Rheumatoid Factors Determined by Fluorescence Immunoassay: Comparison with Qualitative and Quantitative Methods

A. Betts Carpenter¹,² and Celeste D. Bartkowiak²

Fluorescence immunoassay (FIA), a relatively new technique for measuring rheumatoid factors (RF), is automated, quantitative, and calibrated against the Centers for Disease Control reference material for RF. We studied the FIA method in relation to a panel of RF methods, both qualitative [latex (LA) and sheep cell agglutination (SSCA)], and quantitative [nephelometry and enzyme-linked immunoassay (ELISA)]. Regression analysis revealed a highly significant correlation between FIA and either LA (r = 0.90) or nephelometry (r = 0.87). The correlation between FIA and either SSCA (r = 0.62) or ELISA (r = 0.67) was less strong. FIA had the highest sensitivity (91%) of all these methods; the specificity was 86%. FIA provides an accurate, sensitive, and specific measure of RF, and is a good alternative for laboratories wanting to replace titer methods with automated laboratory analysis.

Additional Keyphrase: latex and sheep cell agglutination, nephelometry, ELISA compared

Rheumatoid factors (RF) are determined by various techniques (1,²). Usually, latex beads or sheep cells coated with gamma globulin are used to provide a semiquantitative assessment of RF, reported in a titer unit. Many inherent problems accompany use of these methods, such as subjectivity, difficulty in standardization, and imprecision. Recent reports highlight the variability in RF determination between laboratories and emphasize the need for more quantitative tests, in which values are determined by objective instrument analysis and reported in international units (2, 3). One quantitative technique, nephelometry, was introduced some years ago and provides an accurate assessment of RF (4-6). Enzyme-linked immunoassays (ELISA) have also been developed and evaluated for clinical use (7, 8). Another quantitative technique, fluorescence immunoassay (FIA), provides an alternative to nephelometry (9, 10). Determination of RF by FIA is attractive because the fluorometer can be used for a variety of clinical assays such as anti-nuclear antibodies, complement, and anti-viral antibodies. Only two reports have evaluated RF by FIA (9, 10); in both it was compared with only a single RF methodology. We evaluated FIA in relation to a panel of RF techniques, both the traditional agglutination procedures (latex and sensitized sheep cell agglutination) and the quantitative techniques (ELISA and nephelometry).

Materials and Methods

Patients. Samples from 210 patients were tested in this study. Our sample population comprised 80 individuals with classic or definite rheumatoid arthritis, 51 with degenerative joint disease (DJD), and 76 ostensibly normal blood donors. Rheumatoid arthritis patients were sequential admissions with a diagnosis of classic or definite rheumatoid arthritis (11). DJD patients were sequential hospital admissions for orthopedic surgery. Normal blood donors were hospital employees with no known history of connective tissue disease.

RF assays. We measured rheumatoid factors by five different assays: Latex agglutination (LA; Behring Diagnostics, La Jolla, CA) was performed following the standard procedure as recommended by the manufacturer. Any titer of 80 or greater was considered positive. The sensitized sheep cell agglutination (SSCA) was performed with the Rheumaton™ Kit from Wampole Laboratories, Cranberry, NJ. The manufacturer's specified procedures were followed; a titer of ≥10 was considered positive. For rate nephelometry we used the Immuno-Chemistry System from Beckman Instruments, Inc., Fullerton, CA. Human IgG was the source of antigen and the calibrators were standardized against the Centers for Disease Control (CDC) material for RF. A value <60 × 10⁸ int. units/L was considered a negative result.

The ELISA followed the method of Teitssen and Valdimarsson (7). Rabbit IgG was adsorbed onto the walls of a microtiter plate overnight at 4°C; patient's serum was added and incubated at room temperature, followed by the addition of peroxidase-conjugated anti-human IgM. This assay was also calibrated against the CDC reference standard for RF and results reported in international units. We tested 105 normal individuals for IgM RF, and used as the upper limit of normal the mean +2 SD from these subjects. Values ≥67 × 10⁸ int. units/L were considered positive.

For FIA we used the FIAX™ test kit (Whittaker Bio-products, Walkersville, MD) in which purified human IgG is coated onto a solid-phase stick. We followed the manufacturer's instructions. The standard curve was calibrated against the CDC reference material for RF, and sample results are reported in international units. IgM RF concentrations of <10 × 10⁸ int. units/L were considered negative, 10 to 15 × 10⁸ int. units/L equivocal, and >15 × 10⁸ int. units/L positive.

Data analysis. Data were analyzed by the use of a linear regression model that included a summary of predictors with multiple observations, interval estimates and residuals, correlation coefficient, standard error of regression, and an inverse prediction. Determination of RF by FIA as compared with the other methods was calculated on a logarithmic scale (base 2 for LA and SSCA titers, base 10 for RF by FIA, nephelometry, and ELISA). Chi-square analysis was used to compare the number of positive and negative values by FIA vs other methods.

