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ORIGINAL RESEARCH

10-Year Associations Between Tumor Necrosis Factor Receptors 1 and 2 and Cardiovascular Events in Patients With Stable Coronary Heart Disease: A CLARICOR (Effect of Clarithromycin on Mortality and Morbidity in Patients With Ischemic Heart Disease) Trial Substudy

Axel C. Carlsson, MSc, PhD; Toralph Ruge, MD, PhD; Erik Kjøller, MD, DMSc; Jørgen Hilden, MD, DMSc; Hans Jørn Kolmos, MD, DMSc; Ahmad Sajadieh, MD, DMSc; Jens Kastrup, MD, DMSc; Gorm Boje Jensen, MD, DMSc; Anders Larsson, MD, PhD; Christoph Nowak, MD, PhD; Janus Christian Jakobsen, MD, DMSc; Per Winkel, MD, DMSc; Christian Gluud, MD, DMSc; Johan Ärnlöv, MD, PhD

***Background*—**We aimed to assess the associations and predictive powers between the soluble receptors for tumor necrosis factor (TNF)-α (TNFR1 and TNFR2) and cardiovascular outcomes in patients with stable coronary heart disease.

***Methods and Results*—**CLARICOR (Effect of Clarithromycin on Mortality and Morbidity in Patients With Ischemic Heart Disease) is a randomized clinical trial comparing clarithromycin with placebo in patients with stable coronary heart disease. The primary outcome was a composite of nonfatal acute myocardial infarction, unstable angina pectoris, cerebrovascular disease, and all-cause mortality. Patients were followed up for 10 years; discovery sample, those assigned placebo (1204 events in n=1998); and replication sample, those assigned clarithromycin (1220 events in n=1979). We used Cox regression adjusted for C-reactive protein level, established cardiovascular risk factors, kidney function, and cardiovascular drugs. After adjustments, higher serum levels of TNFR1 and TNFR2 were associated with the composite outcome in the discovery sample (hazard ratio per SD increase, 1.13; 95% confidence interval, 1.05–1.22; P=0.001 for TNFR1; hazard ratio, 1.16; 95% confidence interval, 1.08–1.24; P<0.001 for TNFR2). The associations were similar in the replication sample. The associations with the composite outcome were mainly driven by acute myocardial infarction, cardiovascular mortality, and noncardiovascular mortality. The addition of TNFR1 and TNFR2 to established cardiovascular risk factors improved prediction only modestly (<1%).

***Conclusions*—**Increased concentrations of circulating TNFR1 and TNFR2 were associated with increased risks of cardiovascular events and mortality in patients with stable coronary heart disease. Yet, the utility of measuring TNFR1 and TNFR2 to improve risk prediction in these patients appears limited.

***Clinical Trial Registration*—**URL: https://www.clinicaltrials.gov. Unique identifier: NCT00121550. **(*J Am Heart Assoc*. 2018;7: e008299. DOI: 10.1161/JAHA.117.008299.)**

Key Words: cohort study • coronary atherosclerosis • tumor necrosis factor-α

From the Division of Family Medicine and Primary Care, Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Huddinge, Sweden (A.C.C., C.N., J.A.); Department of Medical Sciences, Cardiovascular Epidemiology (A.C.C.), and Department of Medical Sciences (A.L.), Uppsala University, Uppsala, Sweden; Department of Emergency Medicine, Karolinska University Hospital, Stockholm, Sweden (T.R.); Department of Cardiology, Herlev Hospital (E.K.), and Copenhagen University Hospital (J.C.J.), Copenhagen, Denmark; Section of Biostatistics, University of Copenhagen, Denmark (J.H.); Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark (H.J.K.); Copenhagen University Hospital of Bispebjerg and Frederiksberg, Copenhagen, (A.S.); Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark (J.K.); Copenhagen University Hospital Hvidovre, Copenhagen, Denmark (G.B.J.); Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet, Copenhagen, Denmark (J.C.J., P.W., C.G.); Department of Cardiology, Holbæk Hospital, Holbæk, Denmark (J.C.J.); and School of Health and Social Studies, Dalarna University, Falun, Sweden (J.A.).

Accompanying Tables S1 through S3 and Figures S1, S2 are available at http://jaha.ahajournals.org/content/7/9/008299/DC1/embed/inline-supplementary-material-1.pdf

Correspondence to: Axel C. Carlsson, Division of Family Medicine and Primary Care, Karolinska Institutet, Alfred Nobels Allé 23, 141 83 Huddinge, Sweden. E-mail: axelcefam@hotmail.com

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Clinical Perspective

What Is New?

- Elevated endogenous levels of the soluble receptors for tumor necrosis factor (TNF)-α (TNFR1 and TNFR2) were associated with cardiovascular events and mortality over 10 years of follow-up in patients with stable coronary heart disease at baseline.

What Are the Clinical Implications?

- The utility of measuring TNFR1 and TNFR2 to improve risk prediction in patients with stable coronary heart disease appears limited, and it remains to be shown if the risk associated with circulating levels of TNFR1 and TNFR2 can be lowered by statins, anti-TNF therapy, other pharmaceutical drugs, or lifestyle interventions.

The soluble receptors for tumor necrosis factor (TNF)-α (TNFR1 and TNFR2) have important roles in cellular stress response and inflammatory cascades, both important pathways for the development of cardiovascular disease.[1,2] Higher levels of circulating endogenous TNFR1 and TNFR2 have been associated with increased risk of mortality and adverse cardiovascular outcomes in patients and in the general population after accounting for known inflammatory markers, such as C-reactive protein (CRP).[3–10] To our knowledge, there is no previous study on the association between circulating TNFR1 and TNFR2 and the risk of cardiovascular events and death in patients with stable coronary heart disease. Clarifying these associations and the relationship to established risk markers could highlight potential causal mechanisms and improve risk prediction in this large group of high-risk people.

In the present study, we aimed to assess the association between serum levels of TNFR1 and TNFR2 with fatal and nonfatal cardiovascular events and all-cause mortality using the 10-year follow-up data in the CLARICOR (Effect of Clarithromycin on Mortality and Morbidity in Patients With Ischemic Heart Disease[11]) trial. The CLARICOR trial compared clarithromycin with placebo in patients with stable heart disease and found short-term clarithromycin to be associated with higher mortality.[12]

Methods

Trial Design and Participants

The anonymized data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results or replicating the procedure. The data will be shared at ZENODO (http://zenodo.org), identifiable by the DOI number.

