# TNF-related apoptosis-inducing ligand levels in rheumatoid arthritis, osteoarthritis, and spondyloarthritis

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**Introduction**
Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a tumor necrosis factor (TNF) family member capable of inducing apoptosis in many cell types. Data suggest that TRAIL/TRAIL receptor expression profiles on T cells might be important in rheumatoid arthritis (RA) pathogenesis.

**Objectives**
To characterize the role of TNF-related apoptosis-inducing ligand (TRAIL) in rheumatoid arthritis (RA) and to explore whether TRAIL investigated in serum and synovial fluid were associated with clinical, laboratory, and radiological variables of RA disease activity and severity.

**Methods**
Circulating levels of TRAIL were measured by ELISA in serum samples obtained from 50 patients with RA (during activity and quiescence), 20 patients with osteoarthritis, 15 patients with spondyloarthritis, and 50 normal healthy individuals serving as controls.

**Results**
The median serum TRAIL concentrations were increasingly higher across the following groups: healthy controls (185 pg/ml), and RA patients with active disease (1625 pg/ml; $P=0.0001$ vs. controls) and inactive disease (1750 pg/ml; $P=0.0001$ vs. controls) (inactive vs. active RA; $P=0.07$). It is noteworthy that RA patients had significant higher median TRAIL concentrations as compared with osteoarthritis patients whether during activity or during quiescence. However, the median levels of TRAIL were statistically comparable in RA and spondyloarthritis patients. The median and mean ± SD synovial fluid TRAIL concentrations were 2100 and 1765.8 ± 752 pg/ml, respectively. The levels of TRAIL in synovial fluid from the patients were higher than those in sera from both the patients and the healthy individuals. TRAIL concentrations in paired sera and synovial fluid samples could be related to each other. Serum and synovial concentrations of TRAIL were correlated positively with the total number of joints with active arthritis and with the overall articular severity score. Patients with Larsen index and total radiographic score of at least 1 had significantly higher serum TRAIL levels than patients with indices and scores 1 or less.

**Conclusion**
Upregulated expression of TRAIL might be somewhat useful for the evaluation of RA disease activity and progression, although its increment is not disease specific.

**Keywords:**
activity, apoptosis, rheumatoid arthritis, severity, TNF-related apoptosis-inducing ligand

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## Introduction

Rheumatoid arthritis (RA), a chronic disease of incompletely understood etiology, is characterized primarily by the progressive destruction of articular structures. Its pathogenesis is governed by the concerted action of several cell types that create signs and symptoms characteristic for RA. Accumulating evidence indicates that, in addition to macrophages and T cells, resident fibroblast-like cells have been found to be of utmost importance for joint destruction. Once activated, these cells pass through prominent molecular changes, resulting in an aggressive behavior in both initiating and driving the disease. Not only do activated rheumatoid arthritis synovial fibroblasts (RASFs) with an aggressive phenotype increase in number, their activation also results in the production of proinflammatory mediators and in alterations of programmed cell death. As a result, a hyperplastic synovial tissue is generated that mediates the progressive destruction of articular cartilage and bone [1].

Programmed cell death, or apoptosis, plays a pivotal role in tissue homoeostasis under both physiological and pathological conditions. Some characteristic changes in the composition and structure of the inflamed synovial membrane in RA are linked to an altered apoptotic

response of synovial cells. Research over the past few years has identified different mechanisms that prevent synovial cells in RA from undergoing apoptosis. They include changes in the mitochondrial pathway as well as altered expression of modulators of death receptors [2].

Apoptosis can be induced by members of the tumor necrosis factor (TNF) receptor family through the recruitment of an intracellular membrane-associated complex of proteins (death-inducing signaling complexes), which leads to a cytoplasmic release of active caspase-8 and subsequent activation of the apoptotic cascade. Among these death receptors, Fas/CD95 and its specific ligand FasL/CD95L were found to be important, and it was shown that the stimulation of RASFs with FasL initiates proapoptotic signals. Actually, RASFs undergo less FasL-induced apoptosis than osteoarthritis synovial fibroblasts. It is noteworthy that fibroblasts in RA synovium express both TNF-α receptors and Fas, and their ligands have been detected in colocalized macrophages and T cells [3].

