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Soluble tumor necrosis factor receptor 1 (sTNFR1) is associated with increased total mortality due to cancer and cardiovascular causes – findings from two community based cohorts of elderly

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Abstract

Background: Experimental evidence support soluble receptors for tumor necrosis factor alpha as important mediators of the underlying pathology leading to cardiovascular disease and cancer. However, prospective data concerning the relation between circulating soluble tumor necrosis factor receptor-1 (sTNFR1) and mortality in humans are lacking. We aimed to explore and validate the association between sTNFR1 and mortality, and to explore the influence of other established risk factors for mortality, including other inflammatory markers.

Methods: The association between serum sTNFR1 and the risk for mortality was investigated in two community-based cohorts of elderly: the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS; women 50%, n=1005, mean age 70 years, median follow-up 7.9 years) and the Uppsala Longitudinal Study of Adult Men (ULSAM, n=775, mean age 77 years, median follow-up 8.1 years).

Results: In total, 101 participants in PIVUS and 274 in ULSAM died during follow-up. In multivariable Cox regression models adjusted for inflammation, lifestyle and established cardiovascular risk factors, one standard deviation (SD) higher sTNFR1 was associated with a hazard ratio (HR) for mortality of 1.37, 95% confidence interval (CI) 1.17-1.60, in PIVUS and HR 1.22, 95% CI 1.10-1.37 in ULSAM. Moreover, circulating sTNFR1 was associated with cardiovascular mortality (HR per SD of sTNFR1, 1.24, 95% CI 1.07-1.44) and cancer mortality (HR per SD of sTNFR1, 1.32, 95% CI 1.11-1.57) in the ULSAM cohort. High levels of sTNFR1 identified individuals with increased risk of mortality among those with high as well as low levels of systemic inflammation.

Conclusions: An association between circulating sTNFR1 and an increased risk for mortality was found and validated in two independent community-based cohorts. The future clinical role of sTNFR1 to identify high risk patients for adverse outcomes and mortality has yet to be determined.

Key words: Inflammation; oxidative stress; all-cause mortality; CRP; cytokines; community based cohort; tumor necrosis factor

Introduction

Tumor necrosis factor (TNF)- α , and its highly correlated soluble receptors (sTNFR1 and sTNFR2) are central players in inflammation as well as in stress response cascades, important pathways for the development of cardiovascular disease and cancer [1, 2]. Anti-TNF therapies are widely used in rheumatic diseases and have not been associated with increased cancer risk or cardiovascular risk [3]. In fact, favorable effects in patients with rheumatoid arthritis treated with anti-TNF therapies have been seen on the risk of lymphoma [4], and patients with heart failure treated with anti-TNF therapy have seen improvements in their left ventricular structure [5].

Circulating levels of sTNFR1 and sTNFR2 have been shown to predict cardiovascular and total mortality in patients with rheumatoid arthritis [6], and sTNFR1 has been associated with total mortality in patients with diabetes [7]. Yet, community based data on the effects of circulating levels of sTNFR1 on cardiovascular, cancer and total mortality are sparse [8].

In the present study, we hypothesized that elevated levels of sTNFR1 are causally associated with an increased risk for total mortality. Herein, we primarily aimed to explore and validate the association between standard deviation increments of sTNFR1 and mortality in two community-based cohorts of elderly, and to explore the influence of other established risk factors for mortality, including other inflammatory markers.

Research Design and Methods

Study samples

The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS)

All 70-year old men and women living in Uppsala, Sweden 2001-2004 were eligible for the PIVUS study [9] (described in detail on <http://www.medsci.uu.se/pivus/pivus.htm>). Of 2025 invited individuals, 1016 agreed to participate. Of these, 11 participants were excluded due to missing data on sTNFR1 and CRP, leaving 1005 participants as the present study sample.

The Uppsala Longitudinal Study of Adult Men (ULSAM)

The ULSAM study was initiated in 1970. All 50-year-old men, born in 1920-24 and living in Uppsala, Sweden, were invited to a health survey, focusing at identifying cardiovascular risk factors [10] (described in detail on <http://www.pubcare.uu.se/ULSAM>). These analyses are based on the fourth examination cycle, when participants were approximately 77 years old (1998-2001). Of 1398 invited men, 838 (60%) participated. Of these, 63 were excluded due to missing data on sTNFR1 and CRP, leaving 775 participants as the present study sample.

