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Combined elevation of IgM and IgA rheumatoid factor has high diagnostic specificity for rheumatoid arthritis

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Abstract The diagnostic value of measuring rheumatoid factor (RF) by agglutination or isotype-specific enzyme-linked immunosorbent assay (ELISA) was compared. The study included 70 patients with rheumatoid arthritis (RA) and 205 patients with various other rheumatic conditions. Of the RA patients, 74% were RF-positive by agglutination and 90% had one or more RF isotypes elevated by ELISA compared to 14% and 22%, respectively, of the other patients. Strikingly, 70% of the RF-positive RA patients had an elevation of two or more RF isotypes compared to only 16% of the other RF-positive patients ($P < 0.0001$). Furthermore, a combined elevation of IgM and IgA RF was found in 52% of the RF-positive RA patients, but only in two (4%) of the other RF-positive patients ($P < 0.0001$). It is concluded that a combined elevation of IgM and IgA RF is highly specific for RA and is very rarely found in rheumatic diseases other than RA. Isotype-specific RF assays are therefore diagnostically superior to agglutination tests. The detection of the RA-specific RF isotype pattern may be particularly helpful early in the course of RA even before the disease is fully differentiated.

Key words Rheumatic diseases · Rheumatoid factor · Isotypes · Rheumatoid arthritis diagnosis

Introduction

Rheumatoid factor (RF) is routinely measured by agglutination, using the Rose-Waaler and latex tests, which do not discriminate between individual RF isotypes, but preferentially detected IgM RF. However, individual RF isotypes can be measured by solid-phase assays, and a sensitive enzyme-linked immunosorbent assay (ELISA) screening system has been developed. This screening assay is unbiased in the detection of different RF isotypes [1].

Estimates of the prevalence of raised RF isotypes in rheumatoid arthritis (RA) have been somewhat discordant. It has been reported that combined elevation of IgM RF and IgA RF is the predominant RF pattern in RA [2, 3], although other investigators have found a combined elevation of IgM RF and IgG RF to be more common [4]. However, the diagnostic value of individual RF isotype patterns had, to our knowledge, not been evaluated for RA in the context of other rheumatic diseases, and reports on the frequency of individual RF isotypes in systemic lupus erythematosus (SLE) have been conflicting [5, 6]. This may be due to different methods used for measuring the RF isotypes and, furthermore, to the low number of patients analyzed in each study. Prospective longitudinal studies have shown that elevation of RF often precedes the clinical onset of RA, and that the level and pattern of RF are similar in the pre-rheumatoid phase and during active disease, especially if more than one RF isotype is elevated [4, 7, 8].

It can be difficult to diagnose RA, especially early in the course of the disease. It has furthermore been reported that the onset of this diseases is becoming relatively more common in elderly people [9], in whom it may initially be difficult to distinguish RA from osteoarthritis. In this paper, we compare the diagnostic sensitivity and specificity of RF testing by a conventional agglutination method and by an isotype-specific ELISA system.

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Patients and methods

Patients and samples

Serum samples from 275 patients with different rheumatic conditions were collected. These were from 70 patients with RA, 48 patients with SLE, 18 with scleroderma, 50 with osteoarthritis, 15 with other rheumatic diseases, and 74 patients who were investigated by rheumatologists due to chronic joint pains that could not be attributed to any specific rheumatoid disorder, including RA. Of the 15 pa-

Table 1 Numbers (%) of patients with raised RF by agglutination and ELISA

Diseases	Elevated RF isotypes by ELISA[a]	Positive agglutination (titer ≥ 1:40)
RA ($n=70$)	63 (90%)**	52 (74%)
All other patients ($n=205$)	45 (22%)*	29 (14%)
• SLE ($n=48$)	17 (35%)	8 (17%)
• Scleroderma ($n=18$)	7 (39%)	6 (33%)
• Osteoarthritis ($n=50$)	4 (8%)	7 (14%)
• Other rheumatic diseases ($n=15$)	5 (33%)	2 (13%)
• Joint pains ($n=74$)	12 (16%)	6 (8%)

[a] One or more RF isotypes elevated
* $P<0.05$ or ** $P<0.02$ compared to RF elevation by agglutination

