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. 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 diagnosis approaches (bacterial culture and PCR). Metagenomics is a sensitive pan-pathogen assay for infectious disease diagnosis and the discovery of novel pathogens. 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.

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 COVID-19 patients from southern Vietnam with a population of over 40 million. 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. 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 publicly available pipelines namely IDseq (idseq.net) and DISCVR (https://bioinformatics.cvr.ac.uk/software/discovery). 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. Three were cases of locally acquired infection and five were imported cases, and one was co-infected with rhinovirus. 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. Eight available rhinovirus sequences hindered additional typing effort. Additional analysis of original swabs using multiplex RT-PCR targeted at 15 different respiratory viruses did not identify any additional viral pathogens.

Reference-based mapping of SARS-CoV-2 sequences generated three consensus with genome coverage of ≥70%, and five with genome coverage of <50% (Table 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 consensus, 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. 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. In line with a recent report, 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-
majory 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).

Acknowledgements

This study was funded by the Wellcome Trust of Great Britain (106680/B/14/Z and 204904/Z/16/Z).

We are indebted to Ms Nguyen Thanh Ngoc, Ms Le Kim Thanh, and the OUCRU IT/CTU/Laboratory Management departments for their support.

We thank the patients for their participations in this study, and the doctors and nurses at HTD Cu Chi Hospital, who cared for the patients and provided the logistic support with the study.

OUCRU COVID-19 Research Group

Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam: Nguyen Van Vinh Chau, Nguyen Thanh Dung, Le Manh Hung, Huynh Thi Loan, Nguyen Thanh Truong, Nguyen Thanh Phong,
Dinh Nguyen Huy Man, Nguyen Van Hao, Duong Bich Thuy, Nguyen My Ngoc, Nguyen Phu Huong Lan, Pham Thi Ngoc Thoa, Tran Nguyen Phuong Thao, Tran Thi Lan Phuong, Le Thi Tam Uyen, Tran Thi Thanh Tam, Bui Thi Ton That, Huynh Kim Nhung, Ngo Tan Tai, Tran Nguyen Hoang Tu, Vo Trong Vuong, Dinh Thi Bich Ty, Le Thi Dung, Thai Lam Uyen, Nguyen Thi My Tien, Ho Thi Thu Thao, Nguyen Ngoc Thao, Huynh Ngoc Thien Vuong, Pham Ngoc Phuong Thao, Phan Minh Phuong

**Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam:** Dong Thi Hoai Tam, Evelyne Kestelyn, Donovan Joseph, Ronald Geskus, Guy Thwaites, H. Rogier van Doorn, Huynh Le Anh Huy, Huynh Ngan Ha, Huynh Xuan Yen, Le Nguyen Van Nui, Jeremy Day, Joseph Donovan, Katrina Lawson, Lam Anh Nguyen, Lam Minh Yen, Le Nguyen Truc Nhu, Le Thanh Hoang Nhat, Le Van Tan, Sonia Lewycka Odette, Louise Thwaites, Maia Rabaa, Marc Choisy, Mary Chambers, Motiur Rahman, Ngo Thi Hoa, Nguyen Thanh Thuy Nhien, Nguyen Thi Han Ny, Nguyen Thi Kim Tuyen, Nguyen Thi Phuong Dung, Nguyen Thi Thu Hong, Nguyen Xuan Truong, Phan Nguyen Quoc Khanh, Phung Le Kim Yen, Sophie Yacoub, Thomas Kesteman, Nguyen Thuy Thuong, Tran Tan Thanh, Tran Tinh Hien, Vu Thi Ty Hang

