Rheumatoid factor isotypes in rheumatoid arthritis diagnosis and prognosis: a systematic review and meta-analysis

Francesca Motta, Nicola Bizzaro, Davide Giavarina, Franco Franceschini, Maria Infantino, Boaz Palterer, Gian Domenico Sebastiani, Carlo Selmi

ABSTRACT

Objective The first biomarker associated with rheumatoid arthritis is rheumatoid factor (RF) and since the earliest reports a role has been proposed in the diagnosis and in the prediction of clinical features and outcome. The study of RF isotypes has further attempted to improve diagnostic accuracy and identify specific subgroups of patients. The main objective of this study is to provide an analysis of the literature on the role of RF isotypes in the diagnosis and prognosis of rheumatoid arthritis (RA).

Methods We performed a systematic literature review and meta-analysis on the role of RF isotypes in RA (only in English, from PubMed, search terms: “rheumatoid factor isotypes”, “diagnosis”, “prognosis” and “rheumatoid arthritis”, last search 31 July 2022, two independent assessment of quality and biases, results included in tables and in the meta-analysis).

Results Thirty-six articles were examined (7517 patients). Testing all RF isotypes with latex test or nephelometry allows for the highest sensitivity, while determination of IgA isotype provides the highest specificity (91.4%, 95% CI 90.8% to 92.0%) and the highest positive likelihood ratio (7.7, 95% CI 5.7 to 10.4). When testing IgM isotype the highest specificity (91.4%, 95% CI 90.8% to 92.0%) and the highest positive likelihood ratio (7.7, 95% CI 5.7 to 10.4). When testing IgG isotype the highest specificity (91.4%, 95% CI 90.8% to 92.0%) and the highest positive likelihood ratio (7.7, 95% CI 5.7 to 10.4). When testing IgM isotype the highest specificity (91.4%, 95% CI 90.8% to 92.0%) and the highest positive likelihood ratio (7.7, 95% CI 5.7 to 10.4). When testing IgG isotype the highest specificity (91.4%, 95% CI 90.8% to 92.0%) and the highest positive likelihood ratio (7.7, 95% CI 5.7 to 10.4). When testing IgM isotype the highest specificity (91.4%, 95% CI 90.8% to 92.0%) and the highest positive likelihood ratio (7.7, 95% CI 5.7 to 10.4). When testing IgG isotype the highest specificity (91.4%, 95% CI 90.8% to 92.0%) and the highest positive likelihood ratio (7.7, 95% CI 5.7 to 10.4). When testing IgM isotype the highest specificity (91.4%, 95% CI 90.8% to 92.0%) and the highest positive likelihood ratio (7.7, 95% CI 5.7 to 10.4).

Conclusions Testing RF allows the highest sensitivity, while IgA isotype the highest specificity and positive likelihood ratio for RA diagnosis. On the other hand, determination of RF isotypes does not allow prognostic information, as data are limited and heterogeneous.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting 0.5%–1% of the population worldwide. The disease mainly involves the joints potentially leading to functional disability but can also present extra-articular manifestations in 40% of cases.1 Rheumatoid factor (RF) includes antibodies which recognises the fragment crystallisable region of IgG and represent the first biomarkers detected in RA.2 RF was first described in 1940 by Waaler, as a factor with haemagglutinating activity in the serum of a patient with RA. In fact, RF agglutinates sheep red blood cells sensitised with rabbit IgG (the...
classic Waaler-Rose test). It was then named by Pike in 1949 for its associations with RA.

Agglutination techniques were initially used to detect RF, based on the ability of IgM to induce agglutination. Then, the technique was developed with other IgG carriers such as bentonite and latex particles. Automated methods as nephelometry and ELISA gradually replaced it, for their simplicity and greater reproducibility. Improved techniques for the detection of RF have been developed also for the important role of RF in the diagnosis of RA, as it was included in the 1987 American College of Rheumatology (ACR) classification criteria for RA. However, its relevance has been partly reconsidered as it is not uncommon in other diseases, including Sjögren’s syndrome, systemic lupus erythematosus, in chronic infectious diseases as syphilis, tuberculosis, liver diseases and in healthy population, with increased prevalence in the elderly.

