SARS-COV-2 VIRAL RNA SHEDDING IN DIFFERENT SAMPLES: A CASE STUDY IN VIET NAM

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Summary

Although several papers showing SARS-CoV-2 shedding from different compartments from COVID-19 patients have been published, little has been published from Vietnam. Here, we report the viral RNA shedding of SARS-CoV-2 over time, from those who were confirmed COVID-19, as a part of the national response, including asymptomatic and symptomatic patients admitted at the National Hospital for Tropical Diseases, Hanoi, Vietnam from February to April 2020. All of 14 COVID-19 patients had SARS-CoV-2 RNA detected in nasopharyngeal and throat swabs and 9/13 in fecal specimens; though all urine, blood and rectal swab specimens remained SARS-CoV-2 RNA negativity. The viral RNA was detected in one patient from stool at day 31 onsets of illness. During the time from day to day 20 of hospitalization, 4/13 patients had RNA positivity only from fecal samples, but negativity from nasopharyngeal and throat swabs. From limited findings, we suggest that use of both fecal and respiratory specimen may enhance diagnostic sensitivity, and aid discharge decision.

Key words: viral RNA shedding, SARS-CoV-2.

INTRODUCTION

The severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) emerged in Wuhan, Hubei, China at the end of 2019[1]. The outbreak was declared a public health emergency of concern by the world health organization on 30 January 2020 and a global pandemic on 11 March. Vietnam, a lower middle-income country with a population of 97 million, has confirmed 318 patients with over 270.000 tests, 5 severe cases and 0 deaths.

The clinical spectrum of COVID-19 ranges from entirely asymptomatic to fatal, with high viral load detected across the severity spectrum. Viral shedding is used as an indicator for disease progression and as a marker to allow discharge. Shedding in both respiratory and fecal specimens beyond the duration of clinical symptoms has been reported, but without confirmation of infectivity using viral culture or epidemiological evidence[2].

Here, we report the clinical characteristics and viral shedding from different compartments of 14 infected patients enrolled in National Hospital for Tropical Diseases, Hanoi, Vietnam between February and April 2020.

SUBJECTS AND METHODS

Patients and samples: SARS-CoV-2 PCR positive patients were recruited and sampled between 01/02/2020 and 01/04/2020 at the National Hospital for Tropical Diseases, one of the assigned hospitals for isolation and treatment of SARS-CoV-2 positive cases. Patients were classified into 2 groups: severe and non - severe based on the need of ICU support.

Samples were taken from different compartments: nasopharyngeal and throat swabs (NPS) were taken every three days to monitor shedding; blood, urine, tracheal aspirate and fecal samples were analyzed for presence of

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SARS-CoV-2 when they were taken for other diagnostic purposes as part of routine care.

Molecular detection of SARS-CoV-2: RT - PCR assays were performed following the WHO recommended Charitian protocol[3]. Briefly, viral RNA was extracted using the QIAGEN RNA mini Kit (Qiagen, Hilden, Germany) and PCR targeting the E gene was performed. Stool samples were pre - processed with Proteinase - K for 15 minutes at 50°C before mixing with lysis buffer (Roche, Darmstadt, Germany) and extraction of viral RNA using the MagnaPure96 system. Extracted nucleic acids were tested for SARS-CoV-2 using RT - PCR with Super Script One - Step RT - PCR kit (Invitro gen, Carlsbad (CA), USA) on a Light Cycler 480 real - time PCR system (Roche) in accordance with the manufacturer's instructions. 5ul of extracted DNA was added to 20ul of reaction mix. Reactions were incubated at 50°C for 10 min and 95°C for 5 min followed by 45 cycles at 95°C for 10s and 58°C for 40s. Following national guidelines a cycle threshold value (Ct - value) of less than or equal to 37 was defined as a positive test result, and a Ct - value of more than 40 was defined as a negative test result. A Ct - value of between 37 and 40 required confirmation by retesting and was tentatively reported as inconclusive.

RESULTS

14 patients were recruited in this study with a median age of 49 (IQR: 38 - 59), of whom 7 were male (Table 1). 4/14 (28.5%) participants were classified as severe based on the requirement of ICU support such as mechanical ventilation (3/14) and ECMO (1/14). 5/14 (35.7%) patients were asymptomatic on admission. At the time of writing all except one patient (patient 35) were discharged. All patients were diagnosed by detection of RNA in nasopharyngeal and throat swabs (NS and TS). Serial RT-PCR for SARS-CoV-2 was performed on different specimens, including NS and TS, urine, blood and stool (Table 1). NS, TS and stool specimens were frequently positive (9/13 (69%), but all tested urine (1), blood (2) and rectal swabs (3) for these patients remained negative, respectively. 3/5 (60%) asymptomatic patients had viral RNA detected in both respiratory and fecal specimens.

