Biomarkers of Inflammation in Patients with Unclassified Polyarthritis and Early Rheumatoid Arthritis. Relationship to Disease Activity and Radiographic Outcome

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ABSTRACT. ***Objective.*** To determine plasma interleukin 6 (pIL-6), plasma vascular endothelial growth factor (pVEGF), and serum (s) YKL-40 in patients with early rheumatoid arthritis (RA) and unclassified polyarthritis (PA), and investigate their relationship with radiographic outcome.

Methods. pIL-6 and pVEGF were determined by ELISA and sYKL-40 by an in-house radioimmunoassay in 51 patients with early RA and 21 with PA. Patients were followed with clinical and biochemical measurement every month for 2 years. Conventional radiographs of hands, wrists, and forefeet were scored according to the Larsen method, and magnetic resonance imaging of 2nd to 5th metacarpophalangeal joints of the dominant hand were evaluated for presence or absence of bone erosions.

Results. Baseline pIL-6, pVEGF, sYKL-40, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were elevated in RA patients compared to healthy persons ($p < 0.001$), but were not in patients with PA. Patients with early RA had higher pIL-6 ($p = 0.007$), pVEGF ($p = 0.02$), and sYKL-40 ($p = 0.024$) compared to PA patients. pIL-6, sYKL-40, CRP, and ESR but not pVEGF decreased in patients that responded to treatment after 2 years. The mean value of pIL-6 during the first and second year were higher in patients with early RA with progression in bone erosions ($n = 14$) compared to early RA patients without progression ($n = 30$; first year 8.4 vs 2.8 ng/l, $p = 0.04$; second year 6.1 vs 3.6 ng/l, $p = 0.03$).

Conclusion. Plasma IL-6 was the only biomarker related to treatment response and progressive erosive disease in patients with early RA, but it may not give additional information compared to CRP in relation to disease activity and treatment response. (First Release June 15 2008; J Rheumatol 2008;35:1277–87)

Key Indexing Terms:
BIOMARKERS EARLY RHEUMATOID ARTHRITIS INTERLEUKIN 6 YKL-40
UNCLASSIFIED POLYARTHRITIS VASCULAR ENDOTHELIAL GROWTH FACTOR

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The clinical presentation of early rheumatoid arthritis (RA) is not always characteristic, and the 1987 American College of Rheumatology (ACR) classification criteria are frequently not fulfilled[1,2]. Radiographic bone erosions, a key feature of the ACR classification criteria, are associated with a poor clinical outcome. Early aggressive intervention is superior to later therapy not only regarding control of inflammation but in retarding progression in bone erosions[3]. The conventional biomarkers of inflammation, serum C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), are not always elevated. Normal values of ESR and serum CRP lower the probability of severe disease activity, but do not exclude early RA[1]. Radiographic progression of joint destruction can occur despite normal levels of ESR and serum CRP[4]. The search for new predictive and prognostic biomarkers in patients with RA are therefore of clinical importance.

Interleukin 6 (IL-6) is a multifunctional cytokine that influences various cell types and affects not only the immune system but also many other biological systems[5]. In

the arthritic joint, IL-6 is produced by macrophages, neutrophils, B and T lymphocytes, mast cells and endothelial cells, synoviocytes, fibroblasts, osteoblasts, and chondrocytes[5]. Production of IL-6 by synovial cells is augmented by IL-1, tumor necrosis factor-α (TNF-α), and IL-17, and glucocorticocoids inhibit IL-1-induced IL-6 mRNA expression in synoviocytes[5]. IL-6 inhibits bone formation and stimulates bone resorption[6]. IL-6/soluble IL-6 receptor (sIL-6R) and IL-17 act on osteoblasts to upregulate RANKL expression and promote osteoclastogenesis[6]. Further, IL-6 modulates the balance between matrix metalloproteinases (MMP) and their tissue inhibitors at sites of inflammation in RA[6]. Synovial fluid IL-6 is correlated with the infiltration of inflammatory cells in the synovial membrane[7] and with radiographic joint destruction[8]. IL-6 is a therapeutic target in RA, and a humanized monoclonal IgG1 antibody against the IL-6 receptor, tocilizumab, has shown efficacy in inhibiting disease activity in patients with RA[9]. Circulating IL-6 may be useful as a prognostic biomarker of joint destruction and could have a role in monitoring disease activity in patients with RA during treatment with disease modifying antirheumatic drugs (DMARD)[10] and TNF-α inhibitors[11].

Vascular endothelial growth factor (VEGF) is a specific mitogen for vascular endothelial cells and a potent inducer of angiogenesis and vascular permeability[12]. VEGF induces physiological angiogenesis, and is implicated in pathological angiogenesis associated with RA. Angiogenesis constitutes an early event in the synovial membrane of RA patients and promotes cartilage and bone destruction in later stages of the disease[13]. Sources of VEGF in RA patients are synovial lining cells, macrophages, leukocytes, platelets, fibroblasts, and endothelial cells[14]. VEGF expression is very sensitive to hypoxia that increases VEGF transcription[14]. Several factors including TNF-α, IL-1, and IL-6 stimulate VEGF production[14]. Elevated concentrations of VEGF are found in synovial fluid, serum, and plasma of patients with active RA compared to patients with osteoarthritis or healthy subjects[15,16]. Antiangiogenic therapy inhibits the inflammatory response and articular cartilage and bone destruction in mice, but in RA patients such therapy has not yet been successful[17]. Studies have found that circulating VEGF reflects disease activity in RA[17,18], and one study showed that serum VEGF before treatment of early RA patients could predict progression of joint destruction after 1 year of treatment[19]. Serum VEGF may also be a biomarker for monitoring treatment response, since the concentration decreases in RA patients responding to DMARD therapy[17,18] and TNF-α blockade[13,20].

