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# Levels of chemerin and interleukin 8 in the synovial fluid from patients with inflammatory arthritides and osteoarthritis

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# INTRODUCTION

## OVERVIEW OF PATHOGENESIS OF RHEUMATOID ARTHRITIS AND PSORIATIC ARTHRITIS

**Rheumatoid arthritis** (RA) is a systemic disease in autoimmune pathogenesis, affecting primitively the synovial tissue (ST) of the joints and can have extra-articular manifestations.

The etiology is unknown. The hypothesis is that in a patient with a predisposing genetic makeup, an unknown factor (probably of infectious origin) triggers an autoimmune reaction to the constituents of the self, primarily the ST.

There are several factors that interact with each other, associated with an increased risk of developing RA:

♣ Genetic predisposition: it has been demonstrated that an association exists between RA and antigens of histocompatibility class II HLA-DR4 and DR1. The DR4 positive subjects have a 4-5 times greater risk of contracting the disease than those with negative status [1].

♣ Infectious agents: (streptococci, clostridia, diphtheroids bacteria, mycobacteria, E. coli, mycoplasma) and viruses (including Epstein-Barr virus, but also hepatitis B, varicella, mumps as well as coxsackievirus, adenovirus, papovavirus, reovirus, arbovirus Ross-River, cytomegalovirus, parvovirus, herpes viruses and retroviruses, including HTLV).

♣ Superantigens: different proteins synthesized by streptococci, staphylococci and mycoplasma act as superantigens and are capable of activating T cells with polyclonal mode.

♣ Autoimmunity: an autoimmune phenomenon seems involved in particular in the chronicization of the inflammatory process. The main autoantigens studied are collagen and proteoglycans, resulting from the destruction of the joint cartilage, together with an immunoglobulin of the IgG type, the rheumatoid factor (RF).

♣ Smoking: recent data suggest an increased risk of developing rheumatoid arthritis in smokers HLA-DR4 positive.

A central role in promoting and maintaining joint inflammation is played by the CD4 positive lymphocytes. These cells induce, both through molecules of the surface signal (CD11 and CD69), both through the production of cytokines (IFN-γ and IL-7) the activation of other cells such as monocytes / macrophages, fibroblast-like synoviocytes, dendritic cells, etc..

The TCD4 lymphocytes also stimulate the B cells to produce auto-antibodies, including the rheumatoid factor, a potent inducer of the complement. The rheumatoid factor makes up 70% of the IgM class and binds to synovial IgG immunoglobulins for a defect of glycosylation of the Fc region.

In recent years attention has been placed on citrullinated proteins (fibrin, vimentin and fillagrin) since antibodies directed against the latter (Anti-citrullinated protein antibodies = ACPA) have a great significance from the diagnostic point of view and appear to be associated to the risk of disease and of the forms' aggressiveness.

TCD4 lymphocytes induce the proliferation of osteoclasts, resulting in osteopenia and bone resorption.

The inflammatory process involves an abnormal development of the ST, which in patients with RA is characterized by hyperplasia of synoviocytes, increased vascularization and infiltration by inflammatory cells.

Overall, the T cells amplify immune responses through stimulation of monocytes, fibroblasts, chondrocytes and osteoclasts to release cytokines such as TNF-α, IL-1, IL-6, thus inducing the inflammatory processes at the base of the progressive destruction of articular tissues.

