Published in final edited form as:
Arthritis Care Res (Hoboken). 2010 April ; 62(4): 545–550. doi:10.1002/acr.20042.

Low-Dose Aspirin in the Primary Prevention of Rheumatoid Arthritis: The Women's Health Study

Dr. Nancy A. Shadick, **Dr. Elizabeth W. Karlson**, **Dr. Nancy R. Cook**, **Dr. Nancy E. Maher**, **Dr. Julie E. Buring**, and **Dr. I-Min Lee**
Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Harvard Medical School, Boston, MA (Drs. Karlson and Shadick); Division of Preventive Medicine (Drs. Cook, Buring and Lee), Department of Neurology (Ms. Maher) and Division of Aging (Dr. Buring), Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; Department of Ambulatory Care and Prevention, Harvard Medical School, Boston, MA (Dr. Buring); Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115 (Drs. Cook, Buring and Lee)

Abstract

Background—Low-dose aspirin may reduce the risk of developing rheumatoid arthritis (RA) through its effect on cyclooxygenase activity and its anti-oxidant pathways. Previous randomized trial data have demonstrated a beneficial effect of low-dose aspirin in reducing other inflammatory diseases, such as asthma and colorectal adenomas, but no trial has evaluated the role of aspirin in RA prevention.

Methods—The Women's Health Study is a randomized, double-blind, placebo-controlled trial conducted between 1992 and 2004 designed to evaluate the risks and benefits of low-dose aspirin (100 mg every other day) and vitamin E in the primary prevention of cardiovascular disease and cancer among 39,876 female health professionals age 45 years and older throughout the US. In the present study, 39,144 women were evaluated after excluding women with RA at baseline. A definite diagnosis of RA was assessed during follow-up by self-report and confirmed using a connective tissue disease screening questionnaire (CSQ), followed by medical record review for ACR criteria by a rheumatologist.

Results—During an average follow-up of 10 years, 106 women developed definite RA, 48 women in the aspirin group and 58 in the placebo group. There was a non-significant relative risk (RR) for RA of 0.83 (95% confidence interval [CI] 0.56–1.21, p=0.33) associated with aspirin. There were 64 (60%) seropositive RA cases and 42 (40%) women with seronegative RA. Aspirin also had no significant effect on either seropositive (RR, 1.0; 95% CI, 0.61–1.63) or seronegative RA (RR, 0.62; 95% CI, 0.33–1.15).

Conclusion—100 mg of aspirin taken every other day is not associated with a significant reduction in the risk of developing RA among women in a randomized, double-blind, placebo-controlled trial.

Keywords

rheumatoid arthritis; randomized controlled trial; aspirin; antioxidants

Address for correspondence and reprints: Nancy A. Shadick, 75 Francis St., Boston, MA 02115, telephone (617)-732-5078, FAX (617) 731-9032, nshadick@partners.org.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects approximately 1% of the population [1]. Although the etiology of RA is unclear, abnormal levels of serological markers and asymptomatic synovitis can precede the disease [2–4] potentially allowing for preventive therapy by anti-inflammatory agents such as aspirin.

It is estimated that between 19 and 25% of the general population takes low-dose aspirin prophylactically for the prevention of heart disease [5]. Aspirin and other NSAIDS are used to treat inflammatory symptoms in RA. Aspirin exerts its effect on cyclooxygenase activity, which is linked to inflammation [6]. Aspirin also inhibits IL-4 and nfkB gene expression in non COX-dependent pathways [7]. Its role as an anti-oxidant and modulator of estrogen biosynthesis may also affect RA clinical onset [8]. Previous studies have shown that aspirin has beneficial effects on numerous diseases, including cardiovascular disease [9], stroke [10], and colon cancer [11], but no prior study has evaluated its potential to prevent RA. Evaluating whether a commonly used prophylactic medication such as aspirin can reduce the incidence of RA is an important public health question.

The Women's Health Study (WHS), a large scale randomized, double-blind, placebo-controlled trial of aspirin and vitamin E, provides a unique opportunity to evaluate whether low-dose aspirin decreases the risk of incident RA in apparently healthy women.

