

CXCL 9 and CXCL 10 as Sensitive Markers of Disease Activity in Patients with Rheumatoid Arthritis

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ABSTRACT. *Objective.* To assess whether serum levels of CC and CXC chemokines correlate with disease activity in patients with rheumatoid arthritis (RA), and to determine whether these effects predict clinical response.

Methods. Serum levels of the chemokines CC (CCL2, CCL5) and CXC (CXCL8, CXCL9, CXCL10) were quantified at baseline and after 12 weeks of treatment with disease-modifying antirheumatic drugs or biologic agents in 28 patients using flow cytometry. Serum from 40 healthy individuals was collected for comparison at baseline. Response to treatment was classified according to the European League Against Rheumatism (EULAR) response criteria. Remission of disease was defined as a Disease Activity Score < 2.6.

Results. The baseline serum concentrations of CC and CXC chemokines were significantly elevated in patients with active RA compared to healthy controls ($p < 0.05$) except for CCL2. Significant improvement in all disease activity measurements was observed after 12 weeks of treatment. Seventeen (60.7%) patients achieved good to moderate response based on the EULAR response criteria, and 5 (17.9%) patients achieved remission. The improvement in clinical activity in patients with RA was accompanied by a significant reduction in the serum concentration of CXCL9 and CXCL10 ($p < 0.001$). A significant reduction in the serum level of CXCL10 was also observed in the group that achieved EULAR response. Serum concentration of CCL5 remained significantly elevated in patients with RA ($n = 5$) who achieved remission compared to the healthy controls ($p < 0.05$).

Conclusion. Serum concentration of CXCL9 and CXCL10 may serve as sensitive biomarkers for disease activity in patients with RA. (First Release Dec 23 2009; J Rheumatol 2010;37:257–64; doi:10.3899/jrheum.090769)

Key Indexing Terms:

RHEUMATOID ARTHRITIS

SERUM CHEMOKINES

DISEASE ACTIVITY

DISEASE-MODIFYING ANTIRHEUMATIC DRUG

Rheumatoid arthritis (RA) is an immune-mediated inflammatory disease of unknown etiology that affects synovial tissue in multiple joints¹. Inflamed synovial tissue in RA comprises vastly increased numbers of inflammatory cells including macrophages, lymphocytes, leukocytes, and also resident stromal elements such as fibroblast-like synovio-cytes (FLS)². Chemokines are small chemoattractant cytokines that play a key role in the accumulation of inflammatory cells at the site of inflammation. It is believed that in

rheumatoid synovium, FLS and macrophages are dominant chemokine producers³.

Chemokines are classified into 4 families according to the location of cysteine residues. The 4 chemokine groups are CC, C, CXC, and CX₃C, where C is a cysteine and X is any amino acid residue^{3–6}.

The 2 major subclasses of chemokines in RA include the CC chemokine and the CXC chemokine. CCL2 and CCL5 are chemokines belonging to the CC family, while CXCL8, CXCL9, and CXCL10 belong to the CXC family^{3–6}. These chemokines have been shown to be crucial in selective accumulation of mononuclear cells into synovium, leading to initiation and progression of synovitis, especially in RA^{7–10}. The CXC chemokines mainly act on neutrophils and lymphocytes, while the CC chemokines act on monocytes and lymphocytes without affecting neutrophils¹¹. Besides the chemoattractant property, CXCL8 has an angiogenic property, which enhances synovial proliferation in patients with RA¹². Chemokines also stimulate FLS and chondrocytes in RA to release inflammatory mediators, including matrix metalloproteinase and various inflammatory cytokines, which lead to cartilage degradation and pannus formation¹³.

