ANTI-CYCLIC CITRULLINATED PEPTIDE, RHEUMATOID FACTOR, AND OCULAR SYMPTOMS TYPICAL OF RHEUMATOID ARTHRITIS

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ABSTRACT

Purpose: To correlate the presence of anti-cyclic citrullinated peptide (anti-CCP) antibodies and rheumatoid factor (RF) with ocular symptoms typical of rheumatoid arthritis (RA).

Methods: The records of 451 patients who had been examined by an ophthalmologist and tested for anti-CCP antibodies over a 3-year period at the Mayo Clinic were reviewed. Records of 255 patients with titers of anti-CCP and RF were analyzed for ocular surface and inflammatory disease associated with ocular RA.

Results: Of the 33 anti-CCP+/RF+ patients, all were diagnosed with RA; ocular surface disease was present in 11 (33%) and inflammatory disease in 7 (21%). Of the 17 anti-CCP-/RF+ patients, 4 were diagnosed with an unspecified inflammatory arthritis and 1 with rheumatoid arthritis; a separate 5 (29%) had ocular surface disease. Out of 5 anti-CCP+/RF- patients, 3 were diagnosed with RA but none had ocular symptoms. Out of 200 anti-CCP-/RF- patients, 32 (16%) had ocular surface disease and 2 (1%) had ocular inflammation. Of the 74 patients diagnosed with any form of inflammatory arthritis, anti-CCP+/RF+ patients had more and worse inflammatory ocular RA disease compared to the other groups.

Conclusions: Patients who were both anti-CCP and RF positive tended to have more and worse ocular disease. In patients diagnosed with an inflammatory arthritis, the presence of anti-CCP antibodies and RF provides useful information to ophthalmologists for identifying patients most at risk for inflammatory ocular disease.

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INTRODUCTION

Rheumatoid arthritis (RA) is a systemic inflammatory disease that primarily affects small joints but also commonly affects other organs, including the eye. Ocular involvement is variable and may arise independently of other forms of severe articular or extra-articular disease.[1] Ophthalmic manifestations of RA range from sicca syndromes to severe inflammation involving the sclera and/or cornea, and RA is the most common underlying condition in secondary Sjögren syndrome.[2] Early diagnosis of RA as the cause of ophthalmic symptoms allows for appropriate systemic treatment, which can prevent potentially sight-threatening complications.[3,4]

The diagnosis of RA depends on the combination of symptoms, signs, serologic tests, and radiologic findings as determined by the American College of Rheumatologists.[5] Rheumatoid factors (RFs), autoantibodies against the Fc region of IgG, are found at some time in 70% to 80% of RA patients and are associated with more aggressive joint disease and increased frequency of extra-articular manifestations than seen in RA in patients without RF.[6-10] However, RF may be detected in other connective tissue diseases, certain infections, and healthy persons.[11,12]

Antibodies to cyclic citrullinated peptide (CCP) can be detected by enzyme-linked immunosorbent assay (ELISA) and have been found to be a more specific serum test for RA than the RF titer. Citrulline is an atypical amino acid formed by the deimination of arginine in certain proteins, including fibrin, vimentin, and filaggrin. Citrulline has been implicated in the pathogenesis of RA, as certain isotypes of the enzyme that catalyzes the de-imination reaction, peptidylarginine deiminase, have been found to be up-regulated in the synovium of RA patients. The resulting citrulline residue theoretically fits better in the HLA DR4 antigen-binding grooves, which could lead to autoimmunity.[13]

While it can also be found at lower titers (<50) in patients with active tuberculosis and some mixed connective tissue diseases,[14-19] the presence of anti-CCP antibodies detected by the second-generation test is 95% specific for RA; the specificity of RF is only around 79%. The presence of anti-CCP antibodies has higher predictive value for development of RA in patients with undifferentiated arthritis. Although anti-CCP titers are predictive of radiographic progression and other indicators of disease severity in patients with early polyarthritis, even in patients who have negative IgM-RF titers, it has not been determined how well the presence of anti-CCP predicts ocular manifestations of RA.[20,21]

We investigated the correlation between ocular symptoms typical of RA and the presence of anti-CCP antibodies and RF to determine if their presence provides additional information to ophthalmologists when evaluating patients with inflammatory arthritis.

