

## Research Article

# Patients with Ankylosing Spondylitis and Low Disease Activity because of Anti-TNF-Alpha Therapy Have Higher TRAIL Levels Than Controls: A Potential Compensatory Effect

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**Objective.** TRAIL is a potential biomarker of cardiovascular (CV) disease. Ankylosing spondylitis (AS) is a chronic inflammatory disease associated with metabolic syndrome (MeS) and accelerated atherosclerosis. We assessed whether disease activity, systemic inflammation, and MeS features were associated with circulating TRAIL levels in AS patients undergoing TNF- $\alpha$  antagonist infliximab therapy and if infliximab infusion modified TRAIL levels. **Methods.** We measured TRAIL serum levels in 30 nondiabetic AS patients without CV disease undergoing anti-TNF- $\alpha$  therapy, immediately before and after an infliximab infusion, and in 48 matched controls. Correlations of TRAIL levels with disease activity, systemic inflammation and MeS features, adipokines, and biomarkers of endothelial activation were evaluated. Changes in TRAIL levels following anti-TNF- $\alpha$  infusion were analyzed. **Results.** TRAIL levels were higher in AS patients than controls. TRAIL levels displayed an inverse correlation with total and LDL cholesterol. We observed an inverse correlation with QUICKI and a marginal association with HOMA-IR. We also found an inverse correlation with resistin and a marginal association with apelin and OPN. Anti-TNF- $\alpha$  infusion did not change TRAIL levels after 120'. **Conclusion.** Elevated TRAIL levels in AS patients may be the result of a compensatory mechanism to reduce CV risk in these patients.

## 1. Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease associated with high incidence of cardiovascular (CV) mortality due to accelerated atherosclerosis [1]. In addition,

AS patients often display metabolic syndrome (MeS) features, which include obesity, dyslipidemia, hypertension, alterations in glucose metabolism, including insulin resistance (IR), and also a dysregulation of adipokines [2]. Anti-TNF- $\alpha$  therapy has proven to be effective for the treatment of AS [3].

Interestingly, a single infusion of anti-TNF- $\alpha$  monoclonal antibody infliximab improved insulin sensitivity in nondiabetic AS patients [4]. Additionally, beneficial effects of anti-TNF- $\alpha$  on adipokines, biomarkers of atherosclerosis, and MeS-related biomarkers were also found in AS [5–12]. Since TNF- $\alpha$  blockade may account for biological changes that may slow the progression of atherosclerosis in AS patients [13], the analysis of the potential influence of treatment with anti-TNF- $\alpha$  drugs on disease activity, systemic inflammation, MeS, and new potential CV risk biomarkers might help us to understand the effect of these biologic agents on the mechanism associated with atherosclerosis in AS patients.

TNF-related apoptosis-inducing ligand (TRAIL) belongs to the TNF superfamily and was proposed as a potential biomarker of CV disease [14]. This protein can be expressed on the cell surface, as a transmembrane protein, or secreted as a soluble protein [15]. Apart from being involved in the apoptotic process, TRAIL also exerts anti-inflammatory and antiatherosclerotic functions [16–18].

Taking all these considerations into account, in the present study, we aimed to study if TRAIL serum levels were altered in AS patients undergoing infliximab therapy when compared to controls. We also aimed to assess potential associations between disease activity, systemic inflammation, adipokines, biomarkers of endothelial activation, and MeS features with circulating TRAIL levels in these patients. Finally, we also aimed to determine whether an anti-TNF- $\alpha$  monoclonal antibody infliximab infusion modified TRAIL levels.

## 2. Patients and Methods

**2.1. Patients.** We assessed a series of 30 patients with AS attending hospital outpatient clinics seen over 14 months (January 2009 to March 2010), who fulfilled the modified New York diagnostic criteria for AS [19]. They were treated by the same group of rheumatologists and were recruited from the Hospital Lucus Augusti (Xeral-Calde), Lugo, Spain. For the comparative analysis with AS patients, we used 48 controls matched by age, sex, ethnicity, and traditional CV risk factors, who did not have history of CV events.

