Inflammopharmacology. 1998; 6: 81–89.

A METHODOLOGICAL APPRAISAL OF THE IMPACT OF DIFFERENT CLASSIFICATION PROCEDURES USED IN THREE DIFFERENT PHASES OF THE AUSTRALIAN RHEUMATOID ARTHRITIS TWIN SURVEY

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ABSTRACT

Bellamy N, Duffy DL, Sambrook P, Buchanan RRC, Brooks PM, Dunckley H, Healey SC, Mason S, Martin NG. A methodological appraisal of the impact of different classification procedures used in three different phases of the Australian rheumatoid arthritis twin survey. Inflammopharmacology, 1998;6:81–89.

Aims: To compare methodological aspects of the impact of different classification procedures used in three phases of a twin study examining genetic factors in the aetiopathogenesis of rheumatoid arthritis (RA).

Methods: We have previously reported the results of a study of the aetiopathogenesis of RA based on the Australian Twin Registry (ATR). In the original 258 pairs self-reporting a diagnosis of RA in twin, co-twin or both, a very high false positive self-reporting rate for RA was noted (Phase 1). Subsequent diagnostic information obtained by a disease-specific questionnaire, followed by telephone interviews with subjects and review of information obtained by mail and telephone interview from the patient's general practitioner or musculoskeletal specialist, identified 23 'true' RA pairs (Phase 2). Pairwise concordance percentages for RA based on those 20 discordant and 3 concordant pairs were as follows: monozygotic (MZ) 21% (95% confidence interval (CI) = 6–44%), dizygotic (DZ) 0% (95% CI = 0–25%) (probandwise concordance MZ 35% (8.9–67.3), DZ 0% (0–50.3)). Given the potential effects of misclassification on data interpretation, we have further pursued the accuracy of diagnosis by a systematic clinical, serological and radiographical evaluation of the 23 RA pairs (Phase 3).

Results: In only one instance did more intense diagnostic investigation of the 23 pairs result in recategorization. The probandwise concordance percentages were recalculated: MZ = 37.5%, DZ = 0%.

Conclusions: Our original contention that genetic factors play some part in the aetiopathogenesis of RA, but do not account entirely for its determination, has been substantiated at a higher level of confidence and at almost identical levels of concordance.

Keywords: twins, genetics, rheumatoid arthritis

INTRODUCTION

The part played by genetic factors in the aetiopathogenesis of rheumatoid arthritis (RA) has been the subject of previous studies [1–11]. In particular, there have been a number of twin studies, notably those evaluating one or more pairs of twins [1–8] and the population-based studies by the Arthritis and Rheumatoid Council [9], Aho et al. [10], Bellamy et al. [11] and Silman et al. [12]. The methods used to ascertain subjects, verify diagnosis and define zygosity differ in the latter four studies. We have used a large, population-based sample and applied strict criteria in defining the presence of RA. In this report we describe a methodological appraisal of the impact of different classification procedures used in the three phases of the study, the trade-offs between logistic demands and diagnostic accuracy and the effect on our conclusions regarding the genetic influence on the aetiopathogenesis of RA.

METHODS

Ascertainment of subjects

The study sample was derived from The Australian National Health and Medical Research Council Twin Registry. The methods employed to recruit and identify twins from this Registry are described in our previous publication [11]. In Phase 1 a questionnaire was sent to 5967 adult pairs between November 1980 and March 1982. Of the 3808 twin pairs (response rate = 64%) who returned completed questionnaires, 258 pairs self-reported a diagnosis of RA in twin, or co-twin, or both. Zygosity of the twins was ascertained by response to a number of questionnaire items supplemented in ambiguous cases by examination of photographs sent in by the twins. Of the 258 'RA' pairs, 72 were lost-to-follow-up between 1982 and 1990 (deceased, refused to continue as an active registrant or untraceable).

In Phase 2, a 19 component self-administered Clinical Profile Questionnaire (CPQ) was sent in 1990 to the remaining 186 eligible pairs. The American College of Rheumatology (ACR) Classification Criteria for RA [13] formed one component of the CPQ and were completed by the patient's general practitioner, or specialist, or both following the patient's written consent. Exact details of the survey strategy are described in our previous publication [11]. Based on ACR criteria, a diagnosis of RA was made in 26 individuals (20 discordant and 3 concordant pairs). A subsequent analysis suggested the following: a) there is a high false positive rate (89%) in self-reporting RA, b) the prevalence of RA (0.4%) in Australia may be less than the 0.8–1.0% often quoted, and c) genetic factors play some part in the aetiopathogenesis of RA, but do not account entirely for its determination [11].

