Synovial Biomarkers in Psoriatic Arthritis

UGO FIOCCO, FRANCESCA OLIVIERO, PAOLO SFRISO, FIORELLA CALABRESE, FRANCESCA LUNARDI, ELENA SCAGLIORI, LEOPOLDO RUBALTELLI, ROBERTO STRAMARE, ANTONIO DI MAGGIO, ROBERTO NARDACCHIONE, LUISELLA COZZI, BEATRICE MOLENA, MARA FELICETTI, KATIA GAZZOLA, ALESSANDRO LO NIGRO, BENEDETTA ACCORDI, PASCALE ROUX-LOMBARD, JEAN-MICHEL DAYER, and LEONARDO PUNZI

ABSTRACT. ***Objective.*** To find candidate biomarkers of psoriatic arthritis (PsA). A panel of synovial fluid (SF) and synovial tissue (ST) biomarkers was analyzed in patients with resistant peripheral PsA, in relation to clinical and imaging outcomes of synovitis response following serial intraarticular (IA) etanercept injections (12.5 mg).

Methods. Fourteen PsA patients with resistant knee joint synovitis were treated with 4 IA etanercept injections in a single knee joint, once every 2 weeks. Primary outcome (Thompson's knee index: THOMP) and secondary outcomes were assessed at baseline and end of study: C-reactive protein, Knee Joint Articular Index (KJAI), Health Assessment Questionnaire disability index, maximal synovial thickness (MST) by gray-scale ultrasonography, contrast-enhanced magnetic resonance imaging (C+MRI), ST-cluster differentiation (CD)45+ mononuclear cell, ST-CD31+ vessels, and ST-CD105+ angiogenic endothelial cells, along with levels of SF interleukin 1ß (IL-1ß), IL-1 receptor antagonist (Ra), and IL-6.

Results. At the end of the study, clinical and imaging outcomes, ST and SF biological markers were significantly reduced compared to baseline. There was a significant association between IL-6 and either THOMP or KJAI; between either ST-CD31+ or ST-CD105+ or ST-CD45+; between ST and SF biomarkers expression (CD45+ and IL-1ß) and between ST-CD45+ and both KJAI and MRI-MST. Comparing pre- versus post-IA etanercept injection changes (Δ), Δ IL-1ß was significantly correlated with both Δ IL-6 and with Δ IL-1Ra and Δ IL-6 with Δ IL-1Ra.

Conclusion. The association to disease activity and the changes following IA treatment indicate that ST-CD45+ and ST-CD31+, along with SF-IL-6 and SF-IL-1ß, may represent candidate biomarkers of the knee synovitis response to IA tumor necrosis factor-α blockade. (J Rheumatol 2012;39 Suppl 89:61–64; doi:10.3899/jrheum.120246)

Key Indexing Terms:
PSORIATIC ARTHRITIS SYNOVIAL BIOMARKERS ULTRASOUND
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Psoriatic synovitis, compared to rheumatoid arthritis (RA) synovitis, is characterized by a more abrupt onset and by a high density of tortuous blood vessels at the synovio-entheseal complex from the beginning[1]. Synovial hypervascularity may persist in scattered areas throughout the relapsing course of the disease. However, macroscopic synovitis findings are not correlated with clinical disease measures in psoriatic arthritis (PsA), and histologically the differences between RA and PsA synovium, as regards mononuclear cell (MNC) infiltration and inflammatory cytokines expression, are not well defined.

The available data on reliable biological markers in PsA are limited[2,3], therefore there is an urgent need to develop sensitive synovial biomarkers for the PsA disease process.

From the Department of Medicine, the Department of Medical-Diagnostic Science and Special Therapies, and the Oncohematology Laboratory of the Department of Pediatrics, University of Padua, Padua; the Department of Orthopedics, Leonardo Foundation, Abano Terme General Hospital, Abano Terme, Italy; the Immunology and Allergy Division, Geneva University Hospitals and University of Geneva, Geneva, Switzerland.

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U. Fiocco, MD, PhD; F. Oliviero, PhD; P. Sfriso, MD, PhD; Department of Medicine, University of Padua; F. Calabrese, MD, PhD; F. Lunardi PhD; E. Scagliori, MD; L. Rubaltelli, MD; R. Stramare, MD; A. Di Maggio, MD, Department of Medical-Diagnostic Science and Special Therapies, University of Padua; R. Nardacchione, MD, Department of Orthopedics, Leonardo Foundation, Abano Terme General Hospital; L. Cozzi, MD; B. Molena, PhD; M. Felicetti, MD; K. Gazzola, MD; A. Lo Nigro, MD, PhD, Department of Medicine, University of Padua; B. Accordi, PhD, Oncohematology Laboratory, Department of Pediatrics, University of Padua; P. Roux-Lombard, PhD, Immunology and Allergy Division, Geneva University Hospitals and University of Geneva; J-M. Dayer, MD, PhD, Emeritus Professor, Faculty of Medicine, CMU 1, University of Geneva; L. Punzi, MD, PhD, Department of Medicine, University of Padua.

