ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 5, Issue, 12, pp. 6276-6281, December, 2015

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

A COMPARATIVE STUDY OF VARIOUS SCREENING METHODS TO SCREEN CLINICALLY SUSPECTED RHEUMATOID ARTHRITIS PATIENTS

[1]Singh, T. B., [1,*]Ausaf Ahmad and [2]Usha

[1]Department of Community Medicine, IMS, BHU, Varanasi, U.P., India
[2]Department of Pathology, I.M.S., B.H.U., Varanasi, U.P., India

ARTICLE INFO

Article History:
Received 29th September, 2015
Received in revised form
27th October, 2015
Accepted 08th November, 2015
Published online 30th December, 2015

Key Words:
Epidemiological,
Rheumatoid Arthritis (RA),
Hospital, auto-immune.

ABSTRACT

Background and Objectives: The body mistakenly considers some parts of its own system as pathogen and attack them, are known as autoimmune diseases. The auto-immune diseases are Rheumatoid Arthritis(RA), SLE, etc. are growing day by day worldwide. Till date no aetiological agents are documented for this disease. This study is carried out to with objective to find out the screening accuracy of RF and AntiCCP.
Method: The study is based on 290 clinically suspected subjects. Cross-sectional cohort study design was used. Clinically suspected cases were referred by different OPD's of Sir Sunderlal Hospital for screening. Along with results of these tests other socio-demographic, economic information were also carried through structured pre-tested schedule method.
Result: Rheumatoid factor (RF) test screened 21.8% as positive which was approximately equal to AntiCCP (21.7%). CRP was observed some better test than RF and AntiCCP because it screened approximately one third cases. The sensitivity of RF, AntiCCP , CRP, CRP+AntiCCP, RF+AntiCCP, RF+CRP and RF+AntiCCP+CRP were 77.08, 75.0, 93.75, 97.92, 100, 93.75 and 100 percentages respectively. The overall accuracy was observed highest for RF.
Conclusion: The finding of this study illustrates that there is no single screening test is suitable to diagnose the RA disease. The combination of two screening tests had about 90% sensitivity which is increased up to 100% if three tests combined. Thus, these tests should be performed in all clinically suspected cases to find out the diseased.

INTRODUCTION

The healthy human body is equipped with a powerful set of tools for resisting the onslaught of invading microorganisms (such as viruses, bacteria, fungi and parasites). Unfortunately, this set of tools, known as the immune system, sometimes goes awry and attacks the body itself. These misdirected immune responses are referred to as autoimmunity (Johns Hopkins Center for Autoimmune Disease Research, (2000-2008). The body mistakenly considers some parts of its own system as pathogen and attack them, are known as autoimmune diseases. (Sangha *et al*., 2000) The auto-immune diseases are Rheumatoid Arthritis (RA), SLE, etc. are growing day by day worldwide. Rheumatoid arthritis (RA) is an autoimmune disease that results in a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks flexible (synovial) joints.

Corresponding author: Ausaf Ahmad
Department of Community Medicine, IMS, BHU, Varanasi, U.P., India

It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility if not adequately treated (Alamanos *et al*., 2005 Majithia *et al*., 2007 and Patel *et al*., 2011). The healthy human body is equipped with a powerful set of tools for resisting the onslaught of invading microorganisms (such as viruses, bacteria, fungi and parasites). Unfortunately, this set of tools, known as the immune system, sometimes goes awry and attacks the body itself. These misdirected immune responses are referred to as autoimmunity (Johns Hopkins Center for Autoimmune Disease Research, (2000-2008). The body mistakenly considers some parts of its own system as pathogen and attack them, are known as autoimmune diseases (Sangha *et al*., 2000).

