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

E. Valcamonica[1,2], C.B. Chighizola[3,4], D. Comi[1], O. De Lucia[1], L. Pisoni[1], A. Murgo[1], V. Salvi[5], S. Sozzani[5,6], P.L. Meroni[1,3,4]

[1]Division of Rheumatology, Istituto G. Pini, Milan, Italy;
[2]Doctorate Course in Genetics, Oncology and Clinical Medicine, University of Siena, Siena, Italy;
[3]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy;
[4]Immunorheumatological Research Laboratory, Istituto Auxologico Italiano, Milan, Italy:
[5]Department of Molecular and Translation Medicine, University of Brescia, Brescia, Italy;
[6]Humanitas Clinical and Research Center, Rozzano, Milan, Italy.

Abstract

Objective

Chemerin and interleukin (IL)-8 are pro-inflammatory mediators whose role in joint inflammation and cartilage degradation has been demonstrated in in-vitro findings. Studies on their presence in synovial fluid (SF) samples may offer further information on their pathogenic role. The aim of this study was to investigate SF chemerin and IL-8 levels in patients with different joint diseases.

Methods

37 patients were enrolled: 18 with rheumatoid arthritis (RA), 8 with psoriatic arthritis (PsA) and 11 with osteoarthritis (OA). 41 SF samples were obtained by arthrocentesis in case of knee synovitis. Serum samples were obtained from 13 patients (4 with RA, 6 with PsA and 3 with OA) at the time of arthrocentesis. Chemerin, IL-8, TNF-α and IL-6 levels were measured using commercially available ELISA kits. Immunohistochemical analysis of synovial RA specimens was also performed.

Results

No difference in chemerin SF levels emerged between patients with immune-mediated inflammatory arthritides and those with OA (p=0.0656), while subjects with inflammatory arthritis displayed significantly higher levels of SF IL-8 compared to OA (p=0.0020). No significant difference emerged across the three conditions in the serum levels of both chemerin and IL-8. IL-8 strongly correlated with inflammatory markers as ESR, CRP, IL-6 and TNF-α.

Conclusions

We observed similar chemerin SF and serum levels in the three conditions. Although flawed by some limitations, our findings support the emerging concept of OA as an inflammatory disorder. However the increased IL-8 levels we described in patients with inflammatory arthritis suggest a selective involvement of this pro-inflammatory and angiogenic cytokine in these conditions.

Key words

chemerin, interleukin-8, synovial fluid, rheumatoid arthritis, psoriatic arthritis, osteoarthritis

*Elisabetta Valcamonica, MD**
*Cecilia B. Chighizola, MD**
Daniela Comi, MD
Orazio De Lucia, PhD
Laura Pisoni, MD
Antonella Murgo, MD
Valentina Salvi, PhD
Silvano Sozzani, PhD
Pier Luigi Meroni, MD

**These authors made an equal contribution to this study.*

Please address correspondence to:
Pier Luigi Meroni, MD,
Division of Rheumatology,
Istituto G. Pini,
Pzza. C. Ferrari 1,
20122 Milan, Italy.
E-mail: pierluigi.meroni@unimi.it

Received on November 3, 2013; accepted in revised form on November 12, 2013.

Funding: C.B. Chighizola is supported by by a Research Grant co-financed by University of Milan and Dote Ricerca: FSE, Regione Lombardia;
V. Salvi is the recipient of a fellowship from FIRC (Fondazione Italiana per la Ricerca sul Cancro).
This work was supported by IMI JU-funded project BeTheCure.

Competing interests: none declared.

Introduction

Major achievements in understanding the pathogenesis of arthropathies have recently been made; however, there is still much to unravel. It was recently demonstrated that the adipokine chemerin mediates joint inflammation and cartilage degradation. Initially thought to be restricted to metabolic activities, chemerin was later shown to be a potent chemotactic protein for Chemerin Receptor 23 (ChemR23)-expressing cells as macrophages, natural killer and plasmacytoid dendritic cells (1). Chemerin is expressed mainly by adipocytes and epithelial cells and, to a minor extent, by chondrocytes and fibroblast-like synoviocytes (FLS) (1, 2). It is synthetised as an inactive precursor, then converted into the biologically active form by several proteases from inflammatory and coagulation cascades (3). Chemerin expression is upregulated by lipopolysaccharide (LPS) and tumour necrosis factor (TNF)-α; serum levels of chemerin were shown to be associated with inflammatory markers such as TNF-α, interleukin (IL)-6 and C-reactive protein (CRP) (4). In FLS from patients with rheumatoid arthritis (RA), the pro-inflammatory and stimulatory effects of chemerin were mediated by the activation of ERK1/2, p38MAPK and Akt (2). Moreover, in human articular chondrocytes chemerin has been shown to increase the production of TNF-α, IL-6, IL-1β, metalloproteinases (MMP)-1 and MMP-8. At higher concentrations, chemerin induces MMP-2, MMP-3, MMP-13 and IL-8 (5). IL-8 is a pro-inflammatory C-X-C chemokine previously characterised as the principal agent of the recruitment and activation of neutrophils. In the synovium, IL-8 is constitutively secreted by synovial macrophages while FLS produce IL-8 only in the presence of agonists such as IL-1α, IL-1β, TNF-α and LPS (6). More precisely, IL-8 expression in FLS from RA patients is induced upon activation of Toll-like receptor (TLR) 2 and TLR3, two innate immunity receptors involved in inflammation (7, 8). In animal models, IL-8 injected intra-articularly was shown to induce joint inflammation with synovial histological changes similar to those observed in RA patients (9). IL-8 has also been identified as a pivotal player in angiogenesis, one of the key-mechanisms for the maintenance and perpetuation of chronic synovial inflammation (10, 11). Assessing the concentration in the synovial fluid (SF) of potential pathogenetic mediators may supply further insights into the etiopathogenesis of arthropathies. Therefore, the aim of this study was to investigate chemerin and IL-8 levels in the SF of patients with immune-mediated inflammatory arthritides (RA and psoriatic arthritis, PsA) and osteoarthritis (OA). To better ascertain the local *versus* systemic production of the study cytokines, we also assessed serum chemerin and IL-8 levels. In addition, chemerin and IL-8 were compared to IL-6 and TNF-α, two well-established mediators of joint inflammation.

