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Case Report

Detection of SARS-CoV-2 RNA in exhaled breath and its potential for prevention measures

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SUMMARY

Background: To propose infection prevention measures it is essential to understand the dynamics of SARS-CoV-2 shedding, particularly in asymptomatic patients. This report compares the viral load progression in exhaled breath (EB) with the symptom severity. We aim to evaluate the adequacy of symptom assessment regarding the infectivity level of individuals.

Methods: We observed infected patients since their first positive test during hospitalization. EB samples were collected on days 1, 3, 5, 7, 10, 12 and 14 of hospitalization using a filter-based device. After extraction, viral loads were quantified with qRT-PCR. The infection trajectory was documented after symptom onset.

Case Presentation and Discussion: A 34-year old patient showed mild symptoms, e.g. fever, cough, headache, muscle pain and loss of taste and smell across trajectory of infection (Case 1). The viral loads emitted via exhaling were nearly constant and ranged from 8.6×10^3 and 4.1×10^4 RNA copies per hour. After the infection, the patient developed a pneumonia. The second case of a 65-year old patient depicted an asymptomatic infection trajectory for 14 days after the first diagnosis (Case 2). Nevertheless, the patient exhaled up to 2×10^5 SARS-CoV-2 virus copies hourly, approximately 10 fold higher than measured for Case 1.

Conclusion: Symptomatic and asymptomatic COVID-19 patients exhale distinctive amounts of SARS-CoV-2 not necessarily correlating with symptom severity. Particularly, asymptomatic patients might show higher EB viral shedding. Therefore, EB testing should be included in infection prevention measures as it has high potential to reveal the most infectious individuals regardless of their symptoms during infection.

Background

After almost three years of COVID-19 pandemic, uncontrolled outbreaks still occur making it indispensable to propose optimised and effective prevention and intervention measures. Still, many questions revolving around virus viability, viral shedding and its dynamics, transmissibility and infectiousness of patients remain unanswered. Two key factors to avoid unrestrained continuous spreading of the virus throughout the population are (1) diagnosing SARS-CoV-2 infections immediately, (2) distinguishing and screening most infectious and non-infectious individuals. Even though nasopharyngeal swabs are considered as the gold standard in SARS-CoV-2 diagnostics, the

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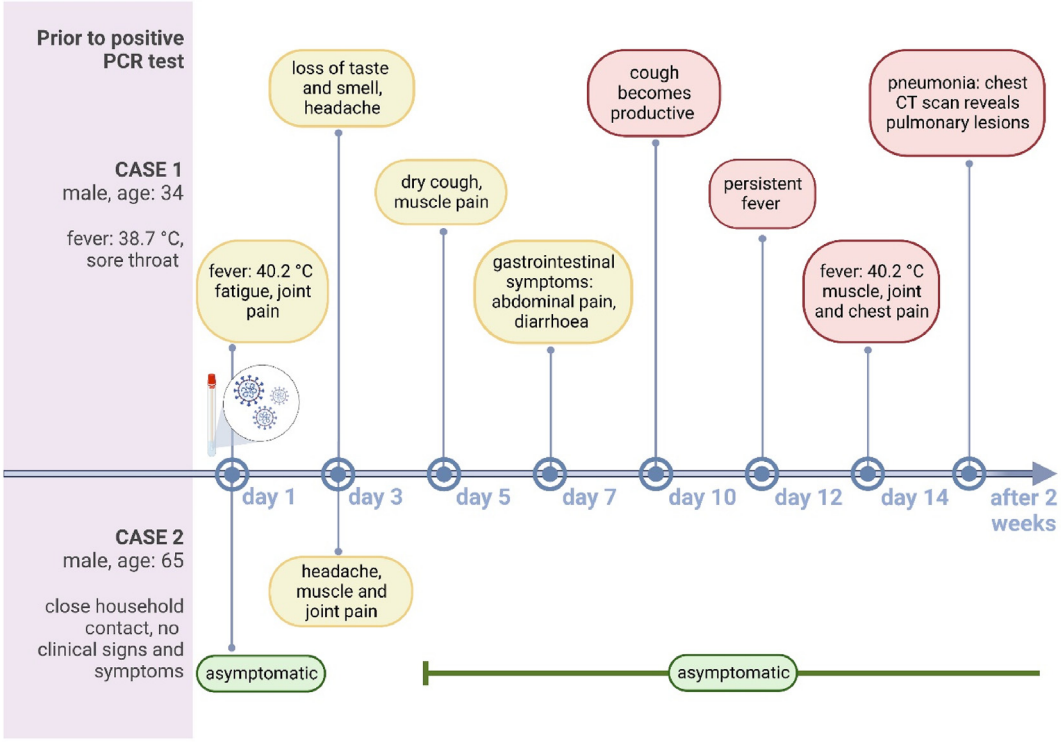

