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False-positive detection of IgM anti-severe acute respiratory syndrome coronavirus 2 antibodies in patients with rheumatoid arthritis: Possible effects of IgM or IgG rheumatoid factors on immunochromatographic assay results

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Abstract

Objectives: The severe acute respiratory syndrome coronavirus 2 causes coronavirus disease 2019. A serological test is conducted to determine prior infection by severe acute respiratory syndrome coronavirus 2. We investigated whether the results of anti-severe acute respiratory syndrome coronavirus 2 antibody tests are modified in patients with rheumatoid arthritis.

Methods: Patients in Japan with rheumatoid arthritis were recruited at Sagamihara Hospital from July 2014 to October 2015 ($n=38$; 2014 cohort) and at Tokyo Hospital from June to October 2020 ($n=93$; 2020 cohort). Anti-severe acute respiratory syndrome coronavirus 2 antibodies were measured by electrochemiluminescence immunoassay or immunochromatographic assay.

Results: Anti-severe acute respiratory syndrome coronavirus 2 antibodies were not detected in any of the samples from rheumatoid arthritis patients tested by electrochemiluminescence immunoassay. Anti-severe acute respiratory syndrome coronavirus 2 antibodies were detected by immunochromatographic assay in the 3 (7.9%) serum samples in the 2014 cohort and 15 (16.1%) serum samples in the 2020 cohort. The IgM rheumatoid factor levels were increased in rheumatoid arthritis patients with IgM anti-severe acute respiratory syndrome coronavirus 2 antibodies detected by immunochromatographic assay (mean $\pm$ standard deviation (IU/ml), 1223.0 ± 1308.7 versus 503.6 ± 1947.2; $P=0.0101$). The levels of IgG rheumatoid factor were also upregulated in rheumatoid arthritis patients with IgM anti-severe acute respiratory syndrome coronavirus 2 antibodies detected by immunochromatographic assay (4.0 ± 0.7 versus 2.4 ± 0.9; $P=0.0013$).

Conclusion: The results of IgM anti-severe acute respiratory syndrome coronavirus 2 antibody testing by immunochromatographic assay are modified by IgM or IgG rheumatoid factors in rheumatoid arthritis patients.

Keywords

Anti-severe acute respiratory syndrome coronavirus 2 antibody, rheumatoid arthritis, rheumatoid factor, electrochemiluminescence immunoassay, immunochromatographic assay

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Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19). An outbreak of COVID-19 was first reported in December 2019 in Wuhan, China.[1] In the diagnosis of COVID-19, quantitative reverse transcription polymerase chain reaction (RT-PCR) testing for SARS-CoV-2 is performed from nasal swab, saliva, or sputum samples. On the other hand, a serological test was conducted to discriminate prior infection of SARS-CoV-2, and cross-reactivity of anti-SARS-CoV-2 antibodies with antibodies against SARS-CoV was reported.[2] The cross-reactivity of autoantibodies in autoimmune disease patients with antibodies against SARS-CoV-2 was reported in some previous studies;[3] false-positive results were detected in enzyme-linked immunosorbent assay (ELISA) or immunochromatographic assay (ICA) in sera with middle to high levels of IgM rheumatoid factors.[4] However, false-positive results were not detected in other studies[5,6]; antibodies against SARS-CoV-2 were not detected in the sera collected before the outbreak of COVID-19 from rheumatoid arthritis (RA), systemic lupus erythematosus, or Sjögren's syndrome.[7] It was suspected that rheumatoid factors were responsible for the false-positive results in these immunological assays. Hence, we investigated whether the results of serological tests of anti-SARS-CoV-2 antibodies would be influenced in patients with RA.