Methods

Study participants

Participants were drawn from the WHS, a randomized clinical trial of the efficacy of low-dose aspirin (100 mg every other day; Bayer Healthcare) and vitamin E (600 IU every other day; Natural Source Vitamin E Association) in the prevention of cardiovascular disease and cancer [12,13]. Details of this trial are provided elsewhere [10]. In brief, beginning in 1992, letters and baseline questionnaires were mailed to 1.7 million female health professionals, including physicians, nurses and dentists. A total of 453,787 women completed the initial questionnaire, 65,169 qualified and were interested in participating. Eligibility criteria included age greater than 45 years; no prior history of coronary heart disease, cerebrovascular disease, cancer (except non-melanoma skin cancer), or other major chronic illness; no reported adverse effects from the study medications; no use of anticoagulants or corticosteroids; and individual supplements of vitamin A, E, or beta carotene, aspirin or any nonsteroidal anti-inflammatory medications (NSAIDS) more than once a week (or willingness to forego their use during the trial). Eligible women were then enrolled into a 3-month run-in period with placebo medications. After the run-in period, 39,876 women were enrolled in the study, and were randomly assigned to low-dose aspirin or placebo and vitamin E or placebo, using a 2x2 factorial design(Figure 1). Written informed consent was obtained from all participants and the Brigham and Women's Hospital Institutional Review Board approved the trial, which was monitored by an external data safety monitoring board.

For the present study, 732 women who reported having RA on their baseline questionnaires, or during follow-up with a diagnosis date that preceded randomization were excluded, leaving 39,144 women.

Study Treatment and Follow-up

Women were followed up about their pill compliance by questionnaire each 6 months during the first year and yearly thereafter. They were also queried about potential side effects to aspirin, diagnoses of outcomes of interest including RA, and risk factors, such as cigarette smoking. At the end of the trial, (March 31, 2004) morbidity and mortality follow-up were

97.2% and 99.4% complete, respectively. Participants were considered compliant if they took at least two-thirds of the study pills (reported on follow-up questionnaires). Non-trial use of aspirin on ≥4 days/month averaged 12% during follow-up, with no significant difference between active and placebo groups.

Validating Self-Reports of RA

The primary end point of this study was definite RA. In the WHS, women self-reported RA on follow-up questionnaires. We then confirmed the diagnosis in a two-stage process. Women were mailed a connective tissue disease screening questionnaire (CSQ). [14,15] Women who screened positive for possible RA on the CSQ –at least 3 RA symptoms (radiographic changes of erosive disease or periarticular osteopenia, morning stiffness, arthritis of 3 or more joints, symmetric arthritis, and arthritis of hand joints or rheumatoid nodules or a positive rheumatoid factor) had their medical records reviewed. They were independently reviewed by two blinded board certified rheumatologists (EWK, NAS) according to the American College of Rheumatology (ACR) classification criteria [16], evidence of RA-specific treatment, and the treating physician's diagnostic impression. Any discrepancies were resolved in person by the two reviewers to determine diagnostic consensus. Definite RA was defined as those who met at least 4 ACR criteria for RA (95%), or had confirmed RA based on clinical symptoms, laboratory tests, medication treatment, and the expert reviewers' consensus (5%). We also noted seropositive and seronegative RA, defined as definite RA with or without a positive rheumatoid factor test documented in the medical record; and inflammatory polyarthritis, defined as ≥2 ACR criteria for rheumatoid arthritis on medical record review. Additionally, we also examined possible RA defined using the CSQ (≥3 RA symptoms on the CSQ), and a patient's self-report of RA (that was not later denied during the validation process).

Statistical Analyses

The SAS 9.1 package (SAS Institute Inc, Cary, NC) was used to perform all analyses. We used chi-square tests for proportions and Students t-tests for continuous variables to determine any demographic differences between the aspirin and placebo groups. Cox proportional hazards regression models were used to calculate relative risks (RR) and 95 % confidence intervals (CI) comparing the event rates of RA between the aspirin and placebo groups. All models were adjusted for age and randomized treatment assignment.

Results

Table 1 outlines the baseline characteristics of women from each of the groups. The mean age of women at study entry was 54.6 years. There were no significant demographic differences between the aspirin and placebo groups with regard to age, smoking status, BMI, parity, age at menarche and menopausal status.

