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Research Article

Screening for SARS-CoV-2 Persistence in Long COVID Patients using Sniffer Dogs and Scents from Axillary Sweats Samples

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ABSTRACT

Background: Dogs can be trained to identify several substances not detected by humans, corresponding to specific Volatile Organic Compounds (VOCs). The presence of VOCs, triggered by SARS-CoV-2 infection, was tested in sweat from long COVID patients.

Patients and methods: An axillary sweat sample of long COVID patients and of COVID-19 negative, asymptomatic individuals was taken at home to avoid any hospital contact. Swabs were randomly placed in olfaction detection cones, and the material sniffed by at least 2 trained dogs.

Results: Forty-five long COVID patients, mean age 45 (6-71), 73.3% female, with prolonged symptoms evolving for a mean of 15.2 months (5-22) were tested. Dogs discriminated in a positive way 23/45 (51.1%) long COVID patients versus 0/188 (0%) control healthy individuals (p<.0001).

Conclusion: Our data provide arguments for the persistence of viral antigens at least in some long COVID patients and raise the possibility of future therapeutic options.

Keywords: Volatile organic compounds; Dogs; SARS-CoV-2; Viral persistence; Long COVID patients

INTRODUCTION

Dogs have olfactory capacities several hundred times greater than humans, likely due to a much larger zone of olfactory epithelium with 40 times more olfactory cells, and to the presence of the Jacobson vomero-nasal organ, which does not exist in humans. Thanks to their intelligence, sociability, and high learning ability, dogs can be trained to detect a comprehensive number of different substances both from biological (plant, animal, or human odors) or non-biological origin (chemical or technological products).

Since April 2020, the veterinarians of the National Veterinary School of Alfort (EnvA) have been training dogs to detect SARS-CoV-2 virus in human sweat, by detecting Volatile Organic Compounds (VOCs) in infected patients [1]. The VOCs exact nature, although specific to SARS-CoV-2 as compared to other viruses [2], is still under identification [3]. During the acute COVID-19 phase, the first results show a detection sensitivity close to 95% and a specificity of 97% for confirmed cases (positive PCR) versus asymptomatic and negative PCR subjects [4,5].

For long COVID patients, the persistence of RNA and/or viral proteins is a widely discussed hypothesis. The literature has documented the viral RNA persistence in olfactory slots [6], digestive tissue sections [7], brain [8] and viral proteins persistence in monocytes [9].

Therefore, it is of great scientific interest to assess whether dogs can identify SARS-CoV-2 persistence in long COVID patients, as they do in the initial phase of the disease.

METHODOLOGY

The included patients had all a symptomatic initial episode of COVID-19, then prolonged symptoms which correspond to the 2021 WHO definition of post-acute COVID syndrome [10].

They were followed at Hotel Dieu Hospital in a descriptive cohort for which an Ethics approval was granted (Institutional Review

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Board of Mondor, Creteil, France, IRB 00011558, Approval number 2020-088).

Patients were asked to take an axillary sweat sample at home to avoid any hospital contact and potential parasite odors. Before any shower in the morning, the patients had to apply a sterile surgical swab under each armpit for 5 minutes, slip it into a freezer bag and send it after sealing it by mail. Controls, recruited by EnvA, were asymptomatic individuals with a negative PCR prior to the testing, without previous history of COVID or recent contact with a confirmed case.

At EnvA, swabs were placed in a glass container in an olfaction detection cone according to the Nosaïs procedure (Figure 1), [1,4]. Each case to be checked was randomly placed with 4 negative swabs from control individuals in 5 different cones. The material was sniffed by 2 different dogs trained and validated to sniff and discriminate positive samples for SARS-CoV-2. The dog and his handler were both blinded to the long COVID sample location.

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RESULTS

Characteristics of the population

Between May and October 2021, 45 Long COVID patients sent their samples to EnvA. Average age was 45 (6-71) and 73.3% were female. No patient had been admitted in intensive care unit during the acute phase.

Prolonged symptoms had been evolving for an average of 15.2 months (range: 5-22). Main symptoms of prolonged phase were intense fatigue (n=37, 82.2%), neurocognitive disorders such as concentration and attention difficulties, immediate memory loss (n=24, 53.3%), myalgias/arthralgias (n=22, 48.9%), cardiopulmonary symptoms (dyspnea, cough, chest pain, palpitations) (n=21, 46.7%), digestive symptoms (diarrhea, abdominal pain, reflux, gastroparesis) (n=18, 40.0%), ENT disorders (hyposmia, parosmia, tinnitus, nasal obstruction, inflammatory tongue, dysphonia, sinusitis) (n=18, 40.0%) in Table 1. 11 (24.4) patients had at least one positive SARS-CoV-2 serology before any vaccination, 29 (64.4%) had a negative SARS-CoV-2 serology and 5 (11.1%) had no serology results.

