Soluble tumor necrosis factor receptors and kidney dysfunction in elderly

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Abstract

Objective- The importance of tumor necrosis factor (TNF)-α and its soluble receptors (sTNFR1 and sTNFR2) for the development of kidney disease is currently being unraveled. Yet, sTNFRs have only been studied in experimental studies or small patient samples and community based data are lacking. Thus, we wanted to explore and validate associations between circulating sTNFRs and different aspects of kidney damage and dysfunction in the community.

Research Design and Methods- Serum sTNFRs and different aspects of kidney damage were assessed cross-sectionally in two independent community-based cohorts of elderly: Prospective Investigation of the Vasculature in Uppsala Seniors, n=815; mean age 75 years, 51 % women) and Uppsala Longitudinal Study of Adult Men (n=778; mean age 78 years).

Results Serum TNFR1 is highly correlated with different aspects of kidney pathology, R: estimated glomerular filtration rate (-0.52); urinary albumin/creatinine ratio (0.22); and urinary kidney injury molecule-1 (0.17) in Uppsala Longitudinal Study of Adult Men ($p<0.001$ for all), and similar numbers were seen in Prospective Investigation of the Vasculature in Uppsala Seniors. These associations were still significant after adjustment for age, gender, inflammatory markers and cardiovascular risk factors and were evident also in participants without diabetes. Serum TNFR2 was associated with all three markers in Prospective Investigation of the Vasculature in Uppsala Seniors ($p<0.001$ for all).

Conclusions Our findings from two independent community-based cohorts confirm and extend previous studies, supporting that circulating sTNFRs are relevant biomarkers for kidney damage and dysfunction in elderly individuals even in the absence of diabetes.

Key words: community based cohort, soluble tumor necrosis factor receptors, glomerular filtration, albumin creatinine ratio (ACR), kidney injury molecule (KIM)-1

Introduction

Tumor necrosis factor (TNF)-α is a central player in the human immune system, and inflammatory and stress response pathways are activated as soon as TNF-α bind to TNF receptors (TNFR).[1] However, the effects of TNF-α are also regulated by soluble TNFR (sTNFR) in plasma: one effect is that they block TNF-α from binding its target cell surface receptor, and another is a prolonged and delayed effect of TNF-α. Additional unique properties of sTNFRs that do not involve TNF-α are also present.[2] Two sTNFRs are known, sTNFR1 and sTNFR2 and their importance in the development of kidney diseases is currently being explored.[3]

Rat models have suggested a causal role for sTNFRs in diabetic nephropathy.[4] Interestingly, two recent studies report that soluble TNFRs predict progression of chronic kidney disease (CKD) and development of end stage renal disease (ESRD) in patients with diabetes.[5, 6] Associations between sTNFRs and progression from microalbuminuria to macroalbuminuria,[7] and of decline in estimated glomerular filtration rate (eGFR)[8] in type 1 diabetes have also recently been reported. Yet, to date community based data on the association between sTNFRs and kidney damage are lacking.

We hypothesize that soluble TNFRs play a causal role in the development of kidney damage and dysfunction. Herein, we aimed to explore and validate the cross-sectional associations between soluble TNFRs and markers of kidney damage and dysfunction used in clinical practice (eGFR) and microalbuminuria (albumin-creatinine ratio (ACR)) in two independent community-based cohorts of elderly. As a secondary aim, we wanted to explore the association between sTNFRs and kidney tubular damage (using the specific tubular damage biomarker urinary kidney injury molecule (KIM)-1).

RESULTS

Baseline characteristics

Baseline characteristics of the study populations are presented in Table 1.

The association between sTNFRs and CKD-stages according to the GFR-strata in the KDIGO guidelines are shown in Table 2. Higher levels of sTNFRs are seen in individuals with lower GFR.

Correlations and linear regression models

Figures 1a-1f shows scatter plots of the association between sTNFR1, and eGFR, ACR and KIM-1 in ULSAM and PIVUS, respectively. In these analyses, higher sTNFR1 was significantly associated with lower eGFR, higher ACR, and higher U-KIM-1, respectively, with salient similarities in the associations between sTNFR1 and each marker in the two cohorts. The Pearson correlation coefficients and p-values are also shown in the figures.

Table 3 shows linear regression models of the association between sTNFR1 and markers of kidney and tubular dysfunction in the ULSAM and PIVUS cohorts. eGFR, ACR and U-KIM-1 were significantly associated with sTNFR1 in all linear regression models tested. The association was strongest with eGFR, followed by ACR and U-KIM-1.

