

Evaluating the Efficacy of a Screening Protocol for Severe Acute Respiratory Syndrome Coronavirus 2 Virus in Asymptomatic Preoperative/Preprocedural Patients at a Military Hospital

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ABSTRACT

Introduction:

Facing the COVID-19 pandemic, many hospitals implemented severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) screening protocols before aerosol-generating procedures (AGPs) in an effort to protect patients and health care workers. Given the limited prior evidence on the effectiveness of such protocols, we report the process improvement experience at a military treatment facility.

Materials and Methods:

We evaluated the outcomes of patients undergoing AGPs from March to September 2020, divided into three cohorts: a preprotocol (PP) cohort who did not receive screening, an early testing (ET) cohort representing the early months of the screening protocol, and a late testing (LT) cohort managed under adaptive modifications to the screening protocol. We recorded identifiable post-procedure COVID-19 diagnoses. The study was approved as a process improvement protocol and was determined not to meet criteria for human subject research through an institutional approval process.

Results:

Across the three cohorts, 4520 procedures were performed: 422 PP, 1297 ET, and 2801 LT. Among 4098 procedures in the ET and LT cohorts, 12 asymptomatic patients tested positive for SARS-CoV-2 (0.29% positivity rate). One left the health system before completing the procedure and another proceeded urgently under COVID precautions, while 10 were rescheduled and completed at a later date; 7 were cleared using a test-based strategy, while 3 were cleared using a time-based strategy. Of 445 patients who had SARS-CoV-2 tests performed within 30 days following their procedures, three patients with negative preoperative tests had a positive test within 30 days, all in the LT cohort but had evidence of acquiring the infection after the procedure or had a false-positive test.

Conclusions:

Our strategy of preprocedural SARS-CoV-2 testing successfully identified asymptomatic infected patients before surgery. Care was delayed for most of these patients without apparent detriment. Adaptation to a time-based strategy for clearance might reduce such delays, but other considerations may still influence how soon procedures should be completed after a positive test.

INTRODUCTION

In the early months of 2020, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread rapidly from its point of origin and was soon detectable throughout the world, prompting the World Health Organization to declare the resultant disease, coronavirus disease 2019 (COVID-19), as an international pandemic.^{1,2} The rapid spread and lethality of COVID-19 drove health organizations worldwide to develop novel approaches toward patient interaction and surgical screening in the interest of provider and patient safety. Implementations of these techniques at various health institutions were rapidly published in the medical literature, but

results of their efficacy and ultimate impact on the patient were limited. Among a wide variety of approaches, the most utilized surgical screening techniques have included temperature measurements, symptom-based questioning, and presurgical reverse transcriptase polymerase chain reaction (RT-PCR) testing.¹⁻⁸ Pulmonary computed tomography scans also emerged as a particularly controversial screening technique, as some research indicated that they provide increased sensitivity to the disease as compared to RT-PCR alone.³⁻⁵

Additionally, the relevant evidence-based literature from past pandemics is limited, with the SARS outbreak of 2002-2004 representing the most comparable event.¹ Previous outbreaks, including SARS-CoV and, differed from COVID-19 in both transmission rates and case fatality rates⁴ but are similar enough to reinforce the necessity of a strong screening program. Findings from the 2002-2004 period demonstrated adequate control of SARS-CoV through a vigorous combination of isolation, contact tracing, quarantining, and robust PPE utilization in health care workers. Notably, advanced diagnostic tests, such as RT-PCR, were not used in

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identifying infected patients. Particularly concerning from the 2002-2004 outbreak was the significant number of health care professionals infected. Research at the time indicated 22% of SARS-CoV-affected persons in Hong Kong and 43% in Toronto were health care workers.¹ These findings, along with the early unknown impact of SARS-2-CoV on the recovery of surgical patients, raised significant concern for the safety of all individuals involved in the surgical process.

