Salivary diagnostic of COVID-19: sailing between Scylla and Charybdis

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Abstract

Coronavirus disease 2019 (COVID-19) is a global health problem which is challenging healthcare worldwide. In this critical review, we discussed the advantages and limitations of the rapid implementation of salivary diagnostic platforms to COVID-19. The diagnostic test of COVID-19 by invasive nasopharyngeal collection is uncomfortable to the patients and requires specialized training to healthcare professionals in order to perform an appropriate collection of samples. Additionally, these professionals are in close contact with infected patients or suspected cases of COVID-19, leading to increased contamination risk for frontline healthcare workers (Scylla). Although there is a colossal demand for novel diagnostic platforms with non-invasive and self-collection samples to COVID-19, the implementation of the salivary platforms remains in debate due to its accuracy (Charybdis). Up to date, clinical trials supports the potential of detecting SARS-CoV-2 RNA in saliva as a biomarker for COVID-19, providing a self-collection, non-invasive, safety, and comfortable procedure. Therefore, the salivary diagnosis is suitable to protect healthcare professionals and others frontline workers, and may encourage patients to be tested due to its advantages over the current invasive methods. Furthermore, we expected that salivary diagnostic devices to COVID-19 continue to be used with austerity without excluding traditional gold standard specimens to detect SARS-CoV-2.

Introduction

In the Greek mythology, Scylla and Charybdis were two immortal monsters who beset the narrow waters traversed by Odysseus, detailed in the Homer's epic Odyssey (Ulysses in Latin). Scylla was a creature of 12 feet and 6 heads on long snaky necks, each head having a triple row of shark-like teeth, which she used to devour whatever ventured within reach. Charybdis, who located on the opposite shore, was most likely the personification of a whirlpool and was fatal to shipping (van de Schoot et al., 2013; Vranic, 2010). Bearing in mind the close location between Scylla and Charybdis, no alive sailor had ever crossed this inescapable way. These narrow waters were avoided by the risk of the dangerous monsters and by the roaring waters. Furthermore, the passage between Scylla and Charybdis was more arduous due to cyclones (Bhatt, 2007).

In this critical review, we discuss the advantages and limitations of the rapid implementation of salivary diagnosis to COVID-19 and point out some recommendations to this potential application. The diagnostic test of COVID-19 by invasive nasopharyngeal

collection is uncomfortable to the patients and requires specialized training for the frontline workers in order to perform an appropriate collection of samples. Additionally, these professionals are in close contact with infected patients or suspected cases of COVID-19, leading to increased morbimortality of healthcare workers (Scylla). It imposes the development of new strategies to COVID-19 diagnosis, however, despite the colossal demand for novel diagnostic platforms with non-invasive and self-collection samples to COVID-19, the accuracy of salivary SARS-CoV-2 platforms has yet to be elucidated (Charybdis). The pivotal impact on social, health, economic and educational fields in a global emergency due to COVID-19 makes it more challenging to compare the advantages and limitations in implementing novel potential salivary platforms (cyclones).

Background

The Coronavirus disease 2019 (COVID-19) is an international public health emergency which also impacts on social, economic and educational aspects worldwide. The outbreak of COVID-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has been spread to more than a hundred countries, in every continent, with more than 17 million cases and ~700.000 deaths (Dong, Du, & Gardner, 2020). The centers for diseases control and prevention around the world have recommended testing for SARS-CoV-2 in upper respiratory specimens.

The COVID-19 diagnostic is mainly based on the detection of SARS-CoV-2 by real time polymerase-chain-reaction (RT-PCR). The sensitivity of this gold standard test is higher in symptomatic than asymptomatic COVID-19 subjects (Gandhi, Lynch, & Del Rio, 2020), besides, the false negative results have uncertain frequency specially in incubation period of disease. Although SARS-CoV-2 RNA detection in nasopharyngeal swab and sputum were reported as the gold standard method for COVID-19 diagnosis, the sample collection by these methods require that healthcare frontline workers are in close contact with infected patients or suspected cases of COVID-19. Besides, this sample procedure is invasive and inconvenient to patients, and requires specialized training of healthcare workers (Chan et al., 2020).