All participants in both studies gave written informed consent and the Ethics Committee of Uppsala University approved the study protocols.

Baseline investigations

The investigations in PIVUS and ULSAM were performed using similar standardized methods, including anthropometrical measurements, blood pressure, blood sampling, and questionnaires regarding socioeconomic status, medical history, smoking habits, medication and physical activity level [9, 10]. Venous blood samples were drawn in the morning after an overnight fast and stored at -70°C until analysis.

The soluble receptor TNFR1 and high sensitivity IL-6 were analyzed using commercially available ELISA kit (DY225 and HS600B, R&D Systems, Minneapolis, MN). The assays had a total coefficient of variation (CV) of approximately 6%. Cystatin C was measured by latex-enhanced reagent (NLatexCystatin C; Siemens, Deerfield, IL, USA) using a BN ProSpec analyser (Siemens) and used to estimate GFR in ULSAM [11], and by latex-enhanced reagents (Gentian, Moss, Norway) using an Architect ci8200 (Abbott Laboratories, Abbott Park, IL, USA) in PIVUS [12]. High-sensitive CRP measurements were performed by latex-enhanced

reagent (Siemens) with the use of a BN ProSpec® analyzer (Siemens) in ULSAM and with the use of an Architect ci8200 in PIVUS [13]. Diabetes mellitus was diagnosed as fasting plasma glucose ≥ 7.0 mmol/l (≥ 126 mg/dl), or use of anti-diabetic medication [14]. Cystatin C-based eGFR was calculated as previously described [11]. Urine albumin was measured by nephelometry (Urine albumin, Dade Behring, Deerfield IL, USA) using a Behring BN ProSpec® analyzer (Dade Behring).

Prevalent cardiovascular disease at baseline was defined as a history of ischemic heart disease or cerebrovascular disease, or Q-, QS-complexes or left bundle-branch block in baseline ECG. Leisure time physical activity was assessed by a questionnaire as previously described [15]. Education level was stratified as low (elementary school, 6-7 years), medium (high school), or high (college/university studies).

End-point definitions

The Swedish Cause-of-Death register was used to define total mortality, cardiovascular mortality (death from ischemic heart disease or cerebrovascular disease [ICD-10] codes I20-I25, I60-I69/G45) and cancer mortality (and [ICD-10] C00-D48). Data on cause-specific mortality was not available in the PIVUS cohort.

Statistical analysis

Primary analyses

We initially investigated distributions of all variables. In our primary analyses, we thereafter investigated cohort-specific associations of serum sTNFR1 modeled as a continuous variable, per standard deviation (SD) with total mortality using Cox proportional hazards regression. We also analyzed the association by quintiles (lowest four quintiles as referent), in order to make a clinical assessment easier. The following multivariable models (using the participants age as the timeline) was used:

- A) Age, inflammation (CRP), and sex-adjusted (PIVUS) model to explore if the association is independent of the most widely used inflammatory marker in clinical practice.
- B) Factors in Model A and lifestyle/socioeconomic factors (BMI, smoking, leisure time physical activity and education level); to explore if the association is independent of common confounders.

C) Factors in Model A and B, and established cardiovascular risk factors (sex, systolic blood pressure, diabetes, smoking, BMI, total cholesterol, HDL-cholesterol, antihypertensive treatment, lipid-lowering treatment and prevalent cardiovascular disease), was explored to determine to what extent associations with these factors can explain our findings.

In the ULSAM cohort, we also investigated the association between serum sTNFR1 and cause-specific mortality from cardiovascular causes or cancer in these models.

Proportional hazards assumptions were confirmed by Schoenfeld's tests. We investigated potential nonlinearity of the associations using penalized splines. After an initial verification of the association between sTNFR1 and the outcomes in individuals without missing data, we imputed the missing data. Multiple imputation methods were used to account for the potential influence of missing data on covariate factors (633 individuals had no missing data, 48 individuals had one variable missing and the individual with most missing data had 7 variables missing in ULSAM. 919 individuals had no missing data, 61 individuals had one variable missing and the individual with most missing data had 9 variables missing in PIVUS). Finally, we calculated the association between sTNFR1 and cancer as well as cardiovascular mortality starting follow-up two years after baseline.

