The Value of HLA-DRB1 Shared Epitope, –308 Tumor Necrosis Factor-α Gene Promoter Polymorphism, Rheumatoid Factor, Anti-Citrullinated Peptide Antibodies, and Early Erosions for Predicting Radiological Outcome in Recent-Onset Rheumatoid Arthritis

SONSOLES RENESES, MARÍA F. GONZÁLEZ-ESCRIBANO, ANTONIO FERNÁNDEZ-SUÁREZ, LUIS PESTANA, BERNABÉ DAVILA, INGEBORG WICHMANN, and ALICIA GARCÍA

ABSTRACT. ***Objective.*** To study the value of HLA-DRB1 shared epitope (SE), –308 tumor necrosis factor-α (TNF-α) gene promoter polymorphism, rheumatoid factor (RF), anti-citrullinated peptide antibodies (anti-CCP), and baseline erosions for predicting radiological outcome at 1 year in patients with recent-onset rheumatoid arthritis (RA).

Methods. Radiological damage was assessed by radiographs at baseline and at 1 year in an inception cohort of 134 RA patients with disease duration ≤ 1 year at study entry. Radiographs were scored with the modified Sharp/van der Heijde (SvdH) erosion score for hands, wrists, and feet. The predictive value of the variables was studied by multiple linear regression analysis, using immunogenetic factors, baseline SvdH erosion score, and type of treatment during the followup period as independent variables, and SvdH erosion score at 1 year as the dependent variable.

Results. The SvdH erosion score increased from the baseline visit to the 1-year visit in 49 patients (36.6%). In multiple linear regression analysis, radiological outcome was significantly predicted by SE homozygosity (ß coefficient 1.75; 95% CI 1.54, 2.96; $p = 0.005$) and baseline SvdH erosion score (ß coefficient 1.56; 95% CI 1.4, 1.71; $p < 0.001$). This model explained 78% of the variability of the dependent variable ($R^2 = 0.779$).

Conclusion. Erosive damage at 1 year in patients with recent-onset RA is significantly influenced by SE homozygosity and the presence of baseline erosions, but not by RF status, anti-CCP status, or –308 TNF-α genotype. (J Rheumatol First Release May 1 2009; doi:10.3899/jrheum.081075)

Key Indexing Terms:
GENETIC MARKERS
IMMUNOLOGIC MARKERS
RADIOLOGY
RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is highly variable in terms of radiological outcome. It is necessary to predict which patients will develop erosive disease so that they can be treated more aggressively from the outset and that patients who are more likely to have a favorable clinical course are spared unnecessary and potentially toxic treatment. The combined role of genes and immunity in the development of erosive damage in RA has been the subject of recent investigations. New data support the hypothesis that the presence of HLA-DRB1 shared epitope (SE) alleles can activate immune reactions such as the production of anti-cyclic citrullinated peptide antibodies (anti-CCP)[1]. Patients with RA who harbor these antibodies in the early stages of the disease could develop more severe erosive damage than those who lack them[2]. Although anti-CCP have been associated with structural damage in RA, it has not been determined whether this is independent of rheumatoid factor (RF)[3].

There are controversial reports that the G-to-A polymor-

From the Department of Rheumatology and Department of Immunology, Virgen del Rocío University Hospital, Seville, Spain; Biotechnology Area, Alto Guadalquivir Hospital, Andújar, Spain; and Language Services, World Health Organization, Geneva, Switzerland.

S. Reneses, MD, PhD, Staff Rheumatologist, Department of Rheumatology; M.F. González-Escribano, MD, PhD, Staff Immunologist, Department of Immunology, Virgen del Rocío University Hospital; A. Fernández-Suárez, MD, Staff Immunologist, Biotechnology Area, Alto Guadalquivir Hospital; L. Pestana, MD, WHO Officer; B. Davila, MD, Immunology Resident; I. Wichmann, MD, Staff Immunologist (retired), Department of Immunology, Virgen del Rocío University Hospital; A. García, MD, PhD, Chief of Rheumatology, Department of Rheumatology, Virgen del Rocío University Hospital.

Address reprint requests to Dr. L. Pestana, GBS/LNG/TRA (Room 3021), World Health Organization, 20 Avenue Appia, 1120 Geneva 27, Switzerland. E-mail: pestanal@who.int

Accepted for publication January 30, 2009.

phism at position 308 of the tumor necrosis factor-α (–308 TNF-α) gene promoter may be an independent marker of radiological damage in recent-onset RA[4]. This association has been suggested in RF-positive patients in particular[5].

Joint destruction in recent-onset RA might be influenced by the presence of very small erosions in the hands and feet at presentation, regardless of the inflammatory activity of the disease[6]. The presence of early erosions is a sound argument for initiating therapy with disease-modifying antirheumatic drugs (DMARD) in patients with recent-onset RA.

