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Short communication

A needle in the haystack? Assessing the significance of envelope (E) gene-negative, nucleocapsid (N2) gene-positive SARS-CoV-2 detection by the Cepheid Xpert Xpress SARS-COV-2 assay

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ABSTRACT

The clinical significance of high crossing threshold (Ct) detection of SARS-CoV-2 by RT-PCR is inadequately defined. In the course of universal admission screening with the Cepheid Xpert Xpress SARS-CoV-2 assay at our institution, we observed that 3.9 % (44/1123) of SARS-CoV-2 positive results were negative for the envelope (E) gene target but positive for the nucleocapsid (N2) target. The overall SARS-CoV-2 positivity rate during the three-month study period was 15.4 % (1123/7285), spanning April-June 2020. The majority of patients with E-negative, N2-positive results were asymptomatic, with 29.5 % of patients symptomatic for COVID-19 at the time of presentation. Asymptomatic patients with E-negative, N2-positive results were significantly younger than symptomatic patients with the same results (average 37.6 vs. 58.4, $p = 0.003$). Similar proportions of prior SARS-CoV-2 positivity were noted among symptomatic and asymptomatic individuals (38.5 % vs. 33.3 %, $p = 0.82$). Among the 16 asymptomatic patients with radiographic imaging performed, four (25 %) had chest radiographic findings concerning for viral pneumonia. Interestingly, we observed an E-negative, N2-positive result in one patient with a previous SARS-CoV-2 by the Xpert Xpress that occurred 71 days prior. Critically, E-negative, N2-positive results were observed in 8 symptomatic patients with a new diagnosis of COVID-19. Thus, though concerns remain about extended SARS-CoV-2 RT-PCR positivity in some patients, the ability of clinical laboratories to detect patients with high Ct values (including E-negative, N2-positive results) is vital for retaining maximal sensitivity for diagnostic purposes. Our data show that a finding of E-positive, N2-negative SARS-CoV-2 should not be used to rule out the presence of subclinical infection.

1. Introduction

Detection of severe acute respiratory syndrome-related coronavirus (SARS-CoV-2) viral RNA by real-time RT-PCR is the gold-standard method for diagnosis of coronavirus disease 2019 (COVID-19) [1,2]. The Cepheid Xpert Xpress SARS-CoV-2 assay is a rapid, sample-to-answer test targeting two distinct regions of the SARS-CoV-2 genome; the envelope (E) and nucleocapsid (N2) genes [3]. Whereas the N2 target is specific for SARS-CoV-2, the E target is shared by other members of the *Sarbecovirus* subgenus (i.e. SARS-CoV). Consequently, when the N2 target alone or both targets are detected, a positive result is reported. If only the E target is detected, the result is reported as "presumptive positive".

Increasing attention is being paid to crossing threshold (Ct) values for SARS-CoV-2 detection as they relate to disease severity and the potential for infectious spread [4,5]. Importantly, Ct values inversely correlate with viral load (i.e. low Ct value = high viral load) but are impacted by several factors including specimen quality, stage of illness, and are not comparable between different assays [6]. The significance of higher Ct values in the diagnosis of COVID-19 is not fully understood. Since beginning universal SARS-CoV-2 screening for new inpatients on hospital admission, we noted E-negative, N2-positive (E-,N2+) results with some frequency. Here, we assess the clinical significance of these molecular findings, particularly with respect to the presence or absence

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of clinical symptoms.

2. Methods

Nasopharyngeal swabs collected into 3 mL of universal transport medium (Copan Diagnostics, Brescia, Italy) or viral transport medium (Hardy Diagnostics, Santa Maria, California) were tested using the Xpert Xpress SARS-CoV-2 assay according to manufacturer's instructions (Cepheid, Sunnyvale, California).