RA is a common rheumatic disease of uncertain aetiology with a significant level of morbidity. Despite decades of study and the development of a series of classification criteria, (Arnett *et al*.,1988) the diagnosis of RA remains empirical and imprecise, particularly early in the course of disease. Because

early initiation of disease modifying treatments can significantly improve long term outcomes for patients with RA, there is considerable motivation to accurately diagnose RA in patients with inflammatory arthritis early in the course of disease (O'Dell *et al*., 2002; Mottonen *et al*., 2002). Serological studies form a cornerstone of laboratory based patient assessment in rheumatology. The presence of "rheumatoid factor" (RF) was identified in patients with RA over 50 years ago (Rose *et al*., 1949); assays for RF remain one of the American College of Rheumatology (ACR) classification criteria for RA. The RF assay, in its current manifestation, remains suboptimal as a diagnostic test, as it lacks sensitivity (54–88%) and specificity (48–92%) (Weinblatt *et al*., 1980; Saraux *et al*., 2002; Bas *et al*., 2002; Bizzaro *et al*., 2001; Schellekens *et al*., 2000), it is present frequently in many other disease states (Mikkelsen *et al*., 1967; Bartfeld *et al*., 1960; Meltzer *et al*., 1966) (reviewed by Shmerling and Delbanco, 1991, Carson *et al*., 1997 and Bridges *et al*., 2001), and its incidence increases with age (Mikkelsen *et al*., 1967; Shmerling *et al*., 1991).

This discovery led to the development of assays employing cyclic citrullinated peptides (CCP) to measure antibodies recognizing citrullinated antigens as a diagnostic test for RA. Initial studies characterising the frequency of antibodies to CCP in mixed cohorts containing patients with rheumatic diseases, infectious diseases, and healthy patients, have shown it to be moderately sensitive (68%) but highly specific (98%) for RA (Schellekens *et al*., 2000). Despite overwhelming evidence of its specificity, anti-CCP antibody detection for the diagnosis of RA is not requested on a regular basis in our country probably due to the lack of comparative studies or the non-availability of this test in routine laboratories (Oommen *et al*., 2012). According to the 2011 census, the population of India stands at 1,210,569,573. The reported prevalence of RA in India has varied from 0.3% to 0.75% (Malaviya *et al*., 1993 and Ganguly, 1997).

Assuming an average prevalence of 0.5% with the adult population of 60%, the projected burden of RA in India is 36 million patients, out of which approximately 25 million patients are from rural community and 11 million patients belongs to urban community (Joshi *et al*., 2013). In 1987, the American College of Rheumatology (ACR) developed classification criteria for RA to improve the uniformity of patient populations enrolled in clinical trials. RA is a common rheumatic disease of uncertain aetiology with a significant level of morbidity. Despite decades of study and the development of a series of classification criteria, (Arnett *et al*., 1988) the diagnosis of RA remains empirical and imprecise, particularly early in the course of disease. Because early initiation of disease modifying treatments can significantly improve long term outcomes for patients with RA, there is considerable motivation to accurately diagnose RA in patients with inflammatory arthritis early in the course of disease (O'Dell *et al*., 2002; Mottonen *et al*., 2002).

Serological studies form a cornerstone of laboratory based patient assessment in rheumatology. The presence of "rheumatoid factor" (RF) was identified in patients with RA over 50 years ago (Rose *et al*., 1949), assays for RF remain one of the American College of Rheumatology (ACR) classification criteria for RA. The RF assay, in its current manifestation, remains suboptimal as a diagnostic test, as it lacks sensitivity (54–88%) and specificity (48–92%) (Weinblatt *et al*., 1980; Saraux *et al*., 2002; Bas *et al*., 2002; Bizzaro *et al*., 2001; Schellekens *et al*., 2000), it is present frequently in many other disease states(Mikkelsen *et al*., 1967; Bartfeld *et al*., 1960; Meltzer *et al*., 1966) (reviewed by Shmerling and Delbanco, 1991, Carson *et al*., 1997 and Bridges *et al*., 2001), and its incidence increases with age, (Mikkelsen *et al*., 1967; Shmerling *et al*., 1991).

