ROC-Based Assessment of ELISA System Used to Detect IgM-RF, IgG-RF and IgA-RF among Iraqi Rheumatoid Arthritis Patients

Mohanad M. Ahmed*, Khalid A. Obaid Al-Ruhaimi**, Suhad H. Mohammed***, Hassan A. Al Saadi*, Shawahd B. Al-Yassiri****, Nidahl Abdul-Ameer****, Salah H. Al-Ali****

*** College of pharmacy/Kerbala University. **Dep. Of Rheumatology and Rehablitation/ Al-Hussain Hospital/Kerbala. *** College of Medicine /Kerbala University. ****Central Public Health laboratory/ Kerbala**

Abstract

Serological tests are mainly evaluated by determining the sensitivity and specificity of the test. These test characteristics were originally meant to be used in making diagnoses. For evaluative purposes their usefulness is weakened by their susceptibility to selection and their dependence on the cut-off points that are used for test positivity. The plotting of a receiver operating characteristic (ROC) curve might be a solution to these problems. The ROC curve yields a measure for the diagnostic power of the test expressed in one number instead of two, namely the area under the curve (AUC). Furthermore, the ROC curve and its AUC permit easy comparison of different tests and the performance of different interpreters of one test. In this study, for direct comparison of the diagnostic values of IgM-RF, IgG-RF and IgA assay in the diagnosis of Rheumatoid Arthritis (RA), we performed an ROC analysis. The calculated areas under curves (AUCs) have shown that each of the three RF-subtypes has a different discriminative value because their curves were located at different distances above the diagonal line. However, the IgG-RF subtype has the highest discriminative value because of that its AUC was 0.937 and, thus, it has a very high clinical value. IgM-RF subtype has a moderate discriminative value (AUC= 0.848), whereas IgA-RF has showed the least discriminative power (AUC= 0.709) that may make it the least clinically useful RF as a diagnostic test. These results clearly demonstrate the superiority of IgG-RF ELISA system as a diagnostic test for RA over the other RFs. Using ROC to determine cutoff values has yielded several cutoff values with variable sensitivities and specificities for each RF subtype. This may allow the choice of the desired cutoff according to the clinical setting in which the test might used.

الخلاصة

ان رسم منحنى تشغيل المتلقي للأختبارات المصلية ينتج قياسا للقوة التشخيصية للاختبارات والتي تعرب عن نفسها برقم واحد بدلا من اثنين (الحساسية والنوعية) وهي المنطقة الواقعة تحت المنحنى مما يتيح سهولة المقارنة للأختبارات المختلفة وأداء مختلف التفسيرات للأختبار الواحد.

أجرينا في هذه الدراسة تحليل منحنى خاصية تشغيل المتلقي لأجراء مقارنة مباشرة للقيم التشخيصية لكل من العوامل الرثوية IgG و IgM و IgA في مرضى التهاب المفاصل الرثوي في العراق. أظهرت المناطق الواقعة تحت المنحنيات المحسوبة أن لكل عامل رثوي قيمة تمييزية مختلفة. ومع ذلك، فأن العامل الرثوي نوع IgG أظهر اعلى قيمة تمييزية (المنطقة تحت المنحنى =0.937) ، وبالتالي فقيمته السريرية عالية جدا. أما العامل الرثوي نوع IgA فقد أظهر اقل قيمة

تمييزية (المنطقة تحت المنحنى =0.709) مما يقلل من قيمته في تشخيص المرض. في حين كان العامل الرثوي نـــوع IgM معتدل القيمة التمييزية (المنطقة تحت المنحنى = 0.848). يتضح مما تقدم تفوق العامل الرثوي نوع IgG بوصـــفه اختبـــارا تشخيصيا لمرض المفاصل الرثوي. كما وأن استعمال منحنى خاصية تشغيل التلقى لتحديد قيم نقاط القطع (cutoffs) اسفر عن تحديد نقاط قطع متعددة لكل عامل رثوي بالاضافة الى تحديد حساسية ونوعية كل نقطة قطع مما يتيح حرية اختيار نقطة القطع حسب الحاجة السريرية.