RF role in RA has been partly overtaken by anti-citrullinated protein antibodies—ACPAs, also named anti-cyclic citrullinated peptide antibodies—with high specificity for the disease. Nonetheless, several analyses have demonstrated the important role of RF, also complementary to ACPAs, as described below.

Consistent with this, in the 2010 ACR/European Alliance of Associations for Rheumatology classification criteria for RA, RF and ACPA are considered equivalent disease markers, and RF positivity allows the same score as ACPA positivity. In fact, in patients with synovitis, ACPA status does not add information for classification as RA beyond those that are provided by a positive RF, despite ACPA being more specific.

Currently, the sensitivity of RF for RA is estimated to be around 41%–66% for early RA and 62%–87% for established RA, while the specificity accounts for 43%–96%, with most studies reporting specificity higher than 70%, as described below. Moreover, the identification of IgM-RF, IgA-RF and IgG-RF isotypes has allowed a more subtle evaluation of the role of RF in different aspects of the disease. Several studies have evaluated whether one or more isotypes could be relevant for the diagnosis and management of RA, but a comprehensive evaluation of these studies has not been performed.

The aim of this systematic literature review and meta-analysis is to provide a comprehensive analysis of the literature reported to date on the role of RF and its isotypes in the diagnosis and prognosis of RA, in order to identify their role in the disease in terms of diagnostic accuracy and prognostic capability.

METHODS

Study search strategy and selection

The Medline database was accessed from PubMed and systematically searched for articles published in English. The search strategy was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses recommendations to increase standardisation and quality in reporting. We followed the search strategy and article selection process illustrated in the flow charts in online supplemental figures 1 and 2, using the search terms: “rheumatoid factor isotypes”, “diagnosis”, “prognosis” and “rheumatoid arthritis”. The search strings used for each association are detailed in the respective flowchart. Only peer-reviewed articles in English accepted for publication were included in this search. The last search was run on 31 July 2022. From the initial search, we excluded papers not appropriate for the study design as ascertained by title and abstract. Reviews and case reports were also excluded. FM and NB have independently assessed the quality of the studies and the risk of biases, screened the full text reports and decided whether these met the inclusion criteria while resolving any disagreement through discussions. Neither of the authors were blind to the journal titles or to the study authors or institutions.

Finally, 36 studies providing data on RF isotypes were considered eligible for inclusion in the data analysis. All papers were assessed for methodological quality and statistical data on diagnostic accuracy of the tests.

Data extraction

Data retrieved from the articles included criteria for patient recruitment; the number of patients and healthy subjects enrolled; demographic and clinical data on the population investigated; the RF isotypes studied; specification of the analytical method used to detect RF isotypes (either in-house or commercial and, in this latter case, subdivided by the manufacturer); the cutoff used; sensitivity and specificity of the assay, and the number of true positive, false negative, true negative and false positive results. Missing data were calculated from the data available. Regarding the role of isotypes in defining prognosis, disease activity, radiographic progression and response to treatment were chosen. Data were reported in online supplemental tables 5–7 and were only described, to avoid risk of biases in a statistical analysis. Any missing or unclear information was not considered. Studies performed on the same population for comparison of two or more analytical methods were maintained. Therefore, the number of studies exceeds the number of selected articles.

Statistical analysis

Data were analysed with Meta-DiSc (V.1.4, Cochrane Colloquium, Barcelona, Spain) using the statistical model previously described. The accuracy of each study was measured as sensitivity, specificity, positive and negative likelihood ratio (LR+ and LR−) and diagnostic OR (DOR). Sensitivity was considered as the proportion of positives among people with disease, and specificity the proportion of negatives among people without disease. The LR express how much more frequent the respective result is among subjects with disease than among subjects without disease; LR+ was calculated by dividing the pooled sensitivity by 1-specificity; LR− was calculated as...
by dividing 1-sensitivity by specificity. The DOR expresses how much greater the odds of having the disease are for subjects with a positive test than for subjects with a negative test result. The overall DOR was calculated by combining each study’s DOR, using a random-effects model. A correction factor of one-half was added to each cell to avoid calculation problems by having a value of 0 in the 2×2 table.

