Lack of cross-reactivity between rheumatoid factor IgM and anti-S1 receptor binding domain of SARS-CoV-2 IgM: a case-control study

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ABSTRACT

Objective. *Since the onset of the COVID-19 outbreak, concern has been raised about reliability of SARS-CoV-2 serological tests in people with serum positivity for rheumatoid factor (RF), due to its ability to interfere during tests carried out with immunoassay techniques, leading to false positive results. The aim of this study was to analyse, on sera from RF seropositive rheumatoid arthritis (RA) patients, the interference between RF IgM and anti-S1 RBD IgM.*

Methods. *The study was conducted on consecutive patients affected by RF seropositive RA and, as control group, COVID-19 patients with SARS-CoV-2 pneumonia hospitalised at Sapienza University of Rome from April 2020 and April 2021. Serum samples from COVID-19 patients during their hospitalisation were collected, while RA subjects' samples were harvested prior to the onset of the COVID-19 pandemic. All samples were tested for RF IgM using nephelometry and ELIA, and for anti-S1 RBD IgM by ELISA.*

Results. *Forty RF seropositive RA and 42 COVID-19 patients were enrolled. In all RA patients, both nephelometric assay and ELIA showed RF IgM positivity, while only one patient of the control group tested positive for RF IgM by nephelometric assay and ELIA.*
IgM directed to S1 RBD were not detected in sera of RA patients, while all COVID-19 patients presented anti-S1 RBD IgM (median anti-S1 RBD IgM COVID-19 vs. RA: 368.5 IU/mL, IQR 654 IU/mL vs. 18.45 IU/mL, IQR 20 IU/mL; p<0.0001).

Conclusion. *This study confirmed the lack of cross-reactivity between RF and anti-S1 RBD IgM, offering to clinicians a valuable tool for a better management of RA patients undergoing SARS-CoV-2 serological tests.*

Introduction

Since the onset of the SARS-CoV-2 outbreak, serological tests have covered the need of easy and quick assays evaluating the SARS-CoV-2 infection, especially during the first phases of the pandemic, when molecular tests were not always available in all countries due to the large demand. Otherwise, SARS-CoV-2 nucleic acid detection, because of its high positive rate, is actually the gold standard for SARS-CoV-2 testing (1).

However, considering the dropping of positive rate of these tests after 6th days from infection, the needs of specialised laboratories and trained personnel is required, although it is a time-consuming procedure, thus it might not be the best choice for screening large-scale populations infected with SARS-CoV-2 (2, 3).

On the other hand, concerns have been raised about the possible cross-reactivity of anti-spike protein S1 receptor-binding domain of SARS-CoV-2 IgM (hereafter referred as anti-S1 RBD IgM) with different autoantibodies, particularly with rheumatoid factor (RF) IgM, and consequently concerns on the reliability of the serological assays in rheumatoid arthritis (RA) patients. RF autoantibodies are directed against the Fc segment of IgG; their main isotype is IgM, which is distinctive of many autoimmune diseases. RF could not specifically bind to the specific antibody Fc segment coated on the solid phase carrier and the labelled antibody Fc segment, resulting in a nonspecific manner, which lead to false positive reaction in sera of patients affected by different infectious diseases, including CoronaVirus Disease-19 (COVID-19) (4, 5).

To date, the reliability of COVID-19 serological tests remains to be defined in RA patients. The aim of the present study was to evaluate RF IgM and anti-S1 RBD IgM cross-reactivity in RA and COVID-19 patients.

Materials and methods

Patients, ELIA and ELISA tests

This is a single-centre, cross-sectional, case-control study on consecutive patients presenting a diagnosis of RA, according to the latest American College of Rheumatology (ACR) classification criteria (6) and tested positive for RF IgM by nephelometry, using N Latex RF kit on BN 100 nephelometer (Siemens Healthcare Diagnostics, München, Germany) and by Enzyme-Linked ImmunoAssay (ELIA), using Fluoroenzyme Immunoassay for Rheumatic Factor on Phadia 100 instrument

(Phadia AB, Uppsala, Sweden), following manufacturer's instructions. All patients were referred to the Rheumatology Unit of Sapienza University of Rome, samples were collected prior to the onset of the COVID-19 pandemic, from January 2015 to December 2019. Furthermore, we enrolled consecutive COVID-19 patients with SARS-CoV-2 pneumonia, hospitalised in the non-intensive care COVID-19 Unit of Sapienza University of Rome, from April 2020 to April 2021. The diagnosis of SARS-CoV-2 infection was confirmed in all the patients by nasopharyngeal swab for the presence of SARS-CoV-2 by real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR), after extraction of viral RNA. All chosen COVID-19 patients were tested for anti-S1 RBD IgM by Enzyme-Linked Immunosorbent Assay (ELISA), following the manufacturer's instructions (COVID-19 S Protein-S1 Receptor-Binding Domain-Human IgM Quantitative ELISA kit, RayBiotech, GA, USA). The sensitivity, specificity, and accuracy of the assay were 79.7%, 98.1%, and 92.4%, respectively.

