

Practice of Epidemiology

Rheumatoid Arthritis in the Women's Health Initiative: Methods and Baseline Evaluation

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Second-generation assays for anti-cyclic citrullinated peptide (anti-CCP), a highly sensitive and specific marker for rheumatoid arthritis (RA), have redefined the epidemiology of RA. In the Women's Health Initiative (WHI) RA study (2009–2011), we evaluated the prevalence of anti-CCP positivity among 15,691 (10.2% of 161,808) WHI participants aged 50–79 years who reported RA. Using stored baseline specimens, we measured serum anti-CCP, rheumatoid factor (RF), and antinuclear antibody in a defined sample of 9,988 of black, white, and Hispanic women. In a subset of women, we measured plasma cytokine levels and number of copies of the human leukocyte antigen (HLA)-DRB1 (*HLA-DRB1*) shared epitope in DNA by means of Luminex polymerase chain reaction typing (Luminex Corporation, Austin, Texas). We validated classification of probable clinical RA in 2 clinics as anti-CCP positivity or self-reported validated use of disease-modifying antirheumatic drugs (DMARDs). The prevalence of anti-CCP positivity was 8.1%, and the prevalence of RF positivity was approximately 16.0%. DMARD use including prednisone was reported by 1,140 (11.4%) participants (841 excluding prednisone) but by 57.5% of anti-CCP-positive women. The prevalence of 2 shared epitopes was also much higher for anti-CCP-positive women (18.2%, as opposed to only 5.5% for women with anti-CCP-negative DMARD-positive RA and 6.6% for anti-CCP-negative, RF-negative DMARD nonusers). Median cytokine levels were much higher for anti-CCP-positive/RF-positive women. Women with anti-CCP-positive RA and anti-CCP-negative RA had different characteristics with regard to HLA shared epitope, cigarette smoking, and inflammation (cytokines).

cytokines; epidemiologic methods; genetics; rheumatoid arthritis

Abbreviations: anti-CCP, anti-cyclic citrullinated peptide; CI, confidence interval; DMARDs, disease-modifying antirheumatic drugs; HLA, human leukocyte antigen; RA, rheumatoid arthritis; RF, rheumatoid factor; WHI, Women's Health Initiative.

Rheumatoid arthritis (RA) is a common inflammatory joint disease that affects about 1% of the adult US population (1–3). RA is much more common in women, approximately 2-fold higher than in men. The specific etiology of RA is not known. The study of the epidemiology of RA has been limited because RA diagnosis previously required clinical evaluation of signs and symptoms, radiographic studies, and the presence of nonspecific markers, including rheumatoid factor (RF), along with an elevated erythrocyte sedimentation rate or C-reactive protein level. A major advance for the study of RA epidemiology came in the 1990s with the identification and development of second-generation assays for

autoantibodies designated anti-cyclic citrullinated peptide (anti-CCP) that are both sensitive (60%–70%) and very specific (90%–98%) for the diagnosis of RA (4–10).

Citrullinated peptides and proteins are produced through posttranslational modification of arginine and occur in many proteins related to both inflammation and cigarette smoking, but antibodies to citrullinated proteins are almost exclusively identified in persons with RA (9, 10). A very important question is whether anti-CCP-negative RA is a unique subcategory of RA or whether, with further advances in the sensitivity of citrullinated peptide antibody assays, more RA cases will be included within the anti-CCP-positive category.

Several alleles of the human leukocyte antigen (HLA)-DRB1 gene (*HLA-DRB1*) are associated with susceptibility to RA. These alleles encode specific amino acid sequences in the hypervariable region of the HLA-DRB1 molecule referred to as the “shared epitope” (11–13). RA patients who are anti-CCP-positive have a much higher prevalence of the shared epitope. Similarly, cigarette smoking is a risk factor for anti-CCP-positive RA. Higher levels of proinflammatory cytokines are also more prevalent among persons with anti-CCP-positive RA (14–16).

The Women’s Health Initiative’s (WHI) included 161,808 women between the ages of 50 and 79 years at baseline (1993–1998) (17), providing a valuable opportunity to evaluate the ability of the combination of serological tests, anti-CCP positivity, and use of disease-modifying antirheumatic drugs (DMARDs) to identify probable clinical RA cases among women from the community rather than from a clinical series. A chart-review validation study carried out in 2 of the WHI centers found a low specificity of self-reported RA, which was improved by coupling it with self-reported use of DMARDs and anti-CCP status (18). Based on this validation study and previous studies of anti-CCP in the literature, we classified women who reported RA and were anti-CCP-positive, or were anti-CCP-negative but reported DMARD use, as likely to have clinical RA. Probable clinical RA cases were then evaluated in relation to the *HLA-DRB1* shared epitope and cytokine levels within the WHI sample. The relationship of anti-CCP status, DMARD use, RF status, antinuclear antibody level, and other risk factors to total mortality over 9–10 years of follow-up has been reported separately (19).

