Received: 24 March 2021 | Revised: 20 March 2022 | Accepted: 21 March 2022

DOI: 10.1111/pan.14448

Pediatric Anesthesia WILEY

PERSPECTIVE

Novel utilization of strand-specific reverse transcription polymerase chain reaction in perioperative clinical decision making for SARS-CoV-2 polymerase chain reaction positive patients

Abstract

In order to prevent in-hospital transmission and potential complications related to SARS-CoV-2 in the perioperative patient, most healthcare institutions require preoperative testing for SARS-CoV-2 prior to proceeding with elective surgery. The Centers for Disease Control and Prevention (CDC) recommends a time and symptom-based duration of isolation for the presumed infectious period. The guidance to avoid retesting of asymptomatic patients in the 90 days following a positive reverse transcription polymerase chain reaction (RT-PCR) test is because of the possibility of detection of non-infectious viral shedding. When to reschedule asymptomatic patients who test RT-PCR positive for SARS-CoV-2 preoperatively is of considerable debate, both from the perspective of ensuring a patient's full preoperative fitness, as well as reducing the risk of viral transmission within the hospital. We describe the novel perioperative use of a strand-specific assay to detect minus strand ribonucleic acid (RNA) in a clinical decision-making algorithm to determine optimal timing of elective surgery after a patient tests RT-PCR positive for SARS-CoV-2. This is the first description in the literature of an attempt to further stratify patients who repeatedly test positive for SARS-CoV-2 into infectious versus non-infectious for perioperative planning.

1 | INTRODUCTION

The COVID-19 pandemic, caused by SARS-CoV-2, has led to mass disruption of healthcare systems and a feeling of uncertainty among healthcare workers, not only in caring for those infected with the virus, but also in learning how to continue the regular work of healthcare and surgeries while not contributing to onward transmission. To that end, many healthcare systems test all patients for SARS-CoV-2 preoperatively and reschedule elective or semi-urgent surgeries if a test results positive.[1] Pediatric patients are more likely to be asymptomatic or mildly symptomatic when infected with SARS-CoV-2 yet may still carry high viral load.[2,3] Therefore, universal preoperative screening of this patient population is especially important to reduce the risk of in-hospital transmission.[4] Reverse transcription polymerase chain reaction (RT-PCR) is the standard method of detecting SARS-CoV-2. However, patients with a positive RT-PCR test for SARS-CoV-2 can shed ribonucleic acid (RNA) and subsequently continue to test positive by RT-PCR for weeks.[5,6] RT-PCR cannot distinguish between inactive virus and actively replicating virus; actively replicating virus is critical for a patient to be infectious. It is thought that the majority of viral shedding is non-infectious, which is the basis for CDC recommendations of a time and symptom-based strategy for clearing isolation as opposed to a test-based strategy.[7]

Emergency surgeries proceed with appropriate precautions regardless of SARS-CoV-2 status, but the optimal timing of when to reschedule patients for urgent or elective surgeries who have previously tested SARS-CoV-2 positive is of considerable interest, especially in asymptomatic patients.[8,9] A test-based strategy for determining non-infectious status of patients has broad appeal to healthcare workers trying to protect themselves and their families from COVID-19. Asymptomatic patients who continue to test positive for SARS-CoV-2 after the recommended isolation period present a conundrum for healthcare workers struggling to balance the need for surgery with the risk of viral transmission and further spread in the hospital. In this report, we describe Stanford Children's Health's work to use a novel laboratory-based test to differentiate patients with active viral infection versus the persistence of viral RNA as a tool to guide rescheduling of elective surgery.

2 | HOW TO DETERMINE IF A PATIENT IS STILL INFECTIOUS

While viral culture is the gold standard for determining the presence of replicating virus, this is largely unavailable outside of research settings given the necessary stringent safety precautions.

There is considerable interest in the development of laboratory tests that can differentiate between the presence of remnant viral RNA (i.e., noninfectious) and replication-competent (i.e., infectious) virus.[10] The Clinical Virology Laboratory at Stanford Health Care and Stanford Children's Health developed a strand-specific assay that detects minus-strand viral RNA as a marker for actively replicating SARS-CoV-2, as a tool to help guide clinical decision-making.[11] SARS-CoV-2 is a positive sense single-stranded RNA virus. The viral replication process involves several steps including transcription, translation, protein formation, and eventual exit from the cell.[12,13] Actively replicating virus produces minus-strand RNA intermediates that can be detected by RT-PCR. The inability to detect minus strand suggests the virus is no longer replicating and the patient is unlikely to be infectious. During test validation, the strand-specific testing demonstrated 100% sensitivity and 72% specificity compared to SARS-CoV-2 viral culture. All culture positive samples had detectable minus strand, as did 28% of non-culturable samples, indicating that strand-specific testing may be more conservative than viral culture as a surrogate for transmissibility.