Case validation

At the end of the trial, with an average of 9.99 years of follow-up, 1110 women reported having RA. These women were sent a CSQ, and asked to provide more details regarding their self-reported diagnosis of RA. Of the 803 (72%) women who responded to this questionnaire, 456 (41%) subsequently denied having a diagnosis of RA. Non-responders to the CSQ were similar to the responders with regards to smoking status and age and were as likely as the responders to have received aspirin or placebo (P= 0.35). A total of 177 (51%) of the remaining 347 responders who did not deny the diagnosis of RA screened positive for RA on the CSQ. Medical record review confirmed RA in 106 women. This calculated out to an annual incidence rate of 27.1 cases per 100,000 person-years. There were 64 (60%) seropositive RA cases and 42 (40%) seronegative RA cases.

Primary Endpoint

A confirmation of definite RA on medical record review was the primary endpoint of the study. Of the 106 subjects with the primary endpoint, 48 were randomized to the aspirin group and 58 to the placebo group. The average duration from the time of randomization to the diagnosis of RA was 5.8 (+2.4) years. There was a non-significant relative risk (RR) of 0.83 for RA (95% CI, 56–1.21, p=0.33) associated with aspirin.

Secondary Endpoints

We also examined the incidence of RA defined as either: seropositive RA, seronegative RA, inflammatory polyarthritis, RA defined using the CSQ, or self-reported RA. When we examined seropositive RA (RR, 1.0; 95% CI, 0.61–1.63), and seronegative RA (RR, 0.62; 95% CI, 0. 33–1.15) separately, aspirin had no effect on either endpoint. There was no significant risk reduction of RA by aspirin for any of the remaining three secondary endpoints: inflammatory arthritis (RR, 0.91; 95% CI, 0.65–1.28); RA defined as screening positive on the CSQ (RR, 1.16; 95% CI, 0.86–1.56); or self-reported RA (RR, 1.01; 95% CI, 0.89–1.13) (Table 2).

Discussion

In this large randomized, double-blind, placebo-controlled study of 100 mg of aspirin or placebo on alternate days we found no significant effect of low-dose aspirin on the incidence of definite RA in women. We also did not find an increased incidence of other types of RA, defined using several different case definitions, including seropositive and seronegative RA, inflammatory polyarthritis, or self reported RA.

We were interested in examining the role of aspirin in preventing the development of RA because several plausible biological mechanisms exist. Previous studies have demonstrated that low-dose aspirin is effective in preventing other diseases where prostaglandin pathways are involved, such as colorectal adenomas and cancers. In colorectal neoplasia, COX-2 is responsible for the production of prostaglandins that impact proliferation of tumor tissues [11]. Inhibition of COX-2 restores apoptosis and inhibits angiogenesis, which also may delay or ameliorate synovitis in early RA[17]. Similarly, prostaglandin E2 increases estrogen production in cultured cells [8] and the use of aspirin may inhibit prostaglandin-driven estrogen and progesterone production. This may explain aspirin's potential ability to reduce hormonally affected cancers such as breast cancer and prostate cancer as well [18,19]. Aspirin may influence RA incidence by modulating estrogen biosynthesis [20] as well as by its ability to restore apoptosis and inhibit angiogenesis [21].

It is also possible that aspirin may reduce the risk of RA via its role as an antioxidant. Salicylate inhibits cytokine-dependent induction of NOS-II gene expression, through nuclear factor-kB activation [22], which reduces the nitrosative stress of cytokine production. Aspirin also has antioxidant effect on proteins and scavenges hydroxyl radicals [6]. An antioxidant effect could prevent or delay RA onset, although a recent report from this trial demonstrated that vitamin E, a dietary antioxidant, did not significantly reduce risk of RA[23]

A recent analysis from the WHS trial demonstrated lower incidence of asthma among women randomized to aspirin, possibly explained by an anti-inflammatory effect of aspirin [24]. Aspirin inhibits IL4 gene expression in T cells, as well as promotes IL-4 and IL-13 induced activation of STAT6 via non-COX dependent pathways, which may also explain its role in reducing asthmatic symptoms[7]. In this report, administration of 100 mg of aspirin reduced the risk of newly reported asthma by 10%. This study had 872 new cases of asthma in the aspirin group, thus providing sufficient power to detect small reductions in risk.