For ethical reasons, patients included in the present study were not randomized to a placebo group. The same procedure has been found acceptable and followed in studies on the short-term effect of infliximab therapy on adipokines and biomarkers of endothelial cell activation in patients with rheumatoid arthritis (RA) [20–22].

Patients on treatment with infliximab seen during the period of recruitment with diabetes mellitus or with plasma glucose levels greater than 110 mg/dL were excluded. None of the patients included in the study had hyperthyroidism or renal insufficiency. Also, patients seen during the recruitment period who had experienced CV events, including ischemic heart disease, heart failure, cerebrovascular accidents, or peripheral arterial disease, were excluded. Patients were diagnosed as having hypertension if blood pressure was  $\geq 140/90$  mmHg or they were taking antihypertensive agents. Patients were considered to have dyslipidemia if they had hypercholesterolemia and/or hypertriglyceridemia (defined

as diagnosis of hypercholesterolemia or hypertriglyceridemia by the patients' family physicians, or total cholesterol and/or triglyceride levels in fasting plasma were  $>220$  mg/dL and  $>150$  mg/dL, resp.). Obesity was defined if body mass index (BMI) (calculated as weight in kilograms divided by height in squared meters) was greater than 30.

In all cases, treatment with the anti-TNF- $\alpha$  monoclonal antibody infliximab was started because of active disease. All patients included in the current study had begun treatment with NSAIDs immediately after the disease diagnosis. All of them were still being treated with these drugs at the time of the study. At the time of this study, most patients were on treatment with naproxen 500–1000 mg/d. Although the 2010 updated recommendations facilitate initiation of TNF- $\alpha$  blockers in AS and only ask for 2 NSAIDs with a minimum total treatment period of 4 weeks [23], for the initiation of anti-TNF- $\alpha$  therapy in these series of patients recruited between January 2009 and March 2010, they had to be treated with at least 3 NSAIDs prior to the onset of infliximab therapy.

A clinical index of disease activity (Bath Ankylosing Spondylitis Disease Activity Index—BASDAI—range of 0 to 10) [24] was evaluated in all patients at the time of the study. Clinical information on hip involvement, history of synovitis in other peripheral joints and peripheral enthesitis, history of anterior uveitis, presence of syndesmophytes, and HLA-B27 status (typed by cell cytotoxicity) was assessed. Moreover, C-reactive protein (CRP), by a latex immunoturbidity method, erythrocyte sedimentation rate (ESR) (Westergren), serum glucose, total cholesterol, HDL and LDL cholesterol, and triglycerides (fasting overnight determinations) were assessed in all the patients at the time of the study.

The main demographic, clinical, and laboratory data of this series of 30 AS patients at the time of the study are shown in Table 1. Since at that time all patients were undergoing periodical treatment with the anti-TNF- $\alpha$  monoclonal antibody infliximab (median duration of periodical treatment with this biologic agent: 23 months), the mean BASDAI  $\pm$  standard deviation (SD) was only  $2.94 \pm 2.11$ .

The local institutional committee approved anti-TNF- $\alpha$  therapy. Also, patients gave informed consent to participate in this study. Neither this study nor the former studies on the short-term effect of infliximab therapy on insulin resistance, adipokines, and biomarkers of endothelial cell activation in AS were supported by any pharmaceutical drug company.

**2.2. Study Protocol.** In all cases, the drug was given to patients as an intravenous infusion in a saline solution over 120 minutes. All measurements were made in the fasting state. Blood samples were taken at 0800 hours for determination of the erythrocyte sedimentation rate (ESR) (Westergren), C-reactive protein (CRP) (latex immunoturbidimetry), lipids (enzymatic colorimetry), plasma glucose, and serum insulin (DPC, Dipesa, Los Angeles, CA, USA). As previously described, insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula (insulin ( $\mu$ U/mL)  $\times$  glucose (mmol/l)  $\div$  22.57) [4]. A commercial ELISA kit was used to measure serum TRAIL levels (R&D Systems, DTRL00); assay sensitivity = 7.87 pg/mL; intra- and interassay coefficients of variation

TABLE 1: Demographic, clinical, and laboratory data of 30 patients with ankylosing spondylitis.