3 | OUR PROCESS

The anesthesia preoperative clinic (PARC) at Stanford Children's Health began using strand-specific RT-PCR testing on September 17, 2020, as part of a protocol to reschedule and retest patients with upcoming scheduled elective surgery who had previously tested SARS-CoV-2 positive within the last 90 days. A follow-up SARS-CoV-2 RT-PCR test was performed after 30 days of patient isolation and parent quarantine in anticipation of most pediatric patients and their caregiver families being off isolation and quarantine at 34 days. This included 20 days of isolation for the patient (assuming much of our patient population is immunocompromised or frequently hospitalized) in addition to 14 days of quarantine for the parent or caregiver who will accompany the patient for their surgical procedure. If the repeat RT-PCR test was positive, a strand-specific RT-PCR assay was added on to the sample. If the strand-specific RT-PCR assay did not detect the presence of the minus strand, surgery could proceed, and the patient was considered SARS-CoV-2 negative. If the strand-specific RT-PCR assay detected the presence of the minus strand, the patient was considered to be SARS-CoV-2 positive and still infectious, and surgery was either further delayed (if elective) or proceeded with airborne precautions. Any sample collected at a Stanford testing site had the capability of being tested via the strand-specific assay. Due to the labor-intensive nature of the strand-specific assay, samples were run 3 days per week, requiring a full day to result; therefore, considerable planning is needed to occur to ensure the strand-specific assay is resulted in time for the rescheduled surgery (Figure 1).

As per our workflow at the time of this data collection, the strand-specific assay was not performed on samples that were a first SARS-CoV-2 positive for a patient, but only on subsequent positive nasopharyngeal swab samples taken 30 days after the initial positive. Therefore, a time-based strategy for classifying a patient as non-infectious had already been fulfilled. As per CDC guidelines, once beyond 90 days, a positive SARS-CoV-2 test is considered a new infection. Nasopharyngeal swabs are the only method of collection deemed sufficiently sensitive for preoperative testing at our institution.