The threshold effect heterogeneity, that is, the differences in sensitivity and specificity occurring for the different cut-offs used in the studies, was explored by representation of accuracy estimates from each study in a summary receiver operating characteristic (sROC) space, and by calculating the Spearman correlation coefficient between the logit of sensitivity and logit of 1-specificity.

The sROC curve analysis was relied on a regression analysis of logit transformation of the data, which plots the difference (D) between the logit of the true-positive (TPR) and the logit of the false-positive (FPR) rates (D=logit TPR–logit FPR) on the y axis and the sum (S=logit TPR+logit FPR) on the x axis. The y axis (D) is equivalent to the log DOR, and the x axis (S) is a measure of how test features vary with test threshold. A regression equation (D = α + β ∙ S) derived from the sROC curve analysis can be used to determine the heterogeneity among study results. If the β coefficient is near zero and not statistically significant, then significant heterogeneity is not present. The symmetric sROC curve was created using the overall DOR of all the studies. The asymmetric sROC curve was generated using the (unweighted) method as described by Moses et al. 12

The heterogeneity between the studies, other than threshold effect, was explored using the Cochran’s Q statistic and the Inconsistency index. DerSimonian and Laird methods13 were used to pool data in a random effects model. Forest plots and summary estimates with their 95% CI were created by constructing sROC regression curves, using each study’s paired sensitivity and specificity. The area under the curve (AUC) was also calculated.

Other potential causes for heterogeneity (such as variations in age of subjects or differences in analytical methods) were evaluated using subgroup and meta-regression analyses using DOR as a global measure of accuracy and also as a method to compare the overall diagnostic accuracy of different tests, according with Littenberg and Moses linear model. This model compares DOR in the subgroups, to evaluate significant differences. The outputs of meta-regression are ratios of DOR. If a specific study level covariate is significantly associated with diagnostic accuracy, then its coefficient will have a low p value, and the DOR ratio will give a measure of magnitude of the association. A relative DOR<1.0 indicates that studies with a particular feature have a lower DOR than studies without this feature.
RESULTS
Diagnostic accuracy of RF isotypes in RA

Our review included 19 articles related to diagnostic accuracy of RF isotypes, which involved 4786 patients and 6994 controls.14–31 The IgM isotype was tested in 17/19 (89.4%) studies, IgA in 19/19 (100%) and IgG in 14/19 (73.6%). A solid phase immunoenzymatic test (ELISA) was used in 15/19 (78.9%) studies, an immunofluorimetric assay (fluorimetric enzyme-linked immunoassay, FEIA) was used in 5/19 (26.3%). In 8/19 (42.1%) articles RF was also tested by latex test (3/8–37.5%) or nephelometry (5/8–62.5%), which include all isotypes. Two articles18 31 described different analytical methods performed on the same population and were therefore considered more than once. Missing data were calculated from the data available. Of the ELISA methods used, 5 were manufactured in-house and 16 by seven different commercial companies. Data regarding RF isotypes retrieved from the articles included are detailed in online supplemental tables 1–4.

A pooled analysis of data from the studies was performed for each isotype.

When considering the detection of all isotypes (by latex test or nephelometry), described in eight articles analysing nine methods, RF had an overall sensitivity of 68.6% (95% CI 66.2% to 71.0%) and a specificity of 88.3% (95% CI 87.1% to 89.4%); DOR was 18 (95% CI 10.7 to 30.5) (figure 1). The overall positive LR for having RA in RF-positive patients was 5.8 (95% CI 3.6 to 9.5) and negative LR was 0.34 (95% CI 0.25 to 0.43). The AUC for symmetric sROC curve was 0.859, while it was 0.853 for the asymmetric sROC curve, indicating that a very moderate threshold effect was present and that the modest heterogeneity was due to the low number of studies, whereas most studies gave homogeneous results (online supplemental figure 3).