METHODS

Participants and data collected in the WHI

Many previous papers have described the WHI. Briefly, between 1993 and 1998, 40 US clinical centers enrolled 161,808 women aged 50–79 years (mean age = 62.8 years) into either a randomized clinical trial ($n = 68,132$) or an observational study ($n = 93,676$) (17). At baseline, 76,192 (47.1%) WHI participants reported a history of arthritis and 7,872 (4.9%) specifically reported RA. At yearly follow-up visits, women were asked whether they had developed any new arthritis, RA, or osteoarthritis. A total of 10,426 women reported a diagnosis of RA during follow-up, of whom 1,829 had previously reported RA at baseline; in addition, 5,783 women had reported other arthritis, not RA, at baseline. Women were not required to re-report previously reported RA. The present study was limited to white, black, and Hispanic women in the WHI with available blood samples (18), leaving 15,188 women who reported RA at baseline or follow-up and were eligible for this study. Of these, 9,998 (66%) were sampled for the current study (Figure 1). A detailed description of phase 1 sampling has been published elsewhere (18).

Based on the anti-CCP results from phase 1 (2009–2010), a phase 2 (2010–2011) sample of 2,993 participants was selected (Figure 1) for measurement of cytokines and HLA-DR typing for shared epitopes. The phase 2 sample included 4 groups—group A: anti-CCP-positive women (100% sample

($n = 774$, excluding 28 with insufficient plasma or DNA samples)); group B: anti-CCP-negative women with DMARD use (100% sample ($n = 649$)); group C: anti-CCP-negative women with no DMARD use who reported RA at baseline (10% random sample plus all deaths in this subgroup ($n = 921$)); and group D: anti-CCP-negative women with no DMARD use who reported RA at follow-up only (10% random sample plus all deaths in this subgroup ($n = 649$)) (Figure 1).

DMARD use was defined as current use of hydroxychloroquine, sulfasalazine, minocycline, methotrexate, leflunomide, azathioprine, cyclosporine, gold, cyclophosphamide, antirheumatic biological agents, or oral steroids (19). DMARD use excluding prednisone was also addressed in the analyses. DMARD use was based on recall of current use by participants and further review by WHI staff of medication bottles brought to the clinic (a point prevalence). Assessment of medication use at baseline was repeated in the WHI observational study arm at year 3 of follow-up and in the clinical trial arm at years 1, 3, 6, and 9 of follow-up. Therefore, DMARD use was defined as reported current DMARD use at baseline or at 1, 3, 6, or 9 years of follow-up. Among women who reported RA at baseline, 634 used DMARDs (excluding prednisone) in the study; 461 (73%) reported DMARD use at baseline, 29 (4%) reported DMARD use for the first time at year 1, 114 (18%) first reported DMARD use at year 3, and 31 (5%) first reported use after year 3. Among women without a history of RA at baseline, 207 used DMARDs during this study, including 31 with first reported use of DMARDs at baseline, 12 with first use at year 1, 101 at year 3, and 63 after year 3. These data excluded the use of prednisone.

Serum biomarkers and *HLA-DRB1* typing

Using baseline serum samples stored at -70°F and not previously thawed, anti-CCP and RF assays were performed in the Rheumatology Clinical Research Laboratory at the University of Colorado, as previously described, with anti-CCP positivity defined as ≥ 5 U/L (19). *HLA-DRB1* typing was carried out in the laboratory of Dr. Massimo Trucco at the University of Pittsburgh (20). Additional details on these methods are presented in the Appendix.

Multiplex cytokine profiling

Multiplex cytokine profiling was performed in the Robinson Laboratory at Stanford University using baseline plasma samples stored at -70°F . The human 22-cytokine Beadlyte kit (Upstate Group Inc., Charlottesville, Virginia) and the Luminex xMAP 100IS platform (Luminex Corporation, Austin, Texas) were used according to the manufacturers’ protocols, except that we used 50% of the recommended serum and buffer volumes. The laboratory ran 100 samples in duplicate at 100% and 50% dilution, and there was little difference in the cytokine levels. All cytokine profiling analyses were performed using 3 $\mu\text{g}/\text{mL}$ HeteroBlock (Omega Biologicals Inc., Bozeman, Montana) in order to minimize nonspecific false elevations in cytokine readouts due to RF (21).

Many of the cytokine levels were very low, resulting in variability of laboratory measurements. In 40% of samples, interleukin-17 was not measurable (i.e., the level was 0).

