Increased levels of soluble Receptor for Advanced Glycation End-Products (RAGE) are associated with a higher risk of mortality in frail older adults

Lee Butcher[1], Jose A. Carnicero[2], David Gomez Cabrero[3], Jean-François Dartigues[4], Karine Pérès[4], Francisco Jose Garcia-Garcia[5], Leocadio Rodriguez-Mañas[2,6], Jorge D. Erusalimsky[1], on behalf of the FRAILOMIC Consortium

[1]*Department of Biomedical Sciences, Cardiff Metropolitan University, Cardiff, UK*

[2]*Fundacion para la investigación biomédica del Hospital Universitario de Getafe, Getafe, Spain*

[3]*Unit of Computational Medicine, Karolinska Institute, Stockholm, Sweden*

[4]*Univ. Bordeaux, Inserm, Bordeaux Population Health Research Center, UMR 1219, 33000 Bordeaux, France*

[5]*Division of Geriatric Medicine, Hospital Virgen del Valle, Complejo Hospitalario de Toledo, Toledo, Spain*

[6]*Hospital Universitario de Getafe, Getafe, Spain*

Address Correspondence to:

Jorge D. Erusalimsky
Department of Biomedical Sciences
Cardiff Metropolitan University
Llandaff Campus, Western Avenue, Cardiff CF5 2YB
United Kingdom

Email: jderusalimsky@cardiffmet.ac.uk
Telephone: +44 (0)29 2041 6853
Fax: +44 (0)29 2041 6982

Abstract

Objective: To evaluate the relationship between serum levels of the soluble Receptor for Advanced Glycation End-products (sRAGE) and mortality in frail and non-frail older adults.

Methods: We studied 691 subjects (141 frail and 550 non-frail) with a median age of 75 years from two population-based cohorts, the Toledo Study of Healthy Aging and the AMI study, who were enrolled to the FRAILOMIC initiative. Multivariate Cox proportional hazards regression and Kaplan-Meier survival analysis were used to assess the relationship between baseline sRAGE and mortality.

Results: During 6 years of follow-up 101 participants died (50 frail and 51 non-frail). Frail individuals who died had significantly higher sRAGE levels than those who survived (median [IQR]: 1563 [1015-2248] vs 1184 [870-1657] pg/mL, *P*=0.006), whilst no differences were observed in the non-frail group (1262 [1056-1554] vs 1186 [919-1551] pg/mL, *P*=0.19). Among frail individuals higher sRAGE levels were associated with an increased risk of death after adjustment for relevant covariates (HR=2.72 per unit increment in ln-sRAGE, 95%CI 1.48-4.99, *P*=0.001). In contrast, in non-frail individuals sRAGE showed no association with mortality. Survival curves demonstrated that among frail individuals the incidence of death was significantly higher in the top sRAGE quartile compared to the three lower quartiles (*P*=0.002). Area under the ROC curve analysis demonstrated that for frail individuals, inclusion of sRAGE in the hazard model increased its predictive accuracy by ~3%.

Conclusions: sRAGE is an independent predictor of mortality among frail individuals. Determination of sRAGE in frail subjects could be useful for prognostic assessment and treatment stratification.

Introduction

Frailty is an age-associated biological syndrome characterized by a decline in physical and mental reserves, a decrease in resistance to external stressors and an enhanced risk of disability, hospitalization and death [1, 2]. Although frailty is known to be a major cause of poorer survival in older adults, and despite the fact that identification of biomarkers associated with excess mortality in this condition could be of clinical prognostic value, these have not been extensively investigated.

The Receptor for Advanced Glycation End-products (RAGE) is a transmembrane glycoprotein of the immunoglobulin superfamily. RAGE binds a variety of damage and stress-associated molecules, including advanced glycation-end products, high-mobility group box 1 and S100 proteins. Upon activation, RAGE elicits pro-inflammatory processes [3]. In addition to membrane-bound RAGE, there are two soluble RAGE (sRAGE) isoforms circulating in blood, both of which lack its membrane and cytoplasmic domains [4]. The predominant form of sRAGE is generated by proteolytic cleavage of membrane-bound RAGE [5, 6]. The second form, called esRAGE, results from alternative splicing of RAGE mRNA and accounts for <25% of total sRAGE [7]. Although the precise function of sRAGE in human biology remains unresolved [4], evidence suggests that its total circulating levels reflect increased RAGE activation [6], thus potentially making it a useful biomarker of underlying inflammatory pathologies [8].

Frailty is associated with a high prevalence of age-related comorbidities [1, 9]. However, these alone do not account for the higher mortality rates seen in people afflicted by this syndrome. Frailty has been also associated with chronic inflammatory mechanisms [10, 11], which could potentially affect its evolution.

Therefore, we surmised that elevated serum sRAGE could be a marker of poor survival in frail older adults. To address this hypothesis we investigated the relationship between sRAGE and mortality in a prospective study of European older adults living in the community.

Methods

Participants

Participants in this study were men and women aged 65 and older from two well characterised population-based European cohorts, namely, Toledo Study of Healthy Ageing (TSHA) [12] and Approche Multidisciplinaire Intégrée (AMI) [13], who were enrolled in 2013 to the exploratory phase of FRAILOMIC, a European project investigating biomarkers of frailty [14] (for cohort details and selection of participants see Appendix 1, available at *Age and Ageing* online).

The TSHA study protocol was approved by the Clinical Research Ethics Committee of the Complejo Hospitalario de Toledo (Spain) and the AMI study was approved by the Ethics Committee of the CHU (University Hospital) of Bordeaux (France). The research followed the principles embodied in the Declaration of Helsinki.

Measurement of frailty

Frailty was evaluated using Fried's frailty phenotype [1], which includes five criteria, namely slow walking speed, weakness, weight loss, self-reported exhaustion, and low physical activity (for a detailed description of the frailty criteria see Appendix 1). In this study individuals meeting three or more criteria were classed as frail and those who met none, one or two criteria were classed as non-frail.

Measurement of sRAGE

Serum levels of sRAGE were determined from fasting blood samples stored at -80°C using a commercially available sandwich ELISA which detects both, cleaved sRAGE and esRAGE (Quantikine Human RAGE Immunoassay, R&D Systems, Abingdon, UK). Measurements were done in the same laboratory with the origin of the samples blinded to the operator. The intra- and inter-assay coefficients of variation were 1.7% and 3.7%, respectively.