TNF-related apoptosis-inducing ligand (TRAIL), also called apoptosis-2 ligand (Apo2L) for its similarity in sequence, structure, and function to FasL/Apo1L, is a tumor necrosis factor superfamily (TNFSF) member designated TNFSF10. TRAIL was cloned from human heart atrium, lymphocyte, and placenta cDNA libraries on the basis of its similarity to regions highly conserved in the TNFSF. TRAIL is a 281 amino acid, ~32 kDa, type II transmembrane protein expressed on the cell surface [4].

TRAIL is expressed in fetal kidney, liver, and lung, as well as in adult colon, heart, kidney, lung, ovary, lymphocytes, placenta, prostate, skeletal muscle, small intestine, spleen, and thymus. TRAIL is variably expressed in tumor cell lines [5].

Yao *et al.* [6] have shown previously that a local, intra-articular injection of an adenoviral vector expressing human TRAIL in a rabbit knee model of inflammatory arthritis stimulated synovial apoptosis and reduced inflammation. Analysis of synovium isolated from the rabbits treated with an intra-articular injection of rTRAIL showed areas of extensive acellular debris and large fibrous regions devoid of intact cells, similar to adenoviral-mediated TRAIL gene transfer. Extensive apoptosis of the synovial lining was observed. In addition, leukocyte infiltration into the synovial fluid of the inflamed knee joints following rTRAIL treatment was reduced more than 50%. These results show that an intra-articular injection of rTRAIL could be therapeutic for the treatment of pathologies associated with RA.

## Participants and methods

### Patient group

This case–control longitudinal study included 50 patients with RA as a stratified nonrandom sample. They were 35 women and 15 men, with a female to male ratio of 2.3 : 1. Their ages ranged from 19 to 52 years, with a mean age ± SD of 37.27 ± 9.79 years. The diagnosis of RA was made on the basis of the 1988-Revised American Rheumatism Association Criteria for Classification of Rheumatoid Arthritis [7]. All patients fulfilled at least four of the seven proposed criteria. Their duration of illness ranged between 1 and 120 months, with a mean disease duration of 46.7 ± 15.45 months. According to Yamanaka *et al.* [8], 20 patients were considered to have early-onset or recent-onset arthritis (arthritis or joint symptoms existing for < 1 year at the time of entry into the present study and none had previously received disease-modifying antirheumatic drugs or systemic steroid therapy). At inclusion in the study, a median duration of 7 months from the onset of symptoms was recorded. The remaining 30 patients were designated to have long-term arthritis (mean disease duration = 86.4 ± 28.8 months).

### Control group

Twenty patients with osteoarthritis, 15 patients with spondyloarthritis, and 50 clinically healthy individuals age and sex matched to RA patients were enrolled for a comparison of laboratory data. The latter group included 30 women and 20 men, with a female to male ratio of 1.5 : 1. Their ages ranged from 20 to 54 years, with a mean age of 37.98 ± 12.71 years. They underwent general and systemic examination to exclude any current illnesses particularly rheumatic diseases, co-existing chronic inflammation, or cancers.

### Study design

Evaluation of RA patients was carried out clinically for active inflammation of all joints and severity of disease. Radiographs were obtained for the hands, wrists, forefeet, and the knees. At this stage, blood samples were obtained from all the patients studied. Aspiration of synovial fluid from the knee joints of 20 RA patients was carried out through arthrocentesis. Paired samples of sera and synovial fluid were collected at the same time to determine the interrelations between TRAIL in both compartments. Follow-up of all RA patients was carried out with close supervision of their compliance to therapy until stabilization of their condition and quiescence of symptoms by treatment (remission or steady state), when at follow-up, second blood samples were obtained for laboratory re-evaluation.

#### *Assessment of clinical activity in rheumatoid arthritis patients*

RA patients were evaluated for active joint inflammation. The clinical indices of articular inflammation used were as follows:

(1) Joint swelling (graded as 0 = none; 1 = mild but obvious synovial swelling or effusion and bony landmarks visible; 2 = moderate swelling and definite obscuring of bony landmarks; 3 = severe swelling and no discernible bony landmarks).
(2) Limitation of motion (graded as 0 = full range of motion; 1 = 25% limitation; 2 = 50% limitation; 3 = 75% limitation; 4 = no motion).
(3) Pain on motion and/or joint tenderness (graded as 0 = none; 1 = mild pain; 2 = moderate pain; 3 = marked pain).