METHODS

Following approval by the Mayo Clinic Institutional Review Board, Mayo Clinic electronic medical record databases were searched for all patients who had both seen an ophthalmologist and had anti-CCP testing from June 1, 2004, through June 1, 2007. Patients were included only if they had a corresponding RF titer within 3 years of their anti-CCP test.

A second-generation ELISA assay is routinely used at the Mayo Clinic to detect anti-CCP antibody. After a 1:50 dilution, the anti-

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CCP antibody titer was determined to be positive if it was >5 arbitrary units. IgM RF was measured by nephelometry and was considered positive at >40 IU/mL.

The patients' medical records were reviewed for demographics, symptoms, examination findings, and diagnoses. Ocular diagnoses were separated into either those directly associated with tear disorders (ocular surface disease) or other ocular inflammatory diseases. Ocular surface disease included dry eye syndrome, meibomian gland dysfunction, and tear film deficiency; inflammatory manifestations included episcleritis, corneal thinning, keratitis, scleritis, and retinal vasculitis. Anterior or posterior uveitis without scleritis was not considered an ocular manifestation of rheumatoid disease. Ocular diagnoses were not considered related to RA if they could be explained by alternate underlying processes, such as infection, or if they were due to drug toxicities from hydroxychloroquine. Diagnoses of systemic inflammatory diseases, such as RA, were recorded according to the diagnosis of Mayo rheumatologists listed in the patient medical record and as reviewed by the study rheumatologist (E.L.M.).

The χ^2 test or the Fisher exact test was used to determine the significance of differences of presence of ocular symptoms between the different groups. The unpaired *t* test was used to compare mean values. A 2-sided probability (*P*) value of less than 5% was considered significant.

RESULTS

A total of 451 patients seen by ophthalmologists had had anti-CCP antibody testing in the past 3 years; of these, 255 had corresponding RF levels and were included in our analysis. Thirty-three patients (13%) were both anti-CCP positive and RF positive. Seventeen patients (7%) were RF positive but were anti-CCP negative. Five patients (2%) were anti-CCP positive and RF negative. The remaining 200 were anti-CCP and RF negative. The age and gender were not significantly different between any groups ($P = .14$ and $P = .59$, respectively).

ANTI-CCP AND RF PRESENCE VS OCULAR SYMPTOMS

Ocular symptoms by group are displayed in Tables 1 and 2.

TABLE 1. PRESENCE OF OCULAR SYMPTOMS IN RELATION TO ANTI-CCP ANTIBODY AND RF SEROLOGY*

GROUP	NO OCULAR SYMPTOMS	OCULAR SYMPTOMS			TOTAL
		SURFACE	INFLAMMATORY	ANY	
Anti-CCP+/RF+	18 (55%)	11 (33%)	7 (21%)	15 (45%)	33
Anti-CCP-/RF+	12 (70.6%)	5 (29%)	0	5 (29%)	17
Anti-CCP+/RF-	5 (100%)	0	0	0 (0%)	5
Anti-CCP-/RF-	168 (84%)	32 (16%)	2 (1%)	32 (16%)	200
Total (n)	203 (80%)	48 (19%)	9 (4%)	52 (20%)	255

Anti-CCP, anti-cyclic citrullinated peptide; RF, rheumatoid factor.
*Values are expressed in n (% within anti-CCP/RF group).

Anti-CCP-/RF-

Of the 200 patients identified to be anti-CCP-/RF-, 73 were diagnosed with an underlying systemic condition. Three patients were diagnosed with seronegative RA, and another 21 (11%) were diagnosed with inflammatory arthritis not otherwise specified (NOS). Also common was the diagnosis of polymyalgia rheumatica, which was made in 23 patients, 4 of whom had biopsy-proven giant cell arteritis. There were also 2 patients with primary Sjögren syndrome, 3 patients with systemic lupus erythematosus, 5 with psoriatic arthritis, 2 with juvenile inflammatory arthritis, 2 with scleroderma, 2 with inflammatory bowel disease, 3 with myasthenic syndromes, 2 with cicatricial pemphigoid, and 1 each with Blau syndrome, dermatomyositis, multiple sclerosis, and relapsing polychondritis.