Mortality data

Dates of death were obtained from the Spanish National Death Index (Ministry of Health and Social Services) for TSHA participants and from the death registries of regional Councils for AMI participants. Where necessary deaths were confirmed by follow-up telephone interviews with relatives of the deceased. Time to death was measured for up to 6 years from baseline, at which point the study was right-censored.

Other variables

Sociodemographic, behavioural and health-related factors recorded at baseline are described in Appendix 1.

Data analysis

Details of the data analysis are described in Appendix 1.

Results

Participant characteristics

A flow chart depicting the selection of participants from the TSHA and AMI cohorts enrolled in FRAILOMIC and their progression through to the current study is shown

in Figure S1 (see Appendix 3, available at *Age and Ageing* online). The 691 participants in the study sample included 550 non-frail and 141 frail individuals. The baseline demographic, behavioural and health characteristics of these two groups are summarized in Table 1. Frail participants were older and had a lower level of education than their non-frail counterparts, with a larger proportion of them being females. Frail participants had a mildly reduced kidney function, higher BMI, higher rates of obesity and a higher dependence for basic ADLs. They also showed a higher prevalence of comorbidities, including diabetes, cardiovascular disease and stroke, but not of cancer or hypertension. In addition, there was a non-significant tendency of frail participants to have higher baseline levels of sRAGE compared with those that were non-frail.

sRAGE levels and mortality

During the six year follow-up 101 deaths were recorded, 51 occurring within the non-frail group (9.3%) and 50 within the frail group (35.5%). The characteristics of frail and non-frail participants according to their survival status are summarized in Table S1 (see Appendix 3). Notably, in the frail group baseline sRAGE levels were significantly higher in individuals who had died compared to those who had survived (1563 [1015-2248] pg/mL vs 1184 [870-1657] pg/mL, P=0.006). In contrast, no difference in sRAGE was seen between non-survivors and survivors of the non-frail group (1262 [1056-1554] pg/mL vs 1186 [919-1551] pg/mL, P=0.19). Additional differences between survivors and non-survivors are described in Appendix 2, available at *Age and Ageing* online.

The relationship between sRAGE and mortality was investigated by Cox proportional hazards regression analysis. The univariate analysis of the full analytical sample

demonstrated that sRAGE (entered as a natural logarithm-transformed continuous variable) was a significant predictor of mortality (HR=2.20, 95%CI 1.43-3.36, *P*<0.001). In addition, a significant interaction between sRAGE and frailty was observed (Table 2). Hence, we repeated the regression analysis after stratification by frailty status (see Table S2 in Appendix 3). In frail participants sRAGE remained a significant predictor of mortality (HR=2.69, 95%CI 1.53-4.76, *P*=0.001), but notably, not in those that were classified as non-frail (HR=1.51, 95%CI 0.81-2.82, *P*=0.196). Several other characteristics were also associated with mortality in the full sample (Table S2). These included age, gender, smoking history, total cholesterol, creatinine, eGFR, inability to perform basic ADLs, frailty and a diagnosis of diabetes, cardiovascular disease or stroke. Except for cholesterol and diabetes, these variables remained associated with mortality both in the non-frail and frail groups.

To examine further the association between sRAGE and mortality, we compared four successive nested multivariate models adjusted for relevant sociodemographic, behavioural and health indicators (for details of model construction see Appendix 1). As shown in Table 2, a significant association between sRAGE and mortality persisted across all models, both for the whole sample and for the frail group, but was absent in the non-frail group. Furthermore, the interaction between sRAGE and frailty was also maintained. Concerning the frail group, adjusting for the cohort origin did not alter the relationship between sRAGE and mortality observed in the unadjusted analysis. Further adjustment for age and gender attenuated the association slightly. Finally, addition of health indicators showed that these had no influence. Altogether, the fully adjusted model indicates that frail individuals are 2.7-fold more likely to die at any given time during the six year follow-up period per unit increment of ln-sRAGE (HR=2.72, 95%CI 1.48-4.99, *P*=0.001). Accordingly, a

secondary analysis where sRAGE levels were divided by subsample quartiles (see Table S3 in Appendix 3), showed that frail participants in the highest quartile of sRAGE had a 3.5-fold greater risk of death than those in the lowest quartile (HR=3.51, 95%CI 1.38-8.91, *P*=0.008). An alternative quartile-based analysis with sRAGE cut-offs set from the entire analytical sample is described in Appendix 2.

Given that renal function is known to affect sRAGE levels [7], we also explored whether there was an interaction effect between eGFR and sRAGE on mortality. However, in this case the interaction term was found to be non-significant (data not shown).

Figure 1 shows the Kaplan-Meier survival curves for non-frail and frail participants by subsample quartiles of sRAGE. In frail participants there was a significant difference in survival rates (*P*=0.002), with ~67% of those in the lowest quartile still being alive at the end of the follow-up, compared to ~36% of those in the highest quartile. Furthermore, the difference in survival became apparent after the first two years of follow-up. In contrast, survival rates of non-frail participants were similar across all quartiles of sRAGE (*P*=0.28). Comparable results were obtained when sRAGE cut-offs were set from the full sample (data not shown).

Performance of sRAGE in a mortality risk model

Area under the ROC curve (AUC) analysis over different time horizons showed that for frail participants inclusion of sRAGE in the regression model consistently increased its predictive accuracy (see Figure S2 in Appendix 3). Accordingly, the average AUC increased from 0.841 (95%CI 0.838-0.845) without sRAGE to 0.870 (95%CI 0.867-0.874) after sRAGE was included. In contrast, for non-frail individuals, inclusion of sRAGE showed no additional discriminatory value over that afforded by

using traditional indicators (AUC=0.825, 95%CI 0.822-0.829 without sRAGE compared to AUC=0.828, 95%CI 0.825-0.831 with sRAGE).

Discussion

In the present study, we found that among older adults diagnosed with frailty the risk of mortality over a six-year period increased significantly with increasing baseline serum sRAGE concentrations. On average, the hazards ratio of mortality in these individuals increased more than 2.5-fold with every unit increase in ln-sRAGE. This association was independent of age, gender, smoking history, cholesterol, renal function, ADLs, comorbidities (diabetes, cardiovascular disease, stroke and cancer) and the cohort origin of the participants. Importantly, we found that the frailty status influences the association between sRAGE and mortality, this relationship being significant only in frail individuals. Thus, our study suggests that sRAGE is an independent predictor of mortality in frail older adults.