This discovery led to the development of assays employing cyclic citrullinated peptides (CCP) to measure antibodies recognizing citrullinated antigens as a diagnostic test for RA. Initial studies characterising the frequency of antibodies to CCP in mixed cohorts containing patients with rheumatic diseases, infectious diseases, and healthy patients, have shown it to be moderately sensitive (68%) but highly specific (98%) for RA (Schellekens *et al*., 2000). Despite overwhelming evidence of its specificity, anti-CCP antibody detection for the diagnosis of RA is not requested on a regular basis in our country probably due to the lack of comparative studies or the non-availability of this test in routine laboratories (Oommen *et al*., 2012). The objective of this study was to find out the screening accuracy of RF, anti-CCP and CRP.

MATERIALS AND METHODS

Study population

The present study is based on prospective cross-sectional cohort study design. In the present study 290 (110 male and 180 female) clinically suspected of rheumatoid arthritis patient were studied. Who were screened at UGC Advanced Immunodiagnostic Training and Research Centre, Department of Pathology, IMS, BHU, Varanasi, U.P. The cases were referred by different OPD's of Sir Sunderlal Hospital. Mostly screened subjects were from eastern Uttar Pradesh, western Bihar, Madhya Pradesh and Jharkhand. About 2-ml of blood samples were collected in plain vial from each patient and each sample were tested by the laboratory person.

Study design

ACR/EULAR 2010 criteria were evaluated at baseline. The baseline assessment included a standardized interview, general physical examination, and standardized rheumatologic evaluation, including the number of swollen and tender joints, the distribution and symmetry of synovitis, the size of involved joints, and the presence of rheumatoid nodules. Laboratory investigations included acute-phase reactants and rheumatoid factor assays.

The ACR/EULAR criteria were considered positive in patients with no other diagnosis explaining the symptoms and with either erosions typical for RA or a score greater or equal to 6/10:

- joint involvement (1 medium-large joint: 0 points; 2 to 10 medium-large joints: 1 point; 1 to 3 small joints: 2 points; 4 to 10 small joints: 3 points; 10 joints with at least one small joint: 5 points);

- serology (no rheumatoid factor [RF] or ACPA: 0 point; low- positive RF and/or ACPA [less than 3 times the upper limit of normal for the laboratory and assay]: 1 point; high-positive RF and/or ACPA [more than 3 times the upper limit of normal for the laboratory and assay]: 3 points);
- synovitis duration (less than 6 weeks: 0 point; greater or equal to 6 weeks: 1 point) and acute-phase reactants (C-reactive protein [CRP] and erythrocyte sedimentation rate [ESR] normal: 0 point; CRP and/or ESR elevated: 1 point).

Statistical analysis

Statistical tests were performed using the Statistical Package for the Social Sciences (SPSS 16.0,). All items of the various criteria sets at baseline. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for the 1987 ACR and 2010 ACR/EULAR criteria. To determine which baseline features best separated patients with and without RA

RESULT AND DISCUSSION

This cross sectional study was done on 290 clinically suspected RA patients. 63 out of 290 samples tested positive for anti-CCP. Of these 63 clinically suspected patients, 36 had RA. This compared with 62 / 290 samples tested positive for RF. In which 37 had RA. Acute- phase reactant CRP showed the highest percentage of positivity among all the clinically suspected RA patients.

