EXTENDED REPORT

Contribution of patient related differences to multidrug resistance in rheumatoid arthritis

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Background: There is a wide variation in responses to standard disease modifying antirheumatic drug (DMARD) treatment in rheumatoid arthritis (RA). Whether multidrug resistance, failure to respond to several DMARDs, is a specific entity over and above that expected by chance alone is unclear.
Objective: To identify patients with RA who demonstrate a multidrug resistant phenotype and to determine what proportion of the variance in drug responses is due to patient related factors.
Methods: Patients with RA (1987 American College of Rheumatology criteria) were identified from clinics at Manchester Royal Infirmary and through the Arthritis Research Campaign National RA Repository. The clinic records were reviewed and multidrug resistance was defined as stopping three or more DMARDs owing to lack of efficacy after an adequate trial of the drug. Logistic regression measured by a random effects model was used to determine the relative contribution of the drug and subject related differences to the multidrug resistance.
Results: 265 patients (210 (79.3%) female) were studied. The mean (SD) age and disease duration were 52.2 (12.9) and 10.7 (8.8) years, respectively. Patients had a median (range) of 2 (1–8) DMARD courses. Failure of at least one DMARD due to inefficacy occurred in 105 (40%) and 13 (5%) were multidrug resistant. Overall, 35% of the variance in drug responses was due to between-subject differences (p=0.02). Rheumatoid factor (RF) status contributed significantly to this (OR=2.15, 95% confidence interval (95% CI) 1.00 to 4.62) but explained only 3% of the total variance in drug inefficacy.
Conclusion: Multidrug resistance occurs in an uncommon (5%) but important subgroup of patients with RA. The between-subject variance is not fully explained by demographics and RF status. Understanding the biological mechanisms that contribute to multidrug resistance may suggest new therapeutic approaches and targets in RA.

Rheumatoid arthritis (RA) is a progressive autoimmune disease, causing increasing pain and disability over many years.[1] Studies have shown that disease modifying antirheumatic drugs (DMARDs) are beneficial, improving pain,[2] disability,[3] and slowing of the progression of joint destruction.[4 5] Treatment regimens have, therefore, shifted towards the increasingly early use of DMARDs,[6–8] either in a sequential approach, as in the "sawtooth" strategy,[9] or in combination, with favourable results.[10] Numerous clinical studies have, however, shown varied response to DMARDs in patients with RA. More than 80% of traditional DMARD courses are discontinued within two years, predominantly because of either toxicity or lack of efficacy.[11 12] Depending on the DMARD chosen, up to 45% of patients discontinue owing to toxicity or side effects. Furthermore, a lack of response to certain DMARDs from the outset of treatment occurs in up to 24% of patients and a further 25% may stop responding after an initial period of response.[11]

Most studies have focused on the incidence of non-response or "resistance" to a single agent and the relative efficacy of one drug over another. Although it is recognised that certain patients will not respond to several drugs, the frequency and reasons for this are unclear. Statistically, if individual drugs carry a certain risk of inefficacy and DMARDs are used sequentially, chance alone will result in a proportion of patients being non-responsive to multiple agents. Alternatively, there may be certain patients who are more likely not to respond to multiple drugs—so called "multidrug resistance". This phenomenon is a recognised feature of certain diseases—for example, solid tumours and HIV disease. Whether and to what extent this phenomenon exists in RA has not been studied. We aimed at identifying, in a series of patients with RA, those patients who display a multidrug resistant phenotype. The specific objective was to determine the contribution of individual patient factors to the occurrence of drug resistance compared with that explained from chance alone.

PATIENTS AND METHODS

Patients and data retrieval

Data from two sources were used retrospectively to ascertain subjects with drug resistance. Patients currently attending clinics at Manchester Royal Infirmary and patients notified by hospital consultants to the Arthritis Research Campaign National RA Repository, as at June 2000, were studied. In the latter, only the index patient of multiplex families was included. All patients satisfied the 1987 revised American College of Rheumatology (ACR) classification criteria for RA.[13] The hospital records were reviewed by trained abstractors. Basic demographic data and clinical and laboratory variables in the ACR criteria list were obtained. Details of the date of starting and stopping each new DMARD course, dosage used and, where applicable, the date and reason for stopping were recorded for each patient. We excluded cyclosporin A courses because this drug is a well characterised P-glycoprotein inhibitor,[14] and may "protect" against drug resistance. As steroids can be used for a variety of reasons other than disease modifying purposes, for the purpose of this study, prednisolone and other corticosteroid use was not classified as a