Introduction

Rheumatoid arthritis (RA) is the most common inflammatory joint disease, with prevalence between 0.5% and 1% worldwide [1]. In most patients, diagnosis of RA is based on the criteria proposed by the American College of Rheumatology (ACR) in 1987 consisting of clinical symptoms and radiological findings, whereas the only laboratory test included is the serum rheumatoid factor (RF) determination [2]. The ACR criteria, however, were primarily developed as classification criteria in established disease, and shortcomings in RA patients with recent-onset disease have now become evident [3]. RFs are antibodies directed to the crystallizable fragment of IgG molecule. They are found in every immunoglobulin subclass (IgM, IgG, IgA and IgE)[4]. Raised levels of IgM, IgG and IgA RF have been frequently reported in patients with RA. The high RF frequency in RA cases makes their detection useful as a diagnostic tool; however these factors are not unique to RA. RF found in 1-4% of the general population, in addition to other autoimmune diseases and infections[5]. Moreover, literatures mentioned different sensitivities and specificities for the detection of each RF subtype. For instance, RF of IgM subtype (IgM-RF) is a traditional laboratory test used to support the clinical diagnosis of RA. It has wide range of sensitivity (50%-85%) but have moderate specificity (80%-95%) for diagnosing RA. This discrepancy may possibly reflect diverse genetic backgrounds and/or methodological differences in diverse antigen preparations and detection techniques applied, in addition to the age and health of the population studied [6]. Therefore, there is a clear need for extensive evaluation and validation of any system used to detect RF in RA patients before its administration for routine use.

Serological tests are mainly evaluated by determining the sensitivity and specificity of the test. These test characteristics were originally meant to be used in making diagnoses. For evaluative purposes their usefulness is weakened by their susceptibility to selection and their dependence on the cut-off points that are used for test positivity. The plotting of a receiver operating characteristic (ROC) curve might be a solution to these problems. The ROC curve yields a measure for the diagnostic power of the test expressed in one number instead of two, namely the area under the curve (AUC). Furthermore, the ROC curve and its AUC permit easy comparison of different tests and the performance of different interpreters of one test[7]. In this study, for direct comparison of the diagnostic values of IgM-RF, IgG-RF and IgA assay in the

diagnosis of Rheumatoid Arthritis (RA), we performed an ROC analysis. Furthermore, there is growing evidence that therapeutic intervention early in the course of RA leads to more efficient disease control, less joint damage, and better prognosis of disease outcome. Therefore, specific laboratory tests are desirable to help in the early differentiation of RA and other forms of rheumatic joint and connective tissue disease [8-11].

The main objective of this study was to use ROC curve for the investigation of the diagnostic accuracy of the recently emerged and commercially available ELISA system for the detection of IgM-RF, IgG-RF and IgA-RF in a moderately large cohort of patients with confirmed diagnosis of RA and non-RA subjects.

Materials and Methods

In order to determine the diagnostic value of IgM-RF, IgG-RF and IgA-RF assays using ELISA system, 102 subjects were enrolled in this study, 65 were with confirmed diagnosis of RA and fulfilling the revised ACR criteria, in addition to 37 carefully chosen non-RA controls. All RA subjects were attendant of the private rheumatology clinic (of the second author) in Kerbala Province during January to April 2008. Non RA subjects were age and sex matched apparently healthy peoples randomly selected from Kerbala Province citizens. Serum were collected from each subject and kept frozen until use. ELISA kits for the quantitative detection of the three RFs were purchased from Aida Gmbh. Serum was incubated in the microplate coated with highly purified Fc fragment of human immunoglobulin (IgG). Patient's antibodies, if present in the specimen, bounded to the antigen. The unbound fraction was washed off in the following step. Afterwards, anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) were incubated and reacted with antigen-antibody complex of the samples in the microplates. Unbound conjugate was washed of in the following step. Addition of TMB-substrate generates an enzymatic colorimetric reaction, which was stopped by diluted acid. The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

