APPENDIX

VIRAL LOAD IN SALIVARY SAMPLES

In the first report the viral load was determined by relative quantification using rRT-PCR. Our group found that *cycle threshold* (*Ct*, the number of cycles required for the fluorescent signal to exceed background level) values were correlated with lactate dehydrogenase (LDH) values, as evaluated by blood chemistry tests. This finding suggested that the viral load could be linked to disease severity and lung tissue damage (Azzi et al., 2020). Williams and colleagues found that the Ct values recorded in the salivary samples were higher than the NPS Ct values, probably indicating a higher viral load in the respiratory samples (Williams et al. 2020). Other studies did not report significant differences between saliva and respiratory samples with respect to Ct values (Iwasaki et al., 2020; Kim et al., 2020).

The first quantitative analysis with an absolute quantification method based on an internal standard of the viral load in the saliva was carried out by To and colleagues on the posterior oropharyngeal saliva (To et al., 2020). The authors found that the viral load was higher during the first week after the onset of symptoms, and it gradually decreased subsequently. Other studies have confirmed these results (Zheng et al., 2020; Zhu et al., 2020). These findings are in contrast to those previously reported during the MERS-CoV and SARS-CoV outbreaks, in which the salivary load appeared to be higher during the second week. The correlation between the salivary viral load and the degree of disease severity is debatable, with several studies reporting that the salivary load shows no direct correlation with a worse clinical progression (To et al., 2020; Zhu et al. 2020).

Another feature that has been highlighted is the lower temporal variability in the saliva than in the NPS (Wyllie et al., 2020). Several case reports have been published about people who had a

negative NPS and then tested positive with a new swab. In these cases, saliva seems to maintain positivity more consistently (Wölfel et al., 2020).

Only a few reports have considered the salivary viral load in asymptomatic subjects (Chau et al., 2020; Willye et al., 2020). Data from these studies suggest that asymptomatic subjects show a viral load similar to that detected in symptomatic subjects, but it is lower in comparison with the NPS, and it is associated with faster viral clearance. However, even when the salivary load was lower, clusters of transmission were described (Chau et al. 2020). Other studies reported higher viral loads in saliva than in oropharyngeal swab (Bosworth et al., 2020). These findings, together with the early detection of high viral loads in infected patients, explain the necessity of a serious discussion about the role played by asymptomatic carriers in the transmission of COVID-19 infection, a role that seems to be more relevant in this pandemic than in the 2003 SARS outbreak (Lavezzo et al. 2020).

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