Table 1. Showing the distribution of positivity of male, female and total of clinically suspected Rheumatoid arthritis separately according to diagnostic tests

	MALE(110 Patients)		FEMALE(180 Patients)		TOTAL(290 Patients)	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
	14(12.7)	96(87.3)	34(18.9)	146(81.1)	48(16.6)	242(83.4)
RF[1]	13(11.8)	97(88.2)	49(27.2)	131(72.8)	62(21.8)	228(78.6)
AntiCCP[2]	26(23.6)	84(76.4)	37(20.6)	143(79.4)	63(21.7)	227(78.3)
CRP[3]	35(31.8)	75(68.2)	61(33.9)	119(66.1)	97(33.4)	193(66.6)
CRP+AntiCCP	44(40)	66(60)	70(38.9)	110(61.1)	114(39.3)	176(60.7)
RF+AntiCCP	30(27.3)	80(72.7)	64(35.6)	116(64.4)	94(32.4)	196(67.6)
RF+CRP	35(31.8)	75(68.2)	76(42.2)	104(57.8)	111(38.3)	179(61.7)
RF +AntiCCP +CRP	44(40)	66(60)	86(47.8)	94(52.2)	130(44.8)	160(55.2)

[1] Rheumatoid factor, [2] Anti-cyclic citrullinated peptide

Table 1 showed that our experience with the serological tests anti-CCP, RF, CRP and the combination of, (CRP and AntiCCP), (RF and AntiCCP), (RF and CRP) and (RF and AntiCCP and CRP) of 290 clinically suspected RA patients, in this study total male subjects was 110 which was approximately 38% of the total study subject. Positivity of RF in male subjects was 11.8%, positivity of anti-CCP was 23.6%, positivity of CRP was 31.8%, and positivity in the combination of serological tests (CRP and AntiCCP), (RF and AntiCCP) and (RF and AntiCCP and CRP) was 40%, 27.3%, 31.8% and 40% respectively. Out of 110 male subjects, 14 had RA, which was about 12.7% and out of total study subject i.e. 290 percentage of male RA patients was nearly equal to 4.8%.

On the other hand total female subjects was 180 which was approximately 62% of total study subject. Positivity of RF in male subjects was 27.2%, positivity of anti-CCP was 20.6%, positivity of CRP was 33.9%, and positivity in the combination of serological tests (CRP and AntiCCP), (RF and AntiCCP) and (RF and AntiCCP and CRP) was 38.9%, 35.6%, 42.2% and 47.8% respectively. Out of 180 male subjects, 34 had RA, which was about 18.9% percentage of RA positivity for female subject to the total study was approximately 11.7%. Consider the gross study subject, found that 48 study subject were positive (16.6%) including male and female according to ACR 2010 criteria. Detection of anti-CCP is very useful for the diagnosis of RA, in fact even RF also very useful for diagnosis of RA and combination of testing for both RF and anti-CCP may be even more useful in comparison to individual test.

Over the past few years, many studies have evaluated the diagnostic performance of anti-CCP on a variety of diagnostic platform (Vander *et al*., 2008; Bizzaro *et al*., 2007; Santiago *et al*., 2008; Correia *et al*., 2008; Vander *et al*., 2009; Jaskowski *et al*., 2010). High levels of C-reactive protein (CRP) are also indicators of active inflammation. Like the ESR, a high result does not indicate what part of the body is inflamed, or what is causing the inflammation (University of Maryl and Medical Center, 2013) Early treatment of RA is important as it can prevent irreversible damage of the joints. Despite the strong diagnostic value of anti-CCP and RF, there is strong demand for novel serological biomarkers to further improve the early diagnostic of this abundant disease (Trouw *et al*., 2012). Sensitivity, specificity, positive predictive value, negative predictive value, accuracy, positive likelihood ratio, negative likelihood ratio along with 95% confidence interval for the diagnosis of RA of female, male, total patients.

Showing in Table 2, Table 3, Table 4 respectively. Table-1 illustrate that sensitivity of male RA patients in combination of diagnostic tests, (CRP and AntiCCP), (RF and AntiCCP) and (RF and AntiCCP and CRP) was maximum (100% ; 95% CI) and was lowest in RF (71.43 ; 95% CI, 41.92 - 91.43) while specificity, positive predictive value, positive likelihood ratio and accuracy of RF was maximum as compared to other diagnostic tests. We also examine the utility of combining the RF and Anti-CCP and CRP diagnostic tests. Table-2 indicates the female RA patients showing maximum (100%; 95% CI) sensitivity and negative predictive value in combination of diagnostic tests, (RF and AntiCCP) and (RF and AntiCCP and CRP). Specificity, positive predictive value, positive likelihood ratio and accuracy of AntiCCP was maximum in all diagnostic tests. Table-3 showing that the result of various screening tests in diagnosis of all (male and female) clinically suspected RA patients.