The linear regression coefficients in a model with all three kidney markers as explanatory variables of sTNFR1 in ULSAM were: eGFR -0.48, 95% confidence interval (CI) -0.54 to -0.42 ($p<0.001$); ACR 0.068, 95% CI 0.037 to 0.14 ($p=0.063$); and U-KIM-1 0.044, 95% CI -0.026 to 0.11 ($p=0.21$). The corresponding linear regression model with all three kidney markers in PIVUS revealed similar regression coefficients and significant associations between sTNFR1 and: eGFR -0.60, 95% CI -0.65 to -0.54 ($p<0.001$); ACR 0.12, 95% CI 0.062 to 0.17 ($p<0.001$); and U-KIM-1 0.062, 95% CI 0.010 to 0.12 ($p<0.020$).

The association between sTNFR1 and eGFR was even more pronounced in participants with eGFR$\leq$60 ml/min/ 1.73 m^2, (significant multiplicative interaction in both ULSAM and PIVUS, $p<0.001$). The regression coefficient for 1-SD increment of eGFR in participants with

eGFR<60 was -1.13 95% CI -1.33 to -0.94 (p<0.001) in ULSAM, and -1.28 95% CI -1.50 to -1.06 (p<0.001) in PIVUS. The corresponding regression coefficients in individuals with eGFR≥60 was -0.38 95% CI -0.48 to -0.28 (p<0.001) in ULSAM and -0.35 95% CI -0.42 to -0.28 (p<0.001) in PIVUS.

Data on sTNFR2 was available in the PIVUS study only and correlated to TNFR1 (r=0.5937, <0.001).

Pearson correlations and linear regression models between sTNFR2 and kidney markers are shown in Table 4. eGFR and ACR were associated with sTNFR2 in all linear regression models. KIM-1 was weakly associated with sTNFR2 and non-significant after adjustments for CRP and cardiovascular risk factors. The linear regression coefficients in a model with all three kidney markers as explanatory variables of sTNFR2 were: eGFR -0.50, 95% (CI) -0.55 to -0.44 (p<0.001); ACR 0.064, 95% CI 0.003 to 0.12 (p<0.05); and U-KIM-1 0.059, 95% CI -0.018 to 0.12 (p=0.057).

The association between sTNFRs and the different aspects of kidney damage and dysfunction were similar after adjustment for level of physical activity (data not shown).

Finally, there were significant differences in medians and their Bonnet-Price confidence intervals in individuals with and without diabetes; p=0.06 for sTNFR1 in ULSAM, as well as p<0.001 for sTNFR1 and p<0.001 for sTNFR2 in PIVUS. The association between the TNFRs and different aspects of kidney damage and dysfunction were also similar in participants with and without diabetes in both cohorts (Supplementary table 1).

Discussion

Main findings

The main finding of this study in two community based cohorts of elderly was that sTNFR are closely associated with the two most relevant clinical markers defining disease stage and progression risk in CKD, namely GFR and ACR. The association between sTNFR and eGFR was, however, of a much higher magnitude than between sTNFRs and ACR even after adjustment for other inflammatory markers and cardiovascular risk factors, and was also more pronounced in participants with GFR < 60 ml/min than in participants with ≥ 60 ml/min. Interestingly, these associations were evident also in participants without diabetes. The

association between sTNFR1 and KIM-1, a marker of tubular damage, was present in both cohorts.

Comparisons with previous studies

Recent studies have shown that higher sTNFRs is associated with a the deterioration of kidney function[5, 8, 9] or progression of microalbuminuria to macroalbuminuria[7] in patients with type 1 diabetes.[5] Interestingly in this patient group, sTNFRs have been shown to be superior to many inflammatory markers including IL-6 and TNF-α as prognostic markers of eGFR decline.[8] Furthermore higher sTNFRs have been shown to predict ESRD in type 2 diabetes.[6] In both cohorts in present study, circulating levels of sTNFRs were significantly higher in participants with diabetes as compared to those without diabetes. But more importantly, the association between sTNFRs and the different aspects of kidney damage and dysfunction were evident also in those without diabetes. Thus, sTNFRs appear to be a marker for kidney pathology also in the absence of diabetes, a finding that has not been reported before.

Moreover, we are, as far as we know, the first to report an association between sTNFRs and a specific marker of kidney tubular damage, U-KIM-1. In ULSAM, the association between sTNFRs and U-KIM-1 was attenuated and no longer significant after adjustments for eGFR and albuminuria while in PIVUS the association between sTNFRs remained statistically significant after adjustment for eGFR and albuminuria. Thus, whether sTNFRs are independent markers for specific damages in the proximal tubuli remains to be established.

The correlation between sTNFR1 and sTNFR2 in the present PIVUS study was 0.59 – confirming a high correlation seen in a study of patients with type 2 diabetes, 0.90,[6] and a study of patients with type 1 diabetes, 0.78,[5] indicating that the strength of association between these two highly correlated markers may be dependent on the population studied.