Variable	n (%)
Mean age (years) $\pm$ SD	
At the time of study	50.47 $\pm$ 14.85
At the time of onset of symptoms	28.23 $\pm$ 10.40
Delay to the diagnosis (years) $\pm$ SD	11.48 $\pm$ 9.01
Men/women	21 (70)/9 (30)
Mean disease duration (years) $\pm$ SD*	21.97 $\pm$ 13.16
History of classic cardiovascular risk factors	
Hypertension (n = 30)	12 (40)
Dyslipidemia (n = 30)	11 (36.67)
Obesity (BMI > 30 kg/m <sup>2</sup> ) (n = 30)	3 (10.00)
Current smokers (n = 30)	13 (43.33)
Mean blood pressure (mm Hg) $\pm$ SD*	
Systolic	123.17 $\pm$ 18.17
Diastolic	75.67 $\pm$ 12.51
Mean body mass index (kg/m <sup>2</sup> ) $\pm$ SD	26.70 $\pm$ 3.26
Mean BASDAI $\pm$ SD*	2.94 $\pm$ 2.11
Mean VAS $\pm$ SD*	31.13 $\pm$ 24.23
Hip involvement, n (%) (n = 30)	6 (20)
Synovitis and/or enthesitis in other peripheral joints, n (%) (n = 27)	11 (36.67)
Anterior uveitis, n (%) (n = 30)	6 (20.00)
Syndesmophytes, n (%) (n = 30)	10 (33.33)
Mean CRP (mg/L) $\pm$ SD**	
At the time of disease diagnosis	24.01 $\pm$ 33.43
At the time of study	6.24 $\pm$ 8.65
Mean ESR (mm/1st hour) $\pm$ SD***	
At the time of disease diagnosis	30.10 $\pm$ 28.23
At the time of study	19.00 $\pm$ 15.18
Mean cholesterol or triglycerides (mg/dL) $\pm$ SD*	
Total cholesterol	199.10 $\pm$ 30.61
HDL cholesterol	53.17 $\pm$ 12.81
LDL cholesterol	126.77 $\pm$ 26.54
Triglycerides	93.97 $\pm$ 56.70
Mean fasting serum glucose (mg/dL) $\pm$ SD*	92.77 $\pm$ 8.63
HLA-B27 positive (n = 27)	20 (74.07)

\*At the time of the study. \*\*Normal value <5 mg/L. \*\*\*Normal value <20 mm/1st hour.

BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BMI: body mass index; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; HDL: high-density lipoprotein; HLA: human leukocyte antigen; LDL: low-density lipoprotein; SD: standard deviation; VAS: visual analogue scale.

were <3.9% and <6%, respectively (Minneapolis, MN, USA) according to the manufacturer's instructions. Serum levels of TRAIL were measured in samples obtained immediately prior to an infliximab infusion and 120 minutes later. Total plasma adiponectin and osteoprotegerin levels, serum resistin, leptin, visfatin, apelin, angiopoietin-2 (Angpt-2), asymmetric dimethylarginine (ADMA), gelsolin, osteopontin (OPN), and retinol-binding protein 4 (RBP-4) levels obtained immediately before infliximab infusion were determined by ELISA as previously described [5–12].

2.3. *Statistical Analyses.* Variables were expressed as mean  $\pm$  SD, median, interquartile range (IQR), or percentages. Correlation between basal TRAIL at time 0 and selected continuous variables was performed, adjusting for age at the time of the study, sex, and classic CV risk factors via estimation of the Pearson partial correlation coefficient ( $r$ ).

The associations between baseline characteristics and serum TRAIL concentrations were assessed by Student's paired  $t$ -test. Differences in TRAIL levels between men and women and patients with hypertension or without were assessed by the Mann-Whitney  $U$  test.

TRAIL serum levels before (at time 0) and after infusion (at time 120 minutes) were compared using the paired Student's  $t$ -test.