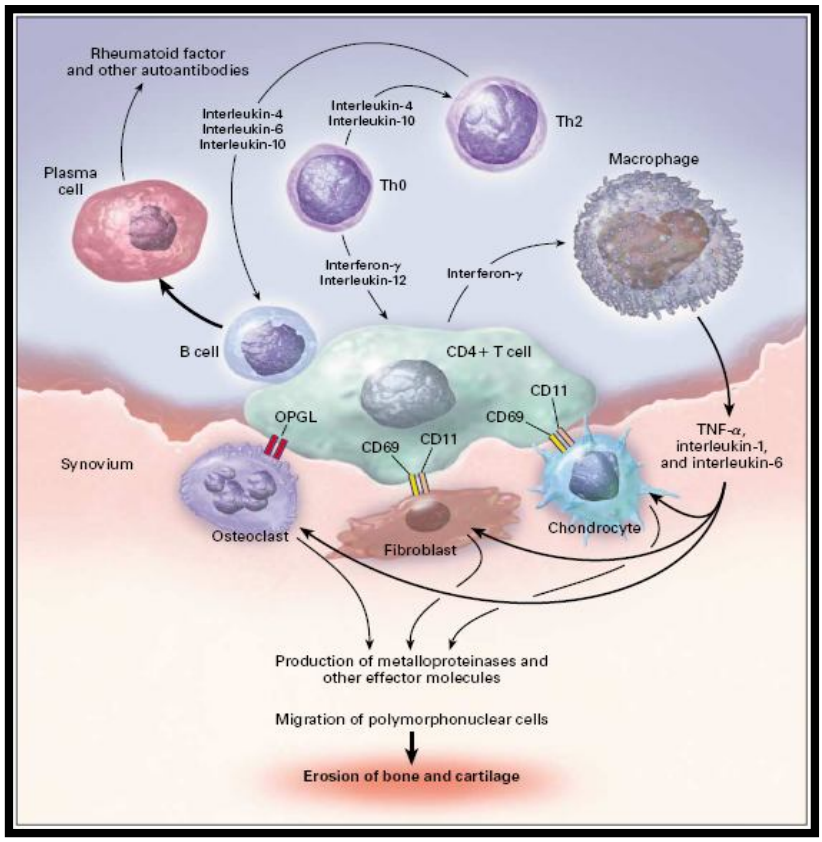



*Figure 1. Cytokine signaling pathways involved in RA [2].*

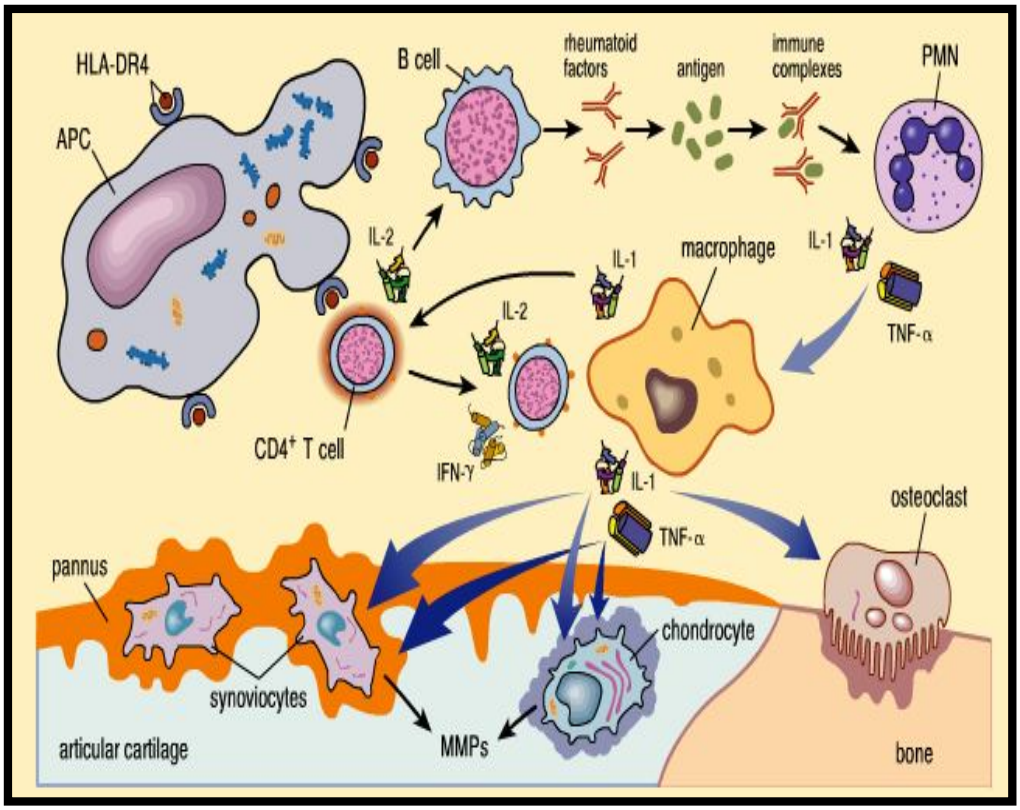



*Figure 2 Pathogenesis of RA: cellular interactions at the level of the ST*

**Psoriatic arthritis** (PsA) is an inflammatory arthropathy associated with psoriasis and is part of the group of seronegative spondyloarthritis. These are represented by a cluster of inflammatory joint diseases characterized by peripheral and / or axial involvement, a significant genetic association, a probable infectious origin and characteristics that differentiate them from RA.

While the main target in RA is the ST, in PsA it is made from enthesis and the ST is involved secondarily.

Even in the case of the PsA immunogenetic studies have found an association with particular HLA alleles, which varies depending on the form of arthritis: in peripheral shape the association with B16, B38 and B39 was highlighted, while in the spondylitic form that of the B27.

Among the factors triggering the disease it has been suggested that cross-reactivity between the components of the self and bacterial antigens (particularly Chlamydia and Klebsiella) is a factor and the micro trauma of the enthesis as predisposing ground.

In recent years, studies have highlighted the possible pathogenetic role of HLA B27 allele. A mechanism of molecular mimicry has been evidenced, which can be explicit or with the propensity of B27 allele to present antigens of the self or with molecular mimicry between bacterial antigens and some sequences of the same HLA B27 [3].

Since there are different subtypes of HLA B27, the latter point would explain why most of the HLA B27 positive subjects do not get sick. Another theory consists in the defect of assembly in the endoplasmic reticulum of the heavy chains of HLA B27 with consequent release of pro-inflammatory cytokines such as TNF-α and IL-23.

Even in PsA the inflammation involves hyperplasia of the ST with increased vascularity and presence of inflammatory infiltrate. In addition to the erosive bone lesions there is, however, an intense reparative reaction, responsible for the abundance of fibrous tissue in the affected sites.

## OVERVIEW OF PATHOGENESIS OF OSTEOARTHRITIS

The pathogenesis of arthritic damage is complex and involves biochemical and mechanical factors.

Under normal conditions the joint cartilage presents visco-elastic properties, thanks to the ability of the extracellular matrix, by means of the negative charges of proteoglycans, to expel and retain water.

In the osteoarthritic process the cartilage progressively loses this function because of the alteration of the integrity of the extracellular matrix (aggrecan and hyaluronic acid) and the dysregulation of interactions between chondrocyte and matrix. The causes of this

process are due both to internal (genetic, inflammatory diseases of the joints) and exogenous factors (obesity, trauma, malalignment), responsible for an increased mechanical stress [4].

While a few years ago, the osteoarthritis (OA) was considered a disease of the cartilage, the latest findings show that the diarthrodial joint has to be regarded as a body composed of different tissues (cartilage, subchondral bone, ST, joint capsule and adjacent soft tissues), each playing a decisive role in the physiopathology of the articular environment.

The alteration of the composition of the cellular matrix involves the suffering of chondrocytes, which are no longer able to synthesize an appropriate matrix, which then in turn loses its damping function, thereby triggering a vicious circle.