Possible mechanisms for observed associations

There are several molecular mechanisms that may explain our observational findings. Micro-inflammation driven by interleukins such as IL-1, IL-6 and IL-18 as well as TNF-α has been shown to be directly involved in the pathogenesis and progression of CKD.[10] Our findings

and findings by others,[5, 6, 9] support that sTNFRs are closely linked to kidney dysfunction and albuminuria,[7, 8] presumably as direct pathogenic mediators and as markers of a high TNF-α activity.[11] The fact that sTNFRs were significant after adjustments for IL-6 and CRP in the present study as well as in other studies of kidney dysfunction and development of ESRD in individuals with diabetes,[6-8] further indicates that sTNFRs mirror an independent inflammatory pathway. Specifically, sTNFRs have been shown to be involved in tubulointerstitial fibrosis and thereby contribute to nephropathy.[12] Inflammation identified by sTNFRs may also trigger and promote loss of kidney function due to TNFR-driven development of atherosclerosis,[13] and malnutrition.[14]

Hyperglycemia has been suggested to affect the levels of oxidative stress and provides one of the main factors explaining the rapid decline in GFR seen in diabetic nephropathy.[15, 16] Oxidative stress has also been shown to increase TNF-α activity,[15] and specifically TNFR2.[17] Our study, in contrast to a study of patients with diabetes where sTNFR1 was more linked to development of ESRD than TNFR2,[6] showed equally strong associations for both sTNFRs with kidney function and microalbuminuria.

Physical inactivity has been shown to be accountable for 15/1000 cardiovascular deaths in individuals with CKD, as important as traditional risk factors including systolic blood pressure 14/1000 and diabetes 14/1000.[18] A possible pathway for the increased mortality in sedentary individuals is a higher inflammatory state, which has been shown to be associated with IL-6 to a greater extent than CRP in patients with CKD.[19] Thus, symptomatic inflammation may decrease ambulation, leading to a viscous circle with more inflammation, progressed CKD and even less activity. Yet, the present association between sTNFRs and kidney damage and dysfunction remained essentially unaltered in models adjusted for IL-6, CRP, and the level of physical activity which would argue against this as a major explanation of our findings

Finally, it is possible that the strong associations between sTNFRs and GFR are partially explained by their impaired renal clearance, however, inflammatory mediators are likely the principal cause of their increase in serum that parallels the decline in kidney function.

Clinical implications

CKD has a major public impact worldwide, with a global prevalence of 10 %.[20] Soluble TNFRs are promising biomarkers of kidney damage and cardiovascular diseases,[21] however, more studies are needed to evaluate the clinical value of sTNFR-measurements for the detection of kidney damage and for the prediction of GFR decline and the development of cardiovascular disease. The higher correlation between sTNFR and markers of kidney and tubular dysfunction in patients with GFR<60 ml/min in the present study indicate that sTNFR measurements may be more clinically relevant in individuals with established CKD. Longitudinal studies of decline of GFR and its association with sTNFR in the community are warranted.

TNF-α inhibition has been shown to reduce albuminuria in rats.[22] Recombinant antibodies against TNF-α and sTNFR have been suggested as potential drugs that may halt decline in kidney function.[23] In fact, studies have demonstrated that progression of CKD can be inhibited by anti-TNF therapy.[24, 25] Additional clinical studies are warranted to elucidate if anti-TNF therapy can halt GFR decline and microalbuminuria in patients with a rapid nephropathy and high circulating levels of sTNFRs.

Strengths and limitations

Strengths of our investigation include the validation of our findings in an independent cohort and the detailed characterization of study participants with regards to kidney phenotypes and cardiovascular risk factors. Limitations include the unknown generalizability to other age-, and ethnic groups. No conclusions regarding causality should be drawn from our cross-sectional observational data; however, the high associations with eGFR and microalbuminuria in the community are of interests as sTNFRs have been shown to be prognostic markers of kidney dysfunction in patient with diabetes.[6-8]

In conclusion, circulating sTNFRs were associated with different aspects of kidney damage in two independent community-based cohorts of elderly, even in the absence of diabetes. Our findings confirm and extend previous studies in patients with diabetes to the community based setting, supporting that circulating sTNFRs are relevant biomarkers for kidney damage and dysfunction, and emphasize the importance of micro-inflammation as a mechanism underpinning kidney damage and nephropathy.

CONCISE METHODS

Study samples

Description of study populations

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

All 70-year old men and women living in Uppsala, Sweden, between 2001-2004 were eligible for the PIVUS study (described in detail on http://www.medsci.uu.se/pivus/pivus.htm).[26] Of 2025 invited individuals, 1016 agreed to participate. In the present study the second examination cycle of PIVUS was used (2006-2009) when participants were 75 years old. Of 964 invited participants, 827 participated (86%) and of these 815 participants had data on sTNFRs.

