2	Hazard ratio (95% CI)	*P*-value	Wald χ^2	Hazard ratio (95% CI)	*P*-value
Baseline model (Framingham variables + BMI and statin use)						
Age (5 years)	247.1	1.66 (1.56–1.76)	<0.0001	798.7	1.79 (1.73–1.87)	<0.0001
Gender	15.0	0.73 (0.63–0.85)	<0.0001	41.4	0.72 (0.65–0.80)	<0.0001
Type 2 diabetes mellitus	12.2	1.40 (1.17–1.67)	<0.0001	29.2	1.39 (1.24–1.56)	<0.0001
Current smoker	18.2	1.58 (1.28–1.94)	<0.0001	58.1	1.67 (1.46–1.90)	<0.0001
Previous smoker	1.2	1.10 (0.94–1.27)	0.5	4.0	1.11 (1.01–1.21)	0.42
Treated systolic blood pressure (20 mmHg)	5.0	0.94 (0.88–1.00)	0.05	1.8	0.98 (0.94–1.02)	<0.05
Untreated systolic blood pressure (20 mmHg)	5.4	0.92 (0.86–0.99)	<0.001	1.9	0.97 (0.93–1.02)	0.05
Total cholesterol (50 mg/dL)	4.1	0.93 (0.85–1.02)	<0.001	11.6	0.90 (0.85–0.96)	<0.0001
HDL cholesterol (20 mg/dL)	2.1	0.94 (0.86–1.03)	<0.001	0.5	0.99 (0.94–1.05)	0.05
Statin use	4.9	0.82 (0.68–1.01)	<0.01	5.2	0.88 (0.78–0.99)	<0.05
Body mass index (kg/m^2)	18.6	0.96 (0.95–0.98)	<0.0001	25.6	0.97 (0.96–0.98)	<0.0001
Biomarkers[a]						
MMP-9 (1 SD)	0.1	1.01 (0.95–1.07)	0.80	1.1	1.02 (0.98–1.06)	0.31
TIMP-1 (1 SD)	83.1	1.32 (1.24–1.40)	<0.0001	125.2	1.28 (1.22–1.33)	<0.0001
Log-hsCRP (1 SD)	22.1	1.17 (1.10–1.26)	<0.0001	11.3	1.08 (1.03–1.12)	0.0008
Log-eGFR (1 SD)	9.0	0.91 (0.85–0.97)	0.003	16.7	0.91 (0.87–0.95)	<0.0001

Age and TIMP-1 were the two strongest multivariable predictors of all-cause mortality when adjusting for baseline model and other biomarkers at both 5 and 10 years of follow-up.
TIMP-1, tissue inhibitor metalloproteinase-1; matrix metalloproteinase-9, MMP-9; hsCRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate; CI, confidence interval.
[a]Both AGE and TIMP-1 are statistically significant ($P < 0.0001$) at 5 and 10 year mortality.

Table 4 Relative strength of Framingham variables and the biomarkers for cause-specific death as assessed in multivariable Cox regression models

All cause death			Cardiovascular death			Cancer death			Other death		
Variable	χ^2	*P*-value	Variable	χ^2	*P*-value	Variable	χ^2	*P*-value	Variable	χ^2	*P*-value
Age	798.7	<0.0001	Age	513.7	<0.0001	Age	49.0	<0.0001	Age	310.4	<0.0001
TIMP-1	**125.2**	**<0.0001**	**TIMP-1**	**67.9**	**<0.0001**	Current Smoker	39.3	<0.0001	Diabetes	29.0	<0.0001
Current Smoker	58.1	<0.0001	Gender	38.9	<0.0001	**TIMP-1**	**37.7**	**<0.0001**	**TIMP-1**	**22.7**	**<0.0001**
Gender	41.4	<0.0001	**eGFR**	**25.2**	**<0.0001**	**hsCRP**	**11.7**	**0.0006**	BMI	18.1	<0.0001
Diabetes	29.2	<0.0001	Current Smoker	21.2	<0.0001	Gender	10.2	0.0014	Total Cholesterol	15.0	0.0001
BMI	25.6	<0.0001	BMI	7.7	0.0054	Prior Smoker	8.1	0.0044	Statins	10.4	0.0012
eGFR	**16.7**	**<0.0001**	**hsCRP**	**6.8**	**0.0092**	BMI	2.2	0.14	**eGFR**	**6.6**	**0.01**
Total cholesterol	11.6	0.0007	Diabetes	5.6	0.018	**eGFR**	**2.2**	**0.14**	Current Smoker	6.3	0.01
hsCRP	**11.3**	**0.0008**	Hypertension	2.1	0.15	Diabetes	2.2	0.14	**MMP-9**	**3.1**	**0.08**
Statin use	5.2	0.0222	HDL	1.1	0.30	Statins	1.7	0.19	Gender	2.8	0.10
Prior smoker	4.0	0.045	Total Cholesterol	0.7	0.40	Hypertension	1.4	0.24	Prior Smoker	2.7	0.10
Hypertension	1.9	0.16	Statin Use	0.4	0.51	Total Cholesterol	1.3	0.25	HDL	0.2	0.63
MMP-9	**1.1**	**0.31**	Prior Smoker	0.2	0.68	HDL	0.4	0.51	Hypertension	0.0	0.95
HDL	0.5	0.47	**MMP-9**	**0.1**	**0.77**	**MMP-9**	**0.1**	**0.77**	**hsCRP**	**0.0**	**0.99**

'Bold print' demonstrates how the biomarkers ranked for cause specific mortality when compared to the baseline model.
TIMP-1, tissue inhibitor metalloproteinase-1; MMP-9, matrix metalloproteinase-9; hsCRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate.

