EXTENDED REPORT

# Serum IL-6 and IL-21 are associated with markers of B cell activation and structural progression in early rheumatoid arthritis: results from the ESPOIR cohort

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Received 27 October 2011
Accepted 23 February 2012
Published Online First 24 April 2012

## ABSTRACT

**Objective** To identify a specific pattern of serum cytokines that correlates with the diagnosis, activity and severity of rheumatoid arthritis (RA) in patients with early RA as well as with the level of serum markers of B cell activation.

**Methods** Serum interleukin (IL)-1β, IL-1 receptor antagonist (IL1-Ra), IL-2, IL-4, IL-6, IL-10, IL-17, IL-21, monocyte chemotactic protein 1 (MCP-1), tumour necrosis factor α and interferon γ levels were measured in the (ESPOIR) Etude et Suivi des POlyarthrites Indifférenciées Récentes early arthritis cohort, which included patients with at least two swollen joints for >6 weeks and <6 months, and no previous corticosteroids or disease-modifying antirheumatic drugs. Serum cytokine levels were compared between patients who met the 1987 American College of Rheumatology criteria for RA (n=578) or had undifferentiated arthritis (UA, n=132) at the 1-year follow-up visit.

**Results** Serum IL-6 and IL-21 were the only cytokines that discriminated RA from UA on univariate analysis. IL-6 level was associated with RA, whereas erythrocyte sedimentation rate and C-reactive protein were not. Higher proportions of rheumatoid factor and anticyclic citrullinated protein (CCP) positivity, levels of markers of B cell activation, and a higher frequency of rapid radiographic progression were observed in patients with RA with detectable IL-6 or IL-21. Multivariate analysis associated IL-6 and anti-CCP levels with radiographic erosions at enrolment with 1-year radiographic progression.

**Conclusion** Serum IL-6 concentration is greater in RA than in UA. Increase in serum IL-6 and IL-21 levels is associated with markers of B cell activation, and IL-6 is associated with radiographic progression in patients with RA.

## INTRODUCTION

Serum markers of B cell activation, such as serum levels of IgG, IgA, free light chains of immunoglobulin (FLCs) and β2-microglobulin, have been reported to be increased in early rheumatoid arthritis (RA).[1] Although this remains controversial, these increases do not seem to be associated with an increase in serum B cell activating factor of the tumour necrosis factor (TNF) family (BAFF), despite its pivotal role in B cell activation.[1–3] This activation may be due to the effects of other cytokines known to drive B lymphocyte activation, such as interleukin (IL)-4, IL-6, IL-10 or IL-21. Cytokines and chemokines are key players in rheumatoid inflammation and joint damage,[4 5] in addition to their potential involvement in B cell activation.

Very few studies have focused on the profile of serum cytokines in patients with untreated early RA. The serum concentrations of these cytokines may be very sensitive to therapeutics and should therefore be assessed before any disease-modifying antirheumatic drugs (DMARDs) or steroid therapy are started.[6 7] Given the tight regulation of cytokine secretion, the concomitant assessment of multiple cytokines should provide a better insight into the pathogenesis of the disease.[8 9] Moreover, their pathogenic role can be evaluated only in a well-characterised series of patients that are followed longitudinally by assessing them simultaneously by sensitive techniques. We have therefore made such measurements in samples from patients with untreated early arthritis prospectively followed-up in the setting of the multicentre ESPOIR cohort. We determined the serum concentrations of 10 cytokines and one chemokine in patients with early arthritis and investigated whether their levels would discriminate patients developing RA from those whose arthritis remained undifferentiated (undifferentiated arthritis (UA)). Subsequently, we analysed patients with RA as to the relationship between these cytokines and immunological features of B cell activation or radiographic progression.