Figure 1. Overview of symptom onset in Case 1 and 2 during SARS-CoV-2 infection trajectory (over 14 days). The subject in Case 1 did not suffer from any diagnosed diseases whereas Case 2 is a type 2 diabetes patient and suffers from dyslipidemia, goitre and gastroesophageal disease. The day on which the infection is confirmed with a valid positive SARS-CoV-2 PCR-test is set as day 1. The symptoms characterise an asymptomatic (green), mild (yellow) and/or moderate (red) infection; "Asymptomatic" is defined as the absence of clinical signs and symptoms of the disease. High temperature (fever) is presented as means (n=10). This figure was created with BioRender.com.

analysis of nasopharyngeal mucus might not be sufficient to address above mentioned questions [1,2].

With growing knowledge about different transmission routes, the SARS-CoV-2 transmissibility via breath (droplets and/or aerosols) has been resurging the interest of exhaled breath (EB) testing [3—8]. Since 2019, there have been many reports about the use of EB as an alternative non-invasive and promising detection method of SARS-CoV-2 [2,9—14]. However, the potential of EB is not limited to detecting SARS-CoV-2. EB testing might also be an efficient tool for estimating the infectiousness among population [1—3].

In order to optimise infection prevention measures it is essential to further investigate the dynamics of SARS-CoV-2 shedding. Evaluating viral shedding in the breath particularly of asymptomatic patients might play a major role achieving this goal.

This case report compares the SARS-CoV-2 viral load progression in EB of two patients with the severity and duration of symptoms. Herein, we aim to discuss exhaled breath testing, which could be a gamechanger for tackling COVID-19.

Methods

Study design

Here we report about two cases that occurred while examining COVID-19 patients for an experimental study [2]. In total, 14 exhaled breath and swab samples were collected from two hospitalized patients diagnosed with COVID-19, respectively, in November 2020. EB specimens were tested for SARS-CoV-2 RNA. We evaluated the course of illness starting with the day of infection (first diagnosis). The symptoms of patients were thoroughly documented with the beginning of the infection (with symptom onset).

This study has been carried out according to the Declaration of Helsinki and was approved (D527/20) by the Ethics Committee of the Faculty of Medicine, Kiel University, Germany. The data protection office of the Faculty of Medicine, Kiel University waived the informed consent for COVID-19 research. Written informed consent was obtained from the patients.

Sample collection, preparation and RT-PCR

EB samples were collected after the first positive COVID-19 diagnosis (= day 1) repeating sample collection every 1—3 days, particularly on days 3, 5, 7, 10 and 14 during hospitalization.

EB samples were collected with an exhaled breath collection device (SensAbues®, Sweden). The unit consists of a mouthpiece, a polymeric electret filter enclosed in the plastic collection chamber, and an attached clear plastic bag [2,15—17]. Nasopharyngeal samples were obtained using a sterile swab. Patients were instructed to not ingest food, smoke, chew gum or brush teeth 30 minutes prior to sample collection. During breath sampling, patients inhaled via the nose and tidally exhaled 20 times through the mouthpiece onto

the filter inside of the collection device. Each EB sample was performed using a new device.

The EB samples were stored at -80 °C until RNA extraction. The extraction and quantification of SARS-CoV-2 RNA was performed as we reported earlier [2]. First, viral RNA extraction was carried out using the QIAamp viral RNA mini kit (QIAGEN GmbH, Hilden, Germany). The filter of the EB collection device was extracted by adding 1 mL of buffer every 5 minutes thrice. 400 µL of the extracted EB samples were then taken for further RNA isolation steps. Finally, the RNA suspension was eluted in 50 µL of buffer of which 10 µL were taken and added to 15 µL of PCR master mix. All qRT-PCR experiments were performed on a BioRAD CFX96 Real-Time Thermal Cycler with Maestro Software (Hercules, California) using the ampliCube Coronavirus SARS-CoV-2 kit (Mikrogen, Neuried, Germany). Thermal cycling conditions were: 50 °C for 8 minutes, 95 °C for 3 minutes, 45 cycles of 95 °C for 10 seconds and 60 °C for 45 seconds. The SARS-CoV-2 RNA detection was determined by amplification of the targeted genes (E, ORF1a) with a cut-off cycle threshold (Ct) of 40. An *in vitro* transcribed-quantified coronavirus 2019 E gene control (European Virus Archive GLOBAL, Charité University Berlin) was used to calculate a calibration curve allowing a precise quantification of the viral load.