In this population of patients who had been evaluated by an ophthalmologist and tested negative for RF and anti-CCP antibodies, 32 (16%) had dry eye syndrome. Of these 32, only 8 had systemic inflammatory disease. Ocular symptoms were not more common in patients with systemic diagnoses than in those without ($P = .84$). Two patients (1%) had inflammatory ocular conditions; both had episcleritis and also dry eye syndrome, but neither of these patients carried a systemic diagnosis.

Anti-CCP+/RF-

Five patients had detectable levels of anti-CCP antibodies but levels of RF within the normal range. Three were diagnosed with RA by rheumatologists: of these patients, 2 had anti-CCP titers >100 along with RF titers <15, and the third patient had an anti-CCP titer of 55.5 and RF titer of 26. Two patients did not have a diagnosis of RA or any other systemic condition and had anti-CCP titers of >100 and 18.1 and RF titers of 16 and <15, respectively. None of the patients were taking disease-modifying antirheumatic drugs

(DMARDs) when tested for anti-CCP antibodies and RF; however, DMARDs were started after testing in the 3 patients with RA. None of the 5 anti-CCP+/RF- patients had ocular RA disease.

TABLE 2. CLINICAL FEATURES OF PATIENTS WITH OCULAR SYMPTOMS

PATIENT	AGE (yr)	SEX	ANTI-CCP TITER (units/mL)	RF TITER (IU/mL)	SYSTEMIC DIAGNOSIS	OCULAR MANIFESTATION
Anti-CCP-/RF+						
1	67	F	<2.0	2630	Primary SS	Dry eye syndrome
2	80	M	<2.0	65	Primary SS	Dry eye syndrome
3	71	F	<2.0	75	Extrahepatic hepatitis C	Meibomian gland dysfunction
4	81	F	<2.0	90	PMR, GCA	Dry eye syndrome
5	50	M	<2.0	145	Monoclonal gammopathy	Dry eye syndrome
Anti-CCP+/RF+						
6	78	F	21.8	896	RA	Dry eye syndrome
7	64	F	>100.0	93	RA	Dry eye syndrome
8	70	F	29.2	53	RA	Episcleritis*
9	45	F	85.3	89	RA	Dry eye syndrome
10	52	F	>100.0	117	RA	Dry eye syndrome
11	83	F	>100.0	122	RA	Peripheral corneal thinning *
12	88	F	>100.0	281	RA	Dry eye syndrome Meibomian gland dysfunction
13	60	F	>100.0	363	RA	Dry eye syndrome
14	52	M	>100.0	417	RA	Dry eye syndrome
15	83	M	>100.0	428	RA	Dry eye syndrome
16	52	F	>100.0	300	RA	Ulcerative keratitis*
17	54	F	>100.0	67	RA	Necrotizing scleritis*
18	85	F	>100.0	114	RA	Scleral melt, corneal erosion*
19	67	M	>100.0	177	RA	Indolent keratitis*
20	76	F	>100.0	448	RA	Scleritis*
Anti-CCP-/RF-						
21	66	F	<2.0	<15	Fibromyalgia	Dry eye syndrome
22	76	F	<2.0	<15	GCA	Dry eye syndrome
23	53	M	<2.0	<15	Inflam arthritis NOS	Dry eye syndrome
24	31	F	<2.0	<15	Juvenile RA	Dry eye syndrome
25	80	M	<2.0	<15	PMR	Dry eye syndrome
26	80	F	<2.0	<15	PMR	Dry eye syndrome
27	70	F	<2.0	<15	PMR	Dry eye syndrome
28	91	F	<2.0	<15	PMR	Dry eye syndrome
29	84	F	<2.0	<15	Psoriatic arthritis	Dry eye syndrome
30	76	F	<2.0	<15	RA	Dry eye syndrome
31	39	F	<2.0	<15	Primary SS	Dry eye syndrome
32	76	F	<2.0	<15	Primary SS	Dry eye syndrome
33	73	F	<2.0	<15	None	Dry eye syndrome
34	86	F	<2.0	<15	None	Dry eye syndrome
35	84	M	<2.0	<15	None	Dry eye syndrome
36	88	F	<2.0	<15	None	Dry eye syndrome