Many studies have shown that these chemokines are

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highly expressed in synovial tissue and synovial fluid of patients with RA^{4,22} and serum levels of CCL2, CCL5, CCXCL8, and CXCL10 were also increased in patients with active RA^{7-10,23-26}. Chemokines are mainly induced by inflammatory cytokines, including interleukin 1 β , tumor necrosis factor- α (TNF- α), and interferon- γ (IFN- γ)^{3,13,27-30}. Infliximab is a chimeric IgG monoclonal antibody that neutralizes both soluble and membrane-bound TNF- α . It works by downregulating the production of cytokines and chemokines, which play an important role in the inflammation cascade. As for leflunomide, the anti-inflammatory activity has been shown to be related to inhibition of neutrophil migration and suppression of proinflammatory cytokines³¹. Changes in the serum level of chemokines in patients with RA have been demonstrated after treatment with infliximab or leflunomide in combination with methotrexate (MTX)^{7-10,23-26}. Reduction of serum concentrations of CCL5, CCL2, and CXCL8 had been found to be associated with improvement in the clinical activity of patients with RA who were treated with leflunomide^{7,8}, MTX⁹, or infliximab in some^{10,23} but not all studies²⁴⁻²⁶. Whether serum levels of these chemokines are useful as markers of disease activity in patients with RA remains controversial.

CXCL9 and its receptor, CXCR3, had been shown in studies to be highly expressed in the synovial tissue of patients with RA. It had been suggested that they might play a significant role in the pathophysiology of RA³². There is also a study suggesting that CXCL9 may be an important chemokine in autoimmune arthritis, including RA³³. Whether serum concentration of CXCL9 could reflect disease activity in patients with RA has never been studied.

Since these chemokines are specific for pathogenesis in RA and are highly expressed in the synovial tissue and synovial fluid of patients with RA, we hypothesized that serum concentration of these chemokines might be useful for monitoring disease activity. The aims of our prospective cohort study were to evaluate whether the changes in serum CC and CXC chemokine levels (CCL2, CCL5, CXCL8, CXCL9, and CXCL10) correlated with disease activity in patients with active RA, and to determine whether these effects predicted clinical response.

MATERIALS AND METHODS

Patient selection. Patients with active RA disease followed at the rheumatology clinic of the Prince of Wales Hospital were recruited for this study. All patients fulfilled the 1987 American College of Rheumatology criteria for RA³⁴. Active disease was defined as a Disease Activity Score (DAS28) > 3.4³⁵ and requiring prednisolone, disease-modifying antirheumatic drugs (DMARD), or biological agents.

Disease activity and clinical response were assessed using DAS28 and the European League Against Rheumatism (EULAR) response criteria³⁶, respectively. After 12 weeks of treatment, patients with moderate to good response according to the EULAR criteria were classified as EULAR responders, while patients who failed to achieve the EULAR response were classified as EULAR nonresponders. Remission was defined as a DAS28 <

2.6 after 12 weeks of treatment³⁶. Serum samples from 40 age-matched healthy controls were obtained for comparison.

The protocol was approved by the Clinical Research Ethics Committee of The Chinese University of Hong Kong-New Territories East Cluster Hospitals, and informed consent was obtained from all participants according to the Declaration of Helsinki.

Followup protocol. Clinical and laboratory assessment was performed at baseline and then every 4 weeks, up to Week 12. Clinical assessment included a visual analog scale for pain (0 = no pain and 10 = worst pain imaginable), the number of swollen and tender joints, the patient's global assessment, a validated version of the Chinese Health Assessment Questionnaire (HAQ), and the physician's global assessment. Laboratory assays included complete blood count, blood chemistry, urinalysis, liver function tests, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). Treatment was adjusted at each visit according to the DAS score with the aim of achieving remission.

Assay of plasma chemokines. Serum concentrations of CCL2, CCL5, CXCL8, CXCL9, and CXCL10 at baseline and 12 weeks after treatment were simultaneously measured with a human chemokine cytometric bead array (CBA) Kit I (BD Pharmingen Corp., San Diego, CA, USA) using a flow cytometer (FACSCalibur, BD Biosciences Corp., San Jose, CA, USA), BD CellQuestTM software, and BDTM CBA software (Ref. Lit 2006).

