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α1-Antichymotrypsin Complex (SERPINA3) Is an Independent Predictor of All-Cause but Not Cardiovascular Mortality in Patients Hospitalized for Chest Pain of Suspected Coronary Origin

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Keywords
α1-antichymotrypsin complex (SERPINA3) · Biomarkers · Acute coronary syndrome · Cardiovascular disease events · Mortality

Abstract
Introduction: SERPINA3 is an acute-phase protein triggered by inflammation. It is upregulated after an acute myocardial infarction (AMI). Data on its long-term prognostic value in MI patients are scarce. We aimed to assess the utility of SERPINA3 as a prognostic marker in patients hospitalized for chest pain of suspected coronary origin. ***Methods:*** A total of 871 consecutive patients, 386 diagnosed with AMI, were included. Stepwise Cox regression models, applying continuous $\log_e$-transformed values, were fitted for the biomarker with all-cause mortality and cardiac death within 2 years or all-cause mortality within the median 7 years as dependent variables. An analysis of MI and stroke, and combined endpoints, respectively, was added. The hazard ratio (HR) (95% CI) was assessed in a univariate and multivariable model. ***Results:*** Plasma samples from 847 patients were available. By 2-year follow-up, 138 (15.8%) patients had died, of which 86 were cardiac deaths. The univariate analysis showed a significant association between SERPINA3 and all-cause mortality (HR 1.41 [95% 1.19–1.68], $p < 0.001$) but not for cardiac death. Associations after adjustment were non-significant. By 7-year follow-up, 332 (38.1%) patients had died. SERPINA3 was independently associated with all-cause mortality from the third year onward. The HR was 1.14 (95% CI, 1.02–1.28), $p = 0.022$. Similar results applied to combined endpoints, but not for MI and stroke, respectively. The prognostic value of SERPINA3 was limited to non-AMI patients. No independent associations were noted among AMI patients. ***Conclusions:*** SERPINA3 predicts long-term all-cause mortality but fails to predict outcome in AMI patients.

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Introduction

The serine protease inhibitor, alpha-1 antichymotrypsin (SERPINA3 or ACT), a member of the serpin superfamily of proteinase inhibitors [1], is involved in a wide range of physiological and pathophysiological processes, such as inflammation, extracellular matrix remodeling, and thrombogenesis [2, 3]. SERPINA3 consists of 423 amino acids and is postranslationally modified by glycosylation, by which it may change its mode of action [4, 5]. It inhibits a wide range of serine proteases, such as chymotrypsins, cathepsin G, and mast cell chymase [1, 4–6]. Of these, cathepsin G is of particular interest. It is contained in neutrophil granules and is released at sites of inflammation, where it activates inflammatory cytokines [2] and protease-activated receptor 4 (PAR-4) [3], initiating platelet aggregation [3]. It also contains a DNA-binding domain and inhibits DNA polymerase, thereby decreasing DNA synthesis, as reflected by inhibition of cellular growth, proliferation, and differentiation [5, 7].

Based on its role in various pathogenic pathways, SERPINA3 has been implicated in the pathology of several disorders, including various types of cancer, neurodegenerative diseases such as Alzheimer's disease, and autoimmune disorders like lupus nephritis [1–3]. To what extent SERPINA3 may influence outcomes in coronary heart disease has not been fully elucidated. Clinically, there are reports with small patient numbers claiming that SERPINA3 may be a predictor of survival in subjects with acute myocardial infarction (AMI) [8] and in a cohort of chronic heart disease [9]. The role of SERPINA3 in coronary heart disease as a marker of outcome and potentially also as a mediator is, however, not clear.

The current study was designed to disclose any existing association between SERPINA3 and outcomes in coronary heart disease in a chest pain population with clinically suspected ACS. Two subpopulations, with and without an AMI, employing a troponin T (TnT) cut-off level of 50 ng/L, were studied separately to assess the influence of an acute TnT response on the prognostic utility of SERPINA3. The patients had a follow-up period of 2 and 7 years, respectively, with respect to pre-defined outcomes involving all-cause mortality, myocardial infarction (MI), stroke, and to cardiac death during the first 2 years.

