# Implication of CXCL5 (epithelial neutrophil-activating peptide 78) in the development of insulin resistance in patients with rheumatoid arthritis

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## Abstract

### Objective

*The chemokine molecule CXCL5 (C-X-C motif chemokine ligand 5, also known as epithelial neutrophil activating peptide 78 -ENA78-) constitutes a link between obesity, inflammation and insulin resistance (IR) in the general population. CXCL5 has also been found to play a role in rheumatoid arthritis (RA) pathogenesis. Since chronic inflammation promotes IR and impairs pancreatic beta cell function in RA patients, we assessed the role of CXCL5 in the development of IR in RA.*

### Methods

*Cross-sectional study that encompassed 141 non-diabetic patients with RA. IR assessed by homeostatic model assessment (HOMA2), insulin and C-peptide serum levels and lipid profile, and CXCL5 serum levels were studied. Regression analysis was performed to evaluate how CXCL5 was related to IR, disease activity, and disease characteristics in RA patients.*

### Results

*HOMA2-IR indexes showed high values for both IR and beta cell production (%B), and low insulin sensitivity (%S) in patients with RA. C reactive protein (beta coef. 0.2 [95%CI -1.5–1.9], p=0.80) and disease activity through DAS28 (beta coef. 13 [95%CI -14–41], p=0.34) revealed no relation with CXCL5. Other disease characteristics, such as disease duration, serological status, or use of methotrexate or anti-TNF alpha therapies, were not associated with CXCL5 serum levels. While glucocorticoids were related to insulin, C-peptide serum levels, and HOMA2-IR and HOMA2-%B-C peptide, the use of prednisone was not associated with CXCL5 serum levels. Insulin and C peptide serum levels and IR indexes showed strong correlations among each other, but not with CXCL5 (insulin r2=-0.034, p=0.69; C peptide r2=-0.050, p=0.56).*

### Conclusion

*CXCL5 is not related to IR in RA patients. Therefore, the mechanisms leading to IR in patients with RA may be different from those in the general population.*

**Key words**
CXCL5, insulin resistance, rheumatoid arthritis

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## Introduction

Chemokines (chemotactic proinflammatory cytokines) are a family of low molecular weight proteins that are potent chemoattractants of leukocytes and may modulate the formation of reactive oxygen species and cytokines. The chemokine molecule CXCL5 (C-X-C motif chemokine ligand 5, also known as epithelial neutrophil activating peptide 78 -ENA78-) is expressed at high levels in the macrophage fraction of white adipose tissue (1). When it binds to the chemokine receptor CXCR2, it reduces insulin-stimulated glucose uptake in muscle, suggesting that CXCL5 plays a role in mediating insulin resistance (IR). Several observations showing that serum levels of CXCL5 are higher in obese *versus* normal weight individuals and in insulin-resistant obese versus noninsulin-resistant obese individuals support this hypothesis (1). A positive correlation between body weight and CXCL5-circulating levels has been demonstrated through calorie restriction experiments, showing that CXCL5 concentrations decrease with weight loss (1). In addition, inhibition of CXCL5 via administration of a neutralising antibody or a selective CXCR2 antagonist in two mice models of IR resulted in an improvement in insulin sensitivity (1). For this reason, CXCL5 has recently been regarded as a new link between obesity, inflammation, and insulin resistance.

Chemokines also play an important role as monocyte and polymorphonuclear neutrophil recruiters in the synovitis and tissue destruction caused by RA (2). Concentrations of the intact form of CXCL5 are significantly higher in RA synovial fluid compared to osteoarthritis or other arthritides (3). Additionally, it has been shown that neutralisation of CXCL5 ameliorates arthritis in a rat adjuvant-induced arthritis model (4), thus supporting the idea that CXCL5 is important in the pathogenesis of RA. Moreover, citrullinated CXCL5 very strongly correlates with disease activity and monocyte recruitment in RA (5). These findings indicate that citrullinated CXCL5 may exert an as yet unrecognised inflammatory function in RA by recruiting monocytes to swollen joint tissue.

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterised by the infiltration of inflammatory cells into the synovial tissue. Chronic inflammation worsens IR and impairs pancreatic beta cell function in RA patients (6). Increased prevalence of IR has been widely observed in patients with RA (6-10); as such, high-grade systemic inflammation is thought to play a part in its onset. It has been observed an increased prevalence of undiagnosed type 2 diabetes and impaired fasting glucose in RA patients when compared with age- and gender-matched control individuals (11, 12). Moreover, TNF-α blockade and other biologics have proved to improve IR in RA patients (13, 14).

