Long Duration and Resurgence of SARS-CoV-2 in Cameroonian Population

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Abstract

Background

Infection with SARS-CoV-2 can lead to a detectable serological immune response even though extent of its protection is still not yet well known. We report long duration and resurgence of SARS-CoV-2 in patients with COVID-19.

Methods

We included a cohort of 99 participants from our non-blinded non-randomized evaluation of COVID-19 tests in Cameroon. Demographic and clinical information was collected from participants including self-reported age, race, ethnicity, and gender. Qualitative data was described as proportions while quantitative data was described with means and accompanying ranges.

Results

Duration of PCR for SARS-CoV-2 positivity was found to range from 4 – 81 days, with mean duration of 32.8 days in patients with PCR-positive. We also identified 4 participants who also had SARS-CoV-2 resurgence within a three-month period.

Conclusion

These observations raise questions on clinical decision to release COVID-19 cases from isolation after 14 days.

Introduction

Infection with SARS-CoV-2 has been reported to lead to a detectable immune response with the production of neutralizing antibodies (1–3) even though the extent of protection is still not very clear. Cases of reinfection with two different phylogenetic strains of SARS-CoV-2 have been reported but it is not yet well understood (4). One of the key questions in predicting the course of the COVID-19 pandemic, caused by SARS-CoV-2, is how well and how long the immune responses protect the host from reinfection. For some viruses like the measles virus, the first infection can provide lifelong immunity (5); for seasonal coronaviruses, protective immunity is short-lived (6). Understanding the immune response and resurgence of SARS-CoV-2 especially in a Sub-Sahara African population will help us understand firstly why Africa have not been hit as predicted (7), and secondly, it will inform how authorities can adapt barrier measures and confinement rules.

Methods

Source of data

This is secondary data gotten from a diagnostic evaluation of the performance characteristics of novel rapid diagnostic tests for SARS-CoV-2 compared to RT-PCR which was carried on consenting individuals aged 21 years or older who presented to any of eight COVID-19 testing sites across the Centre Region of Cameroon between June and August 2020.

Demographic and clinical information was collected from participants including self-reported age, race, ethnicity, and gender. Brief clinical history and case management pertaining to the suspected SARS-CoV-2 infection was recorded, including duration of symptoms, date of symptom onset, date of exposure/infection (if known), symptoms on admission/presentation, disease stage (mild, severe, or critical according to WHO classification), date of admission and discharge (for hospitalized patients), and outcome. At this initial visit (Visit 1), participants were invited return for at least two follow-up visits. Visit 2 was planned for seven days after Visit 1 and Visit 3 was planned within 10–14 days after Visit 1.

At all visits, we collected whole blood by peripheral venepuncture into EDTA-coated and red-top vacutainers. Nasopharyngeal swabs were collected using sterile technique compliant with rigorous infection control guidelines. Nasopharyngeal swab samples for PCR testing were transported in virologic media and stored at the National Laboratory of Public Health at -20° C.

The Innovita (Innovita [Tangshan] Biological Technology Co., Ltd., Beijing, China) test, provided by the Ministry of Health, was the antibody based RDT performed on all participants.

RT-PCR testing was performed at Cameroon's National Public Health Laboratory, where two different protocols were used to amplify SARS-CoV-2 RNA from nasopharyngeal swab samples: an automated extraction protocol and a manual extraction protocol (9). RNA was extracted using a kit for nucleic acid isolation and purification reagent (DAAN Gene, Sun Yat-sen University). Abbott m2000 (Abbot laboratories, Illinois, USA) was then employed and amplification was completed in real-time thermocyclers. The automated extraction protocol was performed using ABBOTT m2000 Real-time SARS-CoV-2 assay. The manual extraction protocol was performed using New RNA detection Coronavirus 2019 Decentralization (SARS-CoV-2; Sun Yat-sen University Protocol), which involves real-time amplification using Taqman probes of purified RNA and was carried out using an ABI 7500 thermocycler (Applied Biosystems, Foster City, USA) following manufacturer's instructions.

Statistical analysis

Data was entered into Microsoft excel spreadsheets and analysis was done using R software.

Tables were used to present data. Qualitative data was described as proportions while quantitative data was described with means and accompanying ranges. Significance was set at $p < 0.05$.

Results

We describe a cohort of 99 patients who had a positive SARS-CoV-2 PCR test and had at least 2 subsequent visits during clinical evaluation of novel SARS-CoV-2 diagnostic tests in Cameroon.

We divided into three groups. The first group includes 56 (56.6%; Group 1) with 66.1% symptomatic on the first visit and with just 1 positive PCR test (Table 1). The second group includes 22 (22.2%) that maintained positivity for less than 14 days (Group 2; Table 1) and the third group includes 21 (21.2%) patients that were positive for 14 days and higher (Group 3; Table 1). The average duration of positivity in Group 2 was 7.9 days (4–12; Table 1) contrasting to Group 3 participants who were positive for an average of 32.8 days (14–81; Table 1) with the longest duration of 81 days. There was no significant difference in the socio-demographic characteristics of the patients in the different groups.