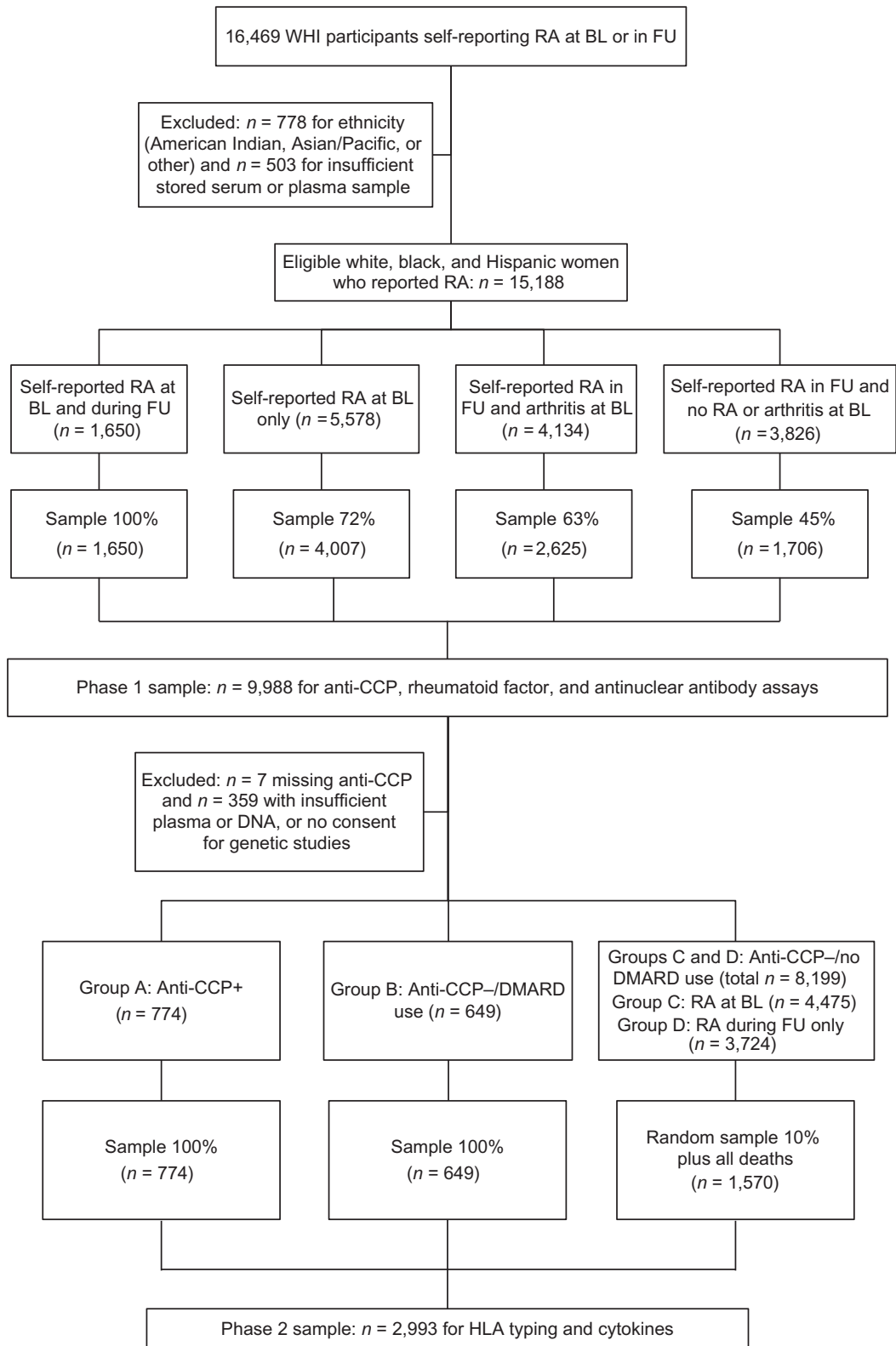


Figure 1. Sampling frame for the Women's Health Initiative (WHI) rheumatoid arthritis (RA) study, 2009–2011. The phase 2 sample of the WHI (2010–2011) included 4 groups—group A: anti-cyclic citrullinated peptide (anti-CCP)-positive women (100% sample ($n = 774$, excluding 28 with insufficient plasma or DNA samples)); group B: anti-CCP-negative women using disease-modifying antirheumatic drugs (DMARDs) (100% sample ($n = 649$)); group C: anti-CCP-negative women with no DMARD use who reported RA at baseline (BL) (10% random sample plus all deaths in this subgroup ($n = 921$)); and group D: anti-CCP-negative women with no DMARD use who reported RA at follow-up (FU) only (10% random sample plus all deaths in this subgroup ($n = 649$)). HLA, human leukocyte antigen.

Levels of interleukin-4 were very low, such that the 25th percentile was only 1 pg/mL. Given the skewed values for most cytokines, the median value and interquartile range (25th–75th percentiles) were better estimates than means. There were 153 blind duplicate samples for cytokine measurements. With high variability classified as >30% variation between blind samples, most cytokine measurements had at least 70% of blind duplicates with <30% variability; the exceptions were interleukin-12 (69%), interleukin-4 (63%), and interleukin-2 (65%).

Statistical analysis

As described above, the study sample consisted of 9,988 women who were sampled from 15,188 eligible WHI participants who reported RA at baseline or during follow-up (Figure 1). “RA at baseline” includes women who reported RA at both baseline and follow-up. Sampling fractions also varied by coronary heart disease status at baseline and by racial/ethnic group. To account for the stratified sampling and to correctly represent the entire population of black, white, and Hispanic women in the WHI who reported RA, we determined sampling weights, defined as 1/sampling fraction, for

each woman and incorporated them into the analyses. Analyses were performed with SAS, version 9.3 (SAS Institute, Inc., Cary, North Carolina). All *P* values were 2-sided at $\alpha = 0.05$.