Salivary biomarkers are an alternative method to surrogate other invasive procedures in the early diagnostic of systemic diseases (Zhang et al., 2016). The collection of saliva samples represents a non-invasive, convenient and easy self-collection method, with no direct contact between healthcare workers and patients. Saliva contains more than 3,000 proteins, 3,000 mRNA, ~50 microRNAs, hundreds of metabolites and more than 700

species of microorganisms such as viruses (Dawes & Wong, 2019). Previously, we detailed the potential of salivary diagnosis for COVID-19 (Sabino-Silva, Jardim, & Siqueira, 2020), which was confirmed in several studies by detecting SARS-CoV-2 in human saliva (Azzi et al. 2020; Pasomsub et al. 2020, Fang et al. 2020; McCormick-Baw et al 2020; Iwasaki et al. 2020 (b); Chau et al. 2020; Ranoa et al. 2020) and in saliva associated with oropharyngeal fluid (To, Tsang, Chik-Yan Yip, et al., 2020; To, Tsang, Leung, et al., 2020). SARS-CoV-2 was also detected in animal models of COVID-19 (Kim et al., 2020). In this context, analysis of SARS-CoV-2 RNA from saliva can provide clues in the early diagnosis of COVID-19. Higher viral loads of SARS-CoV-2 in oropharyngeal fluid mixed with saliva were detected when symptoms onset, which then gradually declined towards the detection limit until 25 days after symptoms started (To, Tsang, Leung, et al., 2020). Besides, an additional study also detected SARS-CoV-2 RNA in saliva and nasal washes from 2 to 8 days post-infection infected ferrets as an animal model of COVID-19 (Kim et al., 2020).

Future directions

As the landscape of SARS-CoV-2 diagnosis comprises limitations for the current gold-standard diagnosis methods and potential benefits for novel applications in COVID-19 diagnosis, a critical evaluation on the advantages and limitations of concurrent emerging salivary diagnosis is mandatory (Table 1).

The Scylla's hazards

The nasopharyngeal collection is performed using a flexible plastic swab with a nylon tip, which is inserted into the nostrils until the healthcare worker observes resistance. Subsequently, the swab is rotated three times in nasopharynx and removed after 5 seconds, procedure which is considered invasive and uncomfortable (Frazee et al., 2018; Li et al., 2013). However, the swab collection protocol can be different in each country. The appropriate nasopharyngeal swab collection is more difficult in children and patients with deviated nasal septum or coagulopathy (Marty, Chen, & Verrill, 2020). Due to the limitations of sampling, the sputum collection was possible in only 1/3 of COVID-19 patients, which reveals a robust restriction of this diagnostic method (Sabino-Silva et al., 2020; To, Tsang, Chik-Yan Yip, et al., 2020). Self-collection of samples from suspected cases of COVID-19 or infected patients are still limited and the direct contact between healthcare workers and patients during the standard collection procedures resulted in about 20% of the healthcare workforce infected, and some deaths reported (Lancet, 2020). The

frontline workers may experience intense anxiety and additional adverse emotion due to the risk of contamination during collection procedure (Qian et al., 2020). Additionally, lower levels of SARS-CoV-2 in nasopharyngeal swabs can results in false negative outcomes due to the inaccurate collection (Qian et al., 2020). The personal protective equipment and creation of exclusive sampling room have been reported as tools capable to enhance protection of frontline workers (Lancet, 2020; Qian et al., 2020). Currently, the COVID-19 cases have been significantly increasing worldwide, overloading national health systems. Furthermore, the situation might be even worse in low- and middle-income countries, since there is scarcity of trained healthcare and others frontline workers to face the COVID-19 pandemic (Nkengasong & Mankoula, 2020). Taken together, these several issues (*Scylla*) demonstrates the critical demand for new approaches for COVID-19 diagnosis.

