Performance characteristics of anti-cyclic citrullinated peptide and rheumatoid factor tests in rheumatoid arthritis and undifferentiated arthritis at Kenyatta National Hospital

Amayo AA[1], Ayunga AO[2] and Oyoo GO[3]

ABSTRACT

Background: The rheumatoid factor (RF) test has been the main serological test for diagnosis of rheumatoid arthritis. Reports of it's low sensitivity and specificity led to the introduction of anti cyclic citrullinated peptide (anti CCP) test, which was added to the diagnostic criteria. The analytical method and cost of the anti CCP test limits its availability in resource constrained environments.
Objective: To determine the analytical performance characteristics of anti CCP in patients with rheumatoid arthritis (RA) and undiffentiated arthritis (UA), and compare with those of RF.
Design: Cross-sectional study.
Methodology: The study subjects comprised 64 RA and 31 UA patients. Serum anti CCP was measured using an automated immunoassay and 3rd generation anti-CCP test. RF was determined using a qualitative particle agglutination method. Manufacturer cut-offs were used for interpretation of results. Sensitivity, specificity, negative and positive predictive values were calculated and compared, for anti-CCP and RF tests.
Results: Anti CCP showed a higher sensitivity than RF (62.5% versus 50%). Specificity was however higher with RF (90.3%) than anti-CCP (83.9%). RF also had a slightly higher positive predictive value (91.4%) than anti-CCP (88.9%). Combining RF and anti-CCP tests led to a slightly higher sensitivity and negative predictive values than those obtained with RF alone but not specificity or positive predictive values.
Conclusion: Although the anti CCP test has shown better sensitivity than RF in RA, there was slightly higher specificity and positive predictive value with RF compared with anti-CCP. The findings show that the latex RF test is an effective test for initial evaluation of patients with arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic auto-immune disease with significant morbidity and mortality, characterized by irreversible joint damage, which affects about 1% of the population globally[1-3].

The diagnostic criteria for RA set by the American College of Rheumatology (ACR) combines clinical, radiological and laboratory features[4]. The most widely used serological test for RA diagnosis is the rheumatoid factor (RF) test[4,5]. Rheumatoid factors are autoantibodies to the Fc portion of human IgG and usually belong to the IgM class of immunoglobulins[5,6]. Several RF assay methods are available ranging from rapid qualitative and semi-quantitative tests to automated immunoassay methods[5-7]. Particle agglutination qualitative RF assays are widely available in resource limited settings because of low cost, easy technique and speed of analysis[8,9].

Limitations of RF have been reported including low specificity and sensitivity[10]. RF positivity has been reported in conditions such as SLE and Hepatitis C which may be part of the differential diagnosis of RA, and RF may be persistently negative in 20% of patients with RA[11]. These performance limitations led to a search for better serological markers for diagnosis and prognostication in RA, and anti cyclic citrullinated peptide (CCP) antibody test was identified as a sensitive and specific biomarker for diagnosis and prognostication in RA[10]. CCP antibodies are derived from deamination of arginine and target the modified amino acid citrulline. Sensitivity of CCP test for RA is reported as 50 to 85% while specificity ranges from 90 to 95%[2,10,11]. Anti-CCP antibodies have been detected in upto 50% of patients with early RA, making them useful for early diagnosis of RA[10]. The features have led to inclusion of anti-CCP test in the revised (year) ACR criteria for RA diagnosis. The analytical method and cost of the anti-CCP test limit it's availability in developing countries as the recommended anti-CCP test is measured on automated immunoassay equipment which is not available in many public health laboratories,where most patients in developing countries access health care[8,9].

[1]Department of Human Pathology, College of Health Sciences, University of Nairobi, Kenya
[2]Garissa Provincial General Hospital, Kenya
[3]Department of Clinical Medicine and Therapeutics, College of Health Sciences, University of Nairobi, Kenya

Corresponding author: *Prof. AA Amayo. Email: aamayo@gmail.com*

Most of the studies reporting better performance characteristics of anti-CCP test compared to RF test were done in Caucasians. Acomparative study of anti CCP antibody test and RF in Black South Africans with early RA found higher specificity with RF (90.7%) versus 84.9% with anti CCP[9]. The sensitivity of the anti-CCP test was 82.5% versus 81.7% for RF alone. The authors concluded that the performance characteristics of anti-CCP test in Africans were not different from RF test. The control group in that study comprised healthy individuals and patients with systemic lupus erythematosus (SLE) and scleroderma. There is a paucity of reports comparing the performance of anti-CCP and RF in African patients with undifferentiated arthritis (UA). In this study we determined the sensitivity and specificity of anti-CCP antibodies for diagnosis of RA in African patients presenting with arthritis, and compared with RF.