Table 2. The results of serology test of clinically suspected Rheumatoid arthritis female patients

Various test FEMALE	Sensitivity(%) 95% CI	Specificity(%) 95% CI	Positive Predictive Value(%) 95% CI	Negative Predictive Value(%) 95% CI	Accuracy(%)	Positive Likelihood Ratio 95% CI	Negative Likelihood Ratio 95% CI
RF[1]	79.41	84.93	55.10	94.66		5.27	0.24
	(62.09 to 91.26)	(78.08 to 90.31)	(40.23to 69.33)	(89.30to97.82)	83.89	(3.46 to 8.03)	(0.12 to 0.47)
AntiCCP[2]	70.59	91.90	64.86	93.01		7.93	0.32
	(52.52 to 84.88)	(85.25 to95.17)	(47.46 to 9.78)	(87.51 to 96.59)	87.22	(4.52 to13.91)	(0.19 to 0.54)
CRP[3]	94.12	80.14	52.46	98.32		4.74	0.07
	(80.29 to 99.11)	(72.73 to 6.27)	(39.27 to 65.40)	(94.05 to 99.75)	82.78	(3.38 to 6.63)	(0.02 to 0.28)
CRP+AntiCCP	97.06	74.66	47.14	99.09		3.83	0.04
	(84.62 to 99.51)	(66.80 to 81.49)	(35.09to 59.45)	(95.02to 99.85)	78.89	(2.88 to 5.09)	(0.01 to 0.27)
RF+AntiCCP	100	79.45	53.12	100		4.87	0.00
	(89.62 to100)	(71.98to 85.68)	(40.23to 65.72)	(96.84to 100)	83.33	(3.54 to 6.70)	
RF+CRP	94.12	69.86	42.11	98.08		3.12	0.08
	(80.29 to 99.11)	(61.73 to77.17)	(30.86 to53.98)	(93.21 to 99.71)	74.44	(2.41 to 4.05)	(0.02 to 0.32)
RF+AntiCCP+CRP	100	64.38	39.53	100		2.81	0.00
	(89.62to100)	(56.04 to72.13)	(29.15 to 50.66)	(96.11 to 100)	71.11	(2.26 to 3.49)	

[1] Rheumatoid factor, [2] Anti-cyclic citrullinated peptide

Table 3. The results of serology test of clinically suspected Rheumatoid arthritis male patients

Various test MALE	Sensitivity(%) 95% CI	Specificity(%) 95% CI	Positive Predictive Value(%) 95% CI	Negative Predictive Value (%) 95% CI	Accuracy (%)	Positive Likelihood Ratio 95% CI	Negative Likelihood Ratio 95% CI
RF[1]	71.43	96.88	76.92	95.88	93.64	22.86	0.29
	(41.9 to 91.43)	(91.1 to 99.31)	(46.20 to 94.69)	(89.77 to 98.84)		(7.15 to 73.06)	(0.13 to 0.68)
AntiCCP[2]	85.71	85.42	46.15	97.62	85.45	5.88	0.17
	(57.16 to 97.80)	(76.74 to 91.79)	(26.61 to 66.61)	(91.64 to 99.64)		(3.46 to 9.98)	(0.05 to 0.61)
CRP[3]	96.86	77.08	37.14	98.67	79.10	4.05	0.09
	(66.06 to 98.81)	(67.38 to 85.04)	(21.49 to 55.07)	(92.77 to 99.78)		(2.73 to 6.01)	(0.01 to 0.61)
CRP+	100	68.75	31.82	100	72.73	3.20	0.00
AntiCCP	(76.66 to 100)	(58.48 to 77.82)	(18.62 to 47.58)	(94.51 to 100)		(2.38 to 4.31)	
RF+	100	83.33	46.67	100		6.00	0.00
AntiCCP	(76.66 to 100)	(74.35 to 90.16)	(28.36 to 65.66)	(95.45 to 100)	85.45	(3.84 to 9.38)	
RF+CRP	92.86	77.08	37.14	98.67	79.10	4.05	0.09
	(66.06 to 98.81)	(67.38 to 85.04)	(21.49 to 55.07)	(92.77 to 99.78)		(2.73 to 6.01)	(0.01 to 0.61)
RF+	100	68.75	31.82	100	72.73	3.20	0.00
AntiCCP+ CRP	(76.66 to100)	(58.48 to 77.82)	(18.62 to 47.58)	(94.51 to 100)		(2.38 to 4.31)	