The CLARICOR trial is an investigator-initiated, randomized, placebo-controlled, blinded, multicenter, superiority trial that involved 4372 Danish patients with stable coronary heart disease who were randomly assigned by a central algorithm in a 1:1 ratio to clarithromycin versus placebo. The trial was inspired by the hypothesis that coronary vulnerability could be attributable to bacterial colonization of the arterial walls.[12–15] The CLARICOR trial complies with the Declaration of Helsinki and has been approved by the local ethics committees and regulatory authorities (Regional Ethics Committee KF 01-076/99 and HB 2009/015; the Danish Data Protection Agency 1999-1200-174 and 2012-41-0757; and the Danish Medicines Agency 2612–975). All residents of Copenhagen, Denmark, with a hospital diagnosis of myocardial infarction or angina pectoris (*International Statistical Classification of Diseases* [*ICD*] codes I20.9–I21.9) between 1993 and 1999, were identified and, if alive, invited by mail in late 1999 to participate in the trial. After providing informed consent, eligible participants with stable coronary heart disease were randomized to a 2-week regimen of either clarithromycin, 500 mg (Klacid Uno), administered orally once daily, or placebo. We excluded participants with short-term events or major chronic disease at the time of randomization, those who had experienced acute myocardial infarction (AMI) or angina pectoris episode during the previous 3 months, and those who had also not been subjected to percutaneous transluminal coronary angioplasty and coronary bypass surgery during the previous 6 months. Participants were followed up for, on average, 10 years from the end of treatment in April 2000 until December 31, 2009, through Danish population registers. Comorbidities and treatment details were obtained from hospital records supported by data from the Register of Causes of Death and the Centrale Person-Register (Table S1). Smoking status was obtained through a questionnaire and coded as never, former, or current smoker.

In the present study, we used the placebo group of the CLARICOR trial as discovery sample and the clarithromycin group as replication sample, although possible distortion of associations by the active intervention cannot be excluded in the latter group.[16] We excluded participants with missing data in any of the variables, leaving n=1998 participants in the discovery group and n=1979 participants in the replication group.

Laboratory Analyses

Laboratory analyses were performed on Mindray BS380 (Mindray, Shenzhen, China) with reagents from Abbott Laboratories (Abbott Park, IL), according to the manufacturer's instructions (Table S1). Estimated glomerular filtration rate was estimated using the creatinine-based Chronic Kidney

Disease Epidemiology Collaboration formula.[17] Blood samples were obtained at baseline and immediately frozen and stored at −70°C. Serum levels of endogenous TNFR1 and TNFR2 were analyzed by ELISA kits DY225 and DY726 by R&D Systems (Minneapolis, MN). The assays had a total coefficient of variation of ≈6% (coefficient of variation data obtained from manufacturer). Laboratory technicians were blinded to participant assignment.

Public Register–Based Outcomes

For every participant, the unique 10-digit Danish personal identity number was linked to the National Patient Register, the Danish Central Civil Register, and the National Register of Causes of Death. For each recorded main diagnosis and for each *underlying cause of death*, we classified the outcome into a priority list of disjoint and exhaustive categories: AMI (*ICD* codes I21.0–I23.9), unstable angina pectoris (*ICD* codes I20.0 and I24.8–I24.9), cerebrovascular disease (*ICD* codes I60.0–I64.9 and G45.0–G46.8), peripheral vascular disease (*ICD* codes I70.2–I70.9), other cardiovascular diseases (*ICD* codes I00.0–I99.9, unless already covered), and noncardiovascular disease (*ICD* codes A00.0–T98.3, unless already covered).[18] We also constructed a composite outcome composed of AMI, unstable angina pectoris, cerebrovascular disease, or all-cause death during follow-up.

Statistical Analysis

Spearman's correlation coefficient was used to determine the crude correlation between the TNFRs and other biomarkers. For all subsequent analyses, TNFR1 and TNFR2 levels were natural logarithm transformed to fulfil the linearity assumption. Log transformation was also applied to CRP and apolipoprotein B. The following multivariable Cox proportional hazards regression models were conducted to study the association between either TNFR and outcomes in the following models:

Model A was adjusted for sex and CRP, to show that the TNFRs provide predictive information beyond the most commonly used inflammatory marker in clinical practice.

Model B was adjusted for factors in model A and established cardiovascular risk factors (hypertension, diabetes mellitus, smoking, apolipoprotein A1, and apolipoprotein B), to determine if either TNFR provides information independent of risk factors assessed in clinical practice.[19,20]

Model C was adjusted for factors in model B and estimated glomerular filtration rate, because the TNFRs have been shown previously to be associated with both kidney function and incident chronic kidney disease,[21,22] to rule out that the TNFRs mirror more than kidney function.

Model D was adjusted for established risk factors, comorbidities and cardiovascular pharmacotherapies, and standard biochemical predictors, as shown in Table S1. Model D was used as the standard model for primary analysis, as predefined in the study protocol.[16]

The proportional hazards assumption for Cox regression was violated for age for the outcomes all-cause death and the composite outcome (ie, age at entry; Bonferroni adjusted $P<0.00056$ for the composite outcome, and $P<0.0044$ for all-cause mortality). Accordingly, we choose to omit age from all Cox models for those 2 outcomes. To provide additional insights into the potential influence of age on the association between TNFR1 and TNFR2 and these outcomes, we also conducted multivariable logistic regression and accelerated failure time models (including age as a covariate because the proportional hazard assumption is not a requisite for these analyses).

Improvement in prediction, relative to that obtained when standard predictors (available from routinely available clinical information model D [Table S1]) were used, was calculated in accordance with the published statistical analysis plan.[16]

We used curves (penalized splines) to see the potential nonlinearity of the associations between either TNFR and the outcome, where the hazard ratio was plotted against the circulating TNFR concentration.

An extension to Stata involving Laplace regression was provided by Professor M. Bottai at Karolinska Institutet (Huddinge, Sweden)[23] and was used to calculate the difference in time for every SD increment of TNFR1 and TNFR2 until 50% of the patients in the respective cohorts were diagnosed with the composite end point. In the Laplace regression calculations we conducted, a negative survival time figure represents a reduction in median time to event for every SD increment in TNFR1 and TNFR2. Analyses were conducted in STATA, version 14.2 (StataCorp, College Station, TX).

Results

Baseline Characteristics

Baseline characteristics of the discovery and replication samples are presented in Table 1.

