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ORIGINAL ARTICLE

Soluble ST2 is a biomarker for cardiovascular mortality related to abnormal glucose metabolism in high-risk subjects

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Abstract

Aims Inflammation plays a role in the development and progression of type 2 diabetes macroangiopathy. Interleukin 33 (IL-33) drives production of Th2-associated cytokines. The soluble form of suppression of tumorigenicity 2 (sST2) acting as a decoy receptor blocks IL-33 and tones down Th2 inflammatory response. We investigated the role of sST2 as a predictor of CV and all-cause mortality in a cohort of patients affected by established atherosclerotic disease.

Methods 399 patients with atherosclerotic disease from the Tor Vergata Atherosclerosis Registry performed follow-up every year by phone interview. The primary endpoint was cardiovascular death and the secondary endpoint was death for any other disease.

Results sST2 plasma levels were significantly increased from normal glucose-tolerant patients to patients with history of type 2 diabetes ($p < 0.00001$). Levels of sST2 were significantly correlated with fasting plasma glucose ($R = 0.16$, $p = 0.002$), HbA1c ($R = 0.17$, $p = 0.002$), and HOMA ($R = 0.16$, $p = 0.004$). Dividing patients in tertiles of sST2 levels, those belonging to the highest tertile showed an increased rate of all-cause and cardiovascular mortality, (all-cause mortality $p = 0.045$ and CVD mortality $p = 0.02$). A multivariate Cox analysis revealed that sST2 increased the risk in cardiovascular mortality per SD by hazard ratio 1.050 (95% CI 1.006–1.097, $p = 0.025$) after adjustment for age and hs-CRP while it did not significantly change the risk for all-cause mortality.

Conclusions High circulating level of sST2 is associated to increased CVD mortality and markers of metabolic dysfunction in subjects with atherosclerotic disease.

Keywords Insulin resistance · Inflammation · Diabetes · Cardiovascular disease

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Background

Cardiovascular diseases (CVD) are still the leading cause of death and disability in type 2 diabetic patients despite the huge efforts to prevent and treat them precociously. In these patients, cardiovascular events typically occur 14.6 years earlier and with greater severity than in individuals without diabetes [1, 2]. The enhanced CVD risk and mortality in type 2 diabetes are due to greater atherosclerotic plaque burden, higher atheroma volume, and smaller coronary artery lumen diameter [3]. Multiple mechanisms underlie the atherogenic process in diabetes such as hyperglycemia, dyslipidemia, inflammation, reactive oxygen species, endothelial dysfunction, hypercoagulability, and vascular calcification [4–7].

Suppression of tumorigenicity 2 (ST2) is a transmembrane receptor expressed mostly on the surface of Th2 and mast cells, and belongs to the Toll-like/IL-1 receptor superfamily. In 2005, Schmitz uncovered the ligand for ST2, interleukin-33 (IL-33), a cytokine involved in Th2-dependent inflammatory processes [8]. The binding of IL-33 to its receptor determines phosphorylation of extracellular signal-regulated kinase (ERK) 1/2, p38 MAPK, JNKs and activation of NF-κB [9]. The soluble form of ST2 (sST2) is generated by alternative splicing and acts as a decoy receptor, and is implicated in the attenuation of the Th2 inflammatory response [10]. The IL33–ST2 pathway was implicated in several inflammatory diseases such as asthma, rheumatoid arthritis, sepsis, autoimmune, fibro-proliferative and cardiovascular diseases [11–13]. IL-33/ST2L signaling has cardioprotective effects; it preserves the myocardium against hypertrophy and cardiac fibrosis following pressure overload [14]. sST2 blocks the binding of IL-33 to his transmembrane receptor and the sequent cardioprotective cascade of events.

In the last decade, several evidences allowed to consider sST2 as a biomarker and predictor in CVD. Its role in acute and chronic heart failure is well demonstrated in numerous studies [15–19] enough to induce the FDA to authorize it for clinical use and the American College of Cardiology/American Heart Association to insert it in the update of HF guidelines in 2017 [20].

The aim of our study is to investigate the role of sST2 as a predictor of CV mortality and all-cause mortality in a cohort of patients affected by established atherosclerotic disease and its association with glucose tolerance.

Methods

Description of patients

The Tor Vergata Atherosclerosis Registry (TVAR) (ISRCTN42405215) was previously described [21]. Here, we show a secondary analysis based on follow-up performed in 2017. The data were available for 399 subjects for an average follow-up of 75 months, 6 patients did not answer to follow-up, and 85 patients had missing data on sST2 levels because of no serum available; however, these subjects had clinical characteristics similar to those included in the analysis.