In secondary analysis, we calculated the risks associated combinations of with high/low sTNFR1 (quintile 1-4 vs 5, based on findings in the present study) and high/low CRP (≤ 3 vs >3 mg/l)[16] in ULSAM and PIVUS, in order to provide further insight to the contribution of sTNFR1 for mortality beyond inflammation reflected by CRP levels.

We performed secondary analyses in the PIVUS cohort in which several inflammatory markers (serum interleukin (IL)-1a, IL-1b, IL-2, IL-4, IL-8 IL-10, and TNF-alpha) were added to model C to explore if the association between sTNFR1 and mortality can be explained by other known cytokines. We investigated effect modification by gender by including multiplicative interaction terms in Model C in PIVUS. Effect modification by CRP was explored in ULSAM and PIVUS.

In the ULSAM-cohort, we further added the following markers of inflammation and oxidative stress to multivariable model C (plasma serum amyloid A and urinary 15-keto-dihydro-PGF_{2 α} [reflecting COX-mediated inflammation] and F₂-isoprostanes [reflecting oxidative stress] to determine if these biomarkers can explain our findings. We also investigated if adjustments for estimated glomerular filtration could explain our findings in ULSAM and PIVUS, which is important as recent studies put forward sTNFR1 as a relevant kidney dysfunction biomarker.

A two-sided p-value <0.05 was regarded as significant in all analyses. Stata11.2 (Stata Corp College Station, TX, USA) was used for all analyses.

RESULTS

Baseline characteristics

Baseline characteristics of the study populations are presented in Table 1. In total, 101 participants in PIVUS and 274 in ULSAM died during follow-up. Among the participants in ULSAM, 122 died of cardiovascular, 84 of cancer and 68 of non-cardiovascular-non-cancer mortality.

The mortality incidence rates per 100 years of follow-up for each quintile of sTNFR1 are shown in Table 2. Having sTNFR1 levels in quintile 5 was associated with a higher risk for total mortality in both PIVUS and ULSAM (Figure 1), as well as cardiovascular and cancer mortality in ULSAM. Lower risks were seen individuals with sTNFR1 levels in quintiles 1-4, suggesting a non-linear association with mortality. Non-cancer, non-cardiovascular mortality was not increased in any quintile of sTNFR1 in ULSAM. Spline curves indicate that the greatest increase in risk for total mortality is seen in the top quintile of sTNFR1 in respective cohorts (Figure 2). In PIVUS, there seems to be a tendency of a U-shaped association, with an increase in risk at the extreme low range of sTNFR1. However, no firm conclusion regarding a potential U-shape should be drawn based on the spline curve given the greater uncertainty and wider confidence intervals at the extreme ends of sTNFR1 levels.

Table 3 shows Cox regression models for total mortality in the PIVUS and the ULSAM studies. For each standard deviation increment in sTNFR1, there is an increased risk of mortality in PIVUS and in ULSAM in models adjusted for standard inflammation (CRP) and age ($p<0.001$ for both). These results remained statistically significant after adjustments for, lifestyle and cardiovascular risk factors as well (Model B and C). Having sTNFR1 levels in quintile 5 verses quintile 1-4 was associated with a 1.6-2.5 fold increased risk for total mortality in all models tested in both cohorts ($p<0.01$, Table 3).

The association between higher sTNFR1 and cause-specific mortality from cancer or cardiovascular mortality in the ULSAM cohort are shown in Table 3. An association between higher sTNFR1 and increased risk for both cardiovascular and cancer mortality was seen in all

multivariable models. There was no association between sTNFR1 and the risk for non-cancer / non cardiovascular mortality.

Secondary analysis

The risk associated with high/low sTNFR1 in individuals with high/low CRP using individuals with low sTNFR1 (quintile 1-4) and low CRP (< 3 mg/l) as referent are shown in Table 4.

Having high sTNFR1 levels (top quintile) was associated with increased risks of mortality in those with low CRP. Individuals with both high sTNFR1 and high CRP were at the highest risk.

The association between sTNFR1 and cancer as well as cardiovascular mortality remained after exclusion of the individuals who died in the first two years after baseline in ULSAM.