Table 2 Numbers (%) of patients with raised IgM, IgG and IgA RF by ELISA

Diseases	Elevation of:		
	IgM RF	IgG RF	IgA RF
RA ($n=70$)	50 (71%)*	31 (44%)*	42 (60%)*
All other patients ($n=205$)	14 (7%)	24 (12%)	14 (7%)
• SLE ($n=48$)	3 (6%)	9 (19%)	9 (19%)
• Scleroderma ($n=18$)	2 (11%)	2 (11%)	3 (17%)
• Osteoarthritis ($n=50$)	2 (4%)	2 (4%)	0 (0%)
• Other rheumatic diseases ($n=15$)	2 (13%)	4 (27%)	0 (0%)
• Joint pains ($n=74$)	5 (7%)	7 (9%)	2 (3%)

* $P<0.0001$ compared to all other patients

tients diagnosed with other rheumatic diseases, 3 had mixed connective tissue disease, 4 psoriatic arthritis, 2 ankylosing spondylitis, 4 juvenile rheumatoid arthritis, 1 polymyalgia rheumatica, and 1 Reiter's disease. All the RA patients had an established disease, and they were recruited both from private practices and hospital clinics. Of the RA patients, 55 (79%) were females and 15 (21%) were males; mean age was 56.4 years (range: 26–82 years). Of the other patients, 174 (85%) were females and 31 (15%) were males; mean age was 49.8 years (range 16–87 years). All patients fulfilled the relevant ACR classification criteria for the diseases in question. Serum samples from 100 adults aged 31–50 years were used as controls for determining cut-off levels for individual RF isotypes. These control sera were from randomly selected individuals participating in an epidemiologic study.

ELISA for measurement of IgM, IgG and IgA RF

The ELISA has been described in detail elsewhere [1]. Briefly, microtiter plates (Dynatech Immulon I) were coated overnight at 4 °C with a 40 μg/ml solution of purified rabbit IgG (Sigma) diluted in carbonate/bicarbonate buffer. Remaining protein binding sites on the plastic were saturated with 1% BSA (Sigma, Fraction V, 99% pure) diluted in PBS for 2–3 h at room temperature (rt). A 1/40 dilution of serum samples and a serial dilution of a local standard were then incubated for 2 h at rt. Three wells on each plate were incubated without serum and served as blanks. After serum incubation, heat-aggregated rabbit IgG diluted in 1% BSA/PBS to 10 μg/ml was incubated for 30 min at rt. This was done in order to block free IgG binding sites on the solid phase bound RF and thus prevent interaction between the RF and Fc fragments of the enzyme conjugates. This step also dissociates some IgM RF-IgG complexes which might cause false-positive IgG RF binding (unpublished). Appropriate dilutions of the alkaline phosphatase-conjugated mouse monoclonal anti-IgM (Sigma, clone MB-11), anti-IgG (Sigma, clone GG-5) or anti-IgA (Oxoid, clone 2D7) were then added and incubated for 2 h at rt, followed by a 1 mg/ml para-nitrophenylphosphate (Sigma) substrate solution. All washings between incubation steps were done three times with PBS containing 0.05% Tween 20. The absorbance was read at 405 nm in a Titertek Multiskan microplate reader (Flow Laboratories) when absorbance of the highest dilution of the standard had reached 1.4–1.5. The mean net absorbance for each triplicate of the standard dilution was calculated and used to determine a standard curve.

When diluted 1/40, most of the test sera could be read well within the linear range of the standard curve for all the RF isotypes, but if required they were diluted further in order to exclude competition for the solid phase bound rabbit IgG. The mean net absorbance for each sample triplicate was calculated and expressed as arbitrary units (AU/ml) based on the standard curve.

RF values above the upper 95% cut-off level (≥25 AU/ml) for the 100 control subjects were considered raised for each RF isotype. The local RF standard was prepared from pooled serum collected from 11 patients with RA and with high levels of RF. The intra-assay variability of this test system has been determined as 6% and the interassay variability is about 15%.

Measurement of RF by conventional agglutination

All patient samples were also tested by a commercial conventional agglutination test (RAPA, Fujirebio, Japan). The RAPA kit is a microtiter version of the Rose-Waaler test and is based upon agglutination of rabbit IgG-coated particles. The resulting values were expressed as titer. Positivity was defined as a titer of ≥1:40 as recommended by the supplier.

