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The soluble receptor for advanced glycation end-products (sRAGE) has a dual phase-dependent association with residual cardiovascular risk after an acute coronary event

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HIGHLIGHTS

- Acute coronary syndrome patients with high S100A12 and sRAGE have worse prognosis.
- Elevated S100A12 and sRAGE are linked to high risk for recurrent coronary events.
- High S100A12 and sRAGE are associated with cardiac dysfunction and heart failure.
- Patients with increasing sRAGE during follow-up are at low risk for re-infarction.

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ABSTRACT

Background and aims: The pro-inflammatory alarmin S100A12 (EN-RAGE) and the soluble form of its receptor, the receptor for advanced glycation endproducts (sRAGE), have diverging roles in cardiovascular disease. In experimental studies, S100A12 promoted atherosclerosis while sRAGE treatment was anti-atherogenic and reduced myocardial infarction size by scavenging RAGE ligands. Here, we aimed to explore the links between S100A12, sRAGE, and long-term prognosis after an acute coronary syndrome (ACS).

Methods: We measured S100A12 and sRAGE in 524 patients within 24 h after an ACS, and again 6 weeks later in a subgroup of 114 patients. This subgroup also completed a follow-up echocardiography after 1 year. The median follow-up time for recurrent major adverse cardiovascular events (MACE), defined as recurrent ACS or cardiovascular death, was 25.7 ± 12.6 months.

Results: In Cox proportional hazard analyses, baseline S100A12 and sRAGE were positively associated with the risk of MACE, independently of traditional cardiovascular risk factors. The association between sRAGE and MACE remained significant after additional adjustment for troponin T, NT-proBNP and hsCRP [HR 95%CI for highest *versus* lowest tertile 3.2 (1.5–6.5), $p = 0.002$]. High sRAGE was also associated with deteriorating left ventricular function and an increased rate of heart failure hospitalization post-discharge. In contrast, patients with increasing sRAGE at 6 weeks compared to baseline had lower incidence of recurrent ACS.

Conclusions: Our data suggest that sRAGE has a dual, phase-dependent association with residual cardiovascular risk after ACS. These findings are important for the design and interpretation of future studies on sRAGE as biomarker and potential treatment in ACS patients.

1. Introduction

Acute myocardial infarction (AMI) and its complications are the most common causes of morbidity and mortality in developed countries [1]. Improved treatment of AMI patients at a high risk to develop further complications and to die due to coronary artery disease (CAD) is an important focus of cardiovascular (CV) research. Myocardial ischemia and necrosis induce potent innate immune responses mediated by rapid

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leukocyte recruitment and activation [2]. The magnitude of these responses impacts post-AMI complications [3], as high white blood cell and neutrophil counts, and a high neutrophil/lymphocyte ratio in blood at the time of the AMI have been associated with increased mortality [4]. These findings suggest that neutrophils and their mediators may play important roles in the pathophysiology of AMI and its complications.

S100A12 is a neutrophil-released pro-inflammatory protein that has emerged in recent years as an important mediator of CV disease [5]. S100A12, also called the extracellular newly identified receptor for advanced glycation end-products binding protein (EN-RAGE), is a member of the calcium binding S100 protein family, and functions as an alarmin. Activated or dying neutrophils are the most important source of S100A12 [6], as S100A12 represents approximately 5% of all cytosolic proteins in these cells [7]. Mice lack the gene for S100A12, but studies on ApoE-deficient mice transgenic for S100A12 have shown that S100A12 expression leads to atherosclerosis progression, aortic calcification and plaque vulnerability [8]. In humans, plasma S100A12 is higher in CAD patients compared to controls [9], and is elevated in stable angina and ACS patients with complex coronary lesions [10]. Furthermore, high S100A12 is associated with increased long-term incidence of coronary events in both chronic CAD patients and in individuals without known CAD [11,12]. The association between S100A12 and post ACS prognosis has not previously been explored.

S100A12 is a ligand of the receptor for advanced glycation end-products (RAGE), an innate immune receptor expressed on the surface of various cell populations, including neutrophils, mononuclear phagocytes, lymphocytes, endothelial cells and smooth muscle cells [13]. RAGE is present at low levels in most tissues but is extensively upregulated in chronic inflammation and in vascular injury [14,15]. Engagement of cellular-bound RAGE by S100A12 and other ligands such as AGEs, other S100 calgranulins, high-mobility group box 1 (HMGB-1), integrins leads to cellular activation and pro-inflammatory cytokine production [5,13,15]. Consequently, RAGE-mediated mechanisms have been shown to be involved in atherosclerosis and post ischemic heart failure [14,16,17]. RAGE has a soluble form that can be detected in the circulation (sRAGE), which is generated through shedding of RAGE from the cell surface by metalloproteinases or through alternative splicing [18–20]. sRAGE functions as an inert RAGE decoy receptor, binding RAGE ligands without activating the downstream signalling pathways [5]. Treatment with sRAGE reduced atherosclerosis in diabetic mice [17], and low levels of sRAGE combined with high S100A12 in type 2 diabetic patients were strongly associated with increased CV risk [21]. These findings suggest a potential protective role of sRAGE in CV disease.