Spearman Correlations at Baseline

TNFR1 and TNFR2 were highly correlated; Spearman's correlation was 0.70 in the discovery cohort and 0.67 in the replication cohort. The Spearman's correlations between TNFR1 and the following biomarkers in the discovery/replication sample were as follows: CRP, 0.28/0.30; creatinine, 0.38/0.37; apolipoprotein B, 0.05/0.09; apolipoprotein A1, −0.07/−0.11 ($P\leq0.005$ when correlation ≥0.06). The

Table 1. Baseline Characteristics in the Discovery (Placebo) and Replication (Clarithromycin) Cohorts

Variable	Discovery Cohort	Replication Cohort
No. of participants	1998	1979
Female sex	624 (31)	603 (30)
Age, y	65±10	65±10
TNFR1, pg/mL	1770±797	1776±836
TNFR2, pg/mL	5386±2011	5416±2099
CRP, mg/L	5.25±7.7	5.76±9.3
Apolipoprotein A1, mg/dL	1.70±0.34	1.70±0.36
Apolipoprotein B, mg/dL	1.21±0.32	1.21±0.33
Diabetes mellitus	300 (15)	301 (15)
Hypertension	805 (40)	790 (40)
eGFR, mL/min	76.3±20	76.5±19
Smoking status		
Never	395 (20)	338 (17)
Former	925 (46)	906 (46)
Current	678 (34)	735 (37)
Previous myocardial infarction	636 (32)	640 (32)
Statin treatment	822 (41)	814 (41)
Aspirin treatment	1764 (88)	1737 (88)
β-Blocker treatment	619 (31)	591 (30)
Calcium antagonist treatment	702 (35)	681 (34)
ACE inhibitor treatment	523 (26)	553 (28)
Long-acting nitrate treatment	412 (21)	411 (21)
Diuretics	691 (37)	702 (35)
Digoxin treatment	117 (7)	140 (7)
Antiarrhythmic treatment	42 (2)	46 (2)

Data are mean±SD for continuous variables and number (percentage) for categorical variables. ACE indicates angiotensin-converting enzyme; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; and TNFR1/TNFR2, soluble receptors for tumor necrosis factor-α.

Table 2. Number of Outcomes, Numbers at Risk, and Incidence Rates With 95% CIs for the Studied Outcomes

Outcomes	Discovery Cohort	Replication Cohort
Composite outcome		
NE, n (%)	1204 (60)	1220 (62)
IR per 100 y	9.23	9.67
95% CI	8.72–9.76	9.15–10.23
Acute myocardial infarction		
NE, n (%)	446 (22)	422 (21)
IR per 100 y	2.97	2.90
95% CI	2.71–3.26	2.63–3.19
Unstable angina pectoris		
NE, n (%)	356 (18)*	356 (18)*
IR per 100 y	2.40	2.50
95% CI	2.16–2.66	2.25–2.77
Stroke		
NE, n (%)	298 (15)	324 (16)
IR per 100 y	1.91	2.18
95% CI	1.71–2.14	1.96–2.43
Cardiovascular mortality		
NE, n (%)	382 (19)	348 (17)
IR per 100 y	2.39	2.10
95% CI	2.15–2.64	1.88–2.32
Noncardiovascular mortality		
NE, n (%)	390	406
IR per 100 y	2.34	2.54
95% CI	2.12–2.59	2.30–2.80
All-cause mortality		
NE, n (%)	738 (37)	788 (40)
IR per 100 y	4.44	4.92
95% CI	4.13–4.77	4.59–5.28

CI indicates confidence interval; IR, incidence rate per 100 person-years of follow-up, estimated rates (per 100) and lower/upper bounds of 95% CIs; and NE, number of events (percentage of participants at risk).
*The number of events was exactly the same in the placebo and the clarithromycin arms.

corresponding correlations for TNFR2 were as follows: CRP, 0.29/0.35; creatinine, 0.39/0.37; apolipoprotein B, 0.06/0.09; and apolipoprotein A1, −0.08/−0.12. The distributions of TNFR1 and TNFR2 in the discovery and replication populations are shown as Figures S1 and S2.

Follow-Up Results at 10 Years

The number of events for all outcomes and the incidence rates (number of events per 100 person-years) are shown for both groups of participants in Table 2. The mean follow-up until censoring or death was 6.5 years in the discovery sample and 6.4 years in the replication sample for the composite outcome. The maximum follow-up was 10.2 years.

The association between TNFR1 and all outcomes is shown in Table 3 and Figure 1 (composite outcome), and the corresponding associations for TNFR2 are shown in Table 4 and Figure 2. Both receptors were significantly associated with the composite outcome, as seen in the spline curves and in models adjusted for inflammation and established cardiovascular risk factors (both in the discovery and replication sample; $P<0.001$ for both). Associations persisted after additional adjustments for kidney function and cardiovascular pharmacotherapies. These associations were similar in

Table 3. Hazard Factors Associated With 1 (Patient-to-Patient) SD TNFR1 Increment

	Discovery Cohort				Replication Cohort			
Variable	Model A	Model B	Model C	Model D	Model A	Model B	Model C	Model D
Composite outcome*								
Hazard ratio	1.32	1.30	1.15	1.13	1.34	1.33	1.18	1.16
95% CI	1.24–1.40	1.23–1.38	1.07–1.23	1.05–1.22	1.27–1.42	1.25–1.41	1.10–1.26	1.08–1.24
P value	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
Acute myocardial infarction								
Hazard ratio	1.30	1.25	1.19	1.19	1.26	1.20	1.18	1.16
95% CI	1.17–1.44	1.13–1.39	1.06–1.34	1.06–1.34	1.13–1.39	1.08–1.34	1.05–1.34	1.03–1.31
P value	<0.001	<0.001	0.003	0.004	<0.001	0.001	0.006	0.017
Unstable angina pectoris								
Hazard ratio	1.06	1.04	1.03	1.01	1.14	1.11	1.11	1.10
95% CI	0.95–1.20	0.92–1.16	0.91–1.17	0.88–1.15	1.01–1.27	0.99–1.25	0.98–1.26	0.97–1.25
P value	0.30	0.56	0.64	0.93	0.03	0.071	0.08	0.14
Stroke								
Hazard ratio	1.05	1.02	0.99	0.96	1.15	1.10	1.06	1.05
95% CI	0.93–1.20	0.90–1.16	0.86–1.15	0.83–1.11	1.02–1.30	0.97–1.24	0.93–1.22	0.92–1.20
P value	0.43	0.72	0.93	0.56	0.024	0.14	0.37	0.48
Cardiovascular mortality								
Hazard ratio	1.34	1.30	1.17	1.14	1.33	1.27	1.22	1.16
95% CI	1.19–1.50	1.15–1.46	1.03–1.35	0.99–1.31	1.19–1.48	1.14–1.43	1.07–1.38	1.02–1.31
P value	<0.001	<0.001	0.018	0.065	<0.001	<0.001	0.002	0.024
Noncardiovascular mortality								
Hazard ratio	1.43	1.41	1.43	1.41	1.35	1.33	1.31	1.29
95% CI	1.28–1.60	1.26–1.58	1.26–1.63	1.25–1.63	1.22–1.50	1.19–1.48	1.16–1.48	1.14–1.46
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
All-cause mortality*								
Hazard ratio	1.66	1.64	1.33	1.31	1.53	1.53	1.25	1.22
95% CI	1.54–1.78	1.53–1.77	1.21–1.45	1.19–1.43	1.43–1.64	1.43–1.64	1.16–1.36	1.13–1.33
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Model A was adjusted for age, sex, and C-reactive protein. Model B was adjusted for factors in model A and established cardiovascular risk factors (hypertension, diabetes mellitus, smoking, apolipoprotein A1, and log[apolipoprotein B]). Model C was adjusted for factors in model B and estimated glomerular filtration rate. Model D was adjusted for established risk factors and comorbidities, standard biochemical predictors, and treatments, as shown in Table S1. CI indicates confidence interval; and TNFR1, the soluble receptor for tumor necrosis factor-α 1.

