Immunogenetic Markers and Seropositivity Predict Radiological Progression in Early Rheumatoid Arthritis Independent of Disease Activity

SYLKE KALTENHÄUSER, ULF WAGNER, ERNST SCHUSTER, RALF WASSMUTH, SYBILLE ARNOLD, WOLFRAM SEIDEL, MICHAEL TRÖLTZSCH, MARKUS LOEFFLER, and HOLM HÄNTZSCHEL

ABSTRACT. ***Objective.*** A prospective clinical study of patients with recent onset rheumatoid arthritis (RA) to examine the relationship between inflammatory disease activity and joint destruction in a 4 year followup, and to evaluate prognostic markers for severe joint erosions early in the disease.

Methods. Eighty-seven patients with RA according to the American College of Rheumatology criteria and a disease duration < 2 years were followed for an observation time of 2 to 4 years (mean 3.1 yrs). Variables of clinical and laboratory disease activity were monitored, and HLA-DRB1 alleles were determined. Hand and foot radiographs were taken every 6 months.

Results. Multivariate analysis of independent contributions of covariates to progression of joint destruction resulted in a mixed effect regression model with significant influences for the presence of a shared epitope (SE) positive DR4 allele (SE+ DR4+; $p = 0.007$), rheumatoid factor (RF) IgA ($p = 0.01$), and sex ($p = 0.059$), but not for clinical variables or acute phase reactants. The odds ratio to reach a Larsen score above 32 during the observation period of 4 years was increased in patients positive for RF IgM (OR 2.7, $p = 0.019$), for the shared epitope on a DR4 allele (OR 8.6, $p < 0.005$), and in patients with erosions already at study entry (OR 11.9, $p = 0.001$). The highest sensitivity and specificity for the prediction of severe bone destruction (84% and 79%) were found when the presence of either a SE+ DR4 allele or of early erosions was used as a prognostic marker (OR 20.4, $p < 0.0001$).

Conclusion. Our results show the pace of joint destruction in RA to be influenced by the presence of SE+ DR4 alleles, RF production, and sex and by the presence of erosive disease at presentation. Those prognostic markers exert their influence independently from the inflammatory disease activity. (J Rheumatol 2001;28:735–44)

Key Indexing Terms:
RHEUMATOID ARTHRITIS IMMUNOGENETICS PROGNOSIS

Clinical management of patients with rheumatoid arthritis (RA) remains a challenging problem due to the potential side effects of immunosuppressive therapy. Treatment decisions would be greatly facilitated by the availability of prognostic markers predictive of both the time course and severity of joint destruction and of complications due to an extraarticular course of the disease[1].

It has been established that immunogenetic markers are predictive for the severity of extraarticular disease. The disease associated HLA-DRB1 alleles (*0401, *0404, *0405, *0408), particularly in homozygous individuals, were shown to be predictive for the development of major organ involvement[2,3]. Aside from an association of the shared epitope (SE) with the course of the extraarticular disease, we and others have shown an influence of immunogenetic markers on the radiological progression of joint destruction in early disease[4-11]. Controversy remains, however, on how sustained this influence remains after more than 2 years of disease duration[12]. Interestingly, the acute inflammatory response does not differ in patients positive or negative for SE positive DRB1*04 alleles as markedly as radiological progression[4,13], or was only increased in patients homozygous for SE+ DR4+[14]. On the other hand, the correlation between C-reactive protein (CRP) and the progression of joint erosions, although detectable in most[15-17] but not all[18] studies, is characterized by wide interindividual variation[19]. In some clinical studies,

From the Department of Medicine IV, University of Leipzig, Leipzig, Germany.

Supported by grants from the Bundesministerium für Bildung und Forschung (Verbundprojekt Struktur und Funktion des Bindegewebes bei rheumatischen und Autoimmunerkrankungen, grant 01ZZ9103/14 and Interdisziplinäres Zentrum für Klinische Forschung Leipzig, Teilprojekt A15).

S. Kaltenhäuser, MD; U. Wagner, MD; S. Arnold, MD; W. Seidel, MD; M. Tröltzsch, MD; H. Häntzschel, MD, Professor of Rheumatology, Department of Rheumatology, University of Leipzig; M. Loeffler, MD, PhD, Professor of Medical Informatics, Statistics and Epidemiology; E. Schuster, PhD, Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig; R. Wassmuth, MD, Institute for Clinical Immunology, Department of Medicine III, University of Erlangen-Nürnberg.

Dr. Kaltenhäuser and Dr. Wagner contributed equally to this article.

Address reprint requests to Dr. H. Häntzschel, Department of Medicine IV, University of Leipzig, Härtelstrasse 16-18, 04107 Leipzig, Germany. E-mail: haentzho@medizin.uni-leipzig.de

Submitted May 24, 2000 revision accepted October 30, 2000.

the association between acute phase response and radiographic progression was most prominent during the first 6 months of disease duration[20] or seemed to be less predictive for the severity of joint destruction than immunogenetic markers[21]. Those observations are consistent with disease models that stem from analysis of cytokine expression in animal models and that propose a dissociation of the inflammatory and the joint destructive component of the disease process in RA[22-24]. In some studies, the clinical analysis of the relationship between synovitis and radiological progression in individual joints also showed that the correlation between the 2 disease components is only weak[25,26].

The objective of our prospective study was to create a multivariate model of the destructive process in the disease that allowed identification of factors prognostic for a more rapid course of joint destruction. We were particularly interested in the inflammatory component of disease activity and its interdependence with the course of bone destruction as seen in hand and feet radiographs.

MATERIALS AND METHODS

Study design. Since 1992 a prospective, observational study of patients with early RA has been carried out. Patients were sequentially recruited from the outpatient clinic of the Department of Rheumatology at Leipzig University. Informed consent was obtained from all patients. Included were patients with RA according to the 1987 American College of Rheumatology (ACR) criteria[27] with onset of symptoms less than 2 years prior to inclusion in the study. Only patients older than 18 years of age were enrolled. To avoid skewing due to the influence of a previous treatment patients who received disease modifying antirheumatic drugs prior to enrolment were excluded. Patients were excluded if a rheumatologic diagnosis other than RA could be established during followup, or if they were lost to followup.

During the study, patients were treated according to standard rheumatological practice. The protocol treatment intended was monotherapy with 2 g sulfasalazine per day or 15 mg methotrexate weekly combined with low dose prednisone in the dosage required to clinically control disease activity. In case of discontinuation of treatment due to insufficient response or adverse side effects, further therapy was modified according to clinical requirements.

Study documentation. Disease activity and joint destruction were documented at initial presentation, after 6 months, and after 1, 2 and 4 years.

For the study of clinical aspects of the disease course, clinical severity of joint affliction was judged by the number of swollen joints, duration of morning stiffness, Health Assessment Questionaire, and a functional mobility score. Joint tenderness was documented using the Ritchie Articular Index.

The relevant laboratory variables consisted of erythrocyte sedimentation rate (ESR), CRP, hemoglobin, complete and differential blood count, and the IgM and IgA rheumatoid factor (RF).

As one major outcome variable of the destructive process, radiological evaluation of joint erosions was carried out. At study entry and at each scheduled visit, hand and foot radiographs were taken and scored by 2 independent radiologists using the Larsen score[28].