Statistical evaluation

The chi-square test, with Yates correction for expected frequencies less than five, and the Mann-Whitney U-test, were used for statistical evaluation of the results. The level of significance was set at $P<0.05$. Sensitivity [true positives (true positives + false negatives)], specificity [true negatives (true negatives + false positives)], positive predictive values [true positives (true positives + false positives)], and negative predictive values [true negatives (true negatives + false negatives)] were calculated for the different RF tests and patterns.

Results

As expected, elevation of RF was much more common in RA than in the other rheumatic diseases. Thus, 90% and 74% of the RA patients were RF-positive by ELISA and agglutination, respectively, compared to 22% and 14% of the patients with the other rheumatic diseases (Table 1). The distribution of individual RF isotypes is shown in Table 2.

As shown in Table 3, 70% (44/63) of the RF-positive RA patients had two or three RF isotypes elevated, compared to only 16% (7/45) of the other RF-positive patients ($P<0.0001$). Furthermore, 52% (33/63) of the RF-positive RA patients had a combined elevation of IgM RF and IgA RF, while this RF pattern was observed in only two (4%) of the other RF-positive patients ($P<0.0001$). Both had SLE and positive SSA antibodies, and one also had features of the sicca syndrome. It should further be noted that the two non-RA patients with combined elevation of IgG RF and IgA RF also had SLE, and in one this was associated with the sicca syndrome. Interestingly, isolated elevation of IgG RF was significantly less common in the RA patients than in the other patients ($P<0.0001$).

The RA patients had higher levels of IgM RF and IgA RF than the other RF-positive patients (Fig. 1). In contrast, IgG RF levels were similar in both groups.

Table 3 RF patterns in the different groups of the RF-positive rheumatic patients

Diseases	Elevation of:					
	IgM RF only	IgG RF only	IgA RF only	IgM RF+ IgG RF	IgG RF+ IgA RF	IgM+IgA RF (±IgG RF)
RA ($n=63$)	8 (13%)	4 (6%)*	7 (11%)	9 (14%)	2 (3%)	33 (52%)*
All other patients ($n=45$)	9 (20%)	19 (42%)	10 (22%)	3 (7%)	2 (4%)[a]	2 (4%)[b]
• SLE ($n=17$)	1 (6%)	7 (41%)	5 (29%)	0 (0%)	2 (12%)	2 (12%)
• Scleroderma ($n=7$)	2 (29%)	2 (29%)	3 (43%)	0 (0%)	0 (0%)	0 (0%)
• Osteoarthritis ($n=4$)	2 (50%)	2 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
• Other rheumatic diseases ($n=5$)	1 (20%)	3 (60%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)
• Joint pains ($n=12$)	3 (25%)	5 (42%)	2 (17%)	2 (17%)	0 (0%)	0 (0%)

[a] Both had the sicca syndrome and one had SSA antibodies
[b] Both had SSA antibodies and the sicca syndrome
* $P<0.0001$ compared to all other patients

Table 4 Comparison of the sensitivity, specificity, and positive and negative predictive values of RF measured by agglutination or isotype-specific ELISA for diagnosing RA

Elevated RF	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Agglutination	74%	86%	64%	91%
By ELISA				
• ≥One isotype	90%	75%	55%	96%
• ≥Two isotypes	63%	97%	86%	88%
• IgM+IgA RF (±IgG RF)	47%	99%	94%	85%

In Table 4 the sensitivity, specificity, and positive and negative predictive values for the agglutination test and the RF isotypes are compared. As can be seen, elevation of at least two RF isotypes, especially IgM and IgA RF, had much higher diagnostic specificity and positive predictive values for RA than a positive agglutination test, but the sensitivity was lower. However, detection of one or more RF isotypes by ELISA gave sensitivity of 90% compared to 74% by agglutination.

