CONCISE REPORTS

Genotyping for disease associated HLA DR β1 alleles and the need for early joint surgery in rheumatoid arthritis: a quantitative evaluation

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Abstract

Objective—**To determine the value of HLA DR β1 disease associated epitope (DAE) and erythrocyte sedimentation (ESR) in predicting the need for major joint replacement in rheumatoid arthritis (RA).**

Methods—**Sixty five RA patients who had undergone hip, knee or shoulder arthroplasty within 15 years of disease onset and 65 who had not. HLA DR β1 genotype was determined by polymerase chain reaction. ESR at first hospital visit was noted.**

Results—**Significantly more patients with two DAE required surgery, (32% *v* 9%), χ^2 = 13.9, p=0.001, odds ratio=5.4 (95% CI: 1.8, 16). Sensitivity was poor, 32%, specificity high, 91%. Presentation ESR was higher in surgery patients compared with non-surgery patients, 52 mm 1st h *v* 25 mm 1st h, p< 0.001, but was independent of DAE status. Sensitivity of an ESR of 30 mm 1st h was 75%, specificity 53%.**

Conclusion—**The presence of two DAE is a risk factor for major joint surgery in RA and is independent of ESR, whereas in those with one or no DAE, a high ESR is an important predictor.**

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Joint destruction because of rheumatoid arthritis (RA) means pain, disability, and socioeconomic cost. The ability to predict future destructive disease at onset is an important but yet unrealised goal. In these patients there is a narrow therapeutic window between the stage of persistent synovitis and irreversible cartilage loss.[1] It is during this interval that disease modifying drugs may confer additional advantage. These treatments however are potentially toxic and require close monitoring.

Several predictors of outcome are recognised, which although useful in epidemiological studies are of limited value in routine clinical practice. Serum rheumatoid factor is useful in diagnosis but is not discriminatory enough to accurately identify individual patients with a poor prognosis.[2] Clinical and laboratory indices of inflammation such as erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) improve with disease modifying treatment but are also influenced by age, coexisting disease and occasionally may be normal in the presence of active disease. Baseline ESR is also a useful indicator of radiological progression over a 5 and 10 year follow up,[3] although the precise value of ESR at presentation as a long term prognostic marker is not accurately quantified. The number of involved joints at presentation is a predictor of mortality. In those with 30 or more joints involved at presentation, five year survival was less than 50%.[4]

HLA DR β1 alleles associated with RA share sequence homology within the third hypervariable region.[5] Phenotypic studies have provided suggestive but not conclusive evidence for more severe disease in HLA DR4 positive patients.[6] Genotypic studies for HLA DR β1 alleles are cheaper, more accurate and informative. In RA it has been suggested that the gene dose results in a hierarchical outcome, one disease epitope resulting in erosive disease and two predisposes to more severe articular as well as extra-articular disease.[7] Theses alleles include HLA DR β1 ★0401, 0404, 0405, 0408, 1402, 1001, 0101, and 0102.[8]

The aim of this study was to accurately quantify the value of genotyping for the disease epitope in identifying those with more severe disease as defined by the need for either shoulder, hip or knee arthroplasty within 15 years of disease onset.

Methods

SELECTION OF CASE PATIENTS AND CONTROL SUBJECTS

A retrospective case-series study was performed to determine the relation between HLA genotype and need for joint surgery before 15 years of disease duration. In a previous survey of 100 consecutive RA patients who had undergone joint replacement surgery attending the same clinic, the median disease duration before requiring surgery was 14.6 years. All study patients were white. One hundred and thirty RA patients consecutively seen by one doctor who satisfied the study entry criteria between March 1995 and April 1996 were included. They were selected from a cohort of 1512 patients attending a hospital based clinic for RA patients. All study patients fulfilled the

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Table 1   Demographic and clinical characteristics of patients who did and did not require joint surgery before 15 years