Two-sided  $P$  values  $\leq$  0.05 were considered to indicate statistical significance. Analyses were performed using Stata 12/SE (StataCorp, College Station, TX).

### 3. Results

3.1. *Differences in TRAIL Serum Levels between AS Patients and Controls.* TRAIL serum levels were higher in AS patients (mean  $\pm$  SD: 160.57  $\pm$  47.48 pg/mL; median (IQR): 160.72 (128.69–195.99)) than in healthy controls (mean  $\pm$  SD: 117.96  $\pm$  48.20 pg/mL; median (IQR): 107.49 (85.32–137.31)) ( $P$  < 0.0003) (Figure 1).

3.2. *Relationship of TRAIL Concentration with Disease Activity and Clinical Features.* Circulating TRAIL levels did not correlate with disease duration, BASDAI, or VAS spinal pain at the time of the study (Table 2). Likewise, no difference in TRAIL concentration was observed when patients with a history of anterior uveitis, presence of syndesmophytes, hip involvement or synovitis in other peripheral joints, and peripheral enthesitis were compared with the remaining patients who did not exhibit these characteristics. It was also the case when patients were compared according to HLA-B27 status (data not shown).

3.3. *Relationship of Demographic Features, Inflammation, Adiposity, and Adipokines with Circulating TRAIL Levels.* TRAIL serum levels did not show significant association with age at the onset of symptoms, BMI, and CRP and ESR at the time of the study and at the time of disease diagnosis (Table 2). Interestingly, we observed an inverse correlation of TRAIL with resistin ( $r = -0.427$ ;  $P = 0.05$ ) and a marginal association with apelin ( $r = 0.373$ ;  $P = 0.06$ ). No association with the rest of adipokines was observed (Table 2). When AS patients were stratified according to sex, no significant differences were found in TRAIL serum levels (data not shown).

3.4. *Relationship of TRAIL Levels with Metabolic Syndrome Features Other Than Adiposity.* TRAIL serum levels displayed an inverse correlation with total cholesterol ( $r = -0.393$ ;  $P = 0.04$ ) and LDL cholesterol ( $r = -0.452$ ;  $P = 0.02$ ). In addition, we observed a negative correlation of TRAIL with insulin sensitivity index (QUICKI:  $r = -0.440$ ;  $P = 0.02$ ) and a marginal association with IR (HOMA-IR:  $r = 0.368$ ;  $P = 0.06$ ) (Table 2). However, we did not observe any association

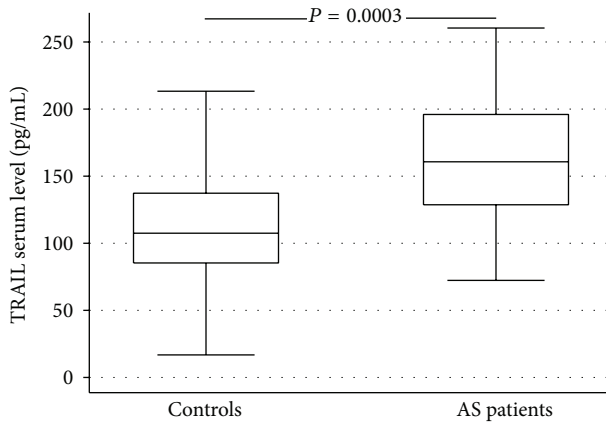


FIGURE 1: Box plot showing differences between AS patients and healthy controls matched by age, sex, and traditional CV risk factors in TRAIL serum concentration.

between TRAIL serum levels and systolic or diastolic blood pressure, HDL cholesterol, triglycerides, or serum glucose levels (Table 2). Likewise, we did not find any correlation between TRAIL and RBP-4 levels (Table 2). Besides, no significant differences in TRAIL serum levels were seen when patients were stratified according to the presence or absence of arterial hypertension (not shown).