The Charybdis' hazards

The enthusiasm in developing new salivary platforms for COVID-19 diagnosis and monitoring is comprehensible, however the true accuracy of this new protocols to detect SARS-CoV-2 in saliva has not yet been established in several scenarios as during the incubation period, the viral response phase and the host inflammatory phase of symptomatic patients. Besides, the diagnostic sensitivity levels in COVID-19 asymptomatic patients also remain unclear (Charybdis). It is important to emphasize that the implementation of salivary diagnosis to COVID-19 before a comprehensive knowledge of its limitation could promotes future issues about the application of salivary diagnostic tests to other systemic diseases. However, the colossal demand for novel diagnostic platforms to COVID-19 with non-invasive and self-collection samples could be used after the creation of well-designed strategic plan for its implementation until this true efficacy had been completely investigated.

Preponderance of reviews and letters over primary clinical trials

The most remarkable data on COVID-19 salivary diagnosis implementation is the unbalanced number of published clinical trials or reviews and letters. PubMed reveals 5 cross-sectional and case-control designed studies, 4 cross-sectional studies with no control subjects and 114 reviews/letters published from February up to June 2020. Additionally, there are 4 additional cross-sectional studies with no control subjects that evaluated the oropharyngeal fluid mixed with saliva as a diagnostic fluid to COVID-19. It suggests that

opinions concerning salivary diagnostic platforms have been consolidated primarily from letters and reviews. On the other hand, it is important to emphasize that the cross-sectional studies with salivary diagnostics indicated higher correlation of sensitivity and specificity than with gold standard samples in COVID-19 diagnosis. We performed this critical review due to the limitations concerning current reviews focusing on the counterbalance between the inevitable obstacles and encouraging results of COVID-19 salivary diagnosis.

Sample size in clinical trials

The main restraint to implementation of salivary diagnosis in COVID-19 is the absence of studies with large sample sizes in order to obtain a more robust comparison with gold standard specimens. The total of samples (non-infected subjects and COVID-19 patients) that compared oral fluids with gold-standard respiratory specimens were 1684, including 202 in the oropharyngeal fluid mixed with saliva samples (Chen et al., 2020; Chu et al., 2020; To, Tsang, Chik-Yan Yip, et al., 2020; To, Tsang, Leung, et al., 2020; Zheng et al., 2020) and 1348 in the salivary fluid samples. Concerning salivary fluid, the total of samples was 625 subjects from published studies (Azzi et al., 2020; Chau et al., 2020; Fang et al., 2020; Han et al., 2020; Jamal et al., 2020; McCormick-Baw et al., 2020; Nagura-Ikeda et al., 2020; E. Pasomsub et al., 2020), 53 subjects from the FDA emergence approved study (Rutgers, 2020), 594 subjects from preprint articles (Griesemer et al., 2020; Miller et al., 2020; Ranoa et al., 2020; Wyllie et al., 2020) and 76 subjects from a letter to the editor (Iwasaki et al., 2020). In the context of the evaluated sensitivity, 448 salivary samples and 208 oropharyngeal fluid mixed with saliva samples from COVID-19 infected patient were used in these studies.

The relevance of specificity in SARS-CoV-2 detection

In general, the absence of analysis in control subjects can be considered a negative condition, however, the main limitation in the use of RT-PCR tests is the detection of RNA in levels near to the sensitivity limits. The detection of unspecific RNA is not a classical limitation of RT-PCR tests (Bustin & Nolan, 2004), which is considered 100% specific due to intrinsic characteristics of this platform (Buonfrate et al., 2018). It must be considered that the presence of SARS-CoV-2 RNA in saliva and negative results in nasopharyngeal samples analyzed by RT-PCR cannot be classified as false positive, but a misclassification of currently gold-standard protocols. This pivotal view is well documented in a previous study that showed 71% of matched detection of SARS-CoV-2

