TNF-related apoptosis-inducing ligand and cardiovascular disease in rheumatoid arthritis

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Abstract

Objective

We examined the association of TNF-related apoptosis-inducing ligand (TRAIL) concentrations with cardiovascular disease (CVD) in rheumatoid arthritis (RA) and, since osteoprotegerin (OPG) can act as a decoy receptor for TRAIL, whether TRAIL concentrations impact on the OPG level-atherosclerotic CVD relation that was recently documented in the present cohort.

Methods

TRAIL concentrations were assessed by ELISA in 151 RA patients of which 75 (49.7%) had CVD comprising ischaemic heart disease (n=27), cerebrovascular accident (n=26), peripheral artery disease (n=9) or/and heart failure (HF) (n=27), and 62 controls.

Results

Mean RA duration was 12 years. In RA patients, C-reactive protein (CRP) levels and cholesterol-HDL cholesterol ratio related to TRAIL concentrations [partial R=-0.222 (p=0.006) and 0.174 (p=0.04), respectively]. TRAIL concentrations were smaller in RA patients compared to controls (median (interquartile range) = 80.2 (60.9–120.4) versus 130.4 (89.4–167.7) pg/ml, p<0.0001)). TRAIL levels were larger in RA patients with compared to those without HF (105.5 (66.5–143.4) versus 79.9 (57.8–110.6), p=0.02); this difference was independent of demographic characteristics and traditional cardiovascular risk factors (p=0.04) but not CRP concentrations (p=0.1). TRAIL levels were consistently unrelated to atherosclerotic CVD. Our previously reported OPG-atherosclerotic CVD relation in RA survived adjustment for TRAIL concentrations in a mixed regression model (p=0.04).

Conclusion

TRAIL concentrations are markedly reduced and associated with HF in established RA, this relationship being explained by CRP levels. OPG may directly enhance CVD risk in RA.

Key words

rheumatoid arthritis, TNF-related apoptosis-inducing ligand, C-reactive protein, cardiovascular disease

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Introduction

TNF-related apoptosis-inducing ligand (TRAIL) belongs to the TNF superfamily (1). TRAIL can be expressed as a transmembrane protein or secreted as a soluble protein (2), which is best known for its capacity to induce apoptosis in cancer cells through interacting with death receptors 4 and 5 (3, 4). TRAIL is also increasingly recognised for its involvement in rheumatoid arthritis (RA) (5). In animal models of RA, TRAIL reduced immune cell hyperactivity and synovial hyperplasia by promoting apoptosis in activated T cells and synoviocytes (5). By contrast, recently reported data suggest that TRAIL has a disease promoting effect in patients with RA (5, 6). TRAIL concentrations and its receptors' polymorphisms also influence the response to disease-modifying agent therapy in early RA (7, 8).

Patients with RA experience a markedly increased risk of atherosclerotic cardiovascular disease (CVD) that includes particularly ischaemic heart disease (IHD) (9, 10) but also stroke (11) and probably peripheral artery disease (PAD) (12). Additionally, independent of conventional cardiovascular risk factors and IHD, RA or RA associated factors increased the risk of heart failure (HF) risk approximately two-fold in a large population study performed by investigators at the Mayo Clinic in Rochester, Minnesota (13). Indeed, both traditional and non-traditional cardiovascular risk factors including genetic factors contribute to CVD in RA (14-17). It is therefore perhaps not surprising that, to date, adequate CVD risk stratification in RA still eludes us (18). In this regard, TRAIL not only participates in the pathophysiology of RA (5) but is also a promising cardiovascular risk biomarker (1). Indeed, TRAIL counteracted the development and extension of atherosclerotic plaques in apoE-null mice (19), which is an experimental model of atherosclerosis. Further, low circulating TRAIL concentrations are associated with poor outcomes in patients with heart disease including those with acute myocardial infarction and HF (20-22). Whether TRAIL influences cardiovascular risk associated with RA is currently unknown.