HLA-DRB1 typing. HLA typing was performed by oligonucleotide hybridization of enzymatically amplified DNA as described[4]. Low resolution HLA-DRB1 typing comprising the DRB1*01 to DRB1*17 specificities was performed by sequence-specific hybridization of a panel of oligonucleotide probes to polymerase chain reaction products. For DRB1*04 positive individuals high resolution subtyping of the HLA-DRB1 locus was performed.

Biometric analyses. Differences in medians or means between groups were analyzed using Mann-Whitney or T test where appropriate. Correlations were evaluated using the Spearman rank correlation coefficient method. Rates and proportions were compared by chi-square or Fisher's exact test. A level of significance of $\alpha = 0.005$ was employed in the univariate tests. For the multivariate regression analysis, a level of significance of $\alpha = 0.05$ was used. For all calculations, SPSS 8.0 (SPSS Inc., Chicago, IL, USA) was used. For evaluation of the predictive diagnostic value, odds ratios were calculated as published[4]. As response variable for statistic modeling, changes in the Larsen score in the first and 2nd year and the averaged yearly change during the 3rd and 4th year of observation were used. The yearly increase in Larsen score was chosen as the outcome measure of disease progression for the multivariate analysis, since the radiological joint destruction measured by the Larsen score is regarded as irreversible. To accommodate the data from repeated time intervals, a linear regression model for repeated measurements was chosen. Due to the considerable variability between patients, a mixed effect regression with a random intercept was introduced that used the main effects as fixed effects.

A step-down elimination procedure using the Bayesian information criterion was applied[29,30]. The Larsen score values after 48 months in patients that had not yet reached that time point of observation can be assumed to be distributed completely at random. Thus all cases could be included in the analysis. These calculations were done using S-PLUS 4.5 (MathSoft, Seattle, WA, USA) with the function lme.

The repeated measurement structure was accounted for by a random intercept, i.e., an estimated individual intercept for each patient, which modifies the common intercept. A normal distribution with the expected value zero was assumed. For serial correlations, a model with equal correlation between residuals at the different time points was adopted (compound symmetry model).

RESULTS

The Patient Cohort

All 87 patients included in the study cohort fulfilled the 1987 ACR criteria at study entry. Five patients that had originally been included in the study, but in whom rheumatological diagnoses other than RA could be established, were excluded from the analysis (2 patients with undifferentiated connective tissue disease, one with psoriatic arthritis, 2 with systemic lupus erythematosus). Six patients were excluded from the analysis since they were lost to followup (2 patients died, and 4 refused further participation in the study). Patients' participation was high, with more than 95% of contacted patients agreeing to participate.

Seventy-three patients in the study cohort were women (84%) and 14 men. The median disease duration before study entrance (time between establishment of the diagnosis and enrolment in the study) was 6.1 months (interquartile range 3.4–11.3 mo). The majority of patients had a disease duration less than one year (77%) before study entry. All 87 study patients had been followed for 2 years, while data over 4 years of followup were available for 48 patients.

A clinical characterization of the study population is given in detail in Table 1; 32% of patients had radiographic evidence of bony erosions at initial presentation. The median swollen joint count at study entry was 9 (interquartile range 5–14) and the median CRP level was 14.7 mg/l (interquartile range 0–44.8 mg/l). Fifty-seven patients (65.5%) had RF IgM seropositive disease, and 53 of them

Table 1. Characteristics of the patient populations at study entry. Clinical and laboratory variables at study entry, Larsen scores and DMARD usage at the end of the observation periods for the study cohorts followed for 24 and 48 months. Disease duration before study entry = time between the establishment of the diagnosis and study enrolment. Number of DMARD = number of successive treatment attempts with different DMARD. Percentages of patients using the different medications refers to current DMARD usage at the time of the 24 and 48 month analysis.

		Patients Followed 24 mo	Patients Followed 48 mo
N		87	48
M/F		14/73	10/38
Age at disease onset, yrs	Median (25–75%)	54 (37.9–63.2)	50.7 (34.9–57.9)
Disease duration before study entry, mo	Median (25–75%)	6.1 (3.4–11.3)	6.0 (3.4–12.3)
ESR, mm Hg	Median (25–75%)	30 (21.3–48.3)	30 (21.0–53.5)
CRP, mg/l	Median (25–75%)	14.7 (0–44.8)	13.4 (0–32.7)
Swollen joint count	Median (25–75%)	9 (5.0–14.0)	10 (5.5–15.0)
Morning stiffness, min	Median (25–75%)	60 (5.0–120)	60 (2.5–120)
Ritchie index	Median (25–75%)	8 (6.0–13.0)	10.5 (6.0–14.5)
Patients positive for RF IgM, %		65.5	62.5
RF IgM, IU/ml	Median (25–75%)	70.6 (0–206)	48.7 (0–162)
Patients positive for RF IgA, %		25.3	16.6
RF IgA, IU/ml	Median (25–75%)	0 (0–31.5)	0 (0–0)
Patients with erosions at study entry, %		32.2	29.2
Larsen score at study entry	Median (25–75%)	0 (0–4.0)	0 (0–5.0)
Larsen score at the end of the observation period	Median (25–75%)	14.0 (0–32.7)	23.5 (0–40.5)
No. of DMARD	Median (25–75%)	2 (1–2)	2 (1–2)
Methotrexate, %		65.6	58.3
Sulfasalazine, %		12.6	10.4
Intramuscular gold salts, %		5.7	4.2
Chloroquine, %		2.3	4.2
Combination of methotrexate and cyclosporin A, %		0	12.5
Steroids only without DMARD therapy, %		13.8	10.4
Steroids in combination with DMARD, %		71.3	56.2

(93%) were already seropositive at initial presentation. In contrast, only 55% of the 40 patients with detectable titers of RF IgA at least once during the observation period (46% of the study population) were already RF IgA positive at study entry. Thirty-three of 40 RF IgA positive patients (82.5%) were also positive for RF IgM (Figure 1A). The results of genotyping of the HLA-DRB1 locus are depicted in Figure 1B. Thirty-two patients (36.8%) expressed the RA associated shared epitope (SE) on a DR4 allele. An additional group of 21 patients (24.1%) typed positive for DR1 (in our ethnically very homogeneous German population almost exclusively DRB1*0101), resulting in a total of 53 patients (60.9%) expressing the RA associated SE on either a DR1 or a DR4 allele. Compound homozygosity for the SE occurred in 12 patients, and 6 patients expressed RA associated DR4 alleles on both chromosomes.

At the final evaluation (for 48 patients after 4 and for 39 patients after 2 years of observation), 52 patients were treated with methotrexate, 10 with sulfasalazine, 5 with intramuscular gold salts, 2 with chloroquine, and 5 with a combination of methotrexate and cyclosporin A. Thirteen patients received low dose prednisone therapy only. A detailed analysis of therapy patients received after 2 and 4 years is given in Table 1.

Clinical and Genetic Variables and Radiological Course of Joint Destruction

Time course of the Larsen score. Despite the short duration of disease before study entry, one-third of the patients already had erosions on hand and foot radiographs when first seen in clinic. This was not, however, associated with a longer time span between onset of symptoms and study entry in those patients or significant differences in any other of the clinical and inflammatory variables analyzed and does compare to or is even lower than the data obtained in other studies[17,31-33]. In addition, we examined the changes in Larsen score occurring during the observation period with respect to their clinical relevance in comparison to the erosive changes already present at study entry. After a disease duration of 2 years, 60 patients (68.9%) had erosive disease. In 50 of those 60, the change in Larsen score over the first 2 years contributed 50% or more of the value reached after 2 years, so that the Larsen score value at study entry was in the vast majority of cases lower than the increase during the study period.

