The Acute-phase Response Is Not Predictive for the Development of Arthritis in Seropositive Arthralgia – A Prospective Cohort Study

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ABSTRACT. ***Objective.*** To evaluate whether markers of the acute-phase response in patients presenting with arthralgia and positive anticitrullinated protein antibodies (ACPA) and/or immunoglobulin M rheumatoid factor (IgM-RF) could be predictive for the development of arthritis.

Methods. In total, 137 ACPA- and/or IgM-RF-positive patients were included. Patients were followed annually for the development of arthritis, defined as presence of 1 or more swollen joints at clinical examination. High-sensitivity C-reactive protein (hsCRP), procalcitonin (PCT), secretory phospholipase A2 (SPLA2), tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), IL-12p70, IL-10, and interferon-γ (IFN-γ) were measured in baseline serum samples. Gene expression focusing on a predefined panel of genes coding for inflammatory molecules was measured by multiplex ligation-dependent probe amplification.

Results. Thirty-five patients (26%) developed arthritis within a median time of 11 months (interquartile range 3.7–18 mo). Circulating levels of cytokines, SPLA2, hsCRP, and PCT were not different between patients with progression to clinical arthritis and those without progression. However, a trend for IL-12p70, TNF-α, IL-10, IL-6, and SPLA2 was observed. No correlation between messenger RNA (mRNA) expression levels of inflammatory genes and progression to arthritis was found. Subgroup analysis of patients with early progression to arthritis showed higher levels of mRNA expression of poly(A)-specific ribonuclease and polycomb complex protein BMI-1 compared to patients without progression to arthritis.

Conclusion. Although low-grade inflammation is present before onset of clinical arthritis in large cohorts and can be detected using consecutive measurements, a single measurement of acute-phase reactants seems to have limited value for prediction of development of arthritis in individual patients.
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Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease, of which the exact etiology remains to be elucidated. In clinically active RA, inflammation is represented in the blood by elevated levels of acute-phase reactants such as high-sensitivity C-reactive protein (hsCRP), which can be used as a diagnostic marker for disease activity.

About half of patients with RA have specific serologic abnormalities several years before the onset of symptoms. The presence of elevated serum level of immunoglobulin M rheumatoid factor (IgM-RF) or anticitrullinated protein antibodies (ACPA) in a healthy individual implies an increased risk for development of RA[1]. Further, a rise in CRP and secretory phospholipase A2 (SPLA2) levels has been associated with the development of RA[2]. Also, several cytokines, cytokine-related factors, and chemokines are elevated prior to diagnosis of RA[3]. However, it remains unclear

whether these serological markers can be used to predict the development of arthritis prior to disease onset.

Our study was conducted to test the hypothesis that acute-phase proteins can be used as markers to predict the development of RA in patients at increased risk.

MATERIALS AND METHODS

Study population. The study population has been described in previous publications[4,5]. Patients with arthralgia and a positive ACPA2 and/or IgM-RF status were recruited at rheumatology clinics in the Amsterdam area. At the first visit, a trained investigator completed a questionnaire on the presenting symptoms. The absence of arthritis was confirmed by 2 independent investigators (WB or LAS and DS) of whom one was a senior rheumatologist blinded for antibody status and medical history. Patients were excluded if one or both investigators observed any swollen joint and/or if chart review revealed past arthritis observed by a rheumatologist. Disagreement resulted in patient's exclusion from the study. Further, patients previously treated with a disease-modifying antirheumatic drug (DMARD) and patients with systemic lupus erythematosus or Sjögren's syndrome were excluded because of the possibility of false-positive RF. During yearly followup visits, development of arthritis was independently confirmed by 2 investigators (WB or LAS and DS).

Details of joint complaints were recorded at baseline and during yearly followup visits. As a subgroup of arthralgia, the presence of symmetric arthralgia in wrists, metacarpophalangeal, proximal interphalangeal, and/or metatarsophalangeal joints was defined as inflammatory arthralgia. Extra visits were planned if the patient developed arthritis. At such visits, soft-tissue swelling in any of the 44 joints was independently confirmed by the same 2 investigators who performed the inclusion visit, and the Disease Activity Score of 28 joints (DAS28) was determined, as well as whether the patient fulfilled the American College of Rheumatology (ACR) criteria for RA. Extra visits were planned if arthritis developed. Median followup was 21 months (range 6–48 mo). Patients recruited between September 2004 and November 2007 were selected for the current study.