Materials and Methods

Study Design and Patient Population

Unselected patients presenting with chest pain of suspected coronary origin were recruited in "Risk Markers in the Acute Coronary Syndrome (RACS)" (ClinicalTrials.gov Identifier: NCT00521976) [10, 11]. They were prospectively followed up for 2 years and retrospectively for additional 5 years. The total material in the RACS study consisted of 871 patients consecutively admitted to Stavanger University Hospital, Norway, from November 2002 until September 2003. Exclusion criteria were age <18 years, unwillingness or incapacity to provide informed consent, and prior inclusion. Patients were sub-classified as having sustained an AMI or not by their TnT level at baseline and 6 h after admission, respectively, employing a cut-off value of 50 ng/L. Patients with a TnT value ≤50 ng/L were classified as having no MI. Coronary angiography during hospitalization was performed in 268 patients, among whom percutaneous coronary intervention (PCI) was performed in 160 patients (10). The TnT-negative group consisted essentially of observational cases, not requiring invasive diagnostics. Correlation studies included high-sensitivity C-reactive protein (hsCRP), pentraxin 3 (PTX3), and B-type natriuretic peptide (BNP) which had previously been studied in the same population [11].

Admission levels of SERPINA3 were available in 847 patients. The flow chart is shown in Supplemental Figure 1 (for all online suppl. material, see https://doi.org/10.1159/000537919). Follow-up was performed at 2 years and median 7 years. Separate endpoints consisted of all-cause mortality, cardiac mortality, MI, and stroke, respectively. Cardiac mortality was recorded up to 2 years, whereas the other endpoints were recorded during the entire follow-up period. Combined endpoints consisted of cardiac mortality, MI, or stroke. The definition of cardiac mortality included death preceded by a definitive MI or by chest pain >20 min in those without a given TnT or a history of ischemic heart disease and no other obvious cause of death [12]. Clinical follow-up data were obtained from hospital and public registries and by telephone interview at 30 days, 6, 12, 24, and 84 months, and, if needed, additional information was obtained from general practitioners and nursing homes [10, 11]. Retrospective follow-up information on mortality beyond 2 years was essentially provided from death registries, and limited to all-cause mortality [11], as retrospective information on cardiac causes during a period of 5 years would be cumbersome and less precise. Baseline data including information on demographics, smoking habits, clinical history, and general laboratory characteristics were collected at hospital admission.

Ethics

Written informed consent was obtained from all patients. The RACS study was approved by the Regional Board of Research Ethics and by the Norwegian Health Authorities and was conducted in accordance with the Helsinki Declaration of 1971, as revised in 1983.

Blood Sampling Procedures and Laboratory Measurements

Blood was drawn by direct venepuncture of an antecubital vein immediately following hospital admission, applying a minimum of stasis. A second blood sample for measurement of TnT was drawn 6–7 h later. Blood samples were centrifuged for 15 min at 2,000 *g* at 20°C and stored at −80°C. Measurement in serum of TnT, estimated glomerular filtration rate (eGFR), glucose, and lipids were performed immediately after centrifugation at the laboratory for Medical Biochemistry at Stavanger University Hospital. Multiple aliquots of ethylene diamine tetra-acetic acid (EDTA) plasma, citrated plasma, and serum were frozen and stored at −80°C for later measurements.

SERPINA3 Measurements

SERPINA3 (given in arbitrary units) was analyzed in duplicate in plasma from 847 patients using antibodies from Abnova (Taipei, Taiwan) diluted as described by the producer in a 384-well format using a combination of a SELMA (Jena, Germany) pipetting robot and a BioTek (Winooski, VT, USA) dispenser/washer. Absorption was read at 450 nm with wavelength correction set to 540 nm using an ELISA plate reader (BioTek). Intra- and inter-assay coefficients of variation (CV) were <10%. EDTA blood was lacking in 65 patients and was replaced by citrated blood. Based on data from our own laboratory, measurements in EDTA and citrated plasma did not differ significantly.