Although SE alleles[6-23], –308 TNF-α gene promoter polymorphism[5], RF[6,21,24-35], anti-CCP[22,23,35-44], and early erosions[6,22,38,45-48] have all been associated with a poor radiological outcome in cohort studies of recent-onset RA, no study to date has investigated the combined effect of this particular set of factors. The combination of several markers could increase the capacity to predict radiological damage in patients with recent-onset RA[49], and identification of markers associated with a poor outcome would facilitate the development of new drug targets[50].

In January 2002, a register for recent-onset inflammatory polyarthritis (IP) was established in Seville, Spain, to address various diagnostic, prognostic, and therapeutic issues[51,52]. In our study, multivariate linear regression was used to find a model that predicts early erosive damage, as seen radiologically, in patients with recent-onset RA.

MATERIALS AND METHODS

We studied a prospective cohort of 134 consecutive patients with RA (disease duration ≤ 1 year) who were referred to our recent-onset IP unit from January 2002 through June 2006. Patients were referred from primary healthcare centers, emergency services, and outpatient rheumatology clinics of the Virgen del Rocio University Hospital Health District in Seville (population 774,619; 2002 census). Details of the case-ascertainment procedure have been described[51].

Subjects. We studied a prospective cohort of 134 consecutive patients with RA (disease duration ≤ 1 year) who were referred to our recent-onset inflammatory polyarthritis (IP) unit from January 2002 through June 2006. Patients referred to the unit and included in the recent-onset IP register had to reside in the hospital health district catchment area, be at least 16 years old, and have at least 2 swollen joints lasting a minimum of 4 weeks and a maximum of 12 months. The 1987 American Rheumatism Association (ARA) revised criteria for RA[53] were used at baseline and in all followup assessments and applied cumulatively. Cases that fulfilled ≥ 4 of these criteria during any visit (at 0, 1, 3, 6, 9, and 12 months) were included in this study; patients with alternative diagnoses were excluded. From January 2002 through June 2006, 897 patients were referred to the recent-onset IP unit. Of these patients, 387 (43%) fulfilled the criteria for inclusion in the register, but 23 (6%) were lost to followup. This left a total of 364 registered patients, of whom 134 (36.8%) fulfilled the 1987 ARA criteria for RA, 62 (46.2%) of them at baseline, and had completed the first year of followup by the time of this analysis. All patients were of Spanish descent. At baseline, no patient had previously received corticosteroids or DMARD. Blood samples for laboratory tests were collected and frozen before treatment was begun.

Radiographic measurements. Posteroanterior radiographs of hands, wrists, and feet were taken at baseline and after 12 months. Radiographs were read by 2 observers blinded to patients' data, and agreement between readers was assessed by means of the interobserver correlation coefficient and its 95% confidence interval (95% CI).

Radiographs were read in concealed time order using the method of Sharp/van der Heijde (SvdH), which includes a joint space narrowing score for hands, wrists and feet, an erosion score for hands, wrists and feet, and a total score[54]. For this study we evaluated only the SvdH erosion score for hands, wrists, and feet; 32 joints were examined in the hands and wrists (graded from 0 to 5) and 12 joints in the feet (graded from 0 to 10); the maximum possible score for erosions was thus 280[54].

Genetic markers. DNA from peripheral blood was obtained using standard methods. HLA-DRB1 SE alleles were genotyped using a reverse dot-blot kit with sequence-specific oligonucleotide probes (Dynal Reli™ SSO HLA-DRB1 typing kits; Dynal Biotech, Bromborough, UK). When necessary, high-resolution typing of HLA-DRB1*01, DRB1*04, and DRB1*14 samples was performed using Dynal AllSet™ SSP DRB1*01, DRB1*04, and DRB1*14, respectively.

For –308 TNF-α, samples were genotyped using TaqMan 5' allelic discrimination (Custom TaqMan SNP genotyping assays method; Applied Biosystems, Foster City, CA, USA). Allele-specific probes were labeled with VIC and FAM fluorescent dyes. Polymerase chain reaction (PCR) was carried out in a total reaction volume of 8 µl with the following amplification protocol: denaturation at 95°C for 10 min, 40 cycles of denaturation at 93°C for 15 s, and annealing and extension at 60°C for 1 min. After PCR, the genotype of each sample was automatically attributed using the SDS 1.3 software for allelic discrimination.