## PATIENTS AND METHODS

### Patients

The French multicentre prospective cohort of patients with early arthritis (ESPOIR) included 813 patients between December 2002 and March 2005 with a 10-year follow-up. These patients had had at least two swollen joints persisting for more than 6 weeks but less than 6 months, and had not been given DMARDs or corticosteroids at inclusion. Their inclusion clinical, immunological and radiological features have been previously published.[10 11]

A total of 83 patients missed the 1-year visit and were not included in this study. Another 20 patients who fulfilled the American College of Rheumatology (ACR) or international consensus group criteria for other arthritides were excluded. RA diagnosis was defined after 1 year of follow-up according to cumulative 1987 ACR criteria for RA (regardless of testing positive for antibodies to cyclic citrullinated protein (CCP)). Patients without a definite diagnosis until the 1-year follow-up visit were diagnosed as having UA. We therefore analysed the 710 patients who completed the first three visits (baseline, 6 months and 1 year) and were diagnosed as having RA or UA after 1 year of follow-up. The Montpellier Ethics Committee approved the study in July 2002, and all the patients and controls gave their informed consent.

### Serum assays

Serum samples were collected from the cohort together with samples from 50 healthy blood donors. All samples were immediately stored at −80°C. One biological resource centre (Paris Bichat, Joelle Benessiano) was responsible for centralising and managing biological data collection. Serum IL-21 was assessed using ELISA (Ebioscience, San Diego, California, USA), with a threshold of detection of 50 pg/ml. The concentrations of IL-1β, IL-1 receptor antagonist (IL1-Ra), IL-2, IL-4, IL-6, IL-10, IL-17, MCP-1, TNFα and interferon (IFN)γ in the serum of all patients and controls were assayed using a commercially available multiplex bead immunoassay, based on the Luminex platform (Fluorokine MAP Multiplex Human Cytokine Panel, R&D Systems, Minneapolis, Minnesota, USA) according to the supplier's instructions, as previously reported.[4]

Thresholds for detecting all these cytokines were 1 pg/ml, except for IL-6 (1.5 pg/ml) and IL-1Ra (10 pg/ml). The methods used to assess serum markers of B cell activation (β2-microglobulin, IgG, IgA, IgM, FLCs and BAFF) and autoantibodies, and the results, have been previously reported.[1]

### Radiological data

At baseline and at 1 year, radiographs were taken of the hands and wrists (anteroposterior view) and of the feet (anteroposterior and oblique views). Their interpretation was standardised as described previously.[10 11] Radiographic progression was defined as an increase in total Sharp/van der Heijde score (SHS) of 1 or more between inclusion and 1 year.

### Statistical analysis

Continuous data are presented as medians with IQR ranges. We used non-parametric tests to analyse continuous variables because the distribution of data was uneven. The Mann–Whitney U test was used to compare continuous data, and the $\chi^2$ test to compare nominal data. Correlations were studied with Spearman's rank test. Biological markers associated by univariate analysis with the type of arthritis or, in RA, with the presence of initial erosions or 1-year radiographic progression were included in multivariate logistic regression models. All statistical analyses were performed with Stata SE V.9.2.

## RESULTS

### Characteristics of the population

The median age of the 710 patients (76.5% females) was 49 (40–64) years; 48.3% of the patients were IgM-rheumatoid factor (RF)-positive and 40.0% anti-CCP-positive. Among patients with anti-CCP, median levels were 516.0 U/ml (215.7–1087.0). The median tender joint count, swollen joint count, patient's disease activity and erythrocyte sedimentation rate (ESR) were 6 (3–12), 6 (3–10) 64 (44–79) and 22 (11.2–38.0), respectively. The median Disease Activity Score 28 (DAS28) was 5.1 (4.3–5.9) and the median Health Assessment Questionnaire (HAQ) was 0.9 (0.4–1.4). At the 1-year follow-up visit, 578 patients (79.2%) had met the 1987 ACR criteria for RA and were classified as having RA, and 132 patients still had UA. Using the new European League Against Rheumatism (EULAR)/ACR criteria for early RA,[12] 83% of the patients fulfilled the criteria of RA.