TnT Measurements

The diagnoses were established 2 decades ago with a less sensitive modified second-generation troponin T assay, and we used a rule-in-rule-out MI protocol with TnT measurements at baseline and 6 h. The assay was based on a high-affinity cardiac-specific TnT isoform antibody (Roche Diagnostics, Germany), which completely abolished the non-specific binding to skeletal muscle known to occur with the first-generation TnT assay [13, 14]. As compared to the conventional second-generation TnT assay used at that time, the performance of a locally modified assay was considerably increased by employing biotin-streptavidin-coated microplates on an Elecsys TM Analyzer [15, 16]. The lower detection limit of the locally modified assay was 10 ng/L, with a cut-off level for MI of 50 ng/L and a coefficient of variation (CV) of 10% [16].

hsCRP, PTX3, and BNP

Measurements of hsCRP, PTX3, and BNP in this patient material have previously been reported [10] and performed as follows: hsCRP was measured in serum using an immunoturbidimetric assay (Tina-quant® C-reactive protein (latex) high sensitive assay, Roche Diagnostics, Germany) performed on a Roche automated clinical chemistry analyzer (MODULAR P). The detection limit was 0.03 mg/L and the measuring range was 0.1–20.0 mg/L, with an extended measuring range with automatic re-run of 0.1–300 mg/L. The between-assay CV was 3.45% at 1.19 mg/L and 2.70% at 0.43 mg/L [10].

PTX3 was analyzed in EDTA plasma using a human PTX3 ELISA kit (Perseus Proteomics Inc., Tokyo, Japan), performed on a BEP III analyzer (Dade Behring, Germany). The PTX3 concentration was measured linearly between 0.1 and 20 ng/mL. The intra- and inter-assay CV were 2.9% at 0.67 ng/mL and 0.8% at 15.91 ng/mL, and 1.8% at 0.66 ng/mL and 4.1% at 15.95 ng/mL, respectively [10].

BNP was analyzed in EDTA plasma using the Microparticle Enzyme Immunoassay (MEIA) Abbott AxSYM® (Abbott Laboratories, Abbott Park, Illinois, USA). The dynamic range was 0–4,000 pg/mL, and the within-run CV was 6.3% at 95 pg/mL and 4.7% at 1,587 pg/mL (10).

Statistical Analysis

Descriptive statistics are presented as medians with an interquartile range (25th–75th percentile) for continuous data and as numbers and percentages for categorical data. Differences in baseline characteristics were assessed by the Kruskal-Wallis test for continuous data and the χ^2 test for categorical data. Due to a skewed distribution, SERPINA3, hsCRP, PTX3, BNP, and eGFR levels were logarithmically transformed to the base-e ($\log_e$) prior to analysis of continuous values and normalized by dividing by the standard deviation (SD). Pearson's correlation coefficient was calculated to identify a possible relation between the admission level of biomarkers.

Patients were divided into quartiles (Q1-4) according to their SERPINA3 concentrations. The Kaplan-Meier product limits were used for plotting the times to event, and the log-rank test was used to test for the equality of the survival curves. Cox regression models, applying both quartiles and continuous $\log_e$-transformed values, were fitted for SERPINA3 with the clinical endpoints as the dependent variables within the defined follow-up periods. In multivariable analysis applying a forced-entry method, risk factors, as shown in the baseline tables, were adjusted for. For continuous $\log_e$-transformed values, we employed hazard ratio (HR) and 95% CI per SD increase of the biomarker. The HRs presented in the results section are 1-SD on the log scale. Subgroup analyses were performed for patients with or without TnT release above 50 ng/L at index hospitalization. The interaction between SERPINA3 and hsCRP was analyzed by fitting a Cox model with their quartiles plus their interaction term for each of the endpoints.

Statistics were performed using the statistical package SPSS version 25 (IBM Corp., Armonk, NY, USA). All tests were 2-sided with a significance level of 5% without multiplicity adjustment.

Results

Characteristics of the Study Population

Exclusions during recruitment are shown in online supplementary Figure 1. No patient was lost to follow-up. Of the 871 subjects included, baseline samples were available in 847 individuals. Median follow-up was 7 years. A peak TnT concentration exceeding 50 ng/L was recorded in 386 patients (44.3%) at inclusion, whereas 485 patients (55.7%) had no or a minor TnT release up to 50 ng/L. The baseline characteristics of the total patient population and for quartiles of SERPINA3 are shown in Table 1, whereas online supplementary Tables 1 and 2, respectively, show the baseline characteristics of the AMI patients and those without AMI, respectively. hsCRP followed the same trend as SERPINA3 across the quartiles ($p < 0.001$). An increase in BNP ($p < 0.001$) and a decrease in eGFR ($p = 0.008$) were noted in the total material, with similar trends for AMI patients.