Materials and methods

Patients

Twenty-six patients with a diagnosis of immune-mediated inflammatory arthritis and 11 with OA were recruited in this study. All 37 patients were diagnosed according to the criteria of the American College of Rheumatology (12-14). SF samples were obtained when an arthrocentesis was performed because of knee synovitis. Four patients (2 with AR, 1 with PsA and 1 with OA) underwent knee arthrocentesis two times six months apart. Clinical data including age, BMI, disease duration, VAS pain, DAS28 (in RA and PsA) and radiographic grade (Kellegren's classification system, (15)) were collected at the time of arthrocentesis. In addition, serum samples were obtained from 13 patients (4 with AR, 6 with PsA and 3 with OA) at the time of arthrocentesis.

Protein assays

The SF and serum levels of chemerin, IL-8, IL-6 and TNF-α were determined by commercially available enzyme-linked immunosorbent assays (ELISAs, R&D Systems, Minneapolis, MN, USA), following manufacturer's instructions. The total protein contents of SF and serum samples (mg/ml) were measured using Bio-Rad Protein assay (Bio-Rad Laboratories, Hercules, CA,

USA), according to manufacturer's instructions. Experiments were all run in triplicates. The SF and serum concentrations of the four cytokines (ng/ml) were then normalised to SF and serum total protein contents respectively and expressed as ng/mg. Chemerin and IL-8 were measured in 41 samples obtained from 37 patients while TNF-α and IL-6 were assessed in 37 samples.

Immunohistochemistry

Synovial tissues for immunohistochemical analysis were obtained from 4 RA patients who underwent knee synovectomy. Tissues were formalin-fixed and paraffin embedded. Sections were incubated with monoclonal antibodies against chemerin (IgG1 clone 14G10; 1:250 dilution) and revealed by Novolink polymer (Leica), followed by DAB as chromogen.

Statistical analysis

Mann-Whitney and Kruskal-Wallis tests were used to compare chemerin, IL-8, TNF-α and IL-6 SF levels in a cohort of 37 patients (41 knees) subgrouped upon diagnosis (RA, PsA and OA), gender and radiological grade (Kellegren's grade II, III and IV). Mann-Whitney and Kruskal-Wallis tests were also used to compare serum levels of chemerin, IL-8, TNF-α and IL-6 levels in a cohort of 13 patients (13 knees) subgrouped upon diagnosis, gender and radiological grade. Associations between SF and serum cytokine levels with demographic, clinical and biochemical variables were determined by Spearman's coefficient (r). SF and serum levels of the four study cytokines normalised to the total SF/serum protein contents were compared using a matched-pair Mann-Whitney test. Univariate, bivariate and multivariate logistic regression analyses were drawn to investigate the relationship between SF and serum levels of the four study cytokines and diagnosis (defined as immune-mediated inflammatory arthritides versus OA). 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 37 subjects recruited in this study, 18 patients were diagnosed with RA, 8 with PsA and 11 with OA. Ten subjects (27%) were of male gender. Demographic and clinical characteristics, biochemical variables and the SF levels of chemerin, IL-8, IL-6 and TNF-α in the cohort of 37 patients are enlisted in Table I. None of the patients with immune-mediated inflammatory arthridites was on biological therapy; 10/18 RA patients were on methotrexate (15 mg/week) and hydroxychloroquine (400 mg/day) and 8/18 on leflunomide (20 mg/day) and hydroxychloroquine (400 mg/day); all were on corticosteroid treatment (<5 mg/die). PsA patients were all on salazopirine (3 gr/day), the OA subjects on non-steroidal inflammatory agents only.

Of the 13 subjects whose serum and SF samples were collected, 4 patients were diagnosed with RA, 6 with PsA and 3 with OA. 9 subjects (69%) were of female gender. Table II reports the demographic and clinical characteristics, the biochemical variables and the serum and SF levels of chemerin, IL-8, IL-6 and TNF-α of the 13 patients.

Demographic, clinical and biochemical variables of 37 patients subgrouped upon diagnosis

A significant age difference emerged across the three subgroups of patients identified upon diagnosis (p=0.0092, Kruskal-Wallis Statistics 9.384), the OA patients being older than subjects with immune-mediated inflammatory arthritides (p=0.0231, Mann-Whitney U=60.50). Conversely, age was comparable between the two immune-mediated conditions (p=0.0723, Mann-Whitney U=34.50). No significant difference emerged between the three groups with respect to disease duration, BMI, VAS pain, ESR and CRP

Table I. Demographic, clinical and biochemical parameters of the 37 patients recruited in our study.

	RA (n=18) median (IQR)	PsA (n=8) median (IQR)	OA (n=11) median (IQR)
Age (years)	64.5 (49-81.25)	48.5 (39.5-63)	70.5 (68-74)
BMI (weight [kg]/height^2 [cm^2])	25 (20-27)	22.7 (21-24.85)	35 (22.65-27.5)
Disease duration (months)	108 (60-180)	72 (42-72)	72 (24-114)
Kellgren's radiographic grade	3	3	3
VAS pain	60 (42.5-70)	60 (45-60)	60 (45-60)
DAS28	3.5 (2.6-4.05)	2.9 (2.8-3.835)	–
ESR	26 (14-48)	10 (7-32)	21 (13.5-26)
CRP	0.59 (0.42-3.1)	0.5 (0.4-0.95)	0.4 (0.3-0.4)
SF Chemerin (ng/ml)	24.79 (14.02-36.49)	37.65 (25.65-48.87)	36.82 (22.22-61.94)
SF IL-8 (ng/ml)	2.175 (0.494-4.724)	1.169 (0.162-2.329)	0.009 (0-0.210)
SF TNF-α (ng/ml)	0.046 (0-0.179)	0 (0-0.107)	ND
SF IL-6 (ng/ml)	6.852 (2.474-20.584)	1.662 (1.276-12.12)	1.217 (0.010-1.551)
SF Chemerin*	0.723 (0.351-1.126)	0.801 (0.504-1.030)	1.658 (1.198-2.262)
SF IL-8*	0.03878 (0.011435-0.16061)	0.01763 (0.00305-0.06658)	0.00028 (0-0.00930)
SF TNF-α*	0.0013 (0-0.00593)	0 (0-0.00262)	–
SF IL-6*	0.1936 (0.0708-0.4394)	0.0525 (0.0346-0.3180)	0.0408 (0.0003-0.0755)