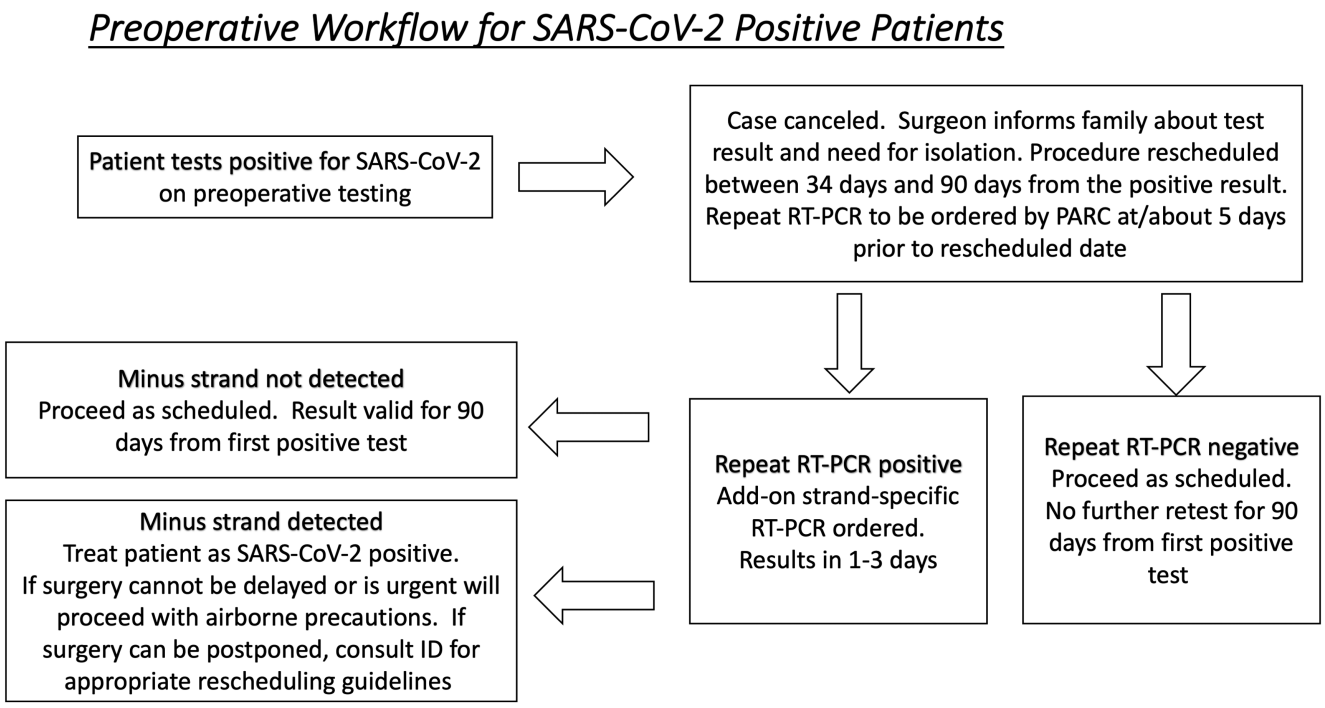

FIGURE 1 PARC Preoperative Workflow for SARS-CoV-2 Positive Patients. Preoperative clinic (PARC) process for patients scheduled for surgery who have had a positive test for SARS-CoV-2 within the past 90 days

4 | OUR EXPERIENCE

As part of a quality and safety initiative, we retrospectively examined all pediatric preoperative patients with surgeries scheduled between September 22, 2020, and February 26, 2021, who had a positive SARS-CoV-2 RT-PCR on initial evaluation for surgery. A secondary query of our electronic medical record was performed to identify all pediatric patients with strand-specific assays ordered at Stanford Children's Health that resulted in a detected minus strand during the same time period. Pediatric patients defined as less than or equal to 21 years of age were included. The Stanford University Institutional Review Board (IRB) determined that this project did not meet the definition of human subject research and therefore did not require further review.

Seventy-three patients were positive on initial RT-PCR testing for SARS-CoV-2. Twenty-five of these patients were symptomatic at initial presentation and all completed the CDC recommended isolation period. Twenty were retested with RT-PCR and again tested RT-PCR positive for SARS-CoV-2. All twenty were asymptomatic on retesting. The strand-specific assay was completed on all twenty repeat positive samples, and the minus strand was not detected in any samples. Three of the initial seventy-three patients were repeat tested only with the strand-specific assay, in lieu of repeat RT-PCR testing first; the minus strand was not detected in any of these samples either. The remaining 50 patients tested negative on repeat RT-PCR and therefore did not undergo a strand-specific test. All patients proceeded to surgery after either their repeat negative RT-PCR or negative strand-specific assay.

Of the seventy-three cases that met inclusion criteria, fifteen were immunocompromised. Nine of those were symptomatic at the time of their initial positive RT-PCR for SARS-CoV-2. The age range was nine months to twenty years. The male to female ratio was 2:1. Fifty-six patients identified as Hispanic, ten as White/Caucasian, four as Asian, and three as Other. Days between the first and repeat test for SARS-CoV-2 were between 20 and 84 days. It is unknown if any of the patients received treatment for SARS-CoV-2 infection (Table 1).

For comparison, we also reviewed strand-specific testing of non-surgical patients in the Stanford Children's Health system during the same time period. We identified eleven patients for whom the minus strand was detected. In some cases, the strand-specific assay was performed and the minus strand detected on a first RT-PCR sample, because teams throughout the healthcare system were utilizing the strand-specific assay in varying clinical contexts. Of these eleven patients, four had detected minus strand at 20 days or beyond their initial positive RT-PCR for SARS-CoV-2 (Table 2). Three of these patients were immunocompromised. Two were symptomatic at the time of their strand-specific assay, one patient's symptoms were unknown. One of the four was an otherwise healthy outpatient who had symptoms of nasal congestion at the time of her initial positive SARS-CoV-2 test, but was asymptomatic at the time of minus-strand detection.

TABLE 1 Descriptive data of perioperative patients RT-PCR positive for SARS-CoV-2

Total SARS-CoV-2 Initial Positive Cases	73
Symptomatic at Presentation	25
Repeat Positive RT-PCR for SARS-CoV-2	20
Strand-Specific Assays	23
Immunocompromised	15
Age at Presentation	9 months to 20 years
Male:Female Ratio	2:1
Ethnicity	
Hispanic™	56
White/Caucasian	10
Asian	4
Other	3

Note: Characteristics of pediatric preoperative patients who tested repeat positive for SARS-CoV-2 20 days or more after their initial positive test.

5 | DISCUSSION

Strategies for the timing of rescheduling asymptomatic patients who test positive for SARS-CoV-2 for elective or semi-elective surgery include time-based, a combination of symptom plus time-based, or RT-PCR test-based. At the time of this data collection, rapid antigen tests were not accepted for pre-procedural testing at our institution. While rapid antigen tests, in general, have excellent specificity, the sensitivity of this testing was not considered sufficient for pre-procedural screening of asymptomatic individuals.