Verification of classification procedures

In Phase 3, which has extended over the last seven years, we have attempted to

determine more exactly the clinical diagnosis, serological and radiographical profile, and zygosity of the 23 twin pairs. However, since 1990 two discordant pairs have declined to participate, and one affected individual of a discordant pair has died. The remaining 41 individuals (17.5 discordant and 3 concordant pairs) have been evaluated. In particular, each has been assessed using a standard questionnaire and undergone a musculoskeletal examination by a consultant rheumatologist. Blood samples have been obtained to determine rheumatoid factor (RF) ($n = 30$), anti-nuclear antibody (ANA) ($n = 29$), serum uric acid (SeUA) ($n = 29$), HLA-DR typing status ($n = 36$), and DNA zygosity determination ($n = 14$ pairs). Hand radiographs have been obtained on 22 individuals diagnosed as having RA and 3 unaffected co-twins (2 osteoarthritis, 1 arthralgia). HLA-DRB1 alleles were determined by a combination of methods including sequence specific oligonucleotide typing as described in the 11th International Histocompatability Workshop (Kimura and Sasazuki [14]): sequencing specific priming (Olerup and Zetterquist [15]): and PCR-RFLP using a modified method from Ota et al. [16]. In cases were the zygosity of the twin was questionable, DNA typing with simple tandem repeat (STR) polymorphism markers was performed. Twins were classified as MZ if they were concordant for at least 8 unlinked markers, while DZ twins were discordant for at least two unlinked markers.

RESULTS

The 26 RA subjects (23 women, 3 men) in the original 3 concordant and 20 discordant pairs had an average age of 43.5 years (range 25–71), mean age of onset of 39 years (range 16–70), and mean disease duration of 15 years (range 2–31). Of those original 46 subjects interviewed by telephone in 1990, subsequent clinical examination by a specialist rheumatologist, review of hand radiographs (RA subjects only) and serological profiles were obtained in forty-one. Of the 5 subjects lost-to-follow-up, 4 declined to participate and one had died. The loss of the latter subject effectively reduced the sample to 20 complete pairs, although the surviving co-twin was examined and had been correctly categorized as non-RA. He in fact showed evidence of osteoarthritis.

When the remaining 40 subjects (20 pairs) were examined by a specialist rheumatologist and categorized by ACR criteria, following review of hand radiographs and serological profiles it was observed that all but one individual had been correctly categorized in 1990. One subject previously classified as having RA from an MZ pair discordant for the disease showed signs only of osteoarthritis when examined by a specialist rheumatologist, but no evidence of rheumatoid arthritis. The final 1994 diagnosis established that the diagnostic accuracy of the 1990 telephone interview/survey process, based on 41 subjects subsequently examined in detail in 1994, was 98% (Table 1).

The clinical profile of the final 19 pairs ultimately verified is illustrated in Table 2. The 22 RA subjects (19 women, 3 men) in the 3 concordant and 16 discordant pairs (Table 3) had a mean disease duration of 19.2 years (range 5–35) and mean number of ACR criteria of 4.9 (range 4–7). Fifty percent of the 18 RA subjects tested were

TABLE 1
Agreement between 1990 survey diagnosis and 1994 diagnosis by rheumatologist*

	RA Present	RA absent
Categorized as RA in 1990	23	17
Categorized as RA in 1994	22**	18**

*Three pairs lost-to-follow-up (two pairs declined, one pair deceased) since 1990

**One RA subject in discordant pair re-categorized as OA

seropositive and 57% of all 23 RA subjects had evidence of erosive changes on hand radiographs. None of the non-RA co-twins fulfilled any ACR criteria for RA. Twelve non-RA co-twins tested for rheumatoid factor were seronegative. Three non-RA co-twins had hand radiographs taken, one being entirely normal, and the other two showing evidence of OA.

The musculoskeletal (MSK) diagnosis in the 16 non-RA co-twins was as follows: normal MSK = 12, OA = 3, fibromyalgia = 1. ANA positivity was recorded in 6/14 (i.e. 43%) of subjects and 3/12 (i.e. 25%) of non-RA subjects. The majority of RA and non-RA subjects were normouricaemic and gouty arthritis was not observed in any subject on physical examination by a specialist rheumatologist.

In all 14 pairs tested using STR polymorphism markers, zygosity assessed by DNA typing was in complete agreement with the original zygosity assessment based on a combination of questionnaire responses and an examination of photographs.

HLA-DR locus antigen frequencies for RA and non-RA subjects are illustrated in Table 4. Although HLA-DR4 was common in RA subjects as a group as well as in RA members of discordant pairs, its frequency was similar in non-RA co-twins. However in discordant pairs HLA-DR3 was more common in affected subjects (44%) than in non-affected co-twins (11%).

Based on the final 19 authenticated RA pairs (Table 3) the probandwise concordance percentages were 37.5% (95% CI = 9.6–70%) among MZ twins and 0% (95% CI = 0–62.9%) among DZ twins. The expected numbers of concordant pairs were calculated separately for MZ and DZ twin pairs. MZ twins were more concordant for RA than DZ twins (Cohen's kappa: MZ = 0.39 (CI = +0.05, +0.73) DZ = 0 (CI = 0.05, +0.04)).