YKL-40 [human cartilage glycoprotein 39 or chitinase-3-like-1 protein (CHI3L1)] is a 40-kDa heparin, collagen, and chitin-binding glycoprotein[21]. YKL-40 is produced in the arthritic joint by activated macrophages, neutrophils, synoviocytes, and chondrocytes[21]. YKL-40 contributes to chondrocyte differentiation by inducing SOX9 and type II collagen expression[22], stimulates proliferation of chondrocytes and fibroblasts, and increases proteoglycan synthesis by chondrocytes[23]. YKL-40 initiates mitogen-activated protein kinases (MAPK) and PI3K signaling cascades in fibroblasts, which are associated with control of mitogenesis and cell survival[24,25]. YKL-40 mRNA expression and protein synthesis in chondrocytes are induced by IL-1 and TNF-α[25]. If TNF-α and IL-1-stimulated chondrocytes and fibroblasts are stimulated with YKL-40, reduction of p38 and SAPK/JNK phosphorylation and production of MMP and IL-8 are observed. This suppressive effect of YKL-40 is dependent on kinase activity and results in AKT-mediated phosphorylation of the apoptosis signal-regulator kinase 1[24]. Further, YKL-40 modulates the collagen fibril formation rate[26]. These observations suggest that YKL-40 plays a role in controlling tissue remodeling. YKL-40 stimulates the migration and tubulogenesis of endothelial cells, suggesting a role in angiogenesis[27]. YKL-40 is a possible autoantigen in RA[28], and a phase I study of RA patients treated with intranasal administration of recombinant YKL-40 demonstrated that disease activity was decreased[29]. Increased levels of YKL-40 in synovial fluid and serum are found in patients with active RA compared to patients with inactive RA and healthy subjects, and changes in serum YKL-40 during DMARD therapy may reflect changes in disease activity[30,31]. In patients with early RA a continuously elevated serum YKL-40 is associated with progression of joint destruction[30].

We evaluated changes in plasma IL-6, plasma VEGF, and serum YKL-40 in patients with early RA and unclassified polyarthritis (PA) during treatment, and investigated their relationships to progression in bone erosions determined by conventional radiography and magnetic resonance imaging (MRI).

MATERIALS AND METHODS

Patients. This was a 24-month prospective, longitudinal study of 75 consecutive patients (60 women, 15 men, aged 20–82 yrs) enlisted between August 1996 and March 1998 at Hvidovre Hospital. Patients were eligible if they had symmetrically swollen and tender 2nd and 3rd metacarpophalangeal (MCP) and proximal interphalangeal joints for at least 4 weeks and < 2 years, or if they had arthralgia in the same joints with a documented nonsteroidal antiinflammatory drug (NSAID) response after 2 weeks. Three patients (1 RA and 2 PA patients) were excluded because they were later diagnosed with ovarian cancer, hepatitis C, or alcoholic liver disease. Patients were classified during the study period as having RA (n = 51), by the ACR 1987 criteria, or unclassified PA (n = 21). Twenty-one patients (7 RA and 14 PA patients) were followed for only 1 year due to the following reasons: one died (pulmonary embolus), 17 dropped out due to lack of compliance, one stopped for personal reasons, and 2 moved to another part of the country.

Therapy. DMARD monotherapy and steroids were first-choice treatment. RA patients were treated with methotrexate (MTX; n = 10), sulfasalazine (n = 39), and hydroxychloroquine (n = 2). At the end of the study, DMARD therapy had been altered in 31 RA patients and the 44 RA patients were treated with MTX (n = 39), sulfasalazine (n = 1), penicillamine (n = 2),

hydroxychloroquine (n = 1), and combination therapy (sulfasalazine, MTX, and hydroxychloroquine, n = 1). The average weekly dosage of MTX was 7.5 mg (range 5.0–10.0 mg) once weekly. Forty-six RA and 10 PA patients were treated with prednisolone; the cumulated mean dose during the study period was 1738 ± 357 mg (SEM) for the RA patients and 351 ± 232 mg (SEM) for the PA patients. All patients were treated with NSAID or analgesics during the study. Further details about patients are as described[32].

Clinical examination. Patients were examined every month for 2 years. At each visit the following measurements were performed: Health Assessment Questionnaire (HAQ score), swollen joint count (SJC), tender joint count (TJC), ESR, serum CRP, and Disease Activity Score (DAS28; ESR-based).

Biochemical methods. Blood samples were collected every month for 2 years; samples were centrifuged at 2000 g for 10 min at room temperature within 3 h after collection. Serum and EDTA plasma were aliquoted and stored at –80°C until analysis. Serum CRP and IgM rheumatoid factor (RF) were determined by nephelometry and ESR by the Westergren method. Plasma IL-6 was measured by ELISA (high-sensitivity; catalogue no. HS600, R&D Systems, Abingdon, UK) following manufacturer's instructions. The sensitivity was 0.10 ng/l, and intra- and inter-assay coefficients of variance (CV) for plasma IL-6 were < 10.5% and < 17.7%. Plasma VEGF was measured by ELISA (catalogue no. SVE00, R&D Systems) following manufacturer's instructions; sensitivity was 12.7 ng/l and intra- and inter-assay CV were < 4.0% and < 12.8%[33]. Serum YKL-40 was determined by an in-house radioimmunoassay[34]. The antiserum was raised in rabbits immunized with purified intact human YKL-40, and homogeneous human YKL-40 was used for standard and tracer. The tracer was prepared by the Iodogen method, and antibody-bound and free ^{125}I-labelled YKL-40 was separated by use of a donkey anti-rabbit antibody-coated cellulose suspension. The sensitivity was 10 µg/l, and the intra- and inter-assay CV were < 6.5% and < 12%. To eliminate inter-assay variation samples from each patient were analyzed in the same assay, and ELISA kits with the same batch number were used.