For quantitative interpretation, a standard curve was established by plotting the optical density (OD) of each of the provided calibrator (Y-axis) with respect to the corresponding concentration values in U/ml (X-axis). From the OD of each sample, the corresponding antibody concentrations expressed in U/ml, was read. Due to the lack of international calibration, this assay was calibrated in arbitrary units (U/ml) for IgG and IgA rheumatoid factors. For IgM rheumatoid factor, the assay was calibrated against the international WHO standard and results were given in IU/ml

For the evaluation of the diagnostic efficiency of IgM-RF, IgG-RF and IgA assays, receiver operating characteristic (ROC) curve was used. In conventional ROC

curve, the true positive fractions (sensitivity) are plotted against the false positive fractions (1-specificity) at a various cutoff levels. The X and Y axes denote the false positive and true positive fractions, respectively. A major diagonal of the ROC space passing from the left lower corner to the right upper corner serves as the baseline for the points on the ROC curve. The X and Y coordinate points corresponding to or below this diagonal provide no diagnostic value. The X and Y coordinates at an ROC curve must be above this diagonal. The further the ROC curve from this diagonal (or closer to the upper left corner of the ROC space), the better is the diagnostic value of the test [10]. For determination of cutoff value, ROC curve analysis was also used. Taking each observed value (RF-titer), as cutoff, ROC gave sensitivity and specificity of the test under investigation. Diagnostic values of IgM-RF, IgG-RF and IgA are described as sensitivity and specificity with 95% confidence interval (CI). Finally, the statistics analyses were performed using SPSS version 15.

Results

The area under the ROC curve (AUC) is widely recognized as the measure of a diagnostic test's discriminatory power [12]. The maximum value for the AUC is 1.0, thereby indicates a (theoretically) perfect test (i.e., 100% sensitive and 100% specific). An AUC value of 0.5 indicates no discriminative value (i.e., 50% sensitive and 50% specific) and is represented by a straight, diagonal line extending from the lower left corner to the upper right (Fig. 1). There are several scales for AUC value interpretation but, in general, ROC curves with an AUC $\leq$0.75 are not clinically useful and an AUC of 0.97 has a very high clinical value, correlating with likelihood ratios of approximately 10 and 0.1. In this study, for direct comparison of the diagnostic values of IgM-RF, IgG-RF and IgA assay, we performed an ROC analysis. The calculated areas under curves (AUCs) have shown that the three RF-subtypes have variable discriminative values because that their curves were located at different distances above the diagonal line (Fig. 1 and table 1). However, the IgG-RF subtype has the highest discriminative value because of that its AUC was 0.937 and, thus, it has a very high clinical value. IgM-RF subtype has a moderate discriminative value (AUC= 0.848), albeit lower than that of IgG-RF. IgA-RF has showed the least discriminative power (AUC= 0.709) that may make it the least clinically useful RF.

A medical diagnostic test, sensitivity, or true positive rate gives the test's ability to detect the presence of a disease. The true positive rate for the diagnostic test is the total number of patients found to be positive by the diagnostic test divided by the total number of patients who actually have the disease that is being tested for (e.g. if 100 people have a disease and a medical diagnostic test for the disease positively identifies 90 of those people as having the disease, its true positive rate is 90/100). The 10 misdiagnosed cases are termed false negatives. On the other hand, a medical diagnostic test's, specificity, or true negative rate gives the test's ability to detect the absence of a

disease. The true negative rate for the diagnostic test is the total number of patients found to be negative by the diagnostic test divided by the total number of patients who do not have the disease that is being tested for (e.g. if 100 people do not have a disease and a medical diagnostic test for the disease indicates that only 80 of these people are free of the disease, its true negative rate is 80/100). The 20 misdiagnosed cases are termed false positives.