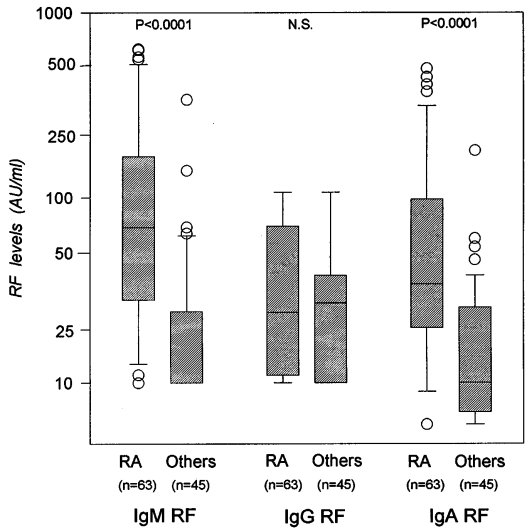

Fig. 1 Levels of IgM, IgG and IgA RF in the RF-positive patients. The *boxes* show medians with 25th and 75th percentiles and the *error bars* indicate the upper and lower 10 percentiles

Discussion

These results show that a combined elevation of IgM RF and IgA RF is the most common RF pattern in RA [2, 3] and agree with a report, published after our study was completed, that this RF pattern is very rarely found in patients with rheumatic diseases other than RA [10].

The findings reported here indicate that simultaneous elevation of IgM RF and IgA RF has very high diagnostic specificity and predictive value for RA, as this combination is virtually never found in other rheumatic diseases. While almost 50% of RA patients have this RF pattern, it was only observed in two non-RA patients who both had SLE associated with SSA antibodies, and one of whom also had features of the sicca syndrome. Elevation of IgA RF is well documented in patients with the sicca syndrome [11, 12]. It should be pointed out that our study cohort included a large proportion of all SLE patients in Iceland, and it is therefore likely that they are representative for the clinical spectrum of this disease. Although the IgM and IgA RF pattern is very characteristic for RA, it has a lower diagnostic sensitivity than a positive agglutination test. However, an ELISA screening system has been developed which is more sensitive for an unbiased detection of a single RF isotype than the conventional agglutination techniques. This screening test, used in combination with isotype-specific ELISA, has a diagnostic sensitivity of 90% compared to 74% for the RAPA agglutination test [1]. Thus, conventional agglutination RF tests which do not distinguish between RF isotypes, but are biased towards the IgM isotype, are clearly diagnostically inferior to methods than can measure individual RF isotypes. It should be particularly emphasized in this context that agglutination does not discriminate between isolated IgM RF and a combination of IgM and IgA RF elevation. Furthermore, some patients with isolated IgA RF are positive by agglutination [12]. It should be pointed out that the ELISA system we use does not distinguish between anti-Fab and RF-type anti-Fc antibodies. However, conventional agglutination tests for RF may also be influenced by anti-Fab antibod-

ies. Furthermore, such antibodies would not affect the diagnostic implications of our findings.

RF may have a physiological immunoregulatory function in healthy individuals, and its increase in various chronic inflammatory diseases may reflect a natural IgM response to immune complexes [13, 14]. However, several reports indicate that RF, including IgA RF, may be raised in RA patients long before the clinical onset of the disease [4, 7, 8]. This strongly indicates that overproduction of RF is not simply a secondary phenomenon in RA and that the etiology of this disease may at least in part involve abnormal regulation of RF production.

TGF-β selectively stimulates IgA production [15]. A marked increase in TGF-β has recently been observed in tissue samples from RA patients [16], and a close correlation has been observed between RF isotype levels in serum and synovial fluid [17]. TGF-β is generally considered to be an anti-inflammatory cytokine, and the association of IgA RF with severe RA may therefore seem paradoxical. However, TGF-β has been reported to be a potent chemoattractant for monocytes and to promote monocyte adhesion and enzymatic digestion of extracellular matrix [18]. Furthermore, TGF-β is produced by osteoclasts, and it may also have an autocrine activity by stimulating osteoclasts and inducing them to secrete proteinases that activate latent TGF-β [19]. It is therefore conceivable that the association of bone erosions with raised levels of IgA RF may result from excessive intra-articular production of TGF-β.

In the present study, most of our RA patients had established disease and many had been treated with drugs that may reduce RF production [20–23]. However, it was not possible to detect any association between previous treatment of the participants and their RF levels or patterns. It is certainly possible that the prevalence of raised IgM and IgA RF may be even higher in untreated patients with early undifferentiated RA, as it has been shown that increased IgM and IgA RF can precede clinical symptoms by several years and is also a very stable phenomenon [4, 8]. Thus, we have recently demonstrated, in an ongoing prospective study of patients with early arthritis, that the great majority of RF-positive RA patients already have a combined elevation of IgM and IgA RF at the time they enter the study [24].

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