Osteoprotegerin is another member of the TNF superfamily that also impacts on the pathophysiology of RA (23). Additionally, OPG can induce endothelial activation and is associated with increased atherosclerotic CVD in non-RA subjects (24-26). We and other investigators have recently documented that OPG concentrations are also associated with enhanced atherosclerosis in RA (27, 28). Moreover, OPG concentrations were related to surrogate markers of early atherogenesis and TNF-α blockade with infliximab resulted in reduced OPG concentrations that were associated with decreased endothelial activation (27). Subsequently, we also found independent relations between OPG concentrations and prevalent atherosclerotic CVD comprising IHD, cerebrovascular accident (CVA) or/and PAD in RA (29). Importantly in the present context, OPG acts as a decoy receptor for TRAIL (20, 24, 30). Therefore, the potential adverse impact of OPG on atherosclerotic CVD risk could conceptually be attributable to its direct effects on the endothelium or, alternatively, neutralising TRAIL (27-29).

In view of these considerations, herein we primarily aimed at examining whether TRAIL concentrations are associated with atherosclerotic CVD and HF in RA. We also determined whether TRAIL concentrations impact on the OPG concentration-atherosclerotic CVD relation in RA, as previously identified by us in the present cohort (29).

Materials and methods

Patients

We investigated 151 white Spanish patients that met the 1987 American College of Rheumatology (31) and 2010 American College of Rheumatology/European League against Rheumatism criteria (32) for RA, and were enrolled at the Hospital Universitario Marques de Valdecilla (Santander, Cantabria, Spain). Sixty-two white control subjects without CVD were also investigated; these formed part of community based, randomly recruited attendants in family physician health care centres of the Cantabria region. Controls had no family history of RA, polymyalgia rheumatica, psoriatic arthritis or any

connective tissue disease. The study was approved by the Ethics Committee of Cantabria for Hospital Universitario Marques de Valdecilla in Santander. Patients and controls gave informed written consent to participate and to the publishing of the results.

Assessments

We calculated the Disease Activity Score in 28 joints (DAS28) (33) and measured the erythrocyte sedimentation rate and concentrations of C-reactive protein (CRP) (latex immunoturbidimetry), lipids (enzymatic colorimetry), glucose, rheumatoid factor (RF) (nephelometry) and anti-cyclic citrullinated peptide (anti-CCP) antibody (enzyme linked immunosorbant assay) and creatinine (colorimetry) in patients with RA. Chronic kidney disease was identified when the Modification of Diet in Renal Disease equation (34) was less than 60ml/min/1.73 m^2. Recorded extra-articular manifestations comprised nodular disease, Felty's syndrome, pulmonary fibrosis, rheumatoid vasculitis and Sjögren's syndrome that were defined as previously reported (16). Cardiovascular drug and antirheumatic agent use was assessed.

We determined the presence of traditional risk factors including hypertension, dyslipidaemia, smoking and diabetes as well as CVD including ischaemic heart disease, cerebrovascular disease, peripheral artery disease and heart failure as previously described (17) in patients and controls. Dyslipidemia was identified when a previous doctor diagnosis of hypercholesterolemia or/and hypertriglyceridaemia was present or total cholesterol or/and triglyceride concentrations were ≥240 and 160 mg/dl, respectively. Hypertension was considered present when there was a previous doctor diagnosis of the respective comorbidity or the systolic blood pressure was ≥140 or/and diastolic blood pressure ≥90 mmHg. Diabetes was identified on the basis of a previous doctor diagnosis or when 2 fasting plasma glucose levels on different days were >125 mg/dl. Participants were stratified as smokers when they had smoked during the previous 10 years. Apart from traditional risk factors, we also calculated body mass index (BMI) and diagnosed obesity when the obtained value was ≥30 kg/m^2.

