Letter to the Editor

SARS-CoV-2 and co-infections detection in nasopharyngeal throat swabs of COVID-19 patients by metagenomics

Dear Editor,

We read with interest a recent article in this journal regarding co-infections in people with COVID-19.[1] Rapid and simultaneous detection of SARS-CoV-2, the cause of COVID-19, and co-infections is essential for clinical management of COVID-19 patients. However, the diversity of possible co-infecting pathogens (bacteria and viruses) challenges conventional diagnostics approaches (bacterial culture and PCR). Metagenomics is a sensitive pan-pathogen assay for infectious disease diagnosis and the discovery of novel pathogens.[2] However, there have been only three studies reporting the application of metagenomics to detect SARS-CoV-2 and co-infections, with a combined sample size of nine patients.[3–5]

Since the beginning of March 2020 an observational study have been conducted at the Hospital for Tropical Diseases (HTD) in Ho Chi Minh City, Vietnam and another one at one of its two designated centers for receiving and treating COVI-19 patients from southern Vietnam with a population of over 40 million (Supplementary Figure 1). We enrolled patients with a confirmed SARS-CoV-2 diagnosis by a WHO recommended assay admitted to the study settings within 48 h. We collected nasopharyngeal throat swabs (NTS), clinical and laboratory data, and travel and contact history from each study participant. The collected NTS was stored at 4 °C at the study sites within four hours and was then transferred to the clinical laboratory of HTD for analysis. The clinical studies received approvals from the Institutional Review Board of the HTD and the Oxford Tropical Research Ethics Committee of the University of Oxford. Study participants gave their written informed consent.

The selected samples were individually analyzed with the inclusion of a molecular grade water sample serving as a non-template control (NTC). Metagenomics was carried out as previously described.[6] DNA libraries of individual samples and NTC were then multiplexed using double unique indexes (i.e. each sample was differentiated by double barcodes) and sequenced on an Illumina MiSeq platform using a 300-cycle MiSeq reagent kit V3 (Illumina). Detection of SARS-CoV-2 and co-infections in the obtained sequence data was carried out using a combination of publically availably pipelines namely IDseq (idseq.net) and DISCVR (https://bioinformatics.cvr.ac.uk/software/discvr/). Reference based mapping approach was applied to assemble SARS-CoV-2 genomes from the obtained sequences using Geneious 11.0.3 (Biomatters, Auckland, New Zealand). SARS-CoV-2 lineage determination and detections of nonsynonymous mutations were carried out using CoV-GLUE (http://cov-glue.cvr.gla.ac.uk), a publically available tool for SARS-CoV-2 sequence analysis (Supplementary Figure 1).

As of March 19th, 2020, a total of 11 PCR confirmed SARS-CoV-2 patients were enrolled in the clinical studies (Supplementary Figure 1). As a pilot, we selected eight with a wide range of viral loads (Table 1) for metagenomics analysis. Information about demographics and clinical status of the eight included patients are presented in Table 1. All were adults and two were asymptomatic carriers identified through contact tracing approach implemented in Vietnam.[7] Three were cases of locally acquired infection and five were imported cases, and one was co-infected with rhihnovirus. Information about duration of stay and clinical and laboratory findings are presented in Table 1.

Metagenomics generated a total of 2–4 million reads per sample in 7/8 included NTS. In the remaining sample, ¼ million reads were obtained (Table 2). SARS-CoV-2 was detected in sequence data obtained from all eight RT-PCR positive NTS samples by both IDseq and DISCVR, but not in the NTS sample. Metagenomics revealed one patient presenting with respiratory infection was co-infected with rhinovirus, which was subsequently confirmed by rhinovirus specific RT-PCR.[8] Three available rhinovirus sequences hindered additional typing effort. Additional analysis of original swabs using multiplex RT-PCR targeted at 15 different respiratory viruses[8] did not identify any additional viral pathogens.

Reference-based mapping of SARS-CoV-2 sequences generated three consensuses with genome coverage of $\geq$70%, and five with genome coverage of <50% (Table 2 and Supplementary Figure 2), with an observed correlation between genome coverage and viral loads (Supplementary Figure 2). A total of 11 nonsynonymous substitutions were detected in three of the eight obtained consensuses, including five being found in two sequences (Supplementary Table 1). Analysis of the obtained consensuses showed all belong to lineage B1.

Here, we demonstrated that when coupled with publically available bioinformatics tools, metagenomics could detect SARS-CoV-2 in RT-PCR positive NTS samples with a wide range of viral loads and rhinovirus in a co-infected patient. The data suggests that metagenomics is a sensitive assay for SARS-CoV-2 diagnosis and detection of co-infection, in line with a recent report.[4] Furthermore, using the obtained sequences, we successfully identified that all the Vietnamese viruses included for analysis belonged to lineage B1, which has been found worldwide.[9] In line with a recent report,[10] we identify several shared nonsynonymous substitutions in the obtained genomes SARS-CoV-2. Further research is needed to ascribe the potential consequences that SARS-CoV-2 evolution may have.