To our knowledge, the relationship between sRAGE and mortality in connection with frailty has not been previously explored. On the other hand, a number of clinical studies have examined the relationship between sRAGE and mortality outcomes in the absence or presence of disease, producing mixed results. Thus for example, high sRAGE levels predicted mortality in sepsis [15], cardiovascular disease [16], type I [17, 18] and type II diabetes [19], but not in patients with advanced chronic kidney disease [20-22], while low levels predicted mortality in people without cardiovascular disease at baseline [23] and in cancer [24]. Therefore, the relationship between sRAGE and mortality appears to be complex and influenced, at least in part, by the pathophysiological context. In this respect, it could be argued that our results merely reflected the high prevalence of diabetes and cardiovascular

disease in the frail group. However, the finding that this relationship was not attenuated after adjusting for those comorbidities, does not support this possibility. Reduced renal function may increase circulating sRAGE levels [7, 25, 26]. In our sample average eGFR values suggested that both frail and non-frail participants had a somewhat reduced kidney function; this is likely normal for their age. Yet, non-frail participants had on average a slightly higher eGFR than those that were frail, and this could explain in part why sRAGE was marginally elevated in the latter. Nevertheless, the association between sRAGE and mortality was independent of the eGFR, indicating that reduced kidney function was not an influencing factor.

The biological mechanisms through which sRAGE might be related to mortality in frail individuals only have yet to be delineated. Non-survivors in that group did not seem to show a higher degree of frailty at baseline. In addition, it is unlikely that sRAGE affects survival directly. Instead, sRAGE may be a biomarker of chronic stress and inflammation. In making this distinction it should be noted that although sRAGE can play a protective role as an extracellular decoy of membrane-bound RAGE, thereby blocking intracellular signaling, its circulating levels may not always be sufficient to neutralize pro-inflammatory RAGE ligands [27]. Alternatively, raised sRAGE levels could simply reflect the overstimulation of cell surface RAGE by stress and damage-associated molecules, which would lead to the induction of further RAGE expression and subsequent increased shedding into the circulation [6]. Thus, stimulation of the RAGE pathway may be a sign of excessive cellular stress, which in frail people could over the long-term exacerbate their underlying vulnerability, leading to an increase in mortality. Conversely, non-frail people may be more resilient to the adverse causes of RAGE stimulation.

In our study adding sRAGE to conventional demographic, lifestyle and clinical parameters improved by 0.029 (~3%) the accuracy with which up to 6 years mortality could be predicted in frail subjects. Another study in adults from England and Wales aged 85 and older has shown that a combination of blood biological markers, which did not include sRAGE, improved the discriminative ability of a clinical deficit frailty index to predict mortality [28]. Thus, our results raise the possibility that adding sRAGE to other biological measures may further improve this estimation.

The current findings could have important implications regarding the clinical management of frail older adults. In this respect, a clinically relevant threshold has so far not been established. Based on the present categorisation of sRAGE concentrations by quartiles, we tentatively suggest that a value of sRAGE above 1800 pg/mL might be a relevant threshold that could be used for risk stratification. Nevertheless, further cohort studies will be required to either validate or modify this value. Similarly, interventional studies in frail people with low and high levels of sRAGE could ascertain the practical utility of this biomarker.

The present study has important strengths, including that it has been performed with participants from well-characterised longitudinal cohorts, for whom large numbers of baseline sociodemographic and health indicators were available. Furthermore, a priori data homogenization within the FRAILOMIC framework and the determination of sRAGE in the same laboratory have enabled data pooling from separate cohorts, thus achieving a sufficiently large sample size to discover meaningful associations. Nonetheless, the study also has a number of limitations. Participants were mostly white Europeans, so the findings might not generalise to other populations, particularly given the fact that sRAGE is known to vary with race [7, 23]. In addition,

esRAGE was not measured in this study and although esRAGE and sRAGE are generally positively correlated [7], we cannot confirm if evaluating different forms of sRAGE will produce similar results. A final limitation of our study is its inability to establish if the observed relationship between sRAGE and mortality is part of a heightened inflammatory state involving RAGE activation, since no data on RAGE ligands was available.

In summary, despite the above-mentioned limitations, our study adds to the growing literature relating biomarkers of inflammation to frailty [10, 29, 30], and might have implications for understanding the biological pathways which influence outcomes in this growing sub-population of older adults. We report for the first time that in older adults living with frailty (and only in that sub-group), increased levels of sRAGE are associated with a higher risk of death. Validation in additional cohorts and interventional studies are needed to establish the utility of this biomarker in the clinical management of frail older adults.

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Conflicts of interest

None declared.

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Table 1: Baseline sociodemographic, behavioural and health characteristics of the study participants

	All (n=691)	**Non-frail (n=550)**	**Frail (n=141)**	***P***
Sociodemographic and behavioural parameters				
Cohort, % (n)				
AMI	41.2 (285)	42.7 (235)	35.5 (50)	0.116
TSHA	58.8 (406)	57.3 (315)	64.5 (91)	
Age in years, median [IQR]	75.0 [71.0-78.0]	74.0 [70.0-77.0]	79.0 [75.5-83.0]	<0.001
Male, % (n)	49.6 (343)	52.9 (291)	36.9 (52)	0.001
Education, % (n)				
Low	87.8 (607)	86.2 (474)	94.3 (133)	0.015
Intermediate	9.8 (68)	11.1 (61)	5.0 (7)	
High	2.3 (16)	2.7 (15)	0.7 (1)	
Smoking history, % (n)	32.9 (227)	32.9 (181)	32.6 (46)	0.949
Laboratory and biomedical parameters				
sRAGE, median [IQR], pg/mL	1211 [923-1609]	1200 [923-1551]	1294 [916-1887]	0.094
Total cholesterol, mean ± SD, mg/dL	199.3 ± 40.0	200.1 ± 39.6	195.9 ± 41.5	0.280
Creatinine, median [IQR], mg/dL	0.9 [0.7-1.0]	0.9 [0.7-1.0]	0.9 [0.7-1.1]	0.160
eGFR, median [IQR], mL/min/1.73m^2	76.6 [61.5-87.8]	78.1 [63.4-88.6]	70.6 [53.6-83.6]	<0.001
BMI, median [IQR], kg/m^2	28.2 [25.5-31.2]	27.9 [25.4-30.8]	29.3 [25.8-32.9]	0.004
Obesity, % (n)	34.9 (241)	32.4 (178)	44.7 (63)	0.007
ADL, % dependent (n)	10.9 (75)	2.5 (14)	43.3 (61)	<0.001
Frailty, % (n)	20.4 (141)	-	-	-
Diabetes, % (n)	17.7 (122)	16.0 (88)	24.1 (34)	0.029
Hypertension, % (n)	61.1 (422)	60.5 (333)	63.1 (89)	0.575
Cardiovascular disease, % (n)	16.6 (115)	14.0 (77)	27.0 (38)	<0.001
Stroke, % (n)	5.5 (38)	4.0 (22)	11.3 (16)	0.001
Cancer, % (n)	8.7 (60)	8.9 (49)	7.8 (11)	0.673