Address correspondence to Dr. U. Fiocco, Rheumatology Unit, Department of Medicine, University of Padua, Via Giustiniani 2, 35128 Padua, Italy. E-mail: ugo.fiocco@unipd.it

Our present study concerns a prospective evaluation of joint inflammation following tumor necrosis factor-α (TNF-α) blockade, based on specific tools to detect quantitative modifications of synovitis processes by serial intraarticular (IA) etanercept injections in patients with resistant peripheral PsA.

MATERIALS AND METHODS

The study protocol was approved by the local ethics committee (Etanercept/TNR-001:n.878P; ClinicalTrials.gov Identifier: NCT00678782). Four IA etanercept injections (12.5 mg/0.5 ml) were administered every 2 weeks to single knee joints of patients with PsA according to the ClASsification for Psoriatic ARthritis criteria[4]. All patients signed consent statements (Table 1).

Efficacy assessment. The primary outcome was the Thompson knee index (THOMP; range 0-9) and the secondary endpoints: local Knee Joint Articular Index (KJAI) score (range 0-14; 4), serum C-reactive protein (CRP; ≤ 5 mg/l), and Health Assessment Questionnaire-Disability Index (HAQ-DI; range 0-3). Outcomes were assessed at baseline and at the end of the study.

Ultrasound (US). US evaluation was carried out using a high-frequency linear transducer (10 MHz Elegra, Siemens, Erlangen, Germany), as described, to assess the maximal synovial tissue (ST) thickening (MST), performed in mm in the area where thickening was worst[5].

Magnetic resonance imaging (MRI). MRI scans were done using a 0.2 T Magnetom unit (Esaote ArtroScan C MR Scanner) to obtain T1-weighted spin–contrast enhanced MRI (C+MR). C+MR-MST measurements were given in mm.

Synovial biopsy and immunohistochemistry. ST specimens were evaluated after immunostaining using cluster differentiation (CD)45 antibodies (MNC infiltrate); CD31 antibodies (vessel density), and CD105 (endoglin transmembrane accessory receptor for transforming growth factor-ß; angiogenic endothelial cells; SN6h)[6] (Dako Cytomation) by computer-assisted morphometric analysis (Image-Pro Plus version 5)[4].

Synovial fluid cytokines. Interleukin 1ß (IL-1ß), IL-1 receptor antagonist (IL-1Ra), and IL-6 were measured in synovial fluid (SF) using a Luminex multiplexed assay platform (Fluorokine MAP7 Multiplex Human Cytokine Panel A, R&D Systems, Minneapolis, MN, USA)[4].

Table 1. Clinical and demographic features of patients with peripheral psoriatic arthritis.

Knee joints, n	14
Age, yrs, mean ± SD	42.86 ± 11.67
Female, n (%)	4 (28.57)
Disease duration, yrs, mean ± SD	8.76 ± 4.73
Duration of KJS, yrs, mean ± SD	6.91 ± 3.87
PASI	≤ 10
CRP, mg/dl, mean ± SD	0.86 ± 0.95
ESR, mm/h	26.97 ± 18.01
Systemic treatment at study entry, n (%)	
DMARD	12 (85.71)
Etanercept	4 (28.57)
Prednisolone*	7 (50.00)
Intraarticular (IA) treatment	
Previous IA-steroid injection, mg, mean ± SD	2.3 ± 1.5
IA etanercept injection: n, mean ± SD	3.93 ± 0.92

* Daily prednisolone dose ≤ 10 mg. KJS: knee joint synovitis; PASI: Psoriasis Area and Severity Index; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DMARD: disease-modifying antirheumatic drugs.

Statistics. All data processing was performed using SPSS software (version 15.0). The Bonferroni correction method was used for multiple comparisons.

RESULTS

IA etanercept injections caused a significant reduction in the local outcomes, systemic disease activity, and changes in imaging outcomes before and after IA tumor necrosis factor-α (TNF-α) blockade. There was a significant reduction in IL-1ß, IL-1Ra, and IL-6 levels in SF (Table 2).

Significant correlations, measured as a whole on the baseline and end of study determinations, were found between US and MRI-MST ($p = 0.02$) and between THOMP and KJAI ($p = 0.0000$). CRP was correlated with HAQ-DI ($p = 0.049$) and with both SF-IL-1ß and IL-1Ra ($p = 0.048$, $p = 0.022$, respectively); both THOMP and KJAI with SF-IL-6 ($p = 0.021$, $p = 0.023$, respectively). There were significant correlations between CD45+ and both KJAI and MRI-MST ($p = 0.044$, $p = 0.045$, respectively), as well as between CD45+ and both CD31+ and CD105+ expression ($p = 0.037$, $p = 0.039$, respectively), in ST and between IL-1ß and both IL-6 and IL-1Ra levels, in SF ($p = 0.003$, $p = 0.0001$, respectively), as well as between ST-CD45+ and SF-IL-1ß ($p = 0.03$; Figure 1).