### Serum cytokine concentrations in patients with early arthritis and healthy controls

IL-2, IL-10 and IL-17 were not detected in the serum of the healthy controls. MCP-1 and IL1-Ra were detected in the serum of all patients with early arthritis, and TNFα and IL-6 were detected in 80% and 65% of them, respectively. The other seven cytokines were found in fewer than 22% of patients. The serum concentrations of all cytokines except IL-1β were higher in patients with early arthritis than in the limited number of healthy controls tested (n=50, table 1).

Serum IL-21 was mainly detected in patients with detectable IL-6, as IL-21 was detectable in 32.0% of patients with detectable IL-6 and in only 14.0% of patients without detectable IL-6 (p=0.007). Three-quarters (74.8%) of the patients with detectable IL-21 had detectable IL-6 levels. However,

**Table 1** Serum markers in patients with early arthritis and controls

| Serum marker | Patients with early arthritis (n=710) | Controls (n=50) | p Value |
|---|---|---|---|
| IL-1β | 1.6 (1.3–2.8) | 1.1 (1.1–1.6) | 0.1 |
| IL-1Ra | 991.4 (729.2–1434.4)* | 397.7 (295.2–562.5)* | <0.0001 |
| IL-2 | 2.3 (1.3–4.5) | NR | NR |
| IL-4 | 2.2 (2.1–4.7) | NA | NR |
| IL-6 | 8.8 (3.5–20.2) | NR | NR |
| IL-10 | 1.7 (1.3–3.1) | NR | NR |
| IL-17 | 1.7 (1.1–3.8) | NR | NR |
| IL-21 | 291.6 (168.7–661.3)* | 94.6 (77.1–128.8)* | <0.0001 |
| IFNγ | 1.9 (1.2–2.6) | 1.0 (1.0–1.0) | <0.0001 |
| TNFα | 2.4 (1.6–3.4) | 1.1 (0.6–1.3) | <0.0001 |
| MCP-1 | 991.4 (729.2–1434.4)* | 392.7 (295.2–562.5)* | <0.0001 |

Results are expressed as median (IQR) ng/ml (when 3% or more subjects had detectable levels of the assessed marker, otherwise NR).
Levels of cytokines were compared using the Mann–Whitney U test when detectable levels of cytokines had been observed in both groups (otherwise NR).
IFN, interferon; IL, interleukin; NA, not assessed; NR, not relevant; TNF, tumour necrosis factor.

no correlation was observed between IL-6 and IL-21 levels (r=−0.03, p=0.7). The serum concentrations of other cytokines did not correlate closely with one another. Of note, no correlation was observed between IL-6 and IL-17 (r=0.04, p=0.8). The concentration of IL-6 correlated significantly with biological markers of inflammation (ESR and C-reactive protein (CRP)) (r=0.6, p<0.0001 for both).

### Serum IL-6 and IL-21 concentrations in patients with RA and UA

No significant differences were observed in the levels of IL-1β, IL-1Ra, IL-2, IL-4, IL-10, IL-17, TNFα, IFNγ or MCP-1 between patients with RA and UA.

The only cytokines with levels significantly different in patients with RA or UA were IL-6 and IL-21, although overlap in their distribution between the two groups was seen. Detection of IL-6 was significantly more common in patients with RA (70.2%) than in those with UA (44.3%, p<0.0001) and, when detected, its serum concentration was higher in RA than in UA (9.6 (3.7–21.5) and 4.9 (2.4–14.0) pg/ml, respectively, p=0.002). Sensitivity, specificity, positive predictive value and negative predictive value of baseline detectable IL-6 and anti-CCP to predict RA versus UA were 70.2% and 47.3%, respectively, 55.6% and 86.7%, respectively, 85.8% and 93.1%, respectively, and 32.8% and 30.0%, respectively. Detection of IL-21 tended to be more common in patients with RA (21.2% vs 14.6% in UA, p=0.08), and, when detected, IL-21 serum concentration was significantly higher (305.2 (183.4–812.0) pg/ml in RA and 178.6 (143.9–285.5) pg/ml in UA, p=0.007).