Surgery	Age (y)	Sex M:F	Disease duration (y)	Age at disease onset (y)	HAQ	DMARD	ESR (mm 1st hr)
Yes (n=65)	59 (51–68)	15:50	**15 (10–17)	43 (37–54)	*2.4 (2–2.6)	3 (2–5)	**52.5 (31–76)
No (n=65)	63 (58–69)	11:54	20 (18–24)	41 (34–48)	2 (1.5–2.5)	2 (1–4)	25.5 (15–43)

Median values are shown with interquartile ranges given in parentheses. M = male; F = female; HAQ = Health Assessment Questionnaire; DMARD = disease modifying anti-rheumatic drugs; ESR = erythrocyte sedimentation rate. ** p < 0.001, * p < 0.01.

1987 American College of Rheumatology criteria for RA,[9] were seropositive for rheumatoid factor and had erosive disease. Patients were selected if they had required either shoulder, hip or knee arthroplasty within 15 years of disease (n=65). Patients with a disease duration greater than 15 years who had not required surgery served as controls (n=65). The extent of disability at the time of study was determined using the Health Assessment Questionnaire (HAQ).[10]

DETERMINATION OF HLA GENOTYPES
Genomic DNA was extracted from venous blood collected in EDTA (Genomix Kit, VH Bio, Newcastle Upon Tyne, UK). Sequence specific primers (SSP) to the HLA DR β1 genes that amplified the alleles 1–16 and 51–53 were used (BSHI, University of Bristol, Bristol UK). Amplification was performed in a final volume of 10 μl (1 × polymerase chain reaction (PCR) buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, primers, 0.125 units of Taq polymerase, 80 ng of genomic DNA). The cycling parameters used for the reaction were those advised by the manufacturer. Patients who were DR4 and DR1 positive were subtyped using SSP primers (Dynal, UK). Genomic DNA (50 ng/μl) was used as a template for amplification with 12 sets of primers to amplify DR β1*0401 to *04019 and five sets of primers to amplify DR β1*0101 to *0104 as advised by the manufacturer. The PCR products were run on a 2% agarose gel and visualised by ethidium bromide staining. A kit containing DR β1 control DNA ((type 2/96), UKTSSA, Bristol) was used to check specificity. Disease associated alleles that give rise to the disease associated epitope (DAE) included HLA DR β1 *0401,* 0404, *0405, *0408,* 0101,* 0102, *1001.

ERYTHROCYTE SEDIMENTATION RATE
When available the initial ESR at first ever visit to the outpatient department at Glasgow Royal Infirmary with presentation of disease was recorded. The ESR had been measured by the Westergren method.

STATISTICAL ANALYSIS
To show a 40% difference (α = 0.02, β = 0.2) 30 patients were required in each group. To correct for potential confounding variables 65 patients per group were recruited. Medians and interquartile ranges are reported. Continuous data were analysed by the Mann-Whitney test, discrete data were analysed by $χ^2$ using 2 × 2 contingency tables. p Values < 0.05 were taken as being significant. To determine the combined influence of HLA genotype and ESR on outcome, logistic regression analysis was performed. In the regression model ESR was a continuous variable.

Results
DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE RA PATIENTS
Table 1 shows the demographic and disease characteristics of the surgery (n=65) and non-surgery (n=65) patients. In the non-surgery group, disease duration was longer than in the surgery group (p < 0.0001). The median disease duration at time of surgery was 8.5 years (IQR: 6–11 years). HAQ score was significantly worse in the surgery group (p = 0.001), but its clinical significance is unclear. In no patient was there evidence of osteoarthritis at disease onset.

DISTRIBUTION OF HLA DR β1 ALLELES
The alleles known to code for DAE were observed in 84 (65%) of the 130 RA patients recruited. The alleles that give rise to DAE that were observed in our study population (both cases and controls) included DR β1* 0401, *0404 , *0405, *0408, *0101, and DR β1 *10, as shown in table 2A.

Twenty seven patients had two DAE, the alleles found included: DR β1*0401/0101, 0401/0401, 0401/0404, 0401/0408, 0404/0408, 0101/0101, see table 2B. Three patients carried either 0408/0408; 1001/1001 or 0404/0405.