Despite increased recent interest in the CV effects of these mediators, the importance of S100A12 and sRAGE release in the context of ACS is poorly understood. In this prospective cohort study, we examined the associations between plasma S100A12 and sRAGE at the time of the acute coronary event and the incidence of recurrent AMI, heart failure and mortality in a population of 524 ACS patients. Further, in a subgroup of 114 of these patients, we investigated whether the dynamics of S100A12 and sRAGE in plasma during the follow-up period are associated with residual CV risk.

2. Patients and methods

2.1. Study population

Initially, 605 patients admitted for suspected ACS to the Coronary Care Unit of Skåne University Hospital Malmö were consecutively included in the study between October 2008 and December 2012. ACS was defined as unstable angina or AMI, diagnosed according to the universal definition of myocardial infarction [22]. Fifty patients did not fulfil the diagnostic criteria for ACS and were excluded. Thirty-one patients were further excluded due to missing samples, leaving a final study population of 524 individuals. The national Swedish Web-based system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies (SWEDEHEART), was used to collect information on smoking, diabetes, hypertension, and previous history of heart failure and ACS. In order to be included into the study, the patients had to be able to provide a written informed consent. No other exclusion criteria were applied.

In order to address the current knowledge gap regarding post-ACS evolution of cardiac function and prognosis in the elderly, patients that were 75 years of age or older at inclusion were invited to a more detailed follow-up. The follow-up consisted in collection of a new plasma sample at 6 weeks after the index event and a follow-up echocardiographic examination at 1 year. This follow-up protocol of elderly patients was predefined before the start of the study. The patients in this subgroup had lower body-mass index (BMI), lower estimated glomerular filtration rate (eGFR), and higher plasma concentration of N-terminal pro-brain natriuretic peptide (NT-proBNP) at baseline compared to the rest of the cohort (Supplementary Table 1). The prevalence of smoking was lower and hypertension was more frequent in this group (Supplementary Table 1).

2.2. Outcomes

Adverse events were recorded prospectively during follow-up. The primary outcome was recurrent MACE, defined as a composite of hospitalization with an ACS diagnosis or CV death. The secondary outcomes were hospitalization for heart failure and total mortality. Events were identified by using data from the Swedish Hospital Discharge Register and the Swedish Cause of death Register. The last follow-up date for all participants was 31st of December 2012 for recurrent AMI, unstable angina, heart failure and CV death, and 31st of December 2013 for all-cause mortality. AMI was defined by ICD 10 codes I21 and I22, unstable angina by code I20, and heart failure by code I50. CV death was defined as death due to AMI, ischemic heart disease, cardiac arrest, atrial fibrillation, heart failure, ischemic or haemorrhagic stroke, defined by the ICD 10 codes I20-25, I46, I48, I50 and I60-69. The study has been approved by the Regional ethics committee in Lund, Sweden and was conducted according to the ethical guidelines of the Declaration of Helsinki. Written informed consent was obtained from all patients.

2.3. Biochemical analysis

Plasma samples were collected in EDTA-coated tubes within 24 h after admission from all patients, and again after 6 weeks in the detailed follow-up group. The blood was centrifuged at 3000 g for 10 min, and plasma was aliquoted and stored at −80 °C before analysis. A plasma aliquot was sent to the certified clinical laboratory of Skane University Hospital Malmö for analysis of high sensitivity c-reactive protein (hsCRP), troponin T (TnT) and cystatin C. Another aliquot was used for analyses of S100A12, sRAGE, NT-proBNP, and IL-6. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula was used to calculate eGFR based on cystatin C, age and sex.

The analysis of S100A12, sRAGE, NT-proBNP and IL-6 in plasma was performed at the Science for Life Laboratory, Uppsala, Sweden, by the Proximity Extension Assay (PEA) technique [23]. Oligonucleotide-labelled antibody pairs were allowed to bind to their respective targets in the plasma samples. A PCR template was formed by adding a DNA polymerase that induced extension and joining of the two oligonucleotides. The DNA template was pre-amplified with universal primers, and the individual DNA sequence for each target protein was thereafter detected and quantified using specific primers. For S100A12, the within-run coefficient of variation was 8% and the between-run coefficient of variation was 22%. The corresponding values for sRAGE were 8% and 13%. All data for S100A12, sRAGE and NT-proBNP are presented as arbitrary units (au).

2.4. Echocardiography

Patients 75 years and older underwent a baseline echocardiography examination during the index hospital stay and were invited for a follow-up echocardiography 1 year after inclusion, as prespecified in the project protocol. The echocardiographic follow-up was completed in 113 patients. Experienced sonographers performed all echocardiography examinations, which were analysed using the Xcelera software (Philips) by a single examiner blinded to the clinical data. Left ventricle ejection fraction (LVEF) was measured according to Simpson's biplane method in the apical 4-chamber and 2-chamber views. Due to poor image quality and missing frames, we were able to perform measurements of acute LVEF in 76 patients, of LVEF at 1-year post-ACS in 99 patients, and of the left ventricle end-systolic volume (LVESV) and left ventricle end-diastolic volume (LVEDV) at 1 year in 108 patients. The delta value of LVEF was calculated as the value of LVEF at 1 year minus the LVEF value at inclusion.