At recruitment, all patients underwent thorough medical history, clinical examination and anthropometric measurements. Patients without history of type 2 diabetes ($n = 325$) performed an oral glucose tolerance test (OGTT) with 75 g of glucose. Patients were classified as normal glucose tolerant (NGT), impaired glucose tolerant (IGT) or type 2 diabetic patients followed ADA classification [22].

Informed consent and ethics committee approval

An informed written consent was obtained from all participants. The study was approved by the local ethics committee and the reported investigations were carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000.

Laboratory measurements

Soluble ST2 was detected by double measurements in one batch by Quantikine ELISA—Human ST2/IL-1 R4 Immunoassay according to manufacturer's instructions (Cat. Num. DST200—R&D Systems, Inc.); the average intra-assay coefficient of variation was 5.6% and interassay coefficient of variation was 17.3%. Other laboratory measurements were previously described [21]. GFR was estimated with the MDRD formula.

Statistical analysis

Baseline parameters were reported as mean and standard deviation (SD) for continuous variables and frequencies, and percentages for categorical ones. Differences among groups were analyzed by one-way ANOVA with Hochberg post hoc analysis and χ^2 test. Association between continuous variables was assessed by means of Spearman and partial correlation. Time-to-event data were analyzed by means of log rank test or univariate Cox models for categorical or continuous variables, respectively. Univariate and multivariate Cox models were also evaluated, to assess the effect of ST2 after adjusting for potential confounders. The final multivariable Cox model was chosen with forward stepwise regression based on Akaike Information Criterion (AIC), which guarantees a final parsimonious model with best prediction performance. All potential predictors were considered for final inclusion in the multivariable Cox model.

p values < 0.05 were considered as statistically significant. All analysis was performed with IBM software SPSS Version 23 (SPSS, Inc., Chicago, IL, USA) and R version 3.4.2 (Vienna, Austria).

Results

Patient clinical characteristics and impaired glucose metabolism

We divided the 399 patients on the basis of the status of glucose tolerance: 118 were NGT, 138 IGT, 69 had a new diagnosis of type 2 diabetes (neoT2D) and 74 were

Table 1 Clinical and biochemical parameters of patients divided on the basis of the degree of glucose impairment

	NGT	IGT	neoT2D	oldT2D	*p*
	118	138	69	74	
Gender (M/W)	89/29	108/30	50/19	50/24	0.377
Age (years)	70.06 ± 9.23	71.72 ± 7.39	71.01 ± 8.61	70.96 ± 7.89	0.466
BMI (kg/m^2)	25.91 ± 4.42	26.36 ± 3.90	26.91 ± 3.84	26.73 ± 3.90	0.373
Systolic blood pressure (mmHg)	139.1 ± 22.6	136.3 ± 18.0	137.1 ± 16.8	141.1 ± 18.7	0.405
Diastolic blood pressure (mmHg)	82.72 ± 9.39	80.04 ± 9.62	78.36 ± 9.66	77.53 ± 7.84	0.002
Total cholesterol (mg/dl)	191.8 ± 41.2	184.0 ± 48.6	191.03 ± 42.8	161.3 ± 48.3	0.00001
HDL cholesterol (mg/dl)	46.21 ± 13.41	42.28 ± 13.57	44.06 ± 13.23	41.94 ± 14.65	0.09
LDL cholesterol (mg/dl)	119.12 ± 36.67	114.25 ± 39.91	115.58 ± 38.06	90.93 ± 33.03	0.000001
Triglycerides (mg/dl)	131.26 ± 60.08	157.74 ± 112.30	182.30 ± 220.87	175.84 ± 145.07	0.046
eGFR	78.05 ± 23.93	82.20 ± 25.90	83.06 ± 40.15	69.48 ± 27.07	0.014
AST (UI/l)	20.43 ± 11.40	19.48 ± 8.11	19.94 ± 7.92	18.58 ± 15.34	0.716
ALT (UI/l)	21.36 ± 15.80	21.53 ± 10.23	25.26 ± 12.88	27.64 ± 21.11	0.011
Fasting plasma glucose (mg/dl)	96.3 ± 9.92	98.22 ± 10.30	113.83 ± 19.79	140.81 ± 45.42	< 0.000001
2 h post-load glucose (mg/dl), *n* = 325	116.63 ± 17.62	167.61 ± 16.99	234.78 ± 34.15		< 0.000001
Fasting plasma insulin (uU/ml)	10.06 ± 7.58	12.25 ± 8.55	18.07 ± 23.56	17.75 ± 15.12	0.0001
2 h post-load plasma insulin (uU/ml), *n* = 325	59.99 ± 45.76	115.70 ± 81.57	164.85 ± 164.82		< 0.000001
HbA1c (mmol/mol)	37.86 ± 3.47	37.706 ± 4.82	42.21 ± 5.70	54.04 ± 11.66	< 0.000001
HOMA index	2.46 ± 2.14	3.03 ± 2.34	5.24 ± 6.58	7.07 ± 6.53	< 0.000001
hs-CRP (mg/dl)	4.08 ± 6.14	5.01 ± 10.73	7.07 ± 20.15	1.01 ± 1.58	0.023
Fibrinogen (mg/dl)	366.8 ± 95.45	406.2 ± 498.2	369.1 ± 94.25	404.9 ± 126.83	0.684
WBC	6901.1 ± 2010.8	6892.3 ± 1899.5	7438.6 ± 2635.8	7416.4 ± 1817.4	0.115
Statins (n/y), %	49.1/50.9	42.3/57.7	45.5/54.5	20/80	0.001
Fibrates (n/y), %	98.8/1.2	98.1/1.9	97.9/2.1	97.2/2.8	0.936
ASA (n/y), %	37.1/62.9	43.9/56.1	43.5/56.5	42.6/57.4	0.74
ACE-i (n/y), %	67.6/32.4	59.7/40.3	64.5/35.5	50.7/49.3	0.86
ATII RB (n/y), %	71/29	66.1/33.9	68.3/31.7	66.2/33.8	0.86
CCB (n/y), %	65.2/34.8	67.7/32.3	59.1/40.9	54.9/45.1	0.275
B blockers (n/y), %	71.4/28.6	73.1/26.9	75.8/24.2	57.7/42.3	0.077
A blockers (n/y), %	81.9/18.1	91.1/8.9	84.1/15.9	85.1/14.9	0.219
Diuretics (n/y), %	61.6/38.4	66.9/33.1	57.6/42.4	60.9/39.1	0.202