Since CXCL5 is not only related to IR in healthy populations but has also been linked to RA pathogenesis and disease activity, we sought to determine in an extensive series of RA patients whether the high prevalence of IR in RA could be mediated by this chemokine.

## Materials and methods

*Study participants*

This was a cross-sectional study that included 141 subjects, all 18 years old or older. They were consecutively recruited at our rheumatology outpatients clinics, and fulfilled the 2010 ACR/EULAR classification criteria (15). They had been diagnosed by rheumatologists and were periodically followed-up at rheumatology outpatient clinics. For purposes of inclusion in the present study, RA disease duration had to exceed 1 year. Although anti-tumour necrosis factor-alpha (TNF) treatment has been associated with changes in IR (16, 17), RA patients undergoing TNF-alpha antagonist therapy were not excluded from the present study. As glucocorticoids are often used in the management of RA, patients taking prednisone or an equivalent dose (10 mg/day or less) were not excluded. However, as RA patients with diabetes mellitus were not included, none of the study subjects were receiving glucose-lowering drugs or insulin therapy. In addition, patients were required to have a glycaemia levels <7 mmol/l. Patients were excluded if they had a history of myocardial in-

farction, angina, stroke, a glomerular filtration rate <60 ml/min/1.73 $m^2$, a history of cancer, or any other chronic disease, or evidence of active infection. The study protocol was approved by the Institutional Review Committees at both Hospital Universitario de Canarias and Hospital Universitario Marqués de Valdecilla (both in Spain), and all subjects provided informed written consent.

*Data collection*

RA subjects completed a cardiovascular risk factor and medication use questionnaire and underwent a physical examination to determine their anthropometrics and blood pressure. Medical records were reviewed to ascertain specific diagnoses and medications. Disease activity was measured using the Disease Activity Score (DAS28) of 28 joints (18), while disease disability was determined using the Health Assessment Questionnaire (HAQ) (19) . Clinical Disease Activity Index (CDAI) (20) and Simple Disease Activity Index (SDAI) (21) scores for RA disease activity were performed as previously described.

*CXCL5 and HOMA assessments*

Fasting serum samples were collected and frozen at -80°C until analysis. Human CXCL5/ENA78 was measured using an enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, Minnesota, USA). The intra- and inter-assay coefficient of variation was <6%. Homeostatic model assessment (HOMA) method was performed to determine IR. Briefly, the HOMA model is used to yield an estimate of insulin sensitivity (%S) and β-cell function (%B) from fasting plasma insulin, C-peptides, and glucose concentrations. In this study, we used HOMA2, the latest computer HOMA model (22). This model can be used to determine insulin sensitivity and β-cell function from paired fasting plasma glucose and specific insulin or C-peptide concentrations across a range of 1–2,200 pmol/l for insulin and 1–25 mmol/l for glucose. In our study, all IR HOMA indexes were calculated with both insulin and C peptide. C-peptide better estimates β-cell