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 (described in detail on http://www.pubcare.uu.se/ULSAM).[27] These analyses are based on the fourth examination cycle, when participants were approximately 77 years old (1997-2001). Of 1398 invited men, 838 (60%) participated and 778 participants had valid data on sTNFRs.

All participants in both studies gave written informed consent and the Ethics Committee of Uppsala University approved the study protocols. The study was conducted according to the Declaration of Helsinki.

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.[26-28] Venous blood samples were drawn in the morning after an overnight fast and stored at –70°C until analysis. In the PIVUS cohort a spot sample of morning urine "first morning void" was used for analyses. In ULSAM a 24-hour collection of urine was used.

sTNFR1 and sTNFR2 were analyzed using commercially available ELISA kit (DY225 and DY726, R&D Systems, Minneapolis, MN). The assays had a total coefficient of variation (CV) of approximately 6%. Soluble TNFR2 was only available in PIVUS.
Cystatin C-based eGFR was calculated as previously described.[29] Urine albumin was measured by nephelometry (Urine albumin, Dade Behring, Deerfield IL, USA) using a Behring BN ProSpec® analyzer (Dade Behring). Urine creatinine was analyzed with a modified kinetic Jaffe reaction on an Architect Ci8200® analyzer (Abbott, Abbot Park, IL, USA) and creatinine related urine albumin (ACR) was calculated. Urinary KIM-1 was analyzed with the commercial sandwich ELISA kit, (DY1750, R&D Systems, Minneapolis, MN, USA) and adjusted for urinary creatinine (IL Test creatinine 181672-00, Monarch 2000 analyzer, Instrumental Laboratories, Lexington, MA).

High-sensitive CRP measurements were performed by latex-enhanced reagent (Siemens) with the use of a BN ProSpec® analyzer (Siemens). Interleukin-6 (IL-6) was analysed in serum using a high sensitivity IL6 assay (Quantikine HS ELISA Kit HS600, R&D Systems, Minneapolis, MN, USA) according to the instructions of the manufacurer in ULSAM.
Diabetes mellitus was diagnosed as fasting plasma glucose ≥7.0 mmol/l (≥126mg/dl), or use of anti-diabetic medication.[30]

Statistical analysis

Pearsons correlation coefficients were calculated between sTNFR and markers of kidney function (logarithmically (ln) transformed variables to promote normal distribution). Linear regression analyses were used to assess cross-sectional associations with log-transformed sTNFR1 and sTNFR2 (PIVUS only) levels as the dependent variable and other parameters as independent variables. The following multivariable models were used:

A-age and gender (PIVUS).

B-inflammation (age, gender (PIVUS), and CRP) to test if sTNFRs add information to models with the clinically most established inflammatory marker, CRP. Additionally, we adjusted for IL-6 in ULSAM.

C- established cardiovascular disease (CVD)-risk factors (age, gender (PIVUS), CRP, IL-6 (ULSAM), systolic blood pressure, cholesterol, HDL, BMI, lipid lowering and

antihypertensive treatment) to test if the association between sTNFRs are independent of CVD risk factors that may promote kidney damage.

Due to non-normal distributions, Spearman correlation coefficients were used to calculate the correlation between sTNFR1 and sTNFR2 in PIVUS (Spearman correlation was also used in order to be able to compare our data with other studies that have used Spearman for this analysis).

In secondary models we also adjusted for leisure time physical activity. We also used a non-parametric metric method to calculate the differences in medians of sTNFRs and between individuals with and without diabetes due to non-normal distributions, by the use of their Bonnett-Price confidence intervals. Stratified multivariable models (Model A) in patients with and without diabetes were also calculated. Finally, we also investigated the association between circulating sTNFR2 and markers of kidney damage in PIVUS using the same multivariable linear regression models.

The statistical software package STATA 11.2 (Stata corp, college Station, TX) was used.

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Author contributions

Author contributions: A.C.C. drafted manuscript and researched data J.Ä. researched data, edited manuscript, contributed to discussion, provided funding. T.L., J.H-K. and L.L. reviewed manuscript, 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 of PIVUS and ULSAM