Further, 20 patients with RA fulfilling the 1987 ACR criteria were selected as positive controls for hsCRP and SPLA2 measurements and 40 healthy blood donors were used as negative controls.

Measurements. Blood samples were obtained by venipuncture. RNA was isolated from PAXgene™ tubes (PreAnalytiX GmbH, Hombrechtikon, Switzerland) containing RNA-stabilizing buffer according to protocol, and gene expression was measured by multiplex ligation-dependent probe amplification (MLPA), focusing on a predefined panel of genes coding for inflammatory molecules, as described[6]. EDTA-anticoagulated plasma was aliquoted and stored at −80°C. The blood was routinely screened for hematological and biochemical variables. hsCRP was measured using a highly sensitive latex-enhanced assay on a Hitachi 911 analyzer (Roche Diagnostics), according to the manufacturer's instructions. Procalcitonin (PCT) levels were measured by a chemiluminescence sandwich immunoassay (Brahms AG, Hennigsdorf, Germany) as described[7]. SPLA2 levels were measured by ELISA[8]. Tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), IL-12p70, IL-10, and interferon-γ (IFN-γ) were measured by cytometric bead array (CBA) multiplex assay (BD Biosciences, San Jose, CA, USA). All samples were measured in a single, blinded fashion without knowledge of the patient's clinical status.

Statistical analysis. Baseline demographic and clinical variables are tabulated and descriptive statistics are presented as numbers (%), medians with interquartile ranges (IQR), or means with SD when a normal distribution could be assumed.

Differences in marker levels between groups were determined with an unpaired T test. Correlations were determined by logistic regression analysis. CBA, MLPA, and biomarker levels were transformed with their natural logarithm prior to statistical analysis to normalize the data.

Subgroup analysis comparing early progressors (defined as patients with development of arthritis within the first IQR after inclusion in the study) with nonprogressors was performed for all markers. Smoking was taken into account as a possible confounder. A 2-tailed p value < 0.05 was considered to indicate statistical significance.

RESULTS

Patient characteristics. A total of 137 ACPA- and/or IgM-RF-positive arthralgia patients were included; patient characteristics are shown in Table 1. Among the patients, 82 (60%) were IgM-RF-positive, 94 (69%) were ACPA-positive, and 39 (29%) were both IgM-RF- and ACPA-positive. Overall, 35 patients (26%) developed arthritis within a median time of 11 months (IQR 3.7–18 mo); 18 patients developed arthritis within the first IQR after inclusion and were considered early progressors. Thirty-three patients were diagnosed with RA according to the American College of Rheumatology/European League Against Rheumatism 2010 criteria[9].

Circulating levels of cytokines. Circulating levels of cytokines were not different between patients with progression to clinical arthritis and those without progression. However, a trend for increased levels of IL-12p70, TNF-α, IL-10, and IL-6 could be observed, with higher levels in patients who progressed to arthritis (p = 0.07, p = 0.07, p = 0.10, and p = 0.05, respectively). Subgroup analysis of circulating levels of cytokines in early progressors as compared to patients without progression to arthritis showed no significant difference (Table 2). Subgroup analysis comparing smokers and nonsmokers showed comparable results: levels of ACPA and RF titers were not independently associated with levels of circulating cytokines (data not shown).

Messenger RNA (mRNA) biomarkers. No significant correlation could be observed between progression to arthritis and mRNA expression of the following inflammatory genes (Table 2): TNF-α, IL-1β, IL-1RA, IL-8, IL-15, nuclear factor (NF)-κB1A, NF-κB1, serpin B9, MYC, cyclin-dependent kinase inhibitor 1A (CDKN1A), small inducible cytokine A4 (SCYa4), platelet-derived growth factor subunit B (PDGFb), poly(A)-specific ribonuclease (PARN), thrombospondin 1 (THBS1), tumor necrosis factor receptor super-

Table 1. Baseline characteristics of patients with arthralgia and positive anticitrullinated antibodies (ACPA) or IgM rheumatoid factor (RF).