Biomarker levels in healthy subjects. The upper normal limit for CRP is 95 nmol/l and for ESR 20 mm/h. Reference intervals for the 3 other biomarkers were determined in different groups of healthy subjects characterized as taking no medication and having no signs of preexisting disorders such as joint, liver, metabolic, or endocrine disease or cancer. The median plasma IL-6 concentration in 318 healthy subjects (122 women, 196 men, median age 47 yrs, range 18–64 yrs) was 1.3 ng/l (range 0.33–26; 10th to 90th 0.75–3.3 ng/l). The median plasma VEGF concentration in 306 healthy subjects (116 women, 190 men, median age 48 yrs, range 18–64 yrs) was 45 ng/l (range 6.1–351; 10th to 90th 17–109 ng/l). The median serum YKL-40 concentration in 260 healthy subjects (144 women, 116 men, median age 48 yrs, range 18–79 yrs) was 102 µg/l (range 38–514; 10th to 90th 71–208 µg/l).

Radiographs. Conventional radiographs of the hands and wrists in the posterior-anterior and Nørgaard projections were obtained at entry and after 3, 6, 12, 18, and 24 months. Radiographs of the feet in posterior-anterior view were taken at entry and after 12 and 24 months. At baseline, radiographs of hands and wrists were obtained from 44 early RA patients and radiographs of feet from 26; after 12 months it was 43 and 39, respectively, and after 24 months 43 and 44, respectively. At baseline, radiographs of hands and wrists were obtained in 20 patients with unclassified PA and 10 had radiographs of feet; after 12 months 10 had radiographs of hands and wrists and 8 of feet; and after 24 months 9 had radiographs of hands and wrists and 3 had radiographs of feet. Each finger, wrist, and foot joint was classified as erosive or nonerosive, and each joint was scored according to the method of Larsen, *et al*[35]. Progression was considered to be any magnitude of increase in the Larsen score or development of bone erosion. All available radiographs were read and scored by the same experienced radiologist, in known chronological order, but blinded to clinical, biochemical, and MRI findings. If a patient did not have a radiograph at baseline or 12/24 months, the patient was excluded from the analysis. Further details about the radiographs have been described[36].

Magnetic resonance imaging. MRI of the 2nd to 5th MCP joints of the dominant hand was done using a 1.0 T Magnetom Impact Unit (Siemens, Erlangen, Germany) at baseline in 39 early RA patients and 16 patients with unclassified PA, after 12 months in 25 early RA patients and 9 patients with unclassified PA, and after 24 months in 3 patients with early RA. Continuous axial and coronal T1-weighted, spin-echo images of the hand were obtained before and after intravenous injection of 0.1 mmol/kg body weight of gadolinium-DTPA. MRI were evaluated for presence or absence of bone erosions. MRI erosions had to be visible on both axial and coronal slices to be diagnosed. MR images were scored by the same reader, in known chronological order, but blinded to clinical, biochemical, and radiographic findings. Further details about the MRI have been described[36].

Our study was approved by the regional scientific ethical committee and conducted according to the Helsinki II Declaration.

Statistical analysis. Statistical analysis was performed with Sigma Stat 3.1 (SPSS Inc., Chicago, IL, USA). Results are given as medians and ranges. Comparisons between and within groups were calculated by Mann-Whitney and Wilcoxon test, respectively. Correlations were calculated using Spearman's test. P values below 0.05 were considered significant. Mean values during the study, defined as the area under the curve (AUC) values, were calculated for each of the parameters using 25 timepoints.

RESULTS

Baseline values. At entry 44 patients fulfilled the ACR 1987 classification criteria for RA, and within the first 8 months of observation 7 more were diagnosed. Twenty-one patients were classified as having unclassified polyarthritis. Table 1 gives the baseline characteristics of patients according to diagnosis at 12 months. Patients with early RA had significantly higher plasma IL-6 (p = 0.007), plasma VEGF (p = 0.020), serum YKL-40 (p = 0.024), and ESR (p = 0.003) compared to the patients with unclassified PA. There was no significant difference in serum CRP (p = 0.074). Patients with early RA had significantly higher (p < 0.001) baseline plasma IL-6, plasma VEGF, and serum YKL-40 compared to healthy subjects, whereas none of the biomarkers was elevated in the PA group compared to healthy subjects (Table 1, Figure 1). Sixty percent of the patients with early RA had elevated plasma IL-6, 62% elevated plasma VEGF, 22% elevated serum YKL-40, 43% elevated ESR, and 41% had elevated serum CRP compared to upper normal levels. Plasma IL-6 was elevated in 35% of patients with unclassified PA, 29% elevated plasma VEGF, 5% elevated serum YKL-40, 10% elevated ESR, and 14% had elevated serum CRP compared to upper normal levels (Figure 1).

In healthy subjects the serum YKL-40 levels increased with age (by a factor of 1.15 per decade). Patients with unclassified PA were median 15 years younger than the early RA patients, and the difference in age alone cannot explain the difference in serum YKL-40 between the 2 groups of patients. There was no significant difference in the sex ratio between the 2 groups of patients (Fisher's exact test). The F/M ratio was 4:1 for RA and 6:1 for PA patients.

In the patients with early RA the baseline concentration of plasma IL-6 correlated with ESR (Spearman's rho = 0.56, p < 0.0001), serum CRP (rho = 0.69, p < 0.0001), DAS28 (rho = 0.45, p = 0.002), serum YKL-40 (rho = 0.29, p =

Table 1. Baseline characteristics of the patients according to the diagnosis at 12 months. Values are median (range) unless otherwise stated.