**Table I.** Characteristics of rheumatoid arthritis patients.

| | RA patients (n=141) |
|---|---|
| Age, years | 52 ± 11 |
| Female, n (%) | 114 (80) |
| Body mass index, kg/$m^2$ | 28 ± 5 |
| Abdominal circumference, cm | 96 ± 13 |
| Systolic blood pressure, mm Hg | 135 ± 17 |
| Diastolic blood pressure, mm Hg | 82 ± 12 |
| *Cardiovascular co-morbidity* | |
| Smoking, n (%) | 25 (13) |
| Hypertension, n (%) | 41 (21) |
| Dyslipidaemia, n (%) | 51 (26) |
| Anti-hypertensive treatment, n (%) | 41 (21) |
| Statins, n (%) | 40 (20) |
| Hormone replacement, n (%) | 0 (0) |
| *Analytical and lipid profile* | |
| ESR, mm/h | 34 ± 22 |
| CRP, mg/dl | 3.2 (1.5-5.5) |
| Cholesterol, mg/dl | 206 ± 36 |
| Triglycerides, mg/dl | 138 ± 88 |
| HDL cholesterol, mg/dl | 57 ± 16 |
| LDL cholesterol, mg/dl | 122 ± 30 |
| Lipoprotein A, mg/dl | 34 (11-119) |
| Apolipoprotein A, mg/dl | 170 ± 29 |
| Apolipoprotein B, mg/dl | 110 ± 64 |
| Apo B/apo A ratio | 0.66 ± 0.30 |
| Atherogenic index | 3.92 ± 1.46 |
| *Glucose homeostasis molecules and HOMA2 indexes* | |
| Glucose, mg/dl | 83 ± 9 |
| Insulin, μU/ml | 11.1 ± 9.7 |
| C-peptide, ng/ml | 2.94 ± 2.23 |
| CXCL-5, pg/ml | 984 ± 196 |
| HOMA2 insulin | |
| HOMA2-IR | 1.40 ± 1.17 |
| HOMA2-%S | 105 ± 53 |
| HOMA2-%B | 135 ± 70 |
| HOMA2 C-peptide | |
| HOMA2-IR | 2.11 ± 1.63 |
| HOMA2-%S | 71 ± 41 |
| HOMA2-%B | 180 ± 83 |
| *Rheumatoid arthritis related data* | |
| Disease duration, years | 7 (4-13) |
| Age of debut, years | 43 ± 13 |
| DAS28 | 3.67 ± 1.20 |
| DAS28-CRP | 2.89 ± 0.99 |
| SDAI | 14 (8-21) |
| CDAI | 9 (5-16) |
| Rheumatoid factor, n (%) | 100 (71) |
| ACPA, n (%) | 84 (60) |
| Prednisone intake, n (%) | 52 (37) |
| Prednisone doses, mg/day | 5 (5-6) |
| NSAIDs intake, n (%) | 66 (47) |
| DMARDs, n (%) | 121 (86) |
| Methotrexate, n (%) | 106 (75) |
| Leflunomide, n (%) | 16 (11) |
| Biologic therapy, n (%) | 32 (23) |
| Anti TNF therapy, n (%) | 17 (12) |

Data expressed as mean (± standard deviation) or median (interquartile range). Dichotomous variables are expressed as n and percentage. DAS28: Disease Activity Score; CXCL5: C-X-C motif chemokine 5; ACPA: Anti-citrullinated peptide/protein antibody; DMARD: disease-modifying anti-rheumatic drug; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; HAQ: Health Assessment Questionnaire; SDAI: Simple Disease Activity Index; CDAI: Clinical Disease Activity Index; HDL-C: high-density cholesterol lipoprotein; LDL-C: low-density cholesterol lipoprotein; NSAIDS: non-steroidal anti-inflammatory drugs; TNF: tumour necrosis factor; HOMA: Homeostasis Model Assessment, IR: insulin resistance; %S insulin sensitivity; %B: beta cell production.

**Table II.** Inflammatory markers, disease-related data, and disease activity scores: univariate relation with insulin and C-peptide serum levels, HOMA indexes and CXCL5.