Materials and methods

Patients *and* sera

Japanese patients with RA were recruited at Sagamihara National Hospital from July 2014 to October 2015 ($n=38$; 2014 cohort) and at Tokyo National Hospital from June to October 2020 ($n=93$; 2020 cohort) for this case-only study. The RA patients fulfilled either American College of Rheumatology criteria for RA[8] or Rheumatoid Arthritis Classification Criteria.[9] All the patients had not been clinically diagnosed with COVID-19 before the collection of sera, including the RA patients with suspected symptoms of COVID-19 (fever, dyspnea, cough, or fatigue) who tested negative by real-time RT-PCR or the RA patients without these symptoms.

Sera from the RA patients were collected and analyzed for anti-SARS-CoV-2 antibodies. The study was reviewed and approved by the Research Ethics Committee of Tokyo National Hospital and Sagamihara National Hospital. Written informed consent was obtained from all of the patients. This study was conducted in accordance with the principles expressed in the Declaration of Helsinki.

Anti-SARS-CoV-2 antibody analyses

IgM and IgG classes of anti-SARS-CoV-2 antibodies were detected by the electrochemiluminescence immunoassay (ECLIA) method (Elecsys Anti-SARS-CoV-2, Roche Diagnostics, Mannheim, Germany) using a cobas 8000 analyzer with e801 module (Roche Diagnostics) according to the manufacturer's instructions. Anti-SARS-CoV-2 antibodies were also detected using ICA (GenBody COVID-19 IgM/IgG, GenBody, Cheonan, Korea) according to the manufacturer's instructions with slight modifications. Briefly, 100 µl of serum was added to the sample port. After the addition of three drops (approximate volume: 100 µl) of buffer to the same sample port, the results were interpreted after 1 h. The presence of only one band in the control line indicated a negative result, while the presence of two bands in the control line and IgM or IgG test line indicated a positive result for IgM or IgG, respectively.

Autoantibody analyses

Rheumatoid factor was detected by N-latex RF kit (Siemens Healthcare Diagnostics, München, Germany) or N-Assay LA RF-K Nittobo (Nittobo Medical, Koriyama, Japan), and IgG rheumatoid factor was measured by Smitest IgG RF ELISA (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan). Anti-citrullinated peptide antibody was measured by Mesacup-2 test CCP (Medical & Biological Laboratories).

Statistical analysis

Differences of demographic features and experimental findings of RA patients were analyzed by Fisher's exact test using 2×2 contingency tables or Mann–Whitney's U test. The 80% statistical power was estimated to be provided when the difference of RF and IgG RF values between IgM anti-SARS-CoV-2 antibody-positive RA and IgM or IgG anti-SARS-CoV-2 antibody-negative RA was 2513.9 and 1.11 [IU/mL], respectively (https://biostat.app.vumc.org/wiki/Main/PowerSampleSize).[10] Statistical significance was defined as $P<0.05$.

Results

Characteristics of the RA patients in the 2014 and 2020 cohorts are shown in Table 1. RA patients in the 2020 cohort were older than those in the 2014 cohort. Age at onset of RA in patients in the 2020 cohort was also older. Anti-citrullinated peptide antibody levels were lower in the 2020 cohort. The percentage of patients treated with infliximab was lower in the 2020 cohort. There were no differences in percentages of males or rheumatoid factor levels between the two cohorts. Anti-SARS-CoV-2 antibody profiles were also analyzed in the 2014 and 2020 cohorts. Anti-SARS-CoV-2 antibodies were not detected in any of the samples from RA patients in both cohorts tested by ECLIA. However, the ICA predicted that serum samples from 3 (7.9%) RA patients were positive for IgM or IgG anti-SARS-CoV-2 antibodies in the 2014

Table 1. Characteristics of RA patients in 2020 and 2014 cohorts.