We also tested and confirmed that the association between sTNFR1 and total mortality remained statistically significant after adjustments for multiple markers of inflammation were added to model C (serum interleukin (IL)-1a, IL-1b, IL-2, IL-4, IL-6, IL-8 IL-10, and TNF-alpha) in PIVUS hazard ratio (HR) per SD increase 1.36 (95% CI 1.14-1.62), as well as markers of oxidative stress, and COX-mediated inflammation, in ULSAM, HR per SD increase 1.21 (95% CI 1.08-1.35). In the PIVUS cohort the association between sTNFR1 and total mortality was essentially unaltered after adjustment for eGFR (HR per SD increase 1.28 95% CI 1.07-1.52) while in ULSAM, the association was attenuated (HR per SD increase 1.12 95% CI 0.99-1.27). This attenuation by eGFR appeared predominantly to be driven by an attenuated association between sTNFR1 and cardiovascular mortality (HR per SD increase 1.06 95% CI 0.89-1.26) rather than by the association between sTNFR1 and cancer mortality (HR per SD increase 1.31 95% CI 1.06-1.62). There was no significant multiplicative interaction with gender in PIVUS ($p=0.674$), and there was no interaction between sTNFR1 and CRP ($p=0.46$ in PIVUS and 0.93 in ULSAM when using total mortality as the outcome). The association between sTNFR1 and mortality was unaltered in both cohorts after further adjustment for the storage time in the biobank freezer (data not shown).

Comments

Main findings

Elevated levels of soluble TNFR1 was associated with increased mortality risk in two independent cohorts of elderly. These associations remained significant after adjustments for lifestyle factors, established cardiovascular risk factors and a large number of inflammatory

markers. Interestingly, high levels of sTNFR1 identified high risk individuals among those with both low as well as high levels of systemic inflammation as evaluated by CRP. When specific causes of death were studied separately in the ULSAM cohort, sTNFR1 appeared to have a stronger association with cancer mortality than with cardiovascular mortality. Our community based data adds to experimental and clinical studies supporting the importance of TNF- α pathways in the development of cardiovascular disease and cancer.

Comparisons with previous studies

Soluble TNFR1 and sTNFR2 have been shown to predict cardiovascular and total mortality in patients with rheumatoid arthritis [6]. A recent study of the community based cohort from Framingham tested many biomarkers of inflammation and oxidative stress, and sTNFR2 was shown to be associated with total mortality [17]. Soluble TNFR1 was recently shown to be associated with total and non-vascular mortality in the Northern Manhattan Study, but cardiovascular mortality was only borderline significant [8], which is in accord with the present study where the point estimate was higher for cancer mortality than cardiovascular mortality.

A few previous retrospective case control studies have previously reported the association between sTNFR1 and incident cancer. The European prospective investigation into cancer and nutrition examined the association between TNF- α , sTNFR1 and sTNFR2 with endometrial cancer in two hundred and seventy cases and over five hundred matched controls [18], and reported higher risks in individuals with elevated levels of all these markers. In contrast, no association was seen between TNF- α and its two soluble receptors with breast cancer, when 142 white women with breast cancer were compared with matched controls [19]. Patients with rheumatoid arthritis and anti-TNF- α therapy have been shown to have equal or slightly elevated risk as the general population as regards lymphoma, whereas a 2-3 fold risk has been shown for RA patients without anti-TNF therapy [4]. Moreover, elevated plasma levels of TNF- α as well as sTNFRs in patients with epithelial ovarian cancer have been associated with increased mortality [20].

Also, some previous studies have reported associations between sTNFR1 and cardiovascular risk. Soluble TNFR1 was shown to be cross-sectionally associated with cardiometabolic risk factors in a study of patients with coronary artery disease: elevated uric acid levels, lower HDL cholesterol, metabolic syndrome, diabetes and heart failure [21]. In accordance with our findings where sTNFR1 was associated with cardiovascular mortality after adjustments for CRP and IL-6, sTNFR1 but not IL-6 and CRP was associated with left ventricular mass in a

study of over 600 patients with stroke [22]. Elevated levels of sTNFR1 and sTNFR2 have also been associated with incident coronary heart disease in women with a low kidney function [23, 24], and elevated levels of sTNFR2 were associated with incident coronary heart disease independently of hyperglycemia a 10-year follow-up of almost one thousand nurses with diabetes [25].