We had limited power to detect moderate reductions in risk of RA, and in seropositive or seronegative disease subtypes because of the relatively small number of incident RA cases. Previous observational studies of low-dose aspirin supplement use in the prevention of cancer and asthma have reported between a 10% and 30% reduction in disease risk. In the present study, with 106 cases of definite RA, we would have only 40% power to detect a 30% reduction in risk but would have 86% power to detect a 50% reduction in risk. For the self reported and CSQ positive endpoints, however, we would have adequate power to detect moderate-sized risk reductions. In addition, while we ascertained RA cases based on self-reports, followed by CSQ screening and the medical record review, our use of strict ACR criteria when reviewing medical records may have resulted in labeling some RA cases as controls. However, analyses of other less stringent diagnostic groups demonstrated no associations as well. Furthermore, our cohort consisted of female health professionals who differ from the general population in some characteristics. For example, study subjects were less likely to smoke than the general population, and more likely to take post-menopausal hormone therapy, but showed similar proportions of elevated blood pressure and obesity. While the differences may affect the generalizability of our findings, they do not by themselves compromise the validity of the results. Finally, the annual incidence rate of definite RA in this study was 27.1 per 100,000 person-years, which is similar to one population based study[25] but lower than rates reported by other studies [1] [26]. Incidence rates in the present study may be lower because individuals who choose to participate in research studies tend to be healthier than the general population.

We were not able to evaluate every participant's medical record to confirm RA. Twenty eight percent of women who self-reported RA did not respond to requests for further information so how many of these participants may have had RA is unknown. However, we did analyze all self-reported RA as a secondary endpoint, where we also observed no association with aspirin. While there were a large number of women who initially reported a diagnosis of RA, but then denied this diagnosis upon further follow-up, this is comparable to two similar studies, the Iowa Women's Health Cohort Study [27] and the Nurse's Health Study where only 7% of original self reports were confirmed as RA[28]. An initial diagnosis of RA later refuted by a rheumatologist is a common experience in community health care settings.

We cannot comment on whether higher doses of aspirin than in the present trial might have a protective effect on the development of RA. Higher doses may mask symptoms of joint pain and delay the diagnosis of RA. Or, higher doses may exert more anti-oxidant, COX-2 inhibition and/or anti-inflammatory effect. Randomized trials using higher doses of aspirin can provide additional data. Observational studies of higher doses of aspirin are limited, in that there is potential for confounding by indication. Individuals with early RA might take NSAIDS more frequently to alleviate their symptoms, potentially masking an inverse association.

In conclusion, low-dose aspirin through anti inflammatory, anti apoptotic or antioxidant effects may potentially reduce the risk of developing RA. Despite these plausible biological mechanisms, as well as data from this trial and others of a reduction in inflammatory diseases such as colorectal neoplasia and asthma, the present study demonstrated no reduction in risk of RA with aspirin. It is possible that higher dose aspirin may prevent the diagnosis of RA or that studies with higher numbers of RA cases may demonstrate a significant result with the modest risk reduction observed in the present study. Given the frequency of aspirin use in the general population, this question deserves further study.