| | beta coefficient (95% confidence interval), *p*-value | | | | |
|---|---|---|---|---|---|
| | Insulin, μU/mL | C-peptide, ng/mL | HOMA2-IR insulin | HOMA2-%B C peptide | CXCL-5 |
| CRP, mg/dL | 0.02 (-0.07-0.10), 0.72 | 0.01 (-0.01-0.03), 0.51 | 0.00 (-0.01-0.01), 72 | 0.44 (-0.28-1.16), 0.23 | 0.22 (-1.49-1.93), 0.80 |
| ESR, mm/hour | -0.03 (-0.10-0.05), 0.50 | -0.00 (-0.02-0.02), 0.84 | -0.00 (-0.01-0.01), 0.51 | -0.16 (-0.79-0.48), 0.63 | 1.00 (-0.50-2.51), 0.19 |
| Disease duration, years | 0.01 (-0.16-0.19), 0.87 | 0.00 (-0.04-0.04), 0.85 | 0.00 (-0.02-0.02), 0.81 | -0.56 (-2.06-0.94), 0.46 | -2.45 (-6.11-1.21), 0.19 |
| ACPA | **-4.49 (-7.83--1.15), 0.009** | **-1.05 (-1.82--0.27), 0.008** | **-0.55 (-0.95--0.14), 0.009** | **-34.75 (-63.89--5.61), 0.020** | 52.35 (-16.53-121.23), 0.14 |
| Rheumatoid factor | -3.26 (-6.91-0.40), 0.080 | -0.41 (-1.26-0.44), 0.34 | -0.38 (-0.82-0.07), 0.095 | -18.12 (-50.02-13.79), 0.26 | 63.95 (-10.98-138.88), 0.09 |
| DAS 28-ESR | -0.32 (-1.66-1.03), 0.64 | -0.02 (-0.33-0.29), 0.89 | -0.04 (-0.20-0.13), 0.64 | -0.07 (-11.64-11.51), 0.99 | 13.41 (-14.28-41.10), 0.34 |
| DAS 28-CRP | 0.20 (-1.43-1.83), 0.81 | 0.07 (-0.30-0.45), 0.70 | 0.02 (-0.17-0.22), 0.82 | 5.20 (-8.80-19.21), 0.46 | 7.93 (-25.88-41.74), 0.64 |
| SDAI | 0.01 (-0.06-0.09), 0.72 | 0.01 (-0.01-0.02), 0.52 | 0.00 (-0.01-0.01), 0.73 | 0.39 (-0.26-1.05), 0.24 | 0.21 (-1.35-1.77), 0.79 |
| CDAI | 0.01 (-0.21-0.22), 0.94 | 0.00 (-0.05-0.05), 0.90 | 0.00 (-0.03-0.03), 0.95 | 0.25 (-1.59-2.08), 0.79 | 0.20 (-4.20-4.59), 0.93 |
| Glucocorticoids intake | 2.56 (-0.31-5.42), 0.080 | **1.69 (1.09-2.28), 0.000** | 0.34 (-0.02-0.70), 0.067 | **51 (31-70), 0.000** | -5.52 (-74.23-63.19), 0.87 |
| DMARDs, intake | 0.03 (-4.50-4.56), 0.99 | 0.47 (-0.57-1.51), 0.38 | -0.00 (-0.55-0.55), 0.99 | 18.04 (-20.86-56.94), 0.36 | 39.63 (-52.33-131.59), 0.40 |
| Methotrexate | -1.83 (-5.55-1.90), 0.33 | -0.16 (-1.02-0.70), 0.72 | -0.23 (-0.68-0.22), 0.31 | -6.54 (-38.68-25.59), 0.69 | -19.41 (-96.24-57.42), 0.62 |
| Biological therapy | 0.89 (-2.96-4.74), 0.65 | -0.09 (-0.98-0.80), 0.84 | 0.10 (-0.37-0.57), 0.67 | -4.17 (-37.32-28.99), 0.80 | -23.66 (-102.06-54.75), 0.55 |
| Anti-TNF alpha drugs | 0.20 (-4.76-5.15), 0.94 | -0.02 (-1.16-1.12), 0.97 | 0.03 (-0.58-0.63), 0.93 | -11.10 (-53.72-31.52), 0.61 | 2.76 (-98.00-103.51), 0.96 |

ACPA: anti-citrullinated peptide/protein antibody; TNF: tumour necrosis factor; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein. DAS28: Disease Activity Score; SDAI: Simple Disease Activity Index; CDAI: Clinical Disease Activity Index; HOMA: Homeostasis Model Assessment, HOMA2-IR: calculated with insulin, HOMA2-%B: beta cell production calculated with C peptide; DMARD: disease-modifying anti-rheumatic drug.

function because C-peptide is a marker of secretion; moreover, insulin data is preferable for calculating %S since HOMA-%S is derived from glucose disposal as a function of insulin concentration. The computer model provides a value for insulin sensitivity expressed as HOMA2-%S (where 100% is normal). HOMA2-IR (insulin resistance index) is simply the reciprocal of %S. Insulin (Architect Abbott, 2000I) and C-peptide (Immulite 2000, Siemens) and was determined by chemiluminescent immunometric assays. Standard techniques were used to measure plasma glucose, C-reactive protein (CRP), the Westergren erythrocyte sedimentation rate (ESR) and serum lipids. Blood collected from all the participants by means of venipuncture was stored at 4°C for less than 4 hours and centrifuged. Serum/plasma was subsequently removed and stored at -80°C.