Significantly more patients in the surgery group carried 2 DAE compared with the non-surgery group, 32 % v 9% $χ^2$ = 13.9, 2 df, p=0.001. The sensitivity and specificity of having two DAE present for predicting requirement for surgery was 32% and 91% respectively, odds ratio (OR) was 5. Comparing those with two DAE with those who had no DAE, OR was 7 and the test sensitivity 58% while specificity was 84 %. There was no statistical

Table 2
A

Allele observed	Surgery	No surgery	Total	%
Numbers of people carrying alleles that give rise to disease associated epitopes				
DR β1*0101	14	9	23	18
DR β1*0401	24	17	41	32
DR β1*0404	17	5	22	17
DR β1*0405	0	2	2	1.5
DR β1*0408	6	3	9	7
DR β1*10	2	0	2	1.5

B

Allele combination	Surgery (n=65)	No surgery (n=65)	Total (n=130)	%
Summary of people carrying two alleles that give rise to the disease associated epitope				
*0101/0101	2	1	3	2
*0101/0401	5	1	6	5
*0401/0401	3	2	5	4
*0401/0404	4	0	4	3
*0401/0408	2	0	2	1.5
*0404/0408	3	1	4	3
*0404/0405	0	1	1	0.8
*0408/0408	1	0	1	0.8
10 / 10	1	0	1	0.8

Table 3 Logistic regression analysis relating HLA status (2DAE) and baseline ESR on requirement for surgery

Variable	Regression coefficient	Standard error	Significance
Two DAE	1.6877	0.5569	0.0024
ESR	0.0343	0.0085	0.001
Constant	−1.776	0.4226	<0.0001

Logit (P) or $l = -1.776 + 1.6877 x_1 + 0.0343 x_2$, were x_1 is the presence (x=1) or absence (x=2) of 2 DAE; x_2 is the ESR at first clinic visit; P = probability of surgery.

difference in need for surgery between those with one DAE and no DAE (66% *v* 47%, $\chi^2 = 3.5$, 1 df p= 0.06).

ERYTHROCYTE SEDIMENTATION RATE

One hundred and twenty four of the 130 patients had an ESR recorded at the first clinic visit. The ESR was significantly higher in the surgery (n=62, median: 52.5 mm 1st h, IQR: 31–76) compared with the non-surgery group (n=62, median: 25.5 mm 1st h, IQR: 15–43), p < 0.001. A ROC curve analysis (data not shown) showed that an ESR of 30 mm 1st h was the most discriminatory cut off value. An ESR of 30 mm 1st h had a high sensitivity of 74% but a low specificity, 53% in identifying those requiring early surgery.

When all patients were considered, those with two DAE (n=27) had a median ESR of 43 mm 1st h (IQR: 15–86) and those with either one DAE or none (n=97), had a median ESR of 37 mm 1st h (IQR: 21–58), p > 0.05, indicating that ESR was independent of HLA status.

PREDICTING SURGERY USING HLA STATUS AND ESR

To predict the need for surgery within 15 years of disease onset using HLA and baseline ESR, a model using logistic regression analysis was derived. The full model is shown in table 3 and confirms that ESR and HLA predict need for surgery independently of each other. Considering HLA and ESR independently the need for early surgery was predicted in 62% and 68% of patients respectively, however when combined the need for surgery was predicted in 73%.

The log probability of surgery, logit (P) or l was calculated using the equation:

$l = -1.776 + 1.6877 x_1 + 0.0343 x_2$ [x_1 is HLA status ($x_1 = 1$ if 2 DAE present, $x_1 = 0$ if 1 or no DAE present) and x_2 is ESR at first clinic visit]. The probability of surgery can be calculated using the equation: $P = e^l / 1 + e^l$

By setting $x_1 = 1$ or $x_2 = 0$, we generated two equations, l (1) and l (0) from which the log odds was calculated: $l(1) - l(0) = 1.6877$, giving an odds ratio of 5.4 (95% CI: 1.8, 16). Thus those with 2 DAE were 5.4 times more likely to require early surgery compared with those with 1 or no DAE, when ESR is not taken into account.