[1] Rheumatoid factor, [2] Anti-cyclic citrullinated peptide

Table 4. The results of serology test of clinically suspected Rheumatoid arthritis

Various test MALE+ FEMALE	Sensitivity(%) 95% CI	Specificity(%) 95% CI	Positive Predictive Value (%) 95% CI	Negative Predictive Value(%) 95% CI	Accuracy(%)	Positive Likelihood Ratio 95% CI	Negative Likelihood Ratio 95% CI
RF1	77.08	89.67	59.68	95.18		7.46	0.26
	(62.68 to 87.95)	(85.13 to 93.20)	(46.45 to 71.95)	(91.53 to 97.56)	87.59	(4.99 to1.15)	(0.15 to 0.43)
AntiCCP2	75.00	88.84	57.14	94.71		6.72	0.28
	(60.40 to 86.35)	(84.18 to 92.52)	(44.05 to 69.54)	(90.95 to 97.24)	86.55	(4.55 to 9.94)	(0.17 to 0.46)
CRP3	93.75	78.51	46.39	98.45		4.36	0.08
	(82.78 to 98.62)	(72.80 to 83.52)	(36.20 to 56.81)	(95.52 to 99.66)	81.03	(3.39 to 5.61)	(0.03 to 0.24)
CRP+	97.92	72.31	41.23	99.43		3.54	0.03
AntiCCP	(88.88 to 99.65)	(66.22 to77.85)	(32.09 to 50.83)	(96.86 to 99.90)	76.55	(2.87 to 4.35)	(0.00 to 0.20)
RF+	100	80.99	51.06	100		5.26	0.00
AntiCCP	(92.53 to100)	(75.47 to 85.73)	(40.54 to 61.52)	(98.12 to 100)	84.14	(4.06 to 6.82)	
RF+CRP	93.75	72.73	40.54	98.32		3.44	0.09
	(82.78 to 98.62)	(66.65 to 78.24)	(31.32 to 50.27)	(95.17 to 99.63)	76.21	(2.76 to 4.28)	(0.03 to 0.26)
RF+	100	66.12	36.92	100		2.95	
AntiCCP+ CRP	(92.53 to100)	(59.78 to 72.05)	(28.63 to 45.83)	(97.70 to 100)	71.72	(2.47 to 3.52)	0.00

[1] Rheumatoid factor, [2] Anti-cyclic citrullinated peptide

Considered the RF values in these tables that sensitivity of RF in female was 79.41% and in male this was decreases upto 71.43%. Sensitivity of total study subjects including male and female was 77.08%. Specificity of male 96.88% which was much higher than female 84.93%. Except sensitivity all the variables were higher in male as compared to female subjects. Most studies showed that RF lacks specificity for RA (Bridges *et al*., 2001) and both sensitivity and specificity of the anti-CCP tests are significantly higher than those of the RF test (Wiik *et al*., 2010). In this study RF showed 89.67% specificity for RA. Which discord with other, However its sensitivity (77.08%). Sensitivity of anti-CCP in male (85.71%) which was much higher with comparison to the female sensitivity. In table 4, although anti-CCP had a somewhat lower sensitivity than the RF test, the specificity of anti-CCP for RA in this study subjects was 88.84%. Presence of both auto-antibodies (RF and anti-CCP) for the diagnosis of RA, decreased specificity to 80.99%.