Univariate analysis also showed that the presence of ESR, CRP and anti-CCP was associated with RA (table 2). In the 25.2% of patients with early arthritis and CRP level <5 mg/dl at enrolment, detection of IL-6 was also significantly more common in those with RA than those with UA (38.3% vs 17.5%, p=0.02). In patients without RF, IL-6 was detectable in 55.0% of those with RA versus 43.2% of those with UA (p=0.04). In patients without anti-CCP, IL-6 was detectable in 55.7% of those with RA versus 40.4% of those with UA (p=0.005).

In multivariate analysis, RA was associated with detectable IL-6 (OR 1.9 (95% CI 1.2 to 2.9), p=0.004) and the presence of anti-CCP at enrolment (OR 5.5 (95% CI 3.2–9.5), p<0.0001), but not with IL-21 level, ESR or CRP (table 2). In a second model of multivariate analysis taking into account baseline levels of IL-6 and anti-CCP, RA was associated with higher levels of IL-6 (OR 1.04 (95% CI 1.02 to 1.08), p=0.001) and higher levels of anti-CCP antibodies (OR 1.001 (95% CI 1.0003 to 1.002), p=0.002), but not with levels of IL-21, ESR or CRP. No combination of cytokines was of better diagnostic value than IL-6 alone. Only IL-6 was associated with RA (71.2% of detectable IL-6 in RA vs 49.2% in UA) according to 2010 EULAR/ACR criteria in multivariate analysis (OR 2.5 (95% CI 1.8 to 3.5), p=0.001).

We subsequently investigated the relationship between serum cytokine concentrations and specific clinical or immunological features in patients with RA.

### Association of serum IL-6 level with disease activity in patients with RA

DAS28 ESR only correlated with IL-6 level (r=0.49, p<0.0001). A raised serum IL-6 concentration correlated with each of the components of this activity score: tender joint count: r=0.09, p=0.02; swollen joint count: r=0.3, p<0.0001; patient's activity visual analogue score: r=0.3, p<0.0001; ESR: r=0.6, p<0.0001. The initial DAS28 score was higher in patients with detectable IL-6 (5.5 (4.8–6.4) vs 4.7 (4.1–5.5), p<0.0001), IL-10 (5.9 (5.2–6.7) vs 5.2 (4.4–6.1), p 0.005) and IFNγ (5.9 (5.5–6.4) vs 5.2 (4.4–6.1), p=0.003).

### Association of serum IL-6 and IL-21 levels with autoantibody secretion and markers of B cell activation in patients with RA

Only serum IL-6 and IL-21 were associated with increased proportions of autoantibodies and higher levels of markers of B cell activation. Patients with both detectable IL-6 and IL-21 were more often positive for IgM-RF or anti-CCP at enrolment than patients with detectable IL-6 but no detectable IL-21 or than patients with detectable IL-21 and no detectable IL-6 or than patients with neither detectable IL-6 nor IL-21 (table 3).