As shown in online supplementary Table 3, in the study group as a whole, baseline levels of SERPINA3 were significantly correlated with PTX3 ($r = 0.282$) and BNP ($r = 0.203$), and particularly with CRP ($r = 0.433$). No significant interaction was observed between SERPINA3 and CRP at any of the endpoints measured.

The results of the univariate and multivariable analyses are presented for 2-year and 7-year follow-up and include (1) all patients, (2) AMI patients, and (3) patients without AMI,

employing separate and combined endpoints. Tables containing results show the total number of patients, the number of patients studied, the number of patients in whom complete information was available for multivariable analysis, the number of events, and the event rates of patients studied.

2-Year Follow-Up on Separate Endpoints

In the univariate analysis for the total patient population at 2 years (online suppl. Table 4A), SERPINA3 remained strongly associated with all-cause mortality ($p < 0.001$), but not with cardiac death ($p = 0.18$), whereas an association between SERPINA3 and MI ($p = 0.016$) and stroke ($p = 0.006$) was noted. However, after adjustment for confounders, only the association with stroke remained significant ($p = 0.035$). The Kaplan-Meier curves comparing quartile levels of SERPINA3 for all-cause mortality and CV mortality at 2 years are depicted in Figure 1.

For the AMI patients, no significant associations were found between SERPINA3 and all-cause mortality, cardiac death, MI, or stroke either, in univariate or multivariable analysis (online suppl. Table 4B). In patients without AMI (online suppl. Table 4c), however, the univariate analysis showed a positive association between SERPINA3 and all-cause mortality ($p < 0.001$), also after adjusting for confounders ($p = 0.023$). The univariate, but not multivariable, analysis also revealed a positive association with stroke ($p = 0.028$), whereas the associations of SERPINA3 with other endpoints remained statistically non-significant (online suppl. Table 4C).

Seven-Year Follow-Up on Separate Endpoints

At 7 years of follow-up (Table 2), the positive association between SERPINA3 and all-cause mortality in the patients as a whole remained highly significant in the univariate analysis ($p < 0.001$), and the association with MI was strengthened ($p = 0.008$), whereas the association with stroke was no longer present ($p = 0.13$). An independent positive association between SERPINA3 and all-cause mortality was already noted in the third year of follow-up ($p = 0.042$) and remained significant at 7-year follow-up ($p = 0.022$). No independent association was noted between SERPINA3 and MI and stroke, respectively.

In the AMI patients, SERPINA3 was found to be significantly associated with all-cause mortality ($p < 0.001$) in univariate analysis but not in the multivariable analysis. No significant associations were found with MI or stroke (online suppl. Table 5).

In subjects without AMI, an independent association with all-cause mortality was observed from the second year of follow-up (HR 1.34 [95% CI: 1.04–1.73], $p = 0.023$). By 7 years (online suppl. Table 6), a positive association was noted between SERPINA3 and all-cause mortality in both univariate ($p < 0.001$) and multivariable analyses ($p = 0.004$) in these patients. Except for a significant association with MI in univariate analysis ($p = 0.027$), no other significant associations with outcome were revealed.

Analysis of Combined Endpoints

In the univariate analysis at 2 years, the association between SERPINA3 and the combined endpoint of cardiac mortality, MI, or stroke in the total population was statistically significant (HR 1.31 [95% CI: 1.15–1.50], $p < 0.001$) but not in the adjusted analysis (HR 1.12 [95% CI: 0.97–1.28], $p = 0.12$).

Second-year results in subgroups are shown in Table 3A. A borderline significant association between SERPINA3 and the combined cardiac endpoint ($p = 0.048$) was noted at 2-year follow-up in the univariate analysis of the AMI patients but was no longer evident after adjustment. A significant univariate association was demonstrated in patients without AMI ($p = 0.002$), although it weakened after adjusting for confounders ($p = 0.049$).

Table 3B shows the univariate and multivariable association between SERPINA3 and the combined endpoint of all-cause mortality, MI, or stroke at 7-year follow-up in AMI and in patients without AMI, respectively. Whereas the univariate association was significant in both populations, it remained significant after adjustment only in patients without AMI ($p = 0.007$).