diabetic (oldT2D) at enrollment, as shown in Table 1. The four groups did not differ for age, sex or BMI. As expected oldT2D patients showed significantly higher levels of fasting plasma glucose and insulin ($p < 0.000001$), HbA1c ($p < 0.000001$) and were more insulin resistant (HOMA, $p < 0.000001$). The oldT2D group had a slight reduction of the glomerular filtration rate ($p = 0.014$) and lower levels of LDL cholesterol ($p < 0.000001$) and triglycerides ($p = 0.05$), probably due to a higher assumption of lipid-lowering therapies compared with the other groups, as shown in Table 1.

Among all the several cytokines and inflammatory markers evaluated, we found that sST2 plasma levels were significantly increased in oldT2D compared with the three other groups (ANOVA $p < 0.00001$, Fig. 1a, post hoc Hochberg analysis NGT vs oldT2D $p < 0.001$, IGT vs oldT2D $p < 0.00001$, newT2D vs oldT2D $p < 0.001$), and the difference remained significant after adjustment for age and GFR ($p < 0.00001$).

Soluble ST2 and impaired glucose metabolism

We observed that sST2 levels were positively correlated with age ($R = 0.23$, $p < 0.0001$) and negatively with GFR ($R = -0.20$, $p < 0.0001$); no correlations were found with BMI, lipid profile, liver function and inflammatory markers (hs-CRP, fibrinogen, TNF-α, white blood cells).

The soluble receptor of IL33 was positively correlated with parameters of glucose metabolism such as fasting plasma glucose ($R = 0.16$, $p = 0.002$), HbA1c levels ($R = 0.17$, $p = 0.002$) and HOMA ($R = 0.16$, $p = 0.004$) after adjustment for age, gender and GFR, as shown in Fig. 1b–d.

We then stratified our cohort according to tertiles of sST2 levels and we found that patients belonging to the highest