Abbreviations: ACR, American College of Rheumatology; DMARDs, disease modifying antirheumatic drugs; IM, intramuscular; Pgp, P-glycoprotein; RA, rheumatoid arthritis; RF, rheumatoid factor

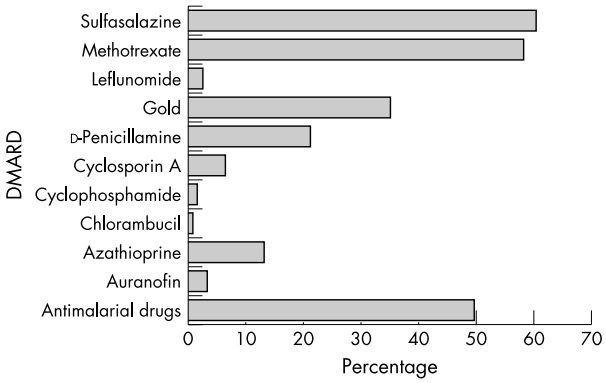

Figure 1 Percentage of patients taking, or who had taken, a DMARD course.

DMARD treatment and data for this drug were not collected. The reason for stopping a DMARD was based on a doctor's statement recorded in the chart. These were categorised as adverse side effect, inefficacy, condition improved/remission, or other. As it was difficult to assign reasons for stopping a combination treatment, combination courses were not included in the analysis. A patient was defined as non-responsive to a specific drug if the DMARD in question was stopped after a trial of ⩾3 months and the doctor stated that the drug had been ineffective. In cases of DMARDs stopped at <3 months the reason for stopping if it was due to toxicity, side effects, improvement of condition, or other was noted. We did not accept a stoppage due to inefficacy during this time period. We subdivided drug failure into primary and secondary failure. A DMARD stopped between 3 and 12 months because of inefficacy was a primary failure whereas drug failure occurring at >12 months because of inefficacy was defined as secondary failure. Multidrug resistance was defined as stopping ⩾3 different DMARDs during the disease course owing to primary and/or secondary inefficacy. For comparative purposes, a "responder" group of patients was identified and defined as patients taking a single DMARD who had continued to receive that single treatment for >2 years.

Statistical analysis

Comparisons of the clinical data and other characteristics between the multidrug resistant group and responder group were undertaken using *t* tests and χ^2 tests as appropriate.

Logistic regression was used to predict the probability of failure of a course of treatment. Age, rheumatoid factor (RF), and sex were added to the logistic model. Both different drugs and individual subjects were treated as random effects to allow for the non-independence of repeated observation on the same person. The importance of the between-subject variation is summarised by the parameter ρ, which estimates the proportion of the variation in outcome that is due to differences between individual patients. This parameter can take values between 0 and 1, with 0 implying that repeated observations within subjects are independent (that is, there is no subject effect), and 1 implying there are some subjects for whom no drug will ever work, and the remainder for whom all drugs will always work.

RESULTS

Two hundred and sixty five patients (210 (79%) female, 55 (21%) male) were studied, 105 patients attending the clinics at the Manchester Royal Infirmary and 160 from the Arthritis Research Campaign National RA Repository. Their mean (SD) age at study was 52.2 (12.9) years and mean (SD) disease duration 10.7 (8.8) years. Of the patient group, 227 (86%) were RF positive.

Drug courses

The 265 patients took 677 courses of DMARDs, of which about 95% were taken between January 1980 and December 1990. We excluded 17 (2.5%) cyclosporin A courses, thus leaving a total of 660 courses analysed. Sulfasalazine was the most commonly prescribed drug, with 61% of patients having taken the drug. The next most commonly used drugs were methotrexate (58%), antimalarial drugs (49%), and intramuscular (IM) gold salts (35%) (fig 1). Over the study period, 62% of methotrexate courses were continued to the end of the study period, compared with 30% of the sulfasalazine, and 29% of the hydroxychloroquine courses. Of those patients taking IM gold salts, 54% of the courses were stopped owing to side effects. Similarly, 36% of the D-penicillamine courses and 29% of the sulfasalazine courses were also stopped owing to side effects (table 1). Thirty eight per cent of antimalarial courses were "ineffective", and a similar proportion of primary and secondary failure occurred with this drug, 20% and 18% respectively. Similarly, 24% of sulfasalazine courses were ineffective owing to primary or secondary inefficacy in 6% and 19% of cases, respectively.