Absolute (ng/ml) and normalised levels (*, ng/mg) of chemerin, interleukin (IL)-8, tumour necrosis factor α (TNF-α) and IL-6 in the synovial fluid (SF). Normalised cytokine levels are calculated as the ratio between the concentration (ng/ml) of the chemokines and the total SF proteins. Values are expressed as median (IQR, interquartile range). RA: rheumatoid arthritis; PsA: psoriatic arthritis; OA: osteoarthritis.

Table II. Demographic, clinical and biochemical parameters of the group of 13 patients.

	RA (n=5) median (IQR)	PsA (n=5) median (IQR)	OA (n=3) median (IQR)
Age (years)	68 (50-82)	50 (40-63)	70 (66.5-70.5)
BMI (weight [kg]/height^2 [cm^2])	24 (23-25)	24.4 (20.4-25.2)	28 (24.7-28.4)
Disease duration (months)	72 (48-360)	72 (63-78)	60 (36-72)
Kellgren's radiographic grade	3	3	3
VAS pain	70 (60-70)	60 (52.5-62.5)	50 (50-55)
DAS28	3.6 (2.5-4.1)	3.1 (2.9-3.5)	–
ESR	31 (14-48)	13.5 (7.5-26.8)	–
CRP	0.5 (0.45-1.45)	0.45 (0.43-0.48)	–
Serum Chemerin (ng/ml)	206.05 (178.14-211.30)	164.30 (133.22-165.51)	172.28 (154.71-226.12)
SF Chemerin	35.22 (16.59-48-03)	51.42 (41.21-56.77)	61.08 (60.45-62.79)
Serum IL-8 (ng/ml)	ND	ND	ND
SF IL-8	4.19 (2.40-8.87)	0.34 (0.03-1.57)	0 (0-0.095)
Serum TNF-α (ng/ml)	0.013 (0-0.020)	0 (0-0.112)	ND
SF TNF-α	0.091 (0.060-0.343)	ND	ND
Serum IL-6 (ng/ml)	0.05 (0.02-0.06)	ND	ND
SF IL-6	27.72 (21.60-69.15)	1.32 (1.00-11.56)	1.46 (1.32-1.55)
Serum Chemerin*	2.97 (2.77-3.17)	2.32 (2.10-2.55)	2.77 (2.33-3.55)
SF Chemerin*	0.735 (0.723-1.152)	1.046 (0.862-1.453)	2.122 (1.894-3.070)
Serum IL-8*	–	–	–
SF IL-8*	0.120 (0.050-0.191)	0.017 (0.002-0.044)	0 (0-0.003)
Serum TNF-α*	0.000199 (0-0.000342)	0 (0-0.0017)	–
SF TNF-α*	0.0026 (0.0019-0.0074)	–	–
Serum IL-6*	0.0007 (0.0004-0.0011)	–	–
SF IL-6*	0.94 (0.57-1.65)	0.03 (0.02-0.22)	0.05 (0.04-0.06)

Absolute (ng/ml) and normalised levels (*, ng/mg) of chemerin, interleukin (IL)-8, tumour necrosis factor α (TNF-α) and IL-6 in the synovial fluid (SF) and in the sera. Normalised cytokine levels are calculated as the ratio between the concentration (ng/ml) of the chemokines and the total SF/serum proteins. Values are expressed as median (IQR, interquartile range). RA: rheumatoid arthritis; PsA: psoriatic arthritis; OA: osteoarthritis; ND: not detectable.

(p=0.1774, Kruskal-Wallis Statistics 3.458; p=0.5188, Kruskal-Wallis Statistics 1.312; p=0.4175, Kruskal-Wallis Statistics 1.747; p=0.4568, Kruskal-Wallis Statistics 1.567 and p=0.0.826, Kruskal-Wallis Statistics 4.989 respectively). Lastly, RA and PsA patients presented similar DAS28 values (p=0.9574, Mann-Whitney U=48.00).

Cytokine SF levels and demographic, clinical and biochemical variables

No significant difference in chemerin, IL-8, TNF-α and IL-6 levels emerged between subgroups of patients identified upon radiographic damage (p=0.436, Kruskal-Wallis Statistics 1.662; p=0.509, Kruskal-Wallis Statistics 1.351; p=0.780, Kruskal-Wallis Statistics 0.498 and p=0.307, Kruskal-Wallis Statistics 2.364 respectively). In particular, patients with more severe damage (Class IV) presented levels of chemerin and IL-8 comparable to subjects with less advanced radiographic progression (Class II and III, p=0.637, Mann-Whitney U=40.000 and p=0.717, Mann-Whitney U=41.000 respectively). The SF concentrations of chemerin, IL-8, TNF-α and IL-6 were similar across subgroups based on gender (p=0.590, Mann-Whitney U=123.00; p=0.782, Mann-Whitney U=131.500; p=0.908, Mann-Whitney U=109.000 and p=0.316, Mann-Whitney U=86.00 respectively) and treatment (p=0.104, Mann-Whitney U=3.746; p=0.097, Mann-Whitney U=9.643; p=0.104, Mann-Whitney U=3.746 and p=0.378, Kruskal-Wallis Statistics=4.689 respectively). Chemerin and TNF-α levels in the SF were not associated with any of the considered demographic, clinical and biochemical variables. In particular, chemerin did not correlate with BMI, not even when considering solely OA patients (r=0.5868, p=0.1262). A significant relationship between the SF concentration of IL-8 and VAS pain was observed; moreover, IL-8 levels significantly correlated with acute phase reactants as CRP and ESR. IL-6 SF levels were found to correlate with VAS pain, CRP and DAS28. The r and p values of the correlations between demographic, clinical and biochemical parameters and the SF levels of the four study cytokines are detailed in Table III.