**Supplementary materials**

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

**References**

1. Lansbury L, Lim B, Baskaran V, Lim WS. Co-infections in people with COVID-19: a systematic review and meta-analysis. J Infect 2020 (in press).
2. Chu CY, Miller SA. Clinical metagenomics. Nat Rev Genet 2019;20(6):341–55.
3. Cao L, Liu W, Zhang Q, Xu K, Ye G, Wu W, et al. RNA-based mNGS approach identifies a novel human coronavirus from two individual pneumonia cases in 2019 Wuhan outbreak. Emerg Microbes Infect 2020.
4. Peddu V, Shen AC, Xie H, Shrestha L, Perchetti GA, Minot SS, et al. Metagenomic analysis reveals clinical SARS-CoV-2 infection and bacterial or viral superinfection and colonization. Clin Chem 2020:1–7.
5. Ai JW, Zhang Y, Zhang HC, Xu T, Zhang WH. Era of molecular diagnosis for pathogen identification of unexplained pneumonia, lessons to be learned. Emerg Microbes Infect 2020;
6. Anh NT, Hong NTT, Nhu LNT, Thanh TT, Lau CY, Linnmathurotsakul D, et al. Viruses in Vietnamese Patients Presenting with Community-Acquired Sepsis of Unknown Cause. J Clin Microbiol 2019;57(9).
7. Dinh L, Dinh P, Nguyen PDM, Nguyen DHN, Hoang T. Vietnam’s response to COVID-19: prompt and proactive actions. J Travel Med 2020:1–3.
8. Jansen RR, Schinkel J, Koekkoek S, Pajkrt D, Beld M, de Jong MD, et al. Development and evaluation of a four-tube real time multiplex PCR assay covering fourteen respiratory viruses, and comparison to its corresponding single target counterparts. J Clin Virol 2011;51(3):179–85.
9. Rambaut A, Holmes EC, Hill V, O’Toole A, McCrone JT, Ruis C, et al. A dynamic nomenclature proposal for SARS-CoV-2 to assist genomic epidemiology. MedRxiv. 2020. doi:10.1101/2020.04.17.20060896.
10. van Dorp L, Acma M, Richard D, Shaw PL, Fird P, Ormond L, et al. Emergence of genomic diversity and recurrent mutations in SARS-CoV-2. Infect Genet Evol 2020;83:1–9.

Le Van Tan*, Nguyen Thi Thu Hong
**Oxford University Clinical Research Unit, 764 Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam**

Nghiem My Ngoc
**Hospital for Tropical Diseases, 764 Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam**

Tran Tan Thanh
**Oxford University Clinical Research Unit, 764 Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam**

Vo Thanh Lam
**Hospital for Tropical Diseases, 764 Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam**

Lam Anh Nguyen, Le Nguyen Truc Nhu, Nguyen Thi Ha Ny
**Oxford University Clinical Research Unit, 764 Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam**

Ngo Ngoc Quang Minh
**Children’s Hospital 1, 341 Su Van Hanh, District 10, Ho Chi Minh City, Vietnam**

Dinh Nguyen Huy Man
**Hospital for Tropical Diseases, 764 Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam**

Vu Thi Ty Hang, Phan Nguyen Quoc Khanh
**Oxford University Clinical Research Unit, 764 Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam**

Tran Chanh Xuan
**Cu Chi Hospital, Ho Chi Minh City, Vietnam**

Nguyen Thanh Phong, Tran Nguyen Hoang Tu
**Hospital for Tropical Diseases, 764 Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam**

Tran Tinh Hien
**Oxford University Clinical Research Unit, 764 Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam**

Nuffield Department of Medicine, Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, United Kingdom

Le Manh Hung, Nguyen Thanh Truong
**Hospital for Tropical Diseases, 764 Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam**

Lamh Min Yen
**Oxford University Clinical Research Unit, 764 Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam**

Nguyen Thanh Dung
**Hospital for Tropical Diseases, 764 Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam**

Guy Thwaites
**Oxford University Clinical Research Unit, 764 Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam**

Nuffield Department of Medicine, Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, United Kingdom

Nguyen Van Vinh Chau
**Hospital for Tropical Diseases, 764 Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam**

*Corresponding author.

E-mail address: tanlv@oucru.org (L. Van Tan)

1 Members of the Group are listed in the acknowledgements.