MATERIALS AND METHODS

The study design and characteristics of the study subjects have been previously described[13]. Adult patients presenting to the outpatient clinic at Kenyatta National Hospital with inflammatory arthritis were studied. Subjects were classified into RA and UA based on ACR criteria. Patients with acute febrile illness, gout and other autoimmune diseases were excluded. Serum samples were collected from all subjects for analysis of RF and anti CCP. RF was measured using a commercial qualitative/semi quantitative particle agglutination method (Accutex RF Latex Test - Thermo Scientific) and reported as either positive or negative. Anti-CCP was estimated on the Abbott Axsym analyser using the third generation Axsym kit (Abbott Laboratories, Abbott Park, IL, USA). Results above the manufacturer's cut-off of 5.0 IU/ml were interpreted as positive. All the analyses were conducted according to the manufacturer's instructions. The study was approved by the Kenyatta National Hospital Ethics and Research Committee.

Statistical analysis: Data was entered into a PC and analysis done using MS Excell software (Microsoft 2007). Two by two tables were created for calculation of sensitivity, specificity and predictive values. The sensitivity, specificity, efficiency, positive predictive value and negative predictive value were calculated as follows:

sensitivity [a/(a+c)] x 100
specificity [d/(b+d)] x 100
positive predictive value = [a/(a+ b)] x 100
negative predictive value = [d/(c+d)] x 100
where a= true positive; b= false positive; c= false negative and d= true negative samples.

RESULTS

Ninety five subjects comprising 87 females and 8 males, were studied. Subjects were classified into RA (60 subjects) and UA (35 subjects) based on ACR clinical criteria. The distribution of anti CCP results in patients with RA and UA are shown in Table 1.

Table 1: *Distribution of anti-CCP values in RA and UA*

	Anti CCP (U/ml) (Percentiles)		
	25th	50th	75th
RA	2.1	20.6	200
UA	0.9	1.7	3.2

Anti-CCP values were higher in RA patients, the median value being 20.6U/ml compared to 1.7U/ml in patients with unclassified arthritis (p=0.000) (Table 1).

Table 2: *Anti-CCP and rheumatoid factor results in study subjects*

	Anti CCP values (U/ml)	RF test	
Subjects	<5 >5	Positive	Negative
Rheumatoid arthritis	24 40	32	32
Undifferentiated arthritis	26 5	3	28

Most RA patients (62.5%) had anti-CCP values above the manufacturer's cut-off value of 5U/ml while only 5 patients classified as UA exceeded the cut-off value giving an anti CCP false positive rate of 16%. Using the Rheumatoid Factor test, 50% of RA and 9.7% of UA patients tested positive hence a false negative RF rate of 50% and a false positive rate of 9.7%. Out of the 32 RA patients who tested positive for RF, 30 also tested positive for anti-CCP antibodies giving a positive concordance rate of 93.7% (Table 3). Patients with UA who tested negative for both RF and anti-CCP were 26 giving a negative concordance of 92.8%.

Table 3: *Cross tabulation of RF and anti-CCP antibody occurrence in RA and UA*

	Rheumatoid arthritis			Undifferentiated arthritis		
	Anti CCP+	Anti CCP -	Total	Anti CCP +	Anti CCP -	Total
RF +	30	2	32	3	0	3
RF -	10	22	32	2	26	28
Total	40	24	64	5	26	31

Table 4: *Performance characteristics of anti-CCP and RF in RA*

	Anti-CCP	RF	Combined anti CCP and RF
Sensitivity, % (95% CI)	62.5 (49.5 – 74)	50.0 (37.2 -62.7)	57.7 (43.2 -71.3)
Specificity, % (95% CI)	83.9 (66.3 -94.5)	90.3 (74.2 – 97.8)	89.7 (72.6 – 97.7)
Positive predictive value (PPV), % (95% CI)	88.9 (75.9 – 96.3)	91.4 (76.9 – 98)	90.9 (75.6 – 98))
Negative predictive value (NPV), % (95% CI)	52 (37.4 – 66.3)	46,7 (33.7 – 60)	54.2 (39.2 – 68.6)

The anti-CCP test had a higher sensitivity (62.5%) than RF which had a sensitivity of 50%. On the other hand, the specificity and positive predictive values (PPV) of RF (90.3% and 91.4% respectively), were slightly higher than those of anti-CCP test which showed specificity of 83.9% and PPV of 88.9% (Table 4). Combining RF and anti-CCP test gave better sensitivity and negative predictive values than RF alone. The specificity and PPV values obtained with RF alone did not however improve when the test was combined with anti-CCP (Table 4).

DISCUSSION

Serological markers play an important role in diagnosis and prognosis of patients with rheumatoid arthritis. An ideal marker should demonstrate high sensitivity and specificity as well as good positive and negative predictive values. RF has been utilized for RA diagnosis for several years, and forms part of the ACR diagnostic criteria. The particle agglutination RF test is relatively cheap and readily available in laboratories in resource limited settings. Anti-CCP test is reported to have superior performance characteristics and was recently added to the ACR criteria[12].