RNA in saliva and nasopharyngeal swabs, 21% only in saliva and 8% only in nasopharyngeal swab (Wyllie *et al.* 2020). It can be related with the limitations in nasopharyngeal swab procedure and/or with low produced nasopharyngeal mucous secretion in COVID-19 patients. In this new pandemic era, the centers for disease control and prevention worldwide took maximal efforts to establish reference standards for COVID-19 diagnosis in a fast and efficient way, based on the outbreak of severe acute respiratory syndrome (SARS) in 2003 (Yang et al., 2020). It is well recognized that updates in COVID-19 diagnosis protocols are crucial and the reference standards are not perfect, specially in samples collected in the first days after infection (Woloshin & Patel, 2020). The procedures related to sample preservation and RNA extraction were reported in all included studies and it seems suitable and presumably that these factors did not influence the results. In this context, it is important to emphasize that the absence of control group in the studies with oropharyngeal fluid mixed with saliva is not a significant limitation (Chen et al., 2020; Chu et al., 2020; To, Tsang, Chik-Yan Yip, et al., 2020; To, Tsang, Leung, et al., 2020; Zheng et al., 2020).

Saliva collection and its correlation with sensitivity

The pioneer study that detected viable SARS-CoV-2 in oral fluid promoted a paradigm shift in diagnosis, monitoring and infection control for COVID-19 (To, Tsang, Chik-Yan Yip, et al., 2020). However, the sensitivity of salivary SARS-CoV-2 RNA to diagnose COVID-19 need to be carefully checked because some data are based on trials designed to evaluate oropharyngeal fluid mixed with saliva (To, Tsang, Chik-Yan Yip, et al., 2020; To, Tsang, Leung, et al., 2020; Zheng et al., 2020). In typical studies with salivary collection, the patient is not required to cough out fluid from their throat. Frequently, total saliva is collected from the mouth under unstimulated or stimulated flow rate (Dawes & Wong, 2019). Some collection devices were also developed to collect saliva specifically from parotid, submandibular/sublingual and minor and palatine glands (Dawes & Wong, 2019). Particularly in three studies (131/273; 48% of total samples from COVID-19 patients), the posterior oropharyngeal saliva was collected by asking to the patient to cough out fluid from their throat into a sterile container (To, Tsang, Chik-Yan Yip, et al., 2020; To, Tsang, Leung, et al., 2020; Zheng et al., 2020), which suggests that the mucous secretion with SARS-CoV-2 from throat, oropharynx and nasopharynx could be mixed with saliva in mouth. The saliva was collected by traditional drooling technique in other three studies (Azzi et al., 2020; Rutgers, 2020; Wyllie et al., 2020). Another study informed

that saliva sample was collect without coughing in container (Ekawat Pasomsub et al., 2020). The SARS-CoV-2 RNA was detected in 85% (381/448) of saliva samples, which supports the potential of salivary SARS-CoV-2 RNA as a biomarker for COVID-19 in a preliminary analysis. The SARS-CoV-2 RNA was also detected in 91% (184/202) of samples of oropharyngeal fluid mixed with saliva. It is worth underlining that the detection of SARS-CoV-2 in oropharyngeal fluid mixed with saliva and total saliva was similar (91% and 85%, respectively), which reinforces the potential of total saliva to be used as a clinical specimen of COVID-19 diagnosis. We also observed that the majority of articles analyzed unstimulated saliva, which avoids a potential dilution of the SARS-CoV-2, as could occur in mouth rinsing or stimulated saliva collection (Arias-Bujanda, Regueira-Iglesias, Balsa-Castro, & Nibali, 2020). One of the studies did not detailed the salivary sample collection procedure (Fang et al., 2020).

The importance of home- and self-sample collection

It was indicated that the primary choice for sampling during illness experience is home based-tests compared with clinic-based strategy. The higher compliance to test for SARS-CoV-2 was verified when lower degree of contact with frontline healthcare workers was required to collect samples: as expected, the home testing was the most preferred, followed by tests in drive-through sets and subsequently, hospital-based testing. It is crucial to provide self-saliva collection and home-based tests to suspected cases of COVID-19 as profitable strategies in order to guarantee the social distance of population. It also contributes to reduce direct contact with frontline workers and also to offers a potential to early diagnosis due to the hierarchy of willingness to COVID-19 test. The self-sample collection and home-based tests should be validated as soon as possible to be applied in public and private healthcare systems (Siegler et al., 2020).