**Table 2** Comparison of baseline clinical and biological characteristics of patients with RA and UA

| Characteristic | Early RA (1) (n=578) | UA (2) (n=132) | Univariate analysis; p value (1) vs (2) | Multivariate Analysis; OR (95% CI), p value (1) vs (2) |
|---|---|---|---|---|
| Sex | 77% | 74% | 0.8 | |
| Age (years) | 50.1 (42.3–65.2) | 47.4 (39.5–62.4) | 0.6 | |
| 1 H ESR (mm) | 23.0 (12.0–40.1) | 17.0 (10.0–32.0) | 0.01 | 0.9 (0.9 to 1.0), 0.3 |
| CRP (mg/l) | 9.5 (3.0–5.0) | 6.0 (0–16.0) | 0.002 | 0.9 (0.9 to 1.0), 0.3 |
| Positivity for anti-CCP | 47.4% | 13.2% | <0.0001 | **5.5 (3.2 to 9.5), <0.0001** |
| IL-1β | 1.8 (1.4–2.9) | 1.4 (1.1–1.9) | 0.1 | |
| IL1-Ra | 1001.3 (719.1–1457.0) | 958.4 (744.2–1354.0) | 0.4 | |
| IL-2 | 2.6 (1.3–4.6) | 1.3 (1.1–2.7) | 0.1 | |
| IL-4 | 3.3 (2.2–4.7) | 2.3 (2.2–2.3) | 0.9 | |
| Detectable IL-6 | 70.2% | 44.3% | <0.0001 | **1.9 (1.2 to 2.9), 0.004** |
| IL-6 | 9.6 (3.7–21.5) | 4.9 (2.4–14 .0) | 0.002 | |
| IL-10 | 1.7 (1.3–2.8) | 3.0 (1.2–25.6) | 0.3 | |
| IL-17 | 1.7 (1.2–3.9) | 1.7 (1.1–2.2) | 0.5 | |
| Detectable IL-21 | 21.2% | 14.6% | 0.08 | |
| IL-21 | 305.2 (183.4–812.0) | 178.6 (143.9–285.5) | 0.007 | 1.01 (0.6 to 1.7), 0.9 |
| TNFα | 2.4 (1.6–3.4) | 2.3 (1.7–3.5) | 0.9 | |
| IFNγ | 1.7 (1.2–2.5) | 1.7 (1.1–4.0) | 0.9 | |
| MCP-1 | 191.0 (140.6–251.0) | 197.1 (139.5–241.9) | 0.9 | |

Results are expressed as median (IQR) pg/ml or %. Pearson's $\chi^2$ test was used to compare proportions of presence of anti-CCP, and the Mann–Whitney U test was applied to compare the other variables. Baseline markers associated on univariate analysis with RA diagnosis were analysed in multivariate analysis; bold type indicates statistical significance.
CCP, cyclic citrullinated protein; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IFN, interferon; IL, interleukin; RA, rheumatoid arthritis; TNF, tumour necrosis factor; UA, undifferentiated arthritis.

**Table 3** Association of IL-6 and IL-21 with markers of B cell activation and RF/anti-CCP positivity in patients with rheumatoid arthritis

| Marker | No detectable IL-6 or IL-21 (n=149) | Detectable IL-6 without detectable IL-21 (n=306) | Detectable IL-21 without detectable IL-6 (n=25) | Both detectable IL-6 and IL-21 (n=98) | p Value |
|---|---|---|---|---|---|
| β2-Microglobulin (mg/l) | 1.8 (1.6–2.2) | 2.1 (1.8–2.4) | 1.9 (1.6–2.1) | 2.2 (1.8–2.7) | <0.0001 |
| IgG (g/l) | 12.6 (10.9–14.8) | 13.7 (11.9–16.4) | 13.5 (11.2–16.0) | 13.7 (12.5–17.3) | 0.0001 |
| IgA (g/l) | 2.3 (1.6–2.9) | 2.8 (2.0–3.7) | 2.7 (2.1–3.1) | 3.0 (2.3–3.8) | <0.0001 |
| IgM (g/l) | 1.3 (1.0–1.8) | 1.5 (1.0–2.0) | 2.0 (1.5–2.6) | 1.7 (1.2–2.3) | 0.003 |
| κ FLCs (mg/l) | 11.0 (8.6–15.0) | 15.2 (11.7–19.3) | 12.6 (10.9–15.1) | 17.3 (13.5–22.4) | <0.0001 |
| λ FLCs (mg/l) | 13.8 (10.9–17.5) | 18.2 (14.0–23.3) | 15.7 (13.9–19.4) | 20.0 (16.7–27.3) | <0.0001 |
| IgM-RF (%) | 27.9% | 58.7% | 60.0% | 84.3% | <0.0001 |
| Anti-CCP (% | 18.4% | 42.4% | 40.0% | 73.1% | <0.0001 |

Results are expressed as medians (IQR) or %. Pearson's $\chi^2$ test was used to compare proportions of presence of IgM-RF and anti-CCP, and the Kruskal–Wallis analysis was applied to compare the other variables.
CCP, cyclic citrullinated protein; FLC, free light chain of immunoglobulins; IL, interleukin; RF, rheumatoid factor.