In addition, negative rapid antigen tests have been shown to yield culturable virus.[14] With increasing attention on the use of antigen testing, both rapid and laboratory-based, as a potential strategy for discontinuing isolation, future research comparing antigen testing with the presence or absence of the minus strand may be of substantial interest.

CDC guidelines on isolation and quarantine periods continue to shift. The time periods followed here reflect the recommendations that were in place from the CDC and our own Infection Prevention and Control department at the time of our data collection. Although the strand-specific RT-PCR test has been validated against viral culture, the most widely accepted surrogate for transmissibility, it is a novel test that is not commonly used in clinical practice. Our institutional clinical experience has been that majority of patients clear minus strand within the expected CDC time-based window but prolonged presence of minus strand can be seen in immunocompromised individuals.[11] Strand-specific RT-PCR can provide an additional valuable data point, especially in immunocompromised patients, to help guide post-infection decision-making related to elective surgeries, as well as patient isolation. This is the first description in the literature of the use of the strand-specific RT-PCR test to further stratify patients who repeatedly test positive for

TABLE 2 Patients in the Stanford children's health system with detected minus strand after time-based isolation period

Patient	Immunocompromised	Symptomatic at Time of Strand-Specific Assay	Age (Years)	Time From First SARS-CoV-2 RT-PCR Positive Test (Days)	Time to Negative RT-PCR or Minus Strand Not Detected (Days)
A	No	No	21	20	30
B	Yes	Yes	10	36	45
C	Yes	Yes	17	55	N/A
D	Yes	Unknown	2	20	N/A

Note: Pediatric patients within the Stanford Children's Health system who had detected minus strand 20 days or more after their initial RT-PCR positive test for SARS-CoV-2.

SARS-CoV-2 into infectious versus non-infectious for perioperative planning purposes.

In this report, we identified one immunocompetent outpatient and three immunocompromised patients with detected minus strand 20 days or more after their initial positive RT-PCR test. (Table 2) Although it is known that immunocompromised patients may have persistent infectivity, the identification of this mildly symptomatic immunocompetent individual is unusual but in congruence with a previously published case report in which an adult with mild illness had specimens with replication-competent virus as long as 18 days after symptom onset.[15] This is one of the few reported cases in the literature of a mildly symptomatic immunocompetent individual with persistent replication-competent virus beyond the period when the CDC time- and symptom-based isolation guidelines would have cleared the patient as non-infectious. An important consideration is that these data were collected before SARS-CoV-2 vaccines were available to most of our patient population, and therefore, information on patient vaccination status was not collected.

Here, we describe the novel utilization of the strand-specific assay as an additional test-based validating tool beyond the CDC time and symptom-based guidelines to prevent inadvertent viral transmission in the hospital. In the future, we may be able to expand its use to also prevent unnecessary cancellation of elective surgeries, for example, in completely asymptomatic patients with no recent infection who incidentally test positive for SARS-CoV-2 on preoperative testing. A strand-specific assay could be performed at the time of their initial positive test, potentially allowing their surgery to proceed without delay if no minus strand is detected, indicating they were likely infected weeks before and therefore past their infectious period. Based on this original data, our hospital has subsequently refined the use of the strand-specific assay to more specific circumstances in consultation with our infectious disease colleagues, such as in immunocompromised patients who have passed their time-based infectious period. While guidelines continue to change based on the emergence of less virulent SARS-CoV-2 strains and an increasingly vaccinated pediatric population, the strand-specific assay provides a useful data point for perioperative clinical decision making in specific cases, such as in immunocompromised patients who can have persistent viable virus. When used in conjunction with a time- and symptom-based preoperative testing algorithm, we believe the strand-specific assay may provide practitioners with a valuable additional tool for perioperative decision making.

ACKNOWLEDGMENTS

The authors would like to thank the PARC staff, Christina S. Kong, MD, Grace Lee, MD, MPH, the Stanford anesthesiology residents, and the Stanford Clinical Virology Laboratory for their tireless work during the pandemic and assistance in implementing this workflow.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Christine G. Jette, Samuel Mireles and Genevieve D'Souza: clinical workflow development, writing, figure creation and final approval of the manuscript. Tammy Wang and Ellen Wang: clinical workflow development, data acquisition, writing and final approval of the manuscript. Janice Y. Man, Birgit Maass and Rebecca Claure: clinical workflow development, writing and final approval of the manuscript. Roshni Mathew: clinical workflow development, manuscript revision and final approval of the manuscript. Benjamin A. Pinsky: developed the laboratory assay, and participated in manuscript revision and final approval of the manuscript.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during this study are available from the corresponding author upon reasonable request.

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