Statistical analysis. Data were analyzed using the Statistical Package for the Social Sciences for Windows, Version 13 (SPSS Inc., Chicago, IL, USA). Demographics data of the subjects were presented as means \pm standard deviation (SD). The differences in clinical outcomes measured between Week 0 and Week 12 were assessed by the Wilcoxon signed-rank test.

The differences in plasma CCL2, CCL5, CXCL8, CXCL9, and CXCL10 before and after treatment were assessed by a test of analysis of variance (ANOVA Friedman test). Results were expressed as either median (interquartile range) or mean \pm SD. Spearman's rank correlation test was used to assess the correlations among reductions in plasma chemokines and clinical outcomes.

RESULTS

Baseline clinical and demographics features. Twenty-eight consecutive patients with active RA disease were recruited for our study, including 26 women and 2 men. The mean age was 49 ± 13 years, and the mean disease duration 7.3 ± 6.0 years. Twenty (71.4%) of them were rheumatoid factor-positive. Bone erosions on radiographs were observed in 14 patients (50%). The median DAS28 score was 5.1 (range 4.5–5.6; Table 1). Six (21.4%) patients were taking prednisolone, with an average dose of 10 mg daily, and 21 patients (75%) were taking MTX, with an average dose of 12.5 mg/week for 3 months after Week 0. Details of other medications are shown in Table 2.

Serum levels of chemokines in patients with active RA and controls. At baseline, the serum concentrations of CCL5, CXCL8, CXCL9, and CXCL10 were all significantly elevated in patients with RA compared to controls (all $p < 0.05$; Table 3). Serum concentrations of CCL2 were similar between patients with RA and controls.

Clinical outcome and serum chemokines. The changes in disease activity measures of patients with RA are summarized in Table 3. Significant improvement was observed in all clinical outcome measures after 12 weeks of immuno-

Table 1. Patient characteristics and demographics.

| Characteristic | All RA, n = 28 | EULAR Responders, n = 17 | EULAR Nonresponders, n = 11 | p |
|---------------------------------------|-------------------|--------------------------------|-----------------------------------|--------|
| Sex (male/female) | 26/2 | 16/1 | 10/1 | |
| Age, yrs (mean \pm SD) | 49 \pm 13 | 48.4 \pm 14.8 | 48.5 \pm 11.7 | NS |
| Disease duration (mean \pm SD), yrs | 7.3 \pm 6.0 | 8.3 \pm 7.5 | 6.0 \pm 5.1 | NS |
| Rheumatoid factor-positive (n, %) | 20 (71.4) | 12 (70.5) | 8 (72.2) | NS |
| Erosion on radiographs, n (%) | 14 (50) | 7 (41.1) | 7 (63.3) | < 0.05 |
| DAS28 ESR | 5.1 (4.5–5.6) | 5.4 (4.8–5.8) | 5.0 (3.7–5.6) | NS |
| CRP, mg/l | 12.2 (5.6–28.3) | 13.1 (5.7–40.7) | 11.3 (5.5–23.4) | NS |
| ESR, mm/h | 38.0 (25.2–78.7) | 40 (25.5–82) | 36 (20.0–75.0) | NS |
| Physician global assessment | 6.2 (5.0–8.0) | 7.0 (5.0–8.0) | 6.0 (5.0–8.0) | NS |
| Swollen joint count | 7.0 (5.0–9.2) | 6 (5.0–9.0) | 8 (5.0–10.0) | NS |
| Tender joint count | 8.0 (4.7–13.2) | 11 (6.0–15) | 5.0 (4.0–9.0) | NS |
| HAQ score | 1.1 (1.0–1.6) | 1.12 (1.0–1.68) | 1.0 (0.87–1.62) | NS |
| Patient global assessment | 6.0 (6.7–7.0) | 7.0 (6.0–8.0) | 6.0 (6.0–7.0) | NS |

Data are expressed as median (interquartile range). EULAR: European League Against Rheumatism; DAS: Disease Activity Score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; HAQ: Health Assessment Questionnaire; NS: not significant.