2.5. Statistical analysis

We compared patient characteristics between the different outcome groups by using the Mann Whitney test for continuous variables and the chi-square test for dichotomous variables. Multivariate Cox proportional hazards analyses were used to assess the associations between biomarkers and outcomes. We used 3 different adjustment models: Model 1 included age and sex; Model 2 included age, sex, hypertension, diabetes mellitus, smoking, eGFR, previous heart failure and/or ACS; Model 3 included the same variables as Model 2, with the addition of the prognostic biomarkers TnT, NT-proBNP and hsCRP. Skewed variables were logarithmically transformed before analysis. The correlations between biomarkers and echocardiographic parameters were analysed with the Spearman rank test. The Wilcoxon signed ranks test was used to evaluate the differences between biomarker levels at baseline and at 6 weeks in the same patient subgroup. *p*-values under 0.05 were considered significant. All calculations were made using SPSS 23.0 (IBM software, Armonk NY).

3. Results

3.1. Baseline characteristics

The baseline characteristics of the study population are presented in Table 1. The median follow-up time to event was 25.7 months (IQR 13.3–38.3) for MACE, and 39.5 months (IQR 27.4–52.2) for total mortality. The patients that suffered a recurrent MACE event during follow-up were older, had a higher prevalence of hypertension, previously known ACS or heart failure, lower eGFR, and higher hsCRP, and NT-proBNP at the time of the index event (Table 1). Additionally, patients who died had a significantly higher prevalence of diabetes and a lower prevalence of smoking (Supplementary Table 3). Baseline S100A12 and sRAGE were significantly higher in patients with recurrent MACE (Table 1), ACS (Supplementary Table 2), or heart failure (Supplementary Table 4) during follow up, and in patients who died (Supplementary Table 3). Baseline S100A12 and sRAGE levels were also higher in the elderly subgroup that underwent detailed follow-up (Supplementary Table 1). In Spearman correlation analyses of biomarkers in the acute phase, S100A12 was directly correlated with age ($r = 0.222$, $p < 0.001$), hsCRP ($r = 0.281$, $p < 0.001$), NT-proBNP ($r = 0.296$, $p < 0.001$), IL-6 ($r = 0.312$, $p < 0.001$), and inversely correlated with eGFR ($r = -0.332$; $p < 0.001$). Similar correlations were observed between sRAGE and age ($r = 0.202$, $p < 0.001$), hsCRP ($r = 0.091$, $P = 0.038$), NT-proBNP ($r = 0.440$, $p < 0.001$), IL-6 ($r = 0.288$, $p < 0.001$), and eGFR ($r = -0.241$; $p < 0.001$). There were no correlations between S100A12, sRAGE and baseline TnT.

3.2. High S100A12 and sRAGE in the acute post-ACS period are associated with recurrent coronary events and heart failure during follow-up

In crude Kaplan-Meier survival analyses with log-rank tests, patients with S100A12 and sRAGE in the highest tertile (T3) at baseline had a significantly higher incidence of recurrent MACE compared to patients in tertiles 1 and 2 (Fig. 1). We used multivariate Cox proportional hazard analyses to investigate the associations between plasma levels of S100A12 and sRAGE at baseline, and the incidence of recurrent MACE,

Table 1
Baseline differences in clinical characteristics between patients with and without recurrent MACE during follow-up.

Characteristics	All patients	Patients with recurrent MACE	Patients without recurrent MACE	*p*
	N = 524	N = 87	N = 437	
Age (years)	67 (59–77)	77 (66–85)	66 (58–74)	< 0.001
Male gender, n (%)	384 (73.3%)	61 (70.1%)	322 (73.7%)	0.493
Hypertension, n (%)	285 (54.4%)	56 (64.4%)	229 (52.4%)	0.041
Smoking, n (%)	132 (25.2%)	18 (20.7%)	114 (26.1%)	0.290
Diabetes, n (%)	126 (24.0%)	24 (27.6%)	102 (23.3%)	0.397
BMI ($kg/m^{2)}$	26.9 (24.3–29.8)	26.0 (23.7–29.7)	27.1 (24.4–29.8)	0.196
eGFR ($mL/min/1.73m^2$)	72.0 (53.1–94.6)	54.9 (34.5–75.8)	74.7 (57.2–96.5)	< 0.001
Index cardiac event				
STEMI	179 (34.2%)	27 (31.0%)	152 (34.8%)	0.501
NSTEMI	295 (56.3%)	56 (64.4%)	239 (54.7%)	0.097
UA	50 (9.5%)	4 (4.6%)	46 (10.5%)	0.086
Previous cardiac event				
HF, n (%)	54 (10.3%)	18 (20.7%)	36 (8.2%)	< 0.001
ACS, n (%)	151 (28.8%)	39 (44.8%)	112 (25.6%)	< 0.001
TnT (ng/L)	365 (62–1291)	379 (96–1290)	364 (55–1297)	0.337
hsCRP (mg/L)	6.7 (2.9–17.8)	8.9 (3.7–29.6)	6.3 (2.6–16.4)	0.005
NT-proBNP (au)	81.0 (49.5–103.6)	107.6 (83.9–121.9)	75.6 (45.7–97.0)	< 0.001
S100A12 (au)	3.7 (3.0–4.8)	4.6 (3.6–6.1)	3.4 (2.9–4.5)	< 0.001
sRAGE (au)	25.5 (20.1–34.8)	34.1 (24.6–45.9)	24.8 (19.2–31.8)	< 0.001