In detail the chondrocytes increase the synthesis and secretion of metalloproteases (MMPs) enzymes that degrade the proteoglycans and the collagen of the matrix. Of particular importance is the MMP-13, that preferentially degrades collagen type II. The cytokines produced by chondrocytes and synoviocytes, especially IL-1β and TNF-α, stimulate the synthesis of MMPs and reduce the production of their inhibitors (TIMPs), in addition to stimulating the production of nitric oxide. The latter plays a role in induction of apoptosis of chondrocytes which further helps to reduce the production of extracellular matrix.

Through these biochemical changes comes the imbalance between synthesis and degradation of cartilage, with loss of integrity of the cartilage itself. Moreover, the production of PGE2 by monocytes-macrophages and synovial cells, induced by IL-1 and TNF-α, inhibits the synthesis of collagen and contributes to the vasodilation and pain.

A vicious cycle of cytokine-stimulating catabolic action is thus established leading to a slow and inexorable loss of cartilage tissue. The process also affects the synthesis of hyaluronic acid, which has both qualitative and quantitative changes, thus contributing to the perpetuation of the damage [5].

## CHEMERIN

Chemerin was recently demonstrated to mediate joint inflammation and cartilage degradation. Chemerin is a potent chemoatactic protein for Chemerin Receptor 23 (ChemR23)-expressing cells as macrophages, natural killer and plasmacytoid dendritic cells (pDCs). Chemerin is expressed mainly by epithelial cells and adipocytes, to a minor extent by chondrocytes and fibroblast-like synoviocytes (FLS). It is synthetized as an inactive precursor, then converted into the biologically active form by several proteases from inflammatory and coagulation cascades [6-7]. Neutrophils are capable of promoting maturation of prochemerin to chemerin, thus suggesting that the chemerin/ChemR23 signalling system may serve as a bridge between innate and adaptative immunity.
Recently the chemerin/ChemR23 signalling system was studied also in autoimmune disease.
Some evidence suggests that chemerin and its receptor may have a role in the recruitment of plasmacytoid dendritic cells, an effective antigen presenting cells.
Dendritic cells are capable of regulating the immune and inflammatory response, promoting a broad spectrum of chemokines that regulate leukocyte recruitment to inflammatory site.
In particular the relationship between dendritc and NK cells is established.
Likewise, dendritc cells are able to regulate the isotypic switch of B cells and to promote the activation of neutrophils and NK cells [8].
The first studies about chemerin/ChemR23 axis concerned psoriatic skin lesion. Psoriasis is a type I interferon-driven T-cell-mediated disease characterized by the recruitment of plasmacytoid dendritic cells into the skin. This involves an iperproduction and a incomplete maturation of keratinocytes, associated with angiogenesis of dermal vessels.

Some studies show that chemerin expression marks the early phases of psoriatic lesions and strongly parallels pDCs and neutrophil dermal infiltration [9-10].

The study of expression of chemerin in inflammatory arthritis has recently attracted great interest, in particular concerning the pathogenetic aspect.

As chemerin is an important chemotactic factor for dendritic cells, early studies have focused on the expression of these cells in inflammatory arthritis.

A study evaluated the expression of plasmocytoid dendritic cells in synovial fluid (SF) and ST of patients with RA, PsA and OA. Dendritic cells were observed more frequently in SF in RA and PsA compared to OA [11].

Subsequently other studies tried to determine chemerin in the SF of inflammatory arthritis and OA by ELISA and to detect the presence of chemerin on the ST of RA by means of immunohistochemical techniques.

The study of Kaneko of 2011 has analyzed the expression of chemerin and its receptor ChemR23 on ST in patients with RA and the effect of chemerin on fibroblast-like synoviocytes.

Chemerin values and its receptor were expressed more in patients with RA compared with controls. Chemerin seemed to be able also to promote the motility of fibroblasts, one of the most important pathogenetic processes in the development of synovial pannus [12].

A study of K.Eisenger in 2010 showed how chemerin and its receptor are also present on chondrocytes. The stimulation by chemerin resulted in an increase of production of inflammatory cytokines, such as IL-6, IL-8, TNF-α, IL-1β and metalloproteases [13], by the chondrocyte activated. At higher concentration, chemerin induced MMP-2, MMP-3, MMP-13 and IL-8 [14].

The study of Ke Huang of 2011, on 124 subjects suffering from OA, has found that the levels of chemerin in SF were directly correlated with the radiographic grade [15].

**IL-8**

IL-8 belongs to the 'superfamily of chemokines', which consists of more than 40 different molecules. IL-8 belongs more precisely to the subfamily of CXC chemokines, molecules with regulatory function on the migration of various types of leukocytes and on the recruitment of hematopoietic progenitor cells and lymphocytes.

The sequence Glu-Leu-Arg (ELR), and the N-terminal region of the IL-8 are crucial for its binding to the receptors, for the action chemo-tactic expressed towards the neutrophils and for angiogenic activity.

IL-8 is secreted by many types of cells, including monocytes, lymphocytes, granulocytes, fibroblasts, endothelial cells, bronchial epithelial cells, keratinocytes, hepatocytes, mesangial cells and chondrocytes. The inhibition of IL-8 by the addition of specific antibodies or the interruption of the gene encoding for the receptor IL-8 drastically reduces the infiltration of neutrophils in tissues during acute inflammation.

IL-8 plays a primary role in acute inflammation through the recruitment and activation of neutrophils.

IL-8 also induces infiltration of T cells in the inflammatory site. The injection of IL-8 causes a massive migration of T cells in the joints. In a reaction of delayed hypersensitivity monoclonal antibodies anti-IL-8 have shown the reduction of the infiltration of neutrophils and lymphocytes.