In this context, our hospital initiated a series of screening approaches for COVID-19 beginning with a nonsystematic, symptom-based screening approach in March. This approach then transitioned toward a multifaceted screening protocol in April 2020, which included basic screening through symptom-based questioning and SARS-2-CoV RT-PCR testing within 48 hours before all possible aerosol-generating outpatient procedures. Because the effectiveness of this protocol in identifying presymptomatic or asymptomatic infected patients in a preprocedural/preoperative population was unknown, as was the potential negative impacts of delaying surgeries based on positive screening results, we evaluated the impact of SARS-CoV-2 testing on patient care through ongoing evolutions of the hospital protocol to inform continued refinements and provide data for future pandemic response.

METHODS

Patients in the study population were identified based on entry into a scheduling system for all patients undergoing procedures that might require anesthesia support. This primarily included procedures taking place in the operating room, endoscopy suite, and select other monitored care settings. Data were collected from an electronic health record system that includes all inpatient and outpatient care received at any location within the health system. Records for care received outside the health system can also be stored in the patient's electronic health record.

The population of interest comprised three cohorts, differentiated by time of procedure relative to protocol implementation and the extent of data collection performed for each cohort. As a baseline comparison group, we reviewed records of patients who underwent surgery during the onset of the pandemic but before the implementation of the protocol (March 18 to April 21) and identified this group as the pre-protocol (PP) cohort. For these patients, screening primarily consisted of symptom screening, and all patients were treated as possible carriers with staff donning full PPE, including N95 masks. Data for this group were collected retrospectively since it preceded initiation of the process improvement protocol.

By April, the hospital had acquired adequate capability to perform large volume RT-PCR testing for SARS-CoV-2, allowing integration into screening. The initial RT-PCR-based screening protocol required patients to be tested within 48 hours before their aerosol-generating procedure (AGP).

Patients with positive tests who had been scheduled for nonurgent procedures were called back for confirmatory repeat testing, were issued quarantine and symptom-monitoring instructions, and had their surgeries rescheduled to a later date. Urgent or emergent procedures proceeded as planned using a designated operating room and strict COVID-19 precautions. Upon implementation of the screening protocol, we began collecting data from the early testing (ET) cohort during the period of April 22 to June 19 to include demographics, SARS-CoV-2 test results, and the impact of these results on the scheduled procedure. Demographic data, test results, procedure type, and impact on procedure were collected prospectively under this protocol.

Finally, for the later testing (LT) cohort who had procedures performed from June 20 to September 25, we continued to track the outcomes of patients with positive SARS-2-CoV tests to better inform the effectiveness of the protocol and monitor the impact of adjustments made in response to internal policy shifts or evolving Centers for Disease Control (CDC) guidelines. Important changes that affected the LT cohort included expanding the preprocedural testing window to 96 hours and a transition from a testing-based protocol to a time-based protocol, per CDC guidelines.⁹ The same depth of demographic and procedure-specific data was not able to be collected on negative patients as compared to the ET cohort as the volume of cases increased and operations were returning to pre-pandemic levels. Data in LT cohort patients with negative tests were collected retrospectively.

For all three cohorts, patient records were retrospectively reviewed for the 30 days following each procedure to identify additional SARS-CoV-2 testing or documented COVID-19 infections. This allowed us to investigate whether preprocedural testing might have yielded false-negative results.

Multiple testing platforms have been used since the availability of testing for SARS-CoV-2. Our facility used RT-PCR platforms from BioFire (bioMérieux, Inc.), Cepheid (Cepheid, Inc.), Roche (Roche Molecular Systems, Inc.), and Hologic (Hologic, Inc.). We characterize tests run on the BioFire and Cepheid platforms as "rapid" tests with expected result turnaround times under one hour, while tests performed on the Roche and Hologic platforms were characterized as "standard" with result turnaround times expected closer to 24-48 hours.