RESULTS

Characteristics of WHI women reporting RA

The 9,988 participants in the current study included 5,712 (57.2%) from the observational study arm of WHI and 4,276 (42.8%) from the clinical trial arm—1,058 (10.6%) Hispanic women, 6,487 (65.0%) white women, and 2,443 (24.4%) black women. In terms of age, 1,167 participants were aged 50–54 years, 1,822 were aged 55–59 years, 4,488 were aged 60–69 years, and 2,511 were aged 70–79 years. A higher percentage of black women as compared with white women reported a history of RA at both baseline and follow-up. Women who reported RA at baseline were older, had less education, and had much higher prevalences of joint pain and poor health, lower levels of physical activity, and greater disability on the Short Form 36 health survey; all differences were significant ($P < 0.0001$) in comparison with no reported RA (not shown).

Table 1. Anti-CCP Positivity and Use of DMARDs at Baseline, by Race and Ethnicity, in a Subsample ($n = 9,988$) of Participants With Self-Reported Rheumatoid Arthritis ($n = 15,188^a$), Women’s Health Initiative Rheumatoid Arthritis Study, 2009–2011

Reported History of RA and Ethnicity	Anti-CCP Positivity			DMARD Use ^b at Baseline			DMARD Use ^b at Any Time		
	Total No.	No.	%	Total No.	No.	%	Total No.	No.	%
RA reported at baseline									
Black	1,160	149	12.8	1,159	77	6.6	1,160	106	9.1
Hispanic	367	41	11.2	367	21	5.7	367	30	8.2
White	4,127	422	10.2	4,130	363	8.8	4,130	498	12.1
Total	5,654 ^c	612	10.8	5,656 ^d	461	8.1	5,657	634	11.2
<i>P</i> value	0.039			0.013			0.003		
RA reported at follow-up only									
Black	1,282	86	6.7	1,283	11	0.9	1,283	51	4.0
Hispanic	691	25	3.6	691	2	0.3	691	18	2.6
White	2,354	89	3.8	2,357	18	0.8	2,357	138	5.9
Total	4,327 ^c	200	4.6	4,331	31	0.7	4,331	207	4.8
<i>P</i> value	0.0001			0.332			0.001		
Grand total									
Black	2,442	235	9.6	2,442	88	3.6	2,443	157	6.4
Hispanic	1,058	66	6.2	1,058	23	2.2	1,058	48	4.5
White	6,481	511	7.9	6,487	381	5.9	6,487	636	9.8
Total	9,981 ^c	812	8.1	9,987 ^d	492	4.9	9,988	841	8.4
<i>P</i> value	0.002			<0.0001			<0.0001		

Abbreviations: anti-CCP, anti-cyclic citrullinated peptide; DMARDs, disease-modifying antirheumatic drugs [excluding prednisone]; RA, rheumatoid arthritis.

^a Includes white, black, and Hispanic Women’s Health Initiative participants who reported RA at baseline or follow-up and had available blood samples.

^b Excluding prednisone.

^c Information on anti-CCP status at baseline was missing for 7 women.

^d Information on DMARD use at baseline was missing for 1 woman.

Table 2. Relationship of Rheumatoid Arthritis at Baseline or Follow-up to Anti-CCP Status,^a DMARD Use, and RF Status in a Subsample of Participants With Self-Reported Rheumatoid Arthritis (Weighted Analyses^b), Women's Health Initiative Rheumatoid Arthritis Study, 2009–2011

Anti-CCP Status	DMARD Use at Any Time	RF Status	History of RA at Baseline		RA at Follow-up Only		Total	
			Weighted <i>n</i>	%	Weighted <i>n</i>	%	No.	%
+	+	+	424	5.9	71	0.9	495	3.3
+	+	–	37	0.5	20	0.3	57	0.4
+	–	+	218	3.0	114	1.4	332	2.2
+	–	–	73	1.0	122	1.5	195	1.3
–	+	+	74	1.0	42	0.5	116	0.8
–	+	–	270	3.8	261	3.3	531	3.5
–	–	+	718	10.0	766	9.6	1,484	9.8
–	–	–	5,396	74.8	6,552	82.4	11,948	78.8
Total			7,210	100.0	7,948	100.0	15,158	100.0
All anti-CCP+			752	10.4	327	4.1	1,079	7.1
	All DMARD+		805	11.2	394	5.0	1,199	7.9
		All RF+	1,434	19.9	993	12.5	2,427	16.0

Abbreviations: anti-CCP, anti-cyclic citrullinated peptide; DMARDs, disease-modifying anti-rheumatic drugs [excluding prednisone]; RA, rheumatoid arthritis; RF, rheumatoid factor.

^a +, positive; –, negative.