The diagnostic accuracy of testing IgM isotype alone (by ELISA or FEIA) was described in 17/19 articles. IgM-RF had an overall sensitivity of 63.4% (95% CI 62.1% to 64.6%) and a specificity of 90% (95% CI 89.5% to 90.7%); DOR was 21.7 (95% CI 16.1 to 29.3) (figure 2). Positive LR for having RA in IgM-RF-positive subjects was 7.09 (95% CI 5.5 to 9.1) and negative LR was 0.35 (95% CI 0.31 to 0.39). The AUC for symmetric sROC curve was
Rheumatoid arthritis

0.855, while it was 0.846 for the asymmetric sROC curve (online supplemental figure 4).

IgA isotype was studied in all the 19 articles. IgA-RF sensitivity was 49.1% (95% CI 48% to 50%) and specificity was 91.4% (95% CI 90.8% to 92.0%); DOR was 16.0 (95% CI 11.3 to 22.7) (figure 3). Positive LR was 7.7 (95% CI 5.7 to 10.4) and negative LR was 0.50 (95% CI 0.47 to 0.55). The AUC for symmetric sROC curve was 0.781, while it was 0.772 for the asymmetric sROC curve (online supplemental figure 5).

IgG-RF was analysed in 14 articles. Its sensitivity was 39.9% (95% CI 38.3% to 41.5%), with a specificity of 78.9% (95% CI 77.7% to 80.0%); DOR was 14.2 (95% CI 7.2 to 28.1) (figure 4). Positive LR was 7.4 (95% CI 3.4 to 16.2), negative LR was 0.56 (95% CI 0.48 to 0.66). The AUC for symmetric sROC curve was 0.831, while it was 0.830 for the asymmetric sROC curve (online supplemental figure 6).

To summarise, nine studies analysed the diagnostic accuracy of testing all the three isotypes by nephelometry or latex test, 17 articles studied IgM-RF, 19 IgA-RF and 14 IgG-RF isotypes tested by ELISA or FEIA. The highest sensitivity was achieved when all three isotypes were tested by nephelometry or latex test (68.6%, 95% CI 66.2% to 71.0%), while the highest specificity was reached by IgA-RF (91.4%, 95% CI 90.8% to 92.0%). DOR was greater when IgM-RF was tested (21.7, 95% CI 16.1 to 29.3). LR+ and LR− were more relevant for IgA-RF (7.7, 95% CI 5.7 to 10.4) and IgM-RF (0.35, 95% CI 0.31 to 0.39), respectively; the ROC AUC was greatest when all three isotypes were tested (0.859) (table 1).

All eight studies that analysed nephelometry or latex test reported significant diagnostic value. Even when compared with ACPA testing alone, RF isotypes increased diagnostic accuracy, as IgM-RF (91.4%, 95% CI 90.8% to 92.0%) and IgA-RF (91.4%, 95% CI 90.8% to 92.0%) added diagnostic value in 8 (61.5%) of them and IgG-RF (91.4%, 95% CI 90.8% to 92.0%) in 7/14 studies (50%). IgG-RF added diagnostic information in only 1/11 (9.1%) of the articles included (table 2).

Role of RF isotypes in RA prognosis

Seventeen articles related to RF isotypes in predicting prognosis were analysed, involving 2731 patients. IgM-RF was tested in 12/17 (70.5%) studies, IgA in 17/17 (100%) and IgG in 11/17 (64.7%). ELISA was used to determine isotypes in all but one study (94.1%),
where FEIA was used. Of the ELISA methods used, 7 were manufactured in-house and 10 by eight different companies.

Correlation of IgM, IgA and IgG-RF isotypes with disease activity, radiographic progression and response to treatment were evaluated for each article. As studies used different criteria to define outcomes, pooled analysis has not been performed and results are detailed in online supplemental tables 5–7. Overall, IgM-RF was associated with disease activity in 3/5 studies (60%), IgA-RF in 4/8 (50%), IgG-RF in 1/3 (33.3%). Radiographic progression was associated to IgM-RF in 3/9 (33.3%) studies, to IgA-RF in 6/12 (50%) and to IgG-RF in 2/8 (25%). IgM-RF isotype predicted response to treatment in none of the 5 articles which analysed the issue, IgA-RF in 3/6 (50%) and IgG-RF in 1/4 (25%).