COVID-19 patients' exclusion criteria were the absence of anti-S1 RBD IgM and/or the presence of autoimmune inflammatory rheumatic diseases (AIRDs).

Each patient underwent venous blood draw and then the whole blood was allowed to clot, by leaving it at room temperature for 15–30 min, and subsequently removed by centrifuging at 2,000 x g for 10 min. The serum samples obtained were stored at -80°C until the assays.

The procedures involving human participants were in accordance with the Declaration of Helsinki. The study protocol was approved by the Bioethics Committee of the Sapienza University of Rome (prot. 0617/2021). A written informed consent was obtained from all individual participants included in the study.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) or median (interquartile range - IQR), according to the distribution of the variables. The Mann-Whitney U-test evaluated continuous variables. Receiver operating characteristic (ROC) analyses were used to evaluate the discriminatory power of the assays. *p*-values <0.05 were considered statistically significant. Prism 6 (GraphPad Software, San Diego, CA, USA) was used for all statistical tests.

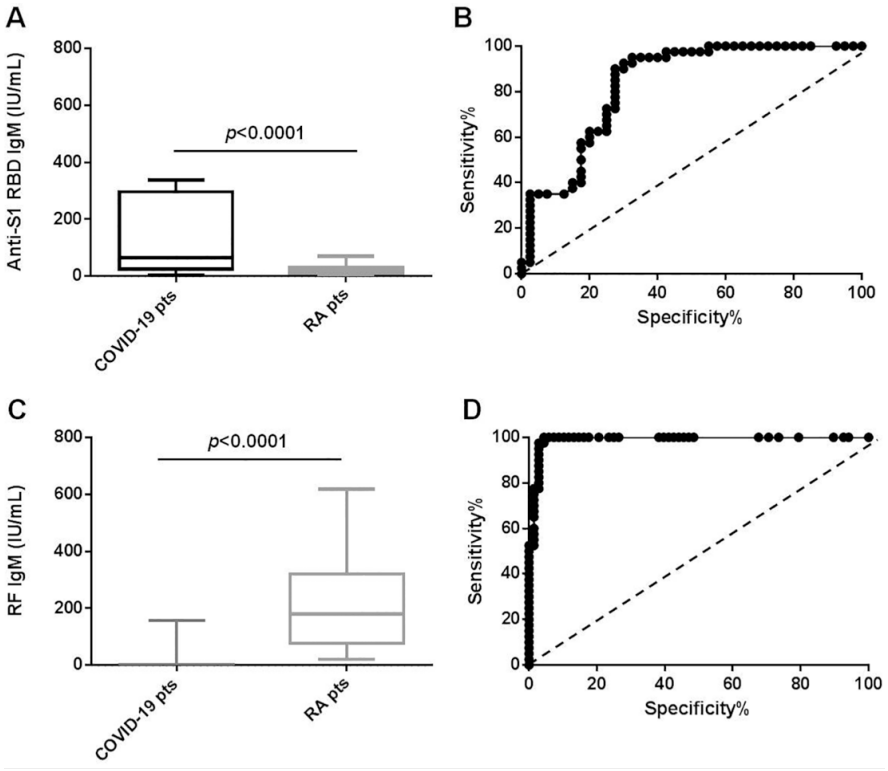

Fig. 1. Anti-S1 RBD and RF IgM serum concentration in COVID-19 and RA patients.
A: ELISA detected anti-S1 RBD IgM in sera from all COVID-19 patients (42/42, 100%), but not in RA patients' sera (0/40) (median of anti-S1 RBD IgM in COVID-19 *vs*. RA patients: 368.5 IU/ml, IQR 654 IU/ml *vs*. 18.45 IU/mL, IQR 20 IU/mL; $p<0.0001$).
B: Receiver operating characteristic (ROC) curve for anti-S1 RBD IgM. COVID-19 *vs*. RA patients: the area under curve (AUC) from ROC analysis is 0.8316 (95% CI 0.7397–0.9234).
C: RF IgM were found in 1/42 (2.4%) COVID-19 patients and in all RA patients (40/40, 100%) by ELIA.
D: ROC curve for RF IgM. COVID-19 *vs*. RA patients: the AUC from ROC analysis is 0.9893 (95% CI 0.9736–1.005).
pts: patients; CI: confidence interval.