ACS, acute coronary syndrome; BMI, body mass index; eGFR, estimated glomerular filtration rate; HF, heart failure; hsCRP, high sensitivity C-reactive protein; MACE, major adverse cardiovascular event; NSTEMI, non ST-elevation myocardial infarction; NT-proBNP, N-terminal pro-brain natriuretic peptide; sRAGE, soluble receptor for advanced glycation endproducts; STEMI, ST elevation myocardial infarction; TnT, Troponin T; UA, unstable angina.

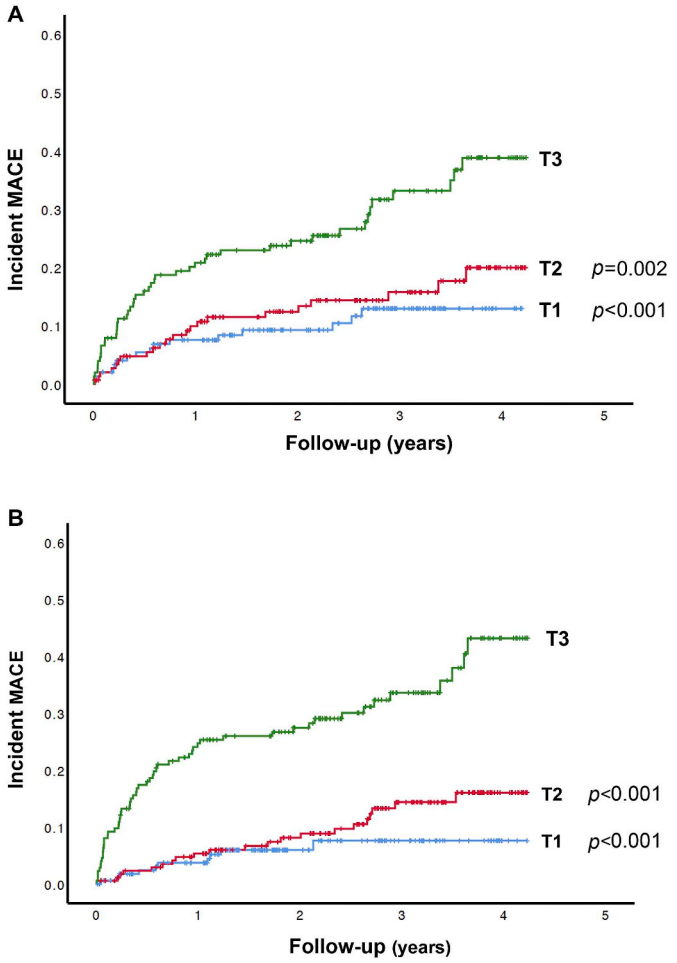

Fig. 1. Kaplan Meyer diagrams of incident MACE after the index acute coronary event.

Survival curves of incident MACE in the entire cohort (n = 524), by tertiles of S100A12 (A) and sRAGE (B) at baseline. The *p*-values refer to the difference in incident MACE between the respective tertile and the highest tertile (T3), calculated with an unadjusted log-rank test.

recurrent ACS (fatal or non-fatal) and total mortality in the cohort. The associations between S100A12, sRAGE and MACE remained significant after adjustment for age, sex, and potential clinical confounders. A significantly higher number of events occurred in the highest compared to the lowest tertile (Table 2, Models 1–2). The association between sRAGE and MACE was independent of TnT, hsCRP, and NT-proBNP, but the relationship between S100A12 and MACE lost significance after further adjustment for these biomarkers (Table 2, Model 3). Similar results were recorded for recurrent ACS (Table 2). S100A12 also showed a weak association with total mortality in Model 1, which was lost after further correction for clinical confounders. We found no associations between baseline sRAGE and total mortality. No significant interactions were found between S100A12, sRAGE and the potential confounders showing collinearity with the biomarkers at baseline (age, hsCRP, NT-proBNP, eGFR) regarding any of the outcomes.

Further, we investigated the relationships between the biomarkers and rehospitalization for heart failure after the index event, as well as with echocardiographic parameters of left ventricle function and remodelling during follow-up. We found that high baseline levels of sRAGE and S100A12 were associated with an increased frequency of heart failure hospitalization (Table 3, Model 1). In line with these findings, high baseline levels of S100A12 were correlated with low LVEF and large LVESV at 1 year (Table 3). High sRAGE was also correlated with a low LVEF at 1 year and also with LVEF deterioration during the first year post-ACS, from baseline to follow-up (Table 4). sRAGE had a stronger association with heart failure hospitalization compared to S100A12, but both associations lost statistical significance when potential confounders and other established biomarkers were considered (Table 3, Models 2 and 3).

