IDCases 20 (2020) e00791

Contents lists available at ScienceDirect

IDCases

journal homepage: www.elsevier.com/locate/idcr

Case report

A cautionary tale of false-negative nasopharyngeal COVID-19 testing

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ARTICLE INFO

Article history:
Received 28 April 2020
Received in revised form 1 May 2020
Accepted 1 May 2020

Keywords:
SARS-CoV-2
COVID-19
Nasopharyngeal swab

ABSTRACT

There remains diagnostic uncertainty regarding the sensitivity of reverse transcription polymerase chain reaction in detection of SARS-CoV-2 from nasopharyngeal specimens. We present a case where two nasopharyngeal specimens were negative, followed by a positive sputum sample. Serial testing for COVID-19 is indicated in patients with high pretest probability of disease.

Case

A 72-year-old gentleman with past medical history of type 2 diabetes mellitus (A1c 7.3 %) and multiple myeloma status-post six cycles of lenalidomide and ixazomib presented to the hospital on March 28, 2020 with six days of worsening malaise, dry cough, anosmia, ageusia and fever. His wife, given similar symptoms, had had a positive nasopharyngeal (NP) swab for SARS-CoV-2 three days before he became symptomatic. Medications on admission were notable for valsartan, albuterol, atorvastatin, ixazomib, and lenalidomide. His social history was notable for previous employment in real estate, minimal alcohol use, and no history of tobacco or recreational drug use. He had a pet dog and had returned from Antigua eight weeks prior.

On initial presentation, vital signs were temperature 38.2 °C, blood pressure 97/55 mmHg, pulse 72 beats per minute, respiratory rate 13 breaths per minute, and oxygen saturation 96 % on room air. Physical examination revealed a fatigued elderly gentleman with clear lung fields. Laboratory results were notable for a lymphopenia, acute kidney injury, and elevated D-dimer, further outlined in Table 1. Two sets of blood cultures were obtained and ultimately negative. Chest X-ray revealed bilateral patchy airspace opacities. He was provided ceftriaxone and azithromycin. Given clinical suspicion for COVID-19, airborne and droplet precautions were put in place and hydroxychloroquine begun empirically. A NP swab on the day of admission returned negative for influenza A and B, respiratory syncytial virus, human metapneumovirus, parainfluenza virus types 1–4, adenovirus, rhinovirus, and SARS-CoV-2 using the CDC-developed RT-PCR assay. Urine antigen testing for *Streptococcus pneumoniae* and *Legionella pneumophilia* was negative. Repeat NP swab for SARS-CoV-2 on hospital day four also returned negative, this time using the automated cobas 6800 system. Over the course of hospital days three through five, he had daily fever as high as 39.6 °C and laboratory results were notable for an LDH of 990 U/L, CPK of 477 U/L, CRP of 166 mg/L, and ferritin >6000 ng/mL. On hospital day five, after the second negative SARS-CoV-2 NP RT-PCR, he was taken off airborne precautions and transferred off the COVID-19 dedicated unit and to the general medical ward. He had persistent fevers and ongoing cough, myalgias, anosmia, and ageusia. The infectious diseases service was consulted for fever of unknown origin, and recommended repeat SARS-CoV-2 testing given his high pretest probability. He was placed back on COVID-19 precautions and the third test, an expectorated sputum sample for SARS-CoV-2 RT-PCR, again using the CDC-developed assay, returned positive Table 2.

Discussion

We present the case of a patient that, based on known exposure to a COVID-19 positive family member, typical symptoms, suggestive labs, and consistent imaging, had a high pre-test probability of having COVID-19, yet tested negative on two

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https://doi.org/10.1016/j.idcr.2020.e00791

Table 1
Laboratory results during hospitalization.

Examination	28 – March	29 – March	30 – March	31 – March	1 – April	2 – April
WBC (K/uL)	1.96	1.38	1.78	2.75	3.27	3.16
ANC	1670	–	–	1870	–	–
ALC	190	–	–	410	–	–
Hgb (gm/dL)	9.1	8.4	7.3	7.2	7.1	6.9
PLT count (K/uL)	47	52	38	44	44	49
AST (U/L)	32	–	–	55	–	–
ALT (U/L)	18	–	–	26	–	–
Creatinine (mg/dL)	2.33	2.08	1.85	1.89	2.17	1.92
D-dimer (ng/mL)	2337	2281	–	–	–	–
CRP (mg/L)	47	49	–	166	–	–
LDH (U/L)	–	691	990	–	–	–
CK (U/L)	–	477	–	–	–	–
Ferritin (ng/mL)	–	–	–	–	–	>6000

Abbreviations: WBC: white-cell count, ANC: absolute neutrophil count, ALC: absolute lymphocyte count, HGB: hemoglobin, PLT: platelet, AST: aspartate aminotransferase, ALT: alanine aminotransferase, CRP: C-reactive protein, LDH: lactate dehydrogenase, CK: creatine kinase.

Table 2
Sensitivities for detection of SARS-CoV-2 by NP swab.