Table 2. Comparison of local and systemic disease activity and imaging indexes and biological synovial markers before and after intraarticular (IA) tumor necrosis factor-α (TNF-α) blockade in psoriatic arthritis knee joint synovitis. All data are mean ± SD.

	Pre-IA TNF-α Block, n = 14	Post-IA TNF-α Block, n = 14	p
Clinical outcomes			
THOMPscore	6.71 ± 1.54	2.07 ± 2.20	0.0010
KJAI score	9.51 ± 1.50	2.92 ± 2.70	< 0.0010
HAQ score	0.58 ± 0.51	0.38 ± 0.56	0.0517
CRP, mg/dl	0.85 ± 0.95	0.30 ± 0.26	< 0.0142
Imaging outcomes			
C+MR-MST, mm	9.37 ± 3.34	7.57 ± 2.65	0.011
US-MST, mm	7.60 ± 3.05	5.15 ± 3.14	0.006
Synovial tissue (cell/2 mm^2)*	n = 5	n = 5	
CD45+	1295 ± 886	481 ± 230	0.0431
CD31+	94.3 ± 27.8	46.1 ± 41.1	0.0499
CD105+	126.2 ± 60.1	58.8 ± 50.1	0.225
Synovial fluid**	n = 14	n = 14	
IL-1Ra, pg/ml	11284 ± 10582	4376 ± 3918	0.006
IL-6, pg/ml	5109 ± 5560	1810 ± 3482	0.018
IL-1ß, pg/ml	8.78 ± 10.85	4.28 ± 0.61	0.006

Significance by Wilcoxon rank test. * Immunohistochemistry computer-assisted morphometric analysis of a 2-mm^2 area. ** Last synovial fluid available for aspiration after intraarticular etanercept injections. C+MR-MST: contrast-enhanced magnetic resonance maximal synovial thickness score; CD: cluster differentiation; CRP: C-reactive protein; HAQ: Health Assessment Questionnaire; IL: interleukin; IL-1Ra: IL-1 receptor antagonist; KJAI: Knee Joint Articular Index score; PsA: psoriatic arthritis; THOMP: Thompson's knee index score; US-MST: ultrasonography maximal synovial thickness.