Patient outcomes

Overall, patients had been treated with a median of 2 (range 1–8) DMARD courses. One hundred and eighty one (68%) patients were treated with two or more drugs over the study period and 72 (27%) had ⩾3 drugs stopped for any reason. Of the 265 patients, 105 (40%) and 30 (11%) found that at least one drug and at least two drugs, respectively, were not efficacious. Multidrug resistance (inefficacy of ⩾3 drug courses) occurred in 13 (5%). In this group there was more secondary than primary inefficacy (fig 2). The commonest drug "failures" in this group were IM gold salt courses (nine), hydroxychloroquine (eight), sulfasalazine (eight), and D-penicillamine (six). We also identified a group of 57 (22%) patients who had responded to and continued to receive the same drug for a period of >2 years. In this group the most commonly drugs used were sulfasalazine 21 (37%) and methotrexate 15 (26%).

Table 1 Reasons for stopping a particular DMARD course

DMARD course (n)	Continuing (%)	Reason for stopping (%)			
		Ineffective	Side effects	Improved/ remission	Other
Antimalarial drugs					
Hydroxychloroquine (109)	29	38	15	1	17
Chloroquine (28)	11	39	25	4	21
D-Penicillamine (56)	23	27	36	0	13
Gold (93)	17	18	54	0	10
Methotrexate (155)	62	9	19	0	10
Sulfasalazine (161)	30	24	29	1	15

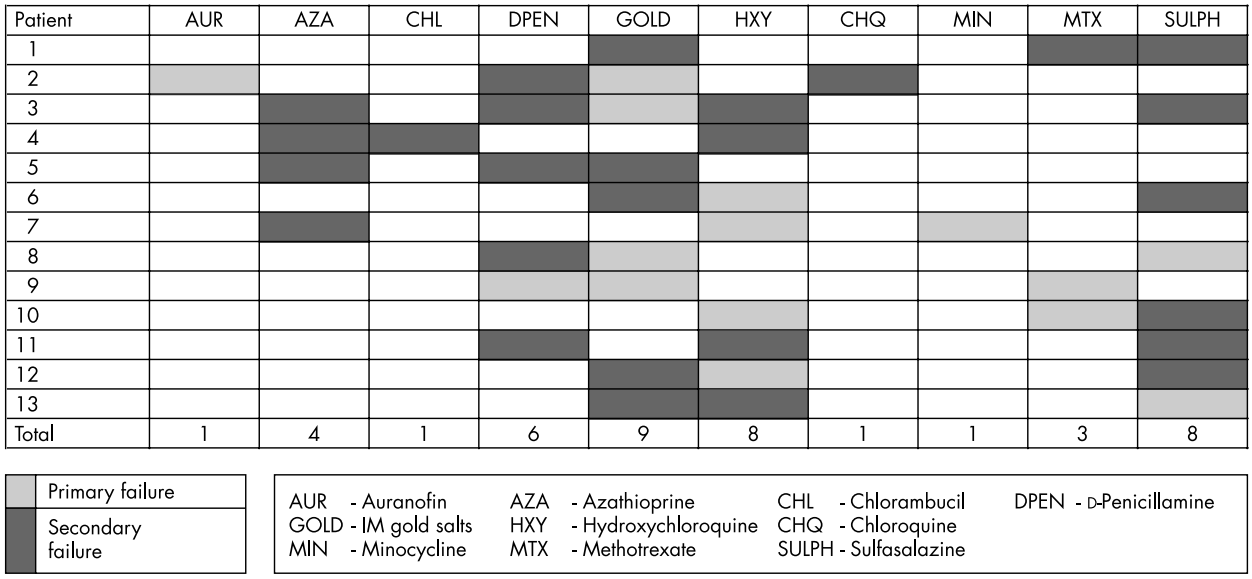

Figure 2 Drug courses stopped owing to inefficacy in those patients for whom ⩾3 DMARDs had failed.