Cytokine SF levels in the three subgroups of patients identified upon diagnosis

In our cohort, no significant difference was observed in the SF concentration of chemerin between patients with RA, PsA and OA (p=0.0656, Kruskal-Wallis Statistics 5.448). No difference in chemerin levels in SF emerged when comparing immune-mediated inflammatory arthritides and OA (p=0.0928, Mann-Whitney U=115.00). Conversely, a significant difference was observed in the SF concentration of IL-8 between patients with RA, PsA and OA (p=0.0020, Kruskal-Wallis Statistics 12.46). In particular, patients with immune-mediated inflammatory arthritides presented significantly higher levels of SF IL-8 compared to OA subjects (p=0.0006, Mann-Whitney U=60.00, Fig. 1). Patients with OA displayed significantly lower SF concentrations of IL-8 compared to both RA and OA patients (p=0.0006, Mann-Whitney U=33.50 and p=0.0246, Mann-Whitney U=26.50, respectively), while no significant difference in IL-8 levels was found in the SF from patients with RA and PsA (p=0.1167, Mann-Whitney

Table III. Correlations between demographic, clinical and biochemical variables and levels of chemerin, IL-8, TNF–α and IL-8 in the synovial fluids obtained from the 37 patients recruited in this study.

	Age	Disease Duration	BMI	VAS pain	ESR	CRP	DAS28	SF Chemerin	SF IL-8	SF TNF-α	SF IL-6
Age	–	r=0.242 p=0.161	r=-0.111 p=0.560	**r=0.544** **p=0.001**	**r=0.566** **p=0.002**	r=0.024 p=0.926	**r=0.730** **p<0.001**	r=0.141 p=0.398	r=0.242 p=0.143	r=0.036 p=0.885	r=0.149 p=0.399
Disease duration	r=0.242 p=0.161	–	**r=-0.445** **p=0.018**	**r=0.404** **p=0.001**	r=-0.053 p=0.793	r=-0.069 p=0.792	r=0.116 p=0.590	r=-0.079 p=0.651	r=0.320 p=0.061	r=0.046 p=0.806	r=0.305 p=0.096
BMI	r=-0.111 p=0.560	**r=-0.445** **p=0.018**	–	r=-0.346 p=0.072	r=-0.367 p=0.093	r=0.331 p=0.247	**r=-0.532** **p=0.016**	r=0.250 p=0.183	r=-0.161 p=0.396	r=-0.126 p=0.541	r=-0.033 p=0.873
VAS pain	**r=0.544** **p=0.001**	**r=0.404** **p=0.001**	r=-0.346 p=0.072	–	**r=0.404** **p=0.037**	r=-0.130 p=0.619	**r=0.655** **p=0.001**	r=0.169 p=0.332	**r=0.402** **p=0.017**	r=0.033 p=0.861	**r=0.589** **p<0.001**
ESR	**r=0.566** **p=0.002**	r=-0.053 p=0.793	r=-0.367 p=0.093	**r=0.404** **p=0.037**	–	r=0.467 p=0.059	**r=0.789** **p<0.001**	r=-0.038 p=0.852	**r=0.662** **p<0.001**	r=0.331 p=0.123	r=0.319 p=0.138
CRP	r=0.024 p=0.926	r=-0.069 p=0.792	r=0.331 p=0.247	r=-0.130 p=0.619	r=0.467 p=0.059	–	r=0.321 p=0.286	r=-0.052 p=0.842	**r=0.737** **p=0.001**	r=0.460 p=0.085	**p=0.515** **p=0.050**
DAS28	**r=0.730** **p<0.001**	r=0.116 p=0.590	**r=-0.532** **p=0.016**	**r=0.655** **p=0.001**	**r=0.789** **p<0.001**	r=0.321 p=0.286	–	r=0.250 p=0.238	**r=0.615** **p=0.001**	r=0.000 p=1.000	**r=0.437** **p=0.048**
SF Chemerin	r=0.141 p=0.398	r=-0.079 p=0.651	r=0.250 p=0.183	r=0.169 p=0.332	r=-0.038 p=0.852	r=-0.052 p=0.842	r=0.250 p=0.238	–	r=-0.33 p=0.839	r=-0.215 p=0.202	r=0.276 p=0.098
SF IL-8	r=0.242 p=0.143	r=0.320 p=0.061	r=-0.161 p=0.396	**r=0.402** **p=0.017**	**r=0.662** **p<0.001**	**r=0.737** **p=0.001**	**r=0.615** **p=0.001**	r=-0.33 p=0.839	–	r=0.251 p=0.134	**r=0.764** **p<0.001**
SF TNF-α	r=0.036 p=0.885	r=0.046 p=0.806	r=-0.126 p=0.541	r=0.033 p=0.861	r=0.331 p=0.123	r=0.460 p=0.085	r=0.000 p=1.000	r=-0.215 p=0.202	r=0.251 p=0.134	–	r=0.323 p=0.052
SF IL-6	r=0.149 p=0.399	r=0.305 p=0.096	r=-0.033 p=0.873	**r=0.589** **p<0.001**	r=0.319 p=0.138	**p=0.515** **p=0.050**	**r=0.437** **p=0.048**	r=0.276 p=0.098	**r=0.764** **p<0.001**	r=0.323 p=0.052	–