In addition to these indices, the total number of joints with active arthritis and the sum of the three clinical indices of articular inflammation referred to as the articular severity score were recorded according to Giannini *et al.* [9].

*Assessment of clinical severity in rheumatoid arthritis patients*
Severity was defined according to Weyand and colleagues [10,11] by the presence of extra-articular manifestations and destructive arthritis. In our study, 20 patients (40%) had extra-articular manifestations, five patients (10%) had subcutaneous nodules, five patients (10%) had pericarditis, five patients (10%) had cutaneous vasculitis, three patients (6%) had pleuritis, and two patients (4%) had amyloidosis.

### Radiological evaluation

Radiographs of the hands, wrists, forefeet, and the knees were obtained at enrollment. The method of Rau and Herbon (a modified version of Larsen's scoring method) was applied for each radiograph. The Larsen index of an individual patient was then expressed as a mean of the grading of all the examined areas of hands, wrists, and feet (32 evaluated joints according to Larsen) as well as of the knees [12,13]. Scoring of joint malalignment was carried out according to Fuchs *et al.* [14]. A composite score was then adopted by merging both scores together as a mean value for each patient and termed the 'total radiographic score'. Two radiographs of the same joint were compared and scores were assigned to reflect the evolution of the joint change (score 00 = unaffected; score 0 = affected, but unchanged; score + 1 = improvement; and score −1 = deterioration) according to van Rossum *et al.* [15].

### Study measurements

(1) Serum and synovial fluid TNF-related apoptosis-inducing ligand (TRAIL/TNFSF10): the Quantikine human TRAIL immunoassay is a 4.5 h solid-phase ELISA. Reagents were supplied by Quantikine (R&D Systems Inc., Minneapolis, Minnesota, USA; Catalog Number: DTRL00). This assay uses a quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TRAIL had been precoated onto a microplate. Standards and samples were pipetted into the wells and any TRAIL present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for TRAIL was added to the wells. Following a wash to remove any unbound antibody–enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of TRAIL bound in the initial step. The color development was stopped and the intensity of the color was measured. For the calculation of results, a standard curve was constructed on a log/log scale by plotting the mean absorbance for each standard on the *y*-axis against the concentration on the *x*-axis and drawing a best-fit curve through the points on the graph. The minimum detectable dose of TRAIL ranged from 0.57 to 7.87 pg/ml. The mean minimum detectable dose was 2.86 pg/ml.

(2) Erythrocyte sedimentation rate first hour [ESR (mm/h)] was determined using the Westergren method. Levels of at least 28 mm/h were considered elevated.

(3) Serum C-reactive protein (CRP) levels were assessed by quantitative turbidimetry (Turbiquant CRP reagent; Behring Werke, Marburg, Germany). Levels of at least 15 mg/l were elevated.

(4) Quantification of serum rheumatoid factor (RF) was carried out by quantitative turbidimetry on a Turbitimer (Turbiquant RF reagent; Behring Werke) (titers < 40 IU/ml = nonreactive; 40–80 IU/ml = weak reactive; and > 80 IU/ml = high reactive).

(5) Serum antinuclear antibodies (ANA) were determined using a standard indirect immunofluorescence technique on HEp-2 cells. Titer more than 1/40 was positive.

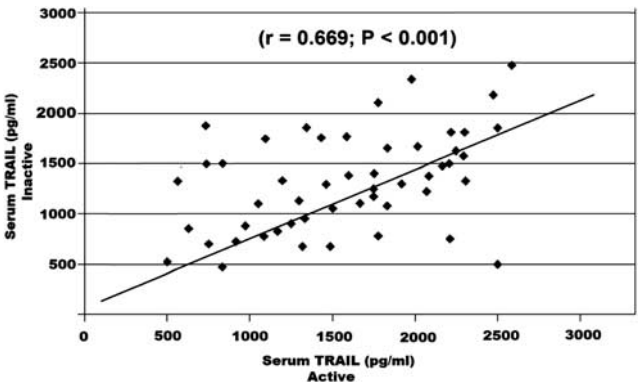
### Statistical analyses

All statistical analyses used the SPSS software for Windows system (version 11.5; SPSS Inc., Chicago, Illinois, USA). Data were expressed as mean, SD, median, and interquartile ranges (25th and 75th percentiles). For nonparametric data, the Mann–Whitney test (*Z*-value) was used to compare between two groups, whereas comparison between the same groups in two repeated measurements was carried out using the Wilcoxon-signed rank test. The Kruskall–Wallis test (*H* value) was used for comparison between more than two groups. For parametric data, the ANOVA test (*F*-value) was used for comparison between more than two groups and Student's *t*-test of significance to compare between two groups. Correlation coefficient (*r*) was used to interrelate the numeric variables.