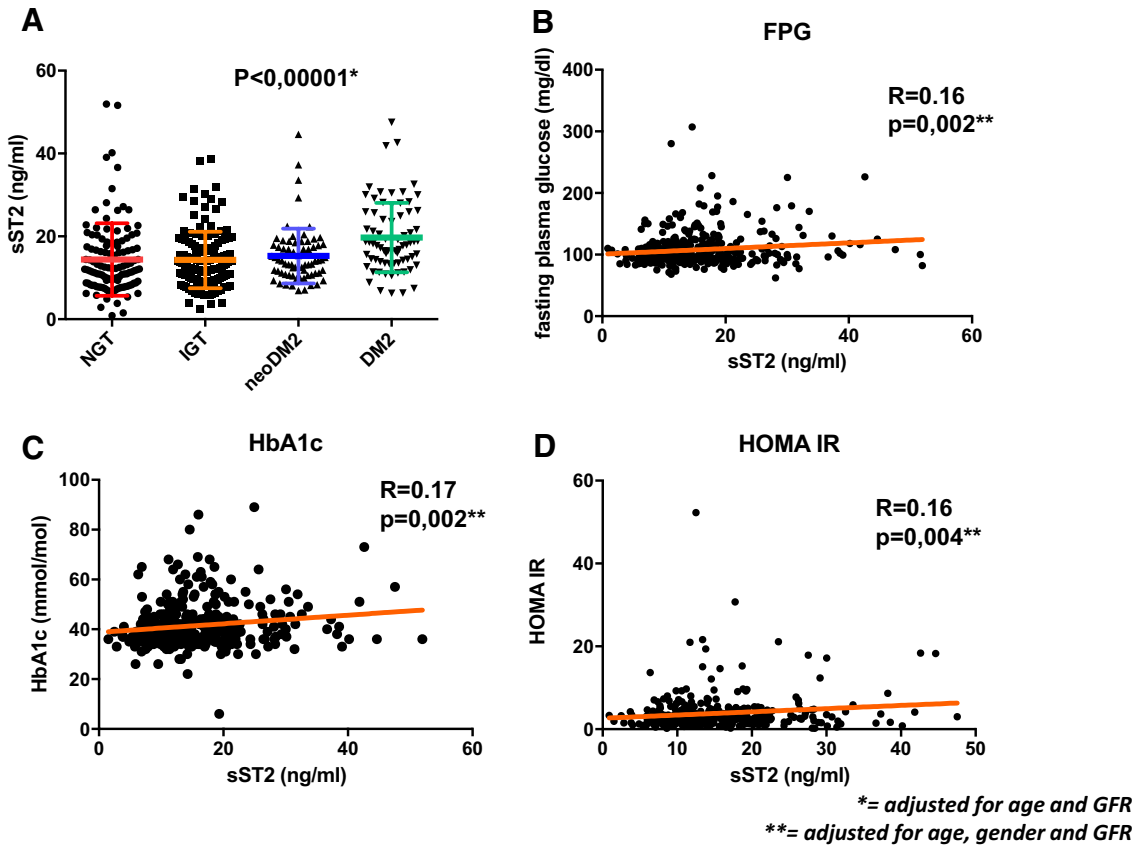

Fig. 1 Correlations of sST2 with glucose tolerance status and metabolic variables. **a** sST2 levels according to glucose tolerance status ANOVA $p<0.00001$, post hoc Hochberg analysis NGT vs oldT2D $p<0.001$, IGT vs oldT2D $p<0.00001$, newT2D vs oldT2D $p<0.004$ after adjustment for age and GFR ($p<0.00001$); **b** correlation of sST2 with fasting plasma glucose levels (partial correlation $R=0.16$, $p=0.002$, after adjustment for age, gender and GFR); **c** correlation of sST2 with HbA1c levels (partial correlation $R=0.17$, $p=0.002$, after adjustment for age, gender and GFR); **d** correlation of sST2 with HOMA-IR (partial correlation $R=0.16$, $p=0.004$, after adjustment for age, gender and GFR)

tertile (sST2 24.18±7.39 ng/ml) were significantly older ($p=0.0001$) and showed lower levels of total ($p<0.0001$) and LDL cholesterol ($p<0.0001$), a worse kidney function measured by GFR ($p=0.03$) and higher fasting plasma glucose ($p=0.05$), HbA1c levels ($p<0.001$) and HOMA index ($p=0.05$) as shown in Table 2.

Results from follow-up

399 patients from our cohort participate to annual follow-up for 74.98 ± 36.27 months. During this period, 99 deaths occurred (24.8%) of which 32 (8%) due to cardiovascular diseases. We did not find significantly differences in all-cause mortality and CVD mortality among the four categories of impaired glucose metabolism.

A Mantel–Cox analysis revealed that patients belonging to the highest tertile of sST2 displayed an increased rate of all-cause and cardiovascular mortality, as shown in Fig. 2 (all-cause mortality, log rank test, $p=0.045$ and CVD mortality log rank test, $p=0.02$).

Among the several parameters and biochemical variables available, sIL6R, TNFa, sVCAM, sICAM, ADMA, only age, sST2 and hs-CRP were found independently associated with all-cause and cardiovascular mortality with univariate Cox analysis, as shown in Table 3.