Figure 1: Dog sniffing setting: A handler is responsible of for the guidance of the dog. The sniffing cone helps the dog sniff the scent of the samples put inside glass containers, which are held in empty boxes.

Table 1: Characteristics of the Long COVID patients included in the study and results of Volatile Organic Compounds (VOCS) detection.

N°	Sex	Long COVID symptoms at the time of sweat samples collection	Date of initial COVID infection	Delay between initial COVID infection and VOCs detection (months)	Volatile organic compounds (VOCs) detection results	SARS CoV-2 serology results
1	F	Sinusitis, digestive pain, headaches, fatigue, cough, shivering	Mar-20	14	POSITIVE	NEG
2	F	Brain fog, headaches, phantosmia, myalgia, ocular troubles, digestive pain	Mar-20	15	POSITIVE	NEG
3	M	Fever, headaches, bone pain, ocular inflammation, eczema,	Mar-20	15	POSITIVE	NEG
4	F	Fever, fatigue, bone and muscle pain, digestive pain, urinary disorders, headaches, visual hallucinations	Mar-20	15	POSITIVE	NEG
5	F	Fatigue, myalgia, dyspnea, brain fog, dizziness	Oct-20	8	NEGATIVE	POS
6	M	Brain fog, dizziness, myalgia, dyspnea, stuffy nose	Apr-20	14	NEGATIVE	POS
7	F	Fatigue, dizziness, arthralgia, dyspnea, contractures	Dec-19	18	POSITIVE	NEG
8	F	Brain fog, fatigue, dizziness, tremor, chest oppression, palpitations, visual and ENT disturbances	Mar-20	16	POSITIVE	NEG
9	F	Fatigue, memory loss, palpitations, postprandial cough, loss of appetite	Aug-20	11	POSITIVE	NEG
10	F	Apex orbital mass, concentration disorders, dizziness, paresthesia, anosmia, palpitations, fatigue	Mar-20	16	POSITIVE	NEG
11	F	Digestive disorders, fasciculations, tremors, stuffy nose, dyspnea, thoracic pain, visual disorders, chills, ageusia, myalgia	Mar-20	16	POSITIVE	NEG
12	M	Fatigue, memory loss, attention and sleep disorders, myalgia, anosmia, digestive disorders	Mar-20	16	POSITIVE	NEG
13	F	Myalgia, burning sensations, nausea, vomiting, dizziness, venous dilatations, temperature changes	Mar-20	16	POSITIVE	NEG
14	M	Fatigue, body aches, attention disorders, swollen tongue, sweats, reflux, irritability	Mar-20	16	NEGATIVE	NEG
14	M	Fatigue, body aches, attention disorders, swollen tongue, sweats, reflux, irritability	Mar-20	16	NEGATIVE	NEG
15	M	Fatigue, fasciculations, tinnitus, nausea, sleep disorders	Mar-20	16	NEGATIVE	NEG
16	F	Pericarditis, brain fog, burning eyes, vision loss, hematomas, desquamation, nausea vomiting, neuropathy, fatigue, myalgia, stuffy nose	Feb-20	17	POSITIVE	NEG
17	F	Fatigue, stuffy nose, hyposmia, lymphangitis, myalgia, arthralgia, back burns, livedo	Mar-20	16	POSITIVE	NEG
18	F	Fatigue, paresthesia, headaches, thoracic pain, tachycardia, anxiety	Mar-20	16	NEGATIVE	NEG
19	F	Fatigue, concentration disorders, loss of weight and appetite, anxiety	Feb-20	17	NEGATIVE	NEG
20	F	Odynophagia, periorbital pain, nausea, permanent cough, dysphonia, gastroparesis, urination disorders	Feb-20	17	NEGATIVE	NEG
21	M	Fatigue, myalgias, weight loss, concentration and memory disorders, tremors, muscular weakness, visual loss, hematomas	Nov-20	9	POSITIVE	NEG
22	F	Fatigue, chills, myalgias, vertigos, neuropathy, tremors, dysphonia, tachycardia, odynophagia, blurred vision, rash	Mar-20	19	POSITIVE	NEG
23	F	Fatigue, arthro-myalgia, chills, fever, headaches, vertigo, paresthesia, neuropathy, memory troubles, urination disorders, dyspnea, palpitations, dysphonia, rhinorrhea, otalgia, venous inflammation, digestive disorders	Sep-20	13	POSITIVE	NEG
24	F	Fatigue, brain fog, insomnia, dyspnea, myalgia, arthralgia, abdominal pain, weight gain	Feb-20	17	POSITIVE	POS
25	F	Fatigue, abdominal pain, insomnia, palpitations, sweats	Mar-20	16	NEGATIVE	POS
26	F	Fatigue, tremors, headaches, brain fog, insomnia	Mar-20	16	POSITIVE	POS
27	M	Fatigue, visual disorders	Dec-20	7	POSITIVE	-