Table 2. Details of therapy.

| Drugs | Week 0 | | Week 12 | | |
|--------------------|----------|-------------------------|----------|-----------------|-------------------------------|
| | N (%) | Median (IQR) | N (%) | No. of Patients | Median (IQR) |
| Prednisolone | 7 (25) | 5 mg/day (5–15) | 6 (21.4) | –1 | 10 mg/day (5–20) |
| Methotrexate | 4 (14.2) | 15 mg/week (11.25–16.8) | 21 (75) | +14 | 12.5 mg/week (10–15) |
| Sulfasalazine | 7 (25) | 1 g/day (1–1.5) | 7 (25) | 0 | 1 g/day (1–1.5) |
| Hydroxychloroquine | 2 (7) | 300 mg/day (200–400) | 1 (3.5) | –1 | 200 mg/day |
| Leflunomide | 2 (7) | 15 mg/day (10–20) | 3 (10.7) | +1 | 20 mg/day (10–20) |
| Infliximab | 0 | | 2 (7) | +2 | 3 mg/kg |
| Ocrelizumab | 0 | | 3 (10.7) | +3 | 200 mg fortnightly \times 1 |
| Single DMARD | 5 | | 17 | | |
| Double DMARD | 3 | | 11 | | |
| Triple DMARD | 1 | | 0 | | |

DMARD: disease-modifying antirheumatic drug. * At baseline and then 2 weeks later.

Table 3. Baseline demographic and serum concentration of chemokines in RA patients and control group.

| | RA, n = 28 | Controls, n = 40 |
|--------------------------|------------------------|------------------|
| Age, yrs (mean \pm SD) | 49 \pm 13 | 38 \pm 9 |
| Sex (female/male) | 26/2 | 39/1 |
| Serum chemokines, pg/ml | | |
| CXCL10 | 2255.7 (1430.7–4196.1) | 737 (597–864)* |
| CCL2 | 55.8 (30.1–106.0) | 33.5 (22.3–47.1) |
| CXCL9 | 3062.0 (1245.7–4899.0) | 203 (159–250)* |
| CXCL8 | 17.9 (13.1–25.7) | 5.2 (3.7–8.9)* |
| CCL5 | 7899.8 (5398.6–9036.9) | 612 (356–1843)† |

Data are expressed as median (interquartile range). † $p < 0.05$, * $p < 0.005$.

suppressive therapy (all $p < 0.05$). Five (18.5%) patients were able to achieve remission, with a DAS28 < 2.6 .

After 12 weeks, a significant reduction in the serum concentration of CXCL10 and CXCL9 ($p < 0.001$) was

observed (both $p < 0.05$; Table 4). The serum concentrations of other chemokines did not show significant changes.

We next analyzed whether serum concentrations of chemokines can predict the clinical response to treatment. At Week 12, 17 (60.7%) patients showed moderate to good response with treatment based on EULAR response criteria (EULAR responders), while 11 (17.7%) patients showed no clinical improvement (EULAR nonresponders). The demographics, clinical characteristics, and disease activity measurements between the 2 groups were similar, except that the prevalence of bone erosion on radiographs was increased in the EULAR nonresponders (Table 1). There were no significant differences in serum concentrations of CXC and CC chemokines between these 2 groups of patients at baseline (Table 5). After 12 weeks of treatment, a significant reduction in serum level of CXCL9 was observed in both groups, while the serum level of CXCL10 was significantly reduced only in the EULAR responders (Table 4; $p < 0.005$). The levels of CCL2, CCL5, and CXCL8 remained unchanged in

Table 4. Changes in disease activity measures and serum chemokine levels of all RA patients (n = 28) before and after 12 weeks of treatment.