On the basis of these results, we performed a multivariate Cox analysis and we observed that sST2, included as continuous variable, increased the risk in cardiovascular mortality per SD by hazard ratio 1.050 [95% confidence interval (CI) 1.006–1.097, $p=0.025$] after adjustment for age and hs-CRP (Table 4), while it did not significantly change the risk for all-cause mortality.

Discussion

In this study, we evaluated whether sST2 may predict all cause and CVD-mortality and its links with glucose tolerance in a cohort with established atherosclerotic vascular disease in secondary prevention. Our results revealed

Table 2 Clinical and biochemical characteristics of patients divided in tertiles of plasma sST2

	Low sST2	Medium sST2	High sST2	*p*
	134	133	132	
Age (years)	68.97 ± 8.29	70.35 ± 8.05	73.61 ± 7.82	0.0001
BMI (kg/m^2)	26.61 ± 4.14	26.54 ± 3.98	25.99 ± 4.04	0.415
Systolic blood pressure (mmHg)	137.46 ± 20.17	136.4 ± 17.86	140.42 ± 20.24	0.263
Diastolic blood pressure (mmHg)	81.09 ± 10.13	80.39 ± 9.46	78.95 ± 8.63	0.203
Total cholesterol (mg/dl)	194.30 ± 51.32	184.06 ± 40.17	171.7 ± 45.06	0.0004
HDL cholesterol (mg/dl)	43.24 ± 12.96	42.93 ± 14.01	44.90 ± 14.22	0.464
LDL cholesterol (mg/dl)	120.62 ± 42.71	112.64 ± 34.78	101.95 ± 35.74	0.0004
Triglycerides (mg/dl)	153.86 ± 114.22	163.74 ± 120.90	155.30 ± 164.56	0.817
eGFR	82.63 ± 24.35	80.26 ± 24.35	73.49 ± 36.04	0.038
AST (UI/l)	18.72 ± 5.04	20.35 ± 12.60	19.86 ± 12.66	0.474
ALT (UI/l)	20.94 ± 8.51	23.66 ± 16.72	25.33 ± 17.41	0.063
Fasting plasma glucose (mg/dl)	103.13 ± 22.27	109.65 ± 28.44	110.77 ± 30.82	0.05
2 h post-load glucose (mg/dl), $n = 325$	155.00 ± 44.18	172.04 ± 52.92	170.14 ± 46.88	0.014
Fasting plasma insulin (uU/ml)	11.61 ± 6.73	14.83 ± 20.48	14.18 ± 10.87	0.145
2 h post-load plasma insulin (uU/ml), $n = 325$	95.12 ± 86.64	117.53 ± 120.42	112.71 ± 108.84	0.239
HbA1c (mmol/mol)	39.41 ± 6.03	42.46 ± 9.73	42.47 ± 9.97	0.006
HOMA index	3.06 ± 2.07	4.16 ± 5.88	4.32 ± 4.68	0.05
hs-CRP	4.01 ± 7.49	4.35 ± 8.15	4.99 ± 16.34	0.79
Fibrinogen	345.2 ± 77.4	376.8 ± 107.18	44.329 ± 513.6	0.032
WBC	6954.8 ± 1819.6	7443.6 ± 2212.1	7086.3 ± 2068.9	0.049
Statins (n/y), %	46.5/53.5	42.9/57.1	32.8/67.2	0.072
Fibrates (n/y), %	99.1/0.9	97.8/2.2	97.4/2.6	0.659
ASA (n/y), %	37.1/62.9	44.7/55.3	43.3/56.7	0.439
ACE-i (n/y), %	63.7/36.3	58.3/40.3	61.3/38.7	0.595
ATII RB (n/y), %	70.2/29.8	61.7/38.3	71.5/28.5	0.218
CCB (n/y), %	73.8/26.2	54.4/45.6	60.5/39.5	0.004
B blockers (n/y), %	66.2/33.8	67.2/32.8	77.4/22.6	0.098
A blockers (n/y), %	83.9/16.1	84.3/15.7	90/10	0.312
Diuretics (n/y), %	70.8/29.2	57.6/42.4	54/46	0.015