^b Weighted numbers of participants are slightly lower than those in the original sample because of missing data on anti-CCP and RF status.

Anti-CCP levels were successfully measured in 9,981 women. Seven women were missing anti-CCP data, and 22 were missing RF data. One person was missing DMARD information at baseline only. Thus, the total numbers shown in the tables vary because of missing data. There were 812 women (8.1%, 95% confidence interval (CI): 7.6, 8.7) who were anti-CCP-positive (Table 1). The mean anti-CCP level for anti-CCP-positive women was 68 U/mL, and approximately 70% had levels over 30 U/mL (not shown). Among women who were anti-CCP-negative ($n = 9,169$), 94% had levels of <1 U/mL, 4% had levels of 1–2 U/mL, 0.9% had levels of 2–3 U/mL, 0.5% had levels of 3–4 U/mL, and 0.3% ($n = 15$) had levels of 4–5 U/mL. These titers did not vary by DMARD use at baseline or by RF status. The prevalence of anti-CCP positivity was higher for black women (9.6%) than for white (7.9%) or Hispanic (6.2%) women (Table 1). Anti-CCP positivity was much higher for participants who reported RA at baseline (612 of 5,654; 10.8%, 95% CI: 10.0, 11.6) than for those who reported RA during follow-up only (200 of 4,327; 4.6%, 95% CI: 4.0, 5.3). In weighted analyses, the prevalence of anti-CCP positivity was higher among women reporting RA at baseline in all age groups (<60 years: 11.7%, 95% CI: 10.4, 13.1 (262 of 2,236 women); 61–69 years: 10.3%, 95% CI: 9.2, 11.4 (313 of 3,032); and ≥ 70 years: 9.1%, 95% CI: 7.8, 10.4 (178 of 1,960)), for a total prevalence of 10.4% (753 of 7,228 women) (data not shown).

DMARD use

Among the 9,988 participants, 9,147 (91.6%, 95% CI: 91.0, 92.1) never reported DMARD use, 492 (4.9%, 95% CI: 4.5, 5.4) reported use of DMARDs at both baseline and follow-up,

and 349 (3.5%, 95% CI: 3.1, 3.9) reported DMARD use at follow-up only, excluding prednisone. With the addition of prednisone as a DMARD, the prevalence of DMARD use at any time would increase from 8.4% to 11.4%. A much higher proportion of anti-CCP-positive women—57.5% ($n = 467$)—were on DMARDs. Excluding prednisone, approximately 51% ($n = 417$) of anti-CCP-positive women were on DMARDs, of whom 275 (33.9%) reported DMARD use at baseline and 142 (17.5%) reported DMARD use at a follow-up visit only. Only 4.6% ($n = 423$) of women who were anti-CCP-negative were on DMARDs (excluding prednisone) at any time: 2.4% ($n = 217$) at baseline and 2.2% ($n = 206$) at follow-up only (not shown). Methotrexate was the most commonly used drug; 36.3% ($n = 295$) of the anti-CCP-positive women were on methotrexate, including 23.5% ($n = 191$) at baseline. Approximately 2.0% ($n = 185$) of anti-CCP-negative women were on methotrexate, including 78 at baseline.

In weighted analysis (Table 2), 1,079 women were anti-CCP-positive and 647 women were anti-CCP-negative and using DMARDs (excluding prednisone) at any time—a total of 1,726 probable RA cases, with a prevalence of 1.1% among 154,673 black, white, and Hispanic women in the WHI. Restricting the analysis to women who reported RA at baseline, 752 were anti-CCP-positive and 344 were anti-CCP-negative and using DMARDs—a total of 1,096 women with probable clinical RA, with a prevalence of 0.71%. The prevalence was consistent with the estimate in the literature of approximately 1%.

Rheumatoid factor

RF was measured successfully in 9,966 women (data were missing for 22 women). In weighted analyses, RF was

positive for 2,427 (16.0%) of the 15,158 participants in the weighted sample, including 19.9% (1,434 of 7,210) who reported RA at baseline and 12.5% (993 of 7,948) who reported RA at follow-up only. RF was positive for 76.6% (827 of 1,079) of anti-CCP-positive women and 89.7% (495 of 552) of women who were both anti-CCP-positive and using DMARDs. In contrast, only 11.4% (1,600 of 14,079) of anti-CCP-negative women were RF-positive, including 116 (17.9%) of 647 women who were anti-CCP-negative and using DMARDs at any time (Table 2).

HLA-DRB1 shared epitope

HLA-DRB1 typing was completed for 2,281 of the 2,993 participants in the phase 2 sample. In weighted analyses restricted to women who reported RA at baseline ($n = 6,737$), 3,736 women (55.4%, 95% CI: 54.3, 56.7) had no shared epitope, 2,598 (38.6%, 95% CI: 37.4, 39.7) had 1 shared epitope, and 403 (6.0%, 95% CI: 5.4, 6.5) had 2 shared epitopes (Table 3). The prevalence of the shared epitope was strongly associated with anti-CCP positivity but not with anti-CCP negativity/DMARD use; that is, the prevalences of 2 and 1 copies of the shared epitope were 18.2% and 50.6%, respectively, for anti-CCP-positive women and only 4.6% and 37.2% for anti-CCP-negative women. Similarly, among women in the anti-CCP-negative/no DMARD use/RF-negative group, only 4.8% had 2 shared epitopes and 36.5% had 1 shared epitope (Table 3).