3.3. Increasing sRAGE in plasma in the post-ACS period is associated with a good prognosis

At 6 weeks post-ACS, high levels of S100A12 were significantly correlated with high hsCRP ($r = 0.372$, $p < 0.001$) and high IL-6 ($r = 0.386$, $p < 0.001$), witnessing an increased systemic pro-inflammatory state. In contrast, there were no associations between sRAGE, hsCRP and IL-6 at this time point. These data suggest that S100A12 might reflect similar pro-inflammatory pathways as hsCRP and IL-6, while sRAGE has different dynamics and possibly a different role during the recovery phase. We investigated whether the dynamics of the biomarkers, i.e. the increase or decrease from the ACS to the 6 weeks follow-up, could better identify patients at high residual risk. The change in biomarker levels was calculated as the value at 6 weeks minus the value at inclusion. Within the detailed follow-up subgroup, the patients who suffered a recurrent MACE were older and had a higher prevalence of previous heart failure at baseline (Supplementary Table 5). The levels of TnT, hsCRP and NT-proBNP were lower at 6 weeks compared to baseline, but S100A12 did not change significantly between the two time points. In contrast, plasma sRAGE increased from baseline to 6 weeks [32.6 (26.7–41.0) vs 28.4 (21.6–37.5), $p < 0.001$], and delta sRAGE was significantly higher in patients who remained MACE-free during follow-up (Supplementary Table 5). The associations between biomarker change and recurrent MACE, recurrent ACS and total mortality were examined in multivariate Cox proportional hazard analyses using the adjustment models described in the Methods section. The patients were divided into tertiles according to the change in biomarker levels. Tertile 3 included patients with an absolute increase in S100A12 and sRAGE, whereas patients within tertile 1 had decreased levels in plasma at 6 weeks compared to baseline (Supplementary Table 6). We found that patients with increasing levels of sRAGE during follow-up had a significantly lower risk for recurrent ACS compared to patients with decreasing sRAGE (Tertile 3 vs Tertile 1, Table 4, Model 1). The association was independent of age, sex, hypertension, smoking, diabetes mellitus, eGFR, previous heart failure and/or ACS, and circulating TnT, NT-proBNP, and hsCRP at 6 weeks (Table 4, Models 2 and 3). The association between sRAGE and incident MACE followed a similar pattern but was only significant in Model 2, suggesting that it is mostly recurrent ACS events that drive the association. We found no significant correlations between the change in S100A12 and outcome. The variation in neither biomarker were associated with mortality.

4. Discussion

In this prospective cohort study, we examined the associations between the pro-inflammatory mediator S100A12, its decoy receptor sRAGE, and long-term prognosis in ACS patients. We found that high plasma levels of both S100A12 and sRAGE at the time of the acute coronary event were associated with lower left ventricular systolic function at 1 year, and with increased risk for recurrent MACE and heart failure during follow-up. Baseline sRAGE had a stronger association with outcome, independently of age, sex, CV risk factors, and the classical risk predictors TnT, hsCRP and NT-proBNP. In contrast to the acute phase, patients with increasing plasma sRAGE at 6 weeks compared to baseline had a very low risk for recurrent ACS. This intriguing change of direction of the association between sRAGE and prognosis from the acute to the recovery phase suggests a phase-dependent role for sRAGE in ACS. In the acute setting, circulating sRAGE might reflect the degree of inflammatory activation triggered by

Table 2
Correlations between biomarkers at baseline and outcome.

Biomarker at inclusion			MACE (N = 87)			ACS (N = 76)			Total mortality (N = 62)		
			HR	CI	*p*	HR	CI	*p*	HR	CI	*p*
S100A12	1[a]	T2 vs T1	1.3	0.7–2.6	0.377	1.4	0.7–2.8	0.331	1.7	0.8–3.7	0.186
		T3 vs T1	2.1	1.2–3.8	0.011	2.2	1.2–4.1	0.014	2.1	1.0–4.3	0.045
	2	T2 vs T1	1.3	0.6–2.5	0.497	1.2	0.6–2.5	0.583	1.5	0.6–3.5	0.340
		T3 vs T1	2.0	1.1–3.7	0.029	1.9	1.0–3.7	0.046	1.9	0.9–4.1	0.114
	3	T2 vs T1	1.2	0.6–2.4	0.609	1.2	0.6–2.4	0.687	1.8	0.7–4.3	0.203
		T3 vs T1	1.8	1.0–3.4	0.071	1.8	0.9–3.5	0.098	2.1	0.9–4.9	0.086
sRAGE	1	T2 vs T1	1.5	0.7–3.3	0.273	1.3	0.6–2.8	0.527	1.1	0.5–2.3	0.875
		T3 vs T1	4.1	2.1–8.1	< 0.001	3.8	1.9–7.5	< 0.001	1.8	0.9–3.6	0.099
	2	T2 vs T1	1.4	0.7–3.1	0.358	1.3	0.6–2.8	0.564	0.9	0.4–2.0	0.773
		T3 vs T1	3.6	1.8–7.3	< 0.001	3.4	1.6–6.8	0.001	1.3	0.6–2.8	0.453
	3	T2 vs T1	1.3	0.6–2.9	0.486	1.2	0.5–2.6	0.688	1.0	0.4–2.3	0.970
		T3 vs T1	3.2	1.5–6.5	0.002	3.0	1.4–6.2	0.003	1.6	0.7–3.6	0.269