Table 1. Characteristics of the patient populations at study entry. Clinical and laboratory variables at study entry, Larsen scores and DMARD usage at the end of the observation periods for the study cohorts followed for 24 and 48 months. Disease duration before study entry = time between the establishment of the diagnosis and study enrolment. Number of DMARD = number of successive treatment attempts with different DMARD. Percentages of patients using the different medications refers to current DMARD usage at the time of the 24 and 48 month analysis.

		Patients Followed 24 mo	Patients Followed 48 mo
N		87	48
M/F		14/73	10/38
Age at disease onset, yrs	Median (25–75%)	54 (37.9–63.2)	50.7 (34.9–57.9)
Disease duration before study entry, mo	Median (25–75%)	6.1 (3.4–11.3)	6.0 (3.4–12.3)
ESR, mm Hg	Median (25–75%)	30 (21.3–48.3)	30 (21.0–53.5)
CRP, mg/l	Median (25–75%)	14.7 (0–44.8)	13.4 (0–32.7)
Swollen joint count	Median (25–75%)	9 (5.0–14.0)	10 (5.5–15.0)
Morning stiffness, min	Median (25–75%)	60 (5.0–120)	60 (2.5–120)
Ritchie index	Median (25–75%)	8 (6.0–13.0)	10.5 (6.0–14.5)
Patients positive for RF IgM, %		65.5	62.5
RF IgM, IU/ml	Median (25–75%)	70.6 (0–206)	48.7 (0–162)
Patients positive for RF IgA, %		25.3	16.6
RF IgA, IU/ml	Median (25–75%)	0 (0–31.5)	0 (0–0)
Patients with erosions at study entry, %		32.2	29.2
Larsen score at study entry	Median (25–75%)	0 (0–4.0)	0 (0–5.0)
Larsen score at the end of the observation period	Median (25–75%)	14.0 (0–32.7)	23.5 (0–40.5)
No. of DMARD	Median (25–75%)	2 (1–2)	2 (1–2)
Methotrexate, %		65.6	58.3
Sulfasalazine, %		12.6	10.4
Intramuscular gold salts, %		5.7	4.2
Chloroquine, %		2.3	4.2
Combination of methotrexate and cyclosporin A, %		0	12.5
Steroids only without DMARD therapy, %		13.8	10.4
Steroids in combination with DMARD, %		71.3	56.2

(93%) were already seropositive at initial presentation. In contrast, only 55% of the 40 patients with detectable titers of RF IgA at least once during the observation period (46% of the study population) were already RF IgA positive at study entry. Thirty-three of 40 RF IgA positive patients (82.5%) were also positive for RF IgM (Figure 1A). The results of genotyping of the HLA-DRB1 locus are depicted in Figure 1B. Thirty-two patients (36.8%) expressed the RA associated shared epitope (SE) on a DR4 allele. An additional group of 21 patients (24.1%) typed positive for DR1 (in our ethnically very homogeneous German population almost exclusively DRB1*0101), resulting in a total of 53 patients (60.9%) expressing the RA associated SE on either a DR1 or a DR4 allele. Compound homozygosity for the SE occurred in 12 patients, and 6 patients expressed RA associated DR4 alleles on both chromosomes.

At the final evaluation (for 48 patients after 4 and for 39 patients after 2 years of observation), 52 patients were treated with methotrexate, 10 with sulfasalazine, 5 with intramuscular gold salts, 2 with chloroquine, and 5 with a combination of methotrexate and cyclosporin A. Thirteen patients received low dose prednisone therapy only. A detailed analysis of therapy patients received after 2 and 4 years is given in Table 1.

Clinical and Genetic Variables and Radiological Course of Joint Destruction

Time course of the Larsen score. Despite the short duration of disease before study entry, one-third of the patients already had erosions on hand and foot radiographs when first seen in clinic. This was not, however, associated with a longer time span between onset of symptoms and study entry in those patients or significant differences in any other of the clinical and inflammatory variables analyzed and does compare to or is even lower than the data obtained in other studies[17,31-33]. In addition, we examined the changes in Larsen score occurring during the observation period with respect to their clinical relevance in comparison to the erosive changes already present at study entry. After a disease duration of 2 years, 60 patients (68.9%) had erosive disease. In 50 of those 60, the change in Larsen score over the first 2 years contributed 50% or more of the value reached after 2 years, so that the Larsen score value at study entry was in the vast majority of cases lower than the increase during the study period.