| | Baseline | Week 12 | p |
|-----------------------------|------------------------|-------------------------|---------|
| CRP, mg/l | 12.2 (5.6–28.3) | 2.1 (1.0–7.7) | < 0.001 |
| ESR, mm/h | 38.0 (25.2–78.7) | 20 (13.5–53.5) | 0.001 |
| Physician global assessment | 6.2 (5.0–8.0) | 3 (1.2–4.2) | 0.001 |
| Swollen joint count | 7.0 (5.0–9.2) | 2 (1.0–6.0) | < 0.001 |
| Tender joint count | 8.0 (4.7–13.2) | 3.0 (1.0–7.0) | 0.002 |
| DAS28 ESR | 5.1 (4.5–5.6) | 3.5 (2.9–4.7) | < 0.001 |
| HAQ score | 1.1 (1.0–1.6) | 1.1 (0.5–1.3) | 0.014 |
| Patient global assessment | 6.0 (6.7–7.0) | 5.0 (3.4–7.0) | 0.04 |
| Serum chemokines, pg/ml | | | |
| CXCL10 | 2255.7 (1430.7–4196.1) | 1713.0 (1318.5–2356.5) | < 0.001 |
| CCL2 | 55.8 (30.1–106.0) | 54 (32.2–93.5) | 0.221 |
| CXCL9 | 3062.0 (1245.7–4899.0) | 1302.9 (836.2–2350.3) | < 0.001 |
| CXCL8 | 17.9 (13.1–25.7) | 17.6 (11.0–31.5) | 0.683 |
| CCL5 | 7899.8 (5398.6–9036.9) | 8299.9 (4657.6–11978.6) | 1.0 |

Data are expressed as median (interquartile range). CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DAS: Disease Activity Score; HAQ: Health Assessment Questionnaire.

Table 5. Changes in serum concentrations of chemokines of the EULAR responders vs EULAR nonresponders before and after 12 weeks of treatment.

| Serum chemokines, pg/ml | EULAR Responders, n = 17 | | EULAR Nonresponders, n = 11 | |
|-------------------------|--------------------------|-------------------------|-----------------------------|--------------------------|
| | Baseline | Week 12 | Baseline | Week 12 |
| CXCL10 | 2161.1 (1463.3–5567.8) | 1628.2 (1020.5–2411.1)* | 2305.7 (1333.1–3603.4) | 1787.8 (1464.1–2338.4) |
| CCL2 | 58.08 (28.1–330.5) | 55.5 (32.4–94.3) | 51.6 (30.8–71.4) | 52.5 (31.6–71.4) |
| CXCL9 | 3605.4 (1408.9–5701.6) | 1511.4 (855.8–2585.1)* | 2811.2 (1142.4–4631.5) | 1179.7 (730.3–1934.9)† |
| CXCL8 | 17.9 (14.9–35.2) | 14.6 (9.5–31.8) | 16.0 (11.0–23.8) | 18.7 (13.8–29.8) |
| CCL5 | 8203.6 (6928.7–9221.1) | 8019.0 (4694.0–11978.6) | 6103.8 (3927.2–8975.6) | 8580.9 (43412.2–11978.6) |

Data are expressed as median (interquartile range). † p < 0.05, * p , 0.005. EULAR: European League Against Rheumatism.

both groups. We also compared the serum concentrations of chemokines at baseline in a group of patients (n = 5) who had achieved remission with a group of patients who did not achieve remission. There was no significant difference between the serum levels of chemokines between these 2 groups (data not shown).

Next we compared the serum concentrations of chemokines in a group of patients (n = 5) who had achieved remission at Week 12 with a healthy control group. The serum concentrations of CCL5 were significantly elevated in this group of patients compared to the controls (p = 0.024). There were no significant differences in the levels of CCL2, CXCL8, CXCL9, and CXCL10 compared to the controls (data not shown).

We also performed a few analyses to assess the possible correlation between the changes of serum chemokines and the usage of DMARD. First, we compared patients who were taking DMARD (n = 9) with those who were not (n = 19) at baseline. Second, we compared patients who were treated with biologic agents (n = 5) with those who were not (n = 23) at Week 12. Third, we compared patients who were treated with MTX (n = 21) with those who were not (n = 7) at Week 12. We could not demonstrate any significant dif-

ference in the serum chemokines in these groups of patients (data not shown).