In this study the sensitivities of RF and anti CCP were 50% and 62% respectively, which are lower than what have been reported from other studies. A study of RA in Black South Africans reported sensitivities of RF and anti CCP to be 81.7% and 82.5% respectively and another study on African Americans found IgM -RF sensitivity of 70% and anti CCP sensitivity of 62%[9,14]. Studies conducted mainly in Caucasian RA patients have shown sensitivity of RF ranging from 59-79%, and anti CCP sensitivity ranging from 64-89%[10,11]. Half of the patients classified as RA in this study tested negative for RF, in keeping with reports which indicate that 30-50% patients with confirmed RA show negative RF results[15].

In this study, specificity of RF was higher than anti CCP (90.3% and 83.9% respectively). Other studies among Africans reported specificities of RF ranging from 77 – 90.7% and that of anti CCP ranged from 84.9% – 98%[9,14]. Studies on Caucasian patients have reported higher specificity for anti CCP, ranging from 88 to 99%, while RF specificity ranged from 80-84%[10,11].

The differences in performance characteristics of these two tests in various studies has been attributed to differences in cut-off values for classifying results into positive and negative, duration and severity of disease in study subjects as well as characteristics of control subjects. One Swedish study showed that in early disease, the sensitivities of both RF and anti CCP were low, ranging from 31%-50% for RF and 39%-50% for anti CCP[16].

Among Caucasians, significantly lower RF specificity compared to anti CCP specificity has been reported in studies which included patients with other rheumatic diseases such as systemic lupus erythematosus[2]. In this study such patients were excluded, leaving only patients classified as undifferentiated arthritis to form the comparative group. One may postulate that this is the possible explanation for the similar specificity obtained for RF and anti CCP in our study but the study on Black South Africans, where patients with SLE and scleroderma were included also reported higher RF specificity (90.7%) than anti CCP (84.9%)[9]. Our findings support suggestions that there may be differences in anti CCP specificity between African and Caucasian patients[9].

Predictive values of diagnostic tests depict the likelihood that a patient has the disease if the test is positive (positive predictive value -PPV), and likelihood that a patient does not have the disease if the test is negative (negative predictive value – NPV). They are important in the clinical utilization of diagnostic tests as they inform the clinician whether additional confirmatory tests are required or treatment can be initiated based on the results. In this study the PPV for RF and anti CCP were 91.5% and 89% respectively, while the NPV were 47% and 52% for RF and anti-CCP respectively. The PPVs found in this study are similar to those reported in a study among Black South Africans where the PPV for RF and anti-CCP were found to be 92.5% and 87.6%[9] and indicate that the likelihood of RA being present was very high if RF or anti-CCP was positive. The NPVs found in this study are however lower than what was reported in the study on Black South Africans (NPV was 78% for RF and 79% for anti-CCP). More than one third of the patients classified as RA in our study tested negative for anti CCP while 50% of them were

negative for RF. The variance may be attributed to the different analytical methods used for measuring RF and anti-CCP in the two studies. In this study, a qualitative latex test and automated immunoassay were used for RF and anti-CCP estimation respectively, whereas in the South African study nephelometry and immunofluorimetric methods were used. In addition, the cut-off for anti-CCP positivity in our study was 5U/ml while the South African study used a cut-off of 10U/ml.

Better diagnostic performance characteristics have been reported if RF and anti CCP are combined and some have advocated for both markers to be used[17]. Among Black South Africans the sensitivity for RA diagnosis increased to 88.3% when both RF and anti CCP are positive from 81.7% using RF alone, and specificity increased to 95.3% from 90.7%[9]. In our study, when both RF and anti CCP were positive, sensitivity increased marginally to 58% and specificity was only 89.6%. These findings, plus the limitation in availability of the analytical technique and cost of anti-CCP test in many resource limited setting suggest that application of an algorithm for anti-CCP testing may be more cost effective than performing both tests concurrently[2,9]. The Royal College of Physicians guidelines indicated that there was need for health economic analysis to determine the cost-effectiveness of anti-CCP test as the differences in performance characteristics were not great[2].

CONCLUSIONS

This study sought to determine the performance characteristics of a third generation anti CCP test in patients presenting with arthritis, and compare with a latex agglutination RF test that is widely available in Kenya. The results show that anti CCP test has better sensitivity than RF in RA, but the specificity of RF was slightly better than that of anti-CCP. The findings show that the latex RF test is useful initial test for excluding RA in patients with arthritis.

STUDY LIMITATIONS

In this study patients with other rheumatic conditions were excluded. This limits the comparison of the study findings. Most of the reported studies on RF utilized quantitative RF assays while in this study a qualitative latex agglutination assay was used. This difference in analytical methods has been reported as one of the causes of differences in clinical performance characteristics of laboratory markers in RA.

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