These results suggest that IL-8 plays a significant role in regulating the migration of T cells in the inflammatory process.

Nowadays, IL-8 is regarded as a pivotal player in angiogenesis one of the key-mechanisms for maintenance and perpetuation of chronic synovial inflammation [16-17]. There is much evidence that RA is closely linked to angiogenesis.

Toll-like receptors (TLRs) comprise a family of germline-encoded type I transmembrane proteins that enable recognition of pathogen-associated molecular patterns (PAMPs) by the innate immune system.

Although only a few studies have investigated the roles of TLRs in RA pathogenesis, they are likely to have complex roles in this disease. TLR2, TLR3, TLR4, and TLR7 are highly expressed in the RA synovium [8]. In fibroblast-like synoviocytes from RA patients, IL-8 expression was induced upon activation of Toll-like receptors TLR2 and TLR3, two innate immunity receptors involved in inflammation [18].

# MATERIALS and METHODS

## OBJECTIVES

The study of the pathogenesis of arthropathies is evolving.
Assessing the concentration in the SF of potential pathogenetic mediators may supply further insights into the pathogenesis of arthropathies.
Among the molecules with a key role in inflammation, chemerin and IL-8 play a leading role.
Therefore, the aim of this study was to investigate chemerin and IL-8 levels in the SF from patients with inflammatory arthritides (RA and PsA), and OA.
In detail, the main objective of the study was to determine the concentrations of chemerin in the SF of patients with inflammatory arthritis (RA, PsA), and OA, correlating them with clinical and radiological data and comparing the data obtained with those of IL-8.
The second objective was to determine chemerin of ST of patients with RA.

## PATIENTS

In this study, 19 patients with a diagnosis of inflammatory arthritis and 8 with OA were recruited.
SF samples were obtained when an arthrocentesis was performed because of severe knee synovitis requiring joint evacuation.
All 27 patients were diagnosed according to the criteria of the American College of Rheumatology [19-20-21].
Table 1 reports the clinical characteristics of the enrolled patients.

Clinical data including age, duration of disease, VAS pain, DAS 28 (in RA and PsA) and radiographic grade (Kellegren's classification system, [22]) were collected at the time of arthrocentesis.

| | RA (n=14) median (IQR) | PsA (n=5) median (IQR) | OA (n=8) median (IQR) |
|---|---|---|---|
| Age (years) | 68 (52.5-76.5) | 46.5 (40.75-59.75) | 73 (70.25-78.75) |
| Duration of disease (months) | 78 (51-120) | 60 (45-72) | 108 (90-114) |
| Kellgren's radiographic grade | 3 | 3 | 3 |
| VAS pain | 60 (40-70) | 55 (47.5-60) | 60 (52.5-60) |
| DAS 28 | 2.8 (2.75-2.82) | 3.6 (2.6-4) | / |

**Table 1.** Clinical characteristics of the 27 patients recruited in our study.

## CHEMOKINE ASSAYS

The SF levels of chemerin and IL-8 were determined by a home-made ELISA as follows: 96-hole plates previously treated with a primary specific coating for chemerin or IL-8 were kept at room temperature for one night; the plates were rinsed (3x) with wash buffer (PBS+0.05%Tween-20), fixed with PBS+1% bovine albumin for 1 hour then rinsed again (3x) with wash buffer.

After addition of the samples, the plates were kept at room temperature for 2 hours, rinsed (3x) with wash buffer.

A specific antibody conjugated with biotin for either chemerin or IL-8 was added, the plates were incubated for 2 hours. After washing to remove unbound antibodies, streptavidin conjugated with peroxidase was added and the plates incubated for 20 minutes.

The reaction was stopped by a $H_2SO_4$ solution 1M; samples were read at 450 nm. A curve was drawn plotting the concentrations of standard

dilutions against their absorbances; the concentrations in the samples were determined by the relevant absorbance values.
The concentrations of the two cytokines (ng/ml) were then normalized to total SF proteins (Bio-Rad Protein assay).

## IMMUNOHISTOCHEMICAL STUDIES

ST samples for immunohystochemical analysis were obtained from 4 RA patients who underwent knee synovectomy.
Sections were incubated with monoclonal antibodies against chemerin (IgG1 clone 14G10; 1:40 dilution) and its main receptor ChemR23 (IgG2b clone 4C7; 1:20 dilution). Secondary biotinylated monoclonal antibodies and staining kits were obtained from Vector Laboratories.
Double immunostaining was developed using the avidin-biotin peroxidase or avidin-biotin-alkaline phosphatase system and the chromogens 3-amino-9-ethyl-carbazole and Blue Vector (Vector Laboratories).

## VAS PAIN (VISUAL ANALOGIC SCALE)

The VAS visually represents the amount of pain that a patient feels.
It consists of a line, usually 10 cm long, in which one end indicates the absence of pain, while the other represents the maximum pain.
The scale is completed by the patient who is asked to draw a sign on the line that represents the level of pain experienced. The distance measured in millimeters is a measure of perceived pain.


Absence of pain Scale of pain Maximum pain

**DAS 28**

The DAS-28 (Disease Activity Score for 28 Joints) is a method used to assess disease activity of inflammatory arthritis, developed within the European League Against Rheumatism (EULAR).

It takes into account four variables:

♣ Number of swollen joints of 28 evaluated.

♣ Number of tender joints on 28 evaluated

♣ ESR (erythrocyte sedimentation rate)

♣ GH: general health on visual analogue scale VAS from 0 to 100.