	2020 cohort	2014 cohort	*P* value
Number	93	38	
Age, years (mean ± SD)	73.4 ± 10.7	66.1 ± 11.0	0.0004
Male, *n* (%)	23 (24.7%)	7 (18.4%)	0.4991[a]
Age at onset, years (mean ± SD)	61.3 ± 17.3	54.1 ± 13.1	0.0110
RF, IU/ml (mean ± SD)	225.6 ± 450.4	1165.8 ± 3260.8	0.1915
ACPA, IU/ml (mean ± SD)	204.6 ± 220.4	674.9 ± 1031.3	0.0013
IgG RF, IU/ml (mean ± SD)	2.4 ± 0.9	2.6 ± 0.9	0.2938
IFX treatment, *n* (%)	1 (1.1%)	6 (15.8%)	0.0024[a]
IgM anti-SARS-CoV-2 Ab positive, *n* (%)	5 (5.4%)	0 (0.0%)	0.3207[a]
IgG anti-SARS-CoV-2 Ab positive, *n* (%)	11 (11.8%)	3 (7.9%)	0.7564[a]
IgM or IgG anti-SARS-CoV-2 Ab positive, *n* (%)	15 (16.1%)	3 (7.9%)	0.2720[a]

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; ICA: immunochromatographic assay; RA: rheumatoid arthritis; RF: rheumatoid factor; ACPA: anti-citrullinated peptide antibody; IFX: infliximab. IgM or IgG anti-SARS-CoV-2 antibodies were detected by ICA. Number or average values of each group are shown. Difference was tested by Fisher's exact test using 2 × 2 contingency tables or Mann–Whitney's *U* test.
[a]Fisher's exact test was employed.

Table 2. Characteristics of RA patients with or without anti-SARS-CoV-2 antibodies detected by ICA.

	IgM or IgG (+)	*P* value	IgM (+)	*P* value	IgG (+)	*P* value	IgM or IgG (−)
Number	18		5		14		113
Age, years (mean ± SD)	74.8 ± 10.2	0.1402	73.6 ± 13.4	0.4072	75.8 ± 9.2	0.1292	70.7 ± 11.3
Male, *n* (%)	3 (16.7%)	0.7630[a]	2 (40.0%)	0.5953[a]	1 (7.1%)	0.3018[a]	27 (23.9%)
Age at onset, years (mean ± SD)	59.9 ± 18.0	0.8650	60.8 ± 23.0	0.8703	61.5 ± 17.7	0.6039	59.0 ± 16.2
RF, IU/ml (mean ± SD)	464.8 ± 812.8	0.0607	1223.0 ± 1308.7	0.0101	181.0 ± 177.9	0.3739	503.6 ± 1947.2
ACPA, IU/ml (mean ± SD)	299.6 ± 277.5	0.5099	219.7 ± 233.5	0.6161	306.8 ± 297.1	0.4857	340.3 ± 659.1
IgG RF, IU/ml (mean ± SD)	2.8 ± 1.0	0.1101	4.0 ± 0.7	0.0013	2.5 ± 0.8	0.7348	2.4 ± 0.9
IFX treatment, *n* (%)	0 (0.0%)	0.5925[a]	0 (0.0%)	1.0000[a]	0 (0.0%)	1.0000[a]	7 (6.2%)

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; ICA: immunochromatographic assay; RA: rheumatoid arthritis; RF: rheumatoid factor; ACPA: anti-citrullinated peptide antibody; IFX: infliximab; IgM or IgG (+): RA patients with IgM or IgG anti-SARS-CoV-2 antibodies; IgM (+): RA patients with IgM anti-SARS-CoV-2 antibodies; IgG (+): RA patients with IgG anti-SARS-CoV-2 antibodies; IgM or IgG (−): RA patients without IgM or IgG anti-SARS-CoV-2 antibodies. Number or average values of each group are shown. Difference was tested in the comparison with IgM or IgG (−) group by Fisher's exact test using 2 × 2 contingency tables or Mann–Whitney's *U* test.
[a]Fisher's exact test was employed.

cohort, and 15 (16.1%) in the 2020 cohort (Table 1). The positive rates of IgM or IgG anti-SARS-CoV-2 antibodies were not significantly different between these two cohorts ($P=0.2720$). Although the sensitivity of ICA could not be calculated in this study, the specificity was 0.86 in the RA populations.