Variable	**PIVUS**	**ULSAM**
Number of subjects	815	778
Female n (%)	414 (51%)	0
Age (years)	75.3 ± 0.2	77.6 ± 0.8
CRP (mg/L)	3.9 ±6.3	3.9 ±2.7
Interleukin-6 (pg/mL)	-	3.9 ±2.7
sTNFR1 (pg/mL)	2455 ±1293	2081 ±865
sTNFR2 (pg/mL)	6332 ±2880	-
Urinary KIM-1/creatinine (ng/mmol)	173 ±1597	118 ± 89
Glomerular filtration rate (ml/min/1.73m2)	68 ±19	73 ±17
Urinary albumin/creatinine ratio (mg/mmol)	6.1 ±29	4.4 ± 19
Body mass index (kg/m^2)	27 ± 4	26 ± 3
Systolic blood pressure (mmHg)	149 ± 19	151 ± 21
Antihypertensive treatment, n (%)	394 (48%)	365 (47%)
S-Cholesterol (mmol/L)	5.4 ±1.1	5.4 ±1.0
HDL (mmol/L)	1.49 ±0.46	1.3 ±0.3
Lipid lowering treatment n (%)	204 (26%)	129 (17%)
Smoking n (%)	50 (6%)	59 (8%)
Diabetes n (%)	112 (14)	107 (14)

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

Table 2. The association between sTNFRs and CKD-stages according to the GFR-strata in the KDIGO guidelines.

CKD-stage	**eGFR**	**ULSAM**		**PIVUS**		
		n	**sTNFR1 mean±SD**	**n**	**sTNFR1 mean±SD**	**sTNFR2 mean±SD**
G1 Normal or high	≥90	108	1613± 507	87	1812±356	4993±3551
G2 Mildly decreased	60-89	511	1973±641	450	2186±449	5727±1692
G3a-b Mildly to severely decreased	30-60	147	2546±834	262	2924±1425	7412±2492
G4 Severely decreased	15-29	11	5504±2315	10	4343±995	10107±2088
G5 Kidney failure	0-14	0	-	0	-	-
ANOVA p-value			<0.001		<0.001	<0.001

Table 3 The association between sTNFR1 and kidney markers in the ULSAM and PIVUS cohorts (ln transformed variables)

Kidney marker	Linear regression models, B-coefficients per standard deviation increment of (95% confidence intervals)					
	ULSAM			PIVUS		
	Model A	Model B	Model C	Model A	Model B	Model C
GFR	-0.51***	-0.48***	-0.48***	-0.62***	-0.59***	-0.58***
	(-0.57, -0.45)	(-0.55, -0.42)	(-0.55, -0.42)	(-0.68, -0.57)	(-0.65, -0.54)	(-0.64, -0.53)
ACR	0.20***	0.18***	0.17***	0.23***	0.21***	0.16***
	(0.13, 0.28)	(0.10, 0.27)	(0.081, 0.25)	(0.16, 0.30)	(0.14, 0.27)	(0.094, 0.23)
U-KIM-1	0.16***	0.14***	0.13**	0.11**	0.080*	0.074*
	(0.087, 0.24)	(0.066, 0.22)	(0.048, 0.20)	(0.045-0.18)	(0.013, 0.15)	(0.0083, 0.14)

Models: A-age and sex (PIVUS), B-inflammation (age, sex (PIVUS), CRP and IL-6 (ULSAM)), C-CVD risk factors (age, sex (PIVUS), CRP, IL-6 (ULSAM), BMI, smoking, systolic blood pressure ,HDL, Cholesterol, diabetes, and antihypertensive and lipid treatment). Significance level ***p<0.001, **p<0.01, *p<0.05

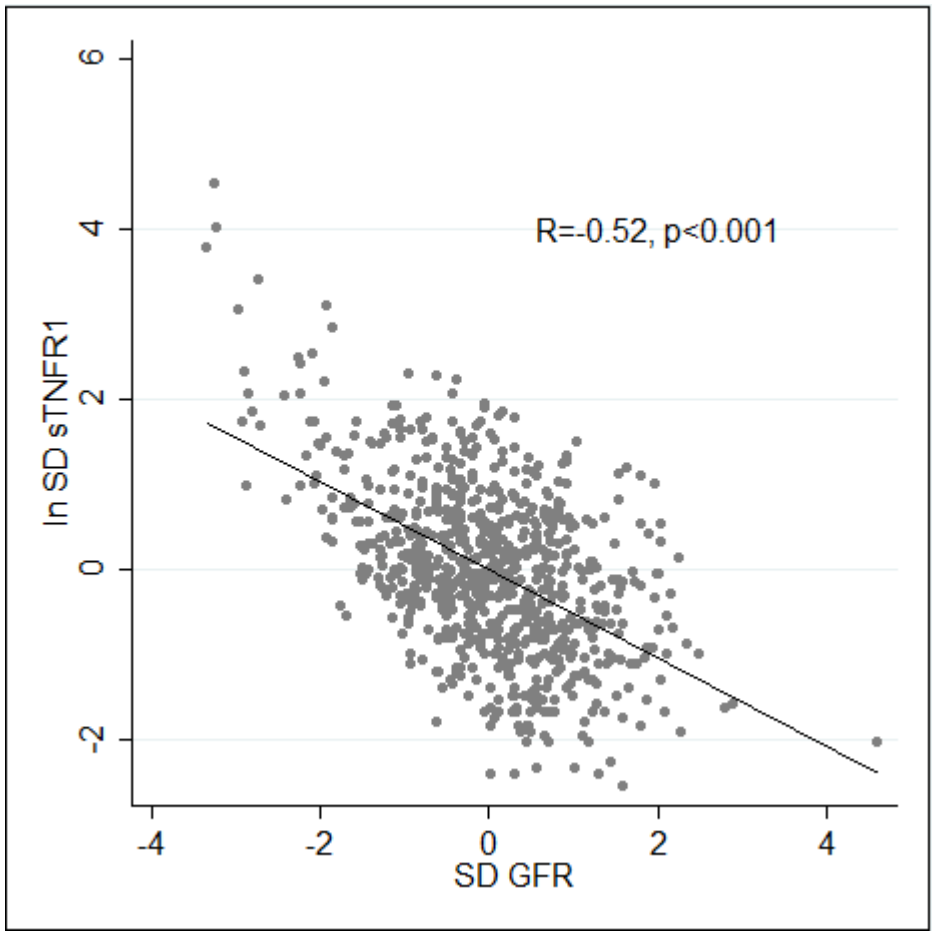
Table 4 The association between sTNFR2 and kidney markers in the PIVUS cohort (ln transformed variables)

