Review 4: "SARS-CoV-2 serological tests can generate false positive results for samples from patients with chronic inflammatory diseases"

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RR:C19 Evidence Scale rating by reviewer:

- **Reliable.** The main study claims are generally justified by its methods and data. The results and conclusions are likely to be similar to the hypothetical ideal study. There are some minor caveats or limitations, but they would/do not change the major claims of the study. The study provides sufficient strength of evidence on its own that its main claims should be considered actionable, with some room for future revision.

Review:

This study evaluated the specificity of available LFA and ELISA kits to measure IgG and IgM antibody titers against SARS-CoV-2, in patients with inflammatory diseases. Serum was a gold standard negative sample taken before 2019, and the results can be useful information for assessing the specificity of the diagnostic kits. However, improvements may be required in the data presentation and interpretation of the findings.

The purpose and results of this study are clear, but the implications for public health are unclear. Generally, it is necessary to identify past COVID-19 morbidity or vaccination by performing serological tests. However, how do false positives in rheumatoid factor-positive RA patients affect an estimate of the seropositive proportion of the general population? Of course, if there are few infections in the general population in the early stages of pandemic (e.g. less than 0.1%), the effect of this misclassification would be large (1). If the proportion of people infected with COVID-19 is relatively high, false positives may not have a significant impact on the estimation.

The authors showed the rate of false positives in Figure 1, up to about 20%. However, such a presentation is not appropriate. The sensitivity and specificity of testing are determined by the target population and situation, and the subjects of their study consist of patients with different diseases, and the proportions also differ from test kits. It is difficult to interpret the false-positive rates shown in this figure unless the composition of patients examined in real practices is similar to the patients examined by the authors. Hence, the illustration in Figure 2 is rather appropriate.

In addition, although the LFA results were graded in five stages, there is a certain difference as to how to handle weak signals among the instruction manual of test kits. Especially in the detection of IgM antibodies, there are several criteria that a weak signal in LFA is not regarded as positives. In this regard, the false positive rate shown in this study needs to be carefully interpreted.

False positives due to RF have been reported not only for SARS-CoV-2 (2). It has also been reported that the removal of RF ruled out false positives of rubella-specific IgM antibodies. It may be difficult to always perform such a procedure for samples of RA patients, but it may need to be considered as a means of retesting.

There is no information of disease activities such as multiple sclerosis, for the purpose of assessing interference with antibody titer measurements in inflammatory diseases. Similarly, RF antibody status is associated with disease activity in rheumatism, but anti-CCP antibodies are not usually associated with disease activity. The authors may show stratified analysis with anti-CCP antibodies.

(1) Lyu Z, Harada Sassa M, Fujitani T, Harada KH. Serological Tests for SARS-CoV-2 Coronavirus by Commercially Available Point-of-Care and Laboratory Diagnostics in Pre-COVID-19 Samples in Japan. *Diseases*. 2020; 8 (4): 36. <https://doi.org/10.3390/diseases8040036>

(2) Meurman O. H., Ziola B. R. IgM-class rheumatoid factor interference in the solid-phase radioimmunoassay of rubella-specific IgM antibodies. *Journal of Clinical Pathology* 1978; 31 (5): 483-487.