Spectrum of patients

In order to provide a suitable spectrum of COVID-19 patients with distinct severity of diseases, it is important to envisage patients searching for the diagnostic test in the onset of symptoms and in late stage of the disease. Bearing in mind that the higher salivary SARS-CoV-2 levels occur during the acute phase of diseases with gradual decline after symptoms onset (To, Tsang, Leung, et al., 2020), it is important to point out the limitations of longitudinal analysis with SARS-CoV-2 level in asymptomatic COVID-19 subjects. Currently, only 2 longitudinal studies evaluated the level of SARS-COV-2 during the

clinical course of COVID-19, which was performed in SARS-CoV-2 infected patients admitted in the hospital. In this context, the temporal analysis of SARS-CoV-2 viral load in saliva should receive more attention among asymptomatic and non-hospitalized COVID-19 patients, which could be pivotal for translation salivary tests to the clinic. However, the currently gold standard protocols are also unable to raise this query (Woloshin & Patel, 2020). The comparison between sensitivity and specificity in different studies reported a limited heterogeneity, which should not be ignored to improve this new potential gold standard protocol. The majority of the collection of samples were performed in patients admitted in hospitals during admission in either the intensive care unit (ICU) or non-ICU (Azzi *et al.*, 2020; Chau *et al.*, 2020; Fang *et al.*, 2020; Han *et al.*, 2020; Jamal *et al.*, 2020; Mccormick-Baw *et al.*, 2020; Nagura-Ikeda *et al.*, 2020; Pasomsub, E. *et al.*, 2020). A critical hurdle for the salivary diagnosis may be the broad-spectrum validation in COVID-19 patients during the incubation period, the viral response phase and the host inflammatory phase to asymptomatic and symptomatic patients. It was proposed that patients could be infected from 24 to 72 hours prior to the symptom onset and that about 50% of cases are performed to transmission from asymptomatic COVID-19 subjects. The viral levels of SARS-CoV-2 RNA are presumably detected in nasopharyngeal swab before or sooner the symptom onset, which is a leading challenge in diagnostic and spread containing of COVID-19 (Gandhi et al., 2020). Therefore, considering the decisive potential of saliva in COVID-19 diagnosis, we suggest that efforts are directed to the organization of a global consortium aiming to validate this biofluid for salivary tests as soon as possible (Table 1).

The COVID-19 Cyclone

The threat to navigate in narrow waters between Scylla and Charybdis is more difficult under cyclone winds, which increase the challenge. In this regard, the potential implementation of salivary SARS-CoV-2 diagnosis under a pandemic situation and social, health, economic and educational issues due to COVID-19 is an additional challenge.

Final remarks

As in Homer's epic Odyssey, we suggest that the best choice is to navigate through these narrow waters in the middle way between Scylla and Charybdis, avoiding the action of these two monsters. It seems to be in agreement with FDA emergence approval which includes a home collection of saliva to diagnose of COVID-19 when indicated by a

healthcare provider. The patients are also informed that a negative result is not the guaranty of the absence of COVID-19 infection. However, due to the high-specificity of RT-PCR analysis, the detection of SARS-CoV-2 in saliva can be acceptable when the diagnostic test for COVID-19 is positive. Besides, the higher compliance to test for SARS-CoV-2 under reduced direct contact, required to collect saliva, may contribute to an early diagnosis of COVID-19, resulting in optimal clinical care, encouraging isolation and reducing the spread of the disease. These results support the potential of SARS-CoV-2 RNA as a biomarker for COVID-19, providing a self-collection, non-invasive, safety and comfortable analysis, suitable to protect healthcare professionals and others frontline workers with self-collection and/or home collection saliva samples. Furthermore, we expected that salivary diagnostic devices to COVID-19 continue to be used with austerity without excluding traditional gold standard specimens to detect SARS-CoV-2.