Immunologic markers. All patients were tested for anti-CCP antibodies by second-generation ELISA (Immunoscan RA anti-CCP2 ELISA; Euro-Diagnostica AB, Malmö, Sweden; positive: > 25 IU/ml) and for RF by nephelometry (BN II nephelometer; Dade Behring, Marburg, Germany, with N Latex RF reactant, Dade Behring, Marburg, Germany; positive: > 50 IU/ml).

Treatments. Treatment with corticosteroids and DMARD during the total followup period was assessed. Treatment with DMARD (methotrexate, sulfasalazine, chloroquine, leflunomide, cyclosporine, or azathioprine) was categorized according to the number of drugs administered (0, 1, 2, or 3).

Statistical methods. The dependent variable was the SvdH score obtained at 12 months. The independent variables were the SvdH score obtained at baseline; the treatment given throughout the 12 months of followup (corticosteroids: yes or no; and DMARD: 0, 1, 2, or 3 drugs); SE, anti-CCP, and RF status; and –308 TNF-α genotype (GG or AG/AA). As there were few AG and AA cases, these 2 categories were collapsed.

All data were recorded in an Access 2000 database and then exported to the Statistical Package for the Social Sciences (SPSS) v.12.0 for analysis.

For an alpha level of 0.05, an anticipated "medium" effect size of 0.15 (according to Cohen's convention for multiple regression), and an assumed 10% rate of attrition, the minimum sample size required to reach a statistical power of 0.80 in a multiple regression model with 8 predictor variables would be 108.

We calculated absolute frequencies and percentages for qualitative variables, and the mean and standard deviation for quantitative variables. Variables that predict the SvdH erosion score at 1 year were identified by univariate and multivariate linear regression models. For univariate analyses we used Student t test or ANOVA, as appropriate. For the multivariate analysis, Student t test was used for stepwise exclusion of variables that had a weak association with the dependent variable, as indicated by a p value ≥ 0.15. As the SE variable is polytomic, it was analyzed creating a dummy variable with the first category (–/–) as the reference. Full and reduced models were compared with the partial multiple F test. The linearity of continuous variables was checked by the Box-Tidwell test. Potential interactions among the variables in the model were studied. Variables with a p value > 0.05 were analyzed as potential confounders, and they were considered such whenever their coefficients showed a percent change > 20%. Multicollinearity among independent variables was assessed by the variance inflation factor, independence by the Durbin-Watson test, normality

by the Shapiro-Wilk test, and homoscedasticity of residues by the dispersion diagram among residues and the estimated values. Outliers were identified by means of Cook's distance. The goodness of fit was assessed with the corrected determination coefficient (R^2). All contrasts were 2-tailed, and the significance level was set at $p < 0.05$.

RESULTS

From January 2002 to June 2006, 364 patients were included in the recent-onset IP register. Of these, 134 (36.8%) fulfilled the 1987 ARA revised criteria for RA[53] and had completed the first year of followup by the time of this analysis.

Interobserver coefficients of correlation for baseline SvdH erosion score were 0.988 (95% CI 0.984–0.991) for hands and wrists, and 0.855 (95% CI 0.840–0.917) for feet. For SvdH erosion score at 1 year they were 0.848 (95% CI 0.796–0.886) for hands and wrists, and 0.842 (95% CI 0.782–0.886) for feet. Since these values were high, we report the mean values for both readers.

The baseline characteristics of the study population are shown in Table 1. At study entry, 48 patients (35.8%) had erosions (SvdH erosion score ≥ 1); only 7 had a baseline SvdH erosion score ≥ 5. At 1 year, 10 additional patients had erosions, for a total of 58 (43.2%). The SvdH erosion score increased from the baseline visit to the 1-year visit not only in these 10 patients, but also in 39 of the 48 who already had erosions at baseline, for a total of 49 patients (36.6%); the increase was ≥ 5 units in 11 of these 49 patients.

G/G genotypes of the –308 TNF-α were present in 115 patients (85.8%), and A/A or A/G genotypes in 19 (14.2%). Fifty-two patients (38.8%) were heterozygous (–/+) for the SE allele, and 12 (8.9%) were homozygous (+/+); 65 (48.5%) were RF-positive and 72 (53.7%) were anti-CCP-positive. Sometime between the baseline visit and the end of the followup period, 126 patients (94.0%) were treated with DMARD and 119 (88.8%) received corticosteroids.

In the univariate analyses, the mean SvdH erosion score at 1 year showed no statistically significant differences linked to –308 TNF-α or SE genotypes, RF or anti-CCP status, or treatment with DMARD or corticosteroids. Only patients with erosions at presentation had a significantly higher mean SvdH erosion score at 1 year ($p < 0.001$; Table 2).