**Table 4** Comparison of baseline characteristics of patients with rheumatoid arthritis with or without radiographic progression at 1 year

| Characteristic | Radiographic progression at 1 year (n=165) | No radiographic progression at 1 year (n=413) | Univariate analysis; p value | Multivariate analysis; OR (95% CI), p value |
|---|---|---|---|---|
| DAS28 | 5.2 (4.4–6.0) | 5.3 (4.5–6.2) | 0.5 | |
| ESR (mm at 1st h) | 29.0 (16.0–47.2) | 20.0 (11.0–38.0) | 0.0001 | 1.0 (0.9 to 1.01), 0.8 |
| CRP (mg/l) | 13.0 (6.0–28.0) | 8.0 (0–25.0) | 0.0005 | 0.9 (0.9 to 1.1), 0.9 |
| IgM-RF positive | 70.9% | 49.2% | <0.0001 | 0.9 (0.5 to 1.7), 0.8 |
| IgA-RF positive | 70.3% | 43.5% | <0.0001 | 1.6 (0.9 to 2.9), 0.1 |
| Anti-CCP positive | **67.9%** | **39.1%** | **<0.0001** | **2.0 (1.1 to 3.7), 0.02** |
| Detectable IL-6 | **87.2%** | **63.5%** | **<0.0001** | **2.5 (1.5 to 4.5), 0.001** |
| Initial erosion | **48.4%** | **15.8%** | **<0.0001** | **3.2 (2.0 to 5.2), 0.001** |
| Detectable IL-21 | 28.6% | 18.2% | 0.009 | 1.3 (0.8 to 2.0), 0.3 |

Results are expressed as median (IQR) or %. Pearson's $\chi^2$ test was used to compare proportions of presence of IgM-RF, IgA-RF and anti-CCP, and the Mann–Whitney U test was applied to compare the other variables. Bold type indicates statistical significance.
CCP, cyclic citrullinated protein; CRP, C-reactive protein; DAS, Disease Activity Score; ESR, erythrocyte sedimentation rate; IL, interleukin; RF, rheumatoid factor.

Patients with RA with detectable baseline levels of IL-6 had significantly higher baseline levels of IgM-RF (14.0 (5.0–78.0) vs 5.0 (4.0–12.0) IU/ml, p<0.0001) and anti-CCP (90.0 (0–573.3) versus 0 (0–0) U/ml, p<0.0001) than patients with no detectable IL-6. Patients with RA with detectable baseline levels of IL-21 had significantly higher baseline levels of IgM-RF (48.0 (7.2–251.0) vs 6.0 (4.0–34.0) IU/ml, p<0.0001) and anti-CCP (138.0 (0–669.5) vs 0 (0–262.2) U/ml, p<0.0001) than patients with no detectable IL-21. Similarly, serum β2-microglobulin, total IgG, κ and λ FLCs were higher in patients with RA with both detectable levels of IL-6 and IL-21 than in those with detectable levels of only IL-6 or IL-21 (table 3).

### Serum IL-6 and IL-21 and radiographic progression in patients with RA

The serum concentrations of IL-1β, IL1-Ra, IL-2, IL-4, IL10, IL-17, MCP-1, TNFα and IFNγ were associated with neither the presence of radiographic features at inclusion nor radiographic progression. Patients with early RA and detectable IL-6 had significantly more erosions typical of RA (30.1% of initial erosions) than patients without detectable IL-6 (17.8%, p=0.004). Multivariate analysis showed an association between radiographic progression at 1 year and detectable IL-6, the presence of anti-CCP antibodies at enrolment, and erosions, but not with IL-21 level, ESR or CRP (table 4). In a second model of multivariate analysis taking into account baseline levels of IL-6 and anti-CCP, radiographic progression at 1 year was associated with higher levels of IL-6 (OR 2.4 (95% CI 1.1 to 5.2), p=0.005), higher levels of anti-CCP (OR 3.1 (95% CI 1.6 to 6.2), p=0.0002), and presence of erosions at enrolment (OR 3.3 (95% CI 2.0 to 5.4), p=0.0001), but not with levels of IL-21, ESR or CRP. Among the 86.7% of patients with RA who had been started on DMARDs within 1 year of follow-up, radiographic progression at 1 year was still associated with higher levels of IL-6 (p=0.009), anti-CCP (p=0.04) and presence of erosions at enrolment (p=0.001).