*Proportional hazards assumption was violated for age; all models in this row are shown without adjustments for age.

logistic regression analyses and accelerated time failure models that also included age as a covariate (Tables S2 and S3).

Higher levels of both the receptors were also significantly associated with a higher risk of myocardial infarction, but for TNFR2, the association was attenuated and no longer significant in the fully adjusted models. Stroke and unstable angina pectoris were not predicted by levels of TNFR1 in any model or sample. In contrast, TNFR2 was significantly associated with stroke in the clarithromycin group, an intergroup discrepancy that is itself weakly significant. Both receptors were associated with noncardiovascular mortality and total mortality in all models tested in both samples ($P<0.001$). TNFR1 was also strongly associated with cardiovascular mortality in all models ($P<0.001$), whereas the association between TNFR2 levels and cardiovascular mortality was attenuated in models adjusted for kidney function and cardiovascular pharmacotherapies and no longer significant in the discovery sample.

There was no significant interaction between TNFR1 or TNFR2 and a previous diagnosis of myocardial infarction or angina pectoris at baseline for the association with the

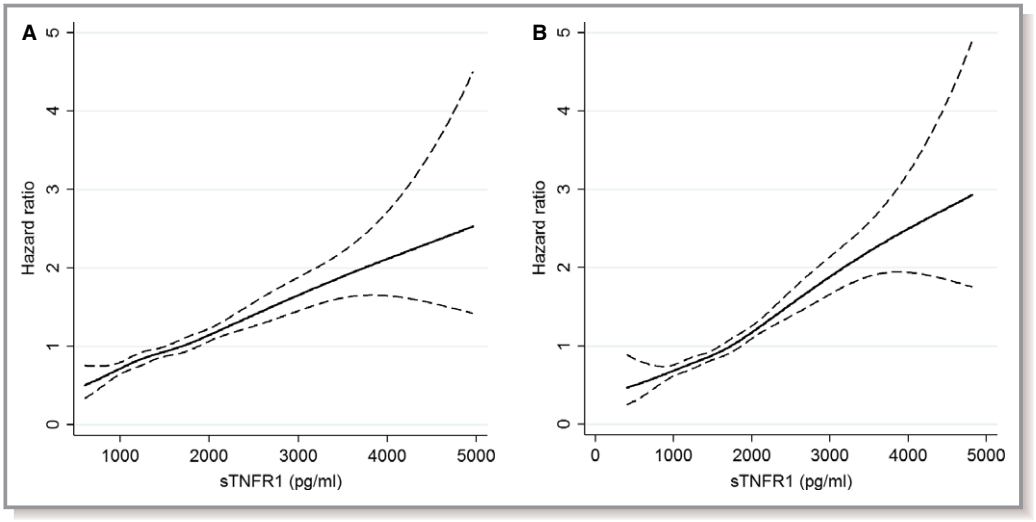

Figure 1. Spline curve of the association between the soluble receptor for tumor necrosis factor-α 1 (sTNFR1) and the composite outcome as hazard ratio with 95% confidence intervals (dotted lines) in the discovery cohort (A) and the replication cohort (B). The mean level of sTNFR1 was 1776±836 pg/mL in the discovery cohort and 1770±797 pg/mL in the replication cohort.

combined outcome (TNFR1, *P*=0.17; TNFR2, *P*=0.13; data not shown in tables).

Prediction Improvement

The number and percentage of correct predictions obtained for the composite outcome and all-cause mortality when TNFR1 and TNFR2 are added to standard predictors are shown in Table 5. Only small improvements were seen (<1%).

Difference in Median Survival Time for the Composite Outcome

In Laplace regression in the placebo group, 1-SD higher levels of log TNFR1 were associated with 0.93 (95% confidence interval, 0.48–1.37) years earlier occurrence of the composite outcome in a model adjusted for age, sex, CRP, and estimated glomerular filtration rate (*P*<0.001). The corresponding number in the clarithromycin group was 1.21 (95% confidence interval, 0.86–1.55) years (*P*<0.001). An SD increment in TNFR2 was associated with 0.89 (95% confidence interval, 0.51–1.28) years reduction of median time in the placebo group and 1.06 (95% confidence interval, 0.78–1.34) years reduction of median time in the clarithromycin group.

Discussion

Main Findings

Elevated endogenous levels of TNFR1 and TNFR2 were associated with cardiovascular events and mortality over 10 years of follow-up in patients with stable coronary heart disease at baseline. The median time until cardiovascular events occurred was ≈1 year earlier for every SD increase in serum concentration of either receptor. These associations were independent of inflammation (CRP), kidney function, established cardiovascular risk factors, and cardiovascular pharmacotherapies. Among subgroups of outcomes, the strongest associations were found between elevated levels of TNFRs and AMI, cardiovascular mortality, and noncardiovascular mortality. These associations were found in both the placebo group and clarithromycin-exposed group of the study. However, only small improvements in prediction of events were seen when TNFR1 and TNFR2 were added to a model with standard predictors. The CLARICOR trial results did not seem to affect the association between TNFR1 and TNFR2 and any of the outcomes.

