Predictive factors for outcome of rheumatoid arthritis

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CHAPTER 12

Joint damage in response to inflammation in rheumatoid arthritis; unraveling underlying mechanisms using extreme discordant phenotypes

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Submitted

ABSTRACT

Introduction

The relation between joint inflammation and destruction is characteristic for RA. Individual patients differ in the amount of joint damage in response to inflammation; the mechanisms underlying coupling/uncoupling are incompletely understood. Evaluation of patients with extreme erosive responses to local inflammation may increase our comprehension. This study explored whether this approach is feasible.

Methods

RA patients included in the Leiden Early Arthritis Clinic with complete 5 years follow-up data (n=159) were studied. Yearly visits included radiographs of hands and feet and swollen joint counts (SJC). The cumulative inflammatory burden was expressed with an AUC of SJCs. Patients with high-inflammatory non-erosive ('resistant') and low-inflammatory high-erosive ('sensitive') phenotypes were identified.

Results

Six patients (4%) had a resistant phenotype; these were rheumatoid factor negative and had short symptom duration. Seventeen patients (11%) had a sensitive phenotype; these patients had a lower SJC at baseline and were often rheumatoid factor positive. Power analyses performed with different risk factor frequencies, different levels of significance and the number of extreme patients identified yielded powers >80%.

Conclusion

Patients with extreme erosive responses to local inflammation were identified. Further evaluations on these patients may elucidate mechanisms contributing to the connection of inflammation and destruction of joints in RA.

INTRODUCTION

Understanding of the mechanisms involved in disease progression or resistance to progression is required to derive strategies to diminish such progression. Generally a whole population of patients is studied to identify such factors. Alternatively, the most and least progressive patients can be compared. This extremes-of-the-phenotype approach reduces the number of patients that need to be studied; this is beneficial when it is impractical or expensive to determine risk factors in large numbers of patients. A third approach, the extreme-discordant-phenotype methodology, studies the response of individuals on an increasing dose of stimuli; the extremes of this gradient are identified as 'sensitive' or 'resistant' phenotypes.[1,2]

This extreme-discordant-phenotype methodology has been successful in the identification of genetic variants involved in responsiveness to drugs, malignancies, and infectious diseases.[3-5] An example of a 'sensitive phenotype' is the observation that some patients with malignancies developed severe toxicity after receiving 5-fluorouracil. Thorough evaluation of these patients led to the association with a complete deficiency of dihydropyrimidine dihydrogenase activity in peripheral blood mononuclear cells, which is caused by diverse genetic alterations.[5] An outstanding example of the identification of a resistant factor is based on the observation that some individuals highly exposed to HIV never developed the infection. This resulted in the identification of a deletion in the gene encoding the chemokine coreceptor CCR-5, which is now a drug target.[4]

Inflammation and destruction of joints are hallmarks of Rheumatoid Arthritis (RA) and the notion that local inflammation leads to destruction of joints is basic to the concept of RA. On the group level, the amount of inflammation is indeed correlated with the amount of erosive joint damage. However, the degree of erosiveness in response to inflammation is highly variable between RA patients and also disconnection has been observed.[6-11] The mechanisms underlying such coupling/uncoupling are incompletely understood. Since the readiness for bone to erode in response to local inflammation appears to be an individual's characteristic, genetic factors may play a role.

Our ultimate aim is to unravel processes contributing to an individual RA-patient's predisposition to develop joint erosions in response to local inflammation. In this study we evaluate whether the extreme-discordant-phenotype methodology is feasible to this end.

PATIENTS AND METHODS

Patients

RA patients included in a population based inception cohort, the Leiden Early Arthritis Clinic (EAC), were studied. For an extensive cohort description see reference.[12] Written informed consent was obtained from all participants. The study was approved by the local medical ethics committee. All RA patients satisfied the 1987 ACR-criteria for RA. From the total number of 695 RA patients, 441 RA patients had achieved 5 years of follow-up. Of these, 159 RA patients

had missed none of the yearly follow-up visits and had complete follow-up data during 5 years. Baseline characteristics were not significantly different between patients with and without missing follow-up visits (data not shown). The 159 RA patients were studied to identify patients with high-inflammatory non-erosive ('resistant') and low-inflammatory high-erosive ('sensitive') phenotypes.