The definition of IHD included acute coronary syndrome with or without persistent ST-segment elevation and chronic coronary heart disease. IHD was diagnosed when any of the following criteria were met: a recorded diagnosis of acute coronary syndromes comprising acute myocardial infarction or unstable angina, the presence of pathological Q waves on an electrocardiogram, and coronary imaging showing >50% stenosis of at least one coronary vessel. Ischaemic dilated cardiomyopathy was also included in this category if systolic function was impaired and the left ventricle dilated with evidence of IHD on electrocardiography or/and catheterisation studies. CVA was diagnosed when a patient had sustained a stroke or/and transient ischaemic attack (TIA). Strokes were confirmed by computer tomography or/and magnetic resonance imaging. TIAs were identified when the symptom duration was self limited to less than 24 hours without residual neurological damage. A diagnosis of PAD required confirmation by Doppler and arteriography. Data on the clinical presentation of heart failure (HF) were also collected in all participants, based on the Framingham Heart Study criteria (35).

Human OPG serum levels were determined by ELISA. Briefly, 96-well microplates were precoated with anti-human OPG antibody (Peprotech). Recombinant human OPG (Peprotech) was used to prepare the standard curve. The standard dilution series ranged from 0.313 to 20 ng/ml. First, 50 µl of each standard or sample was added to the appropriate wells and incubated for 3 hours at room temperature. After discarding the solution and washing 4 times, 50 µl of prepared biotinylated anti-human OPG antibody (Peprotech) was added to each well and incubated for 1 hour. After washing away unbound biotinylated antibody, 50 µl of horseradish peroxidase (HRP)-conjugated avidin (eBioscience) was pipetted into the wells and incubated for 30 minutes. Finally, plates were developed with ABTS Liquid Substrate (Peprotech) and read at 405 and 600 nm (as reference wavelength).

Serum TRAIL concentrations were measured using an ELISA (R&D Systems, DTRLOO). The assay sensitivity was 7.87 pg/ml and the intra- and inter-assay coefficients of variation were <3.9% and <6.0% respectively (Minneapolis, MN, USA) according to the manufacturer's instructions.

Statistical analysis

Results were expressed as mean (SD) and median (interquartile range (IQR)) for normally and non-normally distributed continuous characteristics, respectively, and as proportions for categorical variables.

Associations of baseline characteristics with TRAIL concentrations were assessed in age and sex adjusted mixed regression models.

TRAIL concentrations amongst controls and patients were first compared by the Kruskal-Wallis test, and subsequently in mixed regression models with adjustment for age, sex, traditional cardiovascular risk factors including hypertension, dyslipidaemia, smoking, diabetes and obesity.

TRAIL concentrations amongst patients with and without established CVD were also compared by the Kruskal-Wallis test and in mixed regression models with the additional inclusion of potential confounders as identified in the previous analysis.

We assessed whether TRAIL concentrations were associated with those of OPG in an age and sex adjusted mixed regression model. The OPG level-established CVD relation as previously reported by us in the present cohort (29), was also re-analysed with the additional inclusion of TRAIL concentrations as an independent variable in the respective mixed regression model. Statistical computations were made using the GB Stat™ program (Dynamic Microsystems, Inc, Silverspring, Maryland, USA). Significance was set at a *p*-value of ≤0.05.

Results

Patient characteristics

Seventy-five (49.7%) of the 151 participating patients had established

CVD, which comprised IHD (n=27), CVA (n=26), PAD (n=9) or/and HF (n=27). The proportion of women and mean (SD) age were 61.8% and 62.7% and 70.3 (9.7) and 70.6 (9.9) years in patients without and with established CVD, respectively. Mean (SD) age was 69.2 (11.4) years in the 62 control subjects and 67.7% of them were women. Age and sex did not differ significantly amongst patients and control subjects, and patients with and without CVD. The prevalence of traditional cardiovascular risk factors including hypertension, dyslipidaemia, smoking, diabetes and obesity in patients and controls, were previously reported (29). The same applies to the other characteristics that were recorded in the participating patients, including blood pressure and lipid values, CKD prevalence, cardiovascular drug use, RA features and employed anti-rheumatic agents (29).