Strengths and Limitations

The present study has several strengths, including the large study sample with detailed characterization of the participants, the longitudinal study design, the 10 years of follow-up, and the replication of findings in the clarithromycin group of the study. To our knowledge, the present study is the only cohort study on associations between circulating endogenous levels of TNFR1 and TNFR2 in patients with stable coronary heart disease. The national registers of Denmark are known to be of high completeness and accuracy[24]; however, a small number of events may have been missed because of hospitalization abroad. More important, the results apply to stable coronary disease as ascertained at baseline interview rather than prompted by acute symptoms or made during recovery from a

Table 4. Hazard Factors Associated With 1 (Patient-to-Patient) SD TNFR2 Increment

	Discovery Cohort				Replication Cohort			
Variable	Model A	Model B	Model C	Model D	Model A	Model B	Model C	Model D
Composite end point*								
Hazard ratio	1.33	1.31	1.15	1.16	1.37	1.36	1.20	1.16
95% CI	1.25–1.41	1.23–1.39	1.08–1.24	1.08–1.24	1.29–1.45	1.28–1.44	1.12–1.29	1.08–1.25
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Acute myocardial infarction								
Hazard ratio	1.29	1.24	1.18	1.19	1.17	1.12	1.09	1.06
95% CI	1.16–1.43	1.11–1.37	1.05–1.32	1.06–1.34	1.05–1.31	1.00–1.26	0.96–1.24	0.93–1.20
P value	<0.001	<0.001	0.007	0.004	0.005	0.042	0.164	0.40
Unstable angina pectoris								
Hazard ratio	1.07	1.04	1.04	1.05	1.06	1.03	1.01	0.99
95% CI	0.95–1.21	0.92–1.18	0.91–1.19	0.92–1.20	0.94–1.19	0.91–1.17	0.88–1.16	0.86–1.13
P value	0.24	0.50	0.56	0.48	0.37	0.64	0.87	0.85
Stroke								
Hazard ratio	1.04	1.01	0.98	0.95	1.25	1.20	1.19	1.17
95% CI	0.91–1.19	0.88–1.15	0.84–1.13	0.82–1.10	1.11–1.42	1.06–1.37	1.04–1.37	1.02–1.35
P value	0.53	0.88	0.75	0.51	<0.001	0.004	0.014	0.026
Cardiovascular mortality								
Hazard ratio	1.34	1.29	1.17	1.19	1.27	1.23	1.16	1.10
95% CI	1.19–1.51	1.15–1.45	1.02–1.34	1.04–1.37	1.13–1.42	1.09–1.38	1.02–1.32	0.96–1.25
P value	<0.001	<0.001	0.024	0.012	<0.001	0.001	0.026	0.164
Noncardiovascular mortality								
Hazard ratio	1.47	1.44	1.47	1.47	1.37	1.36	1.35	1.31
95% CI	1.32–1.64	1.29–1.61	1.30–1.67	1.30–1.68	1.23–1.53	1.21–1.51	1.19–1.53	1.16–1.49
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
All-cause mortality*								
Hazard ratio	1.69	1.68	1.35	1.36	1.59	1.59	1.31	1.24
95% CI	1.57–1.82	1.56–1.81	1.24–1.48	1.24–1.49	1.48–1.71	1.48–1.71	1.19–1.43	1.13–1.35
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Model A was adjusted for age, sex, and C-reactive protein. Model B was adjusted for factors in model A and established cardiovascular risk factors (hypertension, diabetes mellitus, smoking, apolipoprotein A1, and apolipoprotein B). Model C was adjusted for factors in model B and estimated glomerular filtration rate. Model D was adjusted for established risk factors and comorbidities, standard biochemical predictors, and treatments, as shown in Table S1. CI indicates confidence interval; and TNFR2, soluble receptor for tumor necrosis factor-α 2.
*Proportional hazards assumption was violated for age; all models in this row are shown without adjustments for age.

major event. Limitations include the unknown generalizability to other ethnic groups and to those unlikely to volunteer. In the replication sample, distortion by the active intervention with clarithromycin cannot be excluded, although associations were broadly similar to those in the placebo group.

Possible Mechanisms for the Observed Associations

TNF-α, TNFR1, and TNFR2 are activated in numerous pathological processes and basic cellular mechanisms,[1,2] making the causal network behind our observational findings challenging to elaborate on. Several mechanisms may explain the association between a long-term state of low-level inflammation, increased TNFR1 and TNFR2 levels, and cardiovascular outcomes and mortality.

First, several heart-specific associations have been reported. Higher levels of both TNFR1 and TNFR2 have been associated with increased infarct size and left ventricular dysfunction in patients with AMI and ST-segment elevation.[25] TNFR1 has been shown to be persistently elevated in patients with coronary artery disease,[26] but the

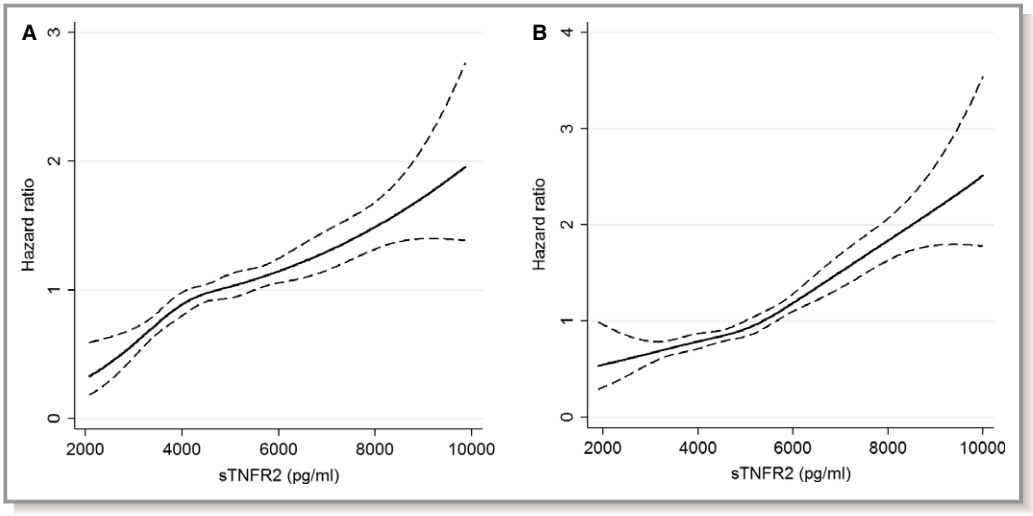

Figure 2. Spline curve of the association between the soluble receptor for tumor necrosis factor-α 2 (sTNFR2) and the composite outcome as hazard ratio with 95% confidence intervals (dotted lines) in the discovery cohort (A) and the replication cohort (B). The mean level of sTNFR2 was 5386±2011 pg/mL in the discovery cohort and 5416±2099 pg/mL in the replication cohort.

levels seem to be normalized by revascularization of the heart.