Acknowledgments

Supported by NIH grants HL43851, CA47988, AR02074, AR49880, AR0524

References

1. Linos A, et al. The epidemiology of rheumatoid arthritis in Rochester, Minnesota: a study of incidence, prevalence, and mortality. Am J Epidemiol 1980;111(1):87–98. [PubMed: 7352462]
2. Aho K, et al. Antifilaggrin antibodies within "normal" range predict rheumatoid arthritis in a linear fashion. J Rheumatol 2000;27(12):2743–6. [PubMed: 11128658]
3. Rantapaa-Dahlqvist S, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003;48(10):2741–9. [PubMed: 14558078]
4. Kraan MC, et al. Asymptomatic synovitis precedes clinically manifest arthritis. Arthritis Rheum 1998;41(8):1481–8. [PubMed: 9704648]
5. Prevalence of aspirin use to prevent heart disease--Wisconsin, 1991, and Michigan, 1994. MMWR Morb Mortal Wkly Rep 1997;46(22):498–502. [PubMed: 9194401]
6. Awtry EH, Loscalzo J. Aspirin. Circulation 2000;101(10):1206–18. [PubMed: 10715270]
7. Perez GM, et al. Aspirin and salicylates inhibit the IL-4- and IL-13-induced activation of STAT6. J Immunol 2002;168(3):1428–34. [PubMed: 11801685]
8. Zhao Y, et al. Estrogen biosynthesis proximal to a breast tumor is stimulated by PGE2 via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene. Endocrinology 1996;137(12):5739–42. [PubMed: 8940410]
9. Fuster V, Cohen M, Halperin J. Aspirin in the prevention of coronary disease. N Engl J Med 1989;321(3):183–5. [PubMed: 2747748]
10. Ridker PM, et al. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. N Engl J Med 2005;352(13):1293–304. [PubMed: 15753114]
11. Asano TK, McLeod RS. Nonsteroidal anti-inflammatory drugs and aspirin for the prevention of colorectal adenomas and cancer: a systematic review. Dis Colon Rectum 2004;47(5):665–73. [PubMed: 15054679]
12. Ridker PM, Cook NR, Buring JE. Use of aspirin as primary prevention of cardiovascular events. Jama 2006;296(4):391. author reply 391–2. [PubMed: 16868289]
13. Cook NR, et al. Low-dose aspirin in the primary prevention of cancer: the Women's Health Study: a randomized controlled trial. Jama 2005;294(1):47–55. [PubMed: 15998890]
14. Karlson EW, et al. A connective tissue disease screening questionnaire for population studies. Ann Epidemiol 1995;5(4):297–302. [PubMed: 8520712]
15. Karlson EW, et al. High sensitivity, specificity and predictive value of the Connective Tissue Disease Screening Questionnaire among urban African-American women. Lupus 2005;14(10): 832–6. [PubMed: 16304727]
16. Arnett FC, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31(3):315–24. [PubMed: 3358796]
17. Thun MJ. Beyond willow bark: aspirin in the prevention of chronic disease. Epidemiology 2000;11(4):371–4. [PubMed: 10874540]
18. Garcia Rodriguez LA, Gonzalez-Perez A. Risk of breast cancer among users of aspirin and other anti-inflammatory drugs. Br J Cancer 2004;91(3):525–9. [PubMed: 15226764]
19. Garcia Rodriguez LA, Gonzalez-Perez A. Inverse association between nonsteroidal anti-inflammatory drugs and prostate cancer. Cancer Epidemiol Biomarkers Prev 2004;13(4):649–53. [PubMed: 15066932]
20. Terry MB, et al. Association of frequency and duration of aspirin use and hormone receptor status with breast cancer risk. Jama 2004;291(20):2433–40. [PubMed: 15161893]
21. Thun MJ, Henley SJ, Patrono C. Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. J Natl Cancer Inst 2002;94(4):252–66. [PubMed: 11854387]

22. Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. Science 1994;265(5174):956–9. [PubMed: 8052854]

23. Karlson EW, et al. Vitamin E in the primary prevention of rheumatoid arthritis: the Women's Health Study. Arthritis Rheum 2008;59(11):1589–95. [PubMed: 18975365]

24. Kurth T, et al. Randomised aspirin assignment and risk of adult-onset asthma in the Women's Health Study. Thorax 2008;63(6):514–8. [PubMed: 18339679]

25. Dugowson CE, et al. Rheumatoid arthritis in women. Incidence rates in group health cooperative, Seattle, Washington, 1987–1989. Arthritis Rheum 1991;34(12):1502–1507. [PubMed: 1747134]

26. Gabriel SE, Crowson CS, O'Fallon WM. The epidemiology of rheumatoid arthritis in Rochester, Minnesota, 1955–1985. Arthritis Rheum 1999;42(3):415–20. [PubMed: 10088762]

27. Criswell LA, et al. Cigarette smoking and the risk of rheumatoid arthritis among postmenopausal women: results from the Iowa Women's Health Study. Am J Med 2002;112(6):465–71. [PubMed: 11959057]

28. Karlson EW, et al. Coffee consumption and risk of rheumatoid arthritis. Arthritis Rheum 2003;48(11):3055–60. [PubMed: 14613266]

Figure 1.