Patients with rapid radiographic progression (increase in SHS ≥5 at the 1-year follow-up visit) had more commonly detectable IL-6 (93.6%) than patients without rapid radiographic progression (60.8%, p=0.001). Detection of IL-21 was not significantly more common in patients with radiographic erosions (26.7% vs 19.8% in patients without erosions, p=0.13), but it was significantly more common in patients with radiographic progression (table 4) and rapid radiographic progression (39.1% vs 20.2% in patients without rapid radiographic progression, p=0.008).

Rapid radiographic progression was observed more often in patients with both detectable IL-6 and IL-21 (17.3%) than in patients with only detectable IL-6 (9.8%) (p=0.05).

## DISCUSSSION

This study of 11 biomarkers in ESPOIR, the prospective French multicentre cohort of patients with early arthritis, demonstrates that only two—serum IL-6 and IL-21 concentrations—were higher in patients with RA than in those with UA.

The first objective of the study was to investigate 11 serum cytokines and chemokines of patients with early arthritis in order to determine their suitability as markers in discriminating those with RA from those with UA. Levels of IL-1β, IL-1Ra,

TNFα, IFNγ, IL-4, IL-10, IL-17 and MCP-1 were not significantly different in these two groups.

Among the 11 assessed cytokines, only baseline serum concentrations of IL-6 were able to discriminate RA from UA in multivariate analysis. Most previous studies analysed IL-6 concentrations in the serum and synovial fluid of patients with established RA, or more recently before the first symptoms of RA, but examined only a limited number of patients with early RA.[13–19] IL-6 remained associated with RA in multivariate analysis, whereas ESR and CRP did not. In addition, IL-6 was also associated with RA in patients without raised CRP, which demonstrates that the association between IL-6 and RA is independent of inflammation. The study also confirms that anti-CCP positivity remains the best predictor of the development of RA. Thus positivity for anti-CCP had a better specificity and positive predictive value than IL-6 for the diagnosis of RA.

Interestingly, the present study suggests the involvement of IL-21 in the pathogenesis of early RA. Thus levels of serum IL-21 were significantly higher in patients with RA than in those with UA, in agreement with previous findings of increased plasma IL-21 in early RA.[20] This increase in IL-21 observed in early RA might originate from follicular T helper (Tfh) cells, since IL-21 is mainly expressed by Tfh and Th17 and considering that serum IL-17 level is not increased. In addition, no correlation was observed between IL-17 and IL-21, also reported in a previous study.[20] The serum increase in IL-21 might be genetically determined, since genetic polymorphisms of the IL-2/IL-21 gene locus predispose to RA.[21] The lower frequency of detection of IL-21 than IL-6 may be related to the higher threshold of detection compared with IL-6, which may explain why IL-21 did not remain associated with diagnosis of RA and structural progression in multivariate analysis.

The second objective of the present study was to analyse the correlation between serum cytokine levels and markers of B cell activation. We previously reported that these markers were not associated with a serum increase in BAFF.[1]

Although correlations between serum cytokine levels and markers of B cell activation cannot be considered as causal relationships, our results suggest that B cell activation may require both IL-6 and IL-21. Previous studies have reported increased IL-6 levels in patients with lymphoid synovial structures,[22] associated with B cell activation, and the correlation between synovial fluid IL-6 and plasma IgG.[23] Similarly, low concentrations of IL-6 in early RA have been associated with lower reactivity against citrullinated epitopes.[24] Thus, as reflected by its association with increased levels of markers of B cell activation, IL-6 may contribute to the activation of B cells.