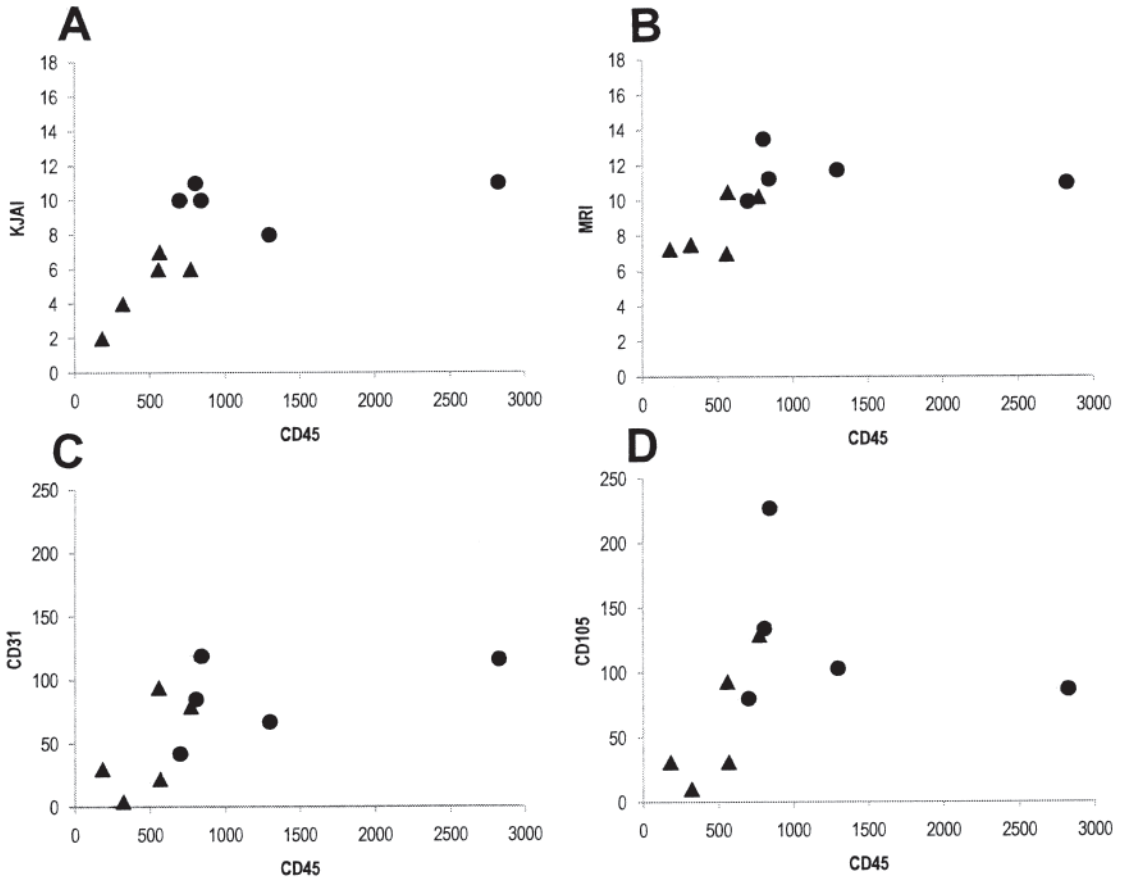

Figure 1. Correlation between synovial tissue CD45+ mononuclear cells with (A) local knee disease activity Knee Joint Articular Index score (KJAI); (B) contrast-enhanced magnetic resonance imaging (MRI) maximal synovial thickness score; (C) synovial tissue CD31+ vessels; and (D) synovial tissue CD105+ mesenchymal-like endothelial progenitor cells.

Comparing pre- versus post-IA etanercept injection changes (Δ), Δ IL-1ß was significantly correlated with both Δ IL-6 and with Δ IL-1Ra ($p = 0.008$, $p = 0.04$, respectively), and Δ IL-6 with Δ IL-1Ra ($p = 0.004$).

DISCUSSION

Very few studies have addressed the issue of the local assessment of inflammation at the single joint level. In spite of the widespread use of TNF-α inhibitors, the early molecular mechanisms by which TNF-α blockade modulates ST inflammation are poorly understood.

The potential usefulness of synovial biomarkers in PsA was recently reported, showing the participation of the IL-6/IL-17 cytokine axis, of IL-1, IL-8, and other chemokines in PsA inflammation, being correlated with systemic disease activity and immunocytochemical ST markers, in resistant peripheral PsA[5]. Our prospective study extends previous analysis based on a translational approach by targeting at single joint level.

Despite the use of the more restrictive Bonferroni correction method, we could confirm the previous association of synovial biomarkers to composite joint functional indexes, disease activity indexes, and quantitative imaging measures, in addition to changes induced by IA TNF-α blockade. Among the biomarkers in SF, both IL-1ß and IL-1Ra were correlated with CRP, IL-6 was associated to both THOMP and KJAI composite knee scores, and IL-1ß and IL-1Ra and IL-6 changes to each other.

Inflammatory cytokines may be induced by synovial hypoxia, taking part in the mechanisms of resistance to TNF-α block[7], while IL-1 and IL-6 in humans may participate in the differentiation of IL-17-producing human T helper (Th17) cells. The association of IL-6 level in SF to local joint inflammation indicates a major role of IL-6 in the mechanism of the resistant PsA synovitis.

The significant reduction after IA etanercept injections and the correlation of synovial tissue CD45+ MNC infiltration with both local knee joint synovitis disease activity score and contrast-MRI synovial thickness measure further support the importance of CD45+ MNC in synovial proliferation in PsA. Early downregulation of CD68+ monocytes and of CD4+ and CD8+ T cell infiltration in ST following alefacept[8] and ustekinumab[9], as well as the decrease of CD3+ T cell number in response to both systemic[10,11] and

local TNF-α blockade[5], were reported. The decrease in synovial MNC infiltration may be explained by an early effect on dendritic/T cell gene expression in responding patients, with a pattern of coordinated deactivation of inflammatory antigen presenting cells and either Th17 or Th1 effector cells in ST, as already observed in psoriatic skin[9,12,13].

Because of the rapid vascular growth in PsA synovitis, CD105+ angiogenic endothelial cells, which are upregulated by hypoxia, may represent a potential target for the local action of IA TNF-α blockers, preferentially affecting immature vessel formation[14,15]. Unlike the findings of systemic treatment[11], the reduction of CD31+ vessels induced by serial IA etanercept injections in ST, being significantly associated to CD105+ angiogenic endothelial cells and to CD45+ MNC infiltration, underlines CD31+ as a potential ST biomarker of the response to IA TNF-α blockade in PsA.

Our study has several important limitations: its single-center, open-label design, its small sample size, and the short followup time.

The association with disease activity and changes following IA treatment suggest that synovial tissue CD45+ and CD31+ expression, along with IL-6 and IL-1ß levels in SF, may represent a panel of candidate biomarkers of the PsA knee synovitis response to IA TNF-α blockade.

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