DISCUSSION

Australia has a land mass of approximately that of the continental United States with a widely distributed population of 18 million. Although the majority of the 186 twin pairs, on whom our research is based, were clustered in or around major urban centres, many were in rural areas and some in excess of 1000 kilometres from the nearest

TABLE 2
Disease and serologic and final categorization profiles of 23 RA twin pairs

Concordant pairs twin identity (A or B)	Zygosity (by DNA)	Gender A	Gender B	MSK diagnosis A	MSK diagnosis B	RA disease duration A	RA disease duration B	Number of ACR criteria A	Number of ACR criteria B	RF status A	RF status B	Erosion A	Erosion B
1	(MZ)	F	F	RA	RA	NK	5	5	4	+	–	–	–
2	(MZ)	F	F	RA	RA	NK	30	4	4	+	+	+	+
3	(MZ)	F	F	RA	RA	7	22	4	5	–	–	+	–
Discordant pairs													
4	(MZ)	F	F	RA	OA	24	NA	5	0	+	ND	–	ND
5	(MZ)	M	M	RA	N	19	NA	6	0	–	–	–	–
6	(MZ)	F	F	RA	N	16	NA	4	0	–	–	–	ND
7	(MZ)	F	F	RA	N	23	NA	6	0	ND	ND	–	ND
8	(MZ)	F	F	RA	FM	17	NA	4	0	ND	ND	–	ND
9	(MZ)	F	F	RA	N	15	NA	4	0	–	–	+	ND
10	(DZ)	F	M	RA	OA	35	NA	6	0	–	–	+	–
11	(DZ)	M	M	RA	OA	20	NA	6	0	+	–	+	–
12	(DZ)	F	M	RA	N	7	NA	5	0	+	–	+	ND
13	(DZ)	F	F	RA	N	20	NA	5	0	–	–	–	ND
14	(DZ)	M	M	RA	N	24	NA	4	0	ND	ND	+	ND
15	(DZ)	F	F	RA	N	23	NA	5	0	ND	–	+	ND
16	(MZ)	F	F	RA	N	NK	NA	4	0	+	–	+	ND
17	(MZ)	F	F	RA	N	15	NA	5	0	–	–	+	ND
18	(MZ)	F	F	RA	N	27	NA	7	0	+	–	+	ND
19	(MZ)	F	F	RA	N	15	NA	5	0	+	–	+	ND
20	No evidence of RA on further investigation												
21	Declined to participate in further investigation												
22	Declined to participate in further investigation												
23	Twin classified as RA deceased prior to further investigations												

MSK, muskuloskeletal; N, normal MSK examination; ND, not done; NA, not applicable; NK, not known

TABLE 3
Monozygotic and dizygotic twin concordances for true rheumatoid arthritis verified by rheumatologist

Type of twin	Concordant (++)	Discordant (+ –)	Concordant (– –)
Monozygotic	3*	10	1785
Dizygotic	0	6	2000

*Numbers refer to pairs

TABLE 4
HLA-DR locus antigen frequencies in RA twin pairs

A. Monozygotic (MZ) twins (concordant, n = 3; discordant, n = 6)

Concordant	Antigen	Fraction	Discordant	Antigen	Fraction
	DR 3	1/3		DR 3	1/6
	DR 4	3/3		DR 4	4/6
	DR 13	1/3		DR 15	2/6
	DR 15	1/3		DR 9	1/6
				DR 11	1/6
				DR 13	1/6
				DR 14	1/6

B. Dizygotic (DZ) (twins, n = 9)

Antigen	RA (fraction)	Non-RA (fraction)
DR 1	1/9	2/9
DR 3	4/9	1/9
DR 4	8/9	8/9
DR 6	1/9	1/9
DR 7	2/9	2/9
DR 11	1/9	1/9
DR 15	–	1/9
DR 16	–	1/9

academic centre. This extremely wide geographic dispersal of study subjects and the necessity for accurate diagnostic categorization created significant methodological and logistical challenges. In essence, the more intense the pursuit of an accurate diagnosis, the higher the cost and the greater the logistical demands of the study. Our recent experience illustrates the trade-offs between study logistics and data accuracy.

Completion of a self-reported questionnaire as used in 1980/82 (Phase 1) is a method of obtaining accurate data at comparatively low cost. This approach is successful in obtaining factual data regarding age, gender, occupation, a variety of

health-related events, and can also be used to assess physical, social and emotional function, human behaviour and aspects of quality of life. However, this approach is less useful in diagnostic categorization since it lacks specificity. In our own experience, we noted an 89% false positive rate for the self-reported diagnosis of RA using the aforementioned method. An additional limitation was that we were unable to define the false negative rate, since twins failing to report a diagnosis of RA were not further assessed. This approach, therefore, is unsatisfactory in accurately identifying subjects with a specific diagnosis unless it is supplemented by other diagnostic strategies.