## Results

At recruitment, the mean total number of joints with active arthritis was 10.7 ± 4.9, and 30 patients (60%) had active

**Figure 1**




Significant positive correlation between serum TNF-related apoptosis-inducing ligand (TRAIL) levels (pg/ml) during disease activity and the corresponding values during disease remission.

joint inflammation with a mean overall articular severity score of 45.65 ± 19.76 (Fig. 1 and Tables 1 and 2).

Serum concentrations of TRAIL were correlated positively with the total number of joints with active arthritis (Pearson's coefficient = 0.41; $P = 0.05$) and with the overall articular severity score (Pearson's coefficient = 0.52; $P = 0.01$; Fig. 2). Similar correlations were found for synovial TRAIL levels (Pearson's coefficient = 0.61 and 0.59 for the number and the score, respectively, $P = 0.01$ for both).

In our study, the median and mean ± SD serum TRAIL concentrations were significantly higher in RA patients with extra-articular manifestations (1550; 1420.58 ± 449 pg/ml) than those without extra-articular manifestations (1050; 1053.5 ± 335 pg/ml; $P < 0.001$).

**Table 1 Characteristics of rheumatoid arthritis patients**

| Variables | RA [activity ($n=50$)] |
|---|---|
| Age (years) | |
| Mean (SEM) | 37.27 (9.79) |
| Range | 19–52 |
| Female/male (*N*) | 35/15 |
| Duration of illness (months) | |
| Mean (SEM) | 46.7 (15.45) |
| Range | 1–120 |
| Early/recent-onset arthritis (months) | |
| *N* | 20 |
| Range; median | 1–12; 7 |
| Long-term arthritis (months) | |
| *N* | 30 |
| Range; mean (SEM) | 13–120; 86.4 (28.8) |
| Extra-articular manifestations [*N* (%)] | 20 (40%) |
| ESR (mm/h) | |
| Range | 40–130 |
| Mean (SEM) | 60.39 (19.54) |
| C-reactive protein (mg/dl) | |
| Range | 4–18 |
| Mean (SEM) | 9 (3.91) |
| Serum antinuclear antibodies (reactive titers ≥ 1/40) [*N* (%)] | 15 (30) |
| Serum rheumatoid factor [*N* (%)] | |
| High-reactive | 10 (20) |
| Nonreactive or weak reactive | 40 (80) |
| Larsen index [mean (SEM)] | 2.51 (0.75) |
| Malalignment score [mean (SEM)] | 2.45 (0.69) |
| Total radiographic score [mean (SEM)] | 2.73 (0.81) |

ESR, erythrocyte sedimentation rate; RA, rheumatoid arthritis.

The median and mean ± SD serum TRAIL concentrations in patients with long-term arthritis (1450; 1340 ± 521 pg/ml) were significantly higher than those of patients with early-onset or recent-onset arthritis (1100; 1075 ± 320 pg/ml; $P < 0.001$). Moreover, serum TRAIL levels could be related to the duration of illness ($r = 0.52$; $P < 0.01$).

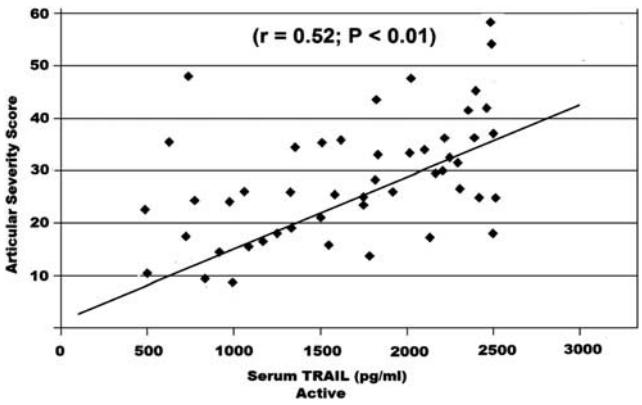
### Serum TNF-related apoptosis-inducing ligand levels in relation to some laboratory variables of rheumatoid arthritis disease activity and severity

Serum concentrations of TRAIL were correlated positively with the levels of CRP (Pearson's coefficient = 0.41; $P = 0.04$) and with ESR (Pearson's coefficient = 0.39; $P = 0.02$) in all RA patients.