Joint damage

All 1908 hand and feet radiographs were scored by one experienced reader (MPMvdL) according to the Sharp-van der Heijde score (SHS) in chronological order. 499 radiographs were rescored; the interclass-observer correlation coefficient was 0.91. The total erosion SHS was used. Based on previous findings,[13] patients whom had a SHS erosionscore ≤1 after 5 years were defined as having non-erosive disease. To select the patients with a high-erosive disease course, the patients with the highest quartile of SHS erosionscores at the 5-years visit were evaluated.

Joint inflammation

Local inflammation of the joints was assessed by the 44-swollen joint count (SJC) at each visit. For classification as 'high-inflammatory' synovitis had to be observed almost persistently during the follow-up period; a SJC of 0 was allowed at only one point in time. For classification of 'low-inflammatory' the SJC during follow-up required to be 0 in three out of the five follow-up time-points and to be ≤5 at the other follow-up timepoint(s). These cut-off values are arbitrary and were chosen based on visual evaluation of the SJCs of the whole RA-population during the follow-up visits. To appraise whether these cut-offs allowed the identification of extreme discordant phenotypes, for each patient the cumulative inflammatory burden over 5 years was estimated by calculating an area under the curve (AUC) and plotted against the SHS-erosionscore at 5 years.

In the evaluation on joint inflammation above, 44 joints (44 SJC) were studied. Although it was observed that in the present dataset the SJC was mainly driven by the number of inflamed small joints, a comparison of joint destruction in the small joints with joint inflammation in small and large joints may be considered inequitable. Therefore we also evaluated inflammation in 32 small joints (the wrist, MCPs, PIPs and MTPs joints that were assessed in the SHS) and again identified patients that were 'high inflammatory' and 'low inflammatory' using the cut-off values as described above.

Statistical analysis

Patient characteristics were compared using crosstabs and Chi-square, Fisher exact, Mantel-Haenszel statistics in SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Power calculations were performed for testing two independent proportions based on the Z-test in Pass 2008 (NCSS, LCC Kaysville, Utah, USA), using two significance levels namely 0.05 and 0.005.

RESULTS

Extreme discordant phenotypes

Applying the definitions of non-erosive, high-erosive, low-inflammatory and high-inflammatory as indicated, resulted in the selection of 6 RA patients with a high-inflammatory non-erosive ('resistant') phenotype and 17 patients with a low inflammatory high-erosive ('sensitive') phenotype (Figure 1A). The remaining 136 patients (85%) were labeled as the reference group. The AUC of the SJC over time was plotted against the erosion score at 5-years (Figure 1B); the patients with the 'sensitive' and 'resistant' phenotype are indicated in red and blue respectively.

Evaluations of inflammation in small and large joints and joint damage in only small joints may be imbalanced when inflammation is predominantly present in large joints. To explore this, analyses were repeated comparing inflammation and destruction in small joints only. Then, 6