This process improvement initiative was submitted prospectively to determine the need for institutional review board approval. Because the initiative was evaluating the implementation of an iterative process based on best available evidence and not initiating what would be considered investigational treatments, it was determined not to meet criteria for human subject research and was given approval under a process improvement designation. Descriptive statistics were generated in SPSS 27 (IBM, Armonk, New York). Because of

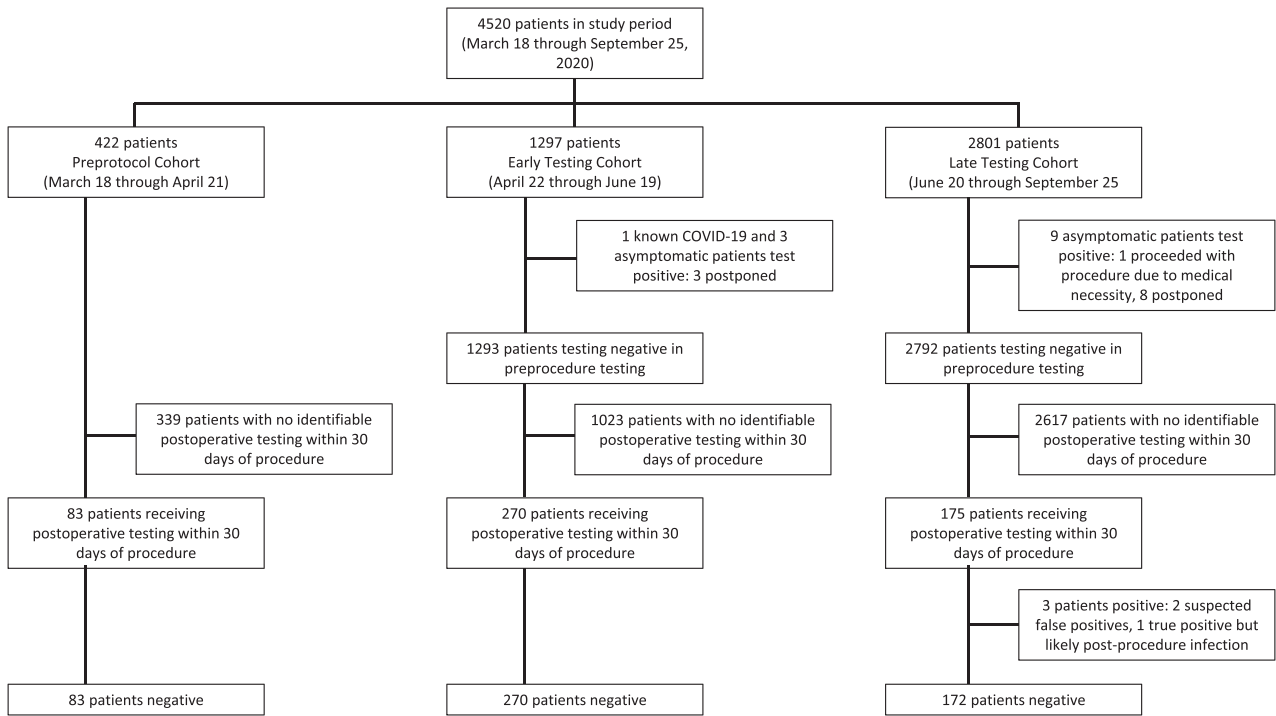


FIGURE 1. Flowchart demonstrating known severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing in different patient cohorts, including preprocedural and post-procedural tests.

the descriptive intent of the project and the very small number of patients in subgroups, inferential statistics were not calculated.

RESULTS

The entire study period from March 18 to September 25, 2020, included 4520 procedures (Fig. 1). The preprotocol cohort included 422 patients, who were screened only on the basis of symptoms. None of them developed a diagnosis of COVID-19 within 30 days of operation. Eighty-three underwent SARS-CoV-2 testing within 30 days following their procedure (Table I) because of either additional procedural screenings or suspicion of infection. All patients tested negative.