Abbreviations: sRAGE, soluble receptor for advanced glycation-end products; eGFR, estimated glomerular filtration rate, BMI, body mass index; ADL, activities of daily living

Table 2: Multivariate Cox proportional hazard models of the relationship between Ln sRAGE and mortality

	All (n=691)			Non-frail (n=550)			Frail (n=141)			
	HR	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	***P* for interaction***
Unadjusted	2.20	1.43-3.36	<0.001	1.51	0.81-2.82	0.196	2.69	1.53-4.76	0.001	<0.001
Model 1	2.25	1.46-3.45	<0.001	1.53	0.82-2.87	0.185	2.65	1.51-4.66	0.001	0.001
Model 2	1.81	1.17-2.80	0.008	1.34	0.70-2.58	0.379	2.30	1.33-3.98	0.003	0.007
Model 3	1.84	1.19-2.84	0.006	1.45	0.73-2.91	0.289	2.73	1.48-5.03	0.001	0.005
Model 4	1.85	1.19-2.86	0.006	1.43	0.71-2.89	0.312	2.72	1.48-4.99	0.001	0.004

* *P* value for the interaction between sRAGE and frailty

Model 1: Multivariate model adjusted for cohort

Model 2: Model 1 additionally adjusted for age and gender

Model 3: Model 2 additionally adjusted for smoking history, total cholesterol, eGFR, ADL, diabetes, cardiovascular disease and stroke

Model 4: Model 3 additionally adjusted for cancer

Figures

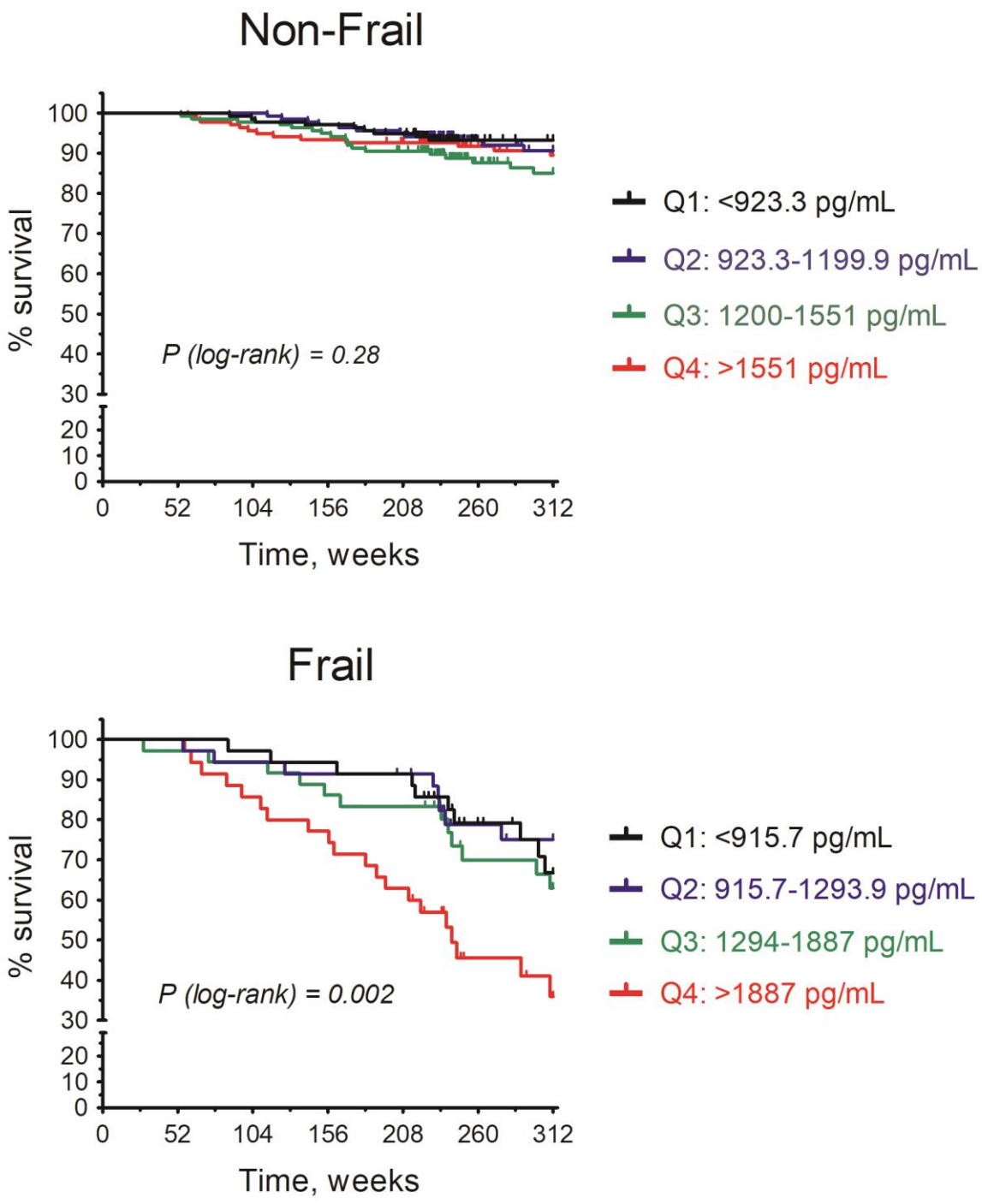

Figure 1: Kaplan-Meier survival curves of non-frail and frail participants by quartiles of sRAGE. Cut-offs were set separately according to the distribution of sRAGE in each individual subsample.