Associations between baseline characteristics and TRAIL concentrations in patients with RA

Table I shows that the total cholesterol-HDL cholesterol and triglycerides-HDL cholesterol ratios were associated with high TRAIL concentrations in demographic characteristic adjusted mixed regression models. CRP concentrations, methotrexate and sulphasalazine use and the number of employed synthetic DMARD were inversely related to TRAIL levels. In a multivariable regression model in which age, sex, CRP concentrations, cholesterol-HDL cholesterol ratio and the number of employed synthetic DMARD or methotrexate and sulphasalazine use were entered as independent variables, only CRP concentrations (partial R=-0.222, p=0.006) and cholesterol-HDL cholesterol ratio (partial R=0.174, p=0.02) remained associated with TRAIL levels.

TRAIL concentrations in controls and patients with RA

As given in Figure 1, TRAIL concentrations were lower in RA patients than controls (median (interquartile range) =80.2 (60.9–120.4) *versus* 130.4 (89.4–167.7) pg/ml) (p<0.0001)). TRAIL levels remained lower in patients than

Table I. Age- and sex-adjusted associations of baseline characteristics with TRAIL concentrations* amongst patients with RA.

Characteristic	Partial R	p-value
Age at time of study	-0.005	1.0
Age at RA onset	-0.015	0.6
Women	0.048	0.6
Traditional cardiovascular risk factors		
Any risk factors	0.089	0.3
Hypertension	-0.023	0.8
Dyslipidaemia	0.097	0.2
Smoking	0.084	0.3
Diabetes	-0.086	0.3
Obesity	0.036	0.8
Number of risk factors	0.028	0.7
Body mass index	0.092	0.3
Blood pressure		
Systolic	-0.004	1.0
Diastolic	0.149	0.07
Lipid parameters		
Total cholesterol	0.053	0.5
LDL cholesterol	0.120	0.2
HDL cholesterol*	-0.153	0.07
Total-HDL cholesterol ratio	**0.174**	**0.04**
Triglycerides*	0.113	0.2
Triglycerides-HDL cholesterol ratio[a]	**0.164**	**0.05**
Non-HDL	0.112	0.2
Chronic kidney disease	0.132	0.1
Cardiovascular drugs		
Anti-hypertensives	-0.023	0.8
Statins	0.035	0.7
Glucose lowering agents		
Any	-0.028	0.7
Oral hypoglycemic agents	0.005	1.0
Insulin	-0.039	0.6
RA disease duration*	0.035	0.7
Rheumatoid factor positive	0.039	0.6
Anti-CCP positive	-0.005	1.0
Extraarticular manifestation(s)	-0.001	1.0
Joint erosion(s) in hands or/and feet	-0.113	0.2
C-reactive protein*	**-0.222**	**0.006**
Erythrocyte sedimentation rate*	-0.107	0.2
Disease Activity Score in 28 joints	-0.154	0.07
Anti-rheumatic agents		
Any DMARDs	0.029	0.7
Synthetic DMARDs		
Any	-0.113	0.2
Methotrexate	**-0.191**	**0.02**
Chloroquine/hydroxychloroquine	0.025	0.8
Leflunomide	-0.026	0.8
Sulphasalazine	**-0.162**	**0.04**
Number	**-0.173**	**0.04**
Biologic DMARDs		
Any	0.013	0.9
TNF-α inhibitor	-0.067	0.4
Non TNF-α inhibitor	0.079	0.3
Prednisone	-0.072	0.4

Relationships were assessed in mixed regression models. Age at disease onset (rather than age at time of study) was entered in the model that included disease duration. Significant associations are shown in bold type.

*Logarithmically transformed. TRAIL: tumour necrosis factor-related apoptosis-inducing ligand; RA: rheumatoid arthritis; LDL: low density lipoprotein; HDL: high density lipoprotein; CCP: cyclic citrullinated peptide; DMARDs: disease-modifying anti-rheumatic drugss for rheumatic disease; TNF: tumour necrosis factor.