TABLE 2. (CONTINUED) CLINICAL FEATURES OF PATIENTS WITH OCULAR SYMPTOMS

PATIENT	AGE (yr)	SEX	ANTI-CCP TITER (units/mL)	RF TITER (IU/mL)	SYSTEMIC DIAGNOSIS	OCULAR MANIFESTATION
37	42	F	<2.0	<15	None	Dry eye syndrome
38	59	F	<2.0	<15	None	Dry eye syndrome
39	79	F	<2.0	<15	None	Dry eye syndrome
40	83	F	<2.0	<15	None	Dry eye syndrome
41	42	F	<2.0	<15	None	*Episcleritis, dry eye syndrome
42	44	F	<2.0	<15	None	*Episcleritis, dry eye syndrome
43	34	F	<2.0	<15	None	Dry eye syndrome
44	59	F	<2.0	<15	None	Dry eye syndrome
45	62	F	<2.0	<15	None	Dry eye syndrome
46	56	M	<2.0	<15	None	Dry eye syndrome
47	64	F	<2.0	<15	None	Dry eye syndrome
48	84	F	<2.0	<15	None	Dry eye syndrome
49	79	F	<2.0	<15	None	Dry eye syndrome
50	67	F	<2.0	<15	None	Dry eye syndrome
51	75	F	<2.0	<15	None	Dry eye syndrome
52	80	F	<2.0	<15	None	Dry eye syndrome

Anti-CCP, anti-cyclic citrullinated peptide; GCA, giant cell arteritis; NOS, not otherwise specified; PMR, polymyalgia rheumatica; RA, rheumatoid arthritis; RF, rheumatoid factor; SS, Sjögren syndrome.

*Inflammation.

Anti-CCP-/RF+

Seventeen patients were identified who had elevated levels of RF but no antibodies to CCP. Of the 17, 1 (6%) was diagnosed with rheumatoid arthritis and 4 with an inflammatory arthritis NOS by a rheumatologist; however, none of these patients had any ocular symptoms of disease. Two patients were diagnosed with primary Sjögren syndrome, 3 with polymyalgia rheumatica, 1 with systemic lupus erythematosus, 1 with extrahepatic hepatitis C, and 1 with polyarticular gout. Seven patients had taken DMARDs at some time in the course of their disease.

Five of 17 anti-CCP-/RF+ patients (29%) had ocular surface disease; all 5 had dry eye syndrome. Two of these patients were diagnosed with primary Sjögren syndrome, 1 with extrahepatic hepatitis C, and 1 with polymyalgia rheumatica; one patient was not diagnosed with a systemic condition.

Anti-CCP+/RF+

There were 33 patients in this category; all were diagnosed with RA by rheumatologists. All of the patients had been taking DMARDs at some time.

Eleven (33%) had ocular surface disease, 7 (21%) had inflammatory disease, and 3 patients had both surface and inflammatory disease. There were higher proportions of ocular surface disease in this group; this was significant compared to the anti-CCP-/RF- group ($P = .03$) but not significant when compared to the anti-CCP+/RF- group ($P = .29$) and the anti-CCP-/RF+ group ($P = 1.00$). Inflammatory ocular disease was significantly more common in anti-CCP+/RF+ patients compared to anti-CCP-/RF- patients ($P < .001$) and compared to patients positive for just anti-CCP or RF ($P = .03$).

For one patient in this group, an ophthalmologist ordered the anti-CCP assay and RF test after diagnosing indolent marginal keratitis and noticing swelling of the proximal interphalangeal joint; both levels were elevated. Oral doxycycline therapy was prescribed, and the patient was referred to a rheumatologist, who diagnosed early rheumatoid arthritis and scheduled yearly follow-up. The patient's ocular symptoms had improved at examination 37 days later.

PATIENTS DIAGNOSED WITH INFLAMMATORY ARTHRITIS

In this series, 72 patients had been diagnosed with some form of inflammatory arthritis (Table 3). Forty of the 72 patients (56%) were diagnosed with rheumatoid arthritis; of these, 33 were categorized as anti-CCP+/RF+, 3 as anti-CCP+/RF-, 1 as anti-CCP-/RF+, and 3 as anti-CCP-/RF-. There were 25 cases of unspecified inflammatory arthritis, of which 21 were anti-CCP-/RF- and 4 were anti-CCP-

/RF+. There were also 5 cases of psoriatic arthritis and 2 cases of juvenile inflammatory arthritis in patients who were categorized as anti-CCP-/RF-.