Discussion

In this follow-up study through 7 years, SERPINA3 was found to be independently associated with all-cause mortality from the second year onwards until the end of the study in patients without AMI, but not in AMI patients. Cardiac mortality was followed prospectively for up to 2 years, and during this period no association was found between SERPINA3 and cardiac death after adjusting for confounders in either AMI or in patients without AMI. Furthermore, in the multivariable analysis, no association was found between SERPINA3 and MI at 2 and 7 years, respectively. Taken together, our findings do not suggest an independent association between SERPINA3 and coronary atherothrombotic events, especially not in AMI as defined by a TnT value >50 ng/L. However, in patients without AMI, SERPINA3 was independently associated with total mortality, suggesting a prognostic impact of SERPINA3 in other subgroups of patients presenting in the emergency department with chest pain.

Table 1. Baseline characteristics in 871 chest pain patients with suspected coronary origin, arranged in quartiles (Q) of SERPINA3, measured in arbitrary units (AU)

	Quartile 1 (n = 211)	Quartile 2 (n = 212)	Quartile 3 (n = 212)	Quartile 4 (n = 212)	p value	Total (n = 847)
SERPINA3 (AU)	39.3 (35.0–41.6)	47.9 (45.6–50.0)	56.6 (54.4–59.0)	69.1 (65.5–76.2)	<0.001[b]	51.8 (43.4–62.4)
Age, years	71.0 (56.3–79.9)	70.2 (57.1–79.2)	71.8 (59.6–80.7)	76.2 (63.4–83.4)	<0.001[b]	72.6 (59.0–81.1)
Risk markers at baseline						
Male gender	133 (63.03)	128 (60.38)	138 (65.09)	120 (56.60)	0.308[a]	519 (61.28)
hsCRP mg/L	2.4 (1.2–4.4)	2.8 (1.2–6.9)	5.1 (2.1–16.2)	12.3 (3.7–33.0)	<0.001[b]	4.0 (1.7–13.5)
BNP, pg/mL	79.0 (36.0–217.0)	72.0 (26.0–226.0)	97.0 (29.0–269.0)	206.0 (58.0–601.0)	<0.001[b]	97.5 (34.0–310.0)
eGFR, mL/min/1.73m^2	65.9 (50.3–77.4)	64.8 (53.0–77.1)	61.3 (46.6–72.9)	59.1 (42.8–74.1)	0.008[b]	63.3 (48.7–75.4)
Total cholesterol, mmol/L	5.2 (4.3–6.0)	5.3 (4.4–6.0)	5.2 (4.3–6.1)	4.9 (4.1–6.0)	0.149[b]	5.2 (4.3–6.0)
TnT release, >0.01 ng/mL*	101 (47.87)	99 (46.70)	118 (55.66)	138 (65.09)	<0.001[a]	456 (53.84)
Risk factors						
Past smoking					0.542[a]	
Never smoked	76 (36.02)	80 (37.74)	74 (34.91)	87 (41.04)		317 (37.43)
Current smoker	48 (22.75)	56 (26.42)	63 (29.72)	52 (24.53)		219 (25.86)
Ex-smoker	87 (41.23)	76 (35.85)	75 (35.38)	73 (34.43)		311 (36.72)
Hypertension	76 (36.02)	92 (43.40)	97 (45.75)	91 (42.92)	0.206[a]	356 (42.03)
Diabetes mellitus type I	3 (1.42)	2 (0.94)	2 (0.94)	1 (0.47)	0.796[a]	8 (0.94)
Diabetes mellitus type II	24 (11.37)	24 (11.32)	31 (14.62)	29 (13.68)	0.664[a]	108 (12.75)
Total cholesterol >6.5 mmol/L	32 (15.17)	35 (16.51)	34 (16.04)	30 (14.15)	0.913[a]	131 (15.47)
History of heart disease						
Angina pectoris	88 (41.71)	87 (41.04)	100 (47.17)	99 (46.70)	0.443[a]	374 (44.16)
Myocardial infarction	78 (36.97)	60 (28.30)	60 (28.30)	82 (38.68)	0.032[a]	280 (33.06)
Previous CABG	23 (10.90)	23 (10.85)	20 (9.43)	21 (9.91)	0.948[a]	87 (10.27)
Previous PCI	23 (10.90)	28 (13.21)	16 (7.55)	20 (9.43)	0.268[a]	87 (10.27)
Heart failure	42 (19.91)	43 (20.28)	58 (27.36)	84 (39.62)	<0.001[a]	227 (26.80)
Treatment prior to admission						
ACEI/ARB	67 (31.75)	58 (27.36)	79 (37.26)	84 (39.62)	0.035[a]	288 (34.00)
Beta-blocker	75 (35.55)	82 (38.68)	69 (32.55)	78 (36.79)	0.608[a]	304 (35.89)
Statins	79 (37.44)	76 (35.85)	71 (33.49)	67 (31.60)	0.603[a]	293 (34.59)