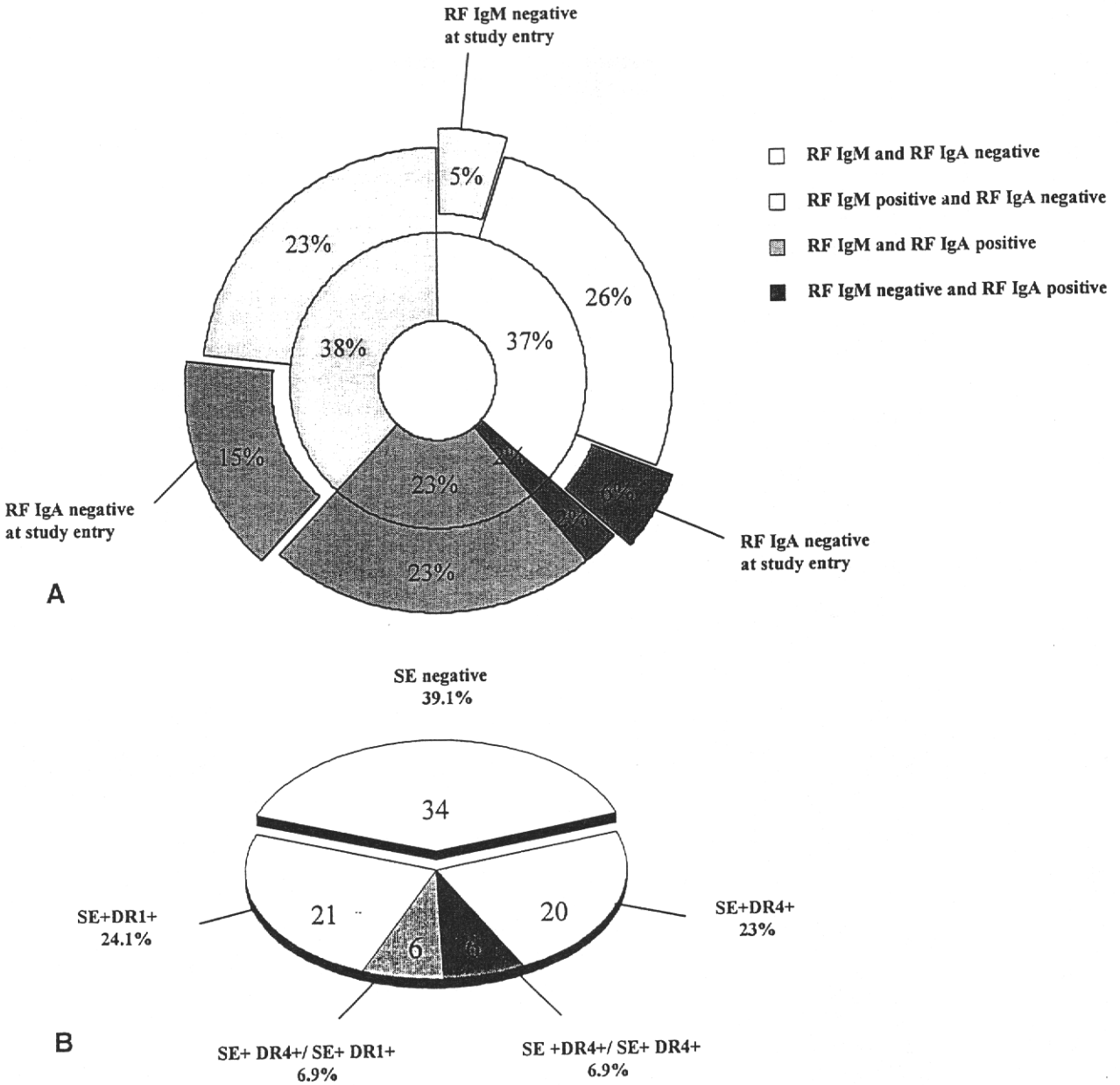

Figure 1. Distribution of rheumatoid factor IgM and IgA production and of immunogenetic markers in the study cohort. A. RF production in 87 patients at initial presentation (inner circle) and development during the course of the study (outer circle). The exploded slices represent the fraction of patients in whom RF status changed during the observation period. Percentages of patients in the respective groups are given. B. Percentages of patients carrying the RA associated shared epitope sequence on one chromosome on a DR1 allele (SE+DR1+), on one chromosome on a DR4 allele (SE+DR4+), on both chromosomes with one DR1 and one DR4 allele (SE+DR1+/SE+DR4+), on both chromosomes on a DR4 allele (SE+DR4+/SE+DR4+), and patients negative for the shared epitope (SE negative).

Larsen scores at study entry showed a significant correlation with the yearly increase in Larsen score during the first (R = 0.3, p = 0.005) and 2nd year of observation (R = 0.29, p = 0.006), and patients with early erosions had a higher increase in Larsen scores during the first and second year of observation (median 4 vs 0; p = 0.001 and median 5 vs 0; p = 0.004, respectively). Patients with the fastest increase in Larsen score during the first year also continued to progress faster later in the study, as indicated by the correlation between the yearly increase in Larsen score during the first year and the yearly increase during the 3rd and 4th years of observation (R = 0.519, p < 0.001).

In summary, the results indicate a continuously faster progression of joint destruction during the first 2 years of observation in patients with very early erosions that were already present at study entry (Figure 2).

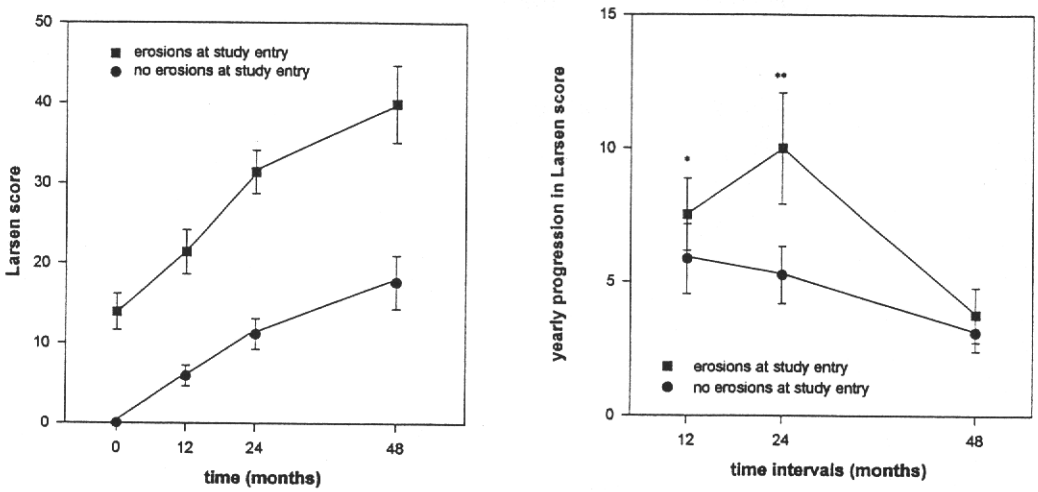

Figure 2. Time course of Larsen scores in patients presenting with erosions at study entry. A. Means and SEM of Larsen scores are given for 0, 12, and 24 months (n = 87) and 48 months (n = 48) for patients negative (●, n = 59) and positive (■, n = 28) for erosions at study entry. B. Means and SEM of the yearly increase in Larsen scores for the time intervals 0 to 12, 12 to 24, and 24 to 48 months are shown for patients negative (●) and positive (■) for erosions at study entry. *p < 0.05, **p < 0.01.

Clinical and laboratory variables and joint destruction. The acute phase response measured by CRP concentrations at different time points showed a significant correlation with the clinical disease activity (number of swollen joints and, to some extent, Ritchie index; Table 2). There was no correlation, however, of ESR or CRP, neither at study entry nor after 2 or 4 years of disease duration, with the Larsen scores after those observation periods (Table 2). Only the increase in Larsen score during the 2nd year of observation was found to correlate with the CRP value obtained after 2 years of observation. While the average CRP values over time were also found to correlate with the increase in Larsen scores during the 2nd year of observation, they did not show a significant relationship with the absolute values of Larsen score reached after 2 or 4 years of observation or the increase during the first, 3rd, and 4th year of the study (Table 2).

With regard to RF production, patients positive for RF IgM were significantly different in their Larsen scores from seronegative patients. They had higher Larsen score values after 2 and 4 years of observation (median 18.8 vs 0; p = 0.017 and median 30.1 vs 2.6; p = 0.031, respectively; Figure 3B). The detection of a positive titer of RF IgA in a patient at least once during the observation period was also associated with a higher Larsen score after 2 and 4 years (median 19.8 vs 5.2; p = 0.006 and median 33.9 vs 9.9; p = 0.005; Figure 3C).

Sex and immunogenetics. At study entry, no difference was seen between the percentage of male and female patients already having bony erosions. The increase in Larsen scores

Table 2. Correlation between variables of disease activity and Larsen scores at different time points. Spearman rank correlation coefficients and radiological findings of the Larsen score are shown for the relationships between variables of disease activity at the time points indicated. Levels of significance shown in parentheses. Significant correlations are printed in bold.