Arthritis Care Res (Hoboken). Author manuscript; available in PMC 2011 April 1.

Table 1

Baseline Characteristics of Women by Group, Women's Health Study

Characteristic	Aspirin N=19,562	Placebo N=19,582	Total N=39,144	P-Value
		No (%)*		
Age				
Mean ± SD (yr)	54.6(7)	54.5(7)	54.6 (7)	0.59
45–54 yr	10945 (56)	10998 (56)	21943 (56)	0.81
55–64 yr	6297 (32)	6244 (32)	12541 (32)	
≥65 yr	2320 (12)	2340 (12)	4660 (12)	
Smoking Status:				
Current	2512 (13)	2572 (13)	5084 (13)	0.61
Past	7048 (36)	6986 (36)	14034 (36)	
Never	9986(51)	10004(51)	19990 (51)	
Body-mass index†				
Mean ± SD	26.1 (5)	26.0 (5)	26.0 (5.1)	0.19
<25.0	9743 (51)	9806 (51)	19549 (51)	0.88
25–29.9	5920 (31)	5923 (31)	11843 (31)	
≥30	3487 (18)	3462 (18)	6949 (18)	
Age at menarche, y				
≤10	1675 (9)	1549 (8)	3224 (8)	0.18
11	3142 (16)	3187 (16)	6329 (16)	
12	5491(28)	5587 (29)	11078 (28)	
13	5619 (29)	5647 (29)	11266 (29)	
≥14	3613 (18)	3587 (18)	7200 (18)	
Parity				
Nulliparous	2463 (13)	2498 (13)	4961 (13)	0.61
Parous	17029 (87)	17002 (87)	34031 (87)	
Menopausal status and hormone therapy (HT)				
Premenopausal	5416 (28)	5450 (28)	10866 (28)	0.94

Characteristic	Aspirin N=19,562	Placebo N=19,582	Total N=39,144	P-Value
Uncertain	3455 (18)	3556 (18)	7011 (18)	
Postmenopausal– no HT	3214 (16)	3211 (16)	6425(16)	
Postmenopausal– past HT use	1422 (7)	1440 (7)	2862 (7)	
Postmenopausal– current HT use	6008(31)	5872 (30)	11880 (30)	

*Numbers do not always sum to group totals due to missing information for some variables. Figures in parentheses represent percentages, unless otherwise stated.

†Weight in kilograms divided by the square of height in meters

Table 2

Relative Risks of Rheumatoid Arthritis (RA) by Group, Women's Health Study

		No.of Events			
Outcome	**No.of cases**	**Aspirin**	**Placebo**	**Relative Risk** ** **(95% Confidence Interval)**	**P- value**
Definite RA*	106	48	58	0.83 (0.56,1.21)	0.33
Seropositive RA*	64	32	32	1.00 (0.61,1.63)	0.99
Seronegative RA	42	16	26	0.62 (0.33,1.15)	0.13
Inflammatory polyarthritis†	134	64	70	0.91 (0.65,1.28)	0.60
RA defined using CSQ ‡	177	95	82	1.16 (0.86,1.56)	0.33
All self-reported RA	1110	557	553	1.01 (0.89,1.13)	0.93
Unrefuted self-reported RA‡‡	654	332	322	1.03 (0.88,1.20)	0.71

*Confirmed on medical record review. Seropositive RA was defined as cases with a positive rheumatoid factor test documented in the medical record.

**Cox proportional hazards models adjusted for age at randomization and randomized treatment assignment

†Defined as ≥ 2 American College of Rheumatology criteria for rheumatoid arthritis[43] on medical record review.

‡Defined as ≥4 RA symptoms on the Connective Tissue Disease Screening Questionnaire (CSQ)[41]

‡‡This analysis considers subjects who reported RA on initial questionnaire, but who subsequently refuted the diagnosis of RA , as non-cases