Conflict of Interest

Sabino-Silva R, Cunha TM, Goulart LR, Cardoso-Sousa L and Martins MM are inventors of patent related to the use of photonic device as COVID-19 diagnostic for saliva and nasopharyngeal samples titled "Spectral profile for COVID-19 diagnostic, use of the same, method, system and platform to COVID-19 diagnosis - BR 10 2020 010992 8". The other authors declare no conflict of interest.

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Table 1. Sensitivity and specificity of COVID-19 salivary diagnosis comparing to gold standard specimens.

Study	Oropharyngeal or total saliva	Patient Characteristic	Sensitivity (%)	Specificity (%)
Rutgers Clinical Genomics Laboratory/FDA, 2020	Total saliva	NR	100% (26/26)	100% (27/27)
Azzi et al. 2020	Total saliva	61.5 y [±11.25] Hospital: Severe	100% (25/25)	100% (NR) **2 patients + in saliva and – in NS
Pasomsub et al. 2020	Total saliva	36 y [29-47] Hospital: Dyspnea 36%	84.2% (16/19)#	98.9% (179/181)
Fang et al. 2020	Total saliva	41 y [34-54] Hospital: 8 ICU and 24 non-ICU	78.1% 25/32)	NR
Wyllie et al. 2020&	Total saliva	Hospital: 19 ICU and 23 non-ICU/ 39 matched-samples / onset of symptoms: 3 days [2-11]	90.3% (28/31)	NR **8 patients + in saliva and – in NS
McCormick-Baw et al 2020	Total saliva	47.8 y [66-90] Hospital: patients not requiring mechanical ventilation	96% (47/49)	98.8% (105/106)
Iwasaki et al. 2020	Total saliva	69 y [30-97] Hospital: mild and moderate disease and not requiring mechanical ventilation	80% (8/10)	100% (66/66)
Chau et al. 2020	Total saliva	29 y [16–60] Hospital: 11 asymptomatic and 16 symptomatic patients	74% (20/27)	NR **1 patient + in saliva and – in NS
Nagura-Ikeda et al. 2020	Total saliva	48 y [36-63] Hospital: 15 asymptomatic and 88 symptomatic patients (61 early phase of onset of symptoms and 27 late phase of onset of symptoms)	81.6% (84/103)	NR
Han et al. 2020	Total saliva	6.5 y [27 days-16 years] Hospital: 9 mildly symptomatic and 3 asymptomatic	73% (8/11)	NR
Miller et al. 2020&	Total saliva	NR	97.1% (33/34)	96.5% (55/57)
Ranoa et al. 2020&	Total saliva	100 individuals	100% (9/9)	100% (91/91)
Jamal et al. 2020	Total saliva	66 y [27-106] Hospital	72% (52/72)	NR

To et al. 2020 (a)	Oropharyngeal saliva	62.5 y [37-75] Hospital	91.6% (11/12)	NR
To et al. 2020 (b)*	Oropharyngeal saliva	62y [37-75] Hospital	81.8% (9/11)	NR
Zheng et al. 2020	Oropharyngeal saliva	55 y [44-64] Hospital: 22 mild disease and 74 severe disease	100% (96/96)	NR
Chen et al. 2020	Oropharyngeal saliva	38 y [31-52] Hospital	84.5% (49/58)	NR **3 patients + in saliva and – in NS
Chu et al. 2020	Oropharyngeal saliva	NR	76% (19/25) [PKH method]	NR

Note: NR: Not Reported * It was reported that 12 of 23 patients were used in another study, thus we removed these patients from this table; ** Report additional patients positive to SARS-CoV-2 in saliva and negative in respiratory samples. *** Report additional patients positive to SARS-CoV-2 in respiratory samples and negative in saliva. # Nucleic acid extraction was performed within 26 min – potential degradation of SARS-CoV-2 RNA; & Preprint.