	At Time Point, mo	Swollen Joint Count	Ritchie Index	Larsen Score			Progression of Larsen Score		
				At Study Entry	After 24 mo	After 48 mo	During 1st yr	During 2nd yr	During 3rd/4th yr
ESR	0	0.23	0.06	0.12	0.21	0.02	–0.09	0.06	0.18
		(0.03)	(0.53)	(0.26)	(0.14)	(0.88)	(0.38)	(0.54)	(0.23)
	24	**0.38**	**0.33**	0.11	–0.01	0.07	0.01	0.28	0.17
		(< 0.001)	**(0.001)**	(0.30)	(0.88)	(0.62)	(0.90)	(0.008)	(0.24)
CRP	0	**0.41**	0.24	0.03	0.05	–0.006	–0.007	0.11	0.01
		(< 0.001)	(0.02)	(0.75)	(0.62)	(0.96)	(0.95)	(0.28)	(0.94)
	24	**0.31**	**0.32**	0.01	0.24	0.22	–0.09	**0.36**	0.23
		(< 0.003)	**(0.002)**	(0.89)	(0.03)	(0.14)	(0.40)	**(< 0.001)**	(0.11)
Average CRP	0–24	0.02	0.12	0.04	0.17	0.08	0.01	**0.33**	0.11
		(0.02)	(0.24)	(0.69)	(0.10)	(0.55)	(0.90)	**(0.001)**	(0.46)

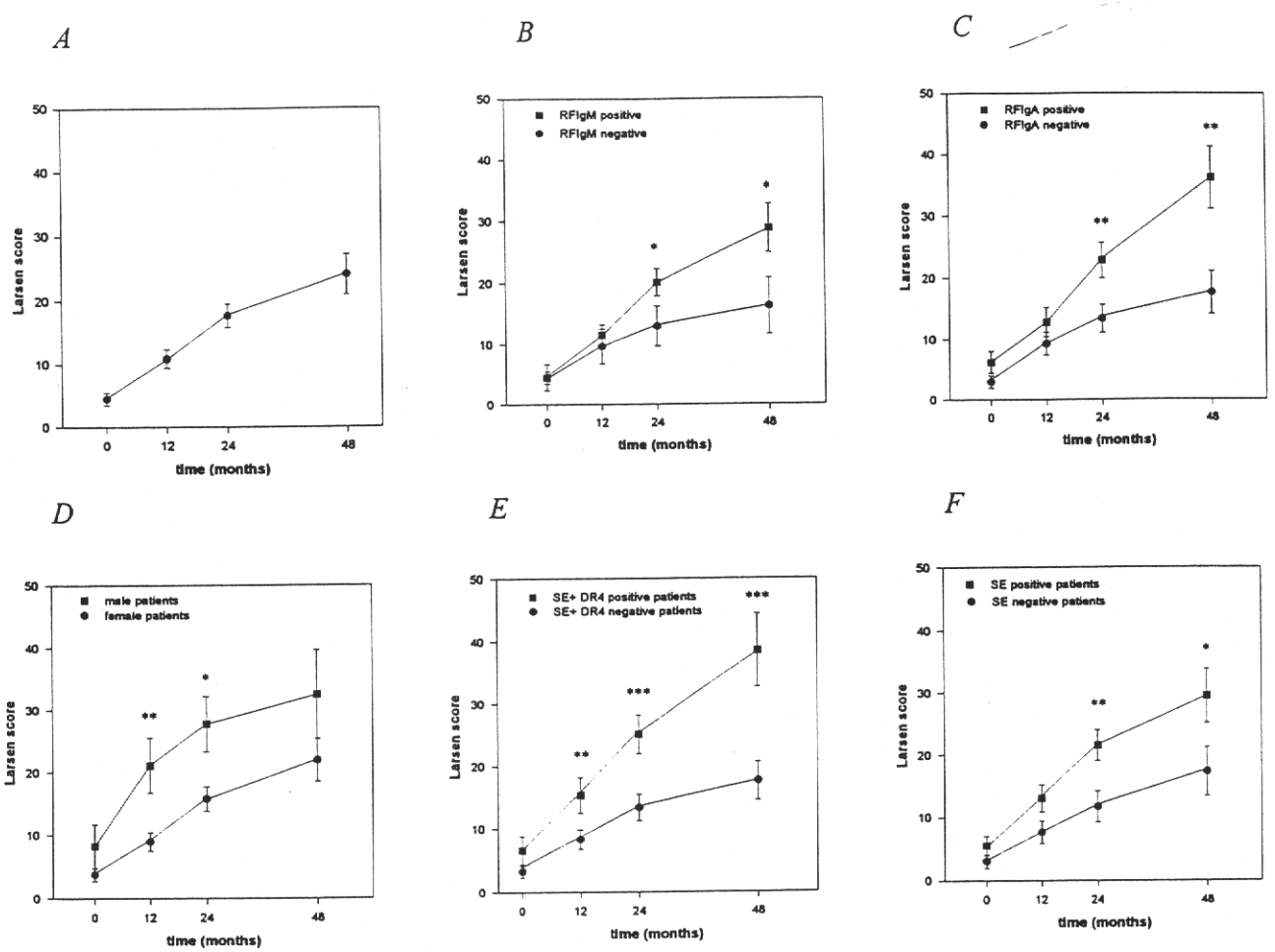

Figure 3. Time course of Larsen scores in patients grouped according to quantitative values of the covariates. Means and SEM of Larsen scores are given for 0, 12, and 24 months (n = 87) and 48 months (n = 48) for groups of patients fulfilling the following criteria. A. Larsen scores in the study population. B. RF IgM negative (●, n = 30) and positive patients (■, n = 57). C. RF IgA negative (●, n = 47) and positive patients (■, n = 40). D. Female (●, n = 73) and male (■, n = 14) patients. E. Patients positive (■, n = 55) and negative (●, n = 32) for the shared epitope sequence on a DRB1*04 allele. F. Patients positive (■, n = 34) and negative (●, n = 53) for the shared epitope sequence. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$.

during the first year of observation was, however, more pronounced in male compared to female patients (median 12 vs 0.5; p = 0.003), resulting in a higher Larsen score after one year (median 19.5 vs 3; p = 0.003). This difference was less pronounced after 2 years (median 27.5 vs 10; p = 0.017) and did not reach statistical significance again in the subsequent course of disease (Figure 3D).

The presence of the immunogenetic markers analyzed had a significant effect on radiological progression. Patients that expressed the SE sequence on a DR4 allele had higher Larsen scores after one, 2, and 4 years of disease duration (median 10.5 vs 0; p = 0.016, median 27.5 vs 4; p = 0.001, and median 39 vs 17.0; p = 0.001; Figure 3E).

The presence of the shared epitope sequence on any DR allele also resulted in higher Larsen scores after 2 and 4 years for positive patients (median 19 vs 2.0; p = 0.006 and median 32 vs 22; p = 0.03; Figure 3F). The influence of this marker, which includes the DR1 allele, only became apparent after 2 years of disease duration, while no difference was seen at initial presentation or after one year. When only patients positive for DR1 were analyzed, they did not differ significantly from SE negative patients. With regard to a possible gene dosage effect, no difference in Larsen score was seen between patients expressing the SE on both chromosomes and the patients positive for the SE sequence on only one chromosome.

Multivariate Analysis of Prognostic Factors for the Radiographic Progression of Joint Destruction

The values of all variables of disease activity at initial presentation and at the second visit after 6 months were included into a multivariate model describing the progression over time as yearly increase in Larsen score and its dependency on the covariates. In addition, sex and the pres-

ence of the RA associated shared epitope as well as age at disease onset, levels of RF IgM and RF IgA, and the Larsen score at initial presentation were included.