	Early Rheumatoid Arthritis	Unclassified Polyarthritis
Women/men	41/10	18/3
Age, yrs	54** (20–82)	39 (27–80)
Disease duration, mo	3 (1–22)	3 (1–24)
ESR mm/h [normal < 20 mm/h]	20** (1–105)	8 (2–70)
Serum CRP nmol/l [normal < 95 nmol/l]	95 (95–1374)	95 (95–247)
IgM RF, % positive	53% (27/51)	10% (2/21)
Plasma IL-6 ng/l [normal level ≤ 3.3 ng/l]	7.0** (0.6–98)	1.4 (0.6–11)
Plasma VEGF ng/l [normal level ≤ 109 ng/l]	139* (15–818)	71 (15–474)
Serum YKL-40 µg/l [normal level ≤ 208 µg/l]	131* (35–900)	91 (60–450)
SJC, 0–28	6 (2–18)	3 (2–13)
TJC, 0–28	15 (2–24)	16 (2–24)
HAQ, 0–3	0.875 (0–2)	0.625 (0–2.375)
DAS28 [ESR]	5.2* (1.6–7.6)	4.5 (2.1–6.5)
Larsen score > 0	13	0
No. patients with bone erosions on x-ray		
On hand x-ray	10	0
On foot x-ray	5	0
On MRI[#]	14	0
No. patients treated with analgesic		
Analgesic	31	10
NSAID	48	18
DMARD	51	0
Glucocorticoid	46	10

[#] 55 patients had MRI at baseline. Difference between groups calculated by Mann-Whitney: * $p < 0.05$, ** $p < 0.01$. CRP: C-reactive protein; DAS28: Disease Activity Score ESR based: DMARD: disease modifying antirheumatic drugs; ESR: erythrocyte sedimentation rate; HAQ: Health Assessment Questionnaire; IL-6: interleukin-6; MRI: magnetic resonance imaging; NSAID: nonsteroidal antiinflammatory drug; RF: rheumatoid factor; SJC: swollen joint count; TCJ: tender joint count; VEGF: vascular endothelial growth factor.

0.05), and IgM RF titer (rho = 0.35, p = 0.02). Baseline serum YKL-40 correlated with ESR (rho = 0.39, p = 0.006), serum CRP (rho = 0.28, p = 0.05), and DAS28 (rho = 0.39, p = 0.005). Baseline ESR correlated with serum CRP (rho = 0.73, p < 0.001). No correlations were found between plasma VEGF and the other biomarkers. In the early RA patients the rho correlations between the biomarkers decreased after start of treatment, and at later timepoints some were not significant.

Baseline plasma IL-6 in unclassified PA patients correlated with ESR (rho = 0.71, p = 0.001), serum CRP (rho = 0.53, p = 0.03), DAS28 (rho = 0.50, p = 0.04), and serum YKL-40 (rho = 0.63, p = 0.007). Baseline serum YKL-40 correlated with ESR (rho = 0.67, p = 0.001), serum CRP (rho = 0.55, p = 0.01), and DAS28 (rho = 0.53, p = 0.02). Baseline ESR correlated with serum CRP (rho = 0.61, p = 0.004). No correlations were found between plasma VEGF and the other biomarkers.

Twenty-nine of the 72 patients were IgM RF-positive and they had higher baseline plasma IL-6 (13 vs 2.1 ng/l; p < 0.001), ESR (20 vs 9 mm/h; p = 0.02), and serum YKL-40 (134 vs 105 µg/l; p = 0.03) compared to IgM RF-negative patients. Among the 51 early RA patients, 53% (n = 27) were IgM RF-positive (median level 101, range 19–1178), and only plasma IL-6 was higher compared to the level in seronegative RA patients (15 vs 3 ng/l; p = 0.01).

Changes in biomarkers during the 24-month study and relation to disease activity. Figure 2 illustrates the changes in the biomarkers and conventional measures of disease activity in the patients with early RA (Figure 2A to 2F) and unclassified PA (Figure 2G to 2K) during the 24-month study. Significant decreases compared to baseline values were found at all timepoints in DAS28 and several timepoints for plasma IL-6 and ESR, whereas plasma VEGF, serum YKL-40, and CRP were unchanged at most visits. In patients with unclassified PA there were no significant decreases in the biomarkers or DAS28.

After 12 months of treatment, 23 of the early RA patients were in remission, defined as DAS28 < 2.6. Significant decreases compared to baseline levels were found in these patients in plasma IL-6 (p = 0.01), plasma VEGF (p = 0.02), serum CRP (p = 0.014), and ESR (p = 0.001), but not in serum YKL-40. After 24 months of treatment 26 of the early RA patients were in remission, and at this timepoint plasma IL-6 had decreased 63% (p < 0.001), ESR 54% (p < 0.001), serum YKL-40 24% (p = 0.028), and serum CRP 23% (p =