The ET cohort included 1,297 scheduled procedures (Table I). Of those, four patients had positive preprocedure RT-PCR tests. Three asymptomatic patients tested positive for SARS-CoV-2 in preprocedural testing and had their procedures postponed until two consecutive negative RT-PCR tests were performed or the patient was determined to be of no infectious risk. The remaining positive patient was already hospitalized for COVID-19 and underwent a tracheostomy as part of their treatment plan (Table II). Ten patients had their procedures cancelled for lack of testing, in accordance with the screening protocol. An additional nine patients proceeded with surgery, despite lack of testing. Of those nine, seven were neonatal procedures on patients recently born in the hospital, and the remaining two were failures of the

system to identify untested status before the surgery was performed. Of the remaining patients in the ET cohort with negative preprocedure tests, 270 received additional SARS-CoV-2 testing within 30 days following their procedure; all were negative.

For the LT cohort, an additional 2,801 procedures were performed with nine patients identified as positive during their presurgical screening (Table I). Four of those patients had their surgery postponed until they received a negative test, as per the initial test-based strategy. Two of the positive patients (as well as one of the persistently positive patients from the ET cohort) had their procedures postponed for a set period of time (20 days) and then proceeded with surgery without further testing, reflecting the CDC-recommended shift to a time-based strategy. One of the positive patients had the surgery performed as scheduled because of the pressing nature of the procedure, and one had the procedure postponed but was lost to follow-up because of departure from our health system (Table II).

Within the LT cohort, 175 patients received post-procedural testing because of routine screening, additional surgical screening, or suspicion of infection within 30 days following their procedure. A total of three were found to be positive (Table III). Based on their clinical details, none of the patients clearly met the criteria for a false-negative on preprocedure testing. The first was asymptomatic but tested positive 16 days after surgery as part of routine occupational screening. However, the patient had a negative test

TABLE I. Study Population Demographics

Characteristic	Preprotocol (PP) cohort (n = 422)	Early testing (ET) cohort preoperative testing negative (n = 1274)	ET cohort preoperative testing positive (n = 4)	Late testing (LT) cohort preoperative testing negative (n = 2792)	LT cohort preoperative testing positive (n = 9)
Mean age (SD)	47.0 (20.0)	47.5 (21.2)	63.8 (3.9)	48.5 (20.3)	35.4 (17.3)
Race (%)					
White	206 (49)	615 (48)	1 (25)	n/a ^a	7 (77)
African American	102 (24)	304 (24)	3 (75)	n/a ^a	2 (22)
Other	60 (14)	183 (14)	0 (0)	n/a ^a	0 (0)
Asian/Pacific Islander	8 (2)	28 (2)	0 (0)	n/a ^a	0 (0)
American Indian/Alaskan	1 (0.2)	3 (0.2)	0 (0)	n/a ^a	0 (0)
Unknown	45 (11)	141 (11)	0 (0)	n/a ^a	0 (0)
Gender (%)					
Male	232 (55)	710 (56)	2 (50)	1428 (51)	4 (44)
Female	190 (45)	564 (44)	2 (50)	1365 (49)	5 (56)
Type of test ^b (%)					
Rapid reverse transcriptase polymerase chain reaction (RT-PCR)	None	283 (22)	0 (0)	n/a ^a	4 (45)
Standard RT-PCR	None	973 (76)	4 (100)	n/a ^a	5 (55)
Unknown	None	18 (1)	0 (0)	n/a ^a	0 (0)
Procedural service (%)					
Surgical					
Orthopedic surgery	74 (16)	209 (16)	0 (0)	419 (15)	2 (22)
General surgery	85 (18)	168 (13)	2 (50)	271 (10)	0 (0)
Otolaryngology	5 (1)	99 (8)	1 (25)	215 (8)	2 (22)
Urologic surgery	26 (6)	98 (8)	1 (25)	152 (5)	1 (11)
Gynecologic surgery	20 (5)	93 (7)	0 (0)	333 (12)	2 (22)
Neurosurgery	36 (8)	90 (7)	0 (0)	174 (6)	0 (0)
Cardiothoracic surgery	23 (5)	68 (5)	0 (0)	19 (1)	0 (0)
Ophthalmology	10 (2)	42 (3)	0 (0)	114 (4)	0 (0)
Oral-maxillofacial	4 (1)	34 (3)	0 (0)	80 (3)	0 (0)
Peripheral vascular	7 (1)	30 (2)	0 (0)	66 (2)	0 (0)
Plastic/reconstructive	13 (3)	24 (2)	0 (0)	85 (3)	0 (0)
Pain management	1 (0.2)	14 (1)	0 (0)	30 (1)	0 (0)
Organ transplant	10 (2)	10 (1)	0 (0)	15 (1)	0 (0)
Podiatry	3 (0.6)	9 (1)	0 (0)	37 (1)	0 (0)
Nonsurgical					
Gastroenterology	73 (16)	188 (15)	0 (0)	452 (16)	2 (22)
Psychiatry (ECT)	8 (2)	35 (3)	0 (0)	50 (18)	0 (0)
Pediatric sedation	13 (3)	31 (2)	0 (0)	14 (1)	0 (0)
Pulmonary	7 (1)	22 (2)	0 (0)	53 (2)	0 (0)
Radiology	1 (0.2)	7 (1)	0 (0)	4 (0.1)	0 (0)
Hematology oncology	3 (0.6)	3 (0.2)	0 (0)	1 (0)	0 (0)
Positive COVID-19 diagnosis within 30 days after procedure (%)	0 (0)	0 (0)	N/A	3 (0.1)	N/A