To recapitulate, results on the role of RF isotypes for RA prognosis are conflicting and not comparable, as not all isotypes were always considered and outcomes were defined differently. Therefore, data

Table 1  Summary of sensitivity, specificity, positive and negative likelihood ratio, diagnostic OR, and AUC described in the included studies for each RF isotype

<table>
<thead>
<tr>
<th>Test</th>
<th>All three isotypes</th>
<th>IgM-RF</th>
<th>IgA-RF</th>
<th>IgG-RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of studies</td>
<td>8</td>
<td>17</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>Sensitivity % (95% CI)</td>
<td>68.6 (66.2 to 71.0)</td>
<td>63.4 (62.1 to 64.6)</td>
<td>49.1 (47.8 to 50.4)</td>
<td>39.9 (38.3 to 41.5)</td>
</tr>
<tr>
<td>Specificity % (95% CI)</td>
<td>88.3 (87.1 to 89.4)</td>
<td>90 (89.5 to 90.7)</td>
<td>91.4 (90.8 to 92.0)</td>
<td>78.9 (77.7 to 80.0)</td>
</tr>
<tr>
<td>Likelihood ratio+ (95% CI)</td>
<td>5.8 (3.6 to 9.5)</td>
<td>7.09 (5.5 to 9.1)</td>
<td>7.7 (5.7 to 10.4)</td>
<td>7.4 (3.4 to 16.2)</td>
</tr>
<tr>
<td>Likelihood ratio− (95% CI)</td>
<td>0.3 (0.25 to 0.43)</td>
<td>0.35 (0.31 to 0.39)</td>
<td>0.50 (0.47 to 0.55)</td>
<td>0.56 (0.48 to 0.66)</td>
</tr>
<tr>
<td>Diagnostic OR (95% CI)</td>
<td>18 (10.7 to 30.5)</td>
<td>21.7 (16.1 to 29.3)</td>
<td>16.0 (11.3 to 22.7)</td>
<td>14.2 (7.2 to 28.1)</td>
</tr>
<tr>
<td>AUC ROC (95% CI)</td>
<td>0.859 (0.856 to 0.862)</td>
<td>0.855 (0.85 to 0.860)</td>
<td>0.781 (0.779 to 0.791)</td>
<td>0.831 (0.826 to 0.836)</td>
</tr>
</tbody>
</table>