**3.5. Relationship of TRAIL Serum Levels with Biomarkers of Endothelial Cell Activation and Atherosclerosis.** No correlation of TRAIL levels with Angpt-2 or ADMA was disclosed. However, we found a marginally negative correlation between TRAIL and OPN levels ( $r = -0.372$ ;  $P = 0.06$ ) (Table 2).

**3.6. Changes in TRAIL Levels upon Infliximab Therapy.** TRAIL serum levels did not change following an infliximab infusion. In this regard, the mean  $\pm$  SD values of TRAIL were  $160.57 \pm 47.48$  pg/mL immediately prior to infliximab infusion (time 0) and  $157.06 \pm 65.90$  pg/mL at the end of the infusion (time 120 minutes) ( $P = 0.772$ ).

## 4. Discussion

TRAIL is a molecule that has been proposed to be involved in the pathophysiology of several autoimmune diseases, not only for its role in the apoptotic process, but also for its functions as an antiatherogenic and anti-inflammatory molecule [25]. Additionally, several pieces of evidence support the potential use of TRAIL as a biomarker of CV disease [14]. Since the implication of TRAIL in AS has not been completely elucidated, in this study we aimed to further clarify the role of this potential biomarker in the pathogenesis and in the mechanisms associated with MeS and CV disease in AS.

In our study, patients with AS displayed low disease activity (mean BASDAI: 2.94) due to prolonged biologic therapy (median duration: 23 months). Interestingly, TRAIL levels were higher in AS patients than in controls. In keeping with our results, increased levels of TRAIL were found in rheumatoid arthritis patients undergoing disease-modifying antirheumatic drug therapy for 1 year when compared to

TABLE 2: Partial correlation of serum TRAIL prior to infliximab infusion (at time 0) with selected continuous variables adjusted for age at the time of the study, sex, and classic cardiovascular risk factors (dyslipidemia, smoking, obesity, and hypertension) in 30 patients with ankylosing spondylitis.

Variable	TRAIL (time 0)	
	<i>r</i>	<i>P</i>
Age at the onset of symptoms	-0.026	0.90
Disease duration*	-0.011	0.96
BMI*	0.042	0.84
Systolic blood pressure*	0.039	0.85
Diastolic blood pressure*	0.087	0.66
BASDAI*	0.089	0.66
VAS spinal pain*	0.135	0.50
ESR* (natural-log-transformed)	0.018	0.93
CRP* (natural-log-transformed)	-0.080	0.69
ESR** (natural-log-transformed)	0.045	0.83
CRP** (natural-log-transformed)	0.011	0.96
<b>Total cholesterol* (natural-log-transformed)</b>	<b>-0.393</b>	<b>0.04</b>
HDL cholesterol* (natural-log-transformed)	-0.111	0.58
<b>LDL cholesterol* (natural-log-transformed)</b>	<b>-0.452</b>	<b>0.02</b>
Atherogenic index* (total cholesterol/HDL)	-0.180	0.38
Triglycerides* (natural-log-transformed)	0.185	0.36
Serum glucose* (natural-log-transformed)	-0.092	0.65
<b>HOMA-IR at time 0*</b>	<b>0.368</b>	<b>0.06</b>
<b>QUICKI at time 0*</b>	<b>-0.440</b>	<b>0.02</b>
<b>Resistin at time 0</b>	<b>-0.427</b>	<b>0.05</b>
Adiponectin at time 0	-0.009	0.97
Leptin at time 0	0.069	0.74
Visfatin at time 0	0.048	0.81
Angpt-2 at time 0	-0.055	0.79
<b>Apelin at time 0</b>	<b>0.373</b>	<b>0.06</b>
ADMA at time 0	-0.058	0.78
Gelsolin at time 0	0.173	0.39
<b>OPN at time 0</b>	<b>-0.372</b>	<b>0.06</b>
RBP-4 at time 0	0.318	0.11
Osteoprotegerin at time 0	0.044	0.83

\* At the time of the study. \*\* At the time of disease diagnosis.