Formula:

DAS-28 = 0.56 * √ (t28) + 0.28 * √ (sw28) + 0.70 * Ln (ESR) + 0.014 * GH

t28 = tender joints count of 28

sw28 = swollen joints count of 28

Ln (ESR) = natural logarithm of the ESR (mm / hr)

GH = overall health (VAS 0-100)

| **Disease activity** |
|---|
| High: > 5,1 |
| Moderate: > 3,2 |
| Low: ≤ 3,2 |
| Remission: ≤ 2,6 |

## RADIOGRAPHIC GRADE

For the radiographic classification, reference was made to the grading system of Kellgren-Lawrence.

| |
|---|
| Grade 0: no OA changes |
| Grade 1: doubtful narrowing of joint space and minute formation of osteophytes |
| Grade 2: minor alterations, defined formation of osteophytes and possible joint space narrowing |
| Grade 3: moderate multiple formations of osteophytes, defined joint space narrowing and some bone sclerosis |
| Grade 4: severe narrowing of the joint space with marked sclerosis, defined bone deformation, large osteophytes |

## STATISTICAL ANALYSIS

Mann-Whitney and Kruskal-Wallis tests were used to compare chemerin and IL-8 levels between subgroups based upon diagnosis gender and radiological grade.

Associations between chemerin and IL-8 levels with several clinical variables were determined by Spearman's coefficient (r), univariate and multivariate linear regression analyses.

Statistical analysis was performed with STATA-10, $p \leq 0.05$ were considered statistically significant.

Continuous variables were expressed as median values (interquartile range, IQR).

# RESULTS

Of the 27 patients recruited in this study, 14 patients were diagnosed with RA, 5 with PsA and 8 with OA.

Demographic and clinical characteristics and the SF levels of chemerin and IL-8 are are enlisted in Table 1.

None of the patients suffering from inflammatory arthridites was on biological therapy; 10/14 RA patients were on methotrexate (15mg/week) and hydroxychloroquine (400mg/day) and 4/14 on leflunomide (20mg/day) and hydroxychloroquine (400mg/day), all were on corticosteroid treatment (<5mg/die). PsA patients were all on salazopirine (3gr/day), the OA subjects on non-steroid inflammatory agents only.

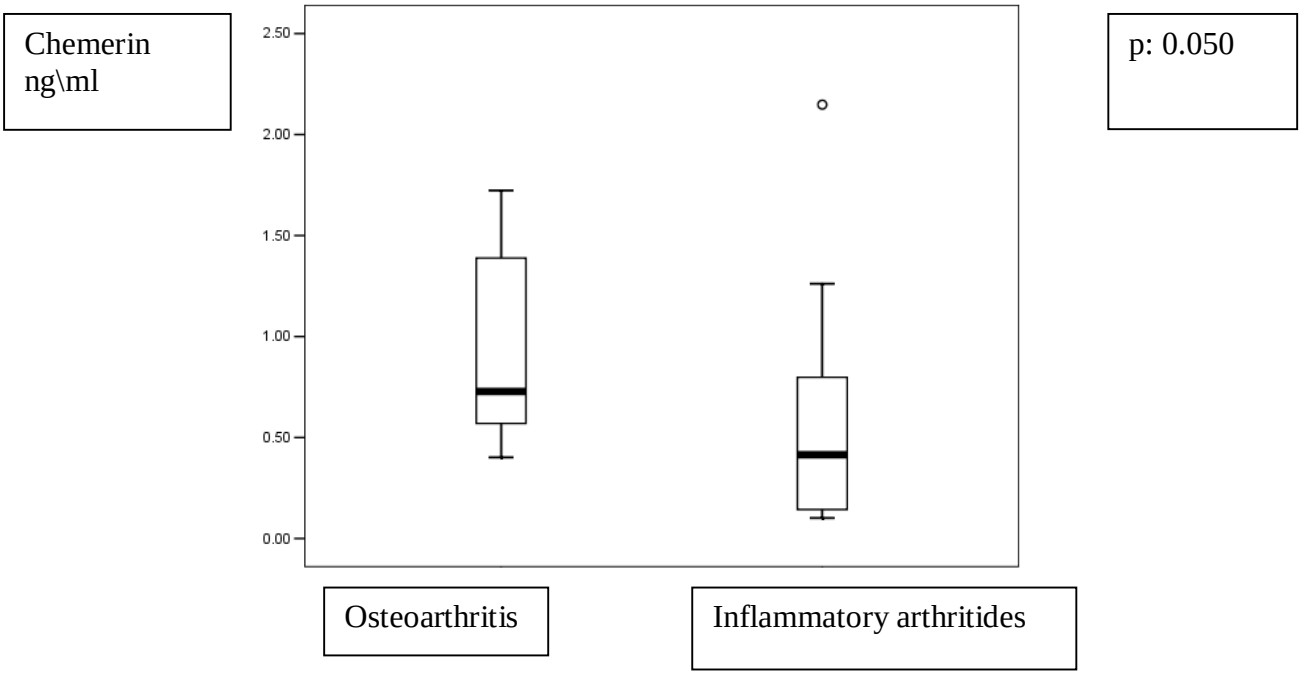
In our cohort, no significant difference was observed in the SF concentration of chemerin between patients with RA, PsA and OA (p=0.1267) (Graphic I and Table 2).

No differences either separately considering RA and PsA (with Kruskal-Wallis) were revealed.

Patients with more severe radiographic damage (Class IV) displayed higher chemerin levels compared to those with less pronounced radiologic damage (Class II and III, p=0.05). Moreover, at linear regression analysis there was a trend towards statistical significance for the radiographic grade to predict chemerin (p=0.07).

No significant association was detected between chemerin and clinical parameters such as gender, age (r=0.3461, p=0.1057), disease duration (r=0.3811, p=0.0974), VAS pain (r=0.2737, p=0.2299) and DAS28 (r=0.1683, p=0.5487).