^aFull demographic data were not collected on test-negative patients in the LT cohort.

^bRapid RT-PCR testing resulted within 1 hour of collection and was generally performed if patient presented through emergency department; standard RT-PCR testing was generally collected 48-96 hours before admission.

just before surgery and five additional negative RT-PCR tests immediately following the sole positive test, raising suspicion of a potential false positive. The second patient tested positive 29 days post-procedure but had an additional negative test shortly after. The final post-procedural positive patient tested positive 6 days after surgery during an emergency room visit because of procedural complications and without COVID symptoms. It was discovered that this patient was living with

an actively positive family member and most likely became infected upon return home.

A total of 10 asymptomatic RT-PCR-positive patients in the ET and LT cohorts received their planned procedure at a later date (Table II). The median delay to procedure completion was 39.5 days (IQR 34-46 days). Seven patients were cleared using a test-based strategy, requiring two negative RT-PCR tests before the rescheduled procedure. Three were cleared

TABLE II. Results of Positive Screening Tests in Early Testing (ET) and Late Testing (LT) Cohorts

Case #	Procedure	Cohort	Reverse transcriptase polymerase chain reaction (RT-PCR) test type ^a	Initial action	Confirmed SARS-2-CoV negative	Surgical completion
1	General surgery	ET	Standard	Postponed	26 days post-cancellation	28 days post-cancellation
2	General surgery	ET	Standard	Postponed	32 days post-cancellation	34 days post-cancellation
3	Otolaryngologic ^b	ET	Standard	Procedure performed	17 days post-test	Completed as scheduled
4	Gastroenterology	ET	Standard	Postponed	Positive as of 30 July	110 days post-cancellation
5	Otolaryngologic	LT	Standard	Postponed	15 days post-cancellation	25 days post-cancellation
6	General surgery	LT	Rapid	Postponed	34 days post-cancellation	37 days post-cancellation
7	Gastroenterology	LT	Standard	Postponed	44 days post-cancellation	46 days post-cancellation
8	Otolaryngologic	LT	Standard	Postponed	76 days post-cancellation	77 days post-cancellation
9	Gynecologic	LT	Rapid	Postponed	N/A—new nontesting guidance	43 days post-cancellation
10	Gynecologic	LT	Rapid	Postponed	N/A—new nontesting guidance	38 days post-cancellation
11	Urologic	LT	Rapid	Postponed	17 days post-cancellation ^c	41 days post-cancellation
12	General surgery	LT	Standard	Cancelled	Unknown—lost to follow-up	Not completed
13	Orthopedic	LT	Standard	Procedure performed	Positive as of 11 Aug	Completed as scheduled

^aRapid RT-PCR testing resulted within 1 hour of collection and was generally performed if patient presented through emergency department; standard RT-PCR testing was generally collected 48-96 hours before admission.