Kidney marker	Pearson Correlation coefficients	Linear regression models, B-coefficients (95% confidence intervals)		
		Model A	Model B	Model C
	All	All	All	All
GFR	-0.51***	-0.51*** (-0.57, -0.45)	-0.48*** (-0.54, -0.43)	-0.47*** (-0.53, -0.41)
ACR	0.17***	0.16*** (0.088, 0.22)	0.14*** (0.071, 0.20)	0.10** (0.029, 0.17)
U-KIM-1	0.090***	0.093** (0.024-0.16)	0.065 (-0.002, 0.13)	0.059 (-0.007, 0.13)

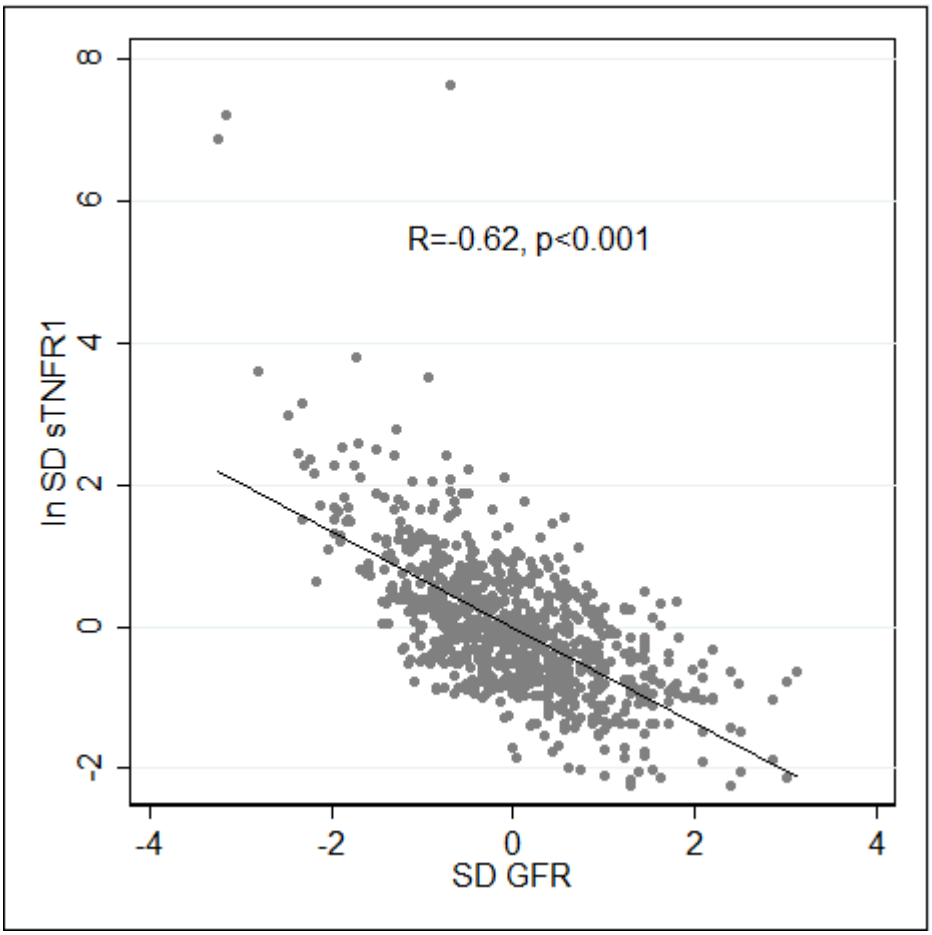
Models: A-age and sex, B-inflammation (age,sex and CRP), C-CVD risk factors (age, sex CRP, BMI, smoking, systolic blood pressure ,HDL, Cholesterol, diabetes, and antihypertensive and lipid treatment) Significance level ***p<0.001, **p<0.01, *p<0.05