No difference in chemerin levels emerged among subgroups based on gender (p=0.656) and treatment (p=0.104).



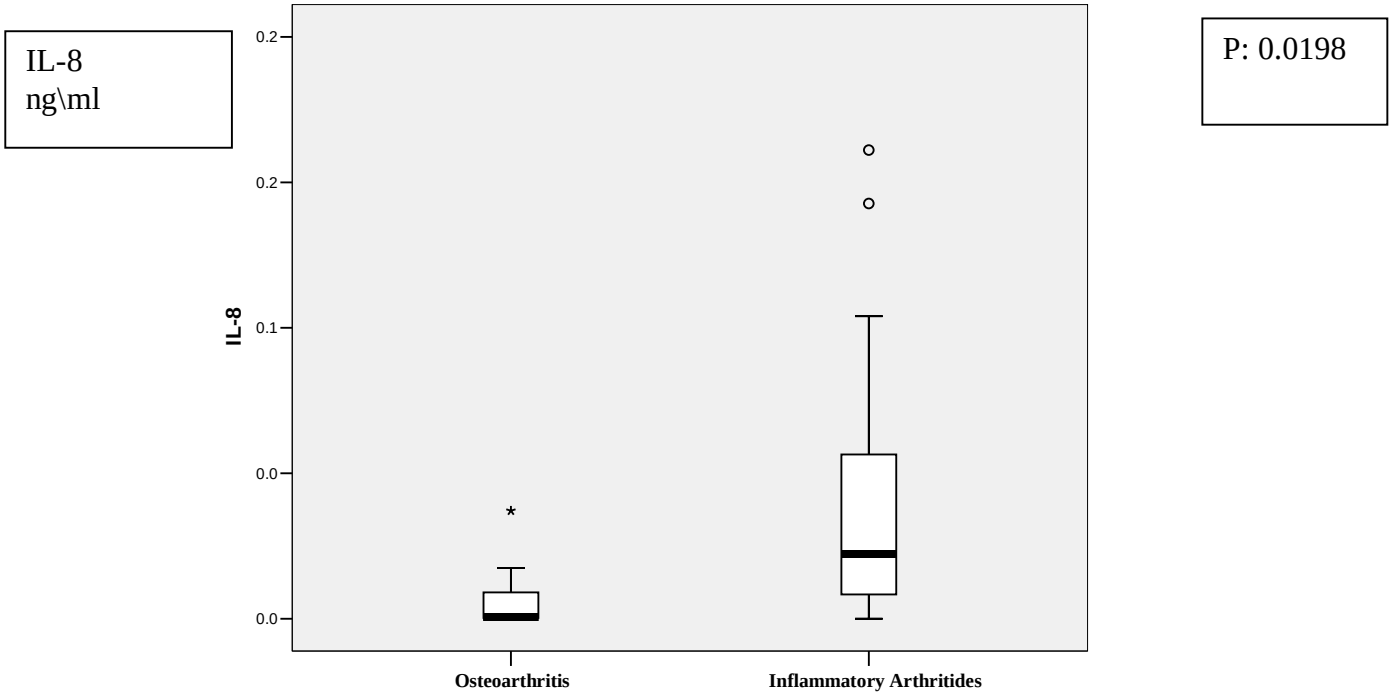

**Graphic I** Box-plot of chemerin levels in the SF of patients with inflammatory arthritides (RA and PsA) and OA. Chemerin concentration (ng/ml) was normalized to the concentration of total proteins in the SF.

Conversely, patients with inflammatory arthritides presented significantly higher levels of IL-8 SF compared to OA subjects (p=0.0198, Graphic II and Table 2).

No significant difference in IL-8 levels was found in the SF from patients with RA and PsA (p=0.307). A significant correlation between the SF concentration of IL-8 and VAS pain was detected (r=0.433, p=0.0495) Graphic III.

At multivariate linear regression analysis, IL-8 levels could be significantly predicted by VAS pain and radiographic grade (p=0.033). IL-8 levels were found not to correlate with any of the other clinical variables as age (r=0.1454, p=0.5080), disease duration (r=0.3083, p=0.1859), DAS 28 (r=0.3421, p=0.2120) and radiographic grade (p=0.889). No difference in IL-8 levels emerged among subgroups based on treatment (p=0.097).

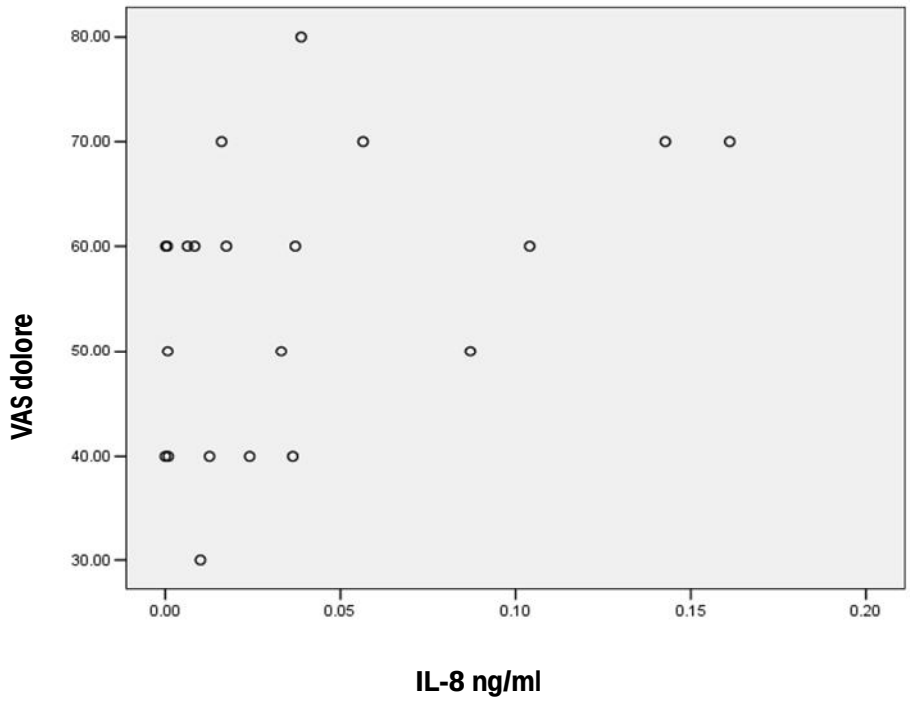
No association could be detected between chemerin and IL-8 SF levels (r=0.1085, p=0.6057).