Table 5 Hazard ratios for all-cause and cause-specific death with 95% confidence interval and Wald χ^2 values for 10-year follow-up

Ten-year multivariable analysis of all cause and cause specific mortality (the model includes biomarkers and Framingham variables, BMI, and statin use)				
Cause of death	**TIMP-1 HR (95% CI)**	**Log-hsCRP HR (95% CI)**	**Log-eGFR HR (95% CI)**	**MMP-9 HR (95% CI)**
All cause	1.28 (1.22–1.33)	1.07 (1.03–1.12)	0.91 (0.87–0.95)	1.02 (0.98–1.06)
CVD	1.31 (1.23–1.39)	1.10 (1.02–1.17)	0.85 (0.79–0.90)	0.99 (0.93–1.06)
Cancer	1.31 (1.20–1.43)	1.16 (1.07–1.27)	1.08 (0.98–1.19)	1.01 (0.93–1.10)
Other	1.21 (1.12–1.30)	1.00 (0.93–1.08)	0.90 (0.84–0.98)	1.06 (0.99–1.13)
Cause of death	**TIMP-1 Wald χ^2 (*P*-value)**	**Log-hsCRP Wald χ^2**	**Log-eGFR Wald χ^2**	**MMP-9 Wald χ^2**
All cause	125.2 (*P*<0.0001)	11.3 (*P* = 0.0008)	16.7 (*P*<0.0001)	1.1 (*P* = 0.31)
CVD	67.9 (*P*<0.0001)	6.8 (*P*<0.0092)	25.2 (*P*<0.0001)	0.1 (*P* = 0.77)
Cancer	37.7 (*P*<0.0001)	11.7 (*P* = 0.0006)	2.2 (*P* = 0.14)	0.1 (*P* = 0.76)
Other	22.7 (*P*<0.0001)	0.0 (*P* = 0.996)	6.6 (*P* = 0.01)	3.1 (*P* = 0.07)

Hazard ratios per 1 SD difference in each variable as assessed by multivariable Cox regression models.
BMI, body mass index; SD, standard deviation; HR, hazard ratio; TIMP-1, tissue inhibitor metalloproteinase-1; MMP-9, matrix metalloproteinase-9; hsCRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate; CVD, cardiovascular death.

Table 6 Net reclassification index and integrated discrimination improvement statistics for individual biomarkers over Framingham risk variables, body mass index, and statin use

	Five-year mortality			**Ten-year mortality**		
	Index	**95% CI**	***P*-value**	**Index**	**95% CI**	***P*-value**
TIMP-1						
NRI	0.28	0.21–0.36	<0.0000	0.19	0.14–0.26	<0.0000
NRI for events	0.02	-0.04 to 0.08		0.01	-0.03 to 0.04	
NRI for non-events	0.27	0.22–0.30		0.19	0.15–0.23	
IDI	0.027	0.019–0.036	<0.0000	0.02	0.01–0.02	<0.0000
hsCRP						
NRI	0.19	0.12–0.28	<0.0000	0.11	0.05–0.17	0.013
NRI for events	0.07	0.02–0.15		0.01	-0.02 to 0.04	
NRI for non-events	0.12	0.08–0.16		0.10	0.06–0.14	
IDI	0.006	0.002–0.01	<0.0000	0.003	0.001–0.007	0.003
eGFR						
NRI	0.12	0.04–0.20	0.0014	0.07	0.01–0.13	0.02
NRI for events	-0.05	-0.12 to 0.01		-0.05	-0.08 to - 0.02	
NRI for non-events	0.17	0.13–0.22		0.12	0.08–0.16	
IDI	0.12	0.007–0.02	<0.0000	0.007	0.004–0.01	<0.0000
MMP-9						
NRI	0.01	-0.07 to 0.07	0.8590	0.01	-0.06 to 0.06	0.87
NRI for events	-0.27	-0.33 to - 0.20		-0.25	-0.28 to - 0.20	
NRI for non-events	0.27	0.23–0.31		0.25	0.19–0.28	
IDI	0.0006	-0.1 to 0.08	0.32	0.0004	-0.0001 to 0.001	0.17

NDI, net reclassification index; IDI, integrated discrimination improvement.

after adding TnT and TnI to the multivariable model. The effect size of TIMP-1 was attenuated by 20% for all-cause mortality and 23.9% for CVD mortality. The χ^2 for TnT and TIMP-1 for 10-year all-cause mortality and for CVD mortality were all individually significant but comparisons between TnT and TIMP-1 were not statistically significant.