In terms of ANA seropositivity, 15 patients (30%) were found to be positive (reactive titers > 1/40). The median and mean ± SD serum TRAIL concentrations in ANA seropositive patients (1395; 1300.5 ± 401 pg/ml) were significantly higher than those of ANA seronegative patients (1050; 1110 ± 510 pg/ml; $P < 0.01$).

The median and mean ± SD serum TRAIL concentrations in high-reactive RF patients were significantly

**Figure 2**




Significant positive correlation between serum TNF-related apoptosis-inducing ligand (TRAIL) concentrations (pg/ml) and the overall articular severity score.

**Table 2 Serum TNF-related apoptosis-inducing ligand levels (pg/ml) in the studied groups**

| | RA | | | | |
|---|---|---|---|---|---|
| TRAIL (pg/ml) | Activity ($n=50$) | Remission ($n=50$) | Spondyloarthritis ($n=15$) | Osteoarthritis ($n=20$) | Controls ($n=50$) |
| Range | 499–2500 | 610–2324 | 380–2009 | 70–682 | 130–450 |
| Median | 1625 | 1750 | 1559 | 225 | 185 |
| IQR | 1050 | 1106 | 1015 | 85 | 70 |
| Mean ± SD | 1667 ± 739 | 1545 ± 643 | 1109 ± 677 | 100.6 ± 25.6 | 167 ± 65.6 |
| Z1 | 3.9** | 4.1** | 3.1** | 1.2# | – |
| Z2 | 1.6# | 1.8# | – | – | – |
| Z3 | 3.6** | 3.1** | – | – | – |
| | Z4 = 0.07# | | Z5 = 3.2** | | – |

IQR, interquartile range; RA, rheumatoid arthritis; TRAIL, TNF-related apoptosis-inducing ligand; Z1, arthritis versus controls; Z2, RA versus spondyloarthritis; Z3, RA versus osteoarthritis; Z4, RA activity versus remission; Z5, spondyloarthritis versus osteoarthritis.
#Nonsignificant.
*Significant ($P < 0.05$).
**Highly significant ($P < 0.01$).

higher than those of weak and nonreactive patients (1402; 1285 ± 325 pg/ml vs. 1015; 1050 ± 455 pg/ml, $P<0.01$). Moreover, serum and synovial fluid concentrations of TRAIL were correlated positively with the RF titers (Pearson's coefficient = 0.54 and 0.55, respectively, $P<0.05$ for both).

### Serum TNF-related apoptosis-inducing ligand levels in relation to the radiological indices of joint destruction

The mean ± SD of Larsen index, malalignment score, and total radiographic score were 2.51 ± 0.75; 2.45 ± 0.69; and 2.73 ± 0.81, respectively. Serum concentrations of TRIL were correlated positively with the Larsen index and the total radiographic score (Pearson's coefficient = 0.62 and 0.51, respectively, $P<0.01$ for both).

Patients with a Larsen index and total radiographic score of at least 1 had significantly higher median serum TRAIL levels than patients with indices and scores less than 1 (1390 vs. 1120 pg/ml; $P<0.01$). In contrast, patients with malalignment scores of less than 2 and those with scores of at least 2 had statistically comparable serum TRAIL levels.

## Discussion

The results of this study confirm the importance of TRAIL in RA pathophysiology. We observed a higher expression of TRAIL in RA patients than controls irrespective of disease activity. Our results are in agreement with those of other reports [16–19]. The difference in serum TRAIL expression between RA patients and controls could not be explained by age or sex distribution as matched controls were used. Elevated production of TRAIL may serve as a potential marker of RA, although its increment is not disease specific as TRAIL involvement has been reported in the pathophysiology of other autoimmune diseases.