Multivariate Cox proportional hazard analyses of the relationship between biomarker tertiles at inclusion, recurrent CV events and mortality.
Number of events per S100A12 tertile (T1/T2/T3): MACE (16/22/46); ACS (14/20/36); Total mortality (10/17/35).
Number of events per sRAGE tertile (T1/T2/T3): MACE (10/21/56); ACS (10/17/49); Total mortality (11/16/36).
ACS, acute coronary syndrome; CI, confidence interval; CV, cardiovascular; HR, hazard ratio; hsCRP, high sensitivity C-reactive protein; MACE, major acute cardiovascular events; NT-proBNP, N-terminal pro-brain natriuretic peptide; sRAGE, soluble receptor for advanced glycation endproducts; TnT, Troponin T.
[a]Adjustment models:
Model 1: age and sex.
Model 2: age, sex and CV risk factors (hypertension, smoking, diabetes mellitus, eGFR, previous heart failure and/or ACS).
Model 3: age, sex, CV risk factors and biomarkers (hsCRP, NT-proBNP and TnT).

myocardial damage. Conversely, in the recovery period, rising sRAGE might prevent recurrent ACS by scavenging pro-inflammatory ligands, including S100A12, and preventing activation of the cellular-bound RAGE receptor. As discussed below, an increasing body of clinical and experimental evidence supports this dual role of sRAGE in CV disease.

Previous clinical studies have shown that sRAGE levels are increased in ACS patients at admission compared to healthy controls [24,25]. Elevated admission sRAGE in these patients has been associated with worse in-hospital prognosis [26] and with lower LVEF at 7 months [27], which is consistent with the results of our study. During AMI, myocardial ischemia triggers danger signals from necrotic cells that activate the innate immune system and mediate a strong inflammatory response in the myocardium [2]. Pro-inflammatory signalling triggered by activation of membrane bound RAGE plays an important role in this process [2,5,16]. sRAGE is mainly produced by proteolytic cleavage of RAGE from the cellular membrane by the proteases ADAM10 and MMP9 [18,19]. Previous work demonstrates that RAGE cleavage and shedding is induced by binding of the pro-inflammatory ligand HMGB1 to the receptor [18]. The alarmin HMGB1 is one of the most important activators of innate immunity and is highly increased during AMI [28,29]. Accordingly, the increased plasma sRAGE in the acute phase of AMI might be the result of RAGE shedding from activated leukocytes to counteract the excessive inflammatory activation, and sRAGE levels might reflect the intensity of the innate immune response. This hypothesis is supported by our data showing that plasma sRAGE at this stage correlates well with the inflammatory biomarker IL-6.

The initial myocardial inflammation is followed by a recovery phase, characterized by resolution of inflammation and fibrous scar formation. This phase is predominantly mediated by reparatory

Table 3
Correlation between biomarkers at baseline, incident heart failure, and cardiac function at 1 year post-ACS.

Biomarker	Models	Heart failure (N = 41)[a]						LVEF 1 year post-ACS		LVEF change[c]		LVESV 1 year post-ACS		LVEDV 1 year post-ACS	
		T2 vs T1			T3 vs T1			r[d]	*p*	r[d]	*p*	r[d]	*p*	r[d]	*p*
		HR[b]	CI for HR	*p*	HR[b]	CI for HR	*p*								
S100A12	1	1.8	0.7–5.0	0.229	2.4	1.0–6.1	0.056	−0.254	0.019	0.031	0.816	0.235	0.023	0.114	0.275
	2	1.3	0.5–3.9	0.584	1.4	0.5–3.9	0.507								
	3	1.0	0.3–3.1	0.993	0.9	0.3–2.5	0.766								
sRAGE	1	1.9	0.6–6.2	0.266	4.6	1.6–13.2	0.005	−0.196	0.054	−0.282	0.021	0.168	0.086	0.055	0.577
	2	1.6	0.5–5.1	0.449	2.4	0.8–7.5	0.126								
	3	1.4	0.4–4.5	0.606	2.0	0.6–6.6	0.252								