6/10 (60%) non - severe and 3/3 (100%) severe patients had RNA detected in fecal samples and viral RNA was still detected in a stool specimen of one (severe) patient after 31 days of illness (patient 68).

4/13 (30.7%) patients had RNA detected in fecal samples while nasopharyngeal and throat swabs were negative (Figure 1). No viral culture or sub - genomic RNA detection was performed to assess infectivity of these stool samples.

Table 1. Patient demographic information and SARS-CoV-2 RNA positivity during hospitalization (P: Positive; N: Negative)

							Admission date					
ID	**Patient ID**	**Age**	**Gender**	**Symptom at admission**	**Severity**	**Sample**	**1 - 7**	**8 - 14**	**15 - 21**	**22 - 28**	**29 - 35**	**36 - 43**
1	Patient 20	60	Male	Yes	Non-severe	Nasal and throat swab	P	P	N			
						Stool	P	P	P			
						Rectal swab		N				
2	Patient 3	24	Female	Yes	Non-severe	Nasal and throat swab						P
						Stool						N
						Rectal swab						N
						Urine						N
3	Patient 35	64	Female	Yes	Severe	Nasal and throat swab	P					
						Stool	P					
						Blood	N					
4	Patient 36	50	Male	Yes	Severe	Nasal and throat swab	P					
						Stool	P					
5	Patient 44	54	Female	Yes	Non-severe	Nasal and throat swab		P				
						Stool		P				
						Rectal swab		N				

							Admission date					
ID	**Patient ID**	**Age**	**Gender**	**Symptom at admission**	**Severity**	**Sample**	**1 - 7**	**8 - 14**	**15 - 21**	**22 - 28**	**29 - 35**	**36 - 43**
6	Patient 37	21	Male	No	Non-severe	Nasal and throat swab	P	N				
						Stool	P	P				
7	Patient 12	55	Male	Yes	Non-severe	Nasal and throat swab				N		
						Stool				N		
8	Patient 54	20	Male	No	Non-severe	Nasal and throat swab		N				
						Stool		N				
9	Patient 46	44	Female	No	Non-severe	Nasal and throat swab		N				
						Stool		P				
10	Patient 8	43	Female	Yes	Non-severe	Nasal and throat swab	N					
						Stool	N					
11	Patient 11	36	Male	Yes	Non-severe	Nasal and throat swab		N				
						Stool		P				
12	Patient 101	48	Female	No	Non-severe	Nasal and throat swab		P				
						Stool		P				
13	Patient 68	88	Female	No	Severe	Nasal and throat swab		P			P	
						Stool					P	
						Tracheal aspirate		P			N	
14	Patient 34	69	Male	Yes	Severe	Nasal and throat swab		P				
						Tracheal aspirate		P				
						Blood		N				