ROC curves display the relationship between sensitivity and 1- specificity across all possible cutoff values that define a positivity of disease or condition. The determination of an "ideal" cut-off value is almost always a trade-off between sensitivity and specificity. As both change with each "cut-off" value it becomes difficult for the reader to imagine which cut-off is ideal. The ROC curve offers a graphical illustration of these trade-offs at each "cut-off" for any diagnostic test that uses a continuous variable [7]. Ideally, the best "cutoff" value provides both the highest sensitivity and the highest specificity, easily located on the ROC curve by finding the highest point on the vertical axis and the furthest to the left on the horizontal axis (upper left corner) (Fig. 1). However, it is rare that this ideal can be achieved, so that, for example, one may opt to choose a higher sensitivity at the cost of lower specificity.

Using ROC curves, all possible cutoff values were examined and found to have variable sensitivities and specificities for each RF subtype. However, some cutoff values have either similar sensitivities or specificities. In those cases only the cutoff values with highest sensitivity and specificity were shown in the Tables [13] and the others were deleted to reduce the size of the tables.

As shown in Tables (2), several cutoff values of IgM-RF have been observed using ROC along with their corresponding sensitivities and specificities. At cutoff value of ≥3.4880 IU/ml showed nearly identical sensitivity (78.5%) and specificity (78.4%). In addition, there are several interesting cutoff values that may be suitable to certain clinical situations. In clinical settings where high specificity is more desired than high sensitivity such as using the test as a confirmatory to another screening test, the cutoff value of ≥11.4175 could be chosen because of its high specificity (94.6%) while maintaining a moderate sensitivity (70.8%). Unfortunately, in clinical situations where the sensitivity is more important (e.g. using the test as screening tool), there is no single cutoff value that showing high sensitivity while maintaining a satisfactory specificity.

As shown in table (3), several cutoff values of IgG-RF have been observed using ROC along with their corresponding sensitivities and specificities. The cutoff value of ≥3.2855 IU/ml showed nearly equal sensitivity (90.8%) and specificity (89.2%). This cutoff value may be considered as the optimal one and may be suitable for different clinical settings. However, there are several other interesting cutoff values that may be more convenient with certain clinical situations. In clinical settings where high specificity is more desired

than high sensitivity such as using the test as a confirmatory to another screening test, the cutoff value of ≥8.1780 U/ml could be chosen because of it displays high specificity (100%) while maintaining a moderate sensitivity (76.9%). Whereas in clinical situations where the sensitivity is more important (e.g. using the test as screening tool), the cutoff value of ≥0.5465 U/ml could be used because of its high sensitivity (93.8%) and satisfactory specificity (86.5%). However, it is not recommended to consider the later cutoff value in clinical practice because of that the sensitivity of the ELISA technique is not reliable for such low titers. Alternatively, the cutoff value of ≥3.2855 IU/ml may be used in this situation.

As shown in Table (4) several cut-off values of IgA-RF have been observed when using ROC analysis along with their corresponding sensitivities and specificities. There is no single cutoff value that shows simultaneous high sensitivity and specificity. With every increase of sensitivity there was a significant drop in the corresponding specificity.

Discussion

This, to the best of our knowledge, is the first study in Iraq to adopt ROC curves analyses for the evaluation of diagnostic performance of serological tests. ROC analysis provides important information about diagnostic test performance: the closer the apex of the curve toward the upper left corner, the greater the discriminatory ability of the test (i.e., the true-positive rate is high and the false-positive [1 – Specificity] rate is low). This is measured quantitatively by the AUC such that a value of >0.96 indicates excellent discriminatory ability [14]. In the current study detection of IgG-RF showed the greater discriminatory ability because of its AUC was very close to the abovementioned value, while the detection of IgA-RF showed the least one. Moreover, IgM-RF was of moderate discriminative power. These results clearly indicate that IgG-RF is the better one in defining RA. We are hopeful that our results can help better understand the value of RF subtypes in RA diagnosis.