Author	Fang et al. (3)	Wang et al. (2)	Ai et al. (4)	Guo et al. (5)
Number of patients	51	205	1014	140
Sensitivity by NP swab alone	71 %	63 %	59 %	52 %

successive NP RT-PCRs. Only on the third COVID-19 sample, taken from sputum, was the patient ultimately correctly diagnosed. As appropriate precautions were stopped after the second negative NP swab, several medical personnel were potentially exposed to SARS-CoV-2 in the process.

In the setting of a high pretest probability for COVID-19, a negative NP RT-PCR result (and in the case presented, multiple negative results) may represent a false negative. Given a growing appreciation in the literature for both heterogeneity of presentation and disease severity, it is critical to have a clear sense of COVID-19 testing performance [2]. It is unclear why our patient's first two nasopharyngeal swabs for SARS-CoV-2 were negative. Possible explanations include improper collection or handling technique, viral load below the detectable limit of the assay, or diminished upper airway viral shedding. The latter possibly reflects the natural history of the disease wherein duration of viral shedding (which may precede symptom onset by several days) was observed to be as few as eight days to as many as 37 [1]; additionally, it is conceivable that the patient's immunocompromised state may have contributed.

The precise test characteristics of a single NP RT-PCR for detection of SARS-CoV-2 are unknown. Available data suggest a range of sensitivities that likely increase with repetition. This may relate to varying assays based on the country of origin, as well as the reference standard used for a positive or presumptive positive test (e.g., viral culture, radiographic findings). Table 1 outlines the observed sensitivities from several recent publications [2–5]. At the early stages of a novel disease when the clinical sensitivity of a given assay is poorly understood, its analytic sensitivity, or limit of detection (LoD), can offer a useful point of reference and comparability. The LoDs reported across the two assays employed in this patient's case however were derived from varying methodologies resulting in entirely different units of measurement (RNA copies/μL vs. TCID50/mL), making early comparisons difficult. The genes targeted in these two assays also differ significantly. While the N1 and N2 genes are included in the CDC assay, Roche® targets the nonstructural ORF1a gene of SARS-CoV-2 in combination with the E gene (envelope protein) of the broader Sarbecovirus group. The relative clinical sensitivity and specificity of these targets are unknown.

In a recent study [2], Wang and colleagues examined a total of 1070 specimens (nasopharyngeal, blood, sputum, feces, urine, and, in those with severe illness, broncheoalveolar lavage (BAL) and fibrobronchoscope brush biopsy specimens) by RT-PCR for SARS-CoV-2 from 205 patients with COVID-19 treated at three hospitals in China. The majority of patients presented with fever, cough, and fatigue, while 39 (19 %) had severe disease. The authors reported positive rates highest for BAL specimens (93 %), followed by sputum (72 %), nasal swabs (63 %), brush biopsy (46 %), pharyngeal swabs (32 %), feces (29 %) and blood (1%), with no positives detected from urine.

Furthermore, a number of studies have attempted to define the natural history of viral shedding in COVID-19 disease. In an analysis of nine patients with mild COVID-19, Wölfel and colleagues found that pharyngeal shedding predominated and reached a peak in the first week of symptomatic disease, with upper airway viral load already declining at the time of presentation [6]. This observation is consistent with our patient's negative NP RT-PCR results from illness on days seven and ten. Shedding in sputum, however, continued through the first week of disease and extended as far as 3 weeks after symptoms began in patients with evidence of lung involvement. Based on these observations, the authors theorized a de-isolation protocol that not only considers date from symptom onset as is suggested by CDC [7], but also viral load. In a similar study, To and colleagues sought to ascertain viral load dynamics of SARS-CoV-2 in the posterior oropharynx and tracheal aspirates, as well as antibody kinetics and the viral genome, by collecting serial swabs from 23 patients [8]. They found that viral loads correlated positively with age and peaked shortly after symptom onset and subsequently declined. Of note, they found that in one third of the cohort viral RNA could still be detected from the posterior oropharynx after 20 days. This observation questions the feasibility of incorporating viral load into de-isolation protocols, as it remains unknown whether these patients are continuing to shed live virus versus inactive virions coated in neutralizing antibody.

Finally, further complicating test interpretation is the notion that RT-PCR positivity may be intermittent as highlighted by Lan and colleagues, where four patients who had recovered from COVID-19 and tested negative by two oropharyngeal swabs separated by 24 h, subsequently tested positive again [9].

Conclusions

The presented case serves as a cautionary tale on the limitations of the current state of diagnostic testing for COVID-19. In patients with a high pre-test probability of COVID-19, a single negative NP RT-PCR may be insufficient to rule-out disease. Additional studies ascertaining precise test characteristics will be essential in correctly identifying cases and avoiding the consequences that accompany improper de-isolation of patients who receive a false-negative result.

Author contributions

Bulls: clinical care, writing of manuscript
Wayne: clinical care, writing of manuscript
Crothers: clinical care, writing of manuscript
Hale: clinical care, writing of manuscript.

Authorship verification

All co-authors have seen and agree with the contents of the manuscript and have contributed significantly to the work

Permissions

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Funding

None.

Declaration of Competing Interest

None of the authors report any conflicts of interest

Acknowledgements

None.

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