On the Need of a Suitable Immunohistochemical Test for Demonstration of SARS-CoV-2 on Formalin-fixed and Paraffin-embedded Tissues

To the Editor:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), inducing coronavirus disease 2019 (COVID-19), has caused over 5 million deaths in the world since February 2020.[1] In their recent paper, Szabolcs et al[2] highlighted the need for an accurate characterization of the histopathologic changes and the usefulness of immunohistochemical (IHC) detection of SARS-CoV-2 for the correct interpretation of histologic results. As they demonstrated, the proper analysis of the IHC reagents used in previous studies was not adequately considered, resulting in only 2 out of the 7 tested antibodies being specifically useful for the IHC detection of SARS-CoV-2 proteins. At our institution we performed the IHC analysis, on a BOND-III fully automated autostainer (Leica Biosystems, Nußloch, Germany), of 16 nasopharyngeal formalin-fixed paraffin-embedded (FFPE) biopsies routinely executed from August 1, 2019 to May 31, 2020. This series included biopsies carry out both before and during the pandemic outbreak, but without evidence of SARS-CoV-2 infection, to demonstrate the viral circulation as early as late 2019 in the Umbria region (Italy). Lung tissue of a patient who died from COVID-19 was used as positive control. The immunostainings were performed using SARS/sars-CoV-2 Coronavirus Nucleocapsid Monoclonal Antibody (B46F) (Thermo Fisher Scientific, Waltham, MA; Cat# MA1-7404, RRID:AB_1018422, dilution 1:100), previously validated for viral nucleoprotein (NP) detection in FFPE tissues.[3] Confirmatory RNAscope in situ hybridization using the V-nCoV2019-S probe (ACD, Newark, CA; Cat No. 848561) on Leica Bond RX (Leica Biosystems) and reverse transcription quantitative polymerase chain reaction for ORF1ab, S protein, and N protein SARS-CoV-2 genes (Thermo Fisher Scientific; PN A47532) were also performed.

Surprisingly, although 10 (62.5%) nasopharyngeal biopsies presented an IHC positivity, particularly as regards the cells cytoplasm of subepithelial glands of the upper airway (Figs. 1A, B), both in situ hybridization and reverse transcription quantitative polymerase chain reaction resulted negative for SARS-CoV-2 RNAs.

The false IHC positivity found in nasopharyngeal specimens could be explained by the lack of adequate specificity of the IHC antibody in detecting the NP. In fact, as widely reported for antigen-detection kits, currently approved for the diagnosis of COVID-19, the NP of SARS-CoV-2, although highly immunogenic and abundantly expressed during the infection, presented a high homology with the NP of other human coronaviruses, leading to relevant cross-link reactivity issues.[4] This aspect has not been properly elucidated yet and we agree with Szabolcs et al[2] about the need for further studies to clarify this issue.

Furthermore, to the best of our knowledge, the monoclonal antibody for SARS-CoV-2 used in our study has not yet been tested on a larger series, to verify both its specificity and sensitivity.[2,5]

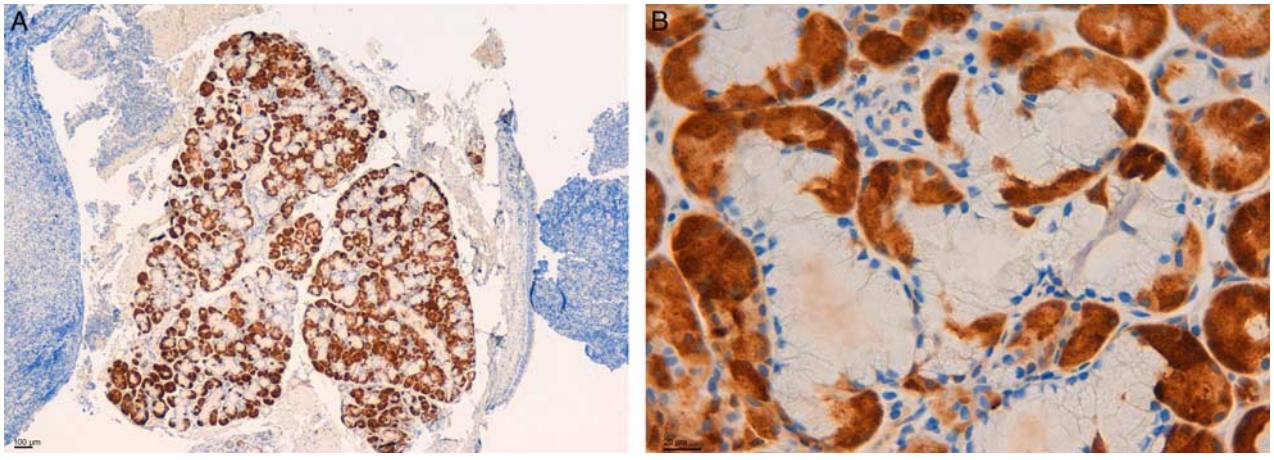

FIGURE 1. Immunohistochemical staining for severe acute respiratory syndrome coronavirus 2. A, Nasopharyngeal biopsies showing cytoplasmic immunohistochemical positivity of subepithelial glands. B, Detail at higher magnification from Figure 1A. Original magnification: A: ×40, B: ×400.

The authors declare no conflict of interest.

The persistent worldwide circulation of SARS-CoV-2 requires the development of an IHC antibody specifically targeting this virus, overcoming the issues related to molecular tests, such as the requirement for specific equipment and adequately trained personnel, which are not always readily available.[4]

Although there are actually few antibodies suitable for the IHC detection of viral proteins on FFPE, an accurate IHC identification of specific SARS-CoV-2 antigens is desirable for a faster, cheaper, and better understanding of the COVID-19 pathogenesis, leading to a more effective diagnostic-therapeutic management of this disease.

Martina Mandarano, MD, PhD*
Cristina Pelliccia, Dr*
Andrea Gianatti, MD†
Angelo Sidoni, MD*
*Section of Anatomic Pathology and Histology, Department of Medicine and Surgery, University of Perugia, Perugia Italy
†Pathology Unit, ASST Papa Giovanni XXIII, Bergamo, Italy

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