Comparison of the responder group (n=57) with the multidrug resistant group of patients (n=13) showed that the multiresistant group were significantly younger at diagnosis (38.6 (7.4) *v* 45.1 (14.4), p=0.03). All of the 13 multidrug resistant patients were female compared with 63% of the responder group (p=0.009). There was no difference in the delay from disease onset to 1st DMARD use between groups. Twelve of 13 multidrug resistant patients were RF positive (table 2). When we compared the responder group with a group of 30 (35%) patients who had experienced inefficacy with ⩾2 drugs, the same subject characteristics were evident. These patients were significantly younger at diagnosis (39.2 (10.5) *v* 45.1 (14.4), p=0.04), 90% were female, and 97% were RF positive.

We then examined the lack of efficacy in all treatment courses to determine the role of patient factors. A total of 646 of the 660 treatment courses were included in the logistic regression analysis using a random effects model. Because treatment of none of the 13 subjects receiving cyclophosphamide (n=4), chlorambucil (n=2), and leflunomide (n=7) was stopped owing to inefficacy, and the only subject treated with minocycline had no response, these drugs had to be excluded from the analysis. There were significant differences between drugs in the proportion of courses ending in inefficacy, from 9% to 50%. Allowing for the different probabilities of failure with different drugs, the random effects model predicted significant differences between subjects for the probability of failure (p=0.026). Thirty five per cent of the variance of probability of failure was due to differences between subjects. The remaining 65% was due to differences between drugs or chance alone. Treatment was more likely to fail if the subject was RF positive (OR=2.15, 95% CI 1.00 to 4.62), although only 3% of the total variation was explained by RF status. After accounting for RF status, the remainder of the between-subject variance remained significant (ρ=0.32, p=0.05). Age and sex did not significantly influence this model.

DISCUSSION

The issue of multidrug resistance has received little attention in RA. This concept is, however, well recognised in other areas of medicine such as oncology and microbiology. It is only recently that a working definition of "resistant RA" has been suggested.[15] Most studies that examine the treatment of RA have tended to concentrate on responses to individual drugs in clinical trials, or overall "drug survival" of individual drugs in observational studies.[11] From these study designs, differences in the efficacy and safety of different treatments can be deduced. Few studies have, however, attempted to determine whether multidrug resistance occurs in RA and, if so, whether patient characteristics have a role in this. In this retrospective study of 265 patients with RA attending hospital clinics, we found that 13 (5%) patients were "multidrug resistant"—that is, had stopped ⩾3 different DMARDs because of their inefficacy. We also found that there were significant differences between subjects in the probability of failure of a particular DMARD. Patient differences accounted for about one third of the variance in probability of failure. This confirms the clinical

Table 2 Comparison of multidrug resistant and responder patients

	Responder patients (n=57)	Multidrug resistant (n=13)	Significance (p value)
Mean (SD) age at diagnosis (years)	45.1 (14.4)	38.6 (7.4)	0.03
Sex			
Male	21	0	0.009
Female	36	13	
Mean (SD) disease duration (years)	7.83 (5.8)	16.8 (7.9)	0.03
Mean (SD) delay before 1st DMARD (years)	3.0 (4.8)	3.3 (3.6)	0.8
RF			
Positive	44	12	0.3
Negative	12	1	

impression that drug resistance in RA is not all due to variations in drug efficacy or chance alone.

A number of methodological issues need to be considered. This was a retrospective observational study of patients treated in routine hospital clinics. The demographic and severity profile of the patients studied is comparable with that of other hospital series of patients with RA and randomised clinical trials.[16] Patient data were not collected prospectively in a standardised way nor were there any predetermined criteria set for discontinuation of treatment. We relied on the treating doctor's statement of the reason for stopping an individual drug course as documented in the patient record. Clearly, the reasons for starting and stopping a particular treatment are guided, in part, by the doctor's clinical opinion and we cannot completely exclude the influence of doctors' preferences and preconceptions. The patients included in this study were, however, treated by a large number of rheumatologists in different sites across the UK, which will minimise this problem. In addition, given the limited therapeutic armamentarium available to rheumatologists, most may be reluctant to stop an individual drug without good reason. Overall, our data on drug continuation, inefficacy, and adverse event rates are comparable with previous reports from other clinic settings.[11] For example, methotrexate was shown to be continued in both studies by over 60%, with side effects reported in 19% and lower inefficacy than with any other drug course. This suggests that although we were unable to define drug inefficacy according to standard criteria, such as the ACR 20 responses,[17] this study nevertheless reflects the proportions of patients for whom a particular drug did not produce a clinically relevant response. The nationwide distribution of the patients involved also suggests that these results do have external validity.