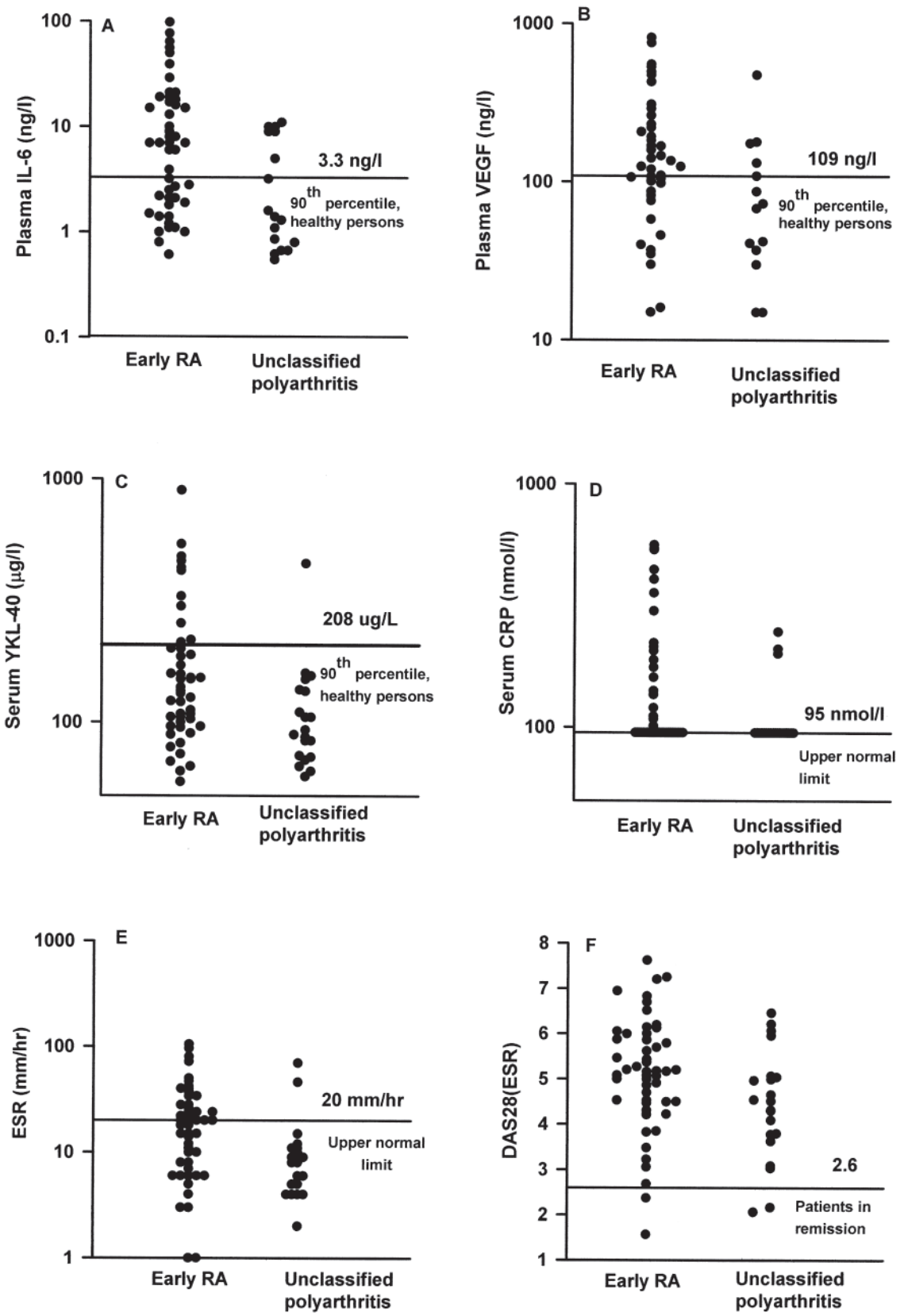

Figure 1. Baseline individual values of biomarkers and DAS28 in the 51 patients with early RA and 21 patients with unclassified polyarthritis. The reference limit for each of the biomarkers is given as the upper normal level (serum CRP and ESR) or as the upper 90th percentile in healthy subjects (plasma IL-6, plasma VEGF, and serum YKL-40). DAS28 remission is 2.6.