Conclusion

The finding of this study illustrates that there is no single screening test is suitable to diagnose the RA disease. The combination of two screening tests had about 90% sensitivity which is increased up to 100% if three tests combined.

To establish the diagnosis of rheumatoid arthritis, we therefore recommend the use of the highly specific anti-cyclic citrullinated peptide antibody test. Especially in ambiguous cases or in rheumatoid factor negative patients with suspected rheumatoid arthritis, this test has proved very helpful and could be an additional diagnostic marker for the diagnosis of rheumatoid arthritis. Our study suggests that the combined use of RF and AntiCCP is the most powerful prognostic and diagnostic tool and as greater value for clinical use than conventional RF tests on their own. This set of diagnostic and prognostic markers would allow the clinician to choose a more powerful disease modifying anti rheumatic drug early in the course of disease, even when clinical judgment might not yet indicate the need for such drugs. No single test available to diagnose the disease, therefore combination of tests would be prefer to find out disease cases Thus, these tests should be performed in all clinically suspected cases to find out the diseased.

REFERENCES

Arnett, F.C., Edworthy, S.M., Bloch, D.A., McShane, D.J., Fries, J.F., Cooper, N.S. *et al*. 1988. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum,* 31:315–24.

Bartfeld, H. 1960. Incidence and significance of seropositive tests for rheumatoid factor in non-rheumatoid disease. *Ann Intern Med*., 52:1059–66.

Bas, S., Perneger, T.V., Kunzle, E., Vischer, T.L. 2002. Comparative study of different enzyme immunoassays for measurement of IgM and IgA rheumatoid factors. *Ann Rheum Dis*., 61:505–10.

Bizzaro, N., Mazzanti, G., Tonutti, E., Villalta, D., Tozzoli, R. 2001. Diagnostic accuracy of the anti-citrulline antibody assay for rheumatoid arthritis. *Clin Chem.,* 47:1089–93.

Bizzaro, N., Tonutti, E., Tozzoli, R., Villalta, D. 2007. Analytical and diagnostic characteristics of 11 2nd- and 3rd-generation immunoenzymatic methods for the detection of antibodies to citrullinated proteins. *Clin Chem*., 53:1527–33.

Bridges, S.L., Lippincott, Williams and wilkins, 2001. Philadelphia Rheumatoid factor. In: Koopman WJ, ed. *Arthritis and Allied Conditions*, pp. 1223–44.

Bridges, S.L. 2001. Rheumatoid factor. In: Koopman WJ, ed. Arthritis and allied conditions. Philadelphia: Lippincott, Williams & Wilkins, 1223–44.

Carson DA. Rheumatoid factor. In: Kelley WN, Ruddy S, Harris ED, Sledge CB, eds. Textbook of rheumatology. Philadelphia: Saunders, 1997:155–63.

Correia, M.L., Carvalho, S., Fortuna, J and Pereira, M.H. 2008. Comparison of three anti-CCP antibody tests and rheumatoid factor in RA and control patients. *Clin Rev AllergyImmunol*., 34:21–5

Jaskowski, T.D., Hill, H.R., Russo, K.L., Lakos, G., Szekanecz, Z., Teodorescu, M. 2010. Relationship between rheumatoid factor isotypes and IgG anti-cyclic citrullinated peptide antibodies. *J Rheumatol*., 37:1582–8.

Johns Hopkins Center for Autoimmune Disease Research, (2000-2008): http://autoimmune.pathology.jhmi.edu/whatisautoimmunity.cfm

Meltzer, M., Franklin, E.C., Elias, K., McCluskey, R.T., Cooper, N. 1966. Cryoglobulinemia—a clinical and laboratory study. *Am J Med*., 40:837–56.