**Graphic II** Box-plot of IL-8 levels in the SF of patients with inflammatory arthritides (RA and PsA) and OA. IL-8 concentration (ng/ml) was normalized to the concentration of total proteins in the SF.

| | RA (n=14) median (IQR) | PsA (n=5) median (IQR) | OA (n=8) median (IQR) |
|---|---|---|---|
| Chemerin # | 0.3168 (0.1436-0.8413) | 0.6269 (0.2228-0.7396) | 0.7277 (0.5695-1.3886) |
| IL-8 # | 0.0240 (0.0126-0.0387) | 0.0063 (0.0008-0.0870) | 0.0005 (0.0002-0.0090) |

**Table 2.** Levels of chemerin and IL-8 in the SF of the 27 patients recruited in our study.
# Values are expressed as the ratio between the concentration (ng/ml) of the chemokines and the total SF proteins.




**Graphic III** – Correlation between IL-8 and VAS pain.

On sections, chemerin reactivity was detected in all 4 synovial biopsies from RA patients in form of cytoplasmic dots in endothelial cells (Figure 3).

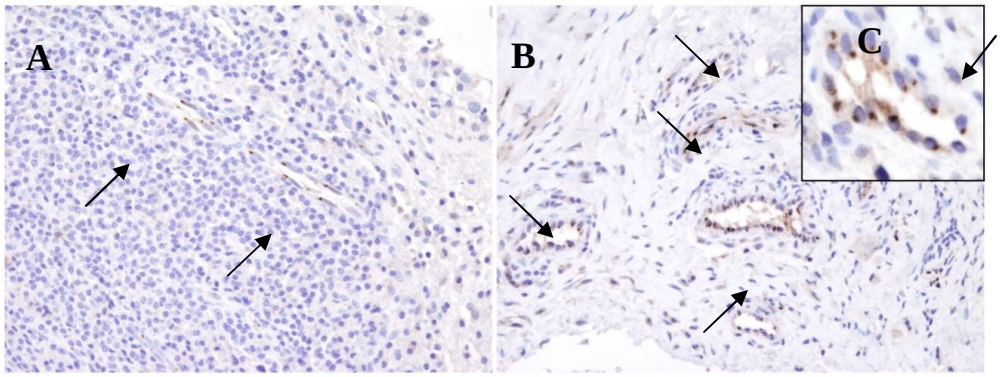



**Figure 3**. Chemerin expression in a ST obtained from a patient with RA as detected by immunochemistry. Sections are counterstained with Meyer's hematoxylin. Original magnification 200x (A and B) and 600x (C).

# CONCLUSIONS

To our knowledge, this is the second study investigating chemerin SF levels in patients with RA, PsA and OA.

In agreement with the previous study, we could not observe any significant difference in SF levels of the pro-inflammatory mediator chemerin among the three subgroups of patients [13].

Chemerin is indeed thought to contribute to chronic synovitis by recruiting inflammatory cells, promoting endothelial cell proliferation and inducing MMPs production. Therefore, our finding is consistent with the emerging concept of OA as an inflammatory disorder and not a merely degenerative disease [5].

It is known that chemerin is activated by proteolytic enzymes; these enzymes increase under conditions of high inflammation.

It should be noted that in our study SF samples were collected in case of severe joint effusion: the acute inflammatory event may have contributed to an increase in chemerin levels.

Moreover, chemerin belongs to the wide family of adipokynes, mediators previously described to exert a pathogenetic role in cartilage degradation [23].

Recent studies have highlighted the role of some adipokines in OA. In particular, it has been suggested a critical metabolic link between obesity and OA, which further strengthens the hypothesis of a metabolic component in OA.

We then confirmed a positive association between chemerin levels and radiographic damage, as already pointed out by Huang [15]. At this regard, it should be considered that our cohort of patients presented a quite severe joint damage.

Similarly, we are the second group investigating IL-8 SF levels from patients with RA, PsA and OA.

IL-8 was chosen to compare the results obtained by chemerin with those of a cytokine pro inflammatory characterized by important pro-angiogenic activity.

In addition, a previous study [14] noted that cultures of chondrocytes stimulated by chemerin showed an increase of proinflammatory cytokines and in particular of IL-6 and IL-8.

Noteworthy, IL-8 SF levels were significantly higher among patients with inflammatory arthritides compared to those with OA. This finding is only partially concordant with what reported by Bertazzolo, who described significantly higher IL-8 levels in the SF from RA patients compared to both OA and PsA subjects [24].

Conversely, in our cohort there was no significant difference in IL-8 levels between RA and PsA. This finding fits well with the histological evidence of abundant synovial hypervascularization described in both RA and PsA, whereas in OA neoangiogenesis is a less prominent finding.