Table 3 gives the effect of all covariates included in the initial model with their respective 95% confidence intervals and the significance for their contribution. Variables without influence on the outcome in yearly increase in Larsen score (LS) were removed from the model by stepwise deletion. By this process, the fit of the model was improved, as indicated by the reduction of the Bayesian information criterion from 1707 to 1605. The resulting final model (Table 3) describes the influence of the covariates on the radiological progression of joint destruction as follows:

Yearly increase in LS = 7.23 + 3.24 [for SE+ DR4+ patients] + 0.0126 * initial RF IgA [IU/ml] + 2.92 [for male patients] – 3.06 [if later than 2 years]

In this model, the progression of disease is indicated by a yearly baseline increase in the Larsen score of 7.23, which is described by the intercept ($p < 0.0001$). The presence of an RA associated DRB1*04 allele had the strongest effect on progression, resulting in an additional yearly increase in LS of 3.24 ($p = 0.007$). In addition, the level of RF IgA measured at study entry had a significant influence on the yearly increase in Larsen score ($p = 0.01$). For example, a RF IgA level of 100 IU/ml at study entry resulted in an additional yearly increase in Larsen score of 1.26.

There was a sex difference, with men having a higher yearly increase of 2.92 compared to women ($p = 0.059$). The covariate sex was included in the final model despite the failed level of significance, since the fit for both Akaike information criterion and Bayesian information criterion was impaired when it was removed. The influence of time indicates that the yearly increase in Larsen score during the 3rd and 4th year was 3.06 ($p = 0.018$), lower than during the first 2 years. Markov chain Monte Carlo (MCMC) methods, which allow for inclusion of cases with missing covariates

Table 3. Initial and final mixed effect regression model for repeated measurements using covariates at study entry and after an observation period of 6 months. Covariates included in the mixed effect regression model for repeated measurements for the radiological progression of joint destruction. As independent variable, the yearly increase in Larsen score (LS) was used. The initial model shows all covariates at study entry and after an observation period of 6 months. In the resulting final model, covariates were reduced to those with a significant effect on radiological joint destruction. Covariates are given with their confidence interval and level of significance in the initial and final model. SE+DR4+: patients positive for the shared epitope on a DR4 allele; SE+ DR1+: patients for the shared epitope on a DR1 allele; intercept: the yearly baseline increase in Larsen score in the study population. Time: factor time for observations during the 3rd and 4th year. Significant findings indicated in bold print.

	Factor	95% CI		p
		Lower Limit	Upper Limit	
Initial model				
Intercept (yearly baseline increase in LS)	4.5248	–2.2336	11.28	0.189
SE+DR4+	3.824	1.0688	6.5792	**0.007**
SE+DR1+	0.9888	–1.8112	3.7856	0.489
Time (1 for 3rd and 4th yr)	–3.152	–5.8016	–0.5024	**0.020**
Male sex	2.8192	6.3328	0.6912	0.115
RF IgA level at study entry, IU/ml	0.016	0	0.0288	**0.036**
RF IgA level after 6 mo, IU/ml	–0.0096	–0.0416	0.0224	0.546
RF IgM level at study entry, IU/ml	0	–0.0032	0.0032	0.634
RF IgM level after 6 mo, IU/ml	–0.0032	–0.0096	0.0032	0.478
CRP level at study entry, mg/l	–0.0096	–0.0512	0.032	0.648
CRP level after 6 mo, mg/l	0.0416	–0.0384	0.1216	0.308
Morning stiffness at study entry, min	0.0064	–0.0096	0.0192	0.488
Morning stiffness after 6 mo, min	–0.0096	–0.0288	0.0128	0.450
Swollen joint count at study entry	0.2528	–0.224	0.7328	0.299
Swollen joint count after 6 mo	0.096	–0.5024	0.6944	0.756
Ritchie index at study entry	–0.1728	–0.3872	0.0416	0.112
Ritchie index after 6 mo	0.2784	–0.0768	0.6336	0.123
Larsen score at study entry	0.016	–0.1344	0.1664	0.835
Age at disease onset, yrs	0.0064	–0.08	0.096	0.875
Final model				
Intercept (yearly baseline increase in LS)	7.2342	4.1984	10.2624	**< 0.001**
Time (1 for 3rd and 4th yr)	–3.0619	–5.5936	–0.5248	**0.018**
SE+DR4+	3.2401	0.8672	5.6128	**0.007**
RF IgA level at study entry, IU/ml	0.0126	0.0032	0.0192	**0.010**
Male sex	2.9180	5.9552	0.1152	0.059

Table 4. Odds ratios (OR) of different markers for patients to experience a severe erosive course of disease after 4 years measured by a Larsen score (LS) of 32. Patients were grouped according to Larsen score reached after 4 years, and sensitivity and specificity for clinical and immunogenetic markers were calculated. Comparisons were made for RF IgM, the presence of erosions already at study entry, the RA associated shared epitope (SE+), the shared epitope on a DRB1*04 allele (SE+DR4+), and combinations of different disease markers as indicated. For each comparison odds ratios are given. Yates corrected chi-square and corresponding p value are given in case of significance.

	Sensitivity, %	Specificity, %	OR	Significance χ^2 p
RF IgM	68	55	2.66	1.71, 0.19
Early erosions	57	89	11.91	10.4, 0.0013
SE+	73	55	3.44	2.80, 0.09
SE+DR4+	57	86	8.6	8.441, 0.0037
SE+DR4+ and/or RF IgM	89	51	9.1	6.81, 0.0091
SE+DR4+ and/or early erosions	84	79	20.44	16.2, < 0.0001

into the analysis, have been applied and are consistent with the model outlined above. Similarly, when MCMC methods were used to calculate a robust model assuming a double exponential distribution instead of a normal distribution of Larsen score, the covariates included in the mixed effect regression model again significantly influenced the Larsen score.

In summary, the mixed effect regression model identified the covariates presence of SE+ DR4, sex, and RF IgA concentration as significantly influencing the progression of the Larsen score over time.

Prognostic Value of the Markers Analyzed

To assess the diagnostic value of those variables that showed a significant influence on radiological progression, the odds ratio of patients for being in the group with the highest Larsen score after 4 years was calculated, and sensitivity and specificity were analyzed. As a cutoff, we used a Larsen score of 32, which was reached by one-third of the study cohort during the observation period[15,17,34].

When used as a prognostic marker, neither RF IgM status nor the presence of the shared epitope was associated with a significantly increased risk for severe outcome (Table 4). Patients carrying the SE on a DR4 allele, however, did have an increased odds ratio to reach a Larsen score > 32 ($p < 0.005$) after 4 years. While there was no additive effect seen in the odds ratio of patients positive for both SE+ DR4 and RF IgM simultaneously (data not shown), the risk of severe erosions was increased to 9.1 ($p = 0.009$) for the group of patients that were positive for either SE+ DR4 or RF IgM or for both markers.

The presence of erosions already at study entry resulted in an almost 12-fold increased odds ratio for severe destruction after 4 years. Again, no additive effect was visible for patients with early erosions at study entry that were simultaneously positive for SE+ DR4. If patients positive for at least one of the 2 markers were compared to those negative for both, however, they were found to have an odds ratio of 20.4 to develop a Larsen score > 32 (Table 4).

In summary, the presence of one of the 2 markers SE+ DR4 and erosions at study entry was sufficient to predict the likelihood of the development of severe erosions in a patient with high sensitivity and moderate specificity.