ADMA: asymmetric dimethylarginine; Angpt-2: angiotensin 2; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BMI: body mass index; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; HDL: high-density lipoprotein; HOMA-IR: homeostasis model assessment of insulin resistance; LDL: low-density lipoprotein; OPN: osteopontin; QUICKI: quantitative insulin sensitivity check index; RBP-4: retinol-binding protein 4; VAS: visual analogue scale.

controls [26]. Likewise, lupus patients with inactive disease showed higher TRAIL levels than those with active disease, even higher than those found in controls [25]. Additionally, a study performed on Chinese AS patients also disclosed higher TRAIL levels when compared to healthy controls [27].

Next, we investigated the potential association of TRAIL with metabolic indices, since AS patients often display MeS features [2]. In this regard, we disclosed an inverse correlation of TRAIL with insulin sensitivity index and a marginal association with IR (HOMA-IR). A similar association between TRAIL and HOMA-IR was observed in diabetic patients [28].



It is well known that IR is associated with inflammation [29]; therefore, an increase in the levels of the anti-inflammatory TRAIL molecule would act to compensate such an inflammatory burden.

Furthermore, we also disclosed an inverse correlation of TRAIL with the proinflammatory adipokine resistin and a marginally negative association with the proinflammatory biomarker of endothelial activation OPN. In line with these results, a marginally positive correlation between TRAIL and apelin, an anti-inflammatory adipokine, was also observed. These results further support the previously described anti-inflammatory role of TRAIL [17].

Unlike patients with diabetes or obese individuals [15, 28], in our study that included nondiabetic and mostly nonobese (27 of 30) AS patients, there was a negative correlation between TRAIL levels and total/LDL cholesterol. Chronic inflammation is associated with a paradoxical decrease of lipid levels [30, 31]. Despite having low disease activity probably due to long-term anti-TNF- $\alpha$  therapy, a chronic inflammatory state is plausible to exist in our patients (CRP 24 at disease diagnosis and 6.24 mg/L at the time of the study). In this regard, it was reported that CRP values greater than 3 mg/L were associated with high risk of future CV events [32]. Therefore, also in this situation, elevated TRAIL levels may act to compensate the chronic inflammatory state that is present in these patients (reflected by the inverse correlation between TRAIL and total/LDL cholesterol as well as by the presence of CRP levels above the normal range).

Finally, although the aim of the study was to evaluate the immediate direct effect of an infliximab infusion on TRAIL levels, adipokines, biomarkers of endothelial activation, and metabolic syndrome, it may constitute by itself a potential limitation since it is possible that the influence of infliximab on TRAIL levels may require several steps before directly reducing TRAIL levels. Therefore, further studies aimed at determining the effect of infliximab on TRAIL levels at different times, especially at later times, are needed to fully establish the effect of anti-TNF- $\alpha$  blockade on TRAIL and other molecules implicated in the mechanisms leading to CV disease in AS.

## 5. Conclusion

In conclusion, our results suggest that elevated TRAIL levels in AS patients may be the result of a compensatory mechanism to reduce CV risk as a result of IR and chronic inflammatory state in these patients.

## Conflict of Interests

The authors declare that they have no conflict of interests.

## Authors' Contribution

Fernanda Genre and Raquel López-Mejías performed the study, contributed to the elaboration of the protocol of study,

and helped in the interpretation of data and in the elaboration of the paper. Javier Rueda-Gotor, Begoña Ubilla, Beatriz Carnero-López, Natalia Palmou-Fontana, Inés Gómez-Acebo, Ricardo Blanco, Trinitario Pina, Rodrigo Ochoa, and Carlos González-Juanatey helped in the interpretation of data and contributed to the elaboration of the paper. José A. Miranda-Fillooy recruited patients for the study, contributed to the elaboration of the protocol of study, and helped in the interpretation of data and the elaboration of the paper. Javier Llorca contributed to the elaboration of the protocol of study, helped in the interpretation of data and the elaboration of the paper, and performed the statistical analysis. Miguel A. González-Gay recruited patients for the study, contributed to the elaboration of the protocol of study, helped in the interpretation of data, and was responsible for the final drafting and elaboration of the paper.

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