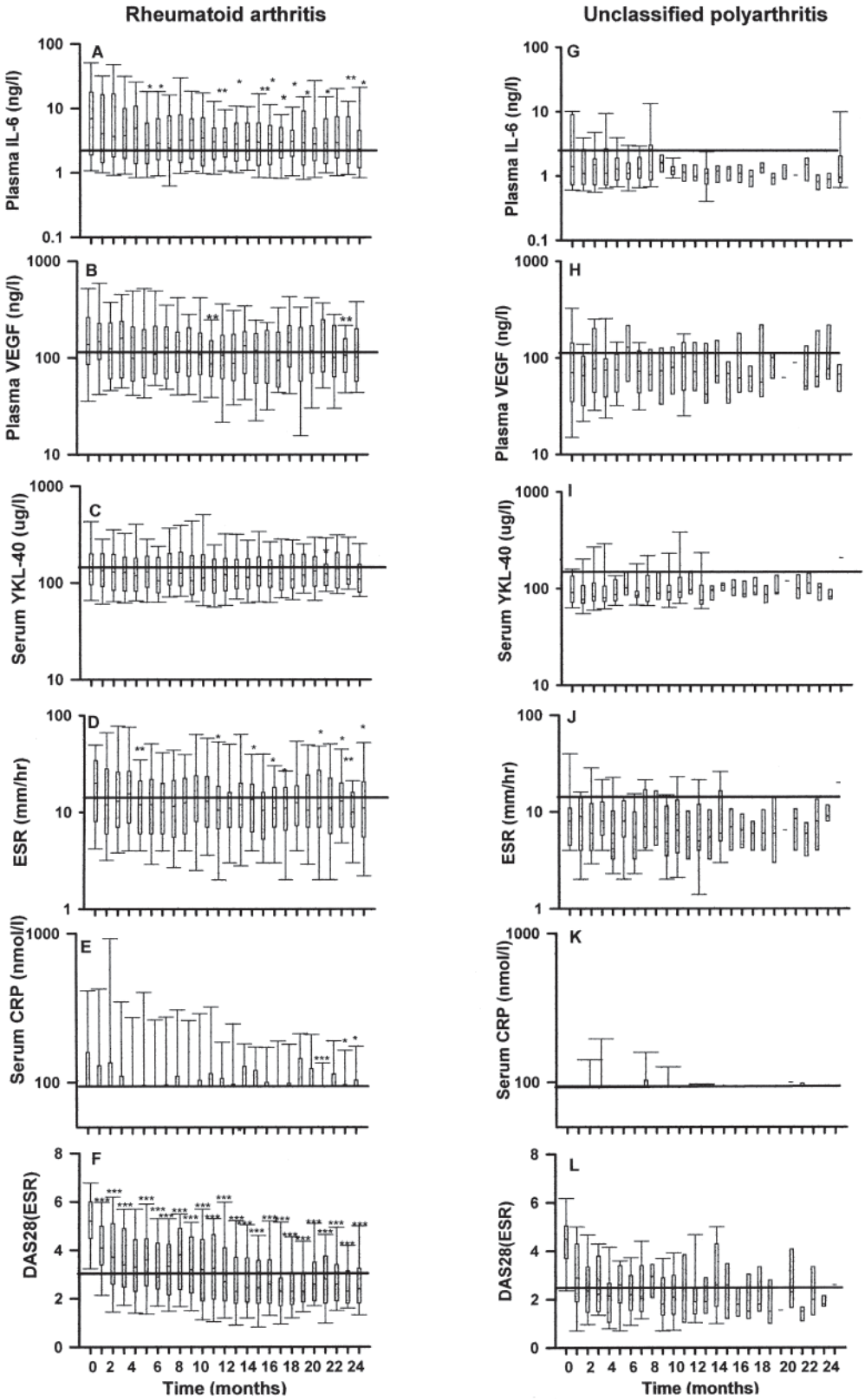

Figure 2. Changes in biomarkers and DAS28 in the 51 early RA (A to F) and 21 PA (G to L) patients during the 24-month study. Box plots represent median, interquartile range, and the 5th to 95th percentile. The reference limit for each biomarker is given as the upper normal level (serum CRP and ESR) or as the upper 90th percentile in healthy subjects (plasma IL-6, plasma VEGF, and serum YKL-40). DAS28 remission is 2.6. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

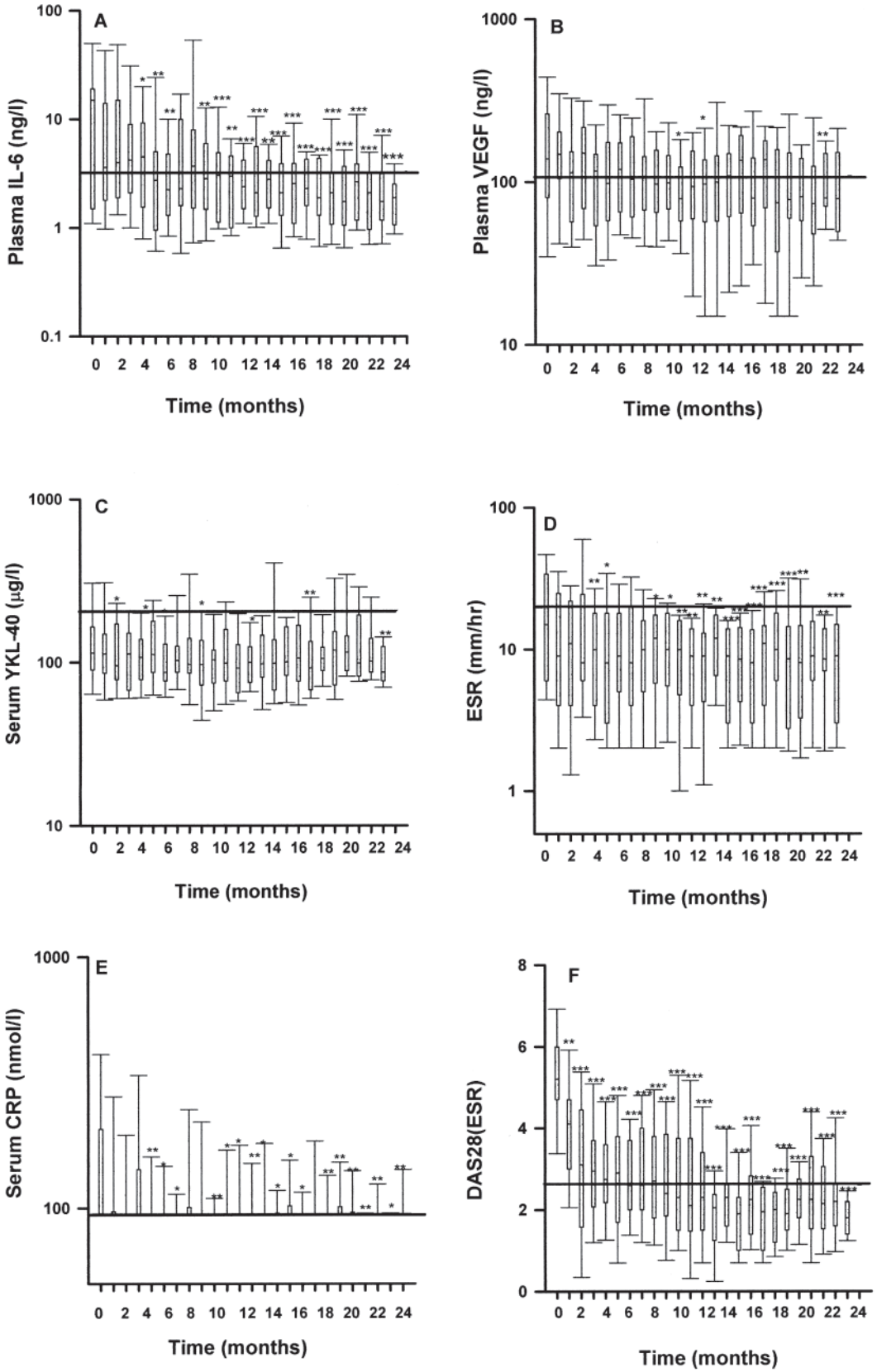

Figure 3. Changes in biomarkers and DAS28 in the 26 early RA patients in remission (DAS28 < 2.6) after 24 months. Box plots represent median, interquartile range, and the 5th to 95th percentile. The reference limit for each biomarker is given as the upper normal level (serum CRP and ESR) or as the upper 90th percentile in healthy subjects (plasma IL-6, plasma VEGF, and serum YKL-40). DAS28 remission is 2.6. $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

0.001) compared to baseline values (Figure 3A to 3F). No significant decrease was found in plasma VEGF.