U=60.50). A trend towards statistical significance emerged when evaluating SF TNF-α levels in the three conditions (p=0.0521, Kruskal-Wallis Statistics 5.908). Indeed, patients with immune-mediated inflammatory arthritides presented significantly higher SF TNF-α compared to OA subjects, whose levels were undetectable (p=0.0194, Mann-Whitney U=78.00). More precisely, RA patients displayed higher TNF-α levels in the SF compared to OA subjects (p=0.0108, Mann-Whitney U=43.50), while no statistically significant difference was observed between RA and PsA patients and between subjects with PsA and OA (p=0.5947, Mann-Whitney U=66.50 and p=0.1869, Mann-Whitney U=34.50 respectively). Conversely, the SF concentrations of IL-6 were significantly dissimilar across patients with RA, PsA and OA (p=0.0020, Kruskal-Wallis Statistics 12.46). In particular, patients with immune-mediated inflammatory arthritides presented higher levels of SF IL-6 compared to OA subjects

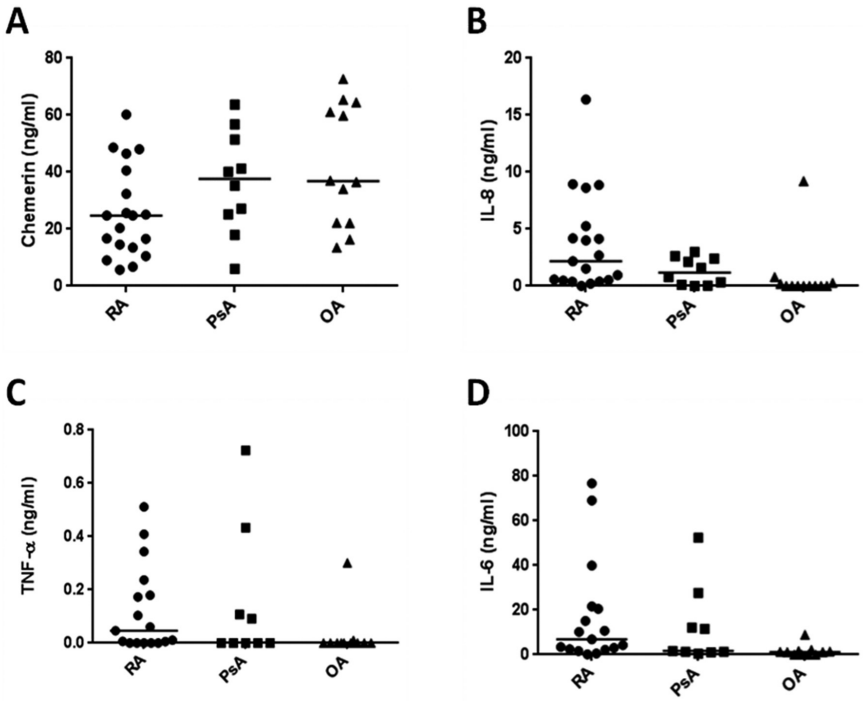

Fig. 1. Dot-plots of chemerin (**A**), IL-8 (**B**), TNF-α (**C**) and IL-6 (**D**) levels (ng/ml) in the synovial fluid of patients with rheumatoid arthritis (RA), psoriatic arthritis (PsA) and osteoarthritis (OA). The horizontal lines refer to the median values in each group.

(p=0.0010, Mann-Whitney U=48.00). Patients with OA displayed significantly lower concentrations of SF IL-6 compared to RA (p=0.0006, Mann-Whitney U=24.0) and almost significantly compared to PsA patients (p=0.0558, Mann-Whitney U=24.00, respectively), while any dissimilarity in IL-6 levels was found in the SF from patients with RA and PsA (p=0.4134, Mann-Whitney U=61.00).

Correlations between SF cytokine levels

SF IL-8 strongly correlated with IL-6 levels (r=0.7637, p<0.0001). A trend towards statistical significance emerged when evaluating the relationship between chemerin and IL-6 levels (r=0.2764, p=0.0977) as well as between IL-6 and TNF-α SF concentrations (r=0.3226, p=0.0515).

No association could be detected between chemerin and IL-8 (r=-0.0328, p=0.8385); similarly, chemerin SF levels did not correlate with TNF-α concentration (r=-0.2147, p=0.2020) and no significant correlation emerged between IL-8 and TNF-α (r=0.2513, p=0.1336).

Demographic, clinical and biochemical variables of 13 patients subgrouped upon diagnosis

No significant age difference emerged between the 13 patients whose SF as well as serum samples were collected, subgrouped upon clinical diagnosis (AR, PsA and OA, p=0.155, Kruskal-Wallis Statistics 3.735). No dissimilarity emerged among the three groups with respect to disease duration, BMI, VAS pain, ESR and CRP (p=0.692, Kruskal-Wallis Statistics 0.737; p=0.678, Kruskal-Wallis Statistics 0.776; p=0.351, Kruskal-Wallis Statistics 2.094; p=0.532, Kruskal-Wallis Statistics 1.264 and p=0.282, Kruskal-Wallis Statistics 2.534, respectively). Moreover, RA and PsA patients presented similar DAS28 values (p=1.000, Mann-Whitney U=10.00).

Cytokine serum levels and demographic, clinical and biochemical variables

No significant difference in serum chemerin, IL-8, IL-6 and TNF-α levels emerged between subgroups of patients identified upon radiographic damage (p=0.667, Mann-Whitney U=3.000; p=0.833, Mann-Whitney U=4.500; p=0.667, Mann-Whitney U=3.000 and p=1.000, Mann-Whitney U=5.000 respectively). Similarly, there was no significant difference in serum chemerin, IL-8, IL-6 and TNF-α levels among subgroups identified upon gender (p=0.503, Mann-Whitney U=16.00; p=0.710, Mann-Whitney U=15.00; p=0.710, Mann-Whitney U=15.00 and p=0.260, Mann-Whitney U=10.00 respectively) and treatment (p=0.235, Mann-Whitney U=7.263; p=0.375, Mann-Whitney U=4.634; p=0.235, Mann-Whitney U=7.263 and p=0.554, Mann-Whitney U=4.653 respectively). No significant association was detected between serum chemerin, IL-8, TNF-α and IL-6 and any of the demographic, clinical and biochemical variables.