Figure 1. Scatter plots of linear regression models of natural logarithm (ln) transformed, standard deviation (SD) increments of sTNFR1 and:

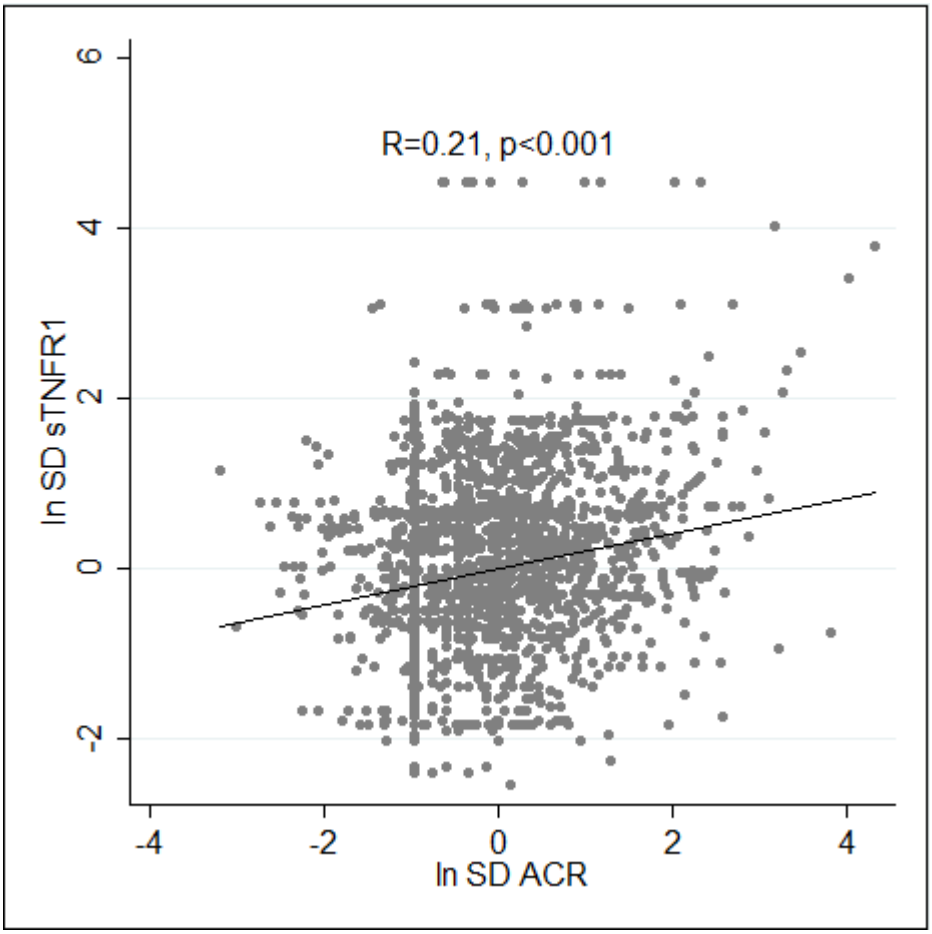