Although the pathogenesis of RA disease is unclear, it is well known that T cells play a major role in both the development and the perpetuation of RA. Evidences for alterations in TRAIL/TRAIL receptor expression on peripheral T lymphocytes in the molecular mechanism of RA development have been widely explored. Bisgin *et al.* [20] reported upregulation of TRAIL and its receptors (both death and decoy) on both CD4+ and CD8+ T cells in rheumatoid patients compared with control individuals [21].

Moreover, the infiltration and accumulation of T cells in RA synovium are hallmarks of disease. Accumulating evidences suggest the functional relevance of APO2L/TRAIL in the persistence of T cells in the rheumatoid synovial fluid. In addition, the presence of an aggressive population of activated synovial fibroblasts and pseudotumoral expansion of fibroblast-like synoviocytes (FLSs) that are prominently involved in the destruction of articular cartilage and bone are constant hallmarks of RA disease.

The median and mean ± SD synovial fluid TRAIL concentrations were 2100 and 1765.8 ± 752 pg/ml, respectively. The levels of TRAIL in synovium fluid from RA patients were higher than those in sera from both patients and controls. TRAIL levels in paired samples of sera and synovial fluid could be related to each other by a correlation coefficient. The high expression of TRAIL suggests its role in RA pathogenesis and that synovial fluid TRAIL levels are believed to be useful in measuring local joint involvement.

Our data are largely in agreement with other reports [3,18,19,21–24] that have reported the presence of TRAIL in the synovial tissues at sites of joint destruction. It is noteworthy that CD3+ lymphocytes infiltrated in synovial fluid have a chronically activated phenotype and are resistant to Fas-induced toxicity; however, they are more susceptible to TRAIL-induced toxicity. The observation on the sensitivity of T cells to rTRAIL could have therapeutic implications because bioactive TRAIL could be beneficial as an RA treatment [6,25].

In our study, the median serum TRAIL concentrations were increasingly higher across the following groups: healthy controls, RA patients with active disease, and patients with inactive disease. It is noteworthy that TRAIL has a very short calculated half-life of only 30 min; this indicates continuous TRAIL production or secretion during activity and quiescence. Lower levels of TRAIL during activity may indicate the inhibition of TRAIL-mediated apoptosis in active rheumatoid synovial tissues despite the stimulation of the intracellular pathway(s) that lead to apoptosis. Inhibited apoptosis occurred downstream of caspase-3 and probably involved caspase-3 inhibitors, survivin, and x-linked inhibitor of apoptosis protein [21]. It is noteworthy that Jin *et al.* [26] found that administration of TRAIL is an effective anti-inflammatory treatment that prevents the development and progression of collagen-induced arthritis in DBA/1 J mice, and they suggest that TRAIL might be considered a potential treatment for human arthritis.

In our study, we found significant correlations of TRAIL levels in serum and synovial fluid with clinical and laboratory parameters of RA activity (total number of joints with active arthritis, the overall articular severity score, and CRP and ESR values). Our data are largely in agreement with other reports [17,20]. These results are not expected on the basis of the anti-inflammatory effect of TRAIL [26]; however, they may be attributed to the dual functionality of TRAIL in stimulating apoptosis and proliferation of FLSs. TRAIL modify the expression of the cell survival regulators and cellular functions associated with different processes in RA synovium particularly the turnover of cartilage matrix during joint inflammation.

Researchers have identified several factors that predict which patients with early RA will experience progression of their disease. Predictors of disease severity and poor outcome include the presence of extra-articular manifestations and ANA and RF seropositivity. RF is the strongest predictor of disease progression in community cases of RA of limited duration [27]. In our study, serum TRAIL levels were significantly higher in RA patients with extra-articular manifestations and in those with ANA

and RF seropositivity. Moreover, serum and synovial fluid concentrations of TRAIL were positively correlated with the RF titers. Our data are largely in agreement with other reports [17,20]. It is noteworthy that Audo *et al.* [22] reported that severity of disease correlated inversely with the susceptibility of FLSs to TRAIL-induced apoptosis and the sensitivity to TRAIL-induced apoptosis varied in FLSs from different patients. TRAIL-sensitive cells expressed significantly lower levels of TRAIL-R1; moreover, silencing of TRAIL-R1 increased TRAIL-induced apoptosis in RA FLS. Therefore, over-expression of TRAIL seems to be in favor of disease activity, severity, and poor outcome.