Instrument records were examined to identify specimens that were E target-negative, but N2-target positive. Medical records were subsequently reviewed to assess clinical significance. Abstracted variables included age, gender, underlying medical conditions, presenting symptoms, as well as laboratory and radiographic findings. Presence or absence of COVID-compatible clinical symptoms was ascertained (i.e. influenza-like illness, fever, cough, shortness of breath, myalgias, diarrhea/abdominal pain, dysgeusia, anosmia). This study was approved by the University of Southern California health sciences campus institutional review board (HS-20-00429). All statistical analysis was carried out using Stata 12.0, College Station, TX. The significance level was set at 0.05.

3. Results

Between March 29th and June 29th 2020, 7285 specimens were tested for SARS-CoV-2 in our laboratory using the Xpert Xpress assay, with universal screening for SARS-CoV-2 performed on all inpatients admitted to our hospital beginning on May 1st. Thirty three specimens were canceled leaving 7252 evaluable results. A total of 1123 specimens were positive for the detection of SARS-CoV-2 during the study period for an overall positivity rate of 15.4 %. Only five presumptive positives were observed. E-negative, N2-positive (E-,N2+) results accounted for 44 specimens (3.9 %) obtained from 44 patients. The median N2 Ct value among these specimens was 41.6 (range: 38.8–44.9, data not shown).

Demographics and clinical findings of patients with E-,N2+ SARS-CoV-2 results are shown in Table 1. Thirteen patients were symptomatic for COVID-19 (29.5 %), with the remaining 31 patients asymptomatic at the time of testing. Asymptomatic E-,N2+ patients were younger in age than symptomatic patients (average age of 37.6 vs. 58.4, p = 0.003). A total of 15 patients had been previously diagnosed with COVID-19 (34.1 %), with similar proportions of prior positivity noted between symptomatic and asymptomatic patients (38.5 % vs. 33.3 %, p = 0.82). Among the five patients who previously tested positive by Xpert Xpress, time from initial detection to E-,N2+ results ranged from 15 to 71 days (Table 2).

Among the 13 symptomatic patients with E-,N2+ results, 11 (84.6 %) had radiographic findings consistent with viral pneumonia. Seven were symptomatic for ≥7 days, 2 had symptoms <7 days, 3 reported no symptoms but were hypoxic on exam, and one patient was a known positive case transferred from an outside hospital for further care. Eight of the thirteen symptomatic patients were febrile (7 with measured fever, 1 with subjective fever).

Of the 31 asymptomatic patients, 51.6 % had imaging performed (n = 16). Eleven (68.8 %) had no evidence of pneumonia, with four patients having abnormal chest radiographic findings: Two patients had documented prior COVID-19 positive results, one had radiographic findings suggestive of viral pneumonia one month prior, and one had a new diagnosis of COVID-19 with concomitant *Klebsiella pneumoniae* bacteremia. Twenty nine percent (9/31) of asymptomatic patients had a fever (8 measured, one subjective): Three were known positive COVID-19 patients; two had concomitant, documented bacterial infection; two were trauma patients and one patient was experiencing heat-stroke. Thus less than 10 % of asymptomatic patients had otherwise unexplained fever likely attributable to COVID-19.

Table 1
Demographics of patients with E -negative, N2-positive SARS-CoV-2 positive results.

	Symptomatic (n = 13)	Asymptomatic (n = 31)	P value
Average Age (±SD)	58.3 (±18.3)	37.6 (±15.4)	0.003
Median Age	50	33	–
Female Gender	4 (30.8 %)	7 (22.6 %)	0.56
Previous COVID-19 positive result	5 (38.5 %)	10 (33.3 %)	0.82
Duration of symptoms	12.4 days (range 1−30)	N/A	–
COVID Symptoms*:			–
Fever	8	N/A	
ILI	2	N/A	
Dyspnea/Hypoxia	11	N/A	
Cough	3	N/A	
Abdominal/ Diarrhea	1	N/A	
Other	0	N/A	
Chest X-ray:			0.001
Normal	1	11	
Unilateral Infiltrates	0	2	
Bilateral Infiltrates	11	2	
Indeterminate	1	1	
Not Performed	0	15	

* Symptoms noted at time of admission.