AUC, area under the curve; FEIA, fluorimetric enzyme-linked immunoassay; RF, rheumatoid factor; ROC, receiver operating characteristic.
<table>
<thead>
<tr>
<th>Study</th>
<th>Nephelometry/latex test</th>
<th>IgM</th>
<th>IgA</th>
<th>IgG</th>
<th>Median age of patients (range or SD)</th>
<th>Time from diagnosis</th>
<th>Median age of controls (range or SD)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visser 1996</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>60 (16–89)</td>
<td>6 months (median)</td>
<td>47 (12–92)</td>
<td>Combination more predictive of RA: nephelometry+IgM RF (sensitivity +15%, specificity –7%).</td>
</tr>
<tr>
<td>Saraux 2002</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>49 (33–65)</td>
<td>n.a.</td>
<td>n.a</td>
<td></td>
</tr>
<tr>
<td>Bas 2002</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>64 (22–87)</td>
<td>n.a.</td>
<td>58 (4–191)</td>
<td>IgM-RF+IgA-RF: increased LR for RA.</td>
</tr>
<tr>
<td>Bas 2003</td>
<td>–</td>
<td>No</td>
<td>Yes</td>
<td>–</td>
<td>54 (22–77)</td>
<td>n.a.</td>
<td>42 (4–101) DC 60 (50–75) HC CCP+IgA-RF: increased specificity for RA diagnosis (CCP specificity 90%, IgA-RF 90%, IgM-RF 82%; CCP+IgA RF 98%; CCP+IgA-RF+IgM RF 98%).</td>
<td></td>
</tr>
<tr>
<td>Rantapää-Dahlqvist 2003</td>
<td>–</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>54 (31–77)</td>
<td>3 years (median)</td>
<td>55 (30–69)</td>
<td>IgA-RF predicts RA diagnosis.</td>
</tr>
<tr>
<td>Vallbracht 2004</td>
<td>–</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>62 (±15)</td>
<td>n.a.</td>
<td>56 (±18) 52 (±19) 50 (±20)</td>
<td></td>
</tr>
<tr>
<td>Greiner 2005</td>
<td>Yes</td>
<td>–</td>
<td>n.a.</td>
<td>–</td>
<td>58.6 (19–84)</td>
<td>n.a.</td>
<td>n.a</td>
<td>CCP sensitivity 62%, specificity 94%. IgM-RF sensitivity 70%, specificity 80%. IgA-RF sensitivity 65%, specificity 87%. IgG-RF sensitivity 38%, specificity 85%. CCP+IgM RF sensitivity 55%, specificity 98%. CCP+IgA RF sensitivity 59%, specificity 98%.</td>
</tr>
<tr>
<td>Mikuls 2006</td>
<td>–</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>51 (±13)</td>
<td>&lt;2 years</td>
<td>45 (±14) HC 39 (±12) SLE</td>
<td></td>
</tr>
<tr>
<td>Bobbio Pallavicini 2007</td>
<td>Yes</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>57 (±12)</td>
<td>8.3 (±6.9)</td>
<td>n.a</td>
<td>In 48 CCP-negative subjects, 57% were FR positive (IgM-RF+IgA RF or triple positive).</td>
</tr>
<tr>
<td>Jaskowski 2010</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>n.a.</td>
<td>8.6 years (mean)</td>
<td>33 (16–70)</td>
<td></td>
</tr>
<tr>
<td>Nell-Duxneuner 2010</td>
<td>Yes</td>
<td>–</td>
<td>No</td>
<td>No</td>
<td>50 (18–83)</td>
<td>&lt; 3 months</td>
<td>43 (18–87)</td>
<td></td>
</tr>
</tbody>
</table>

Continued
<table>
<thead>
<tr>
<th>Study</th>
<th>Nephelometry/latex test</th>
<th>IgM</th>
<th>IgA</th>
<th>IgG</th>
<th>Median age of patients (range or SD)</th>
<th>Time from diagnosis</th>
<th>Median age of controls (range or SD)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brink 2016&lt;sup&gt;21&lt;/sup&gt;</td>
<td>–</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>50.3 (IQR 20.1)</td>
<td>6.2 years (median)</td>
<td>51.1 (IQR 20)</td>
<td>In pre-symptomatic individuals, OR IgM-RF 11.1, IgM-RF+IgA RF 21.9, IgM-RF+IgA-RF+IgG RF 34.5. OR CCP 20.2. OR CCP+IgA RF 50.7, CCP+IgG RF 30.2, CCP+IgM RF 67.6.</td>
</tr>
<tr>
<td>Sieghart 2018&lt;sup&gt;23&lt;/sup&gt;</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>57 (47–66) EA 53 (44–63) RA</td>
<td>&lt;3 months</td>
<td>55 (43–64) DC 50 (42.55) HC</td>
<td>14/290 (4.8%) patients negative at nephelometry resulted positive for IgM-RF. 14/109 (12.8%) ACPA-IgM-RF negative were IgA-RF+.</td>
</tr>
<tr>
<td>Elshafie 2019&lt;sup&gt;15&lt;/sup&gt;</td>
<td>–</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>49 (18–80)</td>
<td>3 years</td>
<td>35 (n.a.)</td>
<td>CCP sensitivity 70.7%. CCP+IgM RF sensitivity 80.1%. CCP+IgM-RF+IgA RF sensitivity 89.5%. CCP+IgM-RF+IgA-RF+IgG RF sensitivity 100%.</td>
</tr>
<tr>
<td>Kelmenson 2020&lt;sup&gt;24&lt;/sup&gt;</td>
<td>–</td>
<td>n.a</td>
<td>n.a</td>
<td>n.a</td>
<td>37 (±8)</td>
<td>5.1 years</td>
<td>37 (±8)</td>
<td></td>
</tr>
<tr>
<td>Janssen 2020&lt;sup&gt;28&lt;/sup&gt;</td>
<td>–</td>
<td>n.a</td>
<td>n.a</td>
<td>–</td>
<td>55 (±11)</td>
<td>5.5 years (3–10)</td>
<td>42 (±15) DC 34 (±15) HC</td>
<td></td>
</tr>
<tr>
<td>Hermosillo-Villafranca 2021&lt;sup&gt;25&lt;/sup&gt;</td>
<td>–</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>n.a.</td>
<td>n.a</td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>Pertsinidou 2021&lt;sup&gt;29&lt;/sup&gt;</td>
<td>–</td>
<td>n.a</td>
<td>n.a</td>
<td>n.a</td>
<td>57 M/54 F (18–70)</td>
<td>&lt;2 months</td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>Van Hoovels 2022&lt;sup&gt;31&lt;/sup&gt;</td>
<td>–</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>57 (18–86)</td>
<td>16.8%&lt;6 weeks 83.2%≥6 weeks</td>
<td>53 (11–92)</td>
<td>IgA RF picked up 0.5% or 2.3% (Thermo Fisher or Orgentec assay, respectively) RA patients that were ACPA and IgM RF negative. Single IgA RF positivity was found in 1.5% or 5.6% (Thermo Fisher or Orgentec assay, respectively) of controls. CCP-high titers RF (IgA or IgM) LR 62-infinity.</td>
</tr>
</tbody>
</table>