Results

We enrolled 40 RA patients, 22 females and 18 males, with a mean age of 62±10 years and 42 COVID-19 patients, 17 females and 25 males, with a mean age of 75±16 years. All RA patients tested positive for RF IgM by nephelometric assay (median 194 IU/ml - IQR 251 IU/ml) and by ELIA (median 175 IU/ml - IQR 242 IU/ml). In the COVID-19 group, only one patient (1/42, 2.4%) resulted positive for RF IgM by nephelometric assay (115 IU/ml) and ELIA (157 IU/ml; Fig. 1C), while 10/42 (14%) COVID-19 patients tested positive for RF IgM by ELIA only at low titre (median 8.8 IU/ml, IQR 8.9 IU/ml). IgM directed to S1 RBD were not detected in sera of RA patients, while all COVID-19 patients presented anti-S1 RBD IgM (Fig. 1A) (median of anti-S1 RBD IgM in COVID-19 *vs*. RA patients: 368.5 IU/ml, IQR 654 IU/ml *vs*. 18.45 IU/mL IQR 20 IU/mL; $p<0.0001$).

ROC analyses showed the statistically significant differences between COVID-19 and RA patients (area under curve, AUC=0.8316, Fig. 1B) for the presence of anti-S1 RBD IgM detected by ELISA and for RF IgM (AUC=0.9893, Fig. 1D) detected by ELIA.

Discussion

This is the first study aimed at evaluating RF IgM and anti-S1 RBD IgM cross-reactivity in RA and COVID-19 patients.

Early enthusiasm for serological tests in the diagnosis of COVID-19 was dampened by reliability concerns, in particular due to the risk of false positives in patients with AIRDs. RF is the first autoantibody reactivity detected in sera from patients with RA, but it cannot be considered specific. Indeed, these autoantibodies are also present in elderly, smokers and in patients with chronic infections (7, 8), thus it is questionable whether it could be falsely positive in patients with SARS-CoV-2 infection. A case report from Lubrano *et al*. showed a patient affected by psoriatic arthritis and seropositive for RF found to be positive for serum anti-SARS-CoV-2 IgM, but not for SARS-CoV-2 nucleic acid test in nasopharyngeal swab, highlighting for the first time the need for greater care in analysing the results of serological tests in RF seropositive patients (9). In order to support this warning, a recent study reported that RA patients with high serum levels of RF IgM and IgG isotypes resulted in a false positive SARS-CoV-2 serological test. In particular, the majority of false positive tests was detected in the IgM assays, due to the IgM lower affinity for the antigen compared to IgG (10).

Besides, Wang and colleagues (11) detected positive SARS-CoV-2 IgM in 22 out of 36 (61%) mid-to-high level RF IgM positive RA sera collected from 25 January 2020 to 15 February 2020, in which SARS-CoV-2 nucleic acid tested negative using RT-PCR. In RA patients, SARS-CoV-2 infection cannot be ruled out only in the light of the negativity of the molecular tests; indeed, molecular tests can result falsely negative in the case of low viral loads in a sample (3).

Similarly to our study, Teng and colleagues considered RA sera collected before SARS-CoV-2 pandemic (2016-2019), and none of the 47 RF positive sera were tested positive for SARS-CoV-2 IgM or IgG, even if they did not consider a COVID-19 control group (12).

Therefore, to date there are no univocal data on the reliability of serological assays in RA patients.

In the present study, we evaluated the presence of anti-S1 RBD IgM in sera from RF IgM positive RA patients collected prior to the onset of the COVID-19 pandemic, abolishing the risk of RA patients' antibodies positivity for SARS-CoV-2 due to the infection. In addition, we included a control group of COVID-19 patients seropositive for anti-S1 RBD IgM, in which we tested the presence of RF IgM.

The lack of cross-reactivity between RF IgM and SARS-CoV-2 IgM was confirmed in a study involving patients with other AIRDs and tested positive for different serum autoantibodies, such as anti-nuclear, anti-dsDNA, anti-Smith, anti-Sjögren's syndrome antigens, anti-citrullinated protein and anti-phospholipid antibodies (12). In fact, in this study the authors reported that none of the 100 systemic lupus erythematosus and the 92 Sjögren's disease patients' sera resulted positive for SARS-CoV-2 IgM or IgG (12).

In conclusion, the results of the present study offer clinicians a valuable tool for managing RA patients, in order to resolve the issue of the cross-reactivity between RF and serologic tests for SARS-CoV-2 S1 RBD-induced antibodies.

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