a) GFR in ULSAM



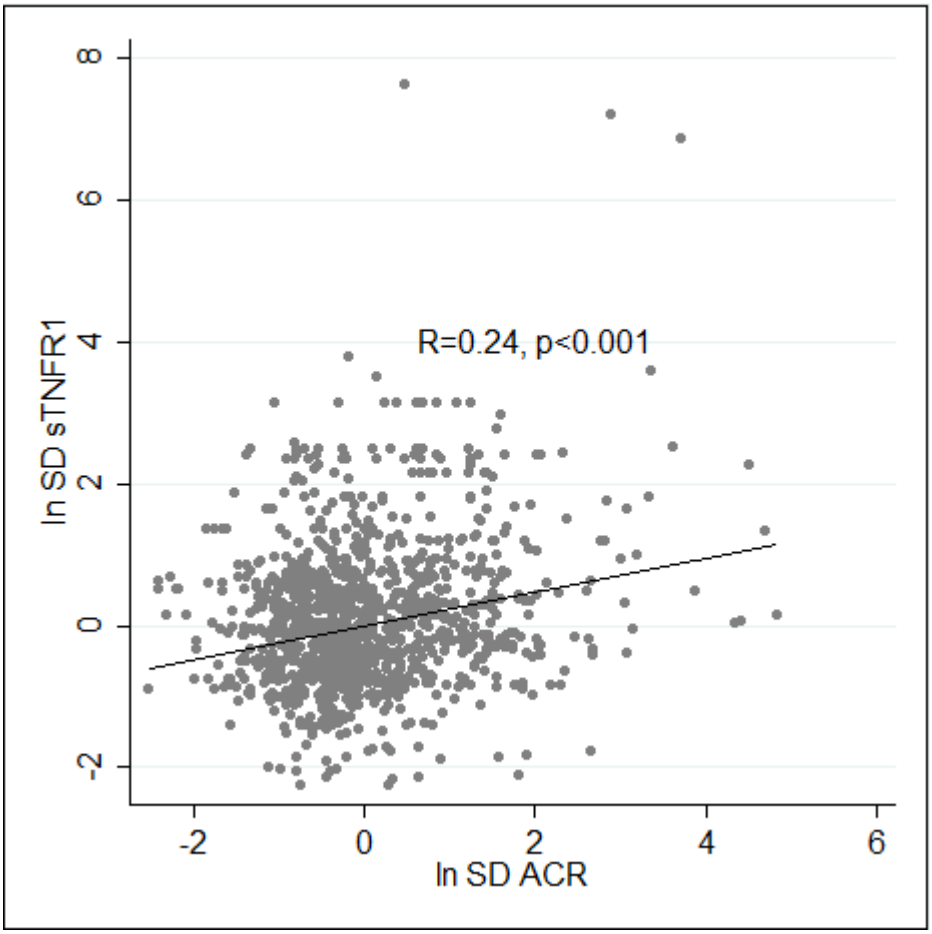
b) GFR in PIVUS



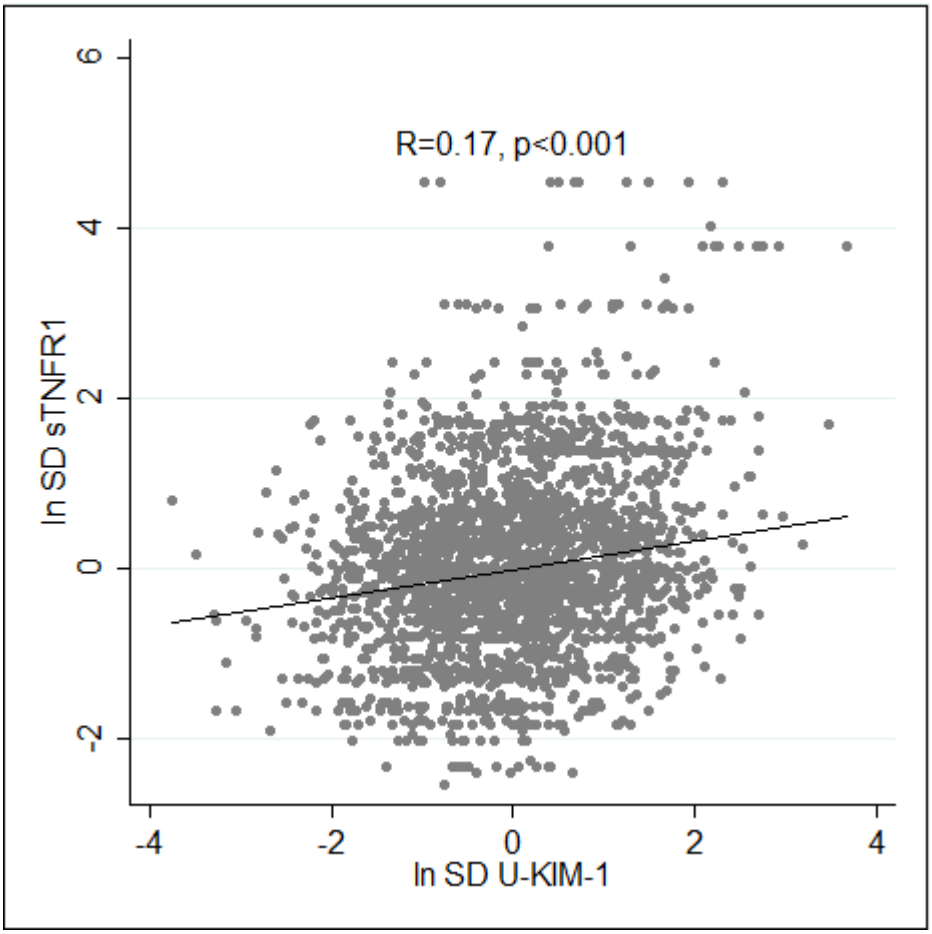
c) ACR in ULSAM



d) ACR in PIVUS



e) U-KIM-1 in ULSAM



f) U-KIM-1 in PIVUS