CCP, cyclic citrullinated peptide antibodies; DC, disease controls; EA, early arthritis; HC, healthy controls; Ig, Immunoglobulin; LR, likelihood ratio; n.a., not applicable; RA, rheumatoid arthritis; RF, rheumatoid factor; SLE, systemic lupus erythematosus.
DISCUSSION

This is the first systematic review and meta-analysis addressing the role of RF isotypes in RA diagnosis and prognosis. This is of relevance since RF is included in the classification criteria for RA with the same importance of ACPA and its levels are relevant for the classification criteria, as values more than three times the upper limit of normal for the laboratory and the assay account for half the score required to classify an arthritis as RA. No specific assay is suggested to determine RF, in agreement with the finding that none proved globally superior to the others. Nonetheless, the isotype determination is not required in the classification criteria but according to the results of this meta-analysis this could be of some relevance for RA diagnosis. Whether testing all RF isotypes with latex test or nephelometry allows for the highest sensitivity (68.6%, 95% CI 66.2 to 71.0), the determination of IgA isotype provides the highest specificity (91.4%, 95% CI 90.8 to 92.0) and the highest LR+ (7.7, 95% CI 5.7 to 10.4), while the determination of IgM isotype provides the higher DOR (21.7, 95% CI 16.1 to 29.3). Further, even when testing ACPA, RF isotype determination appears to increase diagnostic accuracy.

Results on specificity deserve a separate comment, as a few studies show low RF specificity. Poor quality of the assay, low cut-off levels or inappropriate control cohorts could account for these results. Similarly, a paper showed a very high specificity with 95% CI 88% to 100%, likely due to the low number of healthy controls. We included all these studies in our analysis to provide a comprehensive review, despite some data should be critically evaluated. Further, relevant data can be gathered, as prognostic information according to RF isotype presence (online supplemental tables 5–7).

From the results of our study, therefore, a relevant role of RF isotypes could be in the evaluation of seronegative arthritis, adding further information on the serologic state of patients and potentially modifying the diagnosis to RA. In patients with synovitis, ACPA positivity alone allows higher specificity than RF positivity alone. However, when ACPA are negative, positivity for multiple RF isotypes allows higher specificity, despite being rarely seen in ACPA negative patients. This could be particularly relevant for early arthritis, where a portion of patients might be seronegative when testing RF and ACPA, but might reveal positive if tested for RF isotypes, thereby modifying the diagnosis. Reaching a prompt diagnosis, introducing an adequate treatment, and planning a tight control can impact prognosis and late outcome.