Second, the association between TNFR1 and TNFR2 and AMI and cardiovascular mortality might be explained by their atherosclerotic effects as markers of a systemic inflammatory state that is often present in patients with coronary heart disease.[10,27] Inflammation may initiate the reaction leading to an atherosclerotic lesion and may also trigger plaque rupture, the final event behind most thrombotic and atherosclerotic events.[28] TNFR1 and TNFR2 were correlated with CRP ($\rho \approx 0.30$, $P<0.001$), indicating that they portray partly the same aspects in our cohorts. Yet, all Cox regression models we reported were adjusted for CRP, suggesting that the TNFRs mirror pathogenic aspects that are independent of CRP. The models were also adjusted for statins, which have been shown to reduce the inflammation level,[29] and to reduce the risk of adverse events in similar patients as in the present study.[30] We also considered that there may have been an

Table 5. Improvement of Outcome Prediction by Adding TNFR1 and TNFR2, Respectively, to the SP Full Model*

	TNFR1			TNFR2		
Type of Predictions	SPs Included	SPs Plus Biomarker Included	Total Predictions	SPs Included	SPs Plus Biomarker Included	Total Predictions
Composite outcome, n (%)						
True favorable predictions	2910 (48.7)	2908 (48.7)	5972	2910 (48.7)	2922 (48.9)	1996
True unfavorable predictions	1174 (19.7)	1176 (19.7)		1174 (19.7)	1185 (19.8)	1989
Total true predictions	4084 (68.4)	4084 (68.4)		4084 (68.4)	4107 (68.8)	1987 5972
All-cause mortality, n (%)						
True favorable predictions	4585 (76.8)	4580 (76.7)	5971	4585 (76.8)	4383 (73.4)	1996
True unfavorable predictions	392 (6.57)	403 (6.75)		392 (6.57)	408 (6.83)	1989
Total true predictions	4977 (83.4)	4983 (83.5)		4977 (83.4)	4991 (83.6)	1986 5971

For TNFR1, the increase in the number of true predictions when we use the biomarker plus the SPs instead of using only the SPs for the outcome all-cause mortality. This amounts to 4983−4977=6. In percentage of the total number of predictions made (5971), this amounts to 6/5971=0.10%. For TNFR2, the increase in the number of true predictions when we use the biomarker plus the SPs instead of using only the SPs for the outcome all-cause mortality. This amounts to 4991−4977=14. In percentage of the total number of predictions made (5971), this amounts to 14/5971=0.23%. For details of this analysis, the reader is referred to data supplement. SP indicates standard predictor; and TNFR1/TNFR2, soluble receptors for tumor necrosis factor-α 1/2.

*The number of correct predictions of survival at time=3, 6, and 9 years was recorded when the SPs were included in the model and when the SPs plus the biomarker were included in the model, and the percentage improvement was obtained by including the biomarker calculated.

effect modification of the strengths of the associations, depending on if the patient had had a myocardial infarction or angina pectoris before baseline; however, no such interaction was found.

Third, hyperglycemia and diabetes mellitus are common in patients with coronary heart disease and have been shown to affect the levels of oxidative stress.[31,32] Oxidative stress increases the overall TNF-α system activity[31] and has been shown to be associated with TNFR2,[33] which showed the highest risk estimates in the present study. Hyperglycemia may also cause arterial stiffness. Pulse wave velocity is a common surrogate for arterial stiffness and has been shown to be highly correlated with endogenous levels of TNFR1 and TNFR2 in patients with type 2 diabetes mellitus,[34] as well as in patients with coronary atherosclerosis.[35] Another mechanism associated with metabolic disturbances is a procoagulant and hypofibrinolytic state, which is highly prevalent in patients with coronary heart disease.[36] It is thus possible that the elevated levels of TNFR1 and TNFR2 reflect these metabolic disturbances.

Fourth, the increased risk of AMI and cardiovascular mortality in patients with coronary heart disease with elevated TNFR1 and TNFR2 levels could partly be explained by an association with angiogenesis, as has been shown for TNFR1 in ischemic models.[37]

Finally, associations between kidney function and TNFR1 and TNFR2 have been shown repeatedly to be strong.[3,4,21] Thus, a reduced kidney function appears to be an important mediator that may explain the link between the TNFRs and AMI as well as cardiovascular mortality. Moreover, TNFR1 and TNFR2 predict the progression of chronic kidney disease in patients with diabetes mellitus.[38,39] Therefore, it is possible that individuals with elevated endogenous TNFR levels are more likely to be developing chronic kidney disease, which, in turn, substantially increases the risk of cardiovascular events. Accordingly, the risk estimates were somewhat attenuated after adjustment for baseline estimated glomerular filtration rate, indicating that some of the risk that the receptors portray is attributable to kidney function.

Comparisons With Other Studies

Higher levels of TNFR1 and TNFR2 have previously been associated with an increased risk of cardiovascular events and mortality in specific patient groups, such as in people with diabetes mellitus,[4,8,10] chronic kidney disease,[7,40,41] rheumatoid arthritis,[6] or heart failure after myocardial infarction,[42–44] as well as in the general population.[3,9] No association with mortality risk was found in patients undergoing hemodialysis.[45] These previous associations were generally independent of more established inflammatory markers, such as CRP, in most,[3,6,9,10,41,44,45] but not all, studies.[7,8] The present study is unique in that it reports the independent association between the TNFRs and the 10-year risk of adverse outcomes in patients with stable coronary heart disease. The long-term follow-up in the present study resulted in similar risk estimates as in previous studies. Only one of the previous studies assessed prediction improvement and found it to be <1% when CRP and TNFR2 were added to a model with established risk factors,[9] which is in agreement with our study. In line with previous studies,[4,21,45] TNFR1 and TNFR2 were highly correlated to one another ($\rho \approx 0.7$).