TABLE 3. PRESENCE OF OCULAR SYMPTOMS IN PATIENTS WITH ANY INFLAMMATORY ARTHRITIS IN RELATION TO ANTI-CCP ANTIBODY AND RF SEROLOGY*

GROUP	PATIENTS WITH INFLAMMATORY ARTHRITIS n (% OF GROUP)	NO OCULAR SYMPTOMS	OCULAR SYMPTOMS		
			SURFACE	INFLAMMATORY	ANY
Anti-CCP+/RF+ (n=33)	33 (100%)	18 (55%)	11 (33%)	7 (21%)	15 (45%)
Anti-CCP-/RF+ (n=17)	5 (29%)	5 (100%)	0	0	0 (0%)
Anti-CCP+/RF- (n=5)	3 (60%)	3 (100%)	0	0	0 (0%)
Anti-CCP-/RF- (n=200)	31 (16%)	27 (87%)	4 (13%)	0	4 (13%)
Total (n=255)	72 (28%)	53 (74%)	15 (21%)	7 (10%)	19 (26%)

Anti-CCP, anti-cyclic citrullinated peptide antibodies; RF, rheumatoid factor.

*Values are n (% within anti-CCP/RF group with inflammatory arthritis) unless indicated.

Of patients with any form of inflammatory arthritis, 7 (9.7%) had inflammatory ocular disease and all of these patients were anti-CCP+/RF+. Fifteen of 72 (21%) had ocular surface disease, 11 of whom were anti-CCP+/RF+ and 4 of whom were anti-CCP-/RF-. Patients with inflammatory arthritis who were anti-CCP+/RF+, when compared to anti-CCP-/RF- patients with inflammatory arthritis, were more likely to have ocular inflammatory disease and surface disease (P = .01 and P = .08, respectively). When compared to inflammatory arthritis patients who were positive for either anti-CCP or RF, those who were positive for both anti-CCP antibodies and RF were insignificantly more likely to have ocular surface disease (P = .08) and inflammatory ocular disease (P = .31). With the available data comparing the anti-CCP+/RF+ patients with anti-CCP or RF positive patients, there was a 47% power to detect the observed difference for ocular surface disease outcome, and a 44% power to detect the observed difference for inflammatory disease outcome.

DISCUSSION

Ocular manifestations of RA and other systemic inflammatory diseases can cause serious ocular morbidity. Here, out of 255 patients presenting to our eye clinic who were tested for anti-CCP antibodies and RF over a 3-year period, we correlate the presence of the anti-CCP antibody and RF with ocular surface and inflammatory symptoms typical of RA. Patients who were both anti-CCP and RF positive tended to have more surface and worse inflammatory eye disease than patients who were negative for both or positive for just one. Furthermore, in the subset of patients diagnosed with inflammatory arthritis, those who were anti-CCP+/RF+ were more likely to have surface and inflammatory disease.

The presence of anti-CCP antibodies is helpful as a more specific test for RA compared to the RF. As our subset analysis of 72 patients with inflammatory arthritis demonstrates, those with anti-CCP+/RF+ RA were more likely to have ocular disease along with their inflammatory arthritis. Of the 38 patients who were positive for anti-CCP antibodies, 36 (95%) were diagnosed with RA; 11 of these patients had ocular surface disease, and 7 had inflammatory ocular disease. Of the 17 patients who were anti-CCP-/RF+, only 1 was diagnosed with RA, and another 4 were diagnosed with an unspecified inflammatory arthritis; none of these 5 patients had ocular involvement associated with the underlying inflammatory disease. Of the 200 patients in the anti-CCP-/RF- group, only 3 were diagnosed with RA, and another 28 were diagnosed with an unspecified inflammatory arthritis. Only 4 of these patients with any inflammatory disease had ocular symptoms, and all were minor cases of dry eye syndrome. Thus, it seems useful for ophthalmologists to differentiate between inflammatory arthritis and anti-CCP+/RF+ RA, since patients in the latter group tended to have more and worse ocular disease.