Blood samples for measurement of SERPINA3 were available in 847 patients. Data are presented as median (interquartile range) or numbers (%). hsCRP, high-sensitivity C-reactive protein; BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; TnT, troponin T; CABG, coronary artery bypass grafting; PCI, percutaneous coronary intervention; ACEI, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker. *For the diagnosis of an AMI, we applied a cut-off value for TnT of 50 ng/L and the lowest detectable value was 10 ng/L. [a]χ^2 test. [b]Kruskal-Wallis test.

Our results contradict existing small-sized clinical studies claiming an association between SERPINA3 and outcome in AMI patients [8, 17]. Cheow et al. [17] validated the potential diagnostic value of eight novel biomarkers, including SERPINA3, reported to be involved in plaque destabilization [18], in 27 subjects with chronic ischemic heart disease and 26 patients with a MI, and found that SERPINA3 could distinguish myocardial injury from stable angina. Furthermore, Zhao et al. [8], who investigated the utility of SERPINA3 as a predictor of major cardiac events (MACE) in 120 AMI patients, demonstrated that MACE-free survival rate after 9 months was significantly lower in patients with higher SERPINA3 levels. In their study, SERPINA3 was closely associated with increased total leukocyte counts and higher CRP levels. Finally, Li et al. [9] investigated the role of SERPINA3 in 86 patients with coronary artery disease, most of whom were clinically stable and only 14% presented with ACS, compared to 64 patients with a normal angiogram, and demonstrated elevated plasma levels of SERPINA3 in the CAD population as compared to controls.

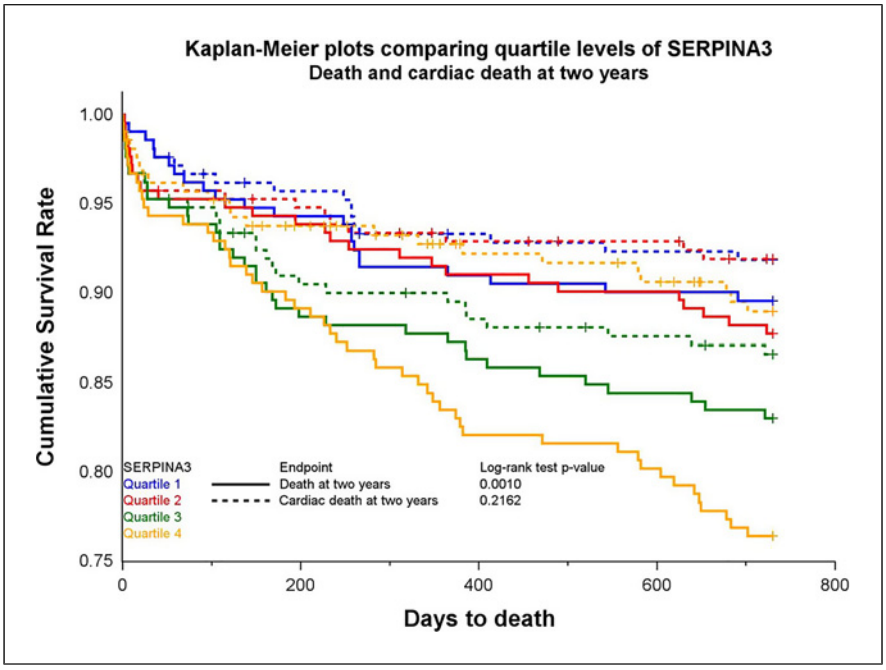

Fig. 1. Kaplan-Meier plots for cardiac and all-cause mortality during 2-year follow-up, comparing quartile levels of SERPINA3. Log-Rank test p value for cardiac death, p = 0.21, and for all-cause mortality, p = 0.001.