Table 3 shows the results of univariate and multivariate linear regression for SvdH erosion score at 1 year. As for the univariate linear regression analyses, only the presence of erosions at baseline was associated with radiographic progression ($p < 0.001$). Multivariate linear regression analysis resulted in a model in which radiographic outcome at 1 year of followup was significantly predicted only by SE homozygosity (ß coefficient 1.75; 95% CI 1.54, 2.96; $p = 0.005$) and the presence of erosions at study entry (ß coefficient 1.56; 95% CI 1.40–1.71; $p < 0.001$), but not by any other variable (partial F test = 0.7206; $p = 0.634$; df = 6,124). SE heterozygosity was not a significant predictor, but it remained in the model since it was a dummy variable.

Table 1. Baseline characteristics of patients (n = 134).

Baseline Variables	N (%)	Mean (SD)
Age, yrs	—	50.4 (1.3)
Sex		
Male	40 (29.8)	—
Female	94 (70.2)	—
Anti-CCP		
Positive	72 (53.7)	—
Negative	62 (46.3)	—
RF		
Positive	65 (48.5)	—
Negative	69 (51.5)	—
–308 TNF-α		
GG	115 (85.8)	—
GA/AA	19 (14.2)	—
Shared epitope		
–/–	70 (52.3)	—
–/+	52 (38.8)	—
+/+	12 (8.9)	—
C-reactive protein, mg/l	—	13.6 (16.7)
Erythrocyte sedimentation rate, mm/h	—	35.6 (23.0)
Swollen joint count	—	12.0 (6.7)
Tender joint count	—	13.1 (7.2)
DAS28	—	6.1 (1.1)
HAQ	—	1.1 (0.6)
SvdH-ES		
Hands	—	0 (1.4)
Feet	—	0 (1.3)
SvdH-ES	—	1 (2.1)
SvdH-ES = 0	86 (64.2)	—
SvdH-ES ≥ 1	48 (35.8)	—
No. DMARD		
0	8 (6.0)	—
1	23 (17.2)	—
2	57 (42.5)	—
3	46 (34.3)	—
DMARD (%)		
Methotrexate (MTX)	116 (86.6)	
Leflunomide (LEF)	58 (43.3)	
Salazopyrin (SLZ)	42 (31.3)	
Chloroquine (CLQ)	51 (38.1)	
Cyclosporine (CYC)	12 (8.9)	
Azathioprine	1 (0.7)	
MTX + SLZ + CLQ	42 (31.3)	
MTX + LEF	30 (22.4)	
MTX + SLZ	15 (11.2)	
MTX + CLQ	13 (9.7)	
MTX + CYC	11 (8.2)	
Corticosteroids		
Yes	119 (88.8)	—
No	15 (11.2)	—

Anti-CCP: anti-cyclic citrullinated peptide antibodies; RF: rheumatoid factor; –308 TNF-α: G-to-A polymorphism at position 308 of the tumor necrosis factor-α gene promoter; DAS28: Disease Activity Score 28; HAQ: Health Assessment Questionnaire; SvdH-ES: Sharp-van der Heijde erosion score; DMARD: disease-modifying antirheumatic drugs.

In this model there were no significant interactions among variables, and no variable was a confounder. All criteria for the use of multivariate linear regression were fulfilled: independence, normality and linearity of the inde-

Table 2. Univariate analyses for predictors of mean Sharp/van der Heijde erosion score (SvdH-ES) at 1 year.

Baseline Variables	SvdH-ES at 1 year Mean (SD)	p (Student t or ANOVA)
Anti-CCP		
Positive	2.5 (4.1)	
Negative	1.6 (3.6)	0.173
RF		
Positive	2.2 (3.5)	
Negative	1.9 (4.2)	0.653
–308 TNF-α		
GG	1.9 (3.8)	
GA/AA	2.2 (3.9)	0.738
Shared epitope		
–/–	1.7 (3.1)	
–/+	2.1 (4.2)	0.532
+/+	3.2 (4.7)	
SvdH-ES		
0	0.2 (0.6)	
≥ 1	5.4 (4.9)	< 0.001
DMARD		
0–1 drugs	2.1 (3.9)	0.808
≥ 2 drugs	1.7 (2.2)	
Corticosteroids		
Yes	1.2 (1.3)	
No	2.1 (3.9)	0.610

Definitions as in Table 1.

pendent variables, no multicollinearity among them, and homoscedasticity of the residues. No patient showed Cook's distance > 1. The model explained 78% of the variability of the dependent variable ($R^2 = 0.779$).

Although the mean SvdH erosion score at 1 year was higher in patients with the –308 TNF-α AG/AA genotype, positive RF, or positive anti-CCP (Table 2), these were not significant predictors of the severity of radiologic changes in our population.

