

**Figure 1 (facing page). Findings from a Mouse Model of Electronic-Cigarette, or Vaping, Product Use–Associated Lung Injury (EVALI).**

Panel A shows levels of vitamin E acetate (VEA) quantified by isotope-dilution mass spectrometry in bronchoalveolar-lavage (BAL) fluid harvested from mice. Values are means and standard deviations for 10 mice. Panel B shows albumin levels measured in BAL fluid from mice exposed to air, a mixture of propylene glycol and vegetable glycerin (PG–VG), or VEA. Values are means and standard deviations for 10 mice. Panel C shows the total number of CD45+ cells infiltrating the lung in mice exposed to air, PG–VG, or VEA. Values are means and standard deviations for 10 mice. The P values in Panels A, B, and C were calculated by two-way analysis of variance in Tukey's post-test comparisons among the exposure groups. Panel D shows BAL fluid from a mouse exposed to VEA, containing lipid-laden macrophages (representative examples are indicated with arrows) with cytoplasmic staining by oil red O in a vesicular pattern. The macrophages are numerous and contain variable amounts of lipid. Background pneumocytes (arrowheads) show comparatively scant cytoplasm and are present as single cells or loose sheets. Panel E shows BAL fluid from a mouse exposed to PG–VG, which contained fewer identifiable macrophages and had minimal to no specific staining by oil red O. Without lipid staining, it is more difficult to distinguish between small alveolar macrophages and pneumocytes in these preparations. Panels F and G show findings in lung sections. In mice exposed to VEA (Panel F), alveolar macrophages (arrowheads and circles) in residence among pneumocytes (P) lining the alveoli (A) contained abundant oil red O–stained lipid. In mice exposed to PG–VG, tiny oil red O–stained granules in the cytoplasm of cells lining the alveoli, including pneumocytes (arrows) and alveolar macrophages (arrowheads), were observed. B denotes bronchiole.

the generated aerosols would be required to identify such by-products. Another limitation is that we did not expose animals to aerosols that contained tetrahydrocannabinol (THC) or nicotine in a dose-dependent manner. Finally, it is possible that aerosols generated from other lipophilic solvents may produce outcomes similar to the outcome seen with vitamin E acetate in this

study. Future studies are needed to address these issues. Our findings, coupled with previous research identifying vitamin E acetate in BAL fluid from patients with EVALI<sup>1,2</sup> and in samples of case-associated product liquids,<sup>5</sup> provide additional evidence for vitamin E acetate as a possible cause of EVALI.

Tariq A. Bhat, Ph.D.

Maciej L. Goniewicz, Ph.D., Pharm.D.

Yasmin M. Thanavala, Ph.D.

Roswell Park Comprehensive Cancer Center  
Buffalo, NY

yasmin.thanavala@roswellpark.org

and Others

Dr. Blount is a member of the Lung Injury Response Lab Task Force; additional members are listed in the Supplementary Appendix, available with the full text of this letter at NEJM.org.

A complete list of authors is available with the full text of this letter at NEJM.org.

The views and opinions expressed in this letter are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention, the National Institutes of Health, or the Food and Drug Administration.

Supported by grants from the National Heart, Lung, and Blood Institute of the National Institutes of Health (R01HL142511), the National Cancer Institute (NCI) (P30CA016056), and the NCI and the Center for Tobacco Products of the Food and Drug Administration (U54CA228110).

Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

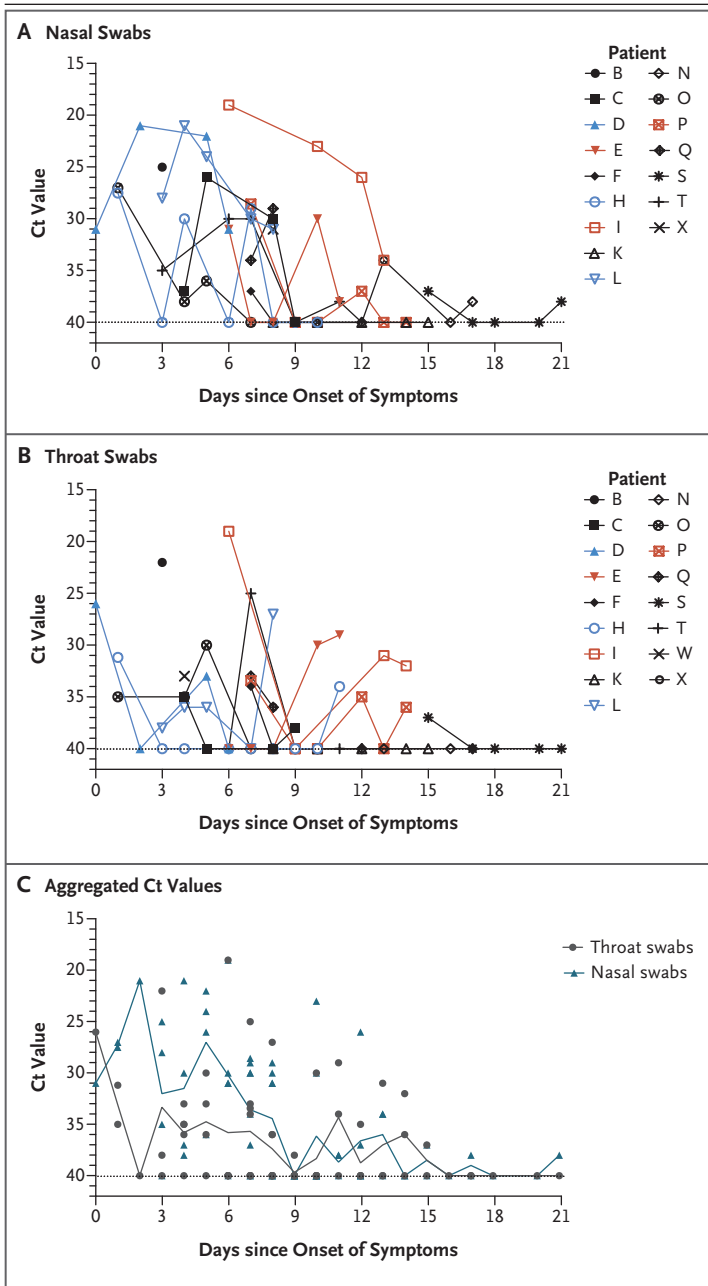
This letter was published on February 26, 2020, at NEJM.org.

1. Blount BC, Karwowski MP, Morel-Espinosa M, et al. Evaluation of bronchoalveolar lavage fluid from patients in an outbreak of e-cigarette, or vaping, product use–associated lung injury — 10 states, August–October 2019. *MMWR Morb Mortal Wkly Rep* 2019;68:1040-1.
2. Blount BC, Karwowski MP, Shields PG, et al. Vitamin E acetate in bronchoalveolar-lavage fluid associated with EVALI. *N Engl J Med* 2020;382:697-705.
3. Layden JE, Ghinai I, Pray I, et al. Pulmonary illness related to e-cigarette use in Illinois and Wisconsin — preliminary report. *N Engl J Med*. DOI: 10.1056/NEJMoa1911614.
4. Maddock SD, Cirulis MM, Callahan SJ, et al. Pulmonary lipid-laden macrophages and vaping. *N Engl J Med* 2019;381:1488-9.
5. Krishnasamy VP, Hallowell BD, Ko JY, et al. Update: characteristics of a nationwide outbreak of e-cigarette, or vaping, product use–associated lung injury — United States, August 2019–January 2020. *MMWR Morb Mortal Wkly Rep* 2020;69:90-4.  
DOI: 10.1056/NEJMc2000231

## SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients

**TO THE EDITOR:** The 2019 novel coronavirus (SARS-CoV-2) epidemic, which was first reported in December 2019 in Wuhan, China, and has been declared a public health emergency of in-

ternational concern by the World Health Organization, may progress to a pandemic associated with substantial morbidity and mortality. SARS-CoV-2 is genetically related to SARS-CoV, which