Cytokines

Cytokine levels were measured in the phase 2 sample. Overall, median levels of most cytokines were significantly

higher for anti-CCP-positive women than for anti-CCP-negative women, whether they used DMARDs or not (data not shown). When results were stratified by RF status (Table 4), cytokine levels were higher for anti-CCP-positive women than for anti-CCP-negative women only for those who were also RF-positive, and were highest for women who were both anti-CCP-positive and RF-positive (Table 4). Current smoking did not explain these results; median cytokine levels were significantly higher for anti-CCP-positive/RF-positive women than for anti-CCP-negative/RF-positive or RF-negative women among both smokers and nonsmokers (not shown). There were no differences in median cytokine levels for smokers as compared with nonsmokers. For example, for never smokers versus smokers, the median interleukin-6 level was 6.2 pg/mL versus 6.1 pg/mL for anti-CCP-negative/RF-negative women and 14.9 pg/mL versus 16.9 pg/mL ($P = 0.24$) for anti-CCP-positive/RF-positive women. Results for other cytokine measures were similar to the results for interleukin-6. Additionally, we previously reported that cytokine levels were higher for anti-CCP-positive participants with 1 or 2 copies of the shared epitope than for those with none (22) (not shown).

Cigarette smoking

Women who were anti-CCP-positive with no DMARD use reported a higher prevalence of current cigarette smoking, about twice as high as the other groups. Women in the anti-CCP-positive/DMARD-use and anti-CCP-negative/DMARD-use groups had prevalences of cigarette smoking similar to those in the remaining WHI sample or the anti-CCP-negative/no-DMARD-use group (Table 5). There was no association between number of copies of the shared epitope and current or former cigarette smoking for either anti-CCP-positive

Table 3. Number of Shared HLA-DRB1 Epitopes, by Anti-CCP Status,^a DMARD Use at Baseline, and RF Status (Weighted), in a Subsample of Participants With Self-Reported Rheumatoid Arthritis at Baseline ($n = 6,737^b$), Women's Health Initiative Rheumatoid Arthritis Study, 2009–2011

Anti-CCP, DMARD, and RF Status	No. of Shared Epitopes						Total	
	0		1		2		No.	Row %
	No.	Row %	No.	Row %	No.	Row %		
Anti-CCP+								
DMARD+	83	26.8	155	50.2	71	23.0	309	100.0
DMARD–	131	34.8	191	50.8	54	14.4	376	100.0
Total	214	31.2	346	50.5	125	18.3	685	100.0
Anti-CCP–								
DMARD+/RF–	96	52.7	74	40.7	12	6.6	182	100.0
DMARD+/RF+	35	66.0	17	32.1	1	1.9	53	100.0
DMARD–/RF–	3,058	58.7	1,900	36.5	251	4.8	5,209	100.0
DMARD–/RF+	333	54.8	261	42.9	14	2.3	608	100.0
Total	3,522	58.2	2,252	37.2	278	4.6	6,052	100.0
Grand total	3,736	55.4	2,598	38.6	403	6.0	6,737	100.0

Abbreviations: anti-CCP, anti-cyclic citrullinated peptide; DMARDs, disease-modifying antirheumatic drugs [excluding prednisone]; HLA, human leukocyte antigen; RF, rheumatoid factor.

^a +, positive; –, negative.

^b Excludes women who reported rheumatoid arthritis at follow-up only.

Table 4. Median^a Cytokine Levels at Baseline, by Anti-CCP and RF Status,^b Among Participants in the Women's Health Initiative Rheumatoid Arthritis Study, 2009–2011