DISCUSSION

Several cohort studies of populations similar to ours have investigated the value of different combinations of variables, including HLA-DRB1 SE alleles, –308 TNF-α gene promoter polymorphism, RF, anti-CCP, and early erosions, for predicting radiological outcome among patients with recent-onset RA[5-48]. These studies differed methodologically in terms of referral and recruitment procedures, inclusion criteria, disease duration, variables measured at presentation, followup until assessment of radiological damage, and radiographic scoring methods. Our study is the first to investigate this particular set of 5 variables using multivariate linear regression models.

Some studies have found a significant association of SE alleles with radiological progression in recent-onset RA[5,6,10,12,13,17,21-23] and some have not[27,31,32]. Some have not used multivariate statistical methods[5,31]. Our results show that homozygosity for HLA-DRB1 SE was significantly associated with greater erosive damage after 1 year (ß coefficient 1.75; 95% CI 1.54, 2.96; p = 0.005). These findings are in agreement with the results of previous studies.

However, since RA is a multigenic inflammatory disorder, it is likely that other factors are involved in its progression. The possibility that –308 TNF-α gene promoter polymorphism may have prognostic implications is currently being debated. In a seropositive RA inception cohort, regression analysis showed that patients with –308 TNF-α AA or AG genotypes had significantly higher rates of progression in erosion scores compared with patients with the TNF-α GG genotype[5]. Like ours, other studies not confined to patients with seropositive RA suggest that the –308 TNF-α gene promoter polymorphism is not a genetic marker for severe radiologic changes in recent-onset RA[55,56].

Numerous studies have reported that RF is a good predictor of radiological severity[6,21,24-35] in recent-onset RA. In our study, baseline RF status was not a predictor of radio-

Table 3. Univariate and multivariate analyses of predictors for median Sharp/van der Heijde erosion score (SvdH-ES) at 1 year.

Baseline Variables	Univariate ß coefficient (95% CI)	p (Student t)	Multivariate ß coefficient (95% CI)*	p (Student t)*
–308 TNF-α (GG vs A/AA)	0.93 (0.85, 1.48)	0.590	—	—
SE (–/– vs –/+)	–0.34 (–0.86, 1.07)	0.629	0.25 (–0.46, 0.96)	0.482
SE (–/– vs +/+)	1.05 (–1.36, 3.46)	0.384	1.75 (1.54–2.96)	0.005
Anti-CCP (neg vs pos)	0.92 (0.41, 2.25)	0.173	—	—
RF (neg vs pos)	0.30 (–1.03, 1.64)	0.653	—	—
Corticosteroids (yes vs no)	0.91 (–2.60, 4.40)	0.610	—	—
DMARD, number (0–1 vs ≥ 2)	0.26 (–0.56, 1.07)	0.535	—	—
SvdH-ES	1.54 (1.39, 1.70)	< 0.001	1.56 (1.40, 1.71)	< 0.001

Linear regression equation: SvdH-ES at 1 year = 0.38 + 0.25 SE (–/– vs –/+) + 1.75 SE (–/– vs +/+) + 1.56 baseline SvdH-ES; ($R^2 = 0.779$; $F = 132.981$; $p < 0.001$). * Values not shown for variables not included in the model, i.e., those with p values ≥ 0.15. The exception is SE (–/– vs –/+), retained in the model as a dummy variable. Definitions as in Table 1.

graphic damage at 1 year. Similar results have been found in other cohorts of patients with recent-onset RA, in Spain[22,31] and elsewhere[11,15,37]. However, it is possible that different results would have been obtained had we used RF concentrations instead of RF status, or had we determined the status repeatedly[26]. For instance, in another inception cohort in our area, RF was significantly associated with the severity of radiological changes at 10 years only when the quantitative measurement was used[31].

The usefulness of anti-CCP for predicting structural joint damage in patients with recent-onset RA has also been evaluated in several cohort studies. The results suggest that this marker is associated with radiographic damage[22,23,35-44]. As noted for RF, the predictive value may depend on whether anti-CCP status or titers are considered. In a prospective study[22], positive anti-CCP status at presentation, but not antibody titers, predicted radiological progression using the modified Larsen method after 2 years of therapy. Conversely, in another study[42], the presence of anti-CCP2 at any time during the first 3 years was associated with radiographic progression at 5 years using the SvdH erosion score, but the presence of anti-CCP2 or RF at baseline was not predictive for this outcome. In our study, patients who were positive for RF or anti-CCP2 at presentation had higher SvdH erosion scores after 1 year than patients who were negative, but the differences were not significant in the univariate analysis (Table 2), and neither marker was a predictor for this outcome in the linear regression model (Table 3).