**Figure 1. Viral Load Detected in Nasal and Throat Swabs Obtained from Patients Infected with SARS-CoV-2.**

Panel A shows cycle threshold (Ct) values of Orf1b on reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay that were detected in nasal swabs obtained from 14 patients with imported cases and 3 patients with secondary cases, and Panel B shows the Ct values in throat swabs. Patient Z did not have clinical symptoms and is not included in the figure. Patients with imported cases who had severe illness (Patients E, I, and P) are labeled in red, patients with imported cases who had mild-to-moderate illness are labeled in black, and patients with secondary cases (Patients D, H, and L) are labeled in blue. A linear mixed-effects model was used to test the Ct values from nasal and throat swabs among severe as compared with mild-to-moderate imported cases, which allowed for within-patient correlation and a time trend of Ct change. The mean Ct values in nasal and throat swabs obtained from patients with severe cases were lower by 2.8 (95% confidence interval [CI], –2.4 to 8.0) and 2.5 (95% CI, –0.8 to 5.7), respectively, than the values in swabs obtained from patients with mild-to-moderate cases. Panel C shows the aggregated Ct values of Orf1b on RT-PCR assay in 14 patients with imported cases and 3 patients with secondary cases, according to day after symptom onset. Ct values are inversely related to viral RNA copy number, with Ct values of 30.76, 27.67, 24.56, and 21.48 corresponding to  $1.5 \times 10^4$ ,  $1.5 \times 10^5$ ,  $1.5 \times 10^6$ , and  $1.5 \times 10^7$  copies per milliliter. Negative samples are denoted with a Ct of 40, which was the limit of detection.

caused a global epidemic with 8096 confirmed cases in more than 25 countries in 2002–2003.<sup>1</sup> The epidemic of SARS-CoV was successfully contained through public health interventions, including case detection and isolation. Transmission of SARS-CoV occurred mainly after days of illness<sup>2</sup> and was associated with modest viral loads in the respiratory tract early in the illness, with viral loads peaking approximately 10 days after symptom onset.<sup>3</sup> We monitored SARS-CoV-2 viral loads in upper respiratory specimens obtained from 18 patients (9 men and 9 women;

median age, 59 years; range, 26 to 76) in Zhuhai, Guangdong, China, including 4 patients with secondary infections (1 of whom never had symptoms) within two family clusters (Table S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). The patient who never had symptoms was a close contact of a patient with a known case and was therefore monitored. A total of 72 nasal swabs (sampled from the mid-turbinate and nasopharynx) (Fig. 1A) and 72 throat swabs (Fig. 1B) were analyzed, with 1 to 9 sequential samples obtained from each patient. Polyester flock swabs were used for all the patients.

From January 7 through January 26, 2020, a total of 14 patients who had recently returned from Wuhan and had fever ( $\geq 37.3^\circ\text{C}$ ) received a diagnosis of Covid-19 (the illness caused by SARS-CoV-2) by means of reverse-transcriptase–polymerase-chain-reaction assay with primers and probes targeting the N and Orf1b genes of SARS-CoV-2; the assay was developed by the Chinese Center for Disease Control and Prevention. Samples were tested at the Guangdong Provincial Center for Disease Control and Pre-

vention. Thirteen of 14 patients with imported cases had evidence of pneumonia on computed tomography (CT). None of them had visited the Huanan Seafood Wholesale Market in Wuhan within 14 days before symptom onset. Patients E, I, and P required admission to intensive care units, whereas the others had mild-to-moderate illness. Secondary infections were detected in close contacts of Patients E, I, and P. Patient E worked in Wuhan and visited his wife (Patient L), mother (Patient D), and a friend (Patient Z) in Zhuhai on January 17. Symptoms developed in Patients L and D on January 20 and January 22, respectively, with viral RNA detected in their nasal and throat swabs soon after symptom onset. Patient Z reported no clinical symptoms, but his nasal swabs (cycle threshold [Ct] values, 22 to 28) and throat swabs (Ct values, 30 to 32) tested positive on days 7, 10, and 11 after contact. A CT scan of Patient Z that was obtained on February 6 was unremarkable. Patients I and P lived in Wuhan and visited their daughter (Patient H) in Zhuhai on January 11 when their symptoms first developed. Fever developed in Patient H on January 17, with viral RNA detected in nasal and throat swabs on day 1 after symptom onset.