Characteristic	Total, n = 137
Female sex, n (%)	104 (76)
Age, median (IQR), yrs	47 (39–55)
Followup, median (IQR), mo	21 (15–31)
Progression to arthritis, n (%)	35 (26)
Time to arthritis, median (IQR), mo	11 (3.7–18)
Tender joint count = 53, median (IQR)	0 (0–2)
RF-positive, n (%)	82 (60)
ACPA-positive, n (%)	94 (69)
RF + ACPA positive, n (%)	39 (29)

IQR: interquartile range.

Table 2. Levels of messenger RNA expression, circulating cytokines, and circulating protein biomarkers in arthralgia patients with and without progression to arthritis. Data are geometric mean (95% CI).

Variable	Arthritis, n = 35	No Arthritis, n = 102	p
Cytometric bead array assay			
IL-12p70	7.08 (2.04–24.55)	1.91 (1.35–2.69)	0.07
TNF-α	2.95 (1.54–5.62)	1.45 (1.15–1.82)	0.07
IL10	2.29 (1.26–4.17)	1.32 (1.15–1.51)	0.10
IL-6	2.69 (1.66–4.37)	1.58 (1.38–1.82)	0.05
IL-1ß	10.0 (3.16–31.6)	5.50 (2.88–10.47)	0.39
IL-8	2.95 (1.70–5.13)	2.69 (2.04–3.55)	0.78
Multiplex ligation-dependent probe amplification assay			
IL-15	0.05 (0.04–0.05)	0.05 (0.04–0.05)	0.54
NF-κB1A	0.39 (0.35–0.44)	0.40 (0.38–0.42)	0.65
TNF	0.02 (0.02–0.02)	0.02 (0.02–0.02)	0.41
IL-1ß	0.11 (0.10–0.13)	0.11 (0.10–0.12)	0.95
IL-1RN	0.35 (0.30–0.42)	0.35 (0.33–0.38)	0.99
IL-8	0.03 (0.03–0.04)	0.03 (0.03–0.04)	0.97
MYC	0.43 (0.38–0.49)	0.39 (0.36–0.42)	0.19
SCYa4	0.09 (0.08–0.10)	0.09 (0.08–0.10)	0.92
Serpin-B9	0.45 (0.39–0.51)	0.43 (0.41–0.45)	0.63
PDGF-ß	0.03 (0.03–0.04)	0.03 (0.03–0.03)	0.23
PARN	0.19 (0.18–0.20)	0.18 (0.17–0.19)	0.09
THBS1	0.05 (0.04–0.05)	0.04 (0.04–0.05)	0.16
LTA	0.05 (0.04–0.05)	0.05 (0.04–0.05)	0.09
CDKN1A	0.13 (0.11–0.16)	0.12 (0.11–0.13)	0.27
TNFRSF1A	0.35 (0.32–0.39)	0.35 (0.34–0.36)	0.80
BMI-1	0.11 (0.10–0.13)	0.10 (0.09–0.11)	0.08
MIF	0.26 (0.22–0.30)	0.25 (0.23–0.27)	0.73
PDE4b	0.34 (0.32–0.37)	0.34 (0.32–0.35)	0.84
PTPN1	0.07 (0.06–0.08)	0.07 (0.06–0.07)	0.53
PTP4A2	0.81 (0.76–0.87)	0.78 (0.74–0.81)	0.20
GSTP1	0.05 (0.04–0.05)	0.04 (0.03–0.05)	0.42
Biomarkers			
Procalcitonin	0.04 (0.03–0.05)	0.04 (0.03–0.05)	0.69
hsCRP, mg/l	2.51 (1.72–3.66)	2.30 (1.82–2.90)	0.71
SPLA2, ng/ml	5.79 (4.75–7.07)	4.20 (3.20–5.53)	0.07
Subgroup analysis	Early progressors, n = 18	No arthritis, n = 102	
PARN	0.20 (0.18–0.22)	0.18 (0.17–0.19)	0.008
BMI-1	0.13 (0.11–0.15)	0.10 (0.09–0.10)	0.02

IL: interleukin; NF: nuclear factor; TNF: tumor necrosis factor; PDGF: platelet-derived growth factor; PARN: poly(A)-specific ribonuclease; THBS: thrombospondin; LTA: lymphotoxin α: CDKN: cyclin-dependent kinase inhibitor; TNFRSF: TNF receptor superfamily member; BMI-1: polycomb complex protein; MIF: macrophage migration inhibitory factor; PDE: phosphodiesterase; PTPN: tyrosine-protein phosphatase non-receptor; PTP4A2: protein tyrosine phosphatase type IVA 2; GSTP1: glutathione S-transferase P; hsCRP: high-sensitivity C-reactive protein; SPLA2: secretory phospholipase 2.

family member 1A (TNFRS1A), polycomb complex protein BMI-1, macrophage migration inhibitory factor (MIF), phosphodiesterase 4B (PDE4B), tyrosine-protein phosphatase non-receptor type 1 (PTPN1), protein tyrosine phosphatase type IVA 2 (PTP4A2), and glutathione S-transferase P (GSTP1).