Erosions are an important determinant of further damage and a strong argument for initiating therapy in patients with recent-onset RA[57]. Radiographic changes in the small joints of the hands, wrists, and feet have been shown to be sufficiently reflective of damage in large joints and to provide an objective outcome measure[57]. Two major systems (Larsen[58] and Sharp[59]) and a number of modifications to both are available for scoring radiographic progression in RA. We used van der Heijde's modification of the Sharp scoring system[54]; it covers a sufficiently broad spectrum of joints to provide sensitivity to change, and erosions and joint space narrowing are scored separately. We evaluated only the subscale for erosions because it is easier to measure than the total Sharp score (erosions and joint space narrowing) in clinical practice, because erosions are widely accepted as an objective and reliable outcome measure, and because the risk of developing erosions is a clear indication for prompt and aggressive treatment[28].

Higher radiographic scores at presentation have been associated with progression of joint destruction[6,22,38,45-48]. We have also found a significant association between the SvdH erosion score at 1 year and its baseline value ($p < 0.001$; Table 3). According to our linear model, a given patient will have a mean increase of 1.56 units (95% CI 1.40, 1.71) in the SvdH erosion score at 1 year for each increase of 1 unit in the baseline SvdH erosion score. The other factor significantly influencing erosiveness at 1 year in our model was SE genotype: given an identical baseline SvdH erosion score, patients homozygous for HLA-DRB1 SE (+/+) will have an SvdH erosion score at 1 year that is 1.75 units (95% CI 1.54, 2.96) greater than that of patients with HLA-DRB1 SE (–/–).

In a similar prospective study of patients with recent-onset RA, Kaltenhäuser, *et al*[6] also used multivariate techniques to analyze the value of several clinical and laboratory variables, including early erosions and HLA-DRB1 alleles, for predicting severe bone destruction at 4 years. The best predictor was the presence of either an SE-positive DR4 allele or early erosions.

Our results show that erosive damage after 1 year of followup in patients with recent-onset RA is significantly influenced by SE homozygosity and the presence of erosive disease at presentation, the 2 factors explaining 78% of the variability of the dependent variable. These 2 markers may be useful in defining a poor outcome in patients with recent-onset RA and in selecting patients for more aggressive therapy.

ACKNOWLEDGMENT

We thank Dr. M.L. Clark for improving the use of English in the manuscript, and Pilar Font for advice in statistical analysis.