Discussion

In this study, TIMP-1 is the strongest predictor of all-cause mortality in the AGES-Reykjavik cohort after age when also considering the variables used to define the Framingham Risk score and statin use. The biomarker, hsCRP, was also a strong predictor of 5-year

all-cause mortality but a weaker predictor of 10-year risk. The metabolic pathways regulating homeostasis in the ECM appear pathologically relevant and fibrogenic processes are prognostically important in this Icelandic population older than 66 years of age.

Prior studies have correlated markers of fibrosis with inflammation[17] or cardiovascular risk factors.[18] Hansson *et al.*[19] demonstrated that higher levels of circulating MMP-9 or TIMP-1 were associated with higher risk of death and higher TIMP-1 levels were related to higher risk of stroke and cardiovascular mortality. TIMP-1 was a predictor for future CVD in patients with known CAD in two other studies.[20,21]

The four TIMPs are the natural endogenous inhibitors of the matrix metalloproteinases (MMPs), of which 22 MMPs are found in humans. Of the TIMPs, TIMP-1 is relatively unique as being present in the plasma and thus accessible as a biomarker. The MMPs regulate the turnover of ECM by regulation of bioactive molecules, chemokines, and growth factors. There are more than 20 zinc (II)-dependent metalloproteinases (MMPs). In our study, we focused on the gelatinase (MMP-9), which regulates pathological remodelling processes that involve inflammation and fibrosis, since TIMP-1 closely associates with MMP-9 and inhibits the protease. Since MMP-9 regulates fibrosis in the extracellular space, it is possible that the circulating levels of MMP-9 do not reflect the biological activity in this population as well as tissue levels or tissue activity.

The fibrotic process is regulated and may be reversible.[22] Initiation of fibrogenesis involves injury and inflammatory activation of monocytes and chemokines that result in profibrotic macrophages. TGF-β plays a central role in activating and promoting proliferation of myofibroblasts. Two processes regulate progression towards healing of fibrosis. Monocyte-derived macrophages play important roles in the resolution of hepatic fibrosis.[23] In addition, MMP-13, MMP-9, TRAIL (TNF-related apoptosis-inducing ligand), and low levels of TIMP-1 result in degradation of the ECM (i.e. scar resolution). Excess levels of TIMP-1inhibit MMPs and result in ECM deposition and fibrotic scar.

There are multiple potential targets for treatment of fibrosis.[1,24] Twenty-five potential treatments to modulate fibrosis are already in clinical trials.[1] Four of these medications are already approved for use in patients, albeit for other indications than modulating fibrosis. Our Kaplan–Meier survival analysis suggests there may be an opportunity to treat patients that is several years in duration. Even the most severe elevations in TIMP-1, it took many years to reach 50% mortality.

In the absence of a specific currently recognized disease, targeting inflammation might have significant effects on the fibrotic process. Acute and chronic inflammatory reactions play an important part in triggering fibrosis in many organ systems. Future clinical trials might take an approach comparable to the JUPITER trial.[11] Statins, anti-inflammatory, or anti-fibrotic agents might be logical choices to reduce fibrosis.

Elevated TIMP-1 is not specific for CVD. TIMP-1 and fibrotic pathways appear important for cancer and potentially other diseases. TIMP-1 and inhibition of MMP-9 may influence anti-apoptotic activity in cancer, tumour growth, angiogenesis, and tissue invasion.[25] TIMP-1 promotes cell proliferation and in many forms of diverse cancers. TIMP-1 is overexpressed in several types of human cancers.[26] As a cytokine and key regulator of ECM degradation, TIMP-1 has multiple functions associated with the tumour micro-environment and cancer progression. Higher levels of TIMP-1 expression in patients with TNBC patients were associated with a poor prognosis.[26] Inhibiting TIMP-1 prevented tumour growth in mice.

Per the European Society Cardiology Guidelines on CVD prevention,[27] circulating and urinary biomarkers have no or only limited value when added to the SCORE system and state that hsCRP contributes little to CV risk assessment.[27] The guidelines do not address TIMP-1 which is a stronger predictor of all-cause mortality and CVD death than hsCRP.

Limitations

Our cohort focused on an aging population. This Icelandic cohort is a Caucasian or European population and thus may not reflect other ethnicities.

Despite being the strongest predictor of mortality after age in this study, the NRI, IDI, and AUC statistics need to be interpreted cautiously. With a large sample size, small changes in re-classification become detectable with high-statistical confidence. Positive likelihood ratios assess the relative strength of elements within the multivariable models.

It is prudent to consider fibrotic pathways important from a pathophysiological perspective. Further work will be needed to understand the best way to use the biomarker or whether the biomarker may help select patients that might benefit from fibrosis modifying therapies.

The concept of treatment of fibrosis based on TIMP-1 or other similar biomarkers will need to be proven by clinical trials. Even with these cautions, it is still quite intriguing that TIMP-1 is as powerful or more powerful predictor of mortality than the other biomarkers and patient characteristics studied.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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