Noteworthy, IL-8 could be predicted by radiographic grade and VAS pain. To this regard, it is well known that, although neovascularization is observed even in the earliest phase, the density of new vessels is significantly increased in patients with more advanced disease.

Radiographic damage could be regarded as a marker of disease damage; moreover, it is well documented that hypoxia, a strong inducer of IL-8 secretion, contributes to the pathogenesis of pain by sensitizing sensory nerves.

This study presents some limitations. Immunochemistry was performed on a limited number of RA synovial tissue samples; unfortunately no bioptic samples from PsA and OA patients were available. Therefore, although chemerin expression in RA synovial specimens is in line with its detection in RA synovial fluid samples, no definitive conclusions can be drawn because of the lack of data from PsA and OA synovial tissue samples.

As a whole, our findings suggest that the SF levels of chemerin do not allow to distinguish between conditions as RA, PsA and OA, supporting a role for chemerin in the pathogenesis of all the three diseases. On the other hand, IL-8 is significantly increased in the SF from patients with inflammatory arthritides, suggesting its selective involvement in these conditions.

It is therefore tempting to postulate that disease-specific factors may contribute to a differential modulation of inflammatory response leading to a selective recruitment of downstream mediators.

Future studies are warranted to better define the role of these chemokines in the pathogenesis of different arthropathies.

# REFERENCES


1. Choy E., Panayi G.S., Cope A.P. (2001). Cytokine pathways and joint inflammation in rheumatoid arthritis. *N. Engl. J. Med.* 344: 907-916.

2. McInnes I.B., Schett G. (2007). Cytokines in the pathogenesis of rheumatoid arthritis. *Nature* 7:429-442.

3. Haroon N. et al. (2010) - Endoplasmic reticulum aminopeptidases: biology and pathogenic potential *Nat. Rev. Rheumatol.*; 6: 461-467.

4. Crow M.K. et al. (2009). Local cytokine profiles in knee osteoarthritis: elevated synovial fluid interleukin-15 differentiates early from end-stages disease. *Osteoarthritis and Cartilage* 17: 1040-1048.

5. Scanzello C. et al. (2008). Innate immune system activation in osteoarthritis: is osteoarthritis a chronic wound? *Current opinion in Rheumatology* 20: 565-572.

6. Parmentier M. et al. (2011). Chemerin and its receptors in leukocyte trafficking, inflammation and metabolism. *Cytokine and Growth factor review* 22: 331-338.

7. Sozzani et al. (2010) Trafficking properties of plasmacytoid dendritic cells in health and disease *Trends in Immunology*, 31(7):270-277.

8. Sozzani S. et al. (2005). Role of ChemR23 in directing the migration of myeloid and plasmacytoid dendritic cells to lymphoid organs and inflamed skin. *The Journal of experimental Medicine* 201: 509-515.

9. Brewer J.M. et al. (2009). Plasmacytoid dendritic cells regulate breach of self-tolerance in autoimmune arthritis. *The Journal of immunology* 182: 963-968.

10. Sozzani S. et al. (2009). Chemerin expression marks early psoriatic skin lesions and correlates with plasmacytoid dendritic cell recruitment. *The Journal of experimental Medicine* 206: 249-258.

11. Lande R. – et al. (2004). Characterization and Recruitment of Plasmacytoid dendritic cells in Synovial Fluid and Tissues of Patients with Chronic Inflammatory Arthritis. *The Journal of immunology* 173: 2815-2824.

12. Kaneko et al. (2011). Chemerin activated fibroblast-like synoviocytes in patients with rheumatoid arthritis. *Arthritis research and therapy* 13:r158.

13. Eisenger K. et al. (2012). Chemerin induces CCL2 and TLR4 in synovial fibroblast of patients with rheumatoid arthritis and osteoarthritis. *Experimental and molecular pathology* 92: 90-96.

14. Berg V. et al. (2010). Human articular condrocytes express ChemR23 and chemerin; ChemR23 promotes inflammatory signaling upon binding the ligand chemerin. *Arthritis research and Therapy* 12:R228.

15. Huang K. et al. (2011). Association of chemerin levels in synovial fluid with thw severity of knee osteoarthritis. *Biomarkers* , 1-5.

16. Sozzani S. et al. (2007). Dendritic cell–endothelial cell cross-talk in angiogenesis *Trends in Immunology;* 28(9): 385-392.

17. Maruotti et al. (2006). Angiogenesis in rheumatoid arthritis. *Histol. Histopathol.* 21(5): 557-66.

18. Cho M.L. et al. (2007) Toll-like receptor 2 ligand mediates the upregulation of angiogenic factor, vascular endothelial growth factor and interleukin-8/CXCL8 in human rheumatoid synovial fibroblasts. *Immunology Letter*;108:121-128

19. Leather D. et al. (2010) Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann. Rheum. Dis;* 69: 1580-1588.

20. Forestier R. et al. (2011) Diagnostic criteria for generalized osteoarthritis: a preliminary study in a population with knee osteoarthritis. *Joint Bone Spine*; 78: 424-426.

21. Taylor W. et al. (2006): Classification criteria for psoriatic arthritis: development of new criteria from a large international study. *Arthritis Rheum.*; 54: 2665-2673

22. Kellgren J.H., Lawrence J.S. (1957). Radiological assessment of osteoarthritis. *Ann. Rheum. Disease* 16:494-512.

23. Zhuo Q. et al. (2012) Metabolic syndrome meets osteoarthritis. *Nat Rev Rheumatol* ; 10.1038

24. Bertazzolo N. et al (1994) Interrelationships between interleukin IL-1, IL-6 and IL-8 in synovial fluid of various arthropaties. *Agent Actions*; 41: 90-92