28	F	Fatigue, dyspnea, visual disorders	Dec-20	7	POSITIVE	-
29	M	Fatigue, brain fog	Mar-20	16	NEGATIVE	NEG
30	F	Pericarditis, myalgia, arthralgia, fatigue, brain fog	Mar-20	16	NEGATIVE	NEG
31	F	Fatigue, dyspnea, digestive disorders, brain fog, dysautonomia	Mar-20	16	NEGATIVE	NEG
32	F	Fatigue, myalgia, insomia, sweats	Mar-20	16	NEGATIVE	NEG
33	M	Fatigue, reflux, ENT inflammation, livedo	Mar-20	16	NEGATIVE	NEG
34	M	Fatigue, tachycardia, thoracic pain, arthralgia, gastric pain	Sep-20	10	NEGATIVE	POS
35	F	Fatigue, digestive disorders, chills, cutaneous disorders, vertigos, brain fog	Mar-20	16	NEGATIVE	NEG
36	F	Brain fog, fatigue, cutaneous burning, weight loss	Mar-20	16	NEGATIVE	POS
37	F	Brain fog, headaches, vertigos, fatigue, tachycardia, thoracic pain, digestive disorders, visual disorders, pains, lymphangitis, fatigue, dysautonomia	Mar-20	17	NEGATIVE	NEG
38	M	Muscular spasms, thoracic pain, skin burn, tongue inflammation, nausea	Mar-20	16	NEGATIVE	POS
39	F	Fatigue, arthromyalgia, brain fog, vertigos, dyspnea, thoracic pain, palpitations, rhinorrhea, visual disorders, digestive disorders	Mar-20	16	NEGATIVE	-
40	F	Fatigue, brain fog, tremors, insomnia, cough, dyspnea, reflux, digestive disorders, anosmia	Mar-20	19	POSITIVE	POS
41	F	Fatigue, digestive disorders, reflux, brain fog, anosmia, myalgias, visual disorders, tinnitus, otalgia, pruritus	Mar-20	19	POSITIVE	POS
42	F	Fatigue, arthromyalgias, brain fog, paresthesia, headaches, depression, dyspnea, dysosmia	Mar-20	19	NEGATIVE	POS
43	M	Fatigue, arthralgias, thoracic pain, dyspnea, aphonia, brain fog, paresthesias, dysautonomia, pruritus, digestive disorders, brain fog	Dec-19	22	NEGATIVE	NEG
44	F	Tremors, paresthesias, dizziness, cerebellum syndrome, visual disorders, myalgia, dyspnea	Nov-20	12	NEGATIVE	-
45	F	Arthromyalgias, brain fog, paresthesias, digestive disorders, fatigue	Apr-21	5	POSITIVE	-

Dog discrimination

23 long COVID samples out of 45 (51.1%), were discriminated in a positive way while none out of 188 (0%), was discriminated among control samples (p<.0001). There was no significant difference in the rate of positive discrimination between subjects with a positive SARS-CoV-2 serology (4/11, 36.4%) and those with a negative serology (16/29, 55.2%), (p=0.478).

DISCUSSION

This study, performed in Long COVID patients, shows for the first time that dogs can detect Volatile Organic Compounds (VOCs) up to 1.5 year after the initial phase of COVID-19. These results strongly suggest that SARS-CoV-2 may persist at least in some Long COVID patients. Furthermore, they show that dogs can detect VOCs in some patients with a negative SARS-CoV-2 serology.

During the acute initial phase of COVID-19, the performance of canine SARS-CoV-2 detection was shown to be excellent. A first study was performed in Recife, Brazil, in which 100 hundred volunteers with COVID-like symptoms participated, and both axillary sweat samples for dog detection and nasopharynx/oropharynx swabs for qPCR were collected. Two dogs, previously trained, detected 97.4% of the sweat samples positive with COVID-19 PCR, including a false-negative qPCR-test, the positive predictive value was 100% and the negative predictive value was 98.2% [4]. A second study was conducted in Lebanon in 256 patients with a proven COVID-19 (PCR positive) and in 203 PCR negative and asymptomatic subjects. Sweat samples were obtained within the 24 hours of the PCR testing and independently tested by two dogs. This detection by dogs showed a sensitivity of 100% and a specificity of 98.6% [5].