Clinical Implications

Anti-TNF therapies are widely used in clinical practice to inhibit the inflammatory cascades in rheumatic diseases and have not been associated with increased cardiovascular risk in these patients.[46] Moreover, improvements in the left ventricular structure have been seen in patients with heart failure when treated with anti-TNF therapy.[47] However, a mouse model study of myocardial infarction reported both protective and harmful effects of TNFR1 administration; there were effects on apoptosis of cells infiltrating the heart tissue and on vascular remodeling of the heart.[48]

Our data imply that circulating levels of endogenous TNFR1 and TNFR2 identify risk independently of established risk factors in patients with stable coronary heart disease. However, the improvement in risk prediction beyond standard predictors was small. Moreover, it remains to be shown if the risk associated with circulating levels of TNFR1 and TNFR2 can be lowered by statins, anti-TNF therapy, other pharmaceutical drugs, or lifestyle interventions. Thus, our data do not support the notion of clinical utility of monitoring endogenous levels of TNFR1 and TNFR2 in high-risk individuals with stable coronary heart disease.

Conclusion

We have shown that endogenous TNFR1 and TNFR2 are associated with incident cardiovascular events and mortality, independently of established risk factors in patients with stable coronary heart disease. We warrant further studies that will help us gain a better understanding about TNF-associated molecules in human disease and to determine a possible future clinical role of endogenous levels of TNFR1 and TNFR2 to monitor and treat patients at risk of cardiovascular events.

Author Contributions

Carlsson performed statistical analyses, researched data, and drafted and revised the text. Ruge researched data and contributed to discussion. Kjøller was chairman of the

outcome committee and critically revised the article. Winkel and Hilden administered the data, performed statistical analyses, and critically revised the article. Kolmos had the original idea to do the CLARICOR (Effect of Clarithromycin on Mortality and Morbidity in Patients With Ischemic Heart Disease) trial and critically revised the article. Sajadieh, Kastrup, and Jensen recruited patients and critically revised the article. Larsson critically revised the article, contributed to discussion, and measured the soluble receptors for tumor necrosis factor-α 1 and 2. Nowak critically revised the article. Gluud provided funding and edited the article. Ärnlöv researched data, edited the article, contributed to discussion, and provided funding.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Table S1. Standard predictors adjusted for in Model D.

Clinical predictors	Current medical treatment	Standard biochemical predictors
Sex, age* at entry, smoking history, history of myocardial infarction compared to angina only, hypertension, and diabetes.	The current medical treatment was included as proxy predictors because information about post infarction heart failure and post-infarction angina pectoris are not available to us. Aspirin (Yes/No), beta-blocker (Yes/No), calcium-antagonist (Yes/No), ACE-inhibitor (Yes/No), long lasting nitrate (Yes/No), diuretic (Yes/No), digoxin (Yes/No), statin (Yes/No), and anti-arrhythmic drugs (Yes/No).	Log transformed high-sensitivity-reactive protein (CRP), glomerular filtration rate (GFR) estimated by creatinine, triglycerides and lipoproteins (total cholesterol, HDL cholesterol, LDL cholesterol, apoprotein A1, and log(apoprotein B)).

*Age was omitted from Model D for the composite outcome as well as for all-cause death outcome due to violation of the proportional hazard assumption.

Table S2. Logistic regression analyses for outcomes that violated the proportional hazard assumption for age for the fully adjusted model (model D).

		Discovery		Replication	
		TNFR1	**TNFR2**	**TNFR1**	**TNFR2**
Composite endpoint	**Odds Ratio**	1.22	1.22	1.23	1.22
	95% CI	1.08 to 1.38	1.07 to 1.38	1.09 to 1.39	1.07 to 1.38
	p-value	0.001	0.003	0.001	0.003
Alltocause mortality	**Odds ratio**	1.39	1.40	1.32	1.29
	95% CI	1.21 to 1.58	1.22 to 1.61	1.16 to 1.51	1.12 to 1.47
	p-value	<0.001	<0.001	<0.001	<0.001

Table S3. Accelerated failure regression analyses for outcomes that violated the proportional hazard assumption for age for the fully adjusted model (model D).

		Discovery		Replication	
		TNFR1	**TNFR2**	**TNFR1**	**TNFR2**
Composite endpoint	**Coefficient**	-0.10	-0.11	-0.14	-0.13
	95% CI	-0.16, to 0.33	-0.18 to 0.048	-0.20 to 0.075	-0.20, to 0.059
	p-value	0.003	0.001	<0.001	<0.001
Alltocause mortality	**Coefficient**	-0.16	-0.18	-0.15	-0.14
	95% CI	-0.22 to 0.10	-0.24 to 0.12	-0.21 to 0.084	-0.20 to 0.069
	p-value	<0.001	<0.001	<0.001	<0.001

Figure S1. Histogram of sTNFR1 in the discovery and replication sample.