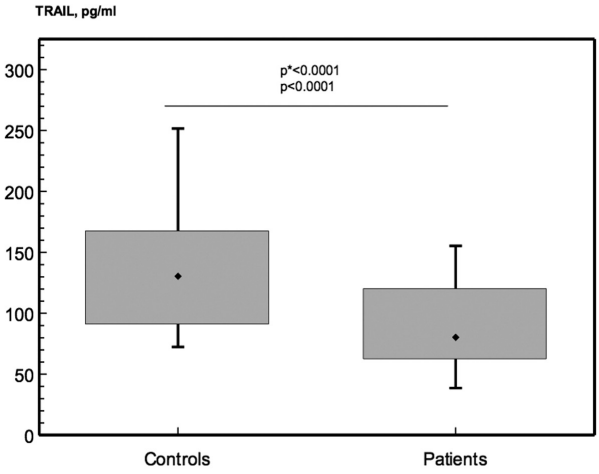

Fig. 1. Box plots showing median and 25–75 and 10–90 percentiles TRAIL concentrations in patients and controls.
*p-adjusted for age at time of the study, sex and traditional cardiovascular risk factors comprising hypertension, dyslipidaemia, smoking, diabetes and obesity.

in controls after adjustment for demographic characteristics and traditional cardiovascular risk factors (p<0.0001).

TRAIL concentrations in RA patients with and without established CVD
Figure 2 shows that TRAIL levels were similar in patients with and without established IHD, CVA or PAD in univariate and adjusted analysis. By contrast, TRAIL concentrations were larger in patients with HF compared to those without HF (median (interquartile range) = 105.5 (66.5–143.4) *versus* 79.9 (57.8–110.6) pg/ml) (p=0.02). TRAIL levels remained related to HF after adjustment for demographic characteristics and traditional cardiovascular risk factors (p=0.04), but not after additional adjustment for CRP concentrations (p=0.1). These results were unaltered upon further adjustment for the number of employed synthetic DMARD, methotrexate and sulphasalazine use or OPG concentrations (data not shown). In this regard, whereas CRP concentrations were similar in those with and without HF (median (interquartile range) = 3.0 (2.0–6.0) *versus* 4.0 (1.5–10.0) mg/l, p=0.2), in age and sex adjusted analysis, CRP concentrations were negatively associated with those of TRAIL in those without (partial R=-0.237, p=0.009) but not with HF (partial R = 0.061, p=0.8).
Notably, TRAIL concentrations were still smaller in RA patients with HF than in control subjects (median (interquartile range) = 105.5 (66.5–143.4) *versus* 130.4 (89.4–167.7) pg/ml, p=0.03).