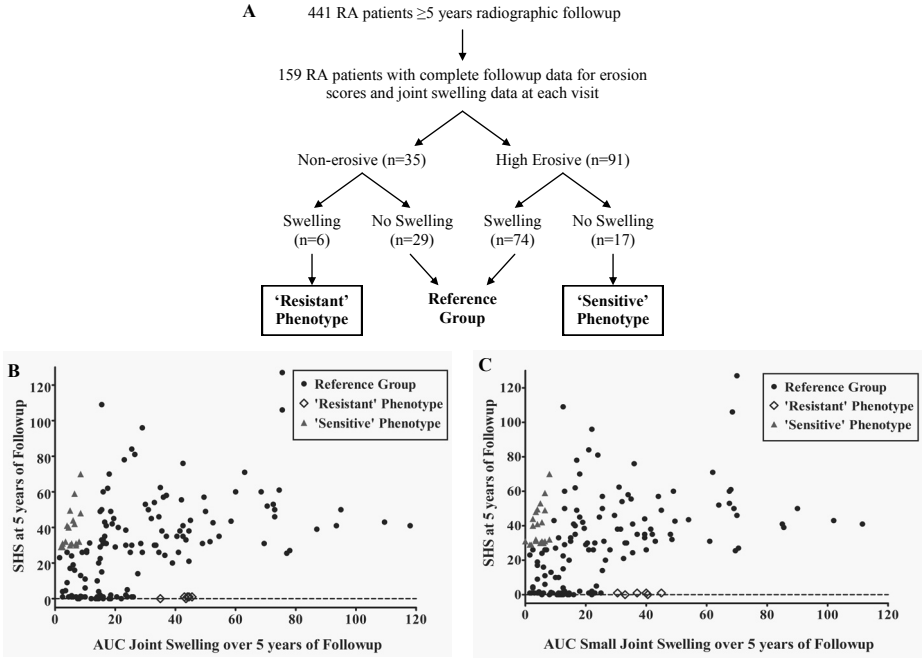

Figure 1. Flow diagram of the selection of extreme discordant phenotypes (A), graphic representation of the cumulative level of inflammation and damage over 5 years of disease evaluating inflammation in 44 joints (B) and evaluating inflammation of small joints only (C). As shown in panel A, patients were used for analysis if they had complete radiographic and SJC data during 5 years of follow-up. Patients were non-erosive when having SHS erosion score ≤1. High-erosive patients were within the highest quartile of erosions scores at year 5. In high-inflammatory patients synovitis had to be present in almost all visits as a SJC of 0 was allowed only at one point in time. Low-inflammatory patients the SJC was 0 for at least three out of five follow-up time points and ≤5 at the other followup time points. For Figure 1A and 1B inflammation was assessed in 44 joints, for Figure 1C inflammation was assessed in 32 small joints (wrists, MCPs, PIPS, and MTP joints that are also evaluated on joint destruction in the Sharp/van der Heijde method)

RA patients were identified with a high-inflammatory non-erosive ('resistant') phenotype and 20 patients with a low-inflammatory high-erosive ('sensitive') phenotype (Figure 1C). The patients with the resistant phenotype were the same individuals as in Figure 1B. Also the patients with the sensitive phenotype were similar but extended with three additional patients. These three additional sensitive patients were "low-inflammatory" when assessing small joints only, but not when assessing 44 joints as they had inflammation in large joints. Therefore, to study the most extreme discordant patients, patients that in both analyses were identified as extreme discordant were evaluated in further analyses.

Patient characteristics

Baseline characteristics of the patients with the 'sensitive' or 'resistant' phenotype were compared to that of patients in the reference group (Table 1). Compared to the reference group, patients with the 'resistant phenotype' were characterized by the absence of IgM-rheumatoid factor, a low frequency of anti-CCP2-positivity, more frequently an acute onset of symptoms, a shorter symptom duration and a higher SJC at first presentation. Patients with the 'sensitive phenotype' were more often rheumatoid-factor positive, had a longer symptom duration and a lower SJC at baseline than the reference group. The erythrocyte sedimentation rate and C-reactive protein (CRP) levels at baseline were not statistically different between the 'sensitive', 'resistant' or reference groups.

Power of extreme discordant phenotype approach

Next we aimed to evaluate the power to identify genetic variants associating with these phenotypes, using the number of patients identified. Our hypothesis is that these extreme discordant phenotypes are multi-factorial and caused by either recessive effects of common genetic variants or rare genetic variants. For the recessive effect we assume that the penetrance is not 100%, i.e. that also in the reference group recessive genotypes occur. Concerning rare variants it is assumed that multiple deleterious and neutral variants are present in the studied genomic region.[14] For comparisons of the number of patients carrying the recessive genotype or the number of patients carrying rare mutations between the two groups the same Z-test can be used; hence one power study is required applying to both situations. For the non-erosive group, the power to detect differences between the high-inflammatory (n=6, 'resistant') and non-high-inflammatory group (n=29) was determined. For an α of 0.005 and for instance carrier frequencies of 0.83 (5 out of 6) in group 1 (P1) and 0.03 (1 out of 26) to 0.10 (3 out of 26) in group 2 (P2), the power to detect a difference is above 90% (Figure 2A). When using an α of 0.05 and similar frequencies, the power is 97% (Figure 2B). For the high-erosive group, the power to detect differences between the low-inflammatory (n=17, 'sensitive') and non-low-inflammatory group (n=74) was determined. In case of carrier frequencies of 0.8 and 0.1, the power to detect a difference is 100%, both for α's of 0.005 and 0.05 (Figure 2C, D). The power for other genotype frequencies is depicted in Figure 2. Overall, it was observed that evaluations on the present number of patients with extreme discordant phenotypes and rare genetic variants have sufficient power.