In this study with long COVID patients, it is difficult to interpret negative discriminations as the sample quality taken at home may have varied, and postal delays may have altered the preservation. Therefore, the sensitivity of canine olfactory test might be underestimated. Nevertheless, in this study, the specificity of SARS-CoV-2 olfactory detection by dogs is strikingly high, like in previous studies [4,5].

Interestingly, the finding that trained dogs can discriminate sweats in long COVID patients corroborates the previous cases of persistence of SARS-CoV-2 RNA and/or antigens, documented in the literature for long Covid patients [6-9]. Up to date, it had not been demonstrated whether it corresponds to the replicative virus or not. This canine detection test, with detection of VOCs, supports the hypothesis that the virus is still actively replicating. Indeed, the nature of these VOCs is currently being identified by several international laboratories in different countries. The training of the dogs now includes the uses of lures-produced by Pasteur Institute (Paris, France)- that are under validation by several international teams (ongoing publications). These lures are derived from supernatants of inactivated SARS-CoV-2 viral cultures, treated with trypsin, containing both viral proteins and volatile compounds, that dogs learn to discriminate.

It has been recently shown that trained dogs can also distinguish SARS-CoV-2-infections from other viral infections causing respiratory infections in humans such as other coronaviruses viruses influenza viruses and parainfluenza viruses with mean specificities above 90% [2].

The strengths of this study are the presence of negative controls during each test, the fact that all controls were negatively discriminated and the easy VOCs detection method. This very sensitive and non-invasive technique, which can be used both during acute and extended phases, is also faster than the RT-PCR and provides a holistic information. Indeed, it gives reliable results on the existence of a persistent infection, even when the precise location of the virus reservoir in the body is not known.

We also recognize some limitations. These results are preliminary with the objective of proving in a population of long COVID-19 subjects the hypothesis of viral persistence in comparison with a control population. Because of the relatively small sample size of our population, this study does not allow a precise calculation of the sensitivity and specificity of the canine olfaction test to detect viral persistence in long COVID.

CONCLUSION

These results reinforce the hypothesis of a SARS-CoV-2 antigens persistence at least for some long COVID patients, possibly with virus actively replicating. It also shows the limited utility of SARS-CoV-2 serological tests made during long COVID. Such results raise the possibility of future therapeutic options and invite to build long COVID antiviral treatments trials. In addition, with a better characterization of the detected VOCs, an improvement of odor sampling methods and the development of point-of-care instruments, this detection by dogs could also help to implement scent-based tests for other major human pathogens.

REFERENCES

1. Grandjean D, Sarkis R, Lecoq-Julien C, Benard A, Roger V, Levesque E, et al. Can the detection dog alert on COVID-19 positive persons by sniffing axillary sweat samples? A proof-of-concept study. PLoS One. 2020;15(12):e0243122.
2. Ten Hagen NA, Twele F, Meller S, Jendrny P, Schulz C, Von Köckritz-Blickwede, et al. Discrimination of SARS-CoV-2 infections from other viral respiratory infections by scent detection dogs. Front Med (Lausanne). 2021;8:749588.
3. Cambau E, Poljak M. Sniffing animals as a diagnostic tool in infectious diseases. Clin Microbiol Infect. 2020;26(4):431-435.
4. Maia RCC, Alves LC, Da Silva JES, Czyba FR, Pereira JA, Soistier V, et al. Canine olfactory detection of SARS-COV2-infected patients: A one health approach. Front Public Health. 2021;9:647903.
5. Sarkis R, Lichaa A, Mjaess G , Saliba M, Selman C, Julien C, et al. New method of screening for COVID-19 disease using sniffer dogs and scents from axillary sweat samples. J Public Health, 2021;215.
6. De Melo GD, Lazarini F, Levallois S, Hautefort C, Michel V, Larrous F, et al. COVID-19-related anosmia is associated with viral persistence and inflammation in human olfactory epithelium and brain infection in hamsters. Sci Transl Med. 2021;13(596):8396.
7. Cheung CCL, Goh D, Lim X, Tien TZ, Lim JCT, Lee JN, et al. Residual SARS-CoV-2 viral antigens detected in GI and hepatic tissues from five recovered patients with COVID-19. Gut. 2022;71(1):226-229.
8. Chertow D, Stein S, Ramelli S, Chung J-Y, Singh M, Yinda K, et al. SARS-CoV-2 infection and persistence throughout the human body and brain. 2021.
9. Patterson BK, Guevara-Coto J, Yogendra R, Francisco EB, Long E, Pise A, et al. Immune-based prediction of COVID-19 severity and chronicity decoded using machine learning. Front Immunol. 2021;12:700782.
10. WHO definition of post-acute COVID syndrome. 2021