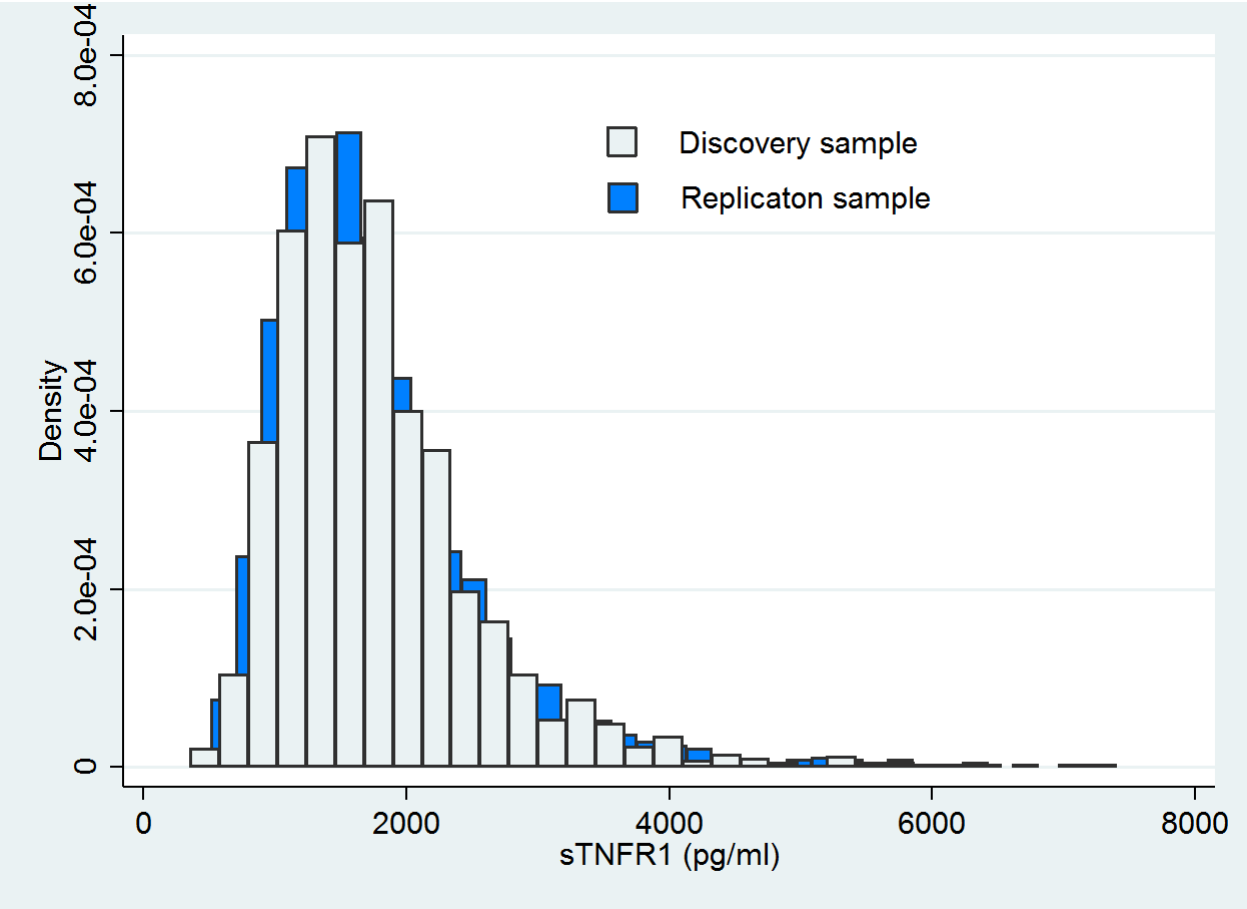
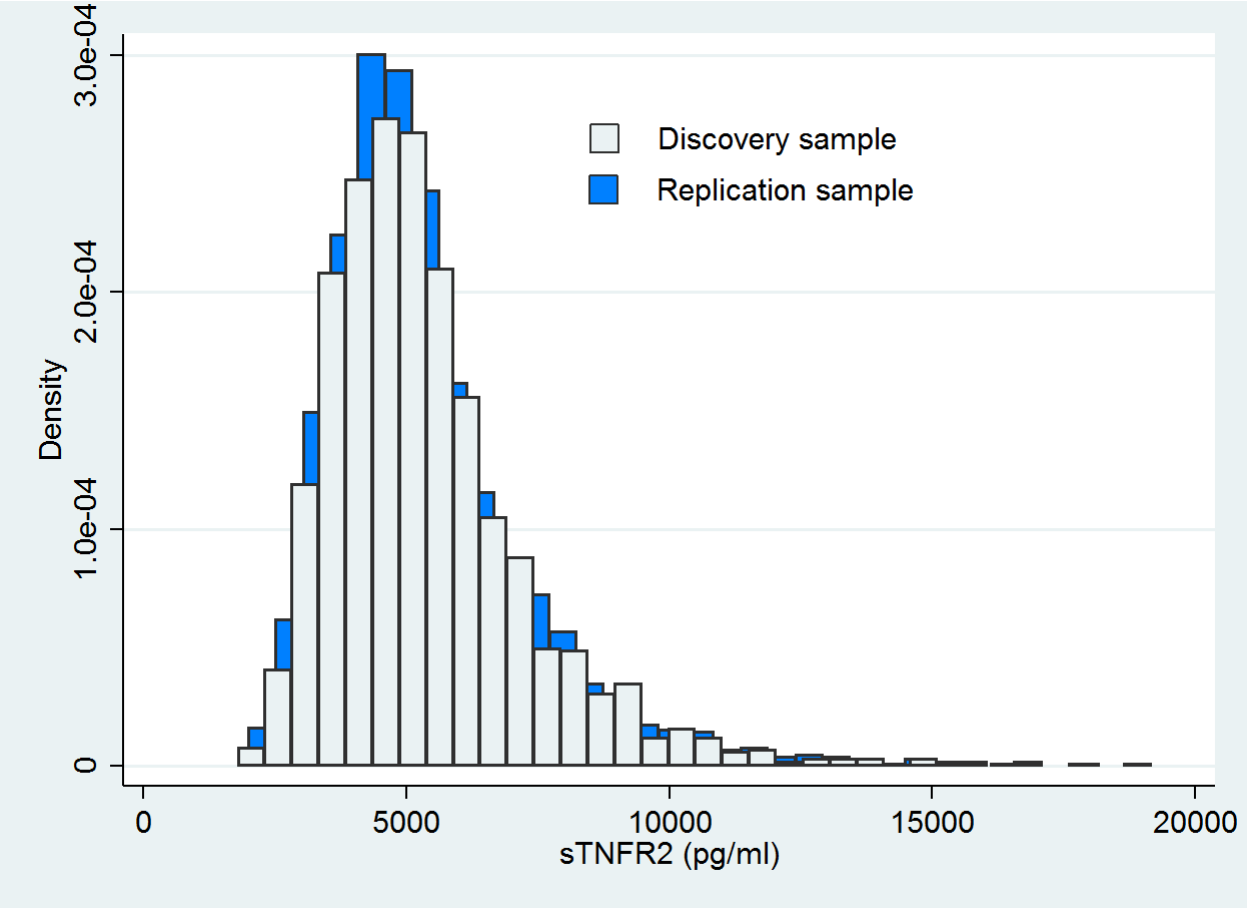


Figure S2. Histogram of sTNFR2 in the discovery and replication sample.



10–Year Associations Between Tumor Necrosis Factor Receptors 1 and 2 and Cardiovascular Events in Patients With Stable Coronary Heart Disease: A CLARICOR (Effect of Clarithromycin on Mortality and Morbidity in Patients With Ischemic Heart Disease) Trial Substudy

Axel C. Carlsson, Toralph Ruge, Erik Kjøller, Jørgen Hilden, Hans Jørn Kolmos, Ahmad Sajadieh, Jens Kastrup, Gorm Boje Jensen, Anders Larsson, Christoph Nowak, Janus Christian Jakobsen, Per Winkel, Christian Gluud and Johan Ärnlöv

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