Cytokine serum levels in the three subgroups of patients identified upon diagnosis

In our cohort, no significant difference was observed in the serum concentration of chemerin, IL-8 and TNF-α among patients with RA, PsA and OA (p=0.300, Kruskal-Wallis Statistics 2.409; p=0.717, Kruskal-Wallis Statistics 6.667 and p=0.323, Kruskal-Wallis Statistics 2.263). In particular, subjects with immune-mediated inflammatory arthritides and OA were comparable with regards to serum chemerin, IL-8 and TNF-α levels (p=0.866, Mann-Whitney U=14.00; p=0.692, Mann-Whitney U=12.00 and p=0.217, Mann-Whitney U=7.50). Of note, IL-8 levels were not detectable in the whole cohort of patients, except one RA and one PsA subject. Serum TNF-α concentrations were below the assay detection limit in all OA patients. A significant difference was observed in the serum concentration of IL-6 among patients with RA, PsA and OA (p=0.032, Kruskal-Wallis Statistics 6.857). In particular, patients with immune-mediated inflammatory arthritides presented similar serum levels of IL-6 compared to OA subjects (p=0.161, Mann-Whitney U=6.00), whose serum levels were below the detection threshold. Similarly, all but one PsA subject displayed undetectable serum IL-6. Patients with OA presented significantly lower SF concentrations of IL-6 compared to RA (p=0.0036, Mann-Whitney U=0.00). A trend towards statistical significance was registered when comparing serum IL-6 in AR and PsA patients (p=0.095, Mann-Whitney U=4.00), while no significant difference in IL-6 levels was found in the SF from patients with PsA and OA (p=0.786, Mann-Whitney U=6.00).

Correlations between serum cytokine levels

Serum chemerin levels did not correlate with any of the considered variables, while a significant correlation emerged between IL-8 and SF IL-6 as well as serum TNF-α and IL-6 concentrations. A significant correlation of serum TNF-α levels with SF TNF-α and serum IL-6 was observed.

Serum/SF gradient

A serum/SF gradient of the four study cytokines was also evaluated. Cytokine levels were considered after normalisation to the protein content of serum and SF. Chemerin levels were significantly higher in serum than in SF (p=0.004, Z=-2.900). Conversely, IL-8 and IL-6 levels were significantly higher in SF samples than in sera (p=0.006, Z=-2.756 and p=0.001, Z=-3.180, respectively). No significant dissimilarity was observed between SF and serum TNF-α levels (p=0.128, Z=-1.521).

Univariate, bivariate and multivariate logistic regression analyses

At bivariate logistic regression analysis, SF chemerin and TNF-α levels corrected by age did not significantly predicted diagnosis, defined as immune-mediated inflammatory arthritides *versus* OA (p=0.081 and p=0.106). Conversely, IL-8 levels in the SF significantly predicted diagnosis (p=0.031, β=0.505, χ^2 Wald 4.632, 95% CI=1.046-2.622) while a trend towards statistical significance was observed for IL-6 in predicting diagnosis (p=0.053, β=0.310, χ^2 Wald 3.757, 95% CI=0.997-1.866).

At univariate logistic regression analysis, none of the serum cytokines significantly predicted diagnosis (p=0.760, p=0.999, p=0.994 and p=0.998 respectively).

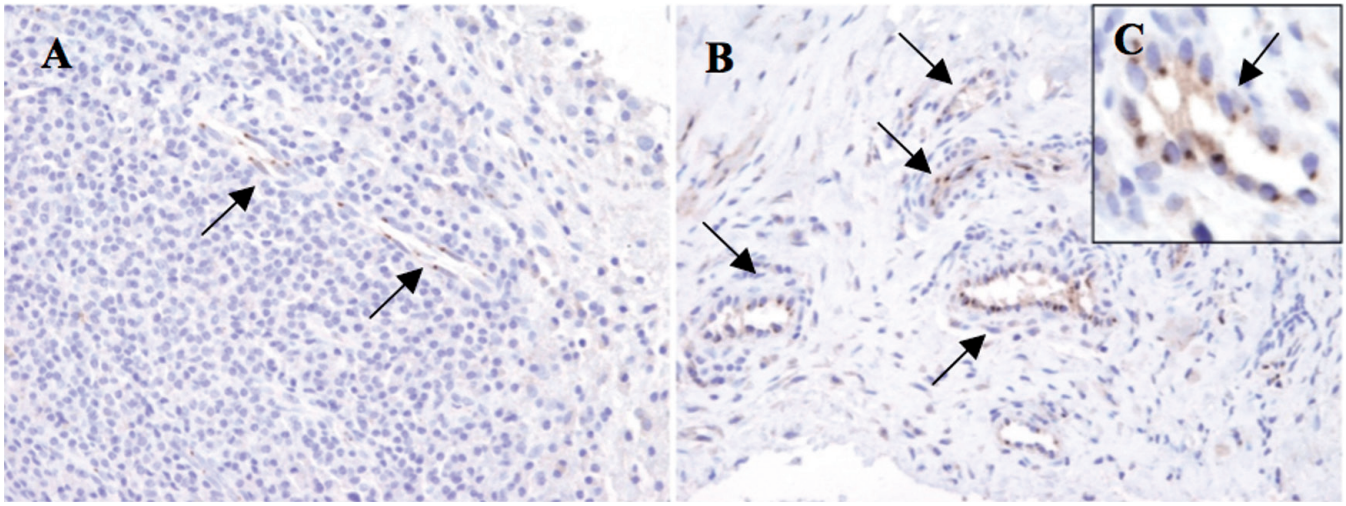

Fig. 2. Chemerin expression in a synovial tissue obtained from a patient with rheumatoid arthritis as detected by immunohistochemistry. Sections are counterstained with Meyer's haematoxylin. Original magnification 200x (**A** and **B**) and 600x (**C**).