DISCUSSION

The purpose of our study involving prospective collection of clinical, laboratory, and immunogenetic data on patients with recent onset RA was the identification of prognostic markers indicative of severe, rapidly progressing joint destruction in recent onset RA. Using the yearly increase in Larsen score as a measure of progression of joint disease, we analyzed the relationship between disease activity, immunogenetic markers, and joint destruction. As a prerequisite for the identification of prognostic indicators of the disease course, we created a stable model of the observed disease progression using the longitudinal data gathered as covariates. The multivariate analysis resulted in a linear mixed effect regression model for repeated measurements, in which the most profound influence was exerted by the presence of an RA associated DR4 allele on at least one chromosome, with significant contributions of only 2 other covariates — sex and the levels of RF IgA. The model confirmed observations of other groups[17,35,36] describing the most rapid progression of joint destruction during the first 2 years. As early as at study entry, we found clinical and inflammatory markers of disease activity correlated with each other. This was true not only for the initial presentation, but continued during the first 2 years of disease, since the swollen joint count showed significant correlations with the CRP level at all time points. Both multivariate analysis and univariate comparisons revealed, however, that the influence of disease activity variables on the radiological outcome was not significant, while the linear model was dominated by fixed variables (sex, immunogenetics, RF

seropositivity) independent of disease activity. This is in contrast to some other studies[17,31,37,38], which found the progression of joint destruction to be dependent upon the acute phase response, while other authors had results similar to ours[20,21,39]. The discrepancies might be explained partly by the wide interindividual variations between patients[19], but also by the usually very quick response of those variables after initiation of immunosuppressive therapy. On the other hand, the time integrated CRP (indicated by the averaged values over time) was found to influence radiological outcome to some extent in our patient population as well as in other studies[40]. While the limited number of cases in the multivariate analysis does not allow ruling out a relevant, although minor effect of time integrated inflammatory variables on radiological progression, we conclude that the dominant covariates like SE positive DR4 alleles, RF IgA, and early erosive disease exert their influence independently from variables of disease activity. Further, the time integrated CRP is not a practical prognostic marker with regard to its predictive value, since serial measurements over a longer time interval are required.

The influence of the production of rheumatoid factors on joint destruction has been described by several groups[16,32,33,38,41-44]. We were able to confirm a faster pace of joint destruction in RF IgM and RF IgA seropositive patients early in the disease process that resulted in significantly higher Larsen scores after 2 and 4 years of disease. When seropositivity for RF IgM was used as a discriminating marker in univariate comparison, the detection of RF of this isotype was associated with higher Larsen scores. However, the influence of seropositivity for RF IgM did not reach significance in the multivariate analysis, possibly due to the greater effect of RF IgA titers in this model, since RF IgA and RF IgM occurred simultaneously in 82.5% of all RF IgA positive patients. Alternatively, the presence of RF IgM seropositivity by itself might be more predictive of faster joint destruction than the serum concentrations of it, which were used in the multivariate model. Since almost all seropositive patients (93%) were positive for RF IgM at initial presentation, the RF IgM status can be used as a prognostic marker that is available at the onset of disease. For RF IgA, we found considerable fluctuations in the individual patients' levels between different visits in clinic. While in the multivariate model initial serum levels for RF IgA had a significant effect on the radiological progression of joint destruction, its usefulness as a prognostic marker is limited because seropositivity frequently developed rather late in the disease course.

The influence of sex in the linear regression analysis and the results of the univariate analysis are consistent with more severe joint destruction in male patients. This might reflect an additional genetic influence on the clinical course of RA, but due to the comparatively small number of men in our study those results need to be confirmed in larger groups.

It has been shown by our group and others that the presence of the RA associated shared epitope, on either a DR4 or a DR1 allele, and the resulting amino acid cassettes QKRAA and QRRAA in the binding groove of the MHC molecule modulate the radiological progression of joint disease early in the disease course[4,7,45]. Our data show that the influence of the RA associated DR4 alleles on the progression of joint destruction is sustained throughout the observation period of 48 months. Patients positive for this marker had an odds ratio of 8.6 to arrive at a Larsen score > 32 after 4 years of disease, which characterizes 34% of the study population with the most severe radiographic changes. The risk of patients carrying the SE sequence on either a DR1 or a DR4 allele to be in this group was only marginally increased and did not achieve statistical significance. This seems to be the result of the weak contribution of the DR1 allele toward an increased risk of more severe joint destruction.

In the univariate analysis, the presence of erosive disease already at study entry was found to be a strong indicator of the most destructive courses of disease. Due to the comparatively low proportion of early erosiveness in our study group (30%), the sensitivity of this marker was somewhat low (Table 4). The multivariate model showed that the effect of this marker was mainly due to a considerable overlap with positivity for the epitope sequence on a DR4 allele, since a high percentage (53.8%) of patients with early erosions were carrying this marker. When both epitope positivity on a DR4 allele and presence of early erosions were used in combination as one marker, the odds ratio was increased 20-fold and sensitivity rose to 84% (Table 4).

Taken together, the results of our prospective study of early RA indicate that the independent factors presence of the shared epitope on a DRB1*04 allele, RF seropositivity, sex, and presence of early erosive disease allow prediction of the progression of joint destruction as early as initial presentation. The influence of these markers remains independent from variables of disease activity during the first 4 years of the disease.

REFERENCES

1. Kirwan JR, Quilty B. Prognostic criteria in rheumatoid arthritis: can we predict which patients will require specific anti-rheumatoid treatment? Clin Exp Rheumatol 1997; 15 Suppl 17:S15-25.
2. Weyand CM, Xie C, Goronzy JJ. Homozygosity for the HLA-DRB1 allele selects for extraarticular manifestations in rheumatoid arthritis. J Clin Invest 1992;896:2033-9.
3. Salvarani C, Macchioni P, Mantovani W, et al. Extraarticular manifestations of rheumatoid arthritis and HLA antigens in northern Italy. J Rheumatol 1992;19:242-6.
4. Wagner U, Kaltenhauser S, Sauer H, et al. HLA markers and prediction of clinical course and outcome in rheumatoid arthritis. Arthritis Rheum 1997;40:341-51.
5. van Zeben D, Hazes JM, Zwinderman AH, et al. Association of HLA-DR4 with a more progressive disease course in patients with rheumatoid arthritis. Results of a followup study. Arthritis Rheum 1991;34:822-30.