We analyzed the viral load in nasal and throat swabs obtained from the 17 symptomatic patients in relation to day of onset of any symptoms (Fig. 1C). Higher viral loads (inversely related to Ct value) were detected soon after symptom onset, with higher viral loads detected in the nose than in the throat. Our analysis suggests that the viral nucleic acid shedding pattern of patients infected with SARS-CoV-2 resembles that of patients with influenza<sup>4</sup> and appears different from that seen in patients infected with SARS-CoV.<sup>3</sup> The viral load that was detected in the asymptomatic patient was similar to that in the symptomatic patients, which suggests the transmission potential of asymptomatic or minimally symptomatic patients. These findings are in concordance with reports that transmission may occur early in the course of infection<sup>5</sup> and suggest that case detection and isolation may require strategies different from those required for the control of SARS-CoV. How SARS-CoV-2 viral load correlates with culturable virus needs to be determined. Identification of patients with few or no symptoms and with modest levels of detectable viral RNA in the oropharynx for at least 5 days suggests that we need better data to

determine transmission dynamics and inform our screening practices.

Lirong Zou, M.Sc.

Guangdong Provincial Center for Disease Control and Prevention  
Guangzhou, China

Feng Ruan, M.Med.

Zhuhai Center for Disease Control and Prevention  
Zhuhai, China

Mingxing Huang, Ph.D.

Fifth Affiliated Hospital of Sun Yat-Sen University  
Zhuhai, China

Lijun Liang, Ph.D.

Guangdong Provincial Center for Disease Control and Prevention  
Guangzhou, China

Huitao Huang, B.Sc.

Zhuhai Center for Disease Control and Prevention  
Zhuhai, China

Zhongsì Hong, M.D.

Fifth Affiliated Hospital of Sun Yat-Sen University  
Zhuhai, China

Jianxiang Yu, B.Sc.

Min Kang, M.Sc.

Yingchao Song, B.Sc.

Guangdong Provincial Center for Disease Control and Prevention  
Guangzhou, China

Jinyu Xia, M.D.

Fifth Affiliated Hospital of Sun Yat-Sen University  
Zhuhai, China

Qianfang Guo, M.Sc.

Tie Song, M.Sc.

Jianfeng He, B.Sc.

Guangdong Provincial Center for Disease Control and Prevention  
Guangzhou, China

Hui-Ling Yen, Ph.D.

Malik Peiris, Ph.D.

University of Hong Kong  
Hong Kong, China

Jie Wu, Ph.D.

Guangdong Provincial Center for Disease Control and Prevention  
Guangzhou, China  
771276998@qq.com

Ms. Zou, Mr. Ruan, and Dr. Huang contributed equally to this letter.

Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

This letter was published on February 19, 2020, and updated on February 20, 2020, at NEJM.org.

1. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. Geneva: World Health Organization, 2004 ([https://www.who.int/csr/sars/country/table2004\\_04\\_21/en/](https://www.who.int/csr/sars/country/table2004_04_21/en/)).

2. Lipsitch M, Cohen T, Cooper B, et al. Transmission dynamics and control of severe acute respiratory syndrome. *Science* 2003;300:1966-70.

3. Peiris JSM, Chu CM, Cheng VCC, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet* 2003;361:1767-72.

4. Tsang TK, Cowling BJ, Fang VJ, et al. Influenza A virus shedding and infectivity in households. *J Infect Dis* 2015;212:1420-8.

5. Rothe C, Schunk M, Sothmann P, et al. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. *N Engl J Med* 2020;382:970-1.

DOI: 10.1056/NEJMc2001737