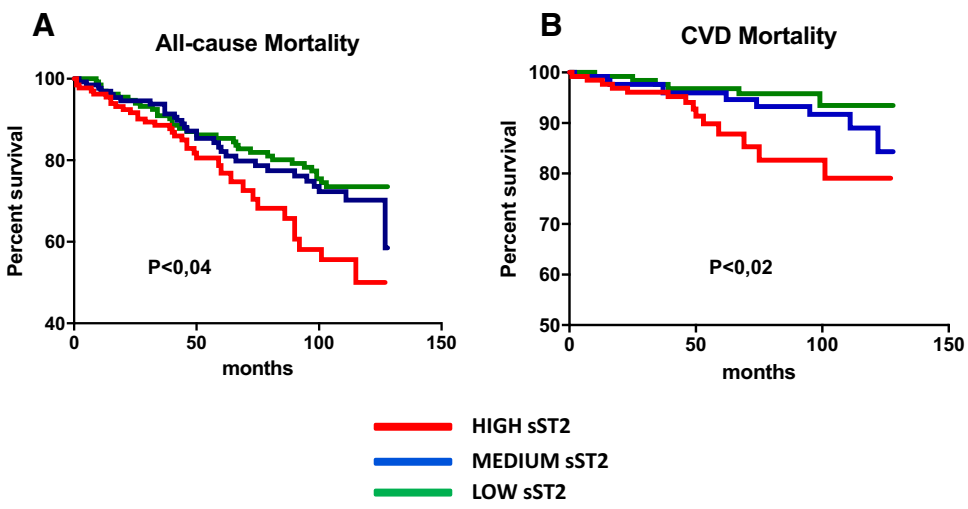

Fig. 2 All cause and cardiovascular mortality according to sST2 tertiles. Mantel–Cox analysis showed that the sST2 highest tertile was significantly associated with all cause (**a**, $p < 0.04$) and cardiovascular mortality (**b**, $p < 0.02$)

Table 3 Univariate Cox analysis for all-cause and cardiovascular mortality

All cause mortality					CVD mortality				
	HD	5%	95%	*p*		HD	5%	95%	*p*
Gender	0.782	0.483	1.266	0.317	Gender	0.659	0.271	1.602	0.357
Age	1.070	1.039	1.102	0.000	Age	1.130	1.068	1.195	0.000
Diabetes status	1.109	0.913	1.346	0.297	Diabetes status	1.197	0.854	1.678	0.297
Family history of diabetes	0.788	0.491	1.264	0.323	Family history of diabetes	0.683	0.294	1.585	0.374
Family history of CVD	0.554	0.361	0.851	0.007	Family history of CVD	0.430	0.198	0.935	0.033
Smoking	1.269	0.828	1.946	0.274	Smoking	0.951	0.432	2.091	0.900
BMI	0.988	0.939	1.040	0.647	BMI	0.957	0.869	1.055	0.378
Hypertension	1.165	0.636	2.136	0.621	Hypertension	0.921	0.354	2.394	0.866
Statin intake	0.631	0.423	0.943	0.024	Statin intake	0.780	0.390	1.563	0.484
HDL	0.992	0.977	1.008	0.313	HDL	0.996	0.970	1.023	0.787
LDL	1.002	0.997	1.007	0.408	LDL	1.002	0.993	1.011	0.657
Tryglicerides	0.999	0.997	1.001	0.464	Tryglicerides	1.000	0.997	1.003	0.814
AST	1.007	0.991	1.024	0.387	AST	0.992	0.952	1.034	0.713
ALT	0.994	0.976	1.012	0.508	ALT	0.990	0.956	1.024	0.553
GFR	0.988	0.978	0.997	0.008	GFR	0.980	0.964	0.997	0.018
Fasting plasma glucose	1.004	0.998	1.010	0.184	Fasting plasma glucose	1.001	0.988	1.014	0.866
Fasting plasma insulin	0.994	0.974	1.014	0.545	Fasting plasma insulin	1.000	0.974	1.028	0.973
HbA1c	1.011	0.988	1.034	0.363	HbA1c	1.020	0.982	1.060	0.307
hsPCR	1.016	1.005	1.027	0.003	hsPCR	1.021	1.005	1.037	0.009
Fibrinogen	1.000	1.000	1.001	0.413	Fibrinogen	1.000	1.000	1.001	0.416
ST2	1.027	1.002	1.052	0.032	st2	1.052	1.015	1.091	0.006