6. Calin A, Elswood J, Klouda PT. Destructive arthritis, rheumatoid factor, and HLA-DR4. Susceptibility versus severity, a case-control study. Arthritis Rheum 1989;32:1221-5.
7. Nepom GT, Gersuk V, Nepom BS. Prognostic implications of HLA genotyping in the early assessment of patients with rheumatoid arthritis. J Rheumatol 1996;23 Suppl 44:5-9.
8. Eberhardt K, Grubb R, Johnson U, Pettersson H. HLA-DR antigens, Gm allotypes and antiallotypes in early rheumatoid arthritis — their relation to disease progression. J Rheumatol 1993; 20:1825-9.
9. Gough A, Faint J, Salmon M, et al. Genetic typing of patients with inflammatory arthritis at presentation can be used to predict outcome. Arthritis Rheum 1994;37:1166-70.
10. MacGregor A, Ollier W, Thomson W, Jawaheer D, Silman A. HLA-DRB1*0401/0404 genotype and rheumatoid arthritis: increased association in men, young age at onset, and disease severity. J Rheumatol 1995;226:1032-6.
11. Westedt ML, Breedveld FC, Schreuder GM, D'Amaro J, Cats A, de Vries RR. Immunogenetic heterogeneity of rheumatoid arthritis. Ann Rheum Dis 1986;45:534-8.
12. Eberhardt K, Fex E, Johnson U, Wollheim FA. Associations of HLA-DRB and -DQB genes with two and five year outcome in rheumatoid arthritis. Ann Rheum Dis 1996;55:34-9.
13. Devlin J, Gough A, Huissoon A, et al. The acute phase and function in early rheumatoid arthritis. C-reactive protein levels correlate with functional outcome. J Rheumatol 1997;24:9-13.
14. Seidl C, Koch U, Buhleier T, et al. HLA-DRB1*04 subtypes are associated with increased inflammatory activity in early rheumatoid arthritis. Br J Rheumatol 1997;36:941-4.
15. Mottonen T, Paimela L, Leirisalo-Repo M, Kautiainen H, Ilonen J, Hannonen P. Only high disease activity and positive rheumatoid factor indicate poor prognosis in patients with early rheumatoid arthritis treated with "sawtooth" strategy. Ann Rheum Dis 1998;57:533-9.
16. Wolfe F, Sharp JT. Radiographic outcome of recent-onset rheumatoid arthritis: a 19-year study of radiographic progression. Arthritis Rheum 1998;41:1571-82.
17. Fex E, Jonsson K, Johnson U, Eberhardt K. Development of radiographic damage during the first 5-6 yr of rheumatoid arthritis. A prospective follow-up study of a Swedish cohort. Br J Rheumatol 1996;35:1106-15.
18. van Zeben D, Hazes JM, Zwinderman AH, Vandenbroucke JP, Breedveld FC. Factors predicting outcome of rheumatoid arthritis: results of a followup study. J Rheumatol 1993;208:1288-96.
19. van Leeuwen MA, van Rijswijk MH, van der Heijde DM, et al. The acute-phase response in relation to radiographic progression in early rheumatoid arthritis: a prospective study during the first three years of the disease. Br J Rheumatol 1993;32 Suppl 3:9-13.
20. Coste J, Spira A, Clerc D, Paolaggi JB. Prediction of articular destruction in rheumatoid arthritis: disease activity markers revisited. J Rheumatol 1997;241:28-34.
21. Eberhardt KB, Rydgren LC, Pettersson H, Wollheim FA. Early rheumatoid arthritis — onset, course, and outcome over 2 years. Rheumatol Int 1990;104:135-42.
22. O'Sullivan FX, Fassbender HG, Gay S, Koopman WJ. Etiopathogenesis of the rheumatoid arthritis-like disease in MRL/l mice. I. The histomorphologic basis of joint destruction. Arthritis Rheum 1985;285:529-36.
23. Tanaka A, O'Sullivan FX, Koopman WJ, Gay S. Etiopathogenesis of rheumatoid arthritis-like disease in MRL/1 mice. II. Ultrastructural basis of joint destruction. J Rheumatol 1988; 151:10-6.
24. van den Berg WB. Joint inflammation and cartilage destruction may occur uncoupled. Springer Semin Immunopathol 1998; 201-2:149-64.
25. Mulherin D, Fitzgerald O, Bresnihan B. Clinical improvement and radiological deterioration in rheumatoid arthritis: evidence that the pathogenesis of synovial inflammation and articular erosion may differ. Br J Rheumatol 1996;3512:1263-8.
26. Kirwan JR. The relationship between synovitis and erosions in rheumatoid arthritis. Br J Rheumatol 1997;362:225-8.
27. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
28. Larsen A. How to apply Larsen score in evaluating radiographs of rheumatoid arthritis in longterm studies. J Rheumatol 1995;22:1974-5.
29. Davidian M, Giltinan DM. Nonlinear models for repeated measurements data. 1st ed. London: Chapman and Hall; 1995.
30. Lindsey JK. Applying generalized linear models. 1st ed. New York: Springer; 1997.
31. Möttönen TT. Prediction of erosiveness and rate of development of new erosions in early rheumatoid arthritis. Ann Rheum Dis 1988;47:648-53.
32. van der Heide A, Remme CA, Hofman DM, Jacobs JW, Bijlsma JW. Prediction of progression of radiologic damage in newly diagnosed rheumatoid arthritis. Arthritis Rheum 1995;38:1466-74.
33. Higami K, Hakoda M, Matsuda Y, Ueda H, Kashiwazaki S. Lack of association of HLA-DRB1 genotype with radiologic progression in Japanese patients with early rheumatoid arthritis. Arthritis Rheum 1997;40:2241-7.
34. Houssien DA, Jonsson T, Davies E, Scott DL. Clinical significance of IgA rheumatoid factor subclasses in rheumatoid arthritis. J Rheumatol 1997;24:2119-22.
35. Plant MJ, Jones PW, Saklatvala J, Ollier WE, Dawes PT. Patterns of radiological progression in early rheumatoid arthritis: results of an 8 year prospective study. J Rheumatol 1998;25:417-26.
36. van der Heijde DM. Joint erosions and patients with early rheumatoid arthritis. Br J Rheumatol 1995;34 Suppl 2:74-8.
37. Graudal N, Tarp U, Jurik AG, et al. Inflammatory patterns in rheumatoid arthritis estimated by the number of swollen and tender joints, the erythrocyte sedimentation rate, and hemoglobin: longterm course and association to radiographic progression. J Rheumatol 2000;27:47-57.
38. Matsuda Y, Yamanaka H, Higami K, Kashiwazaki S. Time lag between active joint inflammation and radiological progression in patients with early rheumatoid arthritis. J Rheumatol 1998; 25:427-32.
39. Richardson C, Emery P. Laboratory markers of disease activity. J Rheumatol 1996;23 Suppl 44:23-30.
40. van Leeuwen MA, van Rijswijk MH, Sluiter WJ, et al. Individual relationship between progression of radiological damage and the acute phase response in early rheumatoid arthritis. Towards development of a decision support system. J Rheumatol 1997;24:20-7.
41. Paimela L, Palosuo T, Leirisalo-Repo M, Helve T, Aho K. Prognostic value of quantitative measurement of rheumatoid factor in early rheumatoid arthritis. Br J Rheumatol 1995;34:1146-50.
42. van Zeben D, Breedveld FC. Prognostic factors in rheumatoid arthritis. J Rheumatol 1996;23 Suppl 44:31-3.
43. Houssien DA, Jonsson T, Davies E, Scott DL. Rheumatoid factor isotypes, disease activity and the outcome of rheumatoid arthritis: comparative effects of different antigens. Scand J Rheumatol 1998;27:46-53.
44. Jonsson T, Steinsson K, Jonsson H, Geirsson AJ, Thorsteinsson J, Valdimarsson H. Combined elevation of IgM and IgA rheumatoid factor has high diagnostic specificity for rheumatoid arthritis. Rheumatol Int 1998;18:119-22.
45. Weyand CM, Hicock KC, Conn DL, Goronzy JJ. The influence of HLA-DRB1 genes on desease severity in rheumatoid arthritis. Ann Intern Med 1992;117:801-6.