Possible mechanisms for the observed associations

The causal effects behind of our observational findings are not easy to explain by specific mechanisms, as numerous basic cellular mechanisms may contribute to our findings. In fact, TNF- α and its soluble receptors are involved in numerous elementary pathological biological processes [1, 2]. When it comes to cancer mortality, the TNF- α biology is involved in virtually all steps of tumor biology, namely: cell transformation, angiogenesis, apoptosis, survival, proliferation, invasion, and metastasis [26]. There are reasons to believe that sTNFR1 has a negative influence on cancer survival in most of these processes [4, 18, 20]. The particular role of sTNFR1, and if high circulating levels result in a higher cancer risk, or if high levels of sTNFR1 propagate tumorigenesis remains to be established. In addition, there is also the possibility that high levels of sTNFR1 reflects a more aggressive immunological response coupled to advanced tumor stage, however, the association between sTNFR1 and cancer mortality remained significant even after adjustments for systemic inflammation (IL-6 and CRP, as well as many interleukins and markers of oxidative stress); which suggest an explicit role of the immune system and specifically sTNFR1 in cancer patients regardless of general inflammatory processes. Yet, given the complex interplay between different inflammatory pathways we cannot rule out substantial residual confounding.

Several studies suggest an association between leukocyte activity and the occurrence of TNF receptors in serum, providing evidence that TNFR1 could constitute potent biomarkers for immunological cell responses, such as severe infections, transplantation-related complications as well as certain malignancies [27, 28]. To exemplify, leukocyte cultures from breast cancer patients has been shown to secrete sTNFRs to a higher extent than cultures from healthy subjects, suggesting that the release of these molecules play a role in the immune systems native anti-tumor defense [29]. Just recently, a study showed that TNF- α induces cell cycle arrest in various cancers through the joint action of interferon-gamma and TNF- α , however this phenomenon specifically required the tumor to exhibit TNFR1 expression [30]. However,

reverse causation due to prevalent but undetected cancer does not appear to explain the association between sTNFR1 and cancer mortality risk completely, since the association with cancer mortality remained unaltered after removal of those dying of cancer within two years of the baseline investigation in the present study.

Our findings indicate that sTNFR1 levels could portray an inflammatory process that is independent of CRP, IL-6, and a wide range of other markers of inflammation and oxidative stress. The association between sTNFR1 and cardiovascular mortality could be explained by its direct atherosclerotic effects by being a marker of a higher systemic inflammatory state [21, 25]. Inflammation does not only initiate the formation of the atherosclerotic lesion, but can also be involved in plaque rupture; the final trigger of most thrombotic and atherosclerotic events [31]. Another potential mechanism to the increased risk of cardiovascular mortality in individuals with elevated sTNFR1 levels could be through angiogenesis, as has been shown in ischemic models [32]. Finally, elevated sTNFR1 could be associated with a procoagulant and hypofibrinolytic state that has been shown to be present in patients with metabolic syndrome [33].

Adjustments for eGFR attenuated the association between sTNFR1 and cardiovascular mortality but not cancer mortality. Thus, a reduced kidney function appears to be an important mediator that may explain the link between sTNFRs and cardiovascular mortality in the present study. This is further supported by some previous studies reporting that TNFRs predict the progression of chronic kidney disease in patients with diabetes [34, 35]. Therefore, it is possible that individuals with elevated sTNFRs are more likely to develop CKD which in turn substantially increases the risk for CVD.

Clinical implications

Systemic inflammation assessed by CRP was used to select high risk individuals with normal cholesterol levels in the JUPITER trial [36]. Our data clearly show that circulating levels of sTNFR1 identify risk not identified by CRP. Yet, it remains to be studied if risk associated with circulating levels of TNFRs can be lowered by statins or by other pharmaceutical drugs or lifestyle interventions. Anti-TNF therapy has also to be studied further in its effects on cancer [4], cardiovascular [5], and kidney disease [37, 38]. Additionally, it remains to be shown if reduction in sTNFRs decrease mortality in general as well as in patients with cardiovascular disease or cancer in clinical settings.

Strengths and limitations

The present study has several strengths. First, to minimize the risk of chance findings, we used two independent community-based study samples with longitudinal data on mortality. Second, National Swedish Registers on causes of death have been shown to be 99.8% complete [39]. Third, the detailed characterization of the study participants enabled us to control for a large number of established risk factors as well as a wide variety of markers reflecting different inflammatory pathways. Fourth, to our knowledge, the ULSAM and PIVUS cohorts are the largest community-based cohorts that have analyzed circulating levels TNFR1 and with follow-up data on mortality.