For the determination of cutoff value to each RF subtype assay upon its administration on our groups of subjects (RA and non-RA), ROC analysis took each observed value (titer) as cutoff and gave sensitivity and specificity. Accordingly, a wide range of sensitivities and specificities for each RF were calculated during ROC curve analysis. Several of those cutoff values are suggested to be useful in specific clinical settings, other were not. Of note, our results regarding sensitivity and specificity of IgM-RF were consistent with previous abroad studies. Some reports has mentioned that sensitivity and specificity of IgM-RF testing for the diagnosis of RA was (68.6%–82.5%) and (72.1%– 82.7%), respectively, whereas in patients with early disease, IgM-RF revealed a sensitivity of (45.1%–86.1%) [15]. For IgA-RF, our recorded sensitivity is below than the reported by some researchres who reported 72% sensitivity [16] and similar to that reported by others (64.3%)[17]. This discrepancy may attributed to that the above cited studies RFs were measured by

qualitative or semiquantitative methods (such as Waaler-Rose or latex fixation tests). However, our results regarding the sensitivity and specificity of IgM and IgA detection do not underestimate their importance as prognostic markers for disease outcome.

It is important to note that ROC performance may change when the diagnostic test is applied to different clinical situations (e.g. patient populations) or under different phases of disease progression (early diagnosed versus long standing RA). Furthermore, the most useful information from a diagnostic test likely originates by pooling the results for several studies examining the same test in different situations, generating averaged specificity, sensitivity and ROC, so as to be able to get a true understanding of the diagnostic test's utility [13,18]. Therefore, we strongly recommend the administration of ELISA system for the detection of RF in more defined groups of RA patients (e.g. early diagnosed versus long standing) with the use of ROC curve analysis to examine the observed values.

In the end, it will be rare for a diagnostic test to have both 100% specificity and sensitivity. The clinician will have to decide which cut-off value will provide the likelihood ratios and sensitivity and specificity values that have the greatest clinical value in the diagnosis of any disorder.

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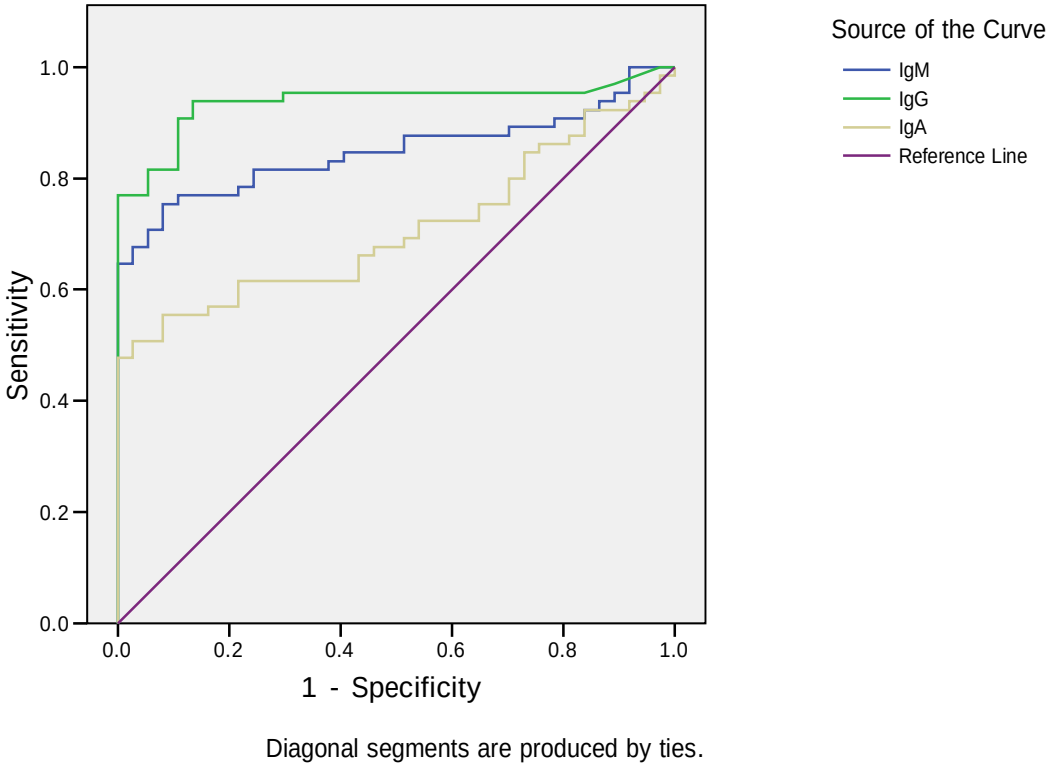