REFERENCES

1. Van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Huizinga TW, Toes RE, de Vries RR. The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. Arthritis Rheum 2006;54:1117-21.
2. Wiik AS. The immune response to citrullinated proteins in patients with rheumatoid arthritis: genetic, clinical, technical, and epidemiological aspects. Clin Rev Allergy Immunol 2007;32:13-22.
3. Mewar D, Coote A, Moore DJ, et al. Independent associations of anti-cyclic citrullinated peptide antibodies and rheumatoid factor with radiographic severity of rheumatoid arthritis. Arthritis Res Ther 2006;8:R128.
4. Rezaieyazdi Z, Afshari JT, Sandooghi M, Mohajer F. Tumour necrosis factor α –308 promoter polymorphism in patients with rheumatoid arthritis. Rheumatol Int 2007;28:189-91.
5. Khanna D, Wu H, Park G, et al. Association of tumor necrosis factor alpha polymorphism, but not the shared epitope, with increased radiographic progression in a seropositive rheumatoid arthritis inception cohort. Arthritis Rheum 2006;54:1105-16.
6. Kaltenhauser S, Wagner U, Schuster E, et al. Immunogenetic markers and seropositivity predict radiological progression in early rheumatoid arthritis independent of disease activity. J Rheumatol 2001;28:735-44.
7. 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 follow-up study. Arthritis Rheum 1991;34:822-30.
8. Emery P, Salmon M, Bradley H, et al. Genetically determined factors as predictors of radiological change in patients with early symmetrical arthritis. BMJ 1992;305:1387-9.
9. 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;20:1825-29.
10. 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.
11. 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.
12. Reveille JD, Alarcón GS, Fowler SE, et al. HLA-DRB1 genes and disease severity in rheumatoid arthritis. Arthritis Rheum 1996;39:1802-7.
13. Wagner U, Kaltenhäuser S, Sauer H, et al. HLA markers and prediction of clinical course and outcome in rheumatoid arthritis. Arthritis Rheum 1997;40:341-51.
14. van Jaarsveld CHM, Otten HG, Jacobs JWG, Kruize AA, Brus HLM, Bijlsma JWJ. Association of HLA-DR with susceptibility to and clinical expression of rheumatoid arthritis: re-evaluation by means of genomic tissue typing. Br J Rheumatol 1998;37:411-6.
15. 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.
16. Harrison B, Thomson W, Symmons D, et al. The influence of HLA-DRB1 alleles and rheumatoid factor on disease outcome in an inception cohort of patients with early inflammatory arthritis. Arthritis Rheum 1999;42:2174-83.
17. Pease CT, Bhakta BB, Devlin J, Emery P. Does the age of onset of rheumatoid arthritis influence phenotype?: A prospective study of outcome and prognostic factors. Rheumatology 1999;38:228-34.
18. Seidl C, Koch U, Buhleier T, et al. Association of (Q)R/KRAA positive HLA-DRB1 alleles with disease progression in early active and severe rheumatoid arthritis. J Rheumatol 1999;26:773-6.
19. El-Gabalawy HS, Goldbach-Mansky R, Smith D, et al. Association of HLA alleles and clinical features in patients with synovitis of recent onset. Arthritis Rheum 1999;42:1696-705.
20. Listing J, Rau R, Müller B, et al. HLA-DRB1 genes, rheumatoid factor, and elevated C-reactive protein: independent risk factors of radiographic progression in early rheumatoid arthritis. Berlin Collaborating Rheumatological Study Group. J Rheumatol 2000;27:2100-9.
21. Combe B, Dougados M, Goupille P, et al. Prognostic factors for radiographic damage in early rheumatoid arthritis. A multiparameter prospective study. Arthritis Rheum 2001; 44:1736-43.
22. Sanmarti R, Gómez-Centeno A, Ercilla G, et al. Prognostic factors of radiographic progression in early rheumatoid arthritis: a two year prospective study after a structured therapeutic strategy using DMARDs and very low doses of glucocorticoids. Clin Rheumatol 2007;26:1111-8.
23. Kaltenhauser S, Pierer M, Arnold S, et al. Antibodies against cyclic citrullinated peptide are associated with the DRB1 shared epitope and predict joint erosion in rheumatoid arthritis. Rheumatology 2007;46:100-4.
24. van der Heijde DM, van Riel PL, van Leeuwen MA, van 't Hof MA, van Rijswijk MH, van de Putte LB. Prognostic factors for radiographic damage and physical disability in early rheumatoid arthritis. A prospective follow-up study of 147 patients. Br J Rheumatol 1992;31:519-25.
25. van Zeben D, Hazes JM, Zwinderman AH, Vandenbroucke JP, Breedveld FC. Factors predicting outcome of rheumatoid arthritis: results of a follow-up study. J Rheumatol 1993;20:1288-96.
26. 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.
27. Suarez-Almazor ME, Tao S, Moustarah F, Russell AS, Maksymowych W. HLA-DR1, DR4, and DRB1 disease related subtypes in rheumatoid arthritis. Association with susceptibility but not severity in a city-wide community based study. J Rheumatol 1995;22:2027-33.
28. Brennan P, Harrison B, Barrett E, et al. A simple algorithm to predict the development of radiological erosions in patients with early rheumatoid arthritis: prospective cohort study. BMJ 1996;313:471-6.
29. Houssien DA, Jónsson 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.
30. 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 the "sawtooth" strategy. Ann Rheum Dis 1998;57:533-9.
31. Valenzuela-Castano A, García-López A, Pérez-Vilches D, Rodríguez-Pérez R, González-Escribano MF, Núñez-Roldán A. The predictive value of the HLA shared epitope for severity of radiological joint damage in patients with rheumatoid arthritis. A 10-year observational prospective study. J Rheumatol 2000;27:571-4.
32. Bukhari M, Lunt M, Harrison BJ, Scott DG, Symmons DP, Silman AJ. Rheumatoid factor is the major predictor of increasing severity of radiographic erosions in rheumatoid arthritis: Results from the Norfolk Arthritis Register Study, a large inception cohort. Arthritis Rheum 2002;46:906-12.
33. Vencovsky J, Machacek S, Sedova L, et al. Autoantibodies can be prognostic markers of an erosive disease in early rheumatoid arthritis. Ann Rheum Dis 2003;62:427-30.
34. Vittecoq O, Pouplin S, Krzanowska K, et al. Rheumatoid factor is the strongest predictor of radiological progression of rheumatoid arthritis in a three-year prospective study in community-recruited patients. Rheumatology 2003;42:939-46.
35. Lindqvist E, Eberhardt K, Bendtzen K, Heinegård D, Saxne T. Prognostic laboratory markers of joint damage in rheumatoid arthritis: Ann Rheum Dis 2005;64:196-201.
36. Kroot EJ, de Jong BA, van Leeuwen MA, et al. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 2000;43:1831-5.
37. Meyer O, Labarre C, Dougados M, et al. Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographic damage. Ann Rheum Dis 2003;62:120-6.
38. Forslind K, Ahlmén M, Eberhardt K, Hafström I, Svensson B, for the BARFOT Study Group. Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides. Ann Rheum Dis 2004;63:1090–5.
39. van Gaalen FA, van Aken J, Huizinga TW, et al. Association between HLA class II genes and autoantibodies to cyclic citrullinated peptides (CCPs) influences the severity of rheumatoid arthritis. Arthritis Rheum 2004;50:2113-21.
40. Berglin E, Johansson T, Sundin U, et al. Radiological outcome in rheumatoid arthritis is predicted by presence of antibodies against cyclic citrullinated peptide before and at disease onset, and by IgA-RF at disease onset. Ann Rheum Dis 2006;65:453-8.
41. Quinn MA, Gough AK, Green MJ, et al. Anti-CCP antibodies measured at disease onset help identify seronegative rheumatoid arthritis and predict radiological and functional outcome. Rheumatology 2005;45:478-80.
42. Meyer O, Nicaise-Roland P, dos Santos M, et al. Serial determination of cyclic citrullinated peptide autoantibodies predicted five-year radiological outcomes in a prospective cohort of patients with early rheumatoid arthritis. Arthritis Res Ther 2006;8:R40.