Table 4 Multivariate cox analysis for all-cause and cardiovascular mortality

	HR	5%	95%	*p*
Cardiovascular mortality				
sST2	1.050	1.006	1.097	0.0256
Age	1.139	1.069	1.214	0.0001
hsPCR	1.018	1.003	1.033	0.0204
All-cause mortality				
sST2	1.018	0.985	1.052	0.284
Age	1.048	1.011	1.086	0.0107
hsPCR	1.010	0.997	1.023	0.124
GFR	0.993	0.983	1.004	0.214
Statin intake	0.663	0.403	0.994	0.046
Family history of CVD	0.557	0.358	0.930	0.024

that increased sST2 is associated with insulin resistance and impaired glucose tolerance. The ST2-IL33 pathway is involved in several chronic inflammatory conditions such as atherosclerosis, obesity and metabolism impairment. In genetically obese diabetic (ob/ob) mice, the administration of recombinant IL-33 reduced adiposity, fasting glucose and improved glucose and insulin tolerance [23]. Furthermore, the selective depletion of fat-resident regulatory T cells via anti-ST2 antibody treatment increased adipose tissue insulin sensitivity in mice [24]. In 2012, Miller and colleagues found in a general population that only fasting plasma glucose, but not insulin resistance, was significantly correlated with ST2 after adjustment for age and sex [25]. Previously another study in 158 subjects revealed that patients with type 2 diabetes exhibited higher sST2 levels compared to healthy controls [26].

We also found that elevated levels of sST2 are associated with increased CVD mortality in our secondary prevention cohort. The role of this decoy receptor as a prognostic biomarker in heart failure is actually well recognized [16–19, 27]. The IL-33–ST2L signaling showed cardioprotective effects: in experimental models, it reduced myocardial fibrosis, prevented cardiomyocyte hypertrophy and improved myocardial function [28, 29]. The IL-33–ST2 pathway is also involved in the atherosclerotic process since IL-33 determined a reduction in aortic atherosclerotic plaque formation and, conversely, sST2 resulted in development of significantly larger atherosclerotic plaque and increased Th1 response in ApoE −/− mice [30]. In our study, all patients suffered from chronic atherosclerotic disease and those with higher levels of sST2 showed an increased CVD mortality at follow-up. To our knowledge, this is the first study demonstrating the possible role for sST2 as a predictor for a second fatal CV event in a high-risk population.

Our study has some limitations. First, we did not observe a specific effect of sST2 on cardiovascular mortality in subjects with impaired glucose metabolism. Given that sST2 levels correlated with insulin resistance, we can hypothesize that the neutral result on mortality in subjects of diabetes may be explained by the sample size that we analyzed. Second, our study observed a correlation between sST2 and GFR but we cannot ascertain whether the increase in the first is explained by the latter or vice versa sST2 may affect kidney function.

In conclusion, our study is suggestive for a role of sST2 in deterioration of cardiovascular function in subjects at high risk.

Author contributions MC and AF performed statistical analysis, interpreted results and generated figures and tables. MF, MC and AF wrote the manuscript. MB, MM, FD, IC, GG, SR, VG, OP, CP, RM, and AI performed experiments. All authors discussed the data and commented on the manuscript before submission.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethics approval The study was approved by the ethics committee of the Policlinico Tor Vergata in Rome, Italy. All the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the principles of the Declaration of Helsinki as revised in 2000.

Informed consent An informed written consent was obtained from all participants.