Table 1
Participant characteristics and Summary of 4 individuals with SARS-CoV-2 resurgence

Item/group		Group 1 (PCR positive once)	Group 2(PCR positive twice PCR < 14days)	Group 3(PCR positive twice PCR14 ≥ days)	P-values
Cases (n)		56	22	21	N/A
Gender (M/F)		30/18	10/12	13/08	0.2797
Age mean (years (range))		42.31 (23–76)	42.23 (25–86)	41.62 (23–80)	0.8973
PCR duration Mean (days(range))		N/A	7.9 (4–12)	32.8 (14–81)	7.961e-06
Symptomatic	1st visit	66.1%	90%	95%	0.7332
	Last visit	24.4%	59.1%	14%	0.03648
Asymptomatic	1st visit	33.9%	10%	5%	0.2206
	Last visit	75.5%	41.9%	86%	0.1438
Resurgence					
	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**	
1st PCR	**Pos**	**Pos**	**Pos**	**N/A**	
Date	26/05	18/05	21/05	N/A	
Status	Symptomatic	Symptomatic	Symptomatic	N/A	
2nd PCR	**Neg**	**Neg**	**Neg**	**Neg**	
Date	6/06	10/06	6/06	10/06	
Status	Symptomatic	Symptomatic	Symptomatic	Asymptomatic	
Antibody	None	**IgM/IgG**	**IgM**	**IgM/IgG**	
3rd PCR	**Pos (Ct 32)**	**Neg**	**Neg**	**Pos (Ct 27)**	
Date	13/06	17/06	20/06	16/06	
Status	Asymptomatic	Asymptomatic	Symptomatic	Symptomatic	
Antibody	None	None	**IgM/IgG**	**IgM/IgG**	
4th PCR	**ND**	**Pos (Ct 29)**	**Pos (Ct 28)**	**Neg**	

ND = Not Done; NA = Not Available

Item/group		Group 1 (PCR positive once)	Group 2(PCR positive twice PCR < 14days)	Group 3(PCR positive twice PCR14 ≥ days)	P-values
Date	ND	29/06	2/09	1/09	
Status	ND	Asymptomatic	Asymptomatic	Asymptomatic	
Antibody	ND	None	IgM/IgG	IgG	
ND = Not Done; NA = Not Available					

We further present the case of 4 individuals from the previously described population among whom 3 had a negative SARS-CoV-2 PCR between 2 positive tests in a period as long as 81 days.

Patient 1 (Table 1) is a 58-year-old male who tested positive and was hospitalized on May 25 with a positive PCR. At the time of inclusion in the evaluation study (06/06/2020), he tested negative for both SARS-CoV-2 PCR and antibodies. During his 2nd study visit, 07 days later, he was asymptomatic, tested positive on PCR and both IgG and IgM were present.

The 2nd patient (Table 1) is a 41-year-old male who tested positive on May 18 and was hospitalized. He was however PCR negative on June 10 at the time of his inclusion but tested positive for IgG and IgM. On his 2nd visit (7 days later), he was negative for PCR and antibodies which was a contrast during a 3rd visit (12 days later) which revealed a positive PCR test, absence of antibodies and an asymptomatic individual.

During his 1st visit on June 6, the 60-year-old male Patient 3 (Table 1) was PCR negative and IgM positive, whereas he was PCR positive and hospitalized before on May 21. In visit 2 (14 days later), PCR stayed negative but both immunoglobulins were present. On September 2, the patient during a control visit presented with symptoms, tested PCR positive and both IgG and IgM were still present.

Patient 4 (Table 1), female, 47-year-old and asymptomatic was included on June 10 and tested PCR negative but both immunoglobulins were positive implying a possible previous infection. She tested PCR positive a week later. During a control test 73 days from her first visit, the still asymptomatic patient had a negative PCR and just IgG was present.

Discussion

These observations have left many unanswered questions strongly suggesting the need for a review of the current guidelines for quarantine, the release of COVID-19 positive cases from isolation as well as the requirement of a negative PCR for COVID-19 patients before resuming their activities. They also highlight the possibility of reinfection that need to be assessed with genome sequencing.

Could the clinical decision (8) to release both symptomatic and asymptomatic COVID-19 cases from isolation after 14 days be ill-advised particularly in Cameroon where more than 20% of patients with positive PCR will carry the virus for more than 14 days and up to 81 days?

Conclusion

This brings a hard puzzle to solve on who should be isolated and how long they should be kept in isolation, especially as no study in Africa was used to inform the decision for quarantine norms. It also brings doubt on whether a negative PCR should be criteria for release or not given that some stay positive for up to 81 days, and whether varied quarantine be applied based on the duration of persistent PCR without applying the cut-across 14 days quarantine.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Cameroon National Ethics Committee (Reference number: 2020/05/1220CE/CNERSH/SP). All participants gave informed consent to participate.

Consent for publication

Authors declare to all be aware of this submission and consent for publication.

Availability of data and materials

Data and materials from the study are available through reasonable requests from the corresponding author.

Competing interests

We declare no competing interests

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Authors' contributions

YB developed the concept. DB, LM, KNF wrote the initial draft. YB did the first revision. BA, LE, NMF, LB, MN, AH, TD, GYC, BN, AA, CT, BT, ER, RN, RN, MF, SE, JPO, MTK, NM, LE, OMC, EE, GAEM and RJ did subsequent revisions and edits.

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