Adjustment models:
Model 1: age and sex.
Model 2: age, sex and CV risk factors (hypertension, smoking, diabetes mellitus, eGFR, previous heart failure and/or ACS).
Model 3: age, sex, CV risk factors and biomarkers (hsCRP, NT-proBNP and TnT).
ACS, acute coronary syndrome; sRAGE, soluble receptor for advanced glycation endproducts; HR, hazard ratio; CI, confidence interval; LVEF, left ventricle ejection fraction; LVEDV, left ventricle end-diastolic volume; LVESV, left ventricle end-systolic volume; eGFR, estimated glomerular filtration rate; hsCRP, high sensitivity C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic peptide; TnT, Troponin T.
[a] Incident hospitalization with a clinical diagnosis of heart failure during follow-up.
[b] Multivariate Cox proportional hazards analyses of the relationship between biomarker tertiles at inclusion and incident heart failure. Number of events per S100A12 tertile (T1/T2/T3): 6/11/22; and per sRAGE tertile (T1/T2/T3): 4/10/27.
[c] Left ventricle ejection fraction at 1 year follow-up minus ejection fraction at inclusion.
[d] Spearman correlation.

Table 4
Correlations between the change in biomarker levels from ACS to the 6-week follow-up and outcome.

Biomarker change[a]			MACE (N = 30)			ACS (N = 25)			Total mortality (N = 19)		
			HR	CI	*p*	HR	CI	*p*	HR	CI	*p*
S100A12	1[b]	T2 vs T1	1.5	0.6–3.9	0.394	1.5	0.5–4.4	0.456	0.5	0.1–1.7	0.261
		T3 vs T1	1.4	0.5–3.5	0.501	1.7	0.6–4.8	0.332	1.1	0.4–3.0	0.875
	2	T2 vs T1	1.6	0.6–4.7	0.381	1.5	0.4–4.8	0.540	0.5	0.1–2.2	0.401
		T3 vs T1	1.1	0.4–3.5	0.837	1.3	0.4–4.5	0.660	1.1	0.3–3.6	0.872
	3	T2 vs T1	2.1	0.7–6.5	0.240	1.7	0.5–6.0	0.394	0.8	0.2–3.4	0.752
		T3 vs T1	0.8	0.2–2.6	0.646	0.9	0.3–3.3	0.862	1.0	0.3–3.5	0.999
sRAGE	1	T2 vs T1	0.8	0.4–1.8	0.581	0.9	0.4–2.1	0.815	0.7	0.2–1.8	0.437
		T3 vs T1	0.4	0.1–1.1	0.088	0.2	0.0–0.8	0.027	0.4	0.1–1.6	0.198
	2	T2 vs T1	0.6	0.2–1.5	0.254	0.7	0.3–1.8	0.454	0.5	0.2–1.6	0.264
		T3 vs T1	0.3	0.1–1.0	0.040	0.1	0.0–0.7	0.013	0.5	0.1–1.8	0.280
	3	T2 vs T1	0.8	0.3–2.2	0.653	0.9	0.3–2.7	0.864	0.7	0.2–2.2	0.516
		T3 vs T1	0.4	0.1–1.2	0.085	0.2	0.0–0.8	0.026	0.5	0.1–2.1	0.352

Multivariate Cox proportional hazard analyses of the relationships between biomarker change from inclusion to the 6-week follow-up time point, incident CV events and mortality.
Number of events per S100A12 tertile (T1/T2/T3): MACE (8/10/10); ACS (6/8/9); Total mortality (7/4/8).
Number of events per sRAGE tertile (T1/T2/T3): MACE (14/11/5); ACS (12/11/2); Total mortality (9/7/3).
Model 1: age and sex.
Model 2: age, sex and CV risk factors (hypertension, smoking, diabetes mellitus, eGFR, previous heart failure and/or ACS).
Model 3: age, sex, CV risk factors and biomarkers (hsCRP, NT-proBNP and TnT).
ACS, acute coronary syndrome; CI, confidence interval; CV, cardiovascular; HR, hazard ratio; hsCRP, high sensitivity C-reactive protein; MACE, major acute cardiovascular events; NT-proBNP, N-terminal pro-brain natriuretic peptide; sRAGE, soluble receptor for advanced glycation endproducts; TnT, Troponin T.
[a] The change in biomarker was calculated as the value at 6 weeks minus the value at the time of the ACS.
[b] Adjustment Models.

macrophages and fibroblasts, and involves production of anti-inflammatory cytokines and profibrotic factors [2]. In our cohort, increasing sRAGE from baseline to 6 weeks identified patients at very low risk to subsequently develop recurrent ACS, independently of all considered baseline predictors. This association cannot prove causality, but suggests that sRAGE might have a favourable role in the recovery phase by possibly dampening the inflammatory response and helping to stabilize potentially vulnerable plaques. Shedding of RAGE from the cell surface might also lead to decreased propensity for immune cell activation. Dutta et al. have shown that inflammatory monocytes that infiltrate the heart in large numbers after an AMI increase the vulnerability of non-culprit coronary atheromas, promoting plaque rupture and recurrent ischemic events [30]. As RAGE-mediated signals are important activators of myeloid cell responses, scavenging of RAGE ligands by sRAGE might have an anti-inflammatory effect, actively contributing to CV protection. In support of this hypothesis, animal studies have consistently demonstrated a protective effect of sRAGE in CV disease. In experimental models of myocardial ischemia, intracoronary and intraperitoneal injection of sRAGE during the first 48 h after induced AMI resulted in significantly decreased infarction size, reduced fibrosis, and preserved LVEDV, LVESV and LVEF compared to controls [31–33]. These experimental results are in line with our current findings in ACS patients. sRAGE treatment has also shown anti-atherosclerotic properties, leading to reversed vascular hyperpermeability and reduced atherosclerosis in diabetic rodents [17,34] and preventing angiotensin II-induced atherosclerosis in ApoE deficient mice [35]. Additionally, sRAGE significantly decreased neointimal expansion, smooth muscle cell proliferation, and increased the luminal area in models of experimentally induced arterial injury [14,36].