4. Discussion

The clinical significance of SARS-CoV-2 detection with high Ct values, and in particular, the relatively unusual finding of E-,N2+ results has previously not been assessed. In our study, E-,N2+ results accounted for almost 4 % of SARS-CoV-2 detections, with asymptomatic patients accounting for 68.2 % of those detections. Importantly, we observed E-,N2+ results in 8 patients presenting with COVID-19 symptoms. These data highlight the importance of retaining maximal sensitivity (40–45 cycles) for the initial diagnosis of COVID-19 infection in hospital settings.

Four asymptomatic E-,N2+ patients (25 %) who underwent radiographic imaging, had abnormal radiographic findings. Importantly, a high prevalence of ground glass opacities among asymptomatic COVID-19 patients by computed tomography (CT) missed by chest X-ray has been noted [7], with patients converting to PCR negative after a median of 13 days (3–19). It is therefore possible that a higher proportion of our asymptomatic patients may have had abnormal radiographic findings, indicating subclinical pulmonary disease, had CT been performed.

The duration of viral RNA detection among pre-symptomatic and asymptomatic patients varies widely among the literature published to date [8,9], however positivity can persist for several weeks [10].

Table 2
Ct values among E-negative, N2-positive SARS-CoV-2 positive patients with prior positive Xpert Xpress results.

	Previous positive Ct values (E;N2)	E-negative, N2-positive Ct values	Time to E-negative, N2-positive result
#7	32.5, 34.2	0.0, 41.1	15 days
#15	26.7, 29.0; 31.4; 33.8	0.0, 39.0	71days
#28*	30.1, 33.5; Negative x3	0.0, 42.4	N/A
#33	30.4, 32.5	0.0, 41.9	29 days
#39 **	25.8, 27.6 BUT then 31.3, 34.4	0.0, 44.7	

* Patient #28 Tested positive one month prior (outside facility), followed by 3 negative tests (each 1–2 weeks apart), then the E-negative, N2-positive result.

** Patient #39 Tested positive (Ct: E = 25.8; N2 = 27.6), with E-negative, N2-positive four weeks later, with a subsequent positive result four weeks thereafter (31.3; 34.4).

Importantly, 34.1 % of patients with E-,N2+ results had a previous diagnosis of COVID-19 and we observed an E-,N2+ result in a patient with an initial positive result obtained 71 days prior (e = 26.7; N2 = 29.0). These data support findings elsewhere [11,12] suggesting that low-level positive results can occur after an extended period in the same patient. It is therefore plausible that high Ct values, and in particular E-, N2+ results may occur among patients with prolonged sub-clinical infection or who have clinically recovered.

We encountered one patient who had an initial positive result, followed by three negative results, before the E-,N2+ result. Of note, SARS-CoV-2 testing was performed on each re-presentation to our facility during the study period. It is possible that an E-,N2+ result represents a stochastic process whereby exceedingly low-level shedding of viral RNA fragments occurred with this patient. Interestingly, one patient tested positive (Ct E = 25.8; N2 = 27.6), followed by an E-,N2+ result four weeks later. Surprisingly, the patient re-presented with a subsequent positive result four weeks thereafter (Ct E = 31.3; N2 = 34.4). It seems unlikely that poor specimen collection could solely account for these differing Ct values given the extended time frame involved. It is therefore possible that this patient may represent a possible re-infection case, or alternatively, a re-escalation of a shouldering or dormant infection.

In the US, clinical laboratories frequently run several assays in order to account for systemic shortages in the national testing supply chain [13]. Because Ct values are not interchangeable between assays, our data cannot necessarily be extrapolated to other assays. Importantly, the Xpert Xpress has emergency use authorization for the testing of symptomatic patients. The performance of the assay for the detection of asymptomatic COVID-19 cases or the duration of positivity by this assay has not been fully established. Thus, while it is possible that searching for the proverbial needle in the haystack may lack clinical significance for asymptomatic patients, it does appear to be warranted for symptomatic patients.

Declaration of Competing Interest

SBW has received consulting honoraria from Cepheid. All other authors have no relevant disclosures.

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