Thereafter, we built a multivariate logistic regression model to predict diagnosis, inserting as predictors the two variables that were significant in the univariate models: IL-8 and IL-6, corrected by age. However, none of these two variables held statistical significance in the multivariate model (p=0.199 and p=0.300, respectively).

Immunohistochemistry

On sections, Chemerin reactivity was detected in all 4 synovial biopsies from RA patients in form of cytoplasmic dots in endothelial cells (Fig. 2).

Discussion

Chemerin is a pro-inflammatory mediator contributing to chronic synovial inflammation by recruiting inflammatory cells, promoting endothelial cell proliferation and inducing MMP production. In our cohort, consistently with the emerging concept of OA as an inflammatory disorder and not a merely degenerative disease (16), OA patients presented similar SF levels of chemerin compared to subjects with immune-mediated inflammatory arthritides. Chemerin was even higher, although non significantly, in OA than in RA and PsA, suggesting chemerin as a player in OA pathogenesis. Chemerin role in OA etiology is further supported by its catabolic effects on cartilage (17). Indeed, in chondrocytes chemerin has been proposed as a downstream mediator of TLR activation, a family of pro-inflammatory receptors exerting a well-characterised inhibitory activity on cartilage biosynthetic activity (18). Moreover, chemerin expression is modulated throughout chondrocyte differentiation, suggesting its role in endochondral ossification (19).

Conversely, patients with immune-mediated inflammatory arthritides presented significantly higher IL-8 SF levels compared to those with OA. This observation fits well with the histological evidence of abundant synovial hypervascularisation described in both RA and PsA, whereas in OA neoangiogenesis is a less prominent finding (11). As a whole, these data significantly contribute to current knowledge, as available reports of chemerin and IL-8 SF levels across the three arthropathic disease were limited to only one study respectively. Our finding of similar chemerin SF concentrations in the three conditions is consistent with the study conducted by Eisinger in a cohort of 36 patients, while some discrepancies with previous data about IL-8 SF levels emerged. Indeed, Bertazzolo *et al*. described higher IL-8 levels in the SF from RA patients compared to both OA and PsA subjects (20, 21).

The anti-inflammatory effects of anti-rheumatic agents such as methotrexate and leflunomide affect the cytokine profile in RA (22). In our study, SF samples were collected in case of knee joint effusion, when disease control was not achieved by medical treatment: this provides an optimal scenario to evaluate the mediators involved in joint inflammation.

Another novel issue investigated in this study is the direct comparison of the circulating concentrations of chemerin and IL-8 across the three conditions. The subgroups of patients displayed comparable serum levels of both chemerin and IL-8. This is a relevant finding, as past studies evaluated serum chemerin in RA and PsA only separately, observing respectively higher and lower levels as compared to healthy subjects (23, 24). On the other hand, serum IL-8 was reported as increased in RA patients compared to OA (2, 25).

Interestingly, our data suggest a serum/SF gradient for chemerin, supporting a systemic rather than local synthesis of this chemokine. Further, we reported an in situ production of IL-8; consistently, many synovial cell types, mainly of macrophagic origin, have been previously shown to secrete IL-8 (6).

This study provides novel insight into the inflammatory orchestra in arthropathies also by assessing the relationship of levels of chemerin and IL-8 with two other proinflammatory cytokines as TNF-α and IL-6, both in serum and SF. To note, we could not confirm the association between chemerin and TNF-α, IL-6 and other markers of inflammatory response as ESR and CRP (26, 27). Similarly, we could not confirm the association of chemerin levels with BMI and OA severity (24, 28). This was a

rather unexpected finding as chemerin and the other members of the adipokine family have been demonstrated to strongly correlate with systemic inflammation, being identified as potential mediators of the emerging link between obesity, low-grade inflammation and OA (29. 30). This could be explained by the rather quite severe joint damage observed in our cohort.

On the other hand, a significant association of IL-8 with disease activity parameters such as ESR, CRP and DAS28 emerged. The pro-inflammatory activity of IL-8 was further documented by the strong relationship with both TNF-α and IL-6 levels, suggesting a close interplay between these three cytokines. The correlation between SF IL-8 and VAS pain is in line with the observation that hypoxia, a strong inducer of IL-8 secretion, contributes to the pathogenesis of pain by sensitising sensory nerves (11).

The expression of chemerin was confirmed also at a synovial level in tissue samples from RA patients. Biopsies of synovial membranes in individuals with osteoarthritis are not justified by clinical purpose, being not routinely performed. Thus, because of the lack of data from PsA and OA synovial tissues, no definitive conclusions can be drawn. We acknowledge the preliminary nature of our study: given the rather small sample size, the above findings need to be confirmed by appropriately powered studies.

As a whole, our data suggest that the SF levels of chemerin do not allow distinguishing between conditions as AR, 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 immune-mediated 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.

Acknowledgements

We thank William Vermi and Silvia Lonardi, University of Brescia, for the immunohistochemical analysis.