Table 1. Characteristics at baseline of all patients, and the patients in the 'sensitive', 'resistant' and reference group

	All patients n=159	Reference group (n=136)	'Sensitive' Phenotype (n=17)	'Resistant' phenotype (n=6)
Female, n (%)	106 (66.7)	90 (66.2)	10 (58.8)	6 (100)
Age at inclusion (yrs), mean (SD)	55.2 (13.8)	55.1 (13.8)	57.5 (13.9)	51.3 (13.8)
Symptom duration at first presentation, weeks mean (SD)	31.9 (26.8)	32.1 (26.5)	37.9 (31.6)	16.1 (20.2)
< 6 weeks, n(%)	36 (24.8)	32 (25.2)	1 (5.9)	3 (50.0)
≥ 6 weeks, n (%)	109 (75.2)	95 (74.8)	11 (91.7)	3 (50.0)
Onset of symptoms				
(Sub)Acute	76 (50.7)	64 (48.5)	8 (61.5)	4 (80.0)
Gradual	74 (49.3)	68 (51.5)	5 (38.5)	1 (20.0)
Morning stiffnes (min), mean (SD)	89.6 (97.7)	88.5 (86.2)	92.5 (171.6)	105.0 (92.5)
44 Swollen joint count, mean (SD)	10.1 (7.7)	10.5 (7.7)	5.2 (3.3)	14.8 (10.1)
1 medium-large joint, n (%)	3 (1.9)	1 (0.7)	1 (5.9)	1 (16.7)
2-10 medium-large joints, n (%)	3 (1.9)	3 (2.2)	0 (0)	0 (0)
1-3 small joints, n (%)	19 (11.9)	15 (11.0)	4 (23.5)	0 (0)
4-10 small joints, n (%)	54 (34.0)	44 (32.4)	9 (52.9)	1 (16.7)
> 10 joints, n (%)	80 (50.3)	73 (53.7)	3 (17.6)	4 (66.7)
ESR (mm/hr), mean (SD)	46.1 (32.7)	46.1 (32.7)	46.3 (32.4)	46.3 (38.1)
CRP (mg/l), mean (SD)	36.6 (40.9)	34.7 (40.4)	52.6 (44.1)	40.7 (43.2)
IgM-RF-positive, n (%)	101 (64.7)	88 (64.7)	13 (92.9)	0 (0)
Anti-CCP2-positive, n (%)	103 (66.5)	91 (67.9)	11 (73.3)	1 (16.7)
HAQ, mean (SD)	0.91 (0.68)	0.92 (0.66)	0.66 (0.88)	1.14 (0.70)

Comparison of 'resistant' phenotype versus reference group: IgM RF p=0.003, anti-CCP p=0.018, symptom duration p=0.057, onset of symptoms p=0.2 and SJC p=0.2. Comparison of 'sensitive phenotype' versus reference group: IgM-RF p=0.036, SJC p=0.004

DISCUSSION

The relation between inflammation and subsequent joint damage is characteristic for RA and is basic to current treatment strategies that aim to prevent or retard joint damage by reducing the inflammatory load. Although this strategy is effective on the group-level, the coupling between inflammation and destruction of joints in individual patients is variable. One method to identify