In 1990 (Phase 2) using a combination of several techniques we were able to more accurately define the presence/absence and nature of musculoskeletal disease in the 186 pairs. The methods employed are described in our previous publication [11] and were as follows:

1. Nineteen component self-administered clinical profile questionnaires (mailed out with subsequent postal and telephone pursuit of non-respondents);
2. Telephone interview by specialist rheumatologist of subjects who either
   a. reported RA
   b. had possible RA or
   c. were of uncertain diagnosis
3. Contact with the patient's general practitioner and/or specialist for clarification purposes and to complete the ACR criteria checklist.

The more intense pursuit, while more costly than that used in Phase 1, resulted in a reduction in the false positive rate from 89% to 2%. Apart from 10 subjects who had marked the 1980/82 questionnaire in error and never had arthritis, the remaining false positives had other forms of chronic musculoskeletal disease, or were currently disease-free. The problem would appear to be that while physicians use the term 'rheumatoid arthritis' to identify a well-defined clinical entity, some subjects may use it in a non-specific fashion for any type of arthritis or rheumatic complaint and may use this response option as the most convenient within which to categorize their complaint. It should not be assumed that physicians and patients share a common language and we would recommend one of the following solutions:

1. The term 'rheumatoid arthritis' be more precisely defined; or
2. A greater variety of alternative categories be provided; or
3. The subject be questioned as to whether this diagnosis was made by a general practitioner or specialist. The phraseology may also be important, since variations in wording may elicit different responses.

The final stage (Phase 3) in this study was to perform a definitive categorization based on the following:

1. Examination by specialist rheumatologist;
2. Determination of serological profile; and
3. Review of hand radiographs.

The logistical requirements of this part of the study were considerable. Clinical examinations required the collaboration of several rheumatologists in different centres. Others were completed by the study rheumatologist travelling into remote parts of rural Australia and performing domicillary assessments. Radiographs and/or the radiographic report of RA subjects were posted to the Queensland Institute of Medical Research for review. Blood samples were taken from all participating pairs and separated for determination of serological profiles, HLA typing and DNA zygosity. For rural areas, samples were appropriately prepared and sent by postal courier for next day delivery. This proved to be an effective method to obtain blood for testing. The costs of this part of the study were considerably higher (per capita) than for the other two phases. However, the benefits derived from these expenditures were twofold: (1) The false positive rate was reduced from 2% to 0%, and (2) We verified that the determination of zygosity based on questionnaire responses and photographs is indeed valid since there was complete agreement between the assigned zygosity and the zygosity determined by DNA analysis. This phase of the study was not very cost effective. The miscategorization of one individual possibly arose because he responded affirmatively to a question regarding rheumatoid nodules. On examination, these were discovered to be Heberden nodes, there being no evidence of RA. We would recommend close attention to the wording of future questionnaires, perhaps including reference to the location and consistency of any nodules. It should be noted that almost all RA subjects had been assessed previously by a specialist rheumatologist who provided relevant clinical, radiographical and serological data. This probably explains why there was little difference in diagnostic accuracy between the second and third phases of the study. While less cost effective than Phase 2, the definitive categorization achieved in Phase 3 did provide an essential confirmation of the conclusions reached in Phase 2, i.e. that genetic factors play some part in the aetiopathogenesis of RA but do not account entirely for its determination.

In conclusion we have observed that self-reporting a diagnosis of RA is unsatisfactory for classification purposes because of a high false positive rate. However, the use of interview/survey techniques to categorize subjects according to ACR criteria markedly reduced the false positive rate. The diagnostic accuracy of the latter approach versus a definitive approach based on specialist examination and additional radiographical and serological investigation is 98% vs. 100%. Previous experience with this type of RA research has been in countries with comparatively small land mass. In contrast, Australia has a land mass similar to that of the continental United States of America, and the logistic demands of conducting individual field interviews is considerable. Therefore, although based on a small sample, we conclude that the interview/survey method used in Phase 2 may be adequate and more cost effective than more intense direct clinical contact for this type of epidemiological work in large countries with widely separated centres of habitation.

ACKNOWLEDGEMENTS

We thank the twins for their cooperation and the enthusiastic collaboration of many rheumatologists and general practitioners throughout Australia who assisted in the successful completion of this project, and acknowledge grant support from the Arthritis Foundation of Australia, the H and L Hecht Visiting Professorship (University of Melbourne) and the University of Western Ontario Department of Medicine, London, Canada.

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Manuscript received 3 Nov. 97.
Accepted for publication 9 Feb. 98.