The mean concentration (AUC) of plasma IL-6 ($IL\text{-}6_{AUC}$) in early RA patients during the 24-month study period correlated with plasma $VEGF_{AUC}$ (rho = 0.32, p = 0.02), serum $YKL\text{-}40_{AUC}$ (rho = 0.39, p = 0.009), serum CRP_{AUC} (rho = 0.51, p < 0.0001), ESR_{AUC} (rho = 0.53, p < 0.0001), and $DAS28_{AUC}$ (rho = 0.32, p = 0.02).

Biomarkers and radiographic outcome in early RA patients. At baseline 13 early RA patients had erosions on conventional radiographs and 14 had erosions on MRI (8 of these had erosions on MRI only). After 12 months, 18 patients had erosions on radiographs and 13 had erosions on MRI (5 had erosions on MRI only), and after 24 months 22 patients had erosions on radiographs and 3 had erosions on MRI (1 had erosions on MRI only). Neither baseline nor 12 or 24-month levels of the biomarkers were significantly different in early RA patients with and those without erosive disease (Table 2).

After 12 months, 14 patients had progression in bone erosions compared to baseline (11 progressed on radiographs and 3 on MRI), and after 24 months 14 patients had progression in bone erosions compared to baseline (13 progressed on radiographs and 1 on MRI). After 24 months 1 patient had progression in bone erosions on radiographs compared to 12 months. Baseline plasma IL-6 was higher in patients with progression in bone erosions compared to patients with no progression after 12 months (15 vs 4.6 ng/l; p = 0.059; Table 3). The plasma $IL\text{-}6_{AUC}$ during the first 12 and 24 months was higher in patients with progression in bone erosions compared to patients with no progression after 12 and 24 months (8.4 vs 2.8 ng/l; p = 0.04 and 6.1 vs 3.6 ng/l; p = 0.03, respectively). There were no significant differences between the patients with and those without progression in bone erosions after 12 and 24 months in any of the other biomarkers at baseline or in their mean values during the study (Table 3).

DISCUSSION

Circulating concentrations of plasma IL-6, plasma VEGF, and serum YKL-40 were measured monthly for 24 months in a cohort of patients with early RA and unclassified PA. Patients with early RA had higher baseline median levels of plasma IL-6, plasma VEGF, and serum YKL-40 than healthy subjects, whereas most patients with unclassified PA had normal levels. However, due to overlap between groups, these biomarkers did not allow discrimination between patients with early RA and PA and between the PA patients and healthy subjects.

In accord with others' results we found a decrease in plasma IL-6[10,37] and serum YKL-40[30,31] concentrations in patients who responded to 24 months of treatment with DMARD and were in remission at the end of the study. This is probably due to the known effects of MTX such as reduction in cytokine production through apoptosis of peripherally active T cells and a decrease in the number of macrophages in the synovial tissue[38]. Plasma IL-6 and serum YKL-40 correlated with the conventional biomarkers of inflammation and the DAS28. In contrast to others' findings[16,18,19], plasma VEGF did not decrease in the patients in remission and was not correlated with clinical or biochemical indicators of disease activity or to radiographic progression. This may be due to the nonaggressive treatment of the RA patients in this study. The majority of the patients with early RA were treated with monotherapy and the mean dose of MTX was 7.5 mg once weekly. This dose is today regarded as low, but at the time of the study (1996-2000), this was

Table 2. Baseline and AUC values of the biomarkers and DAS28 in the early RA patients with bone erosions at baseline, 12 and 24 mo followup compared to RA patients no bone erosions. Values ae median (range) unless otherwise stated.

Characteristic	Erosive RA at Baseline, n = 21	Non-erosive RA at Baseline, n = 29	Erosive RA at 12 mo, n = 23	Non-erosive RA at 12 mo, n = 28	Erosive RA at 24 mo, n = 23	Non-erosive RA at 24 mo, n = 21
Baseline plasma IL-6	7 (0.6–98)	7 (0.8–64)	7 (0.6–98)	7 (1–21)	8 (0.6–98)	3 (1–21)
Plasma $IL\text{-}6_{AUC}$			6.3 (0.8–70)[a]	3.2 (1.4–52)[a]	6.6 (0.8–45)[b]	4.1 (1.2–50)[b]
Baseline plasma VEGF	110 (15–290)	171 (16–818)	136 (15–474)	133 (16–818)	162 (16–818)	162 (16–818)
Plasma $VEGF_{AUC}$			149 (16–561)[a]	135 (60–1126)[a]	130 (25–965)[b]	130 (25–965)[b]
Baseline serum YKL-40	165 (36–460)	108 (35–900)	146 (36–540)	109 (57–480)	145 (57–480)	145 (57–480)
Serum $YKL\text{-}40_{AUC}$			137 (35–1608)[a]	115 (54–417)[a]	127 (54–365)[b]	116 (63–244)[b]
Baseline ESR	20 (3–50)	20 (1–105)	18 (3–50)	19 (1–96)	18 (1–96)	18 (1–96)
ESR_{AUC}			17 (3–85)[a]	14 (2–95)[a]	17 (4–85)[b]	17 (4–85)[b]
Baseline serum CRP	95 (95–445)	95 (95–1374)	95 (95–543)	95 (95–1374)	95 (95–1374)	95 (95–1374)
Serum CRP_{AUC}			110 (95–694)[a]	104 (95–1127)[a]	109 (95–876)[b]	109 (95–876)[b]
Baseline DAS28	5.2 (2.7–7.3)	5.1 (1.6–7.6)	5.2 (2.7–7.3)	4.8 (1.6–7.2)	5.1 (1.6–7.2)	5.1 (1.6–7.2)
$DAS28_{AUC}$			3.4 (1.3–6.2)[a]	3.3 (2.2–5.5)[a]	2.2 (1.2–5.6)[b]	3.3 (2.2–5.5)[b]

[a] The mean value (AUC) of the different biomarkers during the first 12 mo of the study were calculated using 13 time points, and [b] the mean value during the 24 mo study period were calculated using 25 time points. CRP: C-reactive protein; DAS28: disease activity score, ESR based; ESR: erythrocyte sedimentation rate; IL-6: Interleukin-6; RA: rheumatoid arthritis; RF: rheumatoid factor; VEGF: vascular endothelial growth factor.