*Statistical analysis*

In terms of study power, and based on previous reports (1), we expected to find a correlation between CXCL5 and HOMA2-IR of 0.25 (Pearson's correlation coefficient). Therefore, we had to include 120 subjects in our study in order to achieve a power of 80% to detect differences by means of a bilateral Student *t*-test for a Pearson correlation coefficient. These calculations took into account the fact that the level of significance was 5.00% based on an expected correlation of 0.25. Demographic and clinical characteristics of patients with RA are expressed as mean ± standard deviation. For non-normally distributed continuous variables, data were expressed as both a median and interquartile range (IQR). Beta coefficients through univariate regression analysis and Pearson's correlation coefficients were calculated to assess the relation of CXLC5 to glucose homeostasis molecules, HOMA2 indexes and disease characteristics. For all analyses, we used a 5% two-sided significance level, and all analyses were performed using IBM SPSS Statistics v. 21 software (IBM, Armonk, NY, USA) and STATA version 13/SE software (StataCorp, College Station, TX, USA). A *p*-value <0.05 was considered statistically significant.

## Results

*Demographic, analytical and disease-related data*

A total of 141 RA patients with a mean (± standard deviation) age of 52±11 were included in this study. The demographic and disease-related characteristics of the participants are shown in Table I. Patients from our series had moderate-active disease as shown by DAS28 (3.7±1.2) and fifty (37%) were on prednisone (median dose 5 [IQR 5–6] mg/day). Disease duration was 7 (IQR 4–13) years, and 60% and 71% were, respectively, positive for both the anti-citrullinated protein antibodies and rheumatoid factor. Moreover, 86% of the patients were on disease-modifying antirheumatic drugs, while 23% were receiving biologic therapy (12% undergoing anti TNF-alpha treatment).

Regarding glucose homeostasis molecules metabolism, the average glucose in our series was 83±9 mg/dl, and insulin and C-peptide serum levels were, respectively, 11.1±9.7 μU/ml and 2.94±2.23 ng/ml. HOMA2-IR indexes, calculated with both insulin and C-peptide, disclosed high values for IR and beta cell production (%B), as well as low insulin sensitivity (%S), in patients with RA.

*Univariate relation of inflammatory markers and disease-related data with HOMA2 indexes and CXCL5 serum levels*

The associations between laboratory markers, disease activity and therapy with CXCL5 are shown in Table II. DAS28, and other disease activity scores did not correlate with CXCL5 serum levels, nor was any association found using categorised DAS28). No statistically significant association of ACPA ($p$=0.14) or rheumatoid factor

**Table III.** Correlation of CXCL5, glucose, insulin and C peptide with traditional factors related to insulin resistance.

| | Pearson's correlation coefficient, *p*-value | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CXCL5, pg/ml | | glucose, mg/dl | | insulin, µU/ml | | C-peptide, ng/ml | | HOMA2-IR | | HOMA2-%B | |
| Age, years | -0.071 | 0.41 | **0.322** | **<0.001** | 0.145 | 0.086 | **0.249** | **0.003** | 0.154 | 0.067 | 0.121 | 0.15 |
| Systolic pressure, mmHg | -0.023 | 0.79 | 0.132 | 0.12 | 0.038 | 0.65 | 0.089 | 0.30 | 0.046 | 0.59 | 0.037 | 0.66 |
| Diastolic pressure, mmHg | -0.010 | 0.91 | 0.088 | 0.30 | 0.070 | 0.41 | 0.106 | 0.21 | 0.076 | 0.37 | 0.065 | 0.45 |
| BMI, kg/m$^2$ | 0.043 | 0.62 | 0.032 | 0.71 | **0.363** | **<0.001** | **0.294** | **<0.001** | **0.361** | **<0.001** | **0.361** | **<0.001** |
| Abdominal circumference, cm | -0.003 | 0.97 | 0.111 | 0.19 | **0.379** | **<0.001** | **0.321** | **<0.001** | **0.381** | **<0.001** | **0.353** | **<0.001** |
| Hip circumference, cm | 0.097 | 0.26 | -0.054 | 0.53 | **0.261** | **0.002** | 0.165 | 0.051 | **0.256** | **0.002** | **0.246** | **0.003** |
| Waist to hip circumference ratio | -0.104 | 0.23 | **0.244** | **0.003** | **0.314** | **<0.001** | **0.339** | **<0.001** | **0.324** | **<0.001** | **0.298** | **<0.001** |
| Cholesterol, mg/dl | 0.131 | 0.23 | 0.028 | 0.74 | 0.109 | 0.20 | 0.096 | 0.26 | 0.112 | 0.19 | 0.096 | 0.26 |
| Triglycerides, mg/dl | 0.095 | 0.27 | 0.011 | 0.90 | **0.513** | **<0.001** | **0.456** | **<0.001** | **0.509** | **<0.001** | **0.477** | **<0.001** |
| HDL, mg/dl | 0.090 | 0.30 | 0.049 | 0.56 | **-0.322** | **<0.001** | **-0.349** | **<0.001** | **-0.319** | **<0.001** | **-0.402** | **<0.001** |
| LDL, mg/dl | 0.053 | 0.54 | 0.001 | 0.99 | -0.005 | 0.96 | 0.028 | 0.74 | <0.001 | 0.99 | 0.042 | 0.62 |
| ApoA, mg/dl | 0.060 | 0.49 | 0.102 | 0.23 | **-0.166** | **0.049** | **-0.205** | **0.015** | -0.164 | 0.052 | **-0.269** | **0.001** |
| ApoB, mg/dl | -0.092 | 0.29 | -0.074 | 0.39 | 0.018 | 0.83 | 0.012 | 0.89 | 0.017 | 0.84 | 0.025 | 0.77 |
| Lipoprotein A, mg/dl | -0.072 | 0.41 | 0.015 | 0.86 | 0.120 | 0.16 | 0.130 | 0.12 | 0.122 | 0.15 | 0.126 | 0.14 |
| ApoB/ApoA | -0.119 | 0.17 | -0.100 | 0.24 | 0.105 | 0.21 | 0.119 | 0.16 | 0.104 | 0.22 | 0.156 | 0.065 |
| Atherogenic index | -0.005 | 0.95 | 0.019 | 0.82 | **0.396** | **<0.001** | **0.405** | **<0.001** | **0.395** | **<0.001** | **0.425** | **<0.001** |