Characteristics of the RA patients with or without reactivity to the ICA are listed in Table 2. IgM or IgG anti-SARS-CoV-2 antibodies were detected in 18 RA patients. IgM anti-SARS-CoV-2 antibodies were detected in 5 and IgG in 14 of the cohorts, respectively. IgM and IgG anti-SARS-CoV-2 antibodies were found in one patient. Rheumatoid factor levels were increased in RA patients with IgM anti-SARS-CoV-2 antibodies by ICA compared with RA without any anti-SARS-CoV-2 antibodies (mean ± standard deviation (IU/ml) 1223.0 ± 1308.7 versus 503.6 ± 1947.2; $P=0.0101$). The levels of IgG rheumatoid factor were also increased in RA patients with IgM anti-SARS-CoV-2 antibodies detected by ICA (4.0 ± 0.7 versus 2.4 ± 0.9; $P=0.0013$). Rheumatoid factor levels were comparable in RA patients with IgG anti-SARS-CoV-2 antibodies detected by ICA. The levels of IgG rheumatoid factor were not different in RA patients with IgG anti-SARS-CoV-2 antibodies detected by ICA. There were no differences of mean age, percentage of males, or anti-citrullinated peptide antibodies.

Characteristics of the RA patients with or without rheumatoid factor are listed in Table 3. Anti-citrullinated peptide antibody levels were increased in RA patients with rheumatoid factor (mean ± standard deviation (IU/ml) 400.3 ± 671.5 versus 82.7 ± 194.9; $P=3.42\times10^{-6}$). IgG rheumatoid factor levels were also increased in RA patients with rheumatoid factor (mean ± standard deviation (IU/ml) 2.7 ± 0.8 versus 1.5 ± 0.6; $P=7.63\times10^{-10}$). There were no differences of mean age, percentage of males, age at onset, or infliximab

Table 3. Characteristics of RA patients with or without RF.

	RF(+)	RF(−)	*P* value
Number	108	23	
Age, years (mean ± SD)	71.7 ± 10.7	69.1 ± 13.7	0.7278
Male, *n* (%)	24 (22.2%)	6 (26.1%)	0.7850[a]
Age at onset, years (mean ± SD)	58.5 ± 16.3	62.0 ± 16.9	0.4101
IFX treatment, *n* (%)	6 (5.6%)	1 (4.3%)	1.0000[a]
ACPA, IU/ml (mean ± SD)	400.3 ± 671.5	82.7 ± 194.9	3.42×10^{-6}
IgG RF, IU/ml (mean ± SD)	2.7 ± 0.8	1.5 ± 0.6	7.63×10^{-10}
IgM anti-SARS-CoV-2 Ab positive, *n* (%)	5 (4.6%)	0 (0.0%)	0.5858[a]
IgG anti-SARS-CoV-2 Ab positive, *n* (%)	13 (12.0%)	1 (4.3%)	0.4622[a]
IgM or IgG anti-SARS-CoV-2 Ab positive, *n* (%)	91 (15.7%)	1 (4.3%)	0.1959[a]

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; ICA: immunochromatographic assay; RA: rheumatoid arthritis; RF: rheumatoid factor; ACPA: anti-citrullinated peptide antibody; IFX: infliximab; RF(−): RA patients without RF; RF(+): RA patients with RF. IgM or IgG anti-SARS-CoV-2 antibodies were detected by ICA. Number or average values of each group are shown. Difference was tested by Fisher's exact test using 2 × 2 contingency tables or Mann–Whitney's *U* test.
[a]Fisher's exact test was employed.

treatment. RA patients without rheumatoid factor were also negative for IgM anti-SARS-CoV-2 antibodies. However, one RA patient without rheumatoid factor was positive for IgG anti-SARS-CoV-2 antibodies.