43. Machold KP, Stamm TA, Nell VP, et al. Very recent onset rheumatoid arthritis: clinical and serological patient characteristics associated with radiographic progression over the first years of disease. Rheumatology 2007;46:342-9.
44. Syversen SW, Gaarder PI, Goll GL, et al. High anti-cyclic citrullinated peptide levels and an algorithm of four variables predict radiographic progression in patients with rheumatoid arthritis: results from a 10-year longitudinal study. Ann Rheum Dis 2008;67:212-7.
45. Jansen LM, van der Horst-Bruinsma IE, van Schaardenburg D, Bezemer PD, Dijkmans BA. Predictors of radiographic joint damage in patients with early rheumatoid arthritis. Ann Rheum Dis 2001;60:924-7.
46. Lindqvist E, Jonsson K, Saxne T, Eberhardt K. Course of radiographic damage over 10 years in a cohort with early rheumatoid arthritis. Ann Rheum Dis 2003;62:611-6.
47. Goronzy JJ, Matteson EL, Fulbright JW, et al. Prognostic markers of radiographic progression in early rheumatoid arthritis. Arthritis Rheum 2004;50:43-54.
48. Dixey J, Solymossy C, Young A; Early RA Study. Is it possible to predict radiological damage in early rheumatoid arthritis (RA)? A report on the occurrence, progression, and prognostic factors of radiological erosions over the first 3 years in 866 patients from the Early RA Study (ERAS). J Rheumatol 2004;31 Suppl 69:48-54.
49. Moxley G, Cohen HJ. Genetic studies, clinical heterogeneity, and disease outcome studies in rheumatoid arthritis. Rheum Dis Clin North Am 2002;28:39-58.
50. Nepom GT. Preface. Rheum Dis Clin North Am 2002;28:xi-xii.
51. Reneses S, Pestana L, Fernandez-Suarez A, et al. A recent onset inflammatory polyarthritis register in Spain: factors that predict remission. Scand J Rheumatol 2007;36:378-85.
52. Fernandez-Suarez A, Reneses S, Wichmann I, Criado R, Núñez A. Efficacy of three ELISA measurements of anti-cyclic citrullinated peptide antibodies in the early diagnosis of rheumatoid arthritis. Clin Chem Lab Med 2005;43:1234-39.
53. 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.
54. van der Heijde D. How to read radiographs according to the Sharp/van der Heijde method. J Rheumatol 2000;27:261-3.
55. Wilson AG, de Vries N, van de Putte LB, Duff GW. A tumour necrosis factor alpha polymorphism is not associated with rheumatoid arthritis. Ann Rheum Dis 1995;54:601-3.
56. Barton A, Platt H, Salway F, et al. Polymorphisms in the tumour necrosis factor gene are not associated with severity of inflammatory polyarthritis. Ann Rheum Dis 2004;63:280-4.
57. Landewe R, van der Heijde D. Is radiographic progression a realistic outcome measure in clinical trials with early inflammatory arthritis? Clin Exp Rheumatol 2003;21 Suppl 31:S37-41.
58. Larsen A, Dale K. Standardized radiological evaluation of rheumatoid arthritis in therapeutic trials. In: Dumonde DC, Jasani JK, editors. Recognition of anti-rheumatic drugs. Lancaster: MTP Press Ltd.; 1978:285-92.
59. Sharp JT, Young DY, Bluhm GB, et al. How many joints in the hands and wrists should be included in a score of radiologic abnormalities used to assess rheumatoid arthritis? Arthritis Rheum 1985;28:1326-35.