IL-21 was the only other cytokine beside IL-6 to be associated with autoantibodies and markers of B cell activation. Moreover, increased levels of autoantibodies and markers of B cell activation were higher in patients with both detectable IL-6 and IL-21 than in patients with either detectable IL-6 or IL-21. Recently, the decreased generation of IL-21-secreting T follicular cells in a murine model of arthritis resulted in reduced antibody responses.[25] It was also reported that the activation of B lymphocytes by IL-6 required IL-21 produced by T follicular cells[26] and that, in mice, IL-21 and IL-6 were crucial for different aspects of B cell immunity.[27] Last, it has been shown that IL-6 compensates for the lack of IL-21 by signalling through STAT-3.[28] The pathogenic role of IL-21 in B cell activation may therefore be more important in patients without detectable IL-6, as suggested by the increased proportion of autoantibodies in patients with detectable IL-21 but not IL-6 compared with patients with no detectable IL-21 and IL-6.

Baseline serum IL-6 and IL-21 levels were associated with radiographic progression at 1-year in patients with early RA in univariate analysis. IL-6 was the only one of the 11 assessed markers whose serum concentration provided a structural prognosis at 1-year in multivariate analysis, along with radiographic erosions and anti-CCP positivity. DAS28, ESR and CRP concentrations were not associated with radiographic progression, unlike IL-6. This shows that the role of IL-6 in radiographic progression in early RA is independent of inflammation. These results are in agreement with findings based on an animal model of RA[29] suggesting that IL-6 might favour osteoclast formation independently of inflammation.

In conclusion, we measured the serum concentrations of 11 serum cytokines in a prospective cohort of 710 patients with early arthritis and found that only the initial concentrations of IL-6 and IL-21 distinguished RA from UA. The serum IL-6 and IL-21 concentrations were associated with B cell activation and radiographic progression in patients with early RA.

**Contributors** All authors contributed to obtaining the data and data interpretation. JEG and XM wrote the manuscript. BD made the statistical analyses.

**Acknowledgements** We thank all the investigators who recruited and followed the patients (F Berenbaum (Paris-Saint Antoine), M C Boissier (Paris-Bobigny), B Combe (Montpellier), M Dougados (Paris-Cochin), P Fardelonne (Amiens), B Fautrel, P Bourgeois (Paris-La Pitié), R M Flipo (Lille), P Goupille (Tours), F Liote (Paris-Lariboisière), X le Loet and O Vittecoq (Rouen), O Meyer (Paris-Bichat), T Schaeverbeke (Bordeaux), J Sibilia (Strasbourg) and V Devauchelle (Brest) for expert radiograph interpretation, J Benessiano (biological resource center, Paris Bichat) for centralising and managing biological data collection, S Martin (Paris-Bichat) who performed all the assays of CRP, IgA and IgM RF and anti-CCP antibodies, and N Cagnard (Plateforme Bio-informatique Paris Descartes) for the statistical clustering). We also acknowledge the tremendous work of Nathalie Rincheval, who supervised the data collection of the ESPOIR cohort.

**Funding** An unrestricted grant from Merck Sharp and Dohme (MSD) was allocated for the first 5 years of the ESPOIR cohort. Two additional grants from INSERM were obtained to support part of the biological database. The French Society of Rheumatology, Abbott, Amgen and Wyeth have also provided funding for the ESPOIR cohort study. A grant from the French Society of Rheumatology and from Roche allowed us to assay markers of B cell activation.

**Competing interests** None.

**Patient consent** Obtained.

**Ethics approval** Montpellier Ethics Committee.

**Provenance and peer review** Not commissioned; externally peer reviewed.

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*Ann Rheum Dis* 2012 71: 1243-1248 originally published online April 24, 2012
doi: 10.1136/annrheumdis-2011-200975