Discussion

Although anti-SARS-CoV-2 antibodies were not detected by ECLIA in any of the RA samples, some samples were predicted to be positive by ICA in the two cohorts. The positive rates of anti-SARS-CoV-2 antibodies by ICA were comparative between these cohorts. Since samples from 2014 cohort were collected before the outbreak of COVID-19, anti-SARS-CoV-2 antibodies could not be detected in the cohort, indicating false-positive reactions in the sera from these RA patients. In this study, rheumatoid factors were detected by latex fixation tests that mainly measured the IgM class of rheumatoid factor.[11] Because levels of rheumatoid factor were higher in the RA patients with reactivity to the ICA, it was suggested that IgM rheumatoid factor could react with the anti-human IgM antibodies in the ICA kit to cause the false-positive reaction. It was also suggested that IgG rheumatoid factor reacted with the anti-human IgM antibodies in the ICA kit. However, the results of IgG anti-SARS-CoV-2 antibodies by the ICA were not influenced by IgM or IgG rheumatoid factors.

It was reported that assay interference was caused in ELISA by anti-rat immunoglobulin light chain antibodies found in human sera.[12] These results suggested that both IgM and IgG rheumatoid factors might not react with anti-human IgG antibodies in the ICA kit, and that other factors (such as anti-rat immunoglobulin antibodies) would modify the results of IgG anti-SARS-CoV-2 antibodies by the ICA in RA patients. The interference of IgM rheumatoid factor with immunological assays was reported in previous studies,[13–16] including those on anti-SARS-CoV-2 antibody assays,[4] although the effects of IgG rheumatoid factor were poorly investigated. It is still unknown which (IgM or IgG) rheumatoid factor predominantly modified the results of IgM anti-SARS-CoV-2 antibodies by the ICA. Thus, the results of IgM anti-SARS-CoV-2 antibodies detected by ICA would be modified by IgM or IgG rheumatoid factors in RA patients, but the results of IgG would be modified by other factors.

The interference of autoantibodies with immunological assays was suggested in this study. Although use of ICA kit is becoming less frequent, it is still used in some clinics due to its processing speed. Thus, when the subject is suffering from RA, the results on ICA should be confirmed by other methods such as ECLIA.

Since the sample size of this study is modest, large-scale independent studies should be performed to validate the results from this study. Moreover, antibody profiles in patients with other autoimmune diseases than RA were not analyzed in this study; in other autoimmune disease patients, anti-SARS-CoV-2 antibodies should be measured by ICA and ECLIA in future studies. Although the results of IgM anti-SARS-CoV-2 antibodies detected by ICA would be modified by IgM or IgG rheumatoid factors in RA patients, it should be clarified what modified the results of IgG anti-SARS-CoV-2 antibody testing by ICA in RA patients.

Conclusion

To the best of our knowledge, this is the first analysis on the effects of IgG rheumatoid factors on results obtained by ICA for SARS-CoV-2 in RA patients. In RA patients, the results of IgM anti-SARS-CoV-2 antibodies detected by ICA seemed to be influenced by rheumatoid factors, unlike those of IgG anti-SARS-CoV-2 antibodies influenced by other factors. Thus, when anti-SARS-CoV-2 antibodies are measured in RA patients, results detected by ICA should be confirmed by ECLIA or other methods.

Author contributions

HF and ST conceived and designed the experiments, and analyzed the data; SO, TH, and HF performed the experiments; HF, KS, AH, TM, and ST contributed reagents/materials/analysis tools; and SO, HF, and ST wrote the article.

Availability of data and materials

All data are presented in the article.

Declaration of conflicting interests

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Ethics approval

The study was reviewed and approved by the Research Ethics Committee of Tokyo National Hospital (210008) and Sagamihara National Hospital (2009061621).

Informed consent

Written informed consent was obtained from all of the patients. This study was conducted in accordance with the principles expressed in the Declaration of Helsinki.

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