Cytokine	Anti-CCP and RF Status and Baseline Cytokine Level, pg/mL							
	Anti-CCP+ (n=770) ^c			Anti-CCP- (n=2,214) ^d			P Value (Anti-CCP+ vs. Anti-CCP-)	
	RF- (n=157)	RF+ (n=613)	P Value	RF- (n=1,912)	RF+ (n=302)	P Value	RF+	RF-
IL-1b	1.6	2.4	<0.0001	1.5	1.8	<0.0001	<0.0001	0.661
IL-2	3.3	12.6	<0.0001	3.2	5.3	<0.0001	<0.0001	0.587
IL-4	1.6	2.3	<0.0001	1.6	1.8	0.001	<0.0001	0.916
IL-5	3.0	4.1	<0.0001	3.0	3.3	<0.0001	<0.0001	0.641
IL-6	6.3	15.2	<0.0001	6.1	7.5	<0.0001	<0.0001	0.092
IL-7	6.9	8.5	<0.0001	6.9	7.6	<0.0001	0.001	0.908
IL-8	8.9	9.8	0.001	8.6	9.3	0.001	0.303	0.247
IL-10	2.5	3.2	<0.0001	2.4	2.7	<0.0001	<0.0001	0.292
IL-12	12.0	22.9	<0.0001	12.6	16.0	<0.0001	<0.0001	0.756
IL-13	2.4	4.3	<0.0001	2.4	3.0	<0.0001	<0.0001	0.796
Granulocyte-macrophage colony-stimulating factor	144.0	170.2	0.001	147.1	141.9	0.758	0.001	0.800
Monocyte chemotactic protein 1	15.3	20.9	<0.0001	14.5	16.2	<0.0001	<0.0001	0.265
Macrophage inflammatory protein 1 β	45.4	45.9	0.877	41.9	46.5	0.0003	0.530	0.043
Tumor necrosis factor α	21.7	37.7	<0.0001	21.6	25.1	<0.0001	<0.0001	0.605
Interferon γ	40.8	74.8	<0.0001	41.1	50.6	<0.0001	<0.0001	0.749
IL-17	0.8	4.2	<0.0001	0.4	2.3	<0.0001	0.007	0.820
White blood cell count ($\times 10^9$ cells/L)	5.5	6.2	<0.0001	5.8	5.7	0.088	<0.0001	0.041

Abbreviations: anti-CCP, anti-cyclic citrullinated peptide; DMARDs, disease-modifying antirheumatic drugs [excluding prednisone]; IL, interleukin; RF, rheumatoid factor.

^a Wilcoxon test of median values.

^b +, positive; -, negative.

^c Information on RF status was missing for 2 women; information on cytokine levels was missing for 2 women.

^d Information on RF status was missing for 2 women; information on cytokine levels was missing for 3 women.

women or anti-CCP-negative women. For example, among anti-CCP-positive women, 40% with 0 copies of the shared epitope were never smokers as compared with 37% with 1 copy of the shared epitope and 44% with 2 copies.

DISCUSSION

In the current study, we used anti-CCP serological testing to evaluate participant characteristics and classify women

Table 5. Cigarette Smoking Status, by Self-Reported Rheumatoid Arthritis, DMARD Use at Baseline, and Anti-CCP Status,^a Among Participants in the Women's Health Initiative Rheumatoid Arthritis Study, 2009–2011^b

Smoking Status	Anti-CCP+/DMARD Use (n=275)		Anti-CCP+/No DMARD Use (n=537)		Anti-CCP-/DMARD Use (n=217)		Anti-CCP-/No DMARD Use (n=8,951)		No Reported History of RA (n=133,741)		Age-Adjusted P Value
	No.	%	No.	%	No.	%	No.	%	No.	%	
Past smoker	138	50.2	245	45.6	104	48.0	3,557	39.7	56,458	42.2	<0.0001
Current smoker	20	7.3	75	14.0	15	6.9	751	8.4	9,095	6.8	<0.0001

Abbreviations: anti-CCP, anti-cyclic citrullinated peptide; DMARDs, disease-modifying antirheumatic drugs [excluding prednisone]; RA, rheumatoid arthritis.

^a +, positive; -, negative.

^b Totals reflect the fact that information on anti-CCP status was missing for 7 women and information on DMARD use was missing for 1 woman.

reporting RA in the WHI. This study documented that in the WHI, a reported history of RA included a very large percentage of women who probably did not have clinical RA. Second, we found that anti-CCP-positive RA and anti-CCP-negative RA with DMARD use have different epidemiologic and laboratory characteristics, including the shared epitope, cytokine levels, and cigarette smoking, consistent with the findings of other recent clinical studies. Future clinical, genetic, and epidemiologic studies of RA must separate anti-CCP-positive and -negative cases (14, 15, 21).

The prevalence of anti-CCP positivity in the community would be too low for this measure to be a useful screening test for possible RA. The addition of reported new joint symptomatology with anti-CCP measurements may be useful in identifying incident or evolving RA in epidemiologic studies (23).

RA patients are more likely to have 1 or 2 shared *HLA-DRB1* epitopes, but the majority of persons with 2 shared epitopes in the community do not have RA. Therefore, identification of shared epitopes is probably not a useful approach for identifying persons at risk of RA. Possibly the addition of other genetic markers (24) plus the shared epitope would improve the measurement of host susceptibility and aid in the identification of a higher-risk cohort for incident RA.

Inflammation is associated with citrullination of proteins, but antibodies to citrullinated proteins in blood are almost entirely restricted to persons with RA and, to a lesser extent, connective tissue diseases. The association of smoking and possibly other environmental determinants of lung disease with RA risk suggests that inflammation in the lungs may be a contributing cause in the etiology and natural history of RA, especially anti-CCP-positive RA in a genetically susceptible host (25, 26). The relationship between cigarette smoking and the *HLA-DRB1* shared epitope is inconsistent among RA studies (27). Cigarette smoking remains the strongest risk factor for RA, and smoking cessation could possibly prevent incident RA (28).