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; BMI: body mass index; HDL: high-density cholesterol lipoprotein; LDL: low-density cholesterol lipoprotein; Apo: apolipoprotein; HOM2-IR is calculated with insulin; HOMA2-%B is calculated using C peptide; CXCL5: C-X-C motif chemokine 5.

**Table IV.** Correlations between CXCL5 and glucose homeostasis molecules and HOMA2 indexes.

| | Pearson's correlation coefficient, *p*-value | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CXCL5, pg/ml | | C-peptide | | Insulin | | Glucose | | HOMA2-IR | |
| C-peptide. ng/ml | -0.148 | 0.085 | | | | | | | | |
| Insulin. µU/ml | -0.111 | 0.196 | 0.902 | **<0.001** | | | | | | |
| Glucose. mg/dl | -0.082 | 0.343 | 0.349 | **<0.001** | 0.260 | **0.002** | | | | |
| HOMA2-IR | -0.109 | 0.205 | 0.908 | **<0.001** | 0.999 | **<0.001** | 0.300 | **<0.001** | | |
| HOMA2-%B | -0.117 | 0.173 | 0.868 | **<0.001** | 0.817 | **<0.001** | -0.102 | 0.227 | 0.804 | **<0.001** |

HOMA2: Homeostasis Model Assessment, HOMA2-IR: insulin resistance calculated with insulin; HOMA2-%B: beta cell production calculated with C-peptide; CXCL5: C-X-C motif chemokine 5.

(*p*=0.09) with CXCL5 was noted. In addition, the lack of association between ACPA and CXCL5 was confirmed after assessing the interaction of ACPA with disease related variables (data not shown). Glucocorticoids intake was positively associated with insulin (beta coef. 2.56 [95%CI -0.31–5.42 *p*=0.080), C peptide (beta coef. 1.69 [95%CI 1.09–2.28], *p*=0.000), HOMA-IR (beta coef. 0.34 [95%CI -0.02–0.70], *p*=0.067] and HOM2-%B-C peptide (beta coef. 51 [95%CI 31–70], *p*=0.000). However, this relation was not found with CXCL5. Methotrexate, TNF-alpha inhibitors or another non-TNF biologic agent use were not associated with glucose homeostasis molecules including CXCL5. Furthermore, neither the group of DMARDs that includes methotrexate, nor the entire group of biologic therapies exhibited any relation to CXCL5.

*Correlation of CXCL5 and glucose homeostasis molecules with traditional factors related to insulin resistance*

As expected, glucose homeostasis molecules correlated with traditional IR-related factors (Table III). In this sense, body mass index (BMI), abdominal circumference, hip circumference, and waist-to-hip circumference ratio were all strongly and positively correlated with insulin, C-peptide, and sensitivity and secretion HOMA2-IR indexes. This was not the case with CXLC5; for example, it did not show any relation to BMI or abdominal circumference in RA patients. Additionally, when this analysis was performed only in obese patients (BMI >30 kg/m$^2$) CXLC5 did not show any relation with IR indexes.