Correlations of plasma chemokines and clinical outcome measure. At baseline, serum concentration of CCL5 had a significant positive correlation with ESR (r = 0.564, p = 0.003) and CRP (r = 0.495, p = 0.010). The serum concentration of CCL2 also showed significant positive correlation with HAQ (r = 0.508, p = 0.01; data not shown).

As shown in Table 6, changes in the serum concentration of CXCL8 correlated with changes in the tender joint count (r = 0.517, p = 0.014) and the patient global assessment (r = 0.428, p = 0.047) after 12 weeks of treatment. Reduction in the serum concentration of CXCL9 also correlated with changes in the HAQ score (r = 0.533, p = 0.011). There was also a trend suggesting a positive correlation between changes in the serum concentration of CXCL10 and the tender joint count (p = 0.093).

DISCUSSION

This is the first study to examine the effect of immunosuppressive therapy on the serum levels of CXCL9 in patients with active RA. In our study, the baseline serum concentrations of chemokines CCL5, CXCL8, CXCL9, and CXCL10

Table 6. Correlations of changes in plasma chemokines and clinical outcome measures after 12 weeks of treatment in patients with RA.

| Measure | CXCL10 | CXCL9 | CCL2 | CCL5 | CXCL8 |
|-----------------------------|----------------|----------------|----------------|---------------|----------------|
| CRP | 0.005 (0.982) | 0.033 (0.883) | 0.007 (0.974) | 0.238 (0.287) | 0.284 (0.201) |
| ESR | 0.168 (0.454) | 0.019 (0.932) | 0.160 (0.476) | 0.416 (0.54) | 0.282 (0.203) |
| DAS28 ESR | 0.277 (0.212) | 0.094 (0.676) | 0.298 (0.179) | 0.174 (0.431) | 0.400 (0.065) |
| HAQ score | 0.154 (0.494) | 0.533 (0.011)* | 0.296 (0.182) | 0.271 (0.222) | 0.289 (0.192) |
| Physician global assessment | 0.119 (0.598) | 0.091 (0.687) | -0.034 (0.882) | 0.148 (0.511) | 0.172 (0.444) |
| Patient global assessment | -0.043 (0.849) | -0.226 (0.312) | 0.142 (0.528) | 0.263 (0.236) | 0.428 (0.047)* |
| Swollen joint score | 0.189 (0.399) | 0.074 (0.743) | 0.054 (0.813) | 0.162 (0.472) | 0.139 (0.537) |
| Tender joint score | 0.367 (0.093) | 0.122 (0.590) | 0.275 (0.216) | 0.218 (0.330) | 0.517 (0.014)* |

Results are expressed as correlation coefficient *r* (p value). * p value < 0.05 is considered significant using Spearman's rank correlation test. CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; HAQ: Health Assessment Questionnaire.

in patients with active RA were significantly elevated compared to healthy controls. After 12 weeks of treatment, significant reduction in the serum levels of CXCL9 and CXCL10 was observed, in parallel with the improvement in the clinical activity measurements. In the subgroup analysis, a significant reduction in the serum level of CXCL10 was seen only in patients who responded to treatment, compared to CXCL9 levels, which were reduced in both groups of patients. The only significant positive correlation of the reduction of these 2 chemokines with clinical measurements was between serum levels of CXCL9 and HAQ score. However, there was a trend suggesting a positive correlation between changes in the serum concentration of CXCL10 and tender joint count ($p = 0.093$), although it was not statistically significant. We also tried to assess whether the serum concentrations of these chemokines can predict clinical outcomes or can serve as predictors for treatment response in patients with active RA. Thus we compared the serum levels of these chemokines between EULAR responders and EULAR nonresponders and also between patients who were in remission ($n = 5$) and those who were not, at baseline. No significant differences were observed in either comparison.