Increased levels of soluble RAGE are associated with a higher risk of mortality in frail older adults – Supplementary Data

Contents

Appendix 1: Supplementary Methods

Study cohorts and selection of FRAILOMIC participants

The Toledo Study of Healthy Ageing (TSHA) is a prospective cohort study initiated in 2006 aimed at studying the determinants and consequences of frailty in community-dwelling older adults living in the city of Toledo and neighbouring towns, Spain [1]. The Approche Multidisciplinaire Intégrée (AMI) is a population-based prospective cohort started in 2007 to study health and ageing in elderly farmers living in rural South West France [2]. The complete methodologies for recruitment and investigations of participants from the two cohorts have been reported elsewhere [1-3]. In both cohorts, once the participants gave written informed consent, biological samples and a wide range of sociodemographic, behavioural and health-related data were collected between 2006 and 2009 by trained psychologists and/or nurses during home visits. Individuals were considered for inclusion in FRAILOMIC if a stored sample of plasma, serum and/or urine was available for biomarker evaluation and if the frailty status could be determined from case report forms using the frailty criteria proposed by Fried et al. [4] (see below). A total of 1398 participants from TSHA and 695 from AMI fulfilled the aforementioned selection criteria. To ensure adequate numbers of participants for statistical analysis, enrolment to FRAILOMIC was carried out to achieve an approximate ratio 1 frail to 3 non-frail subjects and a similar cardiovascular risk profile in both groups. This resulted in 474 subjects from TSHA (109 frail and 365 non-frail) and 320 subjects from AMI (80 frail and 240 non-frail) in being included in the FRAILOMIC database.

Measurement of frailty

The five frailty parameters were defined as follows: 1) Slow walking speed was defined as the worst quintile in a 3-meter walking speed test, adjusted for gender and height. 2) Weakness was defined as the lowest quintile of grip strength measured with a Jamar hand dynamometer, after adjustment for gender and BMI (in kg/m^2) in TSHA [5] or as having difficulty rising from a chair without using armrests in AMI [6]. 3) Weight loss, was defined as the unintentional loss of at least 4.5 kg in the preceding year in TSHA or ≥3 kg in the previous 3 months in AMI. 4) Self-reported exhaustion was evaluated in both cohorts based on a positive response to any of the following questions from the Center for Epidemiologic Studies Depression Scale [7]: "I felt that anything I did was a big effort" or "I felt that I could not keep on doing things" "at least 3-4 days a week". 5) Low physical activity was defined as the lowest quintile for each gender of the Physical Activity Scale for the Elderly [8] in the case of TSHA, and as <1 hour of exercise/week or <3.5 hours of leisure activities/week in AMI. Individuals from the two cohorts whose case report forms had missing information on one or two parameters, but for whom their frailty status could still be assigned based on the remaining recorded parameters, were also included in FRAILOMIC.

Other variables

Sociodemographic and behavioural information recorded at baseline included age, gender, level of education and smoking history. Participants were also asked to report whether they had previously suffered from any of the following physician-diagnosed diseases: hypertension, diabetes, cardiovascular disease (ischemic heart disease or heart failure), stroke or cancer. Body Mass Index (BMI) was calculated as

weight in kilograms divided by height in meters squared. Limitations in activities of daily living (ADLs) were measured with the use of the Katz ADL Scale [9]. Obesity was defined according to World Health Organization guidelines. Cholesterol and creatinine were measured from fasting blood samples by routine enzymatic methods. The estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI creatinine equation [10].

Analytical sample

Of the original participants included in the FRAILOMIC database, 48 from TSHA and 2 from AMI who did not have enough stored serum to measure sRAGE were excluded from the present study. Similarly, 3 participants from TSHA and 30 from AMI who did not have complete frailty data were also excluded. This left a combined TSHA-AMI sample pool of 711 participants. Of these, we further excluded 16 participants for missing covariates at baseline. We also excluded 4 additional participants for giving extremely high values of sRAGE (over 4 standard deviations above the mean), thus leaving a total of 691 participants in the analytical sample (See Figure S1).

Data analysis

Data obtained from the case report forms were harmonised, thus allowing for data pooling. For comparison of sociodemographic characteristics and health indicators continuous variables are reported as means ± SD for normally distributed data or as medians with IQR for skewed data. Categorical variables are presented as percentages. Differences in characteristics between frail and non-frail groups or between survivors and non-survivors were compared by $\chi 2$ test or Fisher's exact test

for categorical variables, and by Student's t test or Mann-Whitney test for continuous variables, as appropriate.

Unadjusted and adjusted Cox proportional hazards regression models examining the associations between sRAGE and mortality were performed with SSPS v.23 for Windows (SSPS Inc., Chicago, Illinois). Given the skewed distribution of sRAGE, for its evaluation as a continuous variable it was natural log-transformed before further analysis. Multivariate models included those variables identified by univariate analysis as conferring a statistically significant risk of death (P<0.05) in the full analytical sample. In addition, models were adjusted for cohort origin and history of cancer, as these were deemed covariates of potential important demographic or clinical influence, respectively. Accordingly, four successive nested models were constructed: Model 1 was adjusted for cohort origin; model 2 included the variables of model 1 plus those basic demographic characteristics which were identified as statistically significant in the univariate analysis (age and gender); model 3 included all the variables in model 2 plus health and lifestyle covariates which were identified as significant in the univariate analysis (smoking history, total cholesterol, eGFR, ADL, diabetes, cardiovascular disease and stroke); and model 4 included the variables of model 3 plus cancer. All models were also examined for interactions by frailty status and by eGFR. Analyses were initially conducted in the full analytical sample and then repeated for the frail and non-frail groups, given the significant interaction observed between sRAGE and frailty. Division between robust and pre-frail participants, in accordance with the original Fried's classification [4], was deemed unsuitable in this instance due to the relative low number of events registered in each of these categories (16 and 35 deaths, respectively), vis-à-vis the number of covariates included in the regression models [11]. Thus, in order to control

adequately for confounders, robust and non-frail participants were pooled into a single group.

Kaplan-Meier survival curves were plotted with sRAGE categorised by subsample quartiles (frail or non-frail) or by cut-offs set from quartiles of the entire analytical sample. Survival curves were compared by the Mantel-Cox log-rank test using GraphPad Prism (v.5.01). The time interval was calculated as the period between baseline blood sampling and the date of death or being censored after 6 years.

The area under the ROC curve (AUC) was computed for every week over a time interval from two to six years of follow-up using a logistic regression model, which included or excluded sRAGE; this analysis was performed with the Statistical package R v2.15.2 for Windows (Vienna, Austria). The integrated AUC was plotted using these values against time, with Graph Pad Prism (v.5.01).