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³ Nonstandard abbreviations: RF, rheumatoid factor; ELISA, enzyme-linked immunoassay; FIA, fluorescence immunoassay; LA, latex agglutination; SSCA, sensitized sheep cell agglutination; CDC, Centers for Disease Control; and DJD, degenerative joint disease.

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Table 1. Comparison of Predictive Values of 5 Assays of Rheumatoid Factor

<table>
<thead>
<tr>
<th>Subjects</th>
<th>FIA</th>
<th>Latex</th>
<th>Nephelometry</th>
<th>SSCA</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>91% (73/80)</td>
<td>86% (69/80)</td>
<td>83% (66/80)</td>
<td>76% (62/80)</td>
<td>75% (60/80)</td>
</tr>
<tr>
<td>DJD</td>
<td>88% (44/51)</td>
<td>86% (44/51)</td>
<td>86% (44/51)</td>
<td>92% (47/51)</td>
<td>92% (47/51)</td>
</tr>
<tr>
<td>NBD</td>
<td>96% (73/76)</td>
<td>97% (74/76)</td>
<td>96% (73/76)</td>
<td>99% (75/76)</td>
<td>95% (72/76)</td>
</tr>
</tbody>
</table>

Predictive value of result

| DJD      | 91% (73/80) | 91% (69/76) | 90% (68/73) | 94% (62/66) | 94% (60/64) |
| NBD      | 96% (73/76) | 97% (69/71) | 96% (66/69) | 98% (62/63) | 94% (60/64) |

Predictive value of result

| DJD      | 86% (44/51) | 80% (44/55) | 76% (44/58) | 72% (47/65) | 70% (47/67) |
| NBD      | 91% (73/80) | 87% (74/85) | 84% (73/87) | 81% (75/93) | 79% (72/82) |

Efficiency

| DJD      | 88% (117/131) | 86% (113/131) | 84% (110/131) | 83% (109/131) | 82% (107/131) |
| NBD      | 94% (146/156) | 92% (143/156) | 89% (139/156) | 88% (137/156) | 85% (132/156) |

Results

Regression analysis demonstrated excellent correlation between FIA and LA (r = 0.90, P < 0.001; slope (m) = 0.19 (SD = 0.02); intercept (b) = 0.49 (SD = 0.18); SE of estimate = 0.31), and between FIA and nephelometry (r = 0.87, P < 0.001; m = 0.79 (SD = 0.06); b = 0.32 (SD = 0.16); SE of estimate = 0.24). The correlation between FIA and either SSCA (r = 0.82, P < 0.001; m = 0.15 (SD = 0.02); b = 1.57 (SD = 0.16); SE of estimate = 0.35) or ELISA (r = 0.67, P < 0.001; m = 0.54 (SD = 0.07); b = 1.0 (SD = 0.18); SE of estimate = 0.39) was not as good.

Table 1 summarizes the predictive values of the five RF assays. We calculated these values by using two control groups: DJD patients and normal blood donors. FIA detected the presence of RF in 91% of our rheumatoid arthritis patients, making it the most sensitive test. Comparable results were 86%, 93%, 78%, and 75% sensitivity for LA, nephelometry, SSCA, and ELISA, respectively. Interestingly, even though FIA was the most sensitive test, there was no significant decrease in the specificity for DJD patients (86% for LA, FIA, and nephelometry; 92% for SSCA, ELISA) or normal blood donors (FIA 96% vs 97% (LA), 96% (nephelometry), 99% (SSCA), 95% (ELISA)). The predictive value of a positive test for FIA was 91% with DJD patients as controls, 96% with normal blood donors. These predictive values did not differ significantly from that obtained with the other methods (Table 1). Overall, the efficiency of the FIA was the highest of all assays examined: 89% (DJD control group) or 94% (normal blood donor controls).

To examine further the performance of the FIA, we used a regression model and calculated the summary of predictors with multiple observations. This analysis demonstrated little variance (0.015 to 0.062) over the entire range of RF values detected by FIA. The coefficient of variation varied from 6% to 15% across the entire curve.

One difficulty in instituting a new technique in which values are reported in international units is to gain its acceptance from clinicians, who then must adjust to an unfamiliar unit of measurement. One way to ease this transition is to provide a conversion between titer and international units. Reportedly (10), FIA RF values can be converted into an equivalent LA titer by using a log-log plot and a conversion equation generated by estimation of the linear least-squares regression. We attempted this analysis with our data, but found that, even though our correlation coefficient was quite high (r = 0.90, P < 0.001), the standard error of regression (0.31) was too large to allow a meaningful correlation between the two methods. Table 2, which gives the 95% confidence limits for the prediction of an LA value from a given FIA value, demonstrates that we were not able to predict accurately a latex titer from an FIA value. For example, an FIA value of 100 × 10^6 int. units/L could correspond to a latex titer ranging from 20 to 2560, making the conversion value clinically useless.