It would be difficult to state the importance of chemokines CXCL9 and CXCL10 in active RA in our study as there was no positive correlation between the changes of serum levels of these 2 chemokines with most of the clinical measurements except for CXCL9 and HAQ. This might be because the sample size ($n = 28$) of this study was small and the duration was short (12 weeks). A study with a larger sample size could have demonstrated a more significant positive correlation, as there was a trend suggesting a positive correlation between CXCL10 and tender joint count. Nevertheless, we could assume that chemokines CXCL9 and CXCL10 may play an important role in the pathogenesis of RA and that they may be useful as sensitive biological markers for disease activity in patients with RA, based on these findings.

In addition, we could not demonstrate that the changes of these chemokines predict clinical response, as there was no significant difference in serum levels of chemokines at baseline between patients who responded to treatment compared to those who did not.

CXCL9 and CXCL10 are chemokines of the CXC chemokine family, and they share the same receptor: CXCR3⁴. These 2 chemokines and their receptor are predominantly found to be highly expressed in Th1 cells, and Th1/Th2 cytokine imbalance with a predominance of Th1 cytokines is suspected to be of pathogenic importance in RA^{37,38}. Synovium of RA is characterized by infiltration of abundant Th1 cells, and the majority of RA synovial fluid T lymphocytes express CXCR3³⁹⁻⁴¹. This CXCR3 protein was found to be preferentially expressed on mast cells within synovial tissue from patients with RA. These findings suggest that substantial expression of CXCR3 protein on mast cells within RA synovial tissue plays a significant role in the pathophysiology of RA, accompanied by elevated levels of the chemokines CXCL9 and CXCL10³². However, it is also believed that CXCL9 and CXCL10 are secreted by activated synovial fluid leukocytes interacting with fibroblasts that might contribute to the migration of Th1 cells through CXCR3 in the development of RA³². Chemokines CXCL9 and CXCL10 have been shown to be highly expressed in RA synovial tissues and fluids^{32,39-41}, and the concentration gradient of CXCL9 and CXCL10, between the serum and synovial fluid, favors the migration of receptor-expressing cells from the blood into synovium in RA⁴⁰. In addition, a study suggested that CXCL10 plays a critical role in the infiltration of CD4+ T cells and resulted in bone destruction in the inflamed joints of mice⁴².

In our study, the majority of patients were treated with MTX at the end of study ($n = 21$). The average dosage was 12.5 mg/week. MTX itself is a folate analog that inhibits the proliferation of the lymphocytes and other cells responsible for inflammation in the joint. MTX inhibits the enzyme 5-aminoimidazole-4-carboxamide ribonucleotide formyl-

transferase, which leads to intracellular accumulation of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR). AICAR and its metabolites inhibit 2 enzymes important in adenosine metabolism (adenosine deaminase and adenosine monophosphate deaminase), causing intracellular accumulation of adenosine and adenine nucleotides. Dephosphorylation of these nucleotides results in increased extracellular concentrations of adenosine, which is a potent anti-inflammatory agent⁴³. Adenosine was found to be able to dampen adaptive immunity by downregulating the production of 2 pivotal proinflammatory Th1 cytokines, IFN- γ and TNF⁴⁴. Treatment with MTX reduced the serum levels of CCL2 and CCL5 in one study⁹. But serum concentration of CXCL10 was found to remain unchanged after treatment with infliximab infusion in combination with a low dose of MTX, despite improvement in overall clinical activity^{25,26}. It requires further study to determine whether a higher dosage of MTX suppressed the production of certain chemokines.