References for Supplementary Methods

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Appendix 2: Supplementary Results

Characteristics of participants according to their survival status

Table S1 shows the baseline characteristics of non-frail and frail participants by survival status. In both the frail and the non-frail groups, participants who had died were older than those who had survived, with a higher percentage of them being male. Additionally, non-survivors were more likely to have limitations in basic ADLs and a lower eGFR. On the other hand, some phenotypic differences were specific to either the frail or the non-frail group. Non-frail participants who died were more likely to have lower cholesterol levels, a history of smoking, and higher rates of cardiovascular disease and stroke. In contrast, in the frail group, those who died had higher rates of diabetes, but no difference was seen in the prevalence of each frailty criterion. In a further search for quantitative differences in the degree of frailty between survivors and non-survivors of this group, baseline gait speeds were compared. In frail men the mean (SD) gait speed was 0.44 ± 0.19 m/sec for survivors vs. 0.41 ± 0.29 m/sec for non-survivors, P=0.31, whereas in frail women these values were 0.35 ± 0.16 m/sec vs. 0.29 ± 0.15 m/sec, respectively, P=0.09. Taken together, these results suggest that within the frail group non-survivors did not seem to have a more pronounced degree of frailty.

Cox regression analysis according to sRAGE quartiles of the analytical sample

Table S4 shows a quartile-based comparison of the relationship between sRAGE and mortality with sRAGE categorised according to cut-offs set from the entire analytical sample. Consistent with the results obtained using subsample quartiles, this alternative analysis showed that frail participants who fell above the highest cut-off level of sRAGE had a 2.6-fold greater risk of death than those that fell below the

lowest cut-off (HR=2.62, 95%CI 1.07-6.42, *P*=0.036). In contrast, no significant increase in the risk of death was observed using the same sRAGE cut-offs in the non-frail group (HR=1.37, 95%CI 0.56-3.35, *P*=0.497).

Appendix 3: Supplementary Tables and Figures

Table S1: Baseline characteristics of study participants by survival status

	All (n=691)			Non-frail (n=550)			Frail (n=141)		
Variable	**Survivors (n=590)**	**Non-survivors (n=101)**	***P***	**Survivors (n=499)**	**Non-survivors (n=51)**	***P***	**Survivors (n=91)**	**Non-survivors (n=50)**	***P***
Cohort, %									
AMI	41.9	37.6	0.424	43.1	39.2	0.595	35.2	36.0	0.921
TSHA	58.1	62.4		56.9	60.2		64.8	64.0	
Age in years, median [IQR]	74.0 [70.0-78.0]	80.0 [76.0-84.0]	<0.001	73.0 [69.8-77.0]	77.3 [75.0-83.4]	<0.001	78.0 [74.0-82.4]	81.8 [78.1-86.5]	<0.001
Male, %	47.6	61.4	0.011	50.9	72.5	0.003	29.7	50.0	0.017
Education, %									
Low	87.1	92.1	0.181	85.8	90.2	0.423	94.5	94.0	0.698
Intermediate	10.2	7.9		11.2	9.8		4.4	6.0	
High	2.7	0.0		3.0	0.0		1.1	0.0	
Smoking history, %	30.7	45.5	0.003	31.3	49.0	0.010	27.5	42.0	0.078
sRAGE, median [IQR], pg/mL	1185 [916-1559]	1360 [1043-1957]	0.002	1186 [919-1551]	1262 [1056-1554]	0.19	1184 [870-1657]	1563 [1015-2248]	0.006
Total cholesterol, mean ± SD, mg/dL	201.2 ± 39.6	188.2 ± 40.8	0.004	202.0 ± 39.3	181.7 ± 39.0	0.001	196.5 ± 41.5	194.9 ± 41.8	0.824
Creatinine, median [IQR], mg/dL	0.9 [0.7-1.0]	1.0 [0.8-1.3]	<0.001	0.8 [0.7-1.0]	0.9 [0.8-1.2]	0.002	0.9 [0.7-1.0]	1.0 [0.8-1.3]	0.014
eGFR, median [IQR], mL/min/1.73m^2	77.9 [63.5-88.5]	63.0 [48.2-83.2]	<0.001	79.7 [65.2-89.0]	64.4 [50.3-86.6]	0.001	71.5 [58.4-85.4]	63.0 [47.8-80.4]	0.020

Table S1 continued									
BMI, median [IQR], kg/m^2	28.2 [25.5-31.1]	28.1 [25.2-31.3]	0.694	27.9 [25.5-30.8]	27.2 [25.1-30.8]	0.393	29.3 [26.2-33.3]	29.4 [25.2-31.7]	0.266
Obesity, %	34.9	34.7	0.959	32.9	27.5	0.431	46.1	42.0	0.635
ADL, % dependant	7.3	31.7	<0.001	2.0	7.8	0.012	44.0	56.0	0.024
Frailty, %	15.4	49.5	<0.001	-	-	-	-	-	-
Slowness, %	23.2	57.4	<0.001	11.4	23.5	0.013	87.9	92.0	0.451
Weakness, %	22.4	49.5	<0.001	13.2	21.6	0.102	72.5	78.0	0.476
Weight loss, %	12.7	18.8	0.098	9.4	7.8	0.712	30.8	30.0	0.924
Exhaustion, %	13.7	32.7	<0.001	6.6	11.8	0.172	52.7	54.0	0.887
Low physical activity, %	21.4	53.5	<0.001	11.2	29.4	<0.001	76.9	78.0	0.884
Diabetes, %	15.8	28.7	0.002	15.2	23.5	0.124	18.7	34.0	0.042
Hypertension, %	60.8	62.4	0.771	59.9	66.7	0.348	65.9	58.0	0.350
Cardiovascular disease, %	13.7	33.7	<0.001	12.2	31.4	<0.001	22.0	36.0	0.073
Stroke, %	4.1	13.9	<0.001	2.8	15.7	<0.001	11.0	12.0	0.856
Cancer, %	8.3	10.9	0.394	8.6	11.8	0.452	6.6	10.0	0.471

