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Original article

# Synovium CD20 expression is a potential new predictor of bone erosion progression in very-early arthritis treated by sequential DMARDs monotherapy – A pilot study from the VErA cohort

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ABSTRACT

*Objective:* Because available biomarkers (rheumatoid factors [RF], anti-cyclic citrullinated autoantibodies [anti-CCP2], erythrocyte sedimentation rate at 1st hour [ESR]/C-reactive peptide [CRP] and bone erosions) are insufficient to predict rheumatoid arthritis (RA) structural damage, to determine whether synovium expression of greater or equal to 1 markers could constitute new prognostic factor(s).
*Method:* The study was conducted on 18 prospectively enrolled disease-modifying anti-rheumatic drug (DMARD)- and glucocorticoid-naïve, VErA cohort patients with very-early arthritis (median duration: 4 months). Recorded at baseline were: clinical and biological (serum ESR, CRP, RF-isotypes, anti-CCP2, osteoprotegerin, receptor activator of nuclear $\kappa$B-ligand [RANK-L] and cartilage oligomeric matrix protein [COMP] levels) data; synovium expression (HLA-DR, CD163, CD3, CD20, VEGF, osteoprotegerin, RANK-L, Bcl2 and global inflammation index) for a metacarpophalangeal joint-synovium biopsy. Baseline and 3-year hand-and-foot X-rays were graded with the van der Heijde-modified-Sharp score; the judgment criterion was its progression during follow-up. Pearson's product moment correlation statistics were used to test for association between paired samples.
*Results:* A baseline, a significant relationship was found between erosive damage and markers of B-cell activation, notably the synovium CD20 expression ($r = 0.68$; $P = 0.0001$). Quantified by the modified-Sharp erosion score variation, the 3-year structural damage progression was significantly correlated with: serum levels of RF-IgG ($r = 0.75$; $P = 0.0003$), -IgM ($r = 0.69$; $P = 0.001$), anti-CCP2 ($r = 0.53$; $P = 0.02$) and RANK-L ($r = 0.61$; $P = 0.007$); synovium CD20 expression ($r = 0.70$; $P = 0.001$).
*Conclusion:* This analysis of the prognostic value of a large panel of synovium markers in a limited sample of prospectively followed, well-documented patients suggested that both synovial CD20 and serum RANK-L levels might be new predictors of structural damage progression in very-early RA.


## 1. Introduction

Rheumatoid arthritis (RA) is the most frequent chronic inflammatory rheumatism [1]. Its prognosis is mostly linked to the appearance of osteocartilage lesions that, if not controlled, lead to rapid degradation of joint function and the patient's quality of life. Prognosis is usually based on the degree of osteocartilage destruction and, especially, by the progression of these lesions over time. It is widely accepted that 70% of RA patients will develop bone erosions during the first 2 years of disease evolution. Benign (~20% of RA) and severe forms, characterized by rapid joint destruction (10 to 15%), exist [2]. It would be useful to have, at the individual level, prognostic markers that would enable us to determine whether or not the patient will suffer from rapidly progressing osteocartilage destruction.

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During the course of RA, the chronic self-sustaining synovitis is characterized by pseudotumoral synovial hyperplasia susceptible of causing osteocartilage lesions. Synovial pannus formation leads to cellular proliferation, resulting from synoviocyte activation by proinflammatory cytokines and the dysregulation of their apoptosis, as manifested by cell-phenotype and function modifications (p53 mutations, over-expression of proto-oncogenes) [3,4].

RA advances through several phases. The initiation phase is characterized by T-cell recognition of an antigen presented by antigen-presenting cells [5,6]. During the maintenance phase, neoangiogenesis [7] is mediated by growth factors, like vascular endothelial growth factor (VEGF) and others, that recruit peripheral blood cells to the rheumatoid synovium [8]. This infiltration of synovial tissue leads to cell interactions with resident synoviocytes that will create the chronic inflammatory environment in which a dysequilibrium exists between proinflammatory cytokines, like interleukin-1$\beta$ (IL1$\beta$), tumor necrosis factor-alpha (TNF$\alpha$), IL6 and IL15, and anti-inflammatory cytokines, such as soluble TNF$\alpha$ receptors and IL1-receptor antagonist [9,10]. Osteocartilage destruction results from the production of matrix metalloproteinases (MMP) [11] and the activation of the receptor activator of nuclear $\kappa$B (RANK) or its ligand (RANK-L) [12]. Thus, this process is characterized, in the synovium, by the intervention of different cellular and molecular actors (notably cytokines) leading to the typical osteocartilage destruction of RA.

Numerous recent therapeutic advances have improved the prognosis of this destructive rheumatism by slowing the appearance of articular lesions. Hence, beyond making an accurate and early diagnosis, an essential step in RA management is the early prediction of the patient's prognosis at the onset of an inflammatory rheumatism, so as to initiate, as rapidly as possible, an adapted disease-modifying anti-rheumatic drug (DMARD) regimen to block the progression of irreversible lesions.