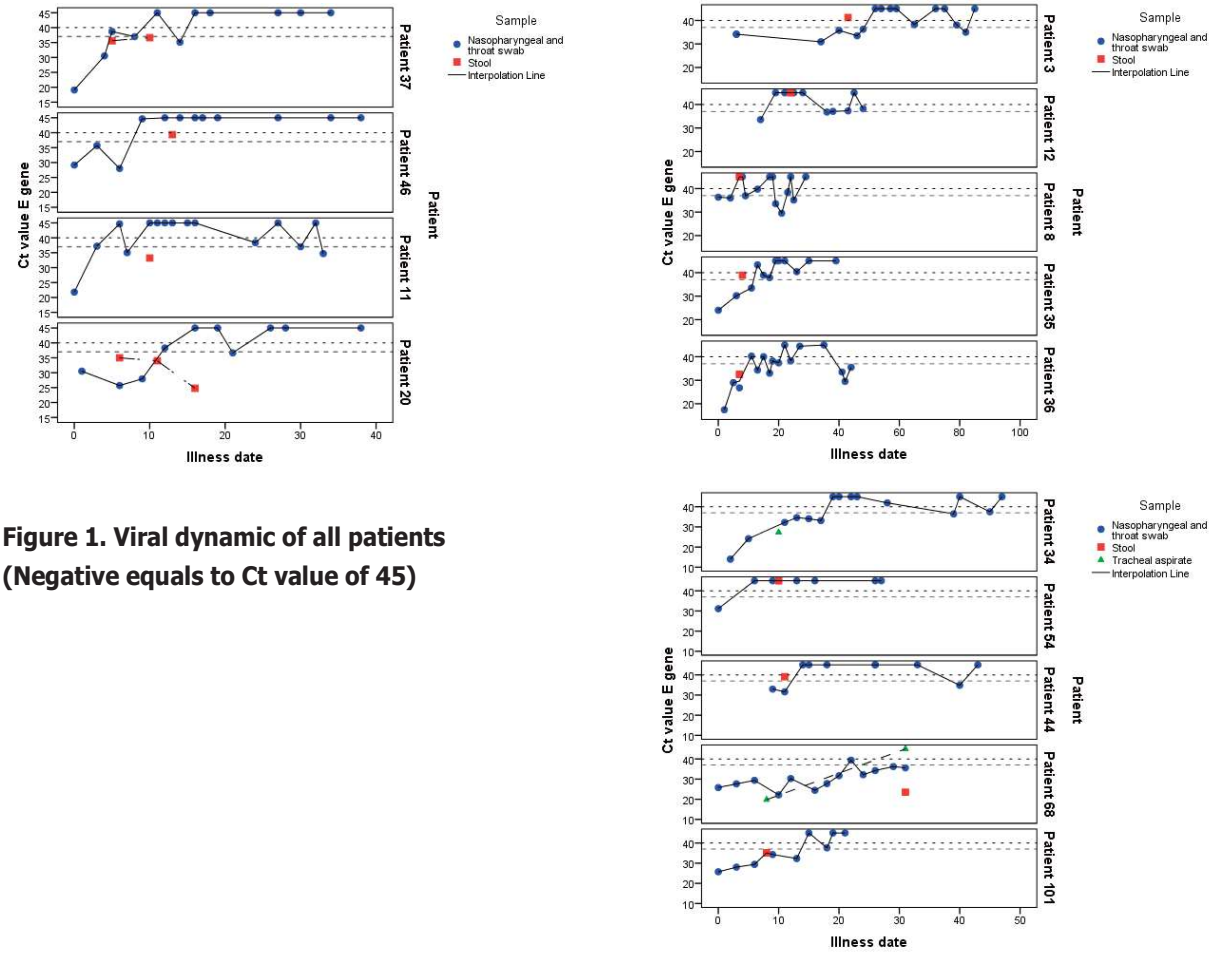

Figure 1. Viral dynamic of all patients (Negative equals to Ct value of 45)

DISCUSSIONS

We report serial viral shedding data from different compartments among 14 SARS-CoV-2 patients admitted to one of the assigned hospitals for isolation and treatment of patients, the National Hospital of Tropical Diseases in Hanoi, Vietnam.

Similar to earlier reports, SARS-CoV-2 was readily detected among patients who were symptomatic, but also among patients who were asymptomatic[4]. Viral shedding was detected among serial respiratory swabs and tracheal aspirates (of mechanically ventilated patients) and viral loads decreased consistently among both symptomatic and asymptomatic patients over time. Both among symptomatic and asymptomatic patients we observed prolonged viral shedding in serial respiratory swabs, and sporadically in stool samples. We also observed samples becoming positive again after having been negative. These shedding patterns have been observed and described by others as well, but shedding in stools and by previously negative cases has not been associated with positivity in viral culture or detection of RNA intermediates indicating active viral replication[2,4-7]. Furthermore, animal models suggest immune - protection against challenge after recovery[8]. However, the study has several limitations such as the sample size was relatively small, i.e. less than 20 patients and no viral culture attempt in those samples.

CONCLUSIONS

Based on our limited number of findings, we suggest that the assessment of both fecal and respiratory specimen may enhance diagnostic sensitivity, and support to discharge decision. Especially, in settings like Vietnam, where SARS-CoV-2 was successfully controlled and community transmission was interrupted, the implications of these shedding patterns are a challenge to clinicians and public health policy makers how to decide when it is safe to release isolated recovered cases. Thus, further studies, such as meta genomics, sub - genomics and viral culture, should be considered to confirm viral - RNR shedding or alive SARS-CoV-2 existed in COVID-19 patients with either clinical asymptoms of symptoms.

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