References

1. BONDUE B, WITTAMER V, PARMENTIER M: Chemerin and its receptors in leukocyte trafficking, inflammation and metabolism. *Cytokine Growth Factors Rev* 2011; 22: 331-8.
2. KANEKO K, MIYABE Y, TAKAYASU A *et al*.: Chemerin activates fibroblast-like synoviocytes in patients with rheumatoid arthritis. *Arthritis Res Ther* 2011; 3: R158.
3. SOZZANI S, VERMI W, DEL PRETE A, FACCHETTI F: Trafficking properties of plasmacytoid dendritic cells in health and disease. *Trends Immunol* 2010; 3: 270-7.
4. PARLEE SD, ERNST MC, MURUNGANANDAN S, SINAL CJ, GORALSKI KB: Serum Chemerin levels vary with time of day and are modified by obesity and tumor necrosis factor **α**. *Endocrinology* 2010; 151: 2590-602.
5. BERG V, SVEINBJÖRNSSON B, BENDIKSEN S, BROX J, MEKNAS K, FIGENSCHAU Y: Human articular chondrocytes express ChemR23 and chemerin; ChemR23 promotes inflammatory signaling upon binding the ligand chemerin. *Arthritis Res Ther* 2010; 12: R228.
6. SZEKANECZ Z, STRIETER RM, KUNKEL SL, KOCH AE: Chemokines in rheumatoid arthritis. *Springer Semin Immunopathol* 1998; 20: 115-32.
7. CHO M-L, JU J-H, KIM H-R *et al*.: Toll-like receptor 2 ligand mediates the upregulation of angiogenic factor, vascular endothelial growth factor and interleukin-8/CXCL8 in human rheumatoid synovial fibroblasts. *Immunol Lett* 2007; 108: 121-8.
8. MOON S-J, PARK M-K, OH H-J *et al*.: Engagement of Toll-like receptor 3 induces vascular endothelial growth factor and interleukin-8 in human rheumatoid synovial fibroblasts. *Korean J Intern Med* 2010; 25: 429-35.
9. ENDO H, AKAHOSHI T, TAKAGISHI K, KASHIWAZAKI S, MATSUSHIMA K: Elevation of interleukin 8 (IL-8) levels in joint fluids of patients with rheumatoid arthritis and the induction by IL-8 of leukocyte infiltration and synovitis in rabbit joints. *Lymphokine Cytokine Res* 1991; 10: 245-52.
10. SOZZANI S, RUSNATI M, RIBOLDI M, MITOLA S, PRESTA M: Dendritic cell-endothelial cell cross talk in angiogenesis. *Trends Immunol* 2007; 28: 385-92.
11. MARUOTTI N, CANTATORE FP, CRIVELLATO E, VACCA A, RIBATTI D: Angiogenesis in rheumatoid arthritis. *Histol Histopathol* 2006; 21: 557-66.
12. ALETAHA D, NEOGI T, SILMAN AJ *et al*.: Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010; 69: 1580–8.
13. FORESTIER R, FRANCON A, BRIOLE V, GENTY MC, CHEVALIER X, RICHETTE P: Diagnostic criteria for generalized osteoarthritis: a preliminary study in a population with knee osteoarthritis. *Joint Bone Spine* 2011; 78: 424-6.
14. TAYLOR W, GLADMAN D, HELLIWELL P, MARCHESONI A, MEASE P, MIELANTS H: Classification criteria for psoriatic arthritis: development of new criteria from a large international study. *Arthritis Rheum* 2006; 54: 2665-73.
15. KELLGREN JH, LAWRENCE JS: Radiological assessment of osteoarthrosis. *Ann Rheum Dis* 1957; 16: 494-512.
16. SCANZELLO CR, PLAAS A, CROW MK: Innate immune system activation in osteoarthritis: is osteoarthritis a chronic wound? *Curr Opin Rheumatol* 2008; 20: 565-72.
17. BERENBAUM F, EYMARD F, HOUARD X: Osteoarthritis, inflammation and obesity. *Curr Opin Rheumatol* 2013; 25: 114-8.
18. GOMEZ R, CONDE J, SCOTECE M, GOMEZ-REINO JJ, LAGO F, GUALILLO O: What's new in our understanding of the role of adipokines in rheumatic diseases? *Nat Rev Rheumatol* 2011; 7: 528-36.
19. CONDE J, GOMEZ R, BIANCO G *et al*.: Expanding the adipokine network in cartilage: identification and regulation of novel factors in human and murine chondrocytes. *Ann Rheum Dis* 2011; 70: 551-9.
20. EISINGER K, BAUER S, SCHÄFFLER A *et al*.: Chemerin induces CCL2 and TLR4 in synovial fibroblast of patients with rheumatoid arthritis and osteoarthritis. *Exp Mol Pathol* 2012; 92: 90-6.
21. BERTAZZOLO N, PUNZI L, STEFANI MP *et al*.: Interrelationships between interleukin (IL)-1, IL-6 and IL-8 in synovial fluid of various arthropathies. *Agent Actions* 1994; 41: 90-2.
22. KRAAN MC, SMEETS TJM, VAN LOON MJ, BREEDVELD FC, DIJKMANS BAC, TAK PP: Differential effects of leflunomide and methotrexate on cytokine production in rheumatoid arthritis. *Ann Rheum Dis* 2004; 63: 1056-61.
23. XUE Y, JIANG L, CHENG Q *et al*.: Adipokines in psoriatic arthritis patients: the correlations with osteoclast precursors and bone erosions. *PloS ONE* 2012; 7: e46740.
24. HUANG K, DU G, LI L, LIANG H, ZHANG B: Association of chemerin levels in synovial fluid with the severity of knee osteoarthritis. *Biomarkers* 2012; 17: 16-20.
25. RAI MF, SANDELL LJ: Inflammatory mediators: tracing link between obesity and osteoarthritis. *Crit Rev Eukaryot Gene Expr* 2011; 21: 131-42.
26. WEIGERT J, NEUMEIER M, WANNINGER J *et al*.: Systemic chemerin is related to inflammation rather than obesity inn type 2 diabetes. *Clinical Endocrinology* 2010; 72: 342-8.
27. LEHRKE M, BECKER A, GREIF M *et al*.: Chemerin is associated with markers of inflammation and components of the metabolic syndrome but does not predict coronary atherosclerosis. *Eur J Endocrinol* 2009; 161: 339-44.
28. BOZAOGLU K, SEGAK D, SHIELDS KA *et al*.: Chemerin is associated with metabolic syndrome phenotypes in a Mexican American population. *J Clin Endocrinol Metab* 2009; 94: 3085-88.
29. ZHUO Q, YANG W, CHEN J, WANG Y: Metabolic syndrome meets osteoarthritis. *Nat Rev Rheumatol* 2012; doi: 10.1038.
30. ERNST MC, SINAL CJ: Chemerin: at the crossroads of inflammation and obesity. *Trends Endocrinol Metab* 2010; 21: 660-7.