^bSymptomatic COVID-19 patient.

^cPatient underwent presurgical RT-PCR testing despite change to CDC guideline.

TABLE III. Characteristics of Patients with Positive Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Testing within 30 Days Post-Procedure

Case #	Surgery	Cohort	Time from surgery to positive test	Persistent positive?	Case details
1	Gynecologic	PT	16 days	No	1 of 9 tests positive, asymptomatic, presumed false positive
2	Gynecologic	PT	29 days	No	1 of 3 tests positive, minimal COVID-type symptoms, presumed false positive
3	Urologic	PT	6 days	Yes	Lived in same house as COVID-positive family member, asymptomatic, presumed true positive

under the time-based strategy (no requirement for repeat RT-PCR testing), after the policy was changed to adapt to CDC guidance. While the time-based strategy only required a 20-day delay, all three had their procedures rescheduled well past 20 days from the original date, at 38, 41, and 43 days.

DISCUSSION

Our preprocedural screening protocol successfully identified asymptomatic SARS-CoV-2 positive patients before surgery, yielding an overall asymptomatic positive test rate of 0.29%. Our data cannot answer whether these asymptomatic positive patients would have experienced worse outcomes if they had not been diagnosed during screening. However, we considered available data on surgical outcomes in infected patients to provide justification for delaying procedures in patients who tested positive.^{7,10-12} We also do not know how much risk these patients posed in transmitting infection to health care workers or other patients, as such risk in this population remains to be determined. The rates of asymptomatic positivity in our population are similar to those observed by

Jou et al. (0.2% in 1491 patients) over a time period that overlapped with our preprotocol and ET cohorts (March 30-May 8, 2020) in a medical system in San Diego, California.¹³ Urban et al. reported a higher rate of 12%, but this was based off of testing in 25 nonelective otolaryngology patients in a practice based in Chicago, Illinois, during the time period of March 23-April 17, 2020.¹⁴ The baseline prevalence rates of infection in the different areas and differences in access for medically time-sensitive care likely influence these disparate results.

All positive patients ultimately received their planned procedures without additional complication (with exception of one patient who exited the military health system), in part because of updated CDC guidance that drove changes to the screening protocol.⁹ As knowledge around the COVID-19 pandemic grew, the CDC recognized that patients can continue to test for the presence of SARS-2-CoV ribonucleic acid fragments for up to 90 days post-infection but with minimal amounts of infective virus shed after approximately 6 days from decline of symptoms. As a result, CDC guidelines

evolved to recommend that planned treatment could proceed 10 days following symptom decline or initial positive test, if asymptomatic. Accordingly, our hospital changed its policy for positively screened surgical patients by rescheduling their procedure to a minimum of 20 days (recently lowered further to 10 days) following either the decline of symptoms or the first positive test, rather than requiring two consecutive negative tests. Since the median delay for all postponed procedures was 39.5 days, it stands to reason that modification of the policy might have reduced delays to performing the procedure. However, of the three positive patients who did receive their surgeries following the revised, no-testing guidelines, their postponement times remained 38, 41, and 43 days, respectively, indicating other factors may have still influenced the decision of performing surgery, including the urgency of the procedure and comfort of the patient in proceeding. It is also important to consider the implication that, if a patient can test positive for up to 90 days post-infection, some of the positively screened patients may have been infected in the months before their procedure and were no longer an infectious risk, making the delay of their surgery unnecessary. However, currently available RT-PCR tests cannot fully discriminate the acutely infectious from the previously infectious, favoring a conservative strategy.