Limitations include the unknown generalizability to other age- and ethnic groups. Also, the mean levels of sTNFR1 appeared higher in PIVUS as compared to ULSAM. As current knowledge on factors that influence circulating levels of sTNFR1 is very limited and we cannot rule out that cohort-specific effects due to differences in handling of the samples (such as freezer time), or differences in gender, age or the time of the baseline examination between the two cohorts may influence the absolute levels of sTNFR1. Thus, additional large-scale studies in other ethnicities and in other age groups are needed to properly validate our findings and to establish optimal thresholds in order to identify individuals at an increased risk.

In conclusion, we have shown that sTNFR1 is associated with total as well as both cancer and cardiovascular mortality in the community independently of established risk factors and a wide variety of inflammatory markers. Our findings accentuate the need for increased understanding regarding TNF-associated molecules in human disease. Further studies are warranted to evaluate the future clinical role and use of sTNFR1 as a marker to identify high risk patients for adverse outcomes and mortality.

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Author contributions

Author contributions: A.C.C. drafted the manuscript and researched data. J.Ä. researched data, edited manuscript, contributed to discussion, and provided funding. C.C.J., T.L., E.I, J.S and L.L. Collected PIVUS data, reviewed manuscript, and contributed to discussion. A.L. reviewed manuscript, contributed to discussion and measured the soluble TNFRs.

The authors of this manuscript have no conflict of interest to disclose.

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Table 1. Baseline characteristics in the PIVUS and ULSAM cohorts

Variable	PIVUS	ULSAM
Number of subjects	1005	775
Female no. (%)	503 (50)	0 (0)
Age (years)	70 ±0.2	77 ±0.8
sTNFR1(pg/mL)	3366 ±971	2081 ±865
CRP (mg/L)	2.5 ±5.2	3.97 ±7.0
Body mass index (kg/m ²)	27.0 ±4.3	26.3 ±3.5
Serum total cholesterol (mg/dL)	5.4 ±1.0	5.4 ±1.0
Serum HDL cholesterol (mg/dL)	1.5 ±0.4	1.3 ±0.3
Systolic blood pressure (mmHg)	150 ±23	151 ±21
eGFR (ml/min)	98 ±25	73 ±17
Smoking – no. (%)	108 (11)	59 (8)
Diabetes no. (%)	87 (9)	107 (14)
Previous cardiovascular disease-no. (%)	165 (16)	210 (27)
Lipid lowering treatment- no. (%)	160 (16)	128 (16)
Antihypertensive treatment – no (%)	311 (31)	365 (47)
Leisure time physical activity (%)		
-Sedentary	115 (12)	60 (8)
-Moderate	593 (59)	272 (35)
-Regular	224 (22)	411 (53)
-Athletic	68 (7)	35 (5)
Education/Social status (%)		
-High	557 (57)	433 (56)
-Middle	182 (18)	223 (29)
-Low	254 (25)	122 (15)

Data are mean ± standard deviation for continuous variables and n (%) for categorical variables

Table 2. Incidence rates in quintiles of serum TNFR1 for total mortality in the PIVUS cohort, and for cardiovascular mortality, cancer mortality, non-cardiovascular/non-cancer mortality and total mortality in the ULSAM cohort

<u>The PIVUS cohort</u>			<u>The ULSAM cohort</u>							
Total mortality			Cardiovascularmortality		Cancer mortality		Non-cardiovascular/ non-cancer mortality		Total mortality	
NE/NR	IR (95% CI)		NE/NR	IR (95% CI)	NE/NR	IR (95% CI)	NE/NR	IR (95% CI)	NE/NR	IR (95% CI)
Q1	17/201	1.1 (0.7–1.7)	20/165	1.6 (1.0–2.5)	15/165	1.2 (0.7–2.0)	13/165	1.1 (0.6–1.8)	48/165	3.9 (2.9–5.2)
Q2	14/201	0.9 (0.5–1.5)	21/146	1.9 (1.3–3.0)	16/146	1.5 (0.9–2.4)	11/146	1.0 (0.6–1.8)	48/146	4.4 (3.3–5.9)
Q3	16/203	1.0 (0.6–1.6)	21/157	1.8 (1.2–2.8)	13/157	1.1 (0.7–2.0)	17/157	1.5 (0.9–2.4)	51/157	4.3 (3.3–5.7)
Q4	18/199	1.2 (0.7-1.8)	25/155	2.0 (1.4–3.0)	13/155	1.1 (0.6–1.8)	16/155	1.3 (0.8–2.1)	53/155	4.4 (3.4–5.7)
Q5	36/201	2.5 (1.8–3.5)	35/152	3.5 (2.5–4.9)	27/152	2.7 (1.8–3.9)	11/152	1.1 (0.6–2.0)	73/152	7.3 (5.8–9.1)