The application of metagenomics for SARS-CoV-2 and respiratory diagnosis would be highly relevant in the near future. This is because SARS-CoV-2 has spread globally, and will likely soon become endemic worldwide. Indeed as of May 21st, 2020 nearly 5 million cases have been reported globally. Notably, the vast ma-

Table 1
Demographics, clinical and real time RT-PCR data of the study participants.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Age in years	33	43	25	30	27	20	48	18
Gender	Male	Male	Male	Male	Female	Female	Female	Male
Arriving in Vietnam from	Spain	UK	NA	NA	NA	US	UK	UK
Local acquired infection (Yes/No)	N	N	Y	Y	Y	N	N	N
Nationality	Spanish	British	Vietnamese	Vietnamese	Vietnamese	Vietnamese	Vietnamese	Vietnamese
Days from confirmed diagnosis to enrolment	2	1	3	2	3	2	1	0
Days from admission to enrolment	2	1	2	1	0	1	2	0
Duration of stay	15	NA	17	18	15	12	13	17
Symptomatic (S)/asymptomatic (A)	S	S	S	A	S	A	S	S
Laboratory results								
White-cell count ($\times 10^3$ per μl)	4.23	6.89	3.96	NA	6.85	4.83	3.46	8.27
Lymphocyte counts ($\times 10^3$ per μl)	0.8	0.54	1.08	NA	2.94	2.50	0.88	2.40
Hemoglobin ((g/dl)	13.6	14.6	16.8	NA	15.7	15.00	11.60	15.60
Hematocrit (%)	42.4	43.4	41.6	NA	37.4	35.78	28.48	38.09
Platelet count (per μl)	140	235	187	NA	414	321.00	178.00	330.00
Glucose (mg/dl)	125.7	112	85	NA	NA	NA	76.80	98.50
Creatinine (mg/dl)	0.96	1.28	1.2	NA	NA	NA	0.99	1.19
Aspartate aminotransferase (U/liter)	24	17	22	NA	NA	NA	17.40	23.30
Alanine aminotransferase (U/liter)	23	16	24	NA	NA	NA	17.40	25.30
SARS-CoV-2 Ct values	26.52	21.47	27.06	32.09	24.81	25.68	24.56	24.13
Clinical signs/symptoms (Yes/No)								
Fever	Y	Y	Y	N	N	N	N	N
Cough	N	Y	N	N	N	N	Y	N
Rhinorrhea	N	N	Y	N	Y	N	N	Y
Fatigue	N	Y	N	N	N	N	N	N
Diarrhea	N	N	N	N	N	N	N	Y
Sore throat	N	N	Y	N	N	N	Y	N
Muscle pain	N	Y	N	N	N	N	Y	N
Headache	N	Y	N	N	N	N	N	N
Abdominal pain	N	N	N	N	N	N	N	N
Lost sense of smell	N	N	N	N	Y	N	N	N

Table 2
Results of mNGS and lineage assignment SARS-CoV-2 sequences.

Patient number	Ct values	Total single reads	No of SARS-CoV-2 reads	% SARS-CoV-2 genome coverage	SARS-CoV-2 lineage	Co-infections
1	26.52	3,182,758	84	16	B1	Not detected
2	21.47	4,218,464	6930	70	B1	Not detected
3	27.06	2,735,464	573	36	B1	Rhinovirus
4	32.09	1,902,512	68	12	B1	Not detected
5	24.81	245,818	14	3	B1	Not detected
6	25.68	3,524,972	995	48	B1	Not detected
7	24.56	2,440,326	16,564	87	B1	Not detected
8	24.13	3,253,308	9095	80	B1	Not detected

jority of SARS-CoV-2 infections are asymptomatic or mild, while COVID-19 patients present with signs/symptoms undistinguished with respiratory diseases caused by other viruses. As such rapid identification of the likely cause of hospitalized patients with respiratory infections is essential for clinical management and outbreak response. Under this circumstance, metagenomics is a preferable method because of its ability to detect both known and unknown pathogens presenting in the tested specimens without the need of pathogen specific PCR primers.[2]

Our study has some limitations. Only eight patients were included for analysis. However during the study period, there were only 14 SARS-CoV-2 confirmed cases reported in our setting. And we did not focus on bacterial co-infection, which warrants further research.

In summary, we show that metagenomics is a sensitive assay for sequence-independent detection of SARS-CoV-2 and co-infections NTS samples. Additionally, metagenomics could generate sufficient sequence data in samples with high viral loads for molecular epidemiological investigation. The utility potential of metagenomics warrants further research.

Declaration of Competing Interest

We, the author of the submitted manuscript declare that we do not have a commercial or other association that might pose a conflict of interest (e.g., pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.06.033.

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