The odds ratio for surgery in those with two DAE did not change with increasing ESR, whereas the odds ratio increased from 1 (95% CI: 0.6, 3.1) at an ESR of 30 mm 1st h to 2 (95% CI: 0.8, 4) at an ESR of 60 mm 1st h in those with one or no DAE. Thus in patients with one or no DAE the risk for surgery increases with increasing ESR. The influence of one DAE was not examined.

Discussion

We show that RA patients homozygous for the DAE are five times more likely to require major joint surgery compared with those with none. As a predictive test we calculated that in those homozygous for the disease epitope the false positive rate was 9% and false negative rate 68%. ESR at presentation gave a false positive rate of 47% and a false negative rate of 24%. We also found that the need for surgery seemed to be directly proportional to the ESR in those with one or no DAE but not in those with two DAE.

Weyand *et al* found that 60% of RA patients homozygous for the disease epitope required a joint replacement, compared with 25% of heterozygotes.[8] This study was in a cohort attending a tertiary referral centre of which only four patients lacked a disease associated allele. Eberhardt *et al* also found that the need for surgery was three times greater in their homozygous group.[11] The numbers were small and in the latter study duration follow up short. The findings in both may have arisen because of bias or statistical error. In neither could the value of the test in clinical practice be accurately quantified. The importance of ESR was not considered in either study.

These important preliminary results lead us to undertake this more specific study in which we made three assumptions. Firstly, at least a 40% difference between the two groups was considered necessary in calculating study size. This difference was deemed appropriate for any future prospective therapeutic study if more aggressive treatments were to be based on this test. It also gave a feasible study size. To further increase the power of the study only patients under the care of one consultant were included. Secondly, although we tested for a large number of alleles we made no correction for the number of alleles as the original hypothesis was based on either the presence or absence of the disease epitope. Finally, although other studies observed differences in the relative effects of the epitopes we assumed a similar influence of each.

Previous studies have demonstrated a direct association between ESR and radiological progression of disease.[4 12] Fex *et al*[12] reported that a high baseline ESR was one of several factors that predicted radiological progression in 57% of patients. In our study the RA patients who required surgery had a median ESR twice that of RA controls, although there was considerable overlap. Using initial ESR and DAE status allows surgery before 15 years duration to be correctly predicted in 73% of patients. This was compared with 62% and 68% correctly predicted when using HLA status or ESR respectively on their own. Thus prediction for surgery is improved by considering both HLA status and baseline ESR. In those with no or one DAE the need for surgery increases in direct proportion to ESR, while in those with two DAE the need is independent of the acute phase response.

Joint surgery was chosen as an end point because it was a well defined and easily quantified outcome and previous studies evaluating

DAE as a predictor had used this parameter. An alternative would have been disability. Serological studies of HLA show DR4 to be associated with a higher radiographic score and rheumatoid factor positivity but not with a worse functional status.[13] Studies examining the influence of the genotype in predicting disability as assessed by disability have been conflicting. In two, inflammatory polyarthritis rather than RA was studied and divergent results were obtained. In both the primary aim was to establish the value of the test in predicting RA rather than HAQ score.[14 15] In the two RA studies, one reported an association and in the other the numbers were too small for confident statistical analysis and period of observation not long enough.[16 17] Disability therefore appears to measure an aspect of the pathogenetic mechanism that is independent of the HLA status. We found that patients who required surgery were significantly more disabled, however no firm conclusion can be drawn and further studies specifically on this issue are necessary.

Few predictive tests of long term outcome in RA are available. The presence of two DAE is a predictor for major joint surgery in RA and is independent of ESR, whereas in patients with one or no DAE, a high ESR is a better indicator in our study. As treatments for RA continue to improve stratifying patients before joint damage being manifest will become increasingly important. We suggest that genotyping for the disease epitope is a useful test for selecting those likely to require early major joint surgery.

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