Discussion

The correlation between FIA and either ELISA or SSCA was not as high as between the other methods, most likely owing to the source of antigen used in these assays: rabbit IgG in ELISA and SSCA, human IgG in FIA. Human RF have a different specificity for the two antigen sources. The reactivity with rabbit IgG is generally thought to represent a cross-reactivity and in many rheumatoid sera constitutes only a minor proportion of the anti-IgG reactivity (1).

FIA and nephelometry are comparable in terms of standardization, automation, and improved reproducibility, but FIA can detect RF concentrations as low as 10 × 10^6 int. units/L. Our nephelometer only measured to 60 × 10^6 int. units/L, equivalent to a latex titer of 160. Therefore, a nephelometric value equivalent to a titer of 80, which is sufficient to satisfy one of the 1956 American Rheumatism Association criteria for rheumatoid arthritis (11), may be reported as negative. In addition, one can also obtain a falsely negative RF by nephelometry in about 2% of specimens because of formation of large aggregating immune complexes (6). Therefore, when the nephelometer is used, our laboratory and others (6) screen all sera initially by latex and use the nephelometer for quantification. Recently, the nephelometric procedure used in this study has been modified to measure as little as 30 × 10^6 int. units of RF per liter. However, this improved technique has not been tested in comparison with the FIA.

We acknowledge the technical assistance of Linda Waxter and Cindy Gubbianelli, and thank Dr. Leslie Kahl for clinical assistance and Dr. Floyd Taylor for help with statistical analysis. The secretarial expertise of Vicky Beyer was also greatly appreciated.

Table 2. Prediction of Latex Titer from a Given FIA Value

<table>
<thead>
<tr>
<th>FIA value, int. units/L × 10^6</th>
<th>Predicted latex value</th>
<th>95% confidence limits, latex titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
<td>10 160</td>
</tr>
<tr>
<td>100</td>
<td>160</td>
<td>20 2560</td>
</tr>
<tr>
<td>1000</td>
<td>5120</td>
<td>640 81 920</td>
</tr>
</tbody>
</table>

* Rounded to closest titer value.
Erythrocyte Sedimentation Rate and C-Reactive Protein Compared in the Elderly

Paul R. Katz,1 Steven I. Gutman,2 Gary Richman,3 Jurgis Karuza,4 William R. Bartholomew,5 and John Baum6

The erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) concentrations were studied in 101 elderly individuals (mean age 72 y) to determine their utility as diagnostic aids in subjects with underlying infection/inflammation. Whereas ESR and CRP were both significantly increased in patients with infection or inflammation, or both, analysis of variance indicated that those subjects still alive six months later had significantly lower ESR values. Analysis of sensitivity, specificity, and positive predictive values indicated that neither test satisfactorily discriminated between patients with and those without ongoing acute or chronic disease. Receiver-operating characteristic curve analysis confirmed the low true-positive/false-positive ratios of both ESR and CRP. In the elderly, neither CRP nor ESR has distinct advantages over the other, and both tests evidently have limited utility.

Additional Keyphrases: geriatric chemistry · distinguishing acute and chronic disease, functional and organic disease · receiver-operating characteristic curves

Diagnosis of disease processes in the elderly is often confounded by subtle and nonspecific symptomatology. As a result, discriminating between functional and organic conditions in this population may be difficult. Two nonspecific measures for disease activity have been advocated for use in this diagnostic setting: the erythrocyte sedimentation rate (ESR) and the C-reactive protein (CRP) (1–4). Although both are widely available and are used interchangeably, whether one is superior in the elderly remains controversial. Furthermore, the increased cost of CRP relative to ESR raises questions of cost effectiveness. Kenny et al. (5), in a small retrospective study comparing ESR and CRP in acutely ill geriatric patients, found CRP to be superior to ESR as an objective, nonspecific maker for disease activity. Nevertheless, sensitivity, specificity, and predictive values were not reported, and problems with both false-negative and false-positive results were noted. Our study was designed to further evaluate the relative value of CRP vs ESR as diagnostic aids for identifying underlying infection/inflammation in an elderly population receiving different levels of care and with acute and chronic disease.

For the efficacy of a clinical test to be adequately evaluated, a "gold standard" needs to be defined. For the purposes of the present study, a thorough chart review, coupled with patient examination where necessary, defined the gold standard for patients with or without infection/inflammation. It is equally important to consider not only the extent to which the clinical tests are able to detect infection/inflammation when truly present (i.e., test sensitivity) but also the extent to which the clinical test correctly identifies the absence of disease (i.e., specificity). Both of these test properties are stable and do not change when different proportions of diseased and well patients are tested (6).

The primary purpose of this study was not to absolutely quantify the diagnostic utility of ESR or CRP. Rather, it was our intention to consider the relative differences in sensitivity and specificity of the ESR and CRP in the same population sample. Because ESR and CRP assays were performed on the same population sample, the prevalence of disease was identical and, therefor, subject classification problems were controlled for the two tests.

1 Nonstandard abbreviations: ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; and ROC, receiver-operating characteristic (curve).