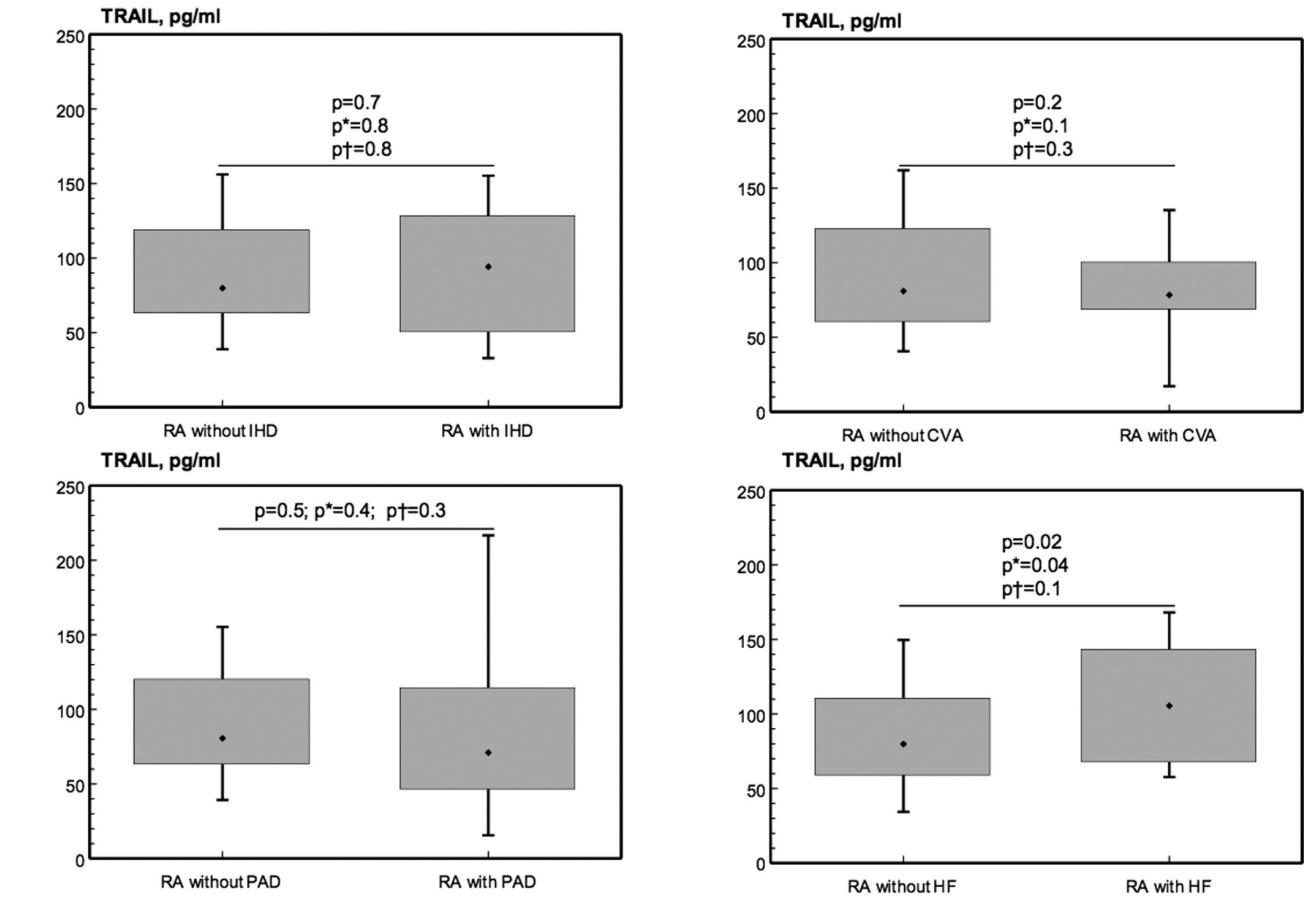

Fig. 2. Box plots showing median and 25-75 and 10-90 percentiles TRAIL concentrations amongst patients with and without IHD, CVA, PAD and HF.
*p-adjusted for age at time of study, sex and traditional cardiovascular risk factors including hypertension, dyslipidemia, smoking, diabetes and obesity; +p additionally adjusted for C-reactive protein. IHD: ischaemic heart disease; CVA: cerebrovascular accident; PAD: peripheral artery disease; HF: heart failure.

The potential impact of TRAIL concentrations on the previously reported OPG level-established CVD relation in the present cohort (29)

In age- and sex-adjusted analysis, TRAIL concentrations were not associated with those of OPG (partial R=-0.075, p=0.4). Also, when we re-analysed our previously reported relationship of established IHD, CVA or/and PAD, with OPG concentrations in a potential confounder (age, sex, traditional risk factors, BMI, anti-cyclic citrullinated peptide antibody, rheumatoid factor and erosive disease) adjusted mixed regression model, with the additional inclusion of TRAIL levels as an independent variable, large OPG concentrations remained associated with established atherosclerotic CVD (p=0.04).