Just as anti-CCP has been shown to be a strong predictor of radiographic progression of RA, our results seem to suggest that the presence of anti-CCP antibodies is associated with worse ocular disease.[21] Two patients (1%) in the anti-CCP-/RF- group did have ocular inflammation, but both had cases of episcleritis that were not associated with any elevated systemic inflammatory markers or

systemic diagnoses; in both cases, the episcleritis resolved without medication. There were no cases of ocular inflammation in the anti-CCP-/RF+ group. In contrast, there were 7 cases of inflammation (21%) in the anti-CCP+/RF+ group, and only 1 was a minor case of episcleritis. Three were cases of scleritis, including a case of scleral melt with corneal erosion, and 3 were cases of corneal inflammation and thinning. Although there were no anti-CCP-/RF+ patients in our study with scleritis or keratitis typical of RA, it is important to realize that these symptoms can be seen in systemic diseases, such as systemic lupus erythematosus, and chronic infections that can be associated with elevated or normal RF levels and no anti-CCP antibodies.

Dry eye syndrome was common in our population of patients who had seen an ophthalmologist and were tested for anti-CCP antibodies and RF. While it was significantly more common in anti-CCP+/RF+ patients compared to anti-CCP-/RF- patients (33% vs 16%, respectively; $P = .03$), ocular surface disease was not significantly more prevalent in the anti-CCP+/RF+ patients compared to the anti-CCP-/RF+ patients (33% vs 29%, respectively; $P = 1.00$). Thus, RF seemed to be a more sensitive test for identifying an underlying systemic disease in patients with dry eye syndrome; this is reasonable when considering that RF is present in various rheumatic disorders separate from RA—many of which are usually associated with an elevated RF.[22,23] Two patients with anti-CCP-/RF+ serology and ocular disease were diagnosed with primary Sjögren syndrome, which is typically associated with an elevated RF and negative or low titers of anti-CCP. There were also 2 patients with anti-CCP-/RF- serology who were diagnosed with dry eye syndrome and Sjögren syndrome, which demonstrates that even RF may be absent in Sjögren syndrome and other systemic diagnoses related to dry eye syndrome.

There were only 5 patients with anti-CCP antibodies but negative RF, and none had ocular disease. Three patients were diagnosed with RA and were probably tested early in their disease, as none were taking DMARDs and all tended to have only minor arthritis symptoms. A number of studies have demonstrated that the presence of anti-CCP antibodies is more sensitive than IgM RF early, before symptoms of RA develop.[24-26] Since ocular involvement is generally associated with severe, seropositive RA, it is reasonable that these symptoms would not be common in anti-CCP+/RF- patients. However, no firm conclusions can be made, since only 5 patients were included in this group.

The presence of anti-CCP antibodies is useful when the diagnosis of RA is still in question, especially early in the disease course. Indeed, this was demonstrated in the patient with indolent marginal keratitis; after discovering an elevated anti-CCP, the ophthalmologist was able to refer the patient to a rheumatologist, who evaluated the patient for systemic disease and diagnosed early RA.

The prevalence of ocular RA disease in our study is comparable to what is generally found in other studies. Dry eye was the most common symptom and was found in 26% of patients diagnosed specifically with RA, which is slightly higher that the reported prevalence of 15% to 25%.[2,27] In our retrospective analysis, we were not able to quantify each patient's dry eye as could be done with the Schirmer's test. Using that test, Punjabi and associates[28] reported that roughly 27% of RA patients had dry eye in an Indian population. Our prevalence in RA patients of scleritis (3 of 40, 7.5%) and episcleritis (1 of 40, 2.5%) is near the reported 4% to 10%.[2] It is slightly higher than the reported prevalences (0.67% and 0.17%, respectively) by McGavin and associates,[29] though their 1976 study did not require serologic diagnosis in the defined RA population.