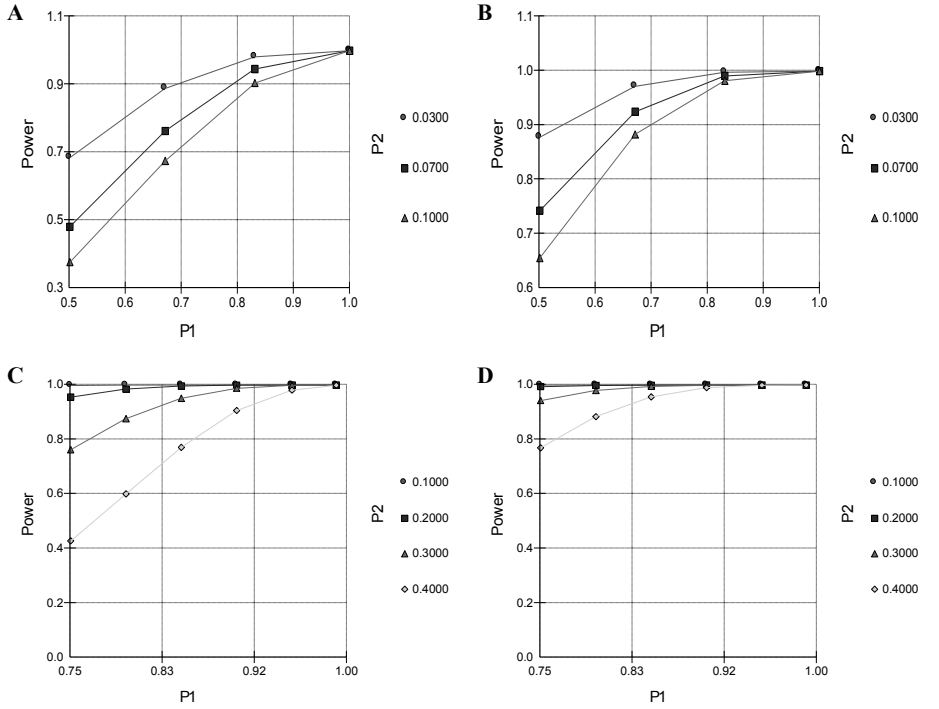

Figure 2. Results of the power analyses. The power is indicated to detect differences between the non-erosive high-inflammatory ('resistant', n=6) and low-inflammatory (n=29) groups (Panel A and B) and high-erosive low-inflammatory, ('sensitive' n=17) and high-inflammatory (n=74) groups (Panel C and D). Power calculations were done for different levels of significance: α of 0.005 (Panel A, C) and 0.5 (Panel B, D). P1 represents the proportion of patients carrying the recessive genotype or the proportion of patients carrying rare mutations in the resistant or sensitive group and P2 represents these proportions in the other group

factors relevant in protection or progression is to appraise patients with extreme responses on inflammation. The present study reports that RA patients with extreme discordant phenotypes can be identified.

We identified two extreme responses on joint inflammation. The 'resistant' phenotype, characterized by the absence of erosive damage despite high cumulative levels of inflammation throughout the studied period, and the 'sensitive' phenotype, characterized by the lowest cumulative inflammation but the highest levels of erosiveness. Considering the 'sensitive' phenotype, a question is whether physical examination on swollen joints was sensitive enough to detect joint inflammation. It is possible that subclinical inflammation was present.[15] However, even in this case, these patients have an extreme sensitive response to subtle local inflammation.

We did not intend to evaluate whether inflammation is associated with joint destruction; this has been studied before on joint level.[16] Moreover, in the present study analyses were performed on patient level to identify patients with extreme responses to inflammation. Because of this aim,

we did not perform analyses on joint level, as this would have resulted in 32 comparisons per patient.

The ultimate question is what processes underlie these extreme phenotypes. Genetic factors may account for an individual's degree of sensitivity to inflammation. It was observed that the power of future genetic studies on rare genetic variants using the number of patients identified is sufficient.

The present study has several limitations. First, the AUC of the SJC was determined using yearly measurements. This may lead to bias since the number of swollen joints at one time-point may not reflect the average number of swollen joints during a year. To prevent misclassification, the medical files of all patients in the high or low inflammatory groups were studied to verify whether the classification fitted with clinical evaluations at time points in between yearly visits. Second, treatment was not taken into consideration. The variety of medications used made adjusting for treatment challenging. It is generally presumed that anti-rheumatic treatment suppresses the level of inflammation. This does not hamper the subject of the present study, which concerns the degree of joint damage in response to inflammation. In case treatment was prescribed that directly affected bone destruction, joint damage may be more diminished than would be the result of suppressing inflammation only. At present, to our knowledge, the only anti-rheumatic treatments that may reduce bone damage to a higher extend than suppressing inflammation are the TNFα inhibitors.[6-9] None of the 6 'resistant' patients were treated with anti-TNF. A third issue is that we studied the SJC and not the level of acute phase reactants or the disease activity score (DAS). We did not study the DAS as it is a composite measure. Pain may increase the DAS also in the absence of synovitis and, vice versa, it is known that a low DAS can be achieved in the presence of inflamed joints. We also chose not to use the CRP as this is a systemic inflammatory-marker, rather than a reflection of local inflammation. A recent study on data from five randomized trials also showed that joint swelling rather than CRP contributes to joint damage.[17]

In conclusion, RA patients with extreme responses in joint destruction to local inflammation are infrequent but prevailing. Further studies in these patients may elucidate mechanisms contributing to the coupling between inflammation and destruction of joints in RA.

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