Subgroup analysis of patients with early progression to arthritis (that is, within 10 weeks after inclusion) showed significantly higher levels of mRNA expression of PARN and BMI-1 compared to patients without progression to arthritis. The same results could be observed comparing levels of mRNA expression of PARN and BMI-1 in early progressors to levels in all other patients.

Smoking and level of ACPA/RF were not independently associated with mRNA expression (data not shown).

Biomarker levels. Mean SPLA2, hsCRP, and PCT levels were not different between patients who developed arthritis and those who did not; a trend toward higher SPLA2 levels in patients with arthritis was observed (p = 0.07; Table 2). SPLA2 levels of patients with arthralgia did not differ from SPLA2 levels of healthy controls [geometric mean (GM) 4.56 (95% CI 3.69–5.64) and GM 4.65 (95% CI 3.84–5.64), respectively; p = 0.93], in contrast to the levels of patients with RA [GM 10.99 (95% CI 6.93–17.42)], which were significantly higher than levels of both healthy controls and patients with arthralgia (p < 0.001). hsCRP levels of patients

with arthralgia, on the other hand, were higher than hsCRP levels of healthy controls [GM 2.35 (95% CI 1.93–2.86) and GM 1.06 (95% CI 0.72–1.55), respectively; $p < 0.001$]. hsCRP levels of patients with RA were even higher [GM 7.58 (95% CI 4.06–14.13); $p < 0.001$].

DISCUSSION

Our study of patients with an increased risk of developing RA was conducted to investigate whether differences in inflammatory patterns in those patients who developed arthritis could be observed before clinical signs of arthritis could be confirmed. We found no evidence of a more pronounced systemic acute-phase response, higher cytokine levels, or higher expression of proinflammatory gene markers in patients who later progressed to arthritis than in those who did not, although a number of the biomarkers showed a trend toward higher levels in those who later developed arthritis. These data indicate that any possible local inflammatory processes in the joints of patients with seropositive arthralgia do not result in a discernible systemic acute-phase response activation.

These results are in contrast with our previous studies, in which we reported elevation of CRP and SPLA2 levels in preclinical RA[2,10]. A possible explanation for this discrepancy could be the difference in study design. In these previous studies, multiple, longitudinally collected serum samples were analyzed, and a slight but significant rise in CRP and SPLA2 levels was observed before RA onset, in contrast to stable levels in healthy controls. However, CRP and SPLA2 levels in preclinical RA were still within the normal range and therefore large numbers of samples are needed to detect such small changes. In our study a trend was observed toward higher levels of IL-12p70, TNF-α, IL-10, IL-6, PARN, lymphotoxin α (LTA), BMI-1, and SPLA2 in arthritis progressors; higher levels of PARN and BMI-1 were observed in early progressors. More significant differences might be observed when larger numbers of samples are analyzed. Also in our study, hsCRP levels of patients with arthralgia were higher than hsCRP levels of healthy controls, indicating that patients with arthralgia have a slightly activated acute-phase response on the group level.

Thus, although evidence exists for an induction of inflammation prior to the onset of RA, our data indicate that acute-phase response markers such as CRP and SPLA2 are not useful in clinical practice as predictors of development of arthritis in individual high-risk patients. This is in accord with results from other studies in which hsCRP and SPLA2 in a single pre-RA serum sample were not increased in comparison to healthy controls[11]. However, considering the likelihood of developing RA in patients with arthralgia and a positive IgM-RF/ACPA, the patients require closer followup than those without positive serology. To date, no early intervention has been proven effective in this high-risk patient group.

Although low-grade inflammation is present before the onset of clinical arthritis in large cohorts and can be detected using consecutive measurements, a single measurement of acute-phase reactants seems to be of limited value for prediction of development of arthritis in individual patients.

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