Q = quintile, IR= incidence rates per 100 person years follow-up, NE/NR= Number of events/ Numbers at risk. Estimated rates (per 100) and lower/upper bounds of 95% confidence intervals (776 records included in the ULSAM and 1003 in the PIVUSanalysis).

Table 3. The associations between serum TNFR1 for total mortality in the PIVUS cohort, and for cardiovascular mortality, cancer mortality, and total mortality in the ULSAM cohort

	<u>PIVUS</u>	<u>ULSAM</u>		
	<u>Total mortality</u> Hazard ratio (95% CI)	<u>CVD mortality</u> Hazard ratio (95% CI)	<u>Cancer mortality</u> Hazard ratio (95% CI)	<u>Total mortality</u> Hazard ratio (95% CI)
Continuous models: per SD higher sTNFR1				
Model A	1.34*** (1.17–1.54)	1.32*** (1.15–1.52)	1.33** (1.13–1.57)	1.28*** (1.16–1.41)
Model B	1.38*** (1.19–1.61)	1.31*** (1.13–1.51)	1.30** (1.10–1.53)	1.26*** (1.14–1.40)
Model C	1.37*** (1.17–1.60)	1.24** (1.06–1.44)	1.32** (1.11–1.57)	1.22*** (1.10–1.37)
Q5 vs Q1–Q4 (referent)				
Model A	2.24*** (1.47–3.40)	1.83** (1.23–2.73)	2.28*** (1.44–3.63)	1.69*** (1.29–2.22)
Model B	2.29*** (1.50–3.48)	1.82** (1.22–2.72)	2.25** (1.41–3.58)	1.71*** (1.30–2.24)
Model C	2.16*** (1.40–3.35)	1.58* (1.05–2.37)	2.32** (1.45–3.72)	1.58** (1.20–2.08)

A) inflammation (CRP), and sex-adjusted (PIVUS) B) Factors in Model A and lifestyle factors (BMI, smoking, leisure time physical activity and education level); C) Factors in Model B, and established cardiovascular risk factors (age, sex, systolic blood pressure, diabetes, smoking, BMI, total cholesterol, HDL-cholesterol, antihypertensive treatment, lipid-lowering treatment and prevalent cardiovascular disease). *p<0.05, **p<0.01 and ***p<0.001

Table 4. The associations between different combinations of sTNFR1 and CRP with mortality in the PIVUS and ULSAM cohorts

Cohort/Outcome	PIVUS		ULSAM					
	NE/NR	Hazard ratio for total mortality (95% CI)	NE/NR	Hazard ratio for cardiovascular mortality (95% CI)	NE/NR	Hazard ratio for cancer mortality (95% CI)	NE/NR	Hazard ratio for total mortality (95% CI)
Normal CRP, low sTNFR1	57/684	referent	48/416	referent	36/416	referent	118/416	referent
High CRP, low sTNFR1	8/120	0.80 (0.38–1.68)	39/207	1.79** (1.17-2.74)	20/207	1.22 (0.71-2.12)	82/207	1.53 ** (1.16–2.05)
Normal CRP, high sTNFR1	19/142	1.63‡ (0.97–2.75)	16/81	1.93* (1.10-3.41)	12/81	1.92* (1.00-3.69)	32/81	1.58* (1.06–2.33)
High CRP, high sTNFR1	17/59	4.33*** (2.52–7.47)	19/71	3.01*** (1.73-5.12)	15/71	3.16*** (1.73-5.77)	41/71	2.66*** (1.86–3.79)

NE/NR= Number of events/ Numbers at risk. High CRP was defined as >3, otherwise normal. Low sTNFR1 was defined as quintile 1-4 and high sTNFR1 as quintile 5. The Cox regression model adjusted for age and established cardiovascular risk factors (known CVD at baseline, antihypertensive treatment, lipid lowering treatment, low-dose aspirin treatment, current smoking, diabetes, systolic blood pressure, BMI, total cholesterol and HDL cholesterol) ‡p=0.06, *p<0.05, **p<0.01 and ***p<0.001