Table S2: Univariate analysis for potential contributing factors of mortality

	All (n=691)			Non-frail (n=550)			Frail (n=141)		
Variable	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Cohort	0.77	0.50-1.18	0.231	0.85	0.47-1.54	0.591	0.58	0.32-1.08	0.084
Age	1.16	1.12-1.19	<0.001	1.16	1.11-1.22	<0.001	1.10	1.05-1.16	<0.001
Male gender	1.88	1.25-2.80	0.002	2.65	1.43-4.90	0.002	2.22	1.28-3.88	0.005
Education	0.71	0.35-1.47	0.361	0.76	0.30-1.92	0.564	1.09	0.34-3.52	0.881
Smoking history	1.84	1.24-2.72	0.002	2.04	1.18-3.53	0.011	1.83	1.04-3.22	0.035
Ln sRAGE	2.20	1.43-3.36	<0.001	1.51	0.81-2.82	0.196	2.69	1.53-4.76	0.001
Total cholesterol	0.99	0.99-1.00	0.006	0.99	0.98-0.99	0.001	1.00	0.99-1.01	0.746
Creatinine	2.84	2.20-3.67	<0.001	2.80	1.95-4.02	<0.001	2.93	1.90-4.53	<0.001
eGFR	0.97	0.96-0.98	<0.001	0.97	0.95-0.98	<0.001	0.98	0.96-0.99	0.003
BMI	0.97	0.93-1.02	0.216	0.96	0.90-1.03	0.234	0.95	0.90-1.00	0.064
Obesity	0.93	0.62-1.41	0.740	0.75	0.41-1.39	0.364	0.81	0.46-1.43	0.471
ADL	4.30	2.82-6.53	<0.001	3.59	1.29-9.97	0.014	1.81	1.04-3.17	0.037
Frailty	4.19	2.84-6.19	<0.001						
Diabetes	1.98	1.28-3.04	0.002	1.61	0.84-3.08	0.148	2.11	1.17-3.79	0.013
Hypertension	1.17	0.78-1.76	0.439	1.41	0.79-2.53	0.244	0.90	0.51-1.57	0.702
Cardiovascular disease	3.07	2.03-4.64	<0.001	3.23	1.78-5.84	<0.001	1.93	1.08-3.44	0.026
Stroke	3.02	1.72-5.32	<0.001	4.99	2.34-10.61	<0.001	1.06	0.45-2.49	0.893
Cancer	1.41	0.75-2.64	0.281	1.46	0.62-3.43	0.382	1.45	0.57-3.65	0.433

Table S3: Multivariate Cox proportional hazard models of the relationship between sRAGE and mortality in frail participants according to sRAGE quartiles

	Model 1			**Model 2**			**Model 3**			**Model 4**		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
*sRAGE quartiles**												
Q1, <915.7 pg/mL	1(ref)			1(ref)			1(ref)			1(ref)		
Q2, 915.7-1293.9 pg/mL	0.86	0.34-2.18	0.746	0.61	0.23-1.61	0.318	0.77	0.26-2.35	0.651	0.73	0.24-2.23	0.584
Q3,1294-1887 pg/mL	1.26	0.54-2.92	0.589	1.06	0.44-2.52	0.903	1.35	0.54-3.39	0.522	1.41	0.56-3.58	0.471
Q4, >1887 pg/mL	2.87	1.34-6.15	0.007	2.53	1.15-5.59	0.021	3.61	1.43-9.12	0.007	3.51	1.38-8.91	0.008

Model 1: Multivariate model adjusted for cohort

Model 2: Model 1 additionally adjusted for age and gender

Model 3: Model 2 additionally adjusted for smoking history, total cholesterol, eGFR, ADL, diabetes, cardiovascular disease and stroke

Model 4: Model 3 additionally adjusted for cancer

* Cut-offs based on quartiles of the frail group

Table S4: Comparison of the relationship between sRAGE and mortality in frail and non-frail participants based on analytical sample quartiles

	All (n=691)				Non-frail (n=550)				Frail (n=141)			
	n	**HR**	**95% CI**	***P***	**n**	**HR**	**95% CI**	***P***	**n**	**HR**	**95% CI**	***P***
*sRAGE quartiles**												
Q1, <923.4 pg/mL	173	1(ref)			138	1(ref)			35	1(ref)		
Q2, 923.4-1211.1 pg/mL	173	0.70	0.36-1.38	0.307	144	1.10	0.45-2.69	0.832	29	0.76	0.24-2.48	0.653
Q3,1211.2-1608.7 pg/mL	172	1.27	0.70-2.31	0.438	143	1.68	0.74-3.86	0.218	29	1.11	0.42-2.94	0.838
Q4, >1608.7 pg/mL	173	1.63	0.92-2.91	0.097	125	1.37	0.56-3.35	0.497	48	2.62	1.07-6.42	0.036

The multivariate model was adjusted for cohort, age and gender, smoking history, total cholesterol, eGFR, ADL, diabetes, cardiovascular disease, stroke and cancer (model 4 in Table S3)