In our study, serum TRAIL concentrations in patients with long-term arthritis were significantly higher than those in patients with early-onset or recent-onset arthritis. Moreover, serum TRAIL levels could be related to the duration of illness, and a positive correlation could be elicited between serum TRAIL levels during disease exacerbations and the corresponding values during remission, indicating that the higher the level during exacerbations, the higher it remained after remission. Audo *et al.* [23] have reported elevated serum and synovial fluids TRAIL levels in the arthritic joints of patients with established RA. They found that a low ratio of the soluble decoy receptor osteoprotegerin to TRAIL in the sera of early RA patients at baseline was associated with a better evolution of disease activity, but high serum levels of TRAIL at follow-up were associated with joint damage and disease-promoting activity.

In the present study, we found that patients with a Larsen index and total radiographic score of at least 1 had significantly higher serum TRAIL levels than patients with indices and scores less than 1. Moreover, serum concentrations of TRAIL were correlated positively with the Larsen index and the total radiographic score. This indicates an association between the level of expression of TRAIL and the radiological stages of the disease progression. Therefore, TRAIL might have a direct relevance in the erosive process to an extent, and the differences in quantification between the two groups of our studied patients highlighted the role of this marker in the pathogenesis of joint damage, reflecting joint destruction that will occur in the near future with disease progression if not aggressively controlled once diagnosed, as radiological progression in RA is a continual process, although it appears to be more rapid during early years of the disease.

Our data are largely in agreement with those of other reports [16,28] that have reported the contribution of interaction of TRAIL and its decoy receptor, osteoprotegerin, toward enhancement of the erosive processes induced by human synovial cells. Thus, we might conclude that TRAIL levels are discriminators of cumulative joint damage and are of prognostic importance. It can predict patients at risk of joint damage that could allow earlier initiation of aggressive anti-inflammatory therapy with a corresponding improvement in outcome. Our findings must be considered as preliminary, pending longitudinal confirmation on larger samples.

In our study, patients with malalignment scores of less than 2 and those with scores of at least 2 had statistically comparable serum TRAIL levels. Radiographic evidence of malalignment is a late finding [29]. Malalignment results from increased joint cartilage destruction that is compatible with loss of cartilage and less turnover; therefore, such comparable levels could be expected.

TRAIL has been proposed as an anti-inflammatory cytokine in animal models of RA. The ability of rTRAIL to induce tumor-specific apoptosis as well as the ability of intra-articular TRAIL gene transfer to induce synovial apoptosis indicates that an intra-articular injection of rTRAIL might also be able to induce apoptosis of hyperplastic synovium. Injection of exogenous rTRAIL was able to induce synovial apoptosis in arthritic joints of rabbits as well as reduce inflammation. In addition, no adverse effect was observed locally on cartilage metabolism or systemically on hepatic function [6]. This suggests that a local injection of rTRAIL could be therapeutic in RA. Recently, efficient therapeutic modalities for RA treatment have been developed in the form of nano-sized complexes (nanocomplexes) based on hyaluronic acid and polyethylene glycol (PEG)-derivatized TRAIL (PEG-TRAIL) formed by N-terminal-specific PEGylation. These results imply that hyaluronic acid/PEG-TRAIL nanocomplex formulations are promising therapeutic modalities for the treatment of RA [30].

## Conclusion

The results of this study confirm the importance of TRAIL in RA pathophysiology. However, the exact mechanism and significance remain to be elucidated in larger prospective studies. Measurement of serum TRAIL, although not a specific diagnostic marker for RA, can still be considered a very informative serological marker in terms of disease activity, severity, and disease progression. We sought to measure TRAIL in serum because it is easier, rapid, and less invasive and would still partially reflect the intra-articular changes. Quantification of such a marker in association with radiographic assessment represents a useful prognostic tool to forecast the development of joint destruction and to identify patients at risk of rapidly progressing disease, enabling a more informed clinical decision to be made, for example, earlier initiation of a more aggressive disease-modifying antirheumatic drugs or biological therapy with a corresponding improvement in RA disease outcome. A prospective longitudinal study is required to investigate serum TRAIL values over time with respect to disease activity and immunosuppressive treatment. Clinical applications of TRAIL as an emerging therapeutic effective anti-inflammatory protein for RA definitely present a new era of immune therapies.

## Acknowledgements

### Conflicts of interest

There are no conflicts of interest.

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