At the group level, four parameters seem to be able to predict the structural outcome of RA: rheumatoid factors (RFs), anti-cyclic citrullinated autoantibodies (anti-CCP2), bone erosions during the first months of RA progression and a persistent inflammatory syndrome (erythrocyte sedimentation rate at 1st hour [ESR] and/or C-reactive peptide [CRP]) [13–16]. However, these factors cannot predict RA prognosis for the individual, as they cannot correctly classify more than 70% of the patients. It is therefore essential to identify new serum and/or synovial tissue markers susceptible of providing complementary information. In this vein, prognostic values of mediators involved in osteocartilage destruction, notably MMPs [17–19] and the RANK–RANK-L–osteoprotegerin (OPG) relationship [20], have been studied for cartilage, while other authors analyzed molecules reflecting subradiological osteocartilage involvement, like cartilage oligomeric matrix protein (COMP) [14], and degradation products of collagen type II CTXII [21] or bone involvement with those of collagen type I. Those results revealed a relationship between initially elevated levels of CTXII, COMP, MMP3 and/or the OPG/RANK-L ratio, and more rapid or severe radiological progression over the first 1 to 5 years of follow-up, independently of the initial radiological involvement. However, some of those markers, notably MMP3, do not seem to provide more information than ESR and/or CRP. In addition, the contributions of the other markers have not yet been confirmed by other teams. Therefore, evaluation of markers expressed in the target tissue that reflect the pathophysiological mechanisms of RA could prove informative.

We examined synovial tissue from patients with very-early inflammatory rheumatisms, focusing on synovium expression of certain markers reflecting RA pathophysiology and affecting subsequent joint outcome for these patients followed for several years.

Thus, some patients from the VErA cohort of treatment-naïve, very-early, inflammatory rheumatisms, well-documented in terms of clinical, biological and radiological findings, were asked, at inclusion, to undergo a metacarpophalangeal (MCP) joint-synovium biopsy. The main objective was to test for correlations between serum level and/or synovium expression of different markers collected at baseline and the structural damage over a 3-year follow-up period in this population.

## 2. Methods

### 2.1. Patients

Our initial analysis was based on 21 patients who accepted to undergo a metacarpophalangeal synovium joint biopsy, among the 310 enrolled in the VErA cohort [22], who had peripheral rheumatisms characterized by the swelling of greater or equal to two joints, lasting greater or equal to 4 weeks and evolving for less than 6 months, and had not received any systemic DMARDs or glucocorticoids. Between October 1998 and January 2002, these patients were recruited prospectively from two French regions (Upper Normandy and the greater Amiens metropolitan area [population-based recruitment]). To recruit a maximum number of patients and obtain a representative sample, a wide-ranging informative media campaign was conducted. This study concerns only patients followed in Rouen. Only 18 patients had a standardized follow-up. Indeed, three were excluded before 3 years (refusal to participate, moved out of the region, psoriatic arthritis). Their clinical, biological and radiological parameters were recorded at inclusion, then every 6 months. Herein, only their inclusion data and the findings of their MCP synovium biopsies were considered, along with their 3-year radiological scores. An orthopedic surgeon (PYM or IAA) took the biopsies from one MCP joint (the most swollen joint) of each consenting outpatient under local anesthesia. There was no side effect after the biopsy. The study was approved by the Upper Normandy Ethics Committee (French law 88-1138; 20 December 1988; file no. 95/138/HP). All patients gave their informed written consent at the time of inclusion.

### 2.2. Inclusion parameters studied

The clinical parameters recorded at inclusion were demographic information (age, sex), rheumatism duration (defined as the date the first symptoms appeared), joint-pain intensity evaluated with a visual analog scale (range: 0–100 mm), and the numbers of painful and swollen joints among the 44 examined.

Among the biological parameters of these patients evaluated, the following were retained: ESR (mm/1st hour); CRP (mg/L); autoantibodies: RFs detected by agglutination (latex-fixation and Waaler–Rose) tests and their isotypes -IgG, -IgM and -IgA by enzyme-linked immunosorbent assay (Elisa, homemade [FJ, OV, FT]); anti-CCP2 detected with a commercialized kit (Euroimmun®, Groβ Grönau, Germany); HLA-DR alleles; markers of bone and cartilage remodeling: serum RANK-L, OPG and COMP levels determined with Elisa kits (Quidel®, San Diego, CA) (MB).

Synovium biopsies were analyzed by two experienced pathologists (CM, FLL), essentially with a semi-quantitative method [23] that has been shown to be not only easier to use and the fastest, but also assured good reading reproducibility, when used by an experienced observer. It uses a series of grades that reflect the levels of the different markers expressed in the synovium biopsy, thereby enabling grading of all the elements in the sample and determination of an overall inflammation score for each biopsy. Then, we used immunohistochemical labeling to determine the expression levels of different markers reflecting the RA pathophysiological processes involved: HLA-DR, for the activation phase; CD3 and CD20 to identify, respectively, T- and B-lymphocyte infiltrations; CD163 for