* Cut-offs based on quartiles of the entire analytical sample

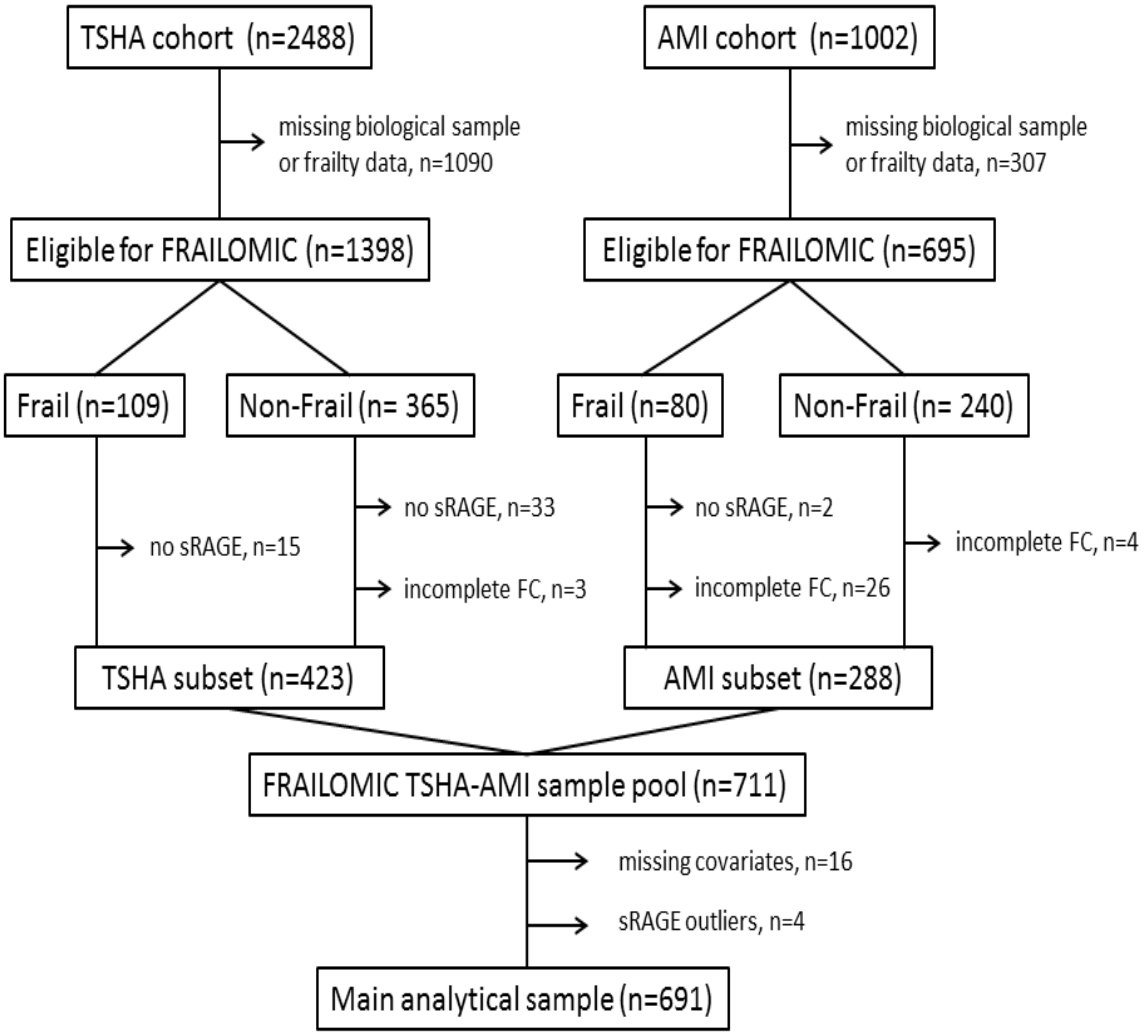

Figure S1: Flow chart of the study sample selection. Details of the selection of participants are described in Appendix 1 above. FC, Fried's criteria

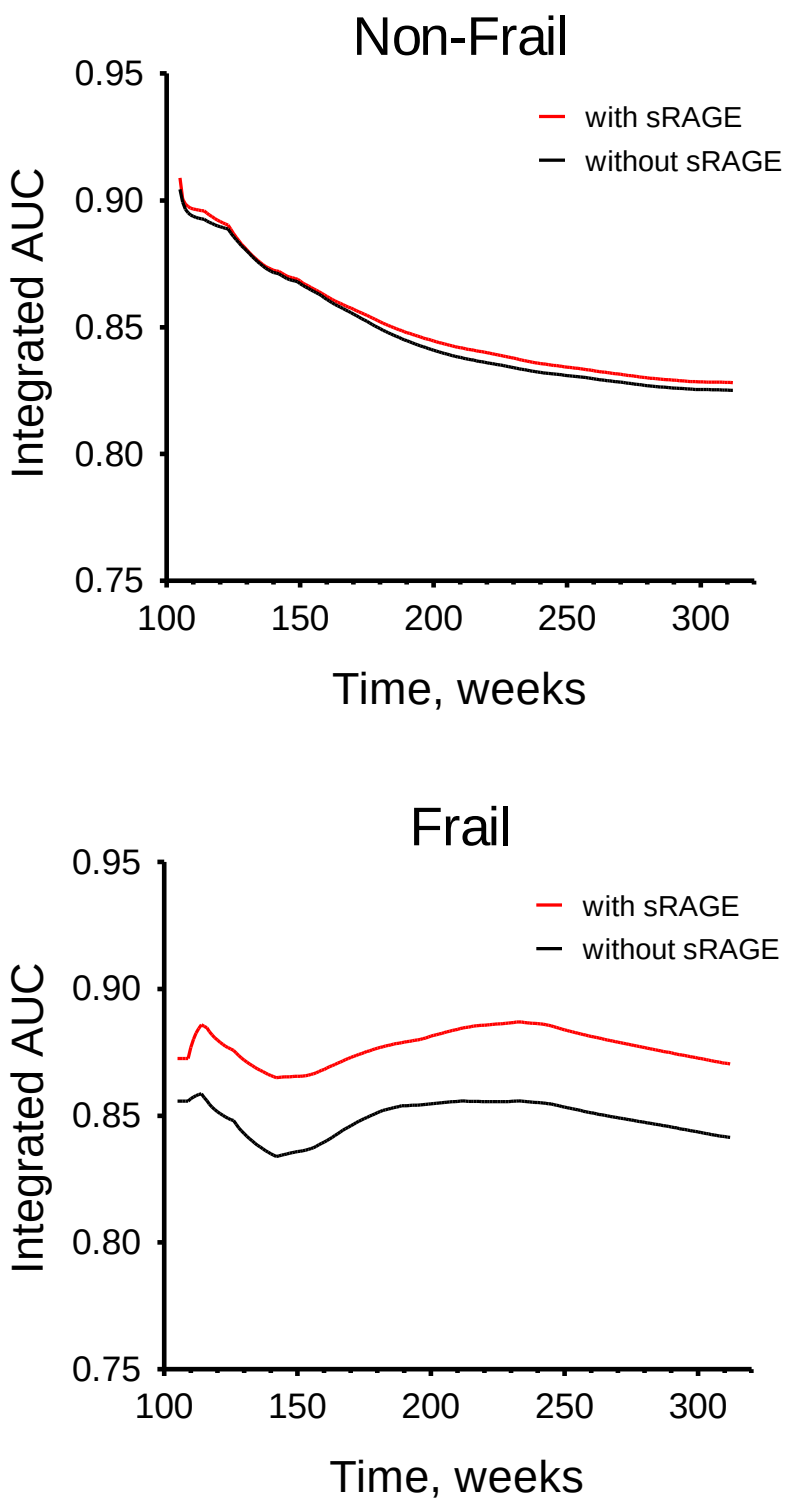

Figure S2: Integrated AUCs for different time horizons. Values were calculated for hazards models that included or excluded sRAGE, as indicated.