Discussion

The present study revealed that TRAIL concentrations are increased in RA patients with compared to those without HF. Increased TRAIL concentrations were previously also reported in non-RA patients with HF (22, 36, 37). TRAIL induces apoptosis in cancer cells (3, 4) but apoptosis also contributes crucially to the development and progression of HF (22). However, in contrast to the effects of soluble TRAIL on cancer cells, this TNF superfamily member protects smooth muscle cells expressing death receptors 4 and 5 from apoptosis (38). Congruently, in non-RA patients with acute myocardial infarction (20, 21), low levels of TRAIL are associated with incident HF and death, and amongst HF patients (22), low TRAIL concentrations predict re-hospitalisation and death. The pro- *versus* anti-apoptotic effect of TRAIL can also be influenced by the presence of soluble decoy receptors for TRAIL that include OPG (22). The TRAIL concentration-HF relation in the present investigation was not impacted upon by OPG levels. Taken together, increased TRAIL concentrations in RA patients with established HF likely represent the consequence of a compensatory mechanism aimed at curtailing adverse outcomes.

Positive as well as negative associations between CRP concentrations and those of TRAIL were previously reported in non-RA studies (19, 39-43). Nevertheless, in line with our results, Zauli *et al*. (43) found an unequivocal inverse relation between CRP levels and those of TRAIL. In strong support of these findings, Secchiero *et al*. recently showed that CRP downregulates TRAIL expression in peripheral monocytes through an Erg-1 dependent pathway (44). In the current study, CRP concentrations were not only negatively associated with TRAIL levels in patients without and not with HF but, strikingly, also explained the TRAIL concentration-HF relation in RA patients. Our results indicate that the absence of inhibitory effects of systemic inflammation on TRAIL production in HF can mediate increases of TRAIL concentrations amongst patients with the respective comorbidity. This finding has clinical implications as it suggests that therapies targeted at reducing the inflammatory burden in patients with RA, can protect against HF. However, this should be subject to future research as the present study was cross-sectionally designed, thereby precluding us from identifying cause-effect relations.

Two previous studies performed in patients with early RA reported unaltered TRAIL concentrations (6, 7). In another investigation amongst 50 RA patients with a median disease duration of 12 years and 50 healthy controls, RA was associated with raised TRAIL levels (45). In the present relatively large investigation that comprised patients with established RA (mean disease duration = 12 years), TRAIL concentrations were markedly reduced, that is, by ~38%, independent of demographic characteristics and traditional cardiovascular risk factors. Whereas the reasons for these discrepancies are unclear, approximately 50% of our patients sustained established CVD. The impact of RA on TRAIL production amongst different settings, as well as the potential role of decreased TRAIL concentrations in the overall enhanced CVD risk associated with RA also requires future study. Of note in this regard, TRAIL concentrations remained still smaller in patients with HF compared to control subjects in our study.

Similar to the results produced by the present RA investigation, a positive association between adverse lipid profiles and TRAIL concentrations was found in non-RA subjects (2). As applies to the TRAIL concentration-HF relation, these findings may reflect the presence of an adaptive mechanism targeted at reducing CVD risk (2).

In the patients that formed part of the present RA cohort, we previously documented an independent relation between OPG concentrations and atherosclerotic CVD (29). In this context, our current analysis showed that the respective relation was not influenced by reduced TRAIL concentrations. This further supports a potentially direct role of OPG in the enhanced atherogenesis amongst patients with RA (27, 29).

With regard to additional limitations of this study, circulating TRAIL concentrations do not necessarily reflect its tissue concentrations. It is however soluble TRAIL that is particularly implicated in attenuating cardiovascular risk (1, 22).

In conclusion, TRAIL concentrations are markedly reduced and associated with HF in patients with established RA. This TRAIL level-HF relationship is explained by CRP concentrations. The involvement of TRAIL in CVD risk and its potential role in enhanced cardiovascular risk stratification amongst RA patients merits further investigation in future mechanistic and longitudinal studies. The present study also suggests that OPG can directly contribute to enhanced atherosclerotic CVD risk in patients with RA.

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