Our results linking elevated S100A12 with a negative prognosis in ACS patients support previously published data demonstrating an important role for S100A12 as a pathogenic and prognostic factor in CV disease. The role of S100A12 as an active mediator in CV disease has been confirmed in animal studies. Transgenic ApoE-deficient mice expressing S100A12 were found to have increased atherosclerotic plaque size, vascular calcifications and plaque vulnerability compared to ApoE-deficient mice not expressing S100A12 [5,8]. Conversely, treatment with a specific S100A12 blocker reversed the phenotype and reduced plaque vulnerability [37]. S100A12 is released from ruptured atherosclerotic plaques in ACS, and from the site of atheroma disruption by percutaneous coronary intervention in stable CAD [9,38]. In ACS patients, S100A12 is increased compared to patients with stable angina pectoris and correlates with coronary lesion complexity [10,39]. In a cohort of 652 stable CAD patients undergoing percutaneous coronary intervention, high S100A12 at the time of the intervention was correlated with incident MACE during a mean follow-up of 2.5 years [11]. Elevated S100A12 levels were also related to incident CAD during a 10-year follow-up in CAD-free individuals randomly selected from the population [12], and with the incidence of re-hospitalization and CV death in patients with chronic heart failure. In contrast to sRAGE, S100A12 does not provide independent prognostic information compared to hsCRP and IL-6 in our study, due to similar dynamics of these biomarkers both in the acute and the post-acute period.

4.1. Study limitations

Our study has some important limitations that need to be considered. Firstly, this association study cannot prove an active role for sRAGE and S100A12 in ACS-related myocardial lesions and coronary plaque inflammation. However, as discussed above, our results are in line with previous findings linking these proteins with CV disease and can potentially be explained by pathogenic mechanisms already demonstrated in experimental settings. Secondly, as predefined in the initial study design, follow-up blood samples and echocardiographic data were only collected from patients older than 75 years of age. The results obtained in this small sub-population have to be interpreted with caution and cannot be directly extrapolated to the entire study cohort due to significant differences in clinical parameters between the whole cohort and the follow-up group. Due to the relatively low number of patients and the selection criterion used, the data generated in this sub-population should only be considered as hypothesis-generating. Thirdly, as the biomarkers were measured by the PLA method and expressed in arbitrary units, we have not been able to determine concrete cut-off values for sRAGE and S100A12 as predictive

biomarkers, and we have not been able to compare the concentrations with the results of other studies. Consequently, studies in larger independent cohorts using conventional measurement methods are necessary to confirm our results.

4.2. Conclusion

In conclusion, we show for the first time that high levels of S100A12 and sRAGE in ACS patients at the time of the acute coronary event are associated with an adverse prognosis.

The associations with the outcomes were stronger for sRAGE, and were independent of age, sex, clinical parameters, and other established biomarkers. In contrast, patients with the highest increase in sRAGE at 6 weeks post-ACS compared to the acute values had a low incidence of recurrent ACS. These results reveal a dual, phase-dependent role of sRAGE in ACS. In the acute phase, elevated levels of plasma sRAGE possibly reflect a high degree of local and systemic inflammatory activation. During the recovery phase, increasing sRAGE might prevent inflammatory activation and destabilization of coronary atheromas by scavenging RAGE ligands. Clinical and experimental studies are needed to confirm the associations found in our study and to explore the potential of sRAGE therapy to improve patient prognosis post-ACS. Interestingly, it has been shown that statins stimulate sRAGE production by cleavage from the cell surface, which may at least indirectly explain some of their beneficial effects on post-ACS prognosis [40].

Conflicts of interest

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

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Author contributions

Helena Grauen Larsen has participated in study design, data analysis and interpretation, and manuscript drafting. Troels Yndigegn has contributed to echocardiography data acquisition and interpretation, and critical revision of the manuscript for intellectual content. Goran Marinkovic has contributed to data acquisition and critical revision of the manuscript for intellectual content. Helena Grufman and Isabel Goncalves have contributed to study design, data acquisition, and critical revision of the manuscript for intellectual content. Razvan Mares has contributed to data interpretation and critical revision of the manuscript for intellectual content. Jan Nilsson has contributed to study design, data interpretation, and critical revision of the manuscript for intellectual content. Alexandru Schiopu has contributed to study conception and design, data analysis and interpretation, and manuscript drafting. All authors have approved the final version of the manuscript.

Appendix A. Supplementary data

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