Figure 1

The association over time between sTNFR1 and total mortality shown as Nelson-Aalen plots in

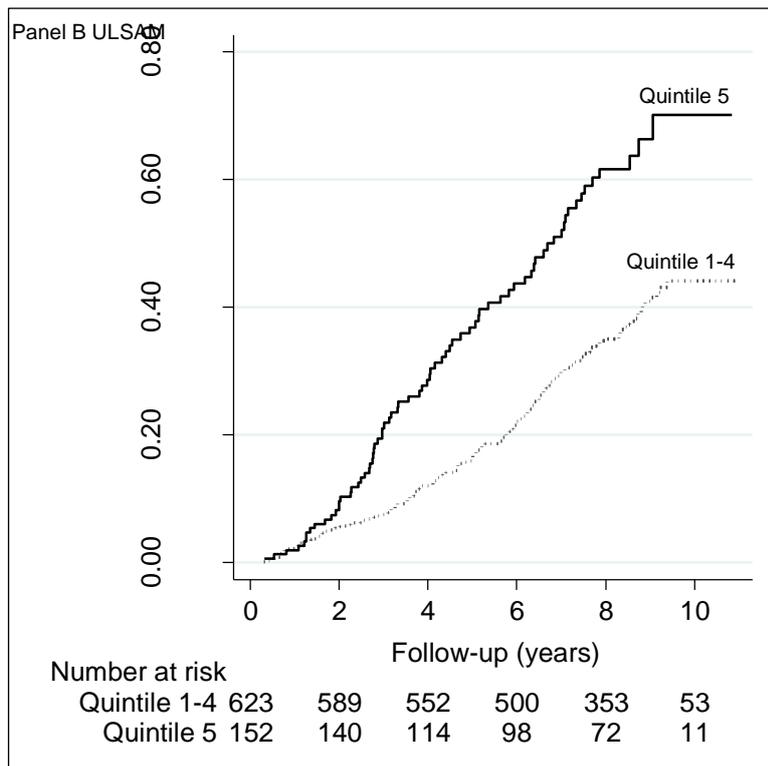
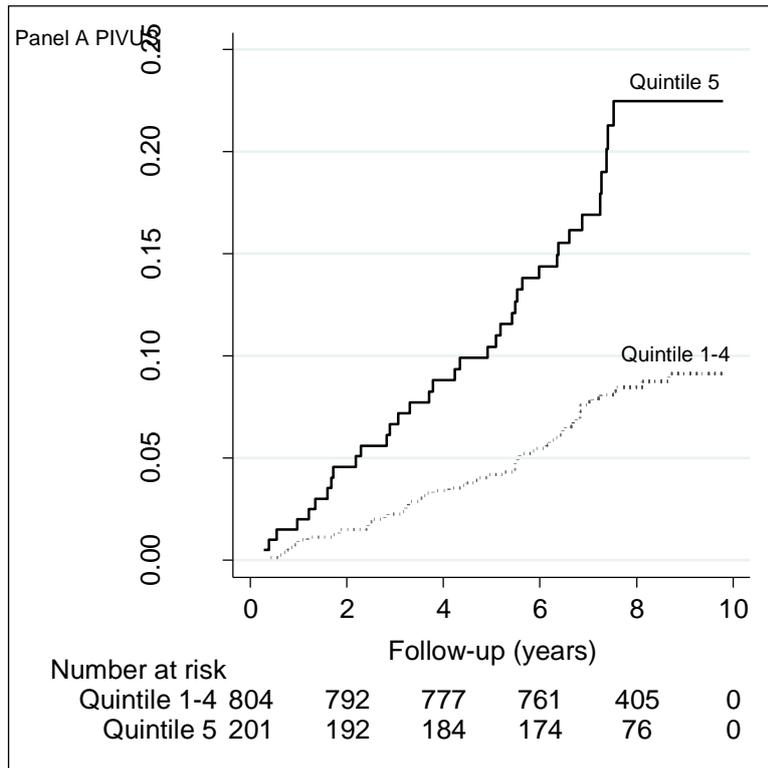


Figure 2. The association between circulating sTNFR1 and total mortality shown as spline curves in:

