Comparison of Rheumatoid Factor Testing Methods: Nephelometric Assay Versus Latex Agglutination Assay

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Abstract : **Rheumatoid factor (RF) is one of the criteria used for diagnosis of rheumatoid arthritis (RA). The method that has been used in our laboratory service for many years is latex agglutination assay that gives semi-quantitative results. We are going to change from this method to nephelometry that gives continuous results. The cut-off point of RF by nephelometry, comparison of these 2 methods and the 4 supplying companies were determined. Serum samples were collected from 70 patients with RA, 22 patients with various collagen diseases and 150 blood donors or normal old people. RF values by nephelometry and 4 commercial latex agglutination assays, that were Latex Alexon, Shield diagnostics, Biosystem, and Behring diagnostics, were determined and compared. The results showed that the cut-off point of RF by nephelometry was 14 IU/ml and the sensitivity and the specificity was 81% and 95% respectively. The sensitivity and the specificity of latex agglutination assays by 4 companies were 76% and 94%, 68% and 97%, 62% and 98% and 64% and 98% respectively. We concluded that nephelometry gave higher sensitivity than latex agglutination assays and Latex Alexon had the highest sensitivity when comparing among the 4 companies.**

เรื่องย่อ : การเปรียบเทียบการตรวจรูมาตอยด์แฟคเตอร์ โดยวิธี Nephelometric assay กับ Latex Agglutination assay

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สารศิริราช 2543; 52: 99-104.

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Received December 2, 1999
Accepted February 4, 2000

รูมาตอยด์แฟคเตอร์ (Rheumatoid Factor = RF) เป็นหนึ่งในหลายข้อที่ถูกกำหนดขึ้นเพื่อช่วยในการวินิจฉัยโรค ข้ออักเสบรูมาตอยด์ การตรวจ RF นั้นมีหลายวิธี สำหรับในหน่วยงานที่ใช้อยู่คือปฏิกิริยาการจับกลุ่ม (Latex agglutination) มาเป็นเวลานานหลายปี และได้พิจารณาที่จะเปลี่ยนการตรวจจากวิธีเดิมมาเป็นวิธีใหม่ซึ่งเรียกว่า Nephelometry ค่าของ RF ที่จะนำมาเพื่อช่วยวินิจฉัยโรคได้ถูกกำหนดหาค่าที่เหมาะสม (cut-off point) และได้ทำการเปรียบเทียบผลการตรวจระหว่าง 2 วิธีดังกล่าวนี้ สำหรับการตรวจโดยวิธีปฏิกิริยาจับกลุ่มได้ทำการเปรียบเทียบระหว่างน้ำยาของ 4 บริษัทด้วยกัน คือ Latex Alexon, Shield Diagnostics, Biosystem, Behring Diagnostics

โดยได้รวบรวมตัวอย่างน้ำเหลือง 70 ตัวอย่างจากผู้ป่วยโรคข้ออักเสบรูมาตอยด์, 22 ตัวอย่างจากผู้ป่วยโรคข้ออักเสบเนื่องจากสาเหตุอื่น และ 150 ตัวอย่างจากผู้บริจาคเลือดหรือผู้ป่วยสูงอายุที่มาทำการตรวจเช็คสุขภาพ ผลการศึกษาพบว่าค่า RF ที่เหมาะสม (cut-off point) โดยวิธี nephelometry คือ 14 หน่วยมาตรฐานต่อลูกบาศก์มิลลิเมตร โดยมีค่าความไวและความจำเพาะอยู่ที่ร้อยละ 81 และร้อยละ 95 ตามลำดับ ขณะที่ค่าความไวและความจำเพาะของวิธีปฏิกิริยาการจับกลุ่มจากอีก 4 บริษัทอยู่ที่ร้อยละ 76 และ 94, ร้อยละ 68 และ 97, ร้อยละ 62 และ 98 และร้อยละ 64 และ 98 ตามลำดับ

สรุปว่าวิธี Nephelomethy จะให้ค่าความไวที่ดีกว่าปฏิกิริยาการจับกลุ่ม และในระหว่างน้ำยาจาก 4 บริษัทที่ตรวจโดยวิธีปฏิกิริยาการจับกลุ่ม Latex Alexon จะให้ค่าความไวสูงที่สุด

INTRODUCTION

Rheumatoid factor (RF) is an immunoglobulin that reacts with the Fc portion of an IgG molecule[1] therefore, RF is an anti-antibody. RF is one of the criterias in American Rheumatism Association (ARA) used for diagnosis of rheumatoid arthritis[2] and also for prognostic prediction[3]. There are several methods that are agglutination (latex agglutination and hemagglutination) and nephelometric methods to determine RF. Latex agglutination has been used in our laboratory service for many years. It takes time to perform the test especially positive results because the tests must be repeated with serial two fold dilution until final titers have been reached before they are reported and needs experience to interpret the positive and negative results (subjective decision). In contrast to latex agglutination, nephelometry is a method used with automated commercial analyzers and the results are quantitative, fast, precise and easy to perform. The results of latex agglutination are reported in titers whereas the ones of nephelometry are reported in continuous values. We are planning to change from latex agglutination assay to nephelometry because of reasons that have been mentioned. Our purpose of the study is to find the cut-off point of RF for diagnosis of patients with suspected of rheumatoid arthritis by nephelometry and to compare the sensitivity and specificity of the tests by nephelometry with the other 4 companies by latex agglutination and among these 4 companies as well.

MATERIALS AND METHODS

Materials

Five milliliter of each serum sample from patients with rheumatoid arthritis(RA), blood donors or normal old people who came to check up (normal control) and patients with various collagen diseases (14 systemic lupus erythematosus, 2 gout, 2 seronegative spondyloarthropathy, 1 reactive arthritis,1 mixed connective tissue disease,1 scleroderma and 1 sjogren disease) were collected and kept at –70°c until examination and each serum sample was examined with tests from all companies at the same time. The sample size was about 70 when expected sensitivity was 80% and acceptable error was 10%. The exclusion criteria was serum samples from adult patients who were diagnosed of rheumatoid arthritis as criteria of ARA including RF positive criteria and from serum samples of blood donors and normal people who had chronic fever or joint arthritis.

Methods

The four companies that commercial latex agglutination assays were purchased from in this study were Latex Alexon, Shield Diagnostics, Biosystem, Behring Diagnostics. Serum dilution was started with titer at 1:20, 1:1, 1:1, and 1:1 for latex agglutination assays from 4 companies respectively and then serial 2-fold dilution was used to continue the tests until the positive results became negative. For nephelometric assay, automated machine from Dade Behring (BN 100, software version N 1.1A) was used in this study. Intra assay variation of 3 defferent RF concentrations at 52, 20, and 510 IU/ml is in the range of 4.2 % to 6.1 % (n = 20) and Inter assay variation (n = 10) of 3 different RF concentrations at 92, 155 and 306 IU/ml) is 9.3 %, 6.2 % and 6.6 % respectively (Data from Dade Behring Inc.). Serum dilution was started with titer at 1:20 and then 1:100, 1:200 and 1:400 by automated machine until RF values could be determined if they were very high. The minimum detection level of RF value was 3 IU/ml and the values less than this level were reported 3 IU/ml.

Principle of the methods

Latex agglutination[4]

This method is an indirect agglutination reaction which in this study latex particles are coated with human gamma globulin. Clumping of antigen and antibody complexes will be seen if a test is positive. They are qualitative tests and semi-quantitative results can be obtained by titration as our study.

Nephelometry[5]

Nephelometry is a direct method of measuring light scattered by particles suspended in solution. The instrument detects the scattered light at an angle different from the incoming light source. The low intensity of scattered light, sometimes not visible to the eye, is measured by a sensitive detector. Latex particles coated with human gamma globulin used in this study bind and form immune complexes with rheumatoid factor, particles capable of scattering light.

Statistical Analysis

The cut-off point of RF values by nephelometry was determined by the optimal point of the highest sensitivity and the specificity which must have been higher than 95% and a likelihood ratio was determined for each level of RF result. The cut-off point of latex agglutination assays used the titer as recommended by each company. Then the sensitivity and specificity of each assay were compared and ROC curve of each test was shown. Mann-Whitney U test was used for comparing RF values from 2 independent groups between male and female and Kruskal-Wallis ANOVA for RF values from among 5 age groups in normal control.

RESULTS

Serum samples from 70 patients with RA, 22 patients with various collagen diseases, and 150 blood donors and normal old people were examined.

Table 1. Baseline characteristics of study groups

	Patients with RA	Patients with non-RA	Blood donors
Number of cases	70	22	150
Age (mean and range)	49.85 (23-81)	34.77 (21-59)	47.85 (21-80)
(SD)	14.02	8.16	12.76
Female/male (%)	65/5 (93%)	19/3 (86%)	71/79 (47%)
RF value (median/range)	104.4 (3-1160)	3 (3-92.5)	3 (3-21.1)

Table 2. Median and Mode of RF values in each age group from blood donors and normal old people

Age (year)	N	Median	Mode
23-30	14	3	3
31-40	27	3	3
41-50	50	3	3
51-60	32	3	3
>60	27	3	3
Total	150	3	3

P value = 0.097

Table 3. Median and mode of RF values in each gender group from blood donors and normal old people

Sex	N	Median	Mode
Female	71	3	3
Male	79	3	3

P=0.951

The sensitivity, specificity and likelihood ratio for each level of RF by nephelometry were shown in table 4 and 5

Table 4. Sensitivity and specificity at each level of RF by nephelomettic assay

RF values (IU/ml)	Sensitivity	Specificity (Blood donor gr)	Specificity (non-RA gr)
4	87 %	77 %	68 %
8	87 %	91 %	77 %
10	85 %	93 %	77 %
14	81 %	95 %	86 %
16	80 %	96 %	86 %
18	78 %	97 %	86 %
20	77 %	99 %	86 %
25	76 %	100 %	86 %

Table 5. Likelihood ratio at each range of RF by nephelometric assay

RF test results	Likelihood ratio
1-13.9	0.2
14-90	3.3
>90	11.3

Table 6. The sensitivity and specificity for each titer level of latex agglutination assay in each company were as follows

Titer	Alexon	Titer	Shield diag	Biosystem	Behring diag
1:20	76 / 94 %	1:1	68 / 97 %	62 / 98 %	64 / 98 %
1:40	70 / 99 %	1:2	60 / 98 %	48 / 99 %	54 / 99 %
1:80	50 / 99 %	1:4	54 / 98 %	37 / 100 %	38 / 100 %
1:160	44 / 100 %	1:8	44 / 99 %	30 / 100 %	31 / 100 %
1:320	24 / 100 %	1:16	27 / 100 %	20 / 100 %	24 / 100 %
1:640	14 / 100 %	1:32	22 / 100 %	8.5 / 100 %	18 / 100 %
1:1,280	1.4 / 100 %	1:64	14 / 100 %	2.8 / 100 %	8.5 / 100 %

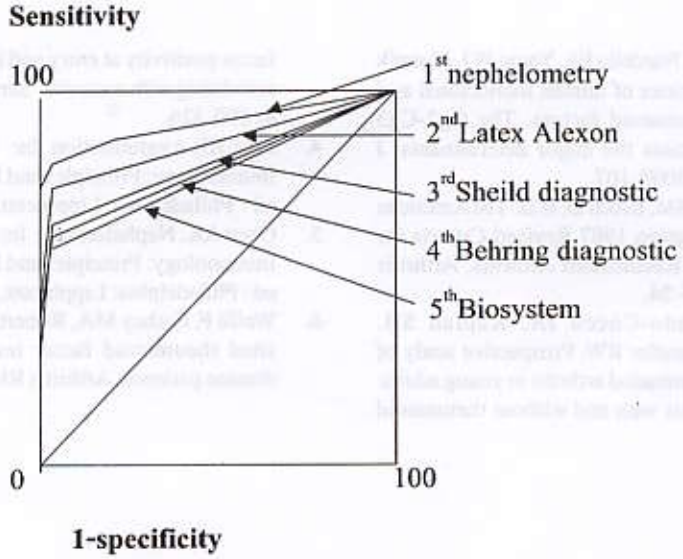

Figure 1. ROC curve

DISCUSSION

As the criteria of ARA for diagnosis of RA, the laboratory test for RF value which will be used to detect the patients who are suspected of RA, should have specificity higher than 95 % in normal people. The cut-off point of RF for diagnosis of RA by nephelometry should be 14 IU/ml (Table 4). We chose the RF value at 14 IU/ml to be the cut-off point, because the specificity was more than 95% as the criteria of ARA and the sensitivity was the highest value among them and the specificity for the non-RA group was also the highest value. Among the 4 companies of latex agglutination assays (Table 6), Alexon had the highest sensitivity, but the specificity was lower than 95 % which was not high enough and if we chose the cut-off point at the titer of 1:40, the specificity was too high so the cut-off point should have been between these two titers and the specificity would be the optimal value. The highest sensitivity of the latex agglutination assays among 4 companies was less than nephelometry even though the specificity of the other three companies were higher than we needed but the serum we had used was the highest concen-tration. So nephelometry had the highest sensitivity and when we made ROC curves (Figure 1), the results were the same.

From the blood donor and normal old people group (Table 2 and 3), the study showed that gender and age had no effect on RF values. It might be that the sample size was not big enough and the sample was not randomized. In the RA group, our data confirms that about 80% of the patients with RA will have positive RF.[6]

CONCLUSION

This study showed us that nephelometry was better than latex agglutination assays when comparing with the 4 companies (Alexon, Shield diagnostics, Biosystem and Behring diagnostics), and the cut-off point of RF value by this method was 14 IU/ml for our patients. Among the 4 companies of latex agglutination assays, Alexon had the highest sensitivity but lowest specificity and it should be the first one to be considered for use if it is necessary to perform tests with latex agglutination assay.

ACKNOWLEDGEMENT

This study was supported by a grant from Siriraj hospital fund.

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