In summary, our study correlating anti-CCP and RF titers with ocular symptoms of rheumatoid diseases suggests that patients who are anti-CCP+/RF+ tend to have more and worse ocular involvement compared to anti-CCP-/RF+, anti-CCP+/RF-, and anti-CCP-/RF- patients. In patients diagnosed with inflammatory arthritis, inflammatory ocular symptoms were present only in patients who were both anti-CCP and RF positive. As previous studies demonstrate, the anti-CCP test is very specific for RA and can be found before typical RA symptoms; thus, the test can be useful for ophthalmologists to order when attempting to confirm RA in patients with typical ocular symptoms of RA. We did find that the RF seemed to be a more sensitive test for ocular surface disease, since it was elevated in non-RA diseases, which can still involve the eye. Our study is limited by lack of power to achieve significance of the differences observed between inflammatory arthritis patient groups; this could be expected with a retrospective study of a relatively new test and binary outcomes. Prospective and case control studies are required to establish whether the presence of anti-CCP predicts more and worse ocular disease, especially in patients with RA.

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PEER DISCUSSION

DR. GARY N. HOLLAND: Laboratory findings can serve a variety of useful functions: as support for a clinical diagnosis; to predict disease outcomes; and as indicators of disease mechanisms. Rheumatologists consider the finding of antibodies against cyclic citrullinated peptide (CCP) to be an important marker of rheumatoid arthritis (RA). In their detailed and carefully prepared manuscript, Mr. Itty and his associates have nicely explained the nature of these antibodies, and have begun to investigate the utility of anti-CCP antibodies in the evaluation of the ophthalmic disorders associated with RA and other connective tissue diseases. The investigators have established a useful database, and have clearly shown a relationship between anti-CCP antibodies and a heterogeneous group of eye problems. The presence of both RF and anti-CCP antibodies appears to be a marker for more severe ocular involvement among patients with RA.

What they have not yet shown is (1) the extent to which the presence of anti-CCP antibodies provides unique information, independent of rheumatoid factor (RF), or (2) whether their presence will predict the development of ophthalmic disorders in patients with connective tissue disease. Information provided by the authors was sufficiently detailed that I was able to investigate the first of these issues, by analyzing their data in a slightly different manner. With the help of Fei Yu, Ph.D. (Senior Statistician and Assistant Scientist, Department of Biostatistics, UCLA School of Public Health), I calculated odds ratios (OR) for the relationships between test results and the presence of ocular involvement among those patients who were tested. For those with RF (regardless of whether or not anti-CCP antibodies were present), 20 (40%) of 50 patients (vs. 32 [16%] of 205 without RF) had eye problems (OR 3.60, 95% confidence interval [CI] 1.83-7.11). For those with anti-CCP antibodies (regardless of whether or not RF was present), 15 (39%) of 38 patients (vs. 37 [17%] of 217 without anti-CCP antibodies) had eye problems (OR 3.17, 95% CI 1.51-6.65). Although the broad CIs preclude firm conclusions, neither test seems to be more strongly related to eye disease than the other. When both tests were positive, however, 15 (45%) of 33 patients (vs. 37 [17%] of 222 patients with only one or no positive tests) had eye problems (OR 4.17, CI 1.93-9.01). Again, the broad CI precludes firm conclusions, but the larger OR suggests that the combination of both tests may be more strongly associated with eye disease than either test alone. Including a test for anti-CCP antibodies in the evaluation of patients with eye disease might be more useful than testing for RF alone, when considering a possible association with connective tissue disease.

The nature of these relationships should be explored further. Even a strong association does not necessarily tell about the predictive value of a test. It is my understanding that laboratory testing and ophthalmic examinations in this study could have been performed at any point during the patients' follow-up care at the Mayo Clinic; thus, the temporal relationships between test results and development of eye disease, if any, is unknown, and the results say little about the predictive value of either test. A longitudinal assessment of the current results could address this issue. Identification of risk factors for disease is critical if one is to manage disease appropriately, especially when dealing with the toxic drugs necessary to treat many patients with RA and similar disorders.

The authors appropriately discuss other limitations that are inherent in most retrospective studies. As they state, tests may have been ordered early in the course of disease; without serial examinations, it is possible that some patients without ocular involvement may eventually have developed an eye problem. There is also the potential problem of unrecognized bias. For example, it is possible that patients with systemic or ocular disease were more likely to be tested. (Or conversely, it is possible that such patients were less likely to be tested, because the diagnosis was not in doubt.)