CCL2 is a member of the CC chemokine family and is produced by leukocytes, fibroblasts, endothelial cells, and chondrocytes¹⁸. Besides attracting monocytes, it also attracts T cells, natural killer cells, and basophils. Serum concentration of CCL2 has been shown to be significantly elevated in patients with active RA^{7,9,22}. In contrast, the serum level of CCL2 in our group of patients with active RA was similar to the control group. Sulfasalazine (SSZ) has been shown to suppress the secretion of CXCL8 and CCL2 in RA synovial tissue and synovial fluid⁴⁵ by inducing the release of adenosine. The binding of adenosine to its receptor has been shown to inhibit the release of CXCL8 by endothelial cells⁴⁶. In addition, a very high concentration of SSZ has been shown to inhibit nuclear factor- κ B (NF- κ B) dependent transcription in colonic epithelial cells⁴⁷. The expression of CXCL8 and CCL2 has been shown to be under the control of NF- κ B⁴⁸. NF- κ B can also induce FLS to produce CCL2⁴⁹. Thus, SSZ may function in the inhibition of chemokine CXCL8 and CCL2 through the production of adenosine and inhibition of NF- κ B⁴⁵. The use of SSZ (n = 7) in our study might have affected the serum level of CCL2. The *in vivo* effect of SSZ on these serum chemokine levels needs to be addressed in future studies.

Glucocorticoids are potent antiinflammatory and immunosuppressive agents that exert effects via a genomic action. They diffuse into the cell and bind with a cytoplasmic glucocorticoid receptor, which moves to the nucleus, where it induces the transcription of I- κ B. This action inactivates NF- κ B, decreasing proinflammatory cytokine production as well as inducing genes to inhibit cyclooxygenase-2, adhesion molecules, and other inflammatory mediators⁵⁰. The effect of glucocorticoids on the serum chemokines has been addressed in studies mainly involving patients with multiple sclerosis (MS), but not in patients with RA. One study demonstrated that intravenous methyl-

prednisolone reduced the serum concentration of CCL5 in patients with active MS⁵¹. In this study, 6 patients were given prednisolone with an average dose of 10 mg/day. However, the serum level of CCL5 remained unchanged at Week 12, perhaps because of the low dose of prednisolone used in this study. Whether prednisolone affects the other chemokines in RA is uncertain and needs to be investigated.

CXCL8 is another member of the CXC chemokine family. It was the first chemokine identified to be involved in leukocyte chemotaxis⁵² and it also has angiogenic activity in the RA joint¹². The reduction in serum concentration of CXCL8 in our study correlated significantly with reduction of tender joint count and patient global assessment score. However, we did not observe any significant changes in the serum level before and after treatment despite improvement in clinical activity, similar to the study by Odai, *et al*²⁶. In contrast, Klimiuk, *et al*^{7,10} reported that the serum level of CXCL 8 was significantly reduced after treatment with either leflunomide or infliximab. However, the significant reduction of CXCL8 could only be seen at Week 24 in the leflunomide group⁷.

CCL5 is a member of the CC chemokine family and it is produced by T cells and synovial fibroblast¹⁹. Interestingly, in patients with RA, this chemokine was shown to be predictive of radiological erosions⁹. In our study, the serum level of CCL5 remained unchanged despite improvement in disease activity of RA, similar to results of the study by Torikai, *et al*²⁴. Other studies demonstrated reduction in the serum level of CCL5 after treatment with leflunomide, MTX, or infliximab^{7,9,10}.

There are some limitations in our study. First, as discussed, the duration was relatively short and the sample size was small. Longer duration of followup may be required for changes of other chemokines to be observable and larger sample sizes would be required to assess whether the concentrations of the various chemokines differ in patients with RA who achieved remission compared with those who did not or compared to controls. Second, the different DMARD and biologic agents used in this study may suppress the inflammatory processes via different mechanisms. This could alter the effect of the chemokines. However, analysis showed no correlation between serum chemokines and treatment. Third, a few other important chemokines that had been shown to play important roles in RA were not included in our study, because samples were running out by the end. Studies have demonstrated that CX3CL1 and CCL18 were also elevated in the synovial tissue and serum of patients with active RA, and treatment with anti-TNF will reduce the serum level of these chemokines^{26,53-56}. Thus, it is essential to include these important chemokines in a future similar study.

Significant reduction of the serum concentration CXCL9 and CXCL10 was observed after 12 weeks of immunomodulating therapy in patients with active RA. Serum concen-

trations of these 2 chemokines might serve as novel biomarkers for disease activity in patients with RA.

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