Limitations to the meta-analysis on the role of isotypes in diagnosis can be that it included retrospective studies, single-centre studies and small case numbers. Further, it considers a period of time during which the classificatory criteria for diagnosis have changed, therefore, disease diagnosis may not be uniform among studies.

As for the significance of RF isotypes for RA prognosis, results are conflicting and this is a limitation of this study. Overall, RF isotypes seem to be associated with disease activity, radiographic progression or response to treatment in 50% or less of the studies analysing the issue, with the exception of IgM-RF, which appeared to be associated with higher disease activity in 60% of the studies. These contrasting findings may in part be due to the scarce homogeneity of the papers analysed, as not all isotypes were always considered and outcomes were defined differently, therefore results are not easily comparable.

There are no previous systematic reviews or meta-analyses to compare the results of our study with; nonetheless, in the literature there are a few reviews addressing the topic. A meta-analysis comparing ACPA and RF stated that ACPA specificity is higher compared with RF, while IgM-RF sensitivity was slightly higher; LR among the three RF isotypes were similar; further, ACPA better predicted erosive disease. A subsequent study reported that ACPA are more specific than RF isotypes but that RF isotypes can be useful in predating the diagnosis; regarding prognosis, limited data did not allow a definite conclusion. In another study, ACPA sensitivity was higher than IgM-RF and IgA-RF, while specificity was similar; LR+ and LR− were better when testing ACPA. Another one concluded that in RA with high disease activity ACPA have higher sensitivity than all RF isotypes.

In conclusion, from the result of our study, RF isotypes may add diagnostic information and prove useful in specific patient population, such as those with seronegative arthritis. From the studies analysed, RF isotypes do not appear to provide relevant prognostic information, as results are conflicting.

Author affiliations

1Division of Rheumatology and Clinical Immunology, IRCCS Humanitas Research Hospital, Rozzano, Italy
2Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Milan, Italy
3Laboratory of Clinical Pathology, Azienda Sanitaria Universitaria Integrata, Udine, Italy
4Department of Laboratory Medicine, St Borbolo Hospital, Vicenza, Italy
5Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy
6Unit of Rheumatology and Clinical Immunology, ASST Spedali Civili di Brescia, Brescia, Italy
7Laboratory of Immunology and Allergology, Ospedale San Giovanni di Dio, Firenze, Italy
8Laboratory of Clinical and Experimental Medicine, University of Florence, Firenze, Italy
9Rheumatology, Azienda Ospedaliera San Camillo Forlanini, Roma, Italy

Contributors FM, NB and CS conceived the study, performed the literature search and wrote the paper. DC performed the statistical analysis. MI, BP and GDS revised the manuscript. All authors approved the final version for submission. NB is the author responsible for the overall content as guarantor.

Funding This systematic review and meta-analysis was supported by ThermoFisher Diagnostics.

Competing interests FM has received grant/research support from ThermoFisher Diagnostics. NB has received speaker honoraria for lectures from Werfen and
ThermoFisher Diagnostics. DG has received speaker honoraria for lectures from DASIT and support for attending meetings and/or travel from Abbott, Stago, DiaSorin, Medical Systems/Snibe, Siemens, Werfen and Roche. FF has acted as a consultant for ThermoFisher Diagnostics. GDS has acted as a consultant for ThermoFisher Diagnostics. CS has received grant/research support from AbbVie, Amgen, and Pfizer Inc. CS has acted as a consultant for and has received lecture fees from AbbVie, Amgen, Afza-Wassermann, Biogen, Eli-Lilly, Galapagos, Janssen, Novartis, Pfizer and SOBI.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

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ORCID iDs
Francesca Motta http://orcid.org/0000-0002-9093-2734
Nicola Bizzaro http://orcid.org/0000-0002-4227-6983
Maria Infantino http://orcid.org/0000-0002-6200-4467
Carlo Selmi http://orcid.org/0000-0002-0323-0376

REFERENCES