Availability of data and material The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. World Health Organization (2015) Cardiovascular diseases (CVDs) Fact sheet No 317. Internet. http://www.who.int/mediacentre/factsheets/ fs317/en/. Accessed 17 Dec 2015
2. Wang CCL, Hess CN, Hiatt WR, Goldfine AB (2016) Atherosclerotic cardiovascular disease and heart failure in type 2 diabetes—mechanisms, management, and clinical considerations. Circulation 133(24):2459–2502. https://doi.org/10.1161/CIRCULATIONAHA.116.022194
3. Nicholls SJ, Tuzcu EM, Kalidindi S et al (2008) Effect of diabetes on progression of coronary atherosclerosis and arterial remodeling: a pooled analysis of 5 intravascular ultrasound trials. J Am Coll Cardiol 52:255–262
4. Kappel BA, Marx N, Federici M (2015) Oral hypoglycemic agents and the heart failure conundrum: lessons from and for outcome trials. Nutr Metab Cardiovasc Dis 25(8):697–705. https://doi.org/10.1016/j.numecd.2015.06.006
5. Stöhr R, Federici M (2013) Insulin resistance and atherosclerosis: convergence between metabolic pathways and inflammatory nodes. Biochem J 454(1):1–11. https://doi.org/10.1042/BJ20130121
6. Rizza S, Cardellini M, Piciucchi G et al (2018) Brachial flow-mediated dilation predicts glycemia worsening in normoglycemic young subjects. Acta Diabetol 55(4):387–389. https://doi.org/10.1007/s00592-018-1108-0
7. Chattopadhyay S, George A, John J, Sathyapalan T (2018) Two-hour post-challenge glucose is a better predictor of adverse outcome after myocardial infarction than fasting or admission glucose in patients without diabetes. Acta Diabetol 55(5):449–458. https://doi.org/10.1007/s00592-018-1114-2
8. Schmitz J et al (2005) IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 23:479–490
9. Kakkar R, Lee RT (2008) The IL-33/ST2 pathway: therapeutic target and novel biomarker. Nat Rev Drug Discov 7(10):827–840. https://doi.org/10.1038/nrd2660
10. Hayakawa H, Hayakawa M, Kume A, Tominaga S (2007) Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. J Biol Chem 282:26369–26380
11. Moulin D et al (2007) Interleukin (IL)-33 induces the release of pro-inflammatory mediators by mast cells. Cytokine 40:216–225
12. Wynn TA (2004) Fibrotic disease and the TH1/TH2 paradigm. Nat Rev Immunol 4:583–594
13. Wynn TA (2007) Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. J Clin Investig 117:524–529
14. Sanada S, Hakuno D, Higgins LJ, Schreiter ER, McKenzie AN, Lee RT (2007) IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. J Clin Investig 117:1538–1549
15. Parikh RH, Seliger SL, Christenson R et al (2016) Soluble ST2 for prediction of heart failure and cardiovascular death in an elderly, community-dwelling population. J Am Heart Assoc 5:e003188. https://doi.org/10.1161/JAHA.115.003188
16. Boman K, Thormark Fröst F, Bergman ACR, Olofsson M (2018) NTproBNP and ST2 as predictors for all-cause and cardiovascular mortality in elderly patients with symptoms suggestive for heart failure. Biomarkers. https://doi.org/10.1080/1354750X.2018.1431692
17. AbouEzzeddine OF, McKie PM, Dunlay SM et al (2017) Suppression of tumorigenicity 2 in heart failure with preserved ejection fraction. J Am Heart Assoc 6:e004382. https://doi.org/10.1161/JAHA.116.004382
18. van Vark LC, Lesman-Leegte I, Baart SJ et al (2017) Prognostic value of serial ST2 measurements in patients with acute heart failure. JACC 19:2378–2388
19. Aimo A, Vergaro G, Passino C et al (2017) Prognostic value of soluble suppression of tumorigenicity-2 in chronic heart failure: a meta-analysis. J Am Coll Cardiol Heart Fail 5:280–286
20. Yancy CW, Jessup M, Bozkurt B et al (2017) 2017 ACC/AHA/HFSA focused update of the 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. J Am Coll Cardiol 70:776–803
21. Cardellini M, Farcomeni A, Ballanti M et al (2017) C-peptide: a predictor of cardiovascular mortality in subjects with established

atherosclerotic disease. Diabetes Vasc Dis Res 14(5):395–399. https://doi.org/10.1177/1479164117710446
22. American Diabetes Association (2014) Diagnosis and classification of diabetes mellitus. Diabetes Care 37(Supplement 1):S81–S90. https://doi.org/10.2337/dc14-S081
23. Miller AM, Asquith DL, Hueber AJ et al (2010) Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice. Circ Res 107(5):650–658. https://doi.org/10.1161/CIRCRESAHA.110.218867
24. Bapat SP, Suh JM, Fang S et al (2015) Depletion of fat-resident Treg cells prevents age-associated insulin resistance. Nature 528(7580):137–141. https://doi.org/10.1038/nature16151
25. Miller AM, Purves D, McConnachie A, Asquith DL, Batty GD (2012) Soluble ST2 associates with diabetes but not established cardiovascular risk factors: a new inflammatory pathway of relevance to diabetes? PLoS One 7(10):e47830. https://doi.org/10.1371/journal.pone.0047830
26. Fousteris E, Melidonis A, Panoutsopoulos G et al (2011) Toll/interleukin-1 receptor member ST2 exhibits higher soluble levels in type 2 diabetes, especially when accompanied with left ventricular diastolic dysfunction. Cardiovasc Diabetol 10:101. https://doi.org/10.1186/1475-2840-10-101
27. McCarthy CP, Januzzi JL Jr (2018) Soluble ST2 in heart failure. Heart Fail Clin 14(1):41–48 https://doi.org/10.1016/j.hfc.2017.08.005 (**Review**)
28. Sanada S, Hakuno D, Higgins LJ, Schreiter ER, McKenzie ANJ, Lee RT (2007) IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. J Clin Investig 117:1538–1549
29. Seki K, Sanada S, Kudinova AY et al (2009) Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling. Circ Heart Fail 2:684e–691e
30. Miller AM, Xu D, Asquith DL et al (2008) IL-33 reduces the development of atherosclerosis. J Exp Med 205(2):339–346. https://doi.org/10.1084/jem.20071868