Table 2. Univariate and multivariable Cox regression model applying continuous $\log_e$-transformed values of baseline SERPINA3 values during median 7-year follow-up in 871 patients with a suspected acute coronary syndrome

	All-cause mortality 7 years *N* = 325 (38.4%) *M* = 315 (38.5%)		MI 7 years *N* = 197 (23.3%) *M* = 191 (23.3%)		Stroke 7 years *N* = 52 (6.1%) *M* = 51 (6.2%)	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Univariate	1.41 (1.26–1.58)	<0.001	1.21 (1.05–1.39)	0.008	1.24 (0.94–1.63)	0.13
Multivariable	1.14 (1.02–1.28)	0.022	1.04 (0.90–1.20)	0.60	1.10 (1.83–1.46)	0.51

	All-cause mortality or MI 7 years *N* = 395 (45.4%) *M* = 383 (44.0%)		All-cause mortality or MI or stroke 7 years *N* = 411 (47.2%) *M* = 398 (45.7%)	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Univariate	1.33 (1.20–1.47)	<0.001	1.30 (1.18–1.43)	<0.001
Multivariable	1.12 (0.01–1.24)	0.031	1.10 (1.00–1.22)	0.060

There were 24 missing values in the univariate and 53 missing values in the multivariable analysis. *N*, number of events in the univariate analysis; *M*, number of events in the multivariable analysis; MI, myocardial infarction; stroke, cerebral stroke; HR, hazard ratio; 95% CI, 95% confidence interval.

Our AMI subpopulation numbered 386 patients observed over a period of 7 years, far more and with a longer duration than the referenced studies above. Our findings do not confirm the suggested prognostic utility of SERPINA3 in patients with AMI. In contrast, in the 485 patients without AMI, an independent

Table 3. Univariate and multivariable Cox regression models

	Troponin T >50 ng/L AMI				Troponin T ≤50 ng/L non-detected AMI			
A	2-year follow-up				2-year follow-up			
	cardiac mortality *N* = 55 (14.8%) *M* = 53 (14.8%)		cardiac mortality or MI or stroke *N* = 126 (33.9%) *M* = 123 (34.3%)		cardiac mortality *N* = 29 (6.1%) *M* = 27 (5.9%)		cardiac mortality or MI or stroke *N* = 89 (18.7%) *M* = 85 (18.5%)	
Statistical analysis	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Univariate	1.02 (0.78–1.33)	0.88	1.19 (1.00–1.42)	0.048	1.34 (0.92–1.94)	0.12	1.40 (1.13–1.73)	0.002
Multivariable	0.83 (0.62–1.13)	0.24	1.01 (0.84–1.21)	0.95	*Did not converge		1.24 (1.00–1.54)	0.049
B	7-year follow-up				7-year follow-up			
	all-cause mortality *N* = 148 (39.8%) *M* = 145 (40.4%)		all-cause mortality or MI or stroke *N* = 197 (53.0%) *M* = 192 (53.5%)		all-cause mortality *N* = 177 (37.3%) *M* = 170 (37.0%)		all-cause mortality or MI or stroke *N* = 214 (45.1%) *M* = 206 (44.9%)	
Statistical analysis	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Univariate	1.32 (1.12–1.55)	<0.001	1.21 (1.05–1.39)	0.008	1.50 (1.28–1.74)	<0.001	1.37 (1.19–1.58)	<0.001
Multivariable	1.03 (0.87–1.23)	0.71	1.00 (0.87–1.16)	0.98	1.25 (1.07–1.44)	0.004	1.21 (1.06–1.39)	0.007

Model A: Univariate and multivariable Cox regression model applying continuous $\log_e$-transformed values of baseline SERPINA3 values during 2 years follow-up in 386 patients with an acute myocardial infarction (AMI) (TnT >50 ng/L), with 14 missing values in the univariate and 27 missing values in the multivariable analysis, and in 485 patients without AMI (TnT ≤50 ng/L), with 10 missing values in the univariate analysis and 26 missing values in the multivariable analysis. Model B: Univariate and multivariable Cox regression model applying continuous $\log_e$-transformed values of baseline SERPINA3 values during 7-year follow-up in 386 patients with AMI and 485 patients without AMI, respectively. *Model did not converge.