Mikkelsen, W.M., Dodge, H.J., Duff, I.F., Kato, H. 1967. Estimates of the prevalence of rheumatic diseases in the population of Tecumseh, Michigan, 1959–1960. *J Chronic Dis*; 20:351–69

Mottonen, T., Hannonen, P., Korpela, M., Nissila, M., Kautiainen, H., Ilonen, J., *et al*. 2002. Delay to institution of therapy and induction of remission using single- drug or combination-disease-modifying antirheumatic drug therapy in early rheumatoid arthritis. *Arthritis Rheum*;46:894–8.

O'Dell, J.R. 2002. Treating rheumatoid arthritis early: a window of opportunity? *Arthritis Rheum*, 46:283–5.

Oommen, S., Appalaraju, B., Sivadarshini, S., Jayashree, 2012. A combined diagnostic approach to rheumatoid arthritis using anti-cyclic citrullinated peptide and rheumatoid factor. *Indian journal of Medical Microbiology,* 29:195-196.

Rose, H.M., Ragan, C., Pearce, E., Lipman, M.O. 1949. Differential agglutination of normal and sensitized sheep erythrocytes by sera of patients with rheumatoid arthritis. *Proc Soc Exp Biol Med*., 68:1–6

Sangha, O., 2000. Epidemiology of rheumatoid diseases, University of Munich, Faculty of medicine, Bavarian public health research center, Munich, Germany. *Rheumatology*, 39(suppl.2):3-12;.

Santiago, M., Baron, M., Miyachi, K., Fritzler, M.J., Abu-Hakima, M., Leclercq, S., *et al*. 2008. A comparison of the frequency of antibodies to cyclic citrullinated peptides using a third generation anti-CCP assay (CCP3) in systemic sclerosis, primary biliary cirrhosis and rheumatoid arthritis. *Clin Rheumatol*., 27:77–83.

Saraux, A., Berthelot, J.M., Chales, G., Le Henaff, C., Mary, J.Y., Thorel, V., *et al*. 2002. Value of laboratory tests in early prediction of rheumatoid arthritis. Arthritis Rheum; 47:155–65.

Trouw, L.A., Mahler, M. 2012. Closing the serological gap: promising novel biomarkers for the early diagnosis of rheumatoid arthritis. *Autoimmunity Reviews* 12 ; 318–322.

University of Maryland Medical Center, Rheumatoid arthritis, 2013. http://umm.edu/health/medical/reports/articles/rheumatoid-arthritis, University of Maryland Medical Center

Van der Linden, M.P., van der Woude, D., Ioan-Facsinay, A., Levarht, E.W., Stoeken-Rijsbergen, G., Huizinga, T.W., *et al*. 2009. Value of anti-modified citrullinated vimentin and third-generation anti-cyclic citrullinated peptide compared with second-generation anti-cyclic citrullinated peptide and rheumatoid factor in predicting disease outcome in undifferentiated arthritis and rheumatoid arthritis. Arthritis Rheum; 60:2232–41.

Vander Cruyssen, B., Nogueira, L., Van Praet, J., Deforce, D., Elewaut, D., Serre, G., *et al*. 2008. Do all anti-citrullinated protein/peptide antibody tests measure the same? Evaluation of discrepancy between anti-citrullinated protein/peptide antibody tests in patients with and without rheumatoid arthritis. *Ann Rheum Dis*., 67:542–6.

Weinblatt, M.E., Schur, P.H. 1980. Rheumatoid factor detection by nephelometry. *Arthritis Rheum*., 23: 777–9.

Wiik, A.S., van Venrooij, W.J., Pruijn, G.J. 2010. All you wanted to know about anti-CCP but were afraid to ask. *Autoimmun Rev*., 10:90–3.

****