We found a 13 (5%) cumulative incidence of patients who had a "multidrug resistance" phenotype. The hospital based nature of these patients means that this may be an overestimate of the true extent of this problem. Conversely, we found that longer disease duration was associated with multidrug resistance, a longer follow up period may therefore show a higher proportion of patients who become resistant. A similar study in a large community based cohort of patients would help to identify the dominant source of bias in an RA population. In our study, 72 (27%) stopped ⩾3 drugs for any reason. Our definition of multidrug resistance was, however, narrow and therefore a low prevalence (5%) of this phenotype was found. In an attempt to ensure that inefficacy was attributed after an adequate period of assessment we only accepted inefficacy if the patient had been receiving the drug at a therapeutic dose for ⩾3 months. Although six months may be necessary to assess the efficacy of some drugs such as gold or D-penicillamine, the commonest drugs in this study will demonstrate most of their anti-inflammatory effects within 12 weeks of full dose treatment. In the case of gold and D-penicillamine, patients stopped because of inefficacy at a median of 26 and 48 months, respectively, suggesting that these drugs were not stopped without an adequate therapeutic trial. Also, in the multidrug resistant group (fig 2), 67% of drug failures were secondary (occurring >12 weeks), which suggests that we did not overestimate inefficacy.

Multidrug resistant patients were more likely to be female, RF positive, and younger at diagnosis. A similar trend was seen when we looked at patients for whom ⩾2 DMARDs had produced no response. The random effects model confirmed that although there were differences between drugs, significant variance was also attributable to between-subject effects. RF status, although significant, only explained about 3% of the overall variance in response. Although age and sex did not contribute significantly to our model, this suggests that additional, as yet undetermined, patient-related factors are also of importance.

Several patient-related factors may be implicated in multiple DMARD failure in RA. Patient non-adherence to treatment may contribute to this problem, but it is impossible to assess this in a retrospective study such as this. Undiagnosed malabsorption states may also interfere with bioavailability and lead to apparent drug resistance.[18] However, because IM gold was a frequent treatment used in the multidrug resistant group (9/13) these are unlikely to be the key explanations. A further explanation may be that immunologically, current treatments for RA may not directly target the relevant inflammatory mechanisms in some RA subgroups. Similarly, the hypoxic environment of the rheumatoid synovium[19] may also render drugs less active in a manner akin to the treatment of solid tumours.[20] O'Dell *et al* noted that in established disease, patients who are HLA-DR4 positive were more likely to respond to a triple combination of methotrexate, sulfasalazine, and hydroxychloroquine than to methotrexate monotherapy.[21] Such an influence is less clear in patients treated early in their disease course.[22] Overall, therefore, mechanisms of relevance to drug resistance in RA may develop or be enhanced over the course of disease as synovitis becomes established. Biological drug efflux pumps may be one of the mechanisms that contribute to this phenomenon. The best characterised of these, results from over expression of the MDR-1 gene that encodes P-glycoprotein (Pgp). Pgp is an ATP dependent efflux pump which can export certain drugs out of cells and impair their therapeutic efficacy. Llorente *et al* noted that a significant proportion of peripheral blood lymphocytes from patients with RA express the MDR-1 gene.[23] Expression of Pgp on lymphocytes is predictive of a poor response to sulfasalazine or bucillamine.[24] In addition, cyclosporin A is an effective Pgp inhibitor,[14] which may, in part, explain its efficacy in RA in combination with methotrexate.[25]

In summary, in a retrospective study of patients with RA a "multidrug resistant" phenotype was relatively uncommon, affecting 5% of the subjects studied. A significant proportion (35%) of variance in drug response was due to between-subject differences. RF status contributed to this, but a large proportion of the between-subject variance remains unexplained. Further understanding of the variance in drug response between subjects may suggest new therapeutic strategies and targets in RA.

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