The results of the WHI should be interpreted carefully considering the limitations of our study. Current DMARD use, as noted, was measured at baseline and at years 1, 3, 6, and 9 for women in the clinical trial and at baseline and year 3 for women in the observational study, and we had no information on history of DMARD use prior to entry into the WHI. It is possible that some anti-CCP-negative women could have been treated with DMARDs for RA in the past and then have become asymptomatic and stopped all DMARD therapy, and yet had clinical RA. However, this should have been rare. In the validation study (19), the prevalence of clinical RA among women who reported RA but were not using DMARDs and were anti-CCP-negative was only 6%.

Another potential source of misclassification is that our assays were conducted only at baseline. It is possible that some anti-CCP-negative participants may have converted to anti-CCP positivity during follow-up, particularly among women who only reported RA at follow-up. However, the literature indicates that seropositivity often predates diagnosis by years (1). Furthermore, of women who reported RA during follow-up, only 4.8% ever reported DMARD use at any visit. It is possible that the development of new antibody tests for citrullinated proteins may result in some of the women who

are anti-CCP-negative converting to a positive antibody test (29). Additional studies are needed to clarify the nature of anti-CCP-negative RA and its clinical characteristics and course, in addition to its potential to convert to anti-CCP-positive RA. Finally, the variability of cytokine laboratory measurements limits interpretation of our results. However, the very large differences in cytokine levels by anti-CCP positivity and RF status are unlikely to have been due to laboratory variability.

In conclusion, there are substantial differences in the characteristics of persons with anti-CCP-positive RA as compared with anti-CCP-negative RA (defined by DMARD use)—for example, a much higher proportion with the shared epitope and higher cytokine levels. Clearly, just obtaining a history of RA is of little value. Ascertainment of RA cases through medical records may be biased by severity of disease, availability of rheumatology services, and other comorbid conditions that identify the patient in the clinical system.

A major question for epidemiologists is whether there are unique agent(s) in the causal pathway of autoimmune diseases such as RA (30, 31). Future epidemiologic studies could probe different populations that have unique lifestyles, environmental exposures, and genetic polymorphisms. Combining such studies with improved techniques for measuring immune function, including both innate and adaptive immune responses at the earliest stage of symptoms or initial conversion to anti-CCP positivity, may be possible in population epidemiologic studies.

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For a list of all of the investigators who have contributed to the science of the WHI, please visit <https://cleo.whi.org/researchers/SitePages/Write%20a%20Paper.aspx>.

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APPENDIX

Expanded Study Methods

Serum biomarkers

Using baseline serum samples stored at -70°F and not previously thawed, second-generation anti-cyclic citrullinated peptide (anti-CCP), rheumatoid factor (RF), and antinuclear antibody assays were carried out in the Rheumatology Clinical Research Laboratory at the University of Colorado. Briefly, anti-CCP (immunoglobulin G) antibodies were measured using commercially available anti-CCP enzyme-linked

immunosorbent assay kits (Diastat; Axis-Shield Diagnostics Ltd., Dundee, United Kingdom). Anti-CCP antibodies were measured in arbitrary units (AU) per mL and were considered positive at a cutoff value ≥ 5 AU/mL, which has been demonstrated to be more than 98% specific for rheumatoid arthritis (RA). RF was measured quantitatively by the reactivity of diluted test serum with heterologous immunoglobulin G in solution via nephelometry, which provided continuously variable quantitative results in international units (IU) (Dade Behring, Newark, Delaware). Per the 1987 American College of Rheumatology RA classification criteria, the positive cutoff value for these tests is set so that 5% of a population of 490 randomly selected healthy anonymous blood donors are positive. Quality control was routinely assessed by means of a procedure in which all autoantibody-positive serum samples (anti-CCP and/or RF) were retested in a blinded fashion, along with 5% of the negative sera. There was greater than 97.5% agreement in repeat testing (18, 32–34).

Human leukocyte antigen (HLA)-DRB1 typing

HLA-DRB1 typing was performed in the laboratory of Dr. Massimo Trucco at the University of Pittsburgh (20). Luminex microspheres (Luminex Corporation, Austin, Texas) were incorporated with infrared and red dyes. These dyes give each microsphere a unique spectral address, allowing for 100 different classifications of beads. Each classification of bead is then bound with a particular oligonucleotide probe. DNA samples are amplified by means of polymerase chain reaction (PCR) using primers that are biotinylated. PCR products are hybridized with a mixture of microspheres that have a predetermined set of oligonucleotide probes bound to them. The PCR product will find complementary sequences and bind with the probe (bead) they recognize. Individual sample reactions are then washed to remove any unbound PCR product. Samples are then stained with R-phycoerythrin-conjugated streptavidin that binds to the biotinylated primer. Samples are then processed through a Luminex flow analyzer. The fluorescent intensity of phycoerythrin on each classification of microsphere is translated into a positive or negative reaction. The assignment of HLA typing is based on the reaction pattern and analyzed using the current HLA sequence data.