Case Presentation and results

The two patients presented in this case study exhibit a distinctive course of disease. The specific symptoms, which appeared across trajectory of infection, were classified as asymptomatic, mild and moderate according to NIH [18]. Both cases occurred during COVID-19 outbreak in late 2020 with the patients not being vaccinated.

Figures 1 and 2 draw a comprehensive overview of the infection trajectory of the patients, considering the time length, temporal viral load decay and symptoms.

Case 1

In our study, a 34-year old individual was subjected to close monitoring. The patient resulted to be positive for SARS-CoV-2 infection. In total, 14 swab and 14 exhaled breath samples were taken during the two weeks after being tested positive for SARS-CoV-2. All of the sampled swabs indicated an infection whereas virus was detectable in exhaled breath just until day 10.

During infection trajectory, the subject showed mild symptoms, e.g. fever, cough, headache, muscle pain and loss of taste and smell (see Figure 1). Figure 2A depicts the viral loads of respective swab and exhaled breath samples during infection. The measured viral load exhaled per hour ranged from 8.6 x 10^3 and 4.1 x 10^4 (Figure 2). After 10 days of infection, the exhaled breath samples were SARS-CoV-2 negative, whereas swab samples still showed a positive result until day 14. At 28 days from first positive test, however, the symptomatic assessment and chest CT scans revealed pulmonary lesions and a nodule in the right upper lobe. The subject had developed a pneumonia, which cured after two weeks of treatment.

Case 2

After 10 days of exposure, a 65-year old diabetes patient was tested positive for SARS-CoV-2. Across the infection, we collected swab and exhaled breath samples, 14 of each

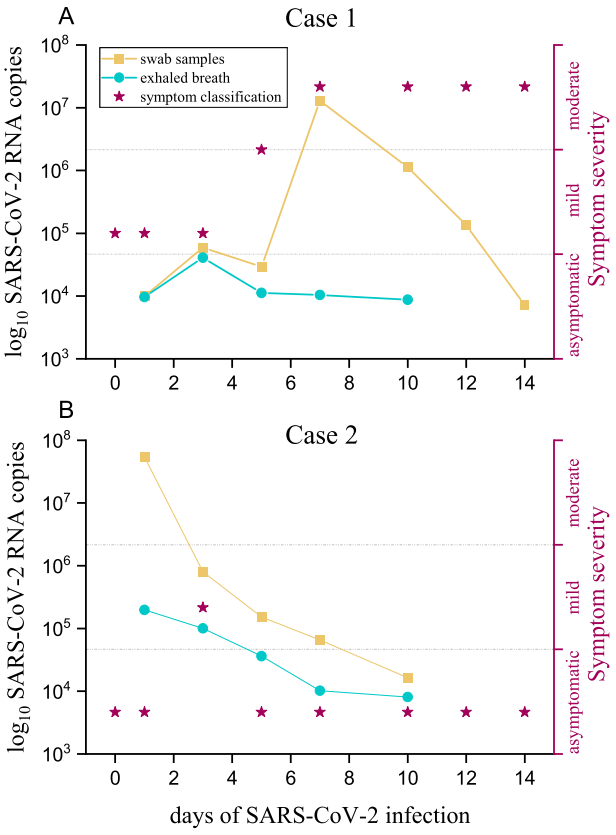

Figure 2. Comparison of temporal viral loads, symptom onset and symptom severity of two patients infected with SARS-CoV-2. Viral loads are presented as $\log_{10}$ RNA copies in one swab sample (yellow, square) and in exhaled breath (blue, circle) emitted per hour across infection trajectory (up to 14 days). Sample collection was repeated every 2—3 days during infection, symptom documentation was performed each day of sample collection. Symptom severity is classified in asymptomatic, mild and moderate (purple, asterisk). The temporal characteristics of respective symptoms of both patients are outlined in Figure 1.

respectively. After 10 days of infection, neither swab nor exhaled breath samples showed a positive SARS-CoV-2 result. The patient emitted up to 2 x 10^5 SARS-CoV-2 virus copies per hour just via regular breathing. Despite the high viral load, the patient was nearly asymptomatic across trajectory of infection. However, the viral loads detected in breath samples were approximately 10 fold higher than those of patient 1.