Table 3. Clinical characteristics and baseline and AUC values of the biomarkers and DAS28 of the early RA patients with progressions in bone erosions at 12 and 24 mo followup compared to RA patients with non-progressive disease. Values are medians (ranges) unless otherwise stated.

Characteristics	Progressive Disease at 12 mo, n = 14	Non-progressive Disease at 12 mo, n = 30	Progressive Disease at 24 mo, n = 14	Non-progressive Disease at 24 mo, n = 30
Age, yrs	59 (29–82)	53 (20–82)	57 (29–82)	54 (20–82)
Women/men	10/4	25/5	10/4	25/5
Disease duration, mo	4 (1–22)	3 (1–17)	4 (1–22)	4 (1–17)
IgM RF positive, %	71 (10/14)	38 (11/29)	71 (10/14)	38 (11/29)
Baseline plasma IL-6	15 (0.6–98)	4.6 (1.0–21)	9 (0.6–98)	6 (1.0–29)
Plasma $IL\text{-}6_{AUC}$	8.4* (0.8–61)[a]	2.8 (0.9–51)[a]	6.1* (0.8–41)[b]	3.6 (1.0–50)[b]
Baseline plasma VEGF	136 (37–474)	136 (15–818)	136 (37–474)	139 (15–818)
Plasma $VEGF_{AUC}$	144 (66–561)[a]	137 (16–1126)[a]	134 (59–469)[b]	132 (15–965)[b]
Baseline serum YKL-40	129 (69–460)	131 (36–540)	119 (69–460)	150 (36–540)
Serum $YKL\text{-}40_{AUC}$	137 (71–1608)[a]	125 (35–460)[a]	123 (72–1328)[b]	125 (47–430)[b]
Baseline serum CRP	95 (95–543)	95 (95–1374)	95 (95–543)	95 (95–876)[b]
Serum CRP_{AUC}	124 (95–1036)[a]	105 (95–1127)[a]	115 (95–694)[b]	109 (95–876)[b]
Baseline ESR	20 (6–50)	15 (1–96)	20 (6–50)	15 (1–96)
ESR_{AUC}	19 (3–85)[a]	13 (2–95)[a]	17 (3–73)[b]	13 (1–85)[b]
Baseline DAS28	5.2 (4.2–7.3)	5.1 (1.6–7.2)	5.2 (3.8–7.3)	5.1 (1.6–7.2)
$DAS28_{AUC}$	3.5 (1.4–5.3)[a]	3.4 (1.3–6.3)[a]	3.0 (1.2–5.0)[b]	3.0 (1.4–5.5)[b]

[a] The mean value (AUC) of the different biomarkers during the first 12 mo of the study were calculated using 13 time points, and [b] the mean value during the 24 mo study period were calculated using 25 time points. Differences between groups were calculated by Mann-Whitney: * $p < 0.05$, ** $p < 0.001$. CRP: C-reactive protein; DAS28: disease activity score, ESR based; ESR: erythrocyte sedimentation rate; IL-6: Interleukin-6; RA: rheumatoid arthritis; RF: rheumatoid factor; VEGF: vascular endothelial growth factor.

the normal dose used to treat RA patients in Denmark. This may explain why the biomarker was not fully suppressed as the disease process in the joints was not fully suppressed, despite low clinical disease activity or even remission. Previous investigations of RA patients analyzed VEGF in serum and not in plasma, as in our study. We chose to analyze plasma because of the time-dependent efflux of VEGF from platelets and neutrophils that takes place during blood clotting, leading to an increased serum VEGF concentration compared to plasma. A substantial amount of VEGF in serum, therefore, does not originate from VEGF production locally in areas with inflammation[34].

Roughly 40% of the patients with early RA had erosions at baseline, and one-third of patients had progressive erosive disease during the 24-month study period. No differences in any of the biomarkers tested were found between the patients with and those without bone erosions at baseline. MRI is considered to be more sensitive regarding detection of erosions compared to conventional radiography[39]. Eight patients had erosions at baseline visible only on MRI, but adding these patients to the group with bone erosions did not change the results, i.e., there was still no difference in the biomarkers between erosive and nonerosive patients. Interestingly, and in agreement with other results[8], the patients with progression in bone erosions during the study period had higher baseline IL-6 and mean plasma IL-6 during the 24-month study compared to patients without progression in bone erosions. It was reported in a study of 20 early RA patients followed for 3 years that time-integrated ESR and serum CRP values were better correlated to radiographic progression than time-integrated plasma IL-6 (determined by an in-house ELISA)[37]. In contrast with an earlier study from our group[30], we could not in the present study find any relation between serum YKL-40 and bone erosions.

A biomarker can be defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention"[40]. Serum or plasma IL-6, VEGF, and YKL-40 concentrations have not yet received US Food and Drug Administration approval for use as biomarkers in patients with RA or any other disease. It remains unknown if circulating concentrations of these biomarkers in an individual patient with early RA are sufficiently reliable to be used to make clinical decisions that will improve outcome for the patient.

Plasma IL-6, plasma VEGF, and serum YKL-40 but not serum CRP were elevated in patients with early RA compared to patients with unclassified polyarthritis and healthy subjects. Plasma IL-6, serum YKL-40, serum CRP, and ESR decreased in patients responding to treatment with DMARD and glucocorticoids, but plasma IL-6 and serum YKL-40 did not provide any additional information regarding disease activity or treatment response, compared to serum CRP and ESR. Plasma IL-6 was related to progression in bone erosions, suggesting that plasma IL-6, probably in combination with other predictors like RA-typical autoantibodies[41], may have clinical value regarding identification of patients with early RA at risk of progressive erosive disease. This remains to be confirmed in larger studies of patients with early RA.

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