Despite these limitations, the authors' results are sufficiently intriguing that additional studies should be undertaken. They may wish to calculate a Kappa statistic for the agreement between RF and anti-CCP antibody test results, as a further refinement of their data. More important, they may wish to look at their data in a longitudinal manner to see whether either test (or the combination of both) predicts the development of eye disease, as discussed above. Because confounding factors may have influenced observed relationships, the results should be confirmed with further investigations.

With additional study, the authors should look more critically at the specific ocular disorders with which test results are associated. In the current analysis, they sought relationships with any ocular involvement, including retinal vasculitis, keratitis, scleritis, dry eye, and blepharitis/meibomitis. Generally, I do not think of blepharitis/meibomitis as a specific manifestation of connective tissue disease. Because blepharitis/meibomitis is so common in the general population, and because disorders such as necrotizing scleritis and ulcerative keratitis can be devastating complications of RA and other connective tissue diseases, they may wish to concentrate on the relationships with these latter eye problems. And eventually, a prospective study of anti-CCP antibodies among patients with connective tissue disease, with close attention to development of ocular involvement, would be warranted.

In summary, the results of the current study suggest the possibility that identification of both RF and anti-CCP antibodies may be clinically useful for the evaluation of patients at risk for eye disease, and it points the way toward additional investigations. I commend Mr. Itty and his colleagues on an interesting manuscript, and thank them for sharing it with me many weeks before its scheduled presentation.

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DR.ALLAN J. FLACH: No conflict of interest. That was amazing presentation for anyone, much less a third year medical student. I only have two questions. First, how did you define "dry eye"? Secondly, let us say this factor was capable of detecting rheumatoid arthritis, extremely early in the course of the disease, perhaps even before any symptoms. Does that really help our patients? I do not want my question to discourage research because I believe understanding mechanisms of disease is important.

SUJIT ITTY: I would like to thank Dr. Holland for his elegant discussion and for his accurate remarks expressing what our study did show and what it did not. I would also like to thank him for sending us his initial thoughts on the manuscript in a timely way so that we could address some aspects of his analysis into the manuscript and presentation. Dr. Holland is absolutely correct; our initial study on the use of the CCP test for ophthalmologists answered some questions, but also left much to be discovered. As he accurately pointed out, our study demonstrated that there is certainly a role to test for both RF and anti-CCP antibodies for ophthalmologists, rather than looking for the better of the two. Since RF and CCP have such a high rate of concordance (the Kappa statistic for our data was calculated to be 0.7, which indicates a "substantial agreement"), the calculated odds ratios for each test would certainly be similar, making it difficult to make conclusions about RF versus CCP. By performing a subgroup analysis, we were able to characterize the patients with discordant results, allowing us to demonstrate the value added by each test for characterizing patients.

Dr. Holland was also absolutely correct that longitudinal studies would be necessary to draw conclusions about the predictive ability of the CCP test, and a prospective study would be of great benefit. I would like to thank Dr. Flach for his questions as well. To answer the first question, we took an inclusive approach in defining dry eye. We looked for any indication of dry eye, particularly looking in charts for dry eye syndrome, Meibomian gland dysfunction or tear film deficiency. As a retrospective review, it would not be possible to expect Schirmer or other diagnostic tests to be performed for every patient suspected of having dry eyes; thus, we were forced to look for any indication of it in patients' charts. Regarding the use of identifying RA early, Dr. Flach would be correct that this would be of little benefit for patients if treatment still hinged on waiting for symptoms to develop. However, more and more studies are demonstrating that using disease modifying anti-rheumatic drugs (DMARDs) early in rheumatoid arthritis is helpful not only for decreasing the progression of symptoms and radiographic erosions, but even also for keeping them from occurring. It is for that reason that rheumatologists are meeting currently to redefine rheumatoid arthritis, since the ACR criteria which came out in 1987 were based on patients with long-standing disease. The CCP test is one of the new tools allowing patients to be diagnosed with RA earlier in their disease course, and it will likely be part of the new criteria. Ophthalmologists can help diagnose RA patients early in disease, which will hopefully lead to early initiation of DMARD therapy, which will hopefully decrease patients' chances of living with the terrible extent of the disease.