Discussion

The non-invasive collection of exhaled breath has gained more interest since the beginning of the pandemic. Several studies have investigated SARS-CoV-2 in exhaled breath proving the potential of EB testing in SARS-CoV-2 diagnostics [2,9—14,19]. However, different devices and collection methods show varying detection rates [19] emphasizing the challenge of standardized EB sample collection. Nevertheless, EB testing could be a gamechanger in assessing the infectiousness of individuals [1,2].

In the presented cases, viral loads determined in EB and nasopharyngeal mucus depict different progressions in both

individuals (Figure 2). Also, no virus could be found in EB samples in both cases after ten days, whereas the swab samples still indicated an infection. Notably, viral loads in EB samples were lower than those in swabs but the measured viral load in EB is the actual amount shed by an infected patient into the environment during tidal breathing. On the contrary, the viral load found in swabs is not fully emitted as pharyngeal mucus with cells containing viral RNA is mainly swallowed [2]. Therefore, breath is likely to be a more promising and accurate biomaterial. Apparently, this paradigm shift is necessary to categorize the infectivity level of individuals. Furthermore, the infectivity level cannot be inferred from symptom assessment sufficiently. Arons *et al*. and Wei *et al*. reported asymptomatic patients being capable of spreading the virus, which is in accordance to our results [20,21].

The infected 65-year old individual presented in case 2 almost had an asymptomatic trajectory of infection. Figure 2B shows decreasing viral loads in exhaled breath during the two weeks after the first diagnosis. Still, the patient exhaled up to 200,000 SARS-CoV-2 RNA copies per hour at the peak of infection. If the patient had not been screened and isolated, he could have infected many other individuals.

Case 1 was closely monitored after being traced as a close contact of an infected critically ill person treated in the ICU. Although the symptoms were classified as mild according to NIH, the patient remained permanently exhausted during the first two weeks of infection. Also, the subject showed all described symptoms that may occur during an infection (Figure 1). The infection and associated symptoms became more severe as almost after 4 weeks of infection chest CT scans revealed that the subject had developed a pneumonia. Interestingly, this infection trajectory was unexpected when evaluating viral shedding via breathing. The measured viral loads exhaled per 1 hour ranged from 8.6×10^3 and 4.1×10^4 (Figure 2). In comparison, the patient exhaled nearly constant viral loads but still not more than the asymptomatic subject (Case 2).

The two cases clearly show no correlation between emitted viral loads and severity of symptoms. Patients exhale distinctive amounts of virus regardless of symptoms caused by the infection. These findings emphasize that the most infectious individuals do not necessarily show a severe infection trajectory.

Nevertheless, it has to be considered that this study investigated the SARS-CoV-2 wildtype. Throughout the pandemic, other variants such as Delta and Omikron appeared, which were discussed to be more infectious. [22—25] It is conceivable that these variants might exhibit distinct characteristics regarding infection and associated symptoms, emitted viral loads and/or infectiousness. Also, the vaccination status of individuals might play a significant role. As the study was performed in 2020, the patients presented here were not vaccinated at all. The vaccination status of infected individuals might have an affect on the results as well. While interpreting these results, it should be considered that this case study only included two cases. Moreover, the pulmonary system and other organs might be differently involved in individuals infected with SARS-CoV-2, thereby contributing to a distinct pattern of viral shedding via breath. Even though other studies support our results [20,21], there is still more data needed to confirm such trends. Future research should also examine the comparative infectiousness of the sampled individuals since no viral culture was performed.

Conclusion

Symptomatic and asymptomatic COVID-19 patients exhale distinctive amounts of SARS-CoV-2. Particularly, asymptomatic patients could show higher viral shedding via breathing. The viral load emitted into the environment of infected patients does not always correlate with the severity of the infection. Hence, EB testing not only has diagnostic potential but also represents a beneficial tool to reveal the most infectious individuals regardless of their symptoms during infection. Therefore, EB testing could be a gamechanger when included in infection prevention measures for tackling pandemic situations caused by airborne pathogens.

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Author contributions

Madiha Malik: conceptualization, methodology, formal analysis, investigation, resources, writing - original draft, visualization, writing - review & editing; **Thomas Kunze:** conceptualization, supervision, project administration, writing - review & editing.

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Competing interests

The authors declare no competing interests.

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