Regarding lipid profiles, our results proved be similar. While triglycerides and atherogenic index, as well as HDL and apolipoprotein A, were respectively, positively and negatively correlated, to different degrees, with insulin, C-peptide and HOMA2-IR indexes, this was not the case for CXCL5. In this regard, CXCL5 did not show any statistically significant association with any of the lipid molecules.

*Correlations between CXCL5 and glucose homeostasis molecules and HOMA2 indexes*

All glucose homeostasis molecules and HOMA2 IR indexes exhibited a high correlation. In this sense, glucose, insulin, and C-peptide displayed high correlation coefficients. In contrast, CXCL5 did not show any association with these molecules or indexes (Table IV).

## Discussion

In the present study, we have studied, for the first time, the potential implication of CXCL5, a molecule associated with IR in healthy populations, in the increased risk of IR found in RA patients. According to our findings, CXCL5 does not play a role in the IR of RA patients. Thus, we hypothesise that the pathogenic mechanisms leading to IR in RA may be different from those occurring in the general population.

In our study, CXCL5 did not show any relation with features of RA. In this regard, CXCL5 did not exhibit any association with acute phase reactants, disease

characteristics, serological status, or the use of glucocorticoids or DMARDs. Previous reports (23) described the 'citrullination' of CXCL5, which is the mechanism that converts CXCL5 from a non-monocyte-recruiting chemokine to a monocyte-recruiting chemokine in RA. The levels of citrullinated CXCL5, but not the non-citrullinated or 'intact' CXCL5 form, were found to directly correlate with C-reactive protein levels and erythrocyte sedimentation rates in RA patients (23). CXCL5 citrullination can change its inflammatory properties, leading to an increased monocyte-recruiting capacity for this chemokine. Taking this into account, we feel that the lack of correlation with RA features in our study may be due to the fact that we measured 'intact' CXCL5.

Insulin, C peptide or IR indexes had some correlations with RA features in our study. In this regard, glucocorticoids intake was associated with higher levels of insulin, C-peptide, as well as with insulin secretion and resistance indexes. These links have also been previously found in RA (6, 24-26).

To our surprise, glucose homeostasis molecules and HOMA2 indexes negatively related with the presence of anti-citrullinated protein antibodies (ACPA). Most previous studies have not reported any link between serological status and IR except for one involving an early inflammatory polyarthritis cohort in which seropositivity for rheumatoid factor or ACPA was positively associated with IR (27).

Moreover, in our study, CXCL5 did not reveal any relation to the traditional factors associated with IR such as BMI or abdominal circumference. Therefore, CXCL5 - in terms of its relation to metabolic syndrome features - behaves in a way different to how it does in the general population. This was not the case for glucose, insulin and C-peptide or HOMA indexes, which did maintain their relation to traditional IR-related factors in RA patients. Although we do not have an exact explanation for this, it should be remembered that CXCL5 is an inflammatory molecule, while insulin and C-peptide are not. For this reason, we believe that CXCL5's relation to traditional IR-related factors is possibly lost or disturbed due to the pro-inflammatory status present in RA. Thus, we believe that insulin and C-peptide could be distorted by an inflammatory state, but not to the point whereby their correlation with traditional IR-related factors is completed lost. With respect to this, in a recent report by our group (*in press*) we demonstrated that the link between glucose homeostasis molecules and traditional IR-related factors, although weaker, is maintained in patients with RA. This finding may also be supported by the fact that, in our study, glucose, insulin, C-peptide and HOMA2-IR indexes, although not CXCL5, maintained their correlations in RA patients.

A potential limitation of our study could be that we measured 'intact' and not citrullinated CXCL5. Nevertheless, the relation of the citrullinated form of CXCL5 with IR has not been demonstrated and our purpose was to show the potential implication of the intact form, known to be related to IR in healthy population, in the IR of RA patients. Another limitation that could have significantly affected our results was that most patients from our series were managed following the treat-to-target strategy, with a good control of the disease activity.

In conclusion, our results suggest that CXCL5 does not represent a link between inflammation and IR in RA patients. Further studies aimed to determine if the mechanisms leading to IR in patients with RA may be different from those of the general population are warranted.

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