positive association between SERPINA3 and all-cause mortality was noted from 2 years onwards. As a predominant number of deaths by 2 years in the current study are assumed to be linked to the index event [9], a cardiovascular cause would be suspected. However, SERPINA3 was not found to be associated with MI during follow-up. Therefore, a role of SERPINA3 as a prognostic indicator of death by an atherothrombotic mechanism is not obvious but still a possibility, as demonstrated for the combined cardiac endpoint in the cohort without AMI at 2-year follow-up. The reason for the link between SERPINA3 and all-cause mortality is at present not clear, but high expression of SERPINA3 has been associated with an increased mortality rate in cancer [1, 19], and further studies are needed to clarify which disease categories contribute to the association between SERPINA3 and all-cause mortality in patients without AMI. Nonetheless, our data may indicate that patients attending to hospital with chest pain are at risk for adverse outcome even in the absence of AMI.

SERPINA3 increases on a similar scale as that of CRP during an acute-phase reaction [20]. In the current setting, SERPINA3 and CRP [21], both essentially produced in the liver, were significantly and positively correlated, but even if they correlate, their utility as prognosticators may differ, which may provide grounds for future clinical research.

Limitations

The registry was established in 2002, in a clinical setting with on-site invasive treatment, when required. Coronary angiography based on clinical criteria was performed in approximately one-third of the population during hospitalization. Medical management may have changed somewhat, but symptoms are universal and remain unchanged, underscoring the relevance of the study

results. Patients presented with clinically suspected ACS and were sorted by their TnT values. In this study, we did not use the high-sensitivity "fifth-generation" TnT assay which was implemented in 2010 (13). As compared to our locally modified "second-generation" TnT assay (15, 16), assays with a high sensitivity would pick up minor myocardial injury with TnT values below our cut-off level of 50 ng/L, down to a level of 14 ng/L. However, the fact remains that SERPINA3 in the present setting with a well-defined AMI did not serve as a predictor of future atherothrombotic events. Sampling of the biomarkers was limited to one draw at hospital admission. However, as SERPINA3 is an acute phase reactant and is upregulated following an AMI, this may have affected its prognostic utility, despite its measurement in early samples collected at admission. Due to some missing EDTA samples, citrated plasma was used instead for measurement of SERPINA3. No definite diagnosis of the chest pain origin in patients without AMI limits our interpretation of these data. Measurement of SERPINA3 was run as a modification of the original assay kits, which may affect the absolute biomarker values obtained. Furthermore, all subjects were recruited from a Norwegian population. Therefore, our results may not necessarily be generalizable.

Conclusions

SERPINA3 served as a predictor of all-cause mortality in the third year of follow-up in the total population with suspected ACS. Its utility as an independent predictor of all-cause mortality and combined cardiovascular events was limited to the cohort without AMI and did not apply to patients with diagnosed AMI. The lack of associations with cardiac events (i.e., cardiac mortality and MI) further suggests that SERPINA3 is not a good marker of atherothrombotic events.

Statement of Ethics

The RACS study was approved by the Regional Board of Research Ethics (approval No. 118.02, 2010/1074-3) and by the Norwegian Health Authorities. Written informed consent was obtained from participants to participate for 2 years and for long-term follow-up. The study was conducted in accordance with the Helsinki Declaration of 1971, as revised in 1983.

Conflict of Interest Statement

The authors have no conflicts of interest to declare. All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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Author Contributions

D.N., T.U., and P.A. were responsible for conception. V.P., T.B.-A., H.G., and R.A.A. were responsible for acquisition of data. D.N., H.S., P.A., T.U., and A.E.M. were responsible for analysis and interpretation of data. D.N. was responsible for design and drafting the article. P.A. and A.E.M. were responsible for revising the draft critically. All authors contributed to final approval of the final version of the paper prior to submission.

Data Availability Statement

The data that support the findings of this study are not publicly available due to privacy reasons but are available from the corresponding author.

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