Figure 1 Comparison of the diagnostic values of the Rheumatoid IgM-RF, IgG-RF and IgA-RF subtypes measured using ELISA technique. Receiver operating curves (ROC) of the three tests were shown. The sensitivity of each test is plotted against one minus specificity for varying cutoffs (values lower than the cutoff were considered negative, and other values were considered positive (n =102)

Table 1 Measurements of the Areas Under the Curves of the IgM-RF, IgG-RF and IgA-RF measured using ELISA system.

Test Result Variable(s)	Area under curve (AUC)	Std. Error(a)	Asymptotic Sig.(b)	Asymptotic 95% Confidence Interval	
				Upper Bound	Lower Bound
IgM	.848	.038	.000	.773	.924
IgG	.937	.026	.000	.886	.987
IgA	.709	.050	.000	.610	.807

a Under the nonparametric assumption
b Null hypothesis: true area = 0.5

Table 2 Cut-off values observed using ROC curve analysis of the IgM-RF detected using ELISA system along with their corresponding sensitivity and specificity.

Test Result Variable	Positive if Greater Than or Equal To (Cutoff value)	Sensitivity	Specificity
IgM	.0055	100%	8.1%
	.0200	95.4%	11.8%
	.0270	93.8%	13.5%
	.0380	92.3%	16.2%
	.0630	90.8%	21.6%
	.1395	89.2%	29.7%
	.6825	87.7%	48.6%
	1.0250	84.6%	59.5%
	1.3440	83.1%	62.2%
	3.2315	81.5%	75.7%
	3.4880	78.5%	78.4%
	5.6550	76.9%	89.2%
	6.9985	75.4%	91.9%
	11.4175	70.8%	94.6%
	12.8285	67.7%	97.3%
	13.4945	64.6%	100%

Table 3 Cut-off values observed using ROC curve analysis of the IgG-RF detected using ELISA system along with their corresponding sensitivity and specificity.

Test Result Variable	Positive if Greater Than or Equal To(Cutoff value)	Sensitivity	Specificity
IgG	.0005	100%	2.7%
	.0015	96.9%	10.8%
	.0445	95.4%	70.3%
	.5465	93.8%	86.5%
	3.2855	90.8%	89.2%
	5.1980	81.5%	94.6%
	8.1780	76.9%	100%

Table 4 Cutoff values observed using ROC curve analysis of the IgA-RF detected using ELISA system along with their corresponding sensitivity and specificity.

Test Result Variable	Positive if Greater Than or Equal To(Cutoff value)	Sensitivity	Specificity
IgA	.0245	98.5%	2.7%
	.0805	95.4%	5.4%
	.2355	93.8%	8.1%
	.4615	92.3%	16.2%
	1.4525	87.7%	18.9%
	2.3055	86.2%	24.3%
	2.8285	84.6%	27.0%
	3.5935	80.0%	29.7%
	3.8390	75.4%	35.1%
	4.3180	72.3%	45.9%
	4.5245	69.2%	48.6%
	4.8710	67.7%	54.1%
	5.1465	66.2%	56.8%
	7.9265	61.5%	78.4%
	10.2130	56.9%	83.8%
	10.9640	55.4%	91.9%
	13.6710	50.8%	97.3%
	14.3640	47.7%	100%