The sparse number of positive tests in the 30 days post-procedure supports the effectiveness of the screening protocol in an asymptomatic population. We only identified three patients with positive SARS-CoV-2 testing in the 30 days post-procedure, and none had clear evidence of a false-negative test on preprocedural testing. While it must be considered that individuals can continue to test positive for up to 90 days after infection,^{9,15} the clinical context for these patients suggest that they were not miscategorized by preprocedural testing. It is worth noting that there were no post-procedural positive tests or COVID diagnoses in the pre-protocol cohort. This finding may be related to the conservative procedural selection criteria during the period of peaking COVID-19 diagnosis rates in the local area.¹⁶ We also cannot rule out the possibility that patients asymptomatic at the time of their procedure remain unlikely to manifest symptoms of COVID-19 infection, even after procedural stress.

As an additional balancing measure of our protocol, we assessed the number of patients cancelled because of the logistical issue of not being able to get tested preprocedurally, and we found that an acceptably small percentage of patients had their procedures rescheduled for this reason. Based on these results and additional research confirming the risks of asymptomatic transmission and aerosolization of the virus,^{17,18} our hospital continues to perform preprocedural testing for asymptomatic patients at the time of this writing, a practice that was increasingly facilitated as testing sites within the health system have expanded. However, we remain cognizant that false-negative testing is still a risk, and our results should not be interpreted to suggest that any patient with a negative test can be treated without transmission precautions.

Current hospital policies still stress treating all patients as at-risk for carrying asymptomatic infection, regardless of testing results.

While this data supports the effectiveness of this testing protocol, our ability to assess its effectiveness fully is limited in the following ways. The unique nature of the military population in comparison to our surrounding community should be considered when generalizing this data. Access to the hospital is mostly limited to a defined beneficiary population and thus restricts much of the surrounding community from seeking care at our facility. Comparison data¹⁶ of the local Maryland community where our hospital is located confirms the apex of new cases occurred between April 1 and June 15, peaking with 533 confirmed new cases on May 15, but this may not completely reflect prevalence in our population. As a result, the ability to generalize our results to other populations is likely influenced by the prevalence of the virus. This reality is illustrated by the higher rates of positive tests seen by Bloom et al. in their evaluation of preprocedural testing during a period of high community prevalence.¹⁹ Additionally, when evaluating effectiveness, the possibility of not capturing positive patients in follow-up must also be considered, since we did not have a formal postoperative screening plan. However, the military health care system is notable in its ability to capture comprehensive treatment data. The closed system allows for direct surveillance of all appointments and testing within any military facility, worldwide. Additionally, the low barriers to medical care and the reporting responsibility of service members contribute to a high likelihood of capturing information from COVID-19 hospitalizations, even if the care occurs outside the military health care system. Of the 4,520 procedures examined in this study, only one patient was lost to 30-day follow-up after separating from the service. Finally, the inability to continue collecting the same depth of information for test-negative patients in the LT phase limits our ability to understand demographic changes that may have occurred as operative volumes returned to pre-pandemic levels.

CONCLUSION

Our results demonstrate that the use of a RT-PCR-based, preprocedure screening protocol for AGPs successfully identified a significant number of asymptomatic SARS-CoV-2-positive patients, allowed us to modify treatment plans to ensure the safe delivery of patient care, and did not result in high numbers of patients experiencing delayed care from the logistical burden of preprocedure testing. As we have gained more information on COVID-19 and CDC guidance has evolved, our protocol has adjusted accordingly and continues to demonstrate an effective capture of those patients who present risk of viral transmission to staff and other patients. Additionally, ongoing adjustments provided an opportunity to reduce the negative impacts of surgical delay in asymptomatic positive patients, although this was not fully realized in our observational cohort. It should be noted, however, that the

recent literature suggests increased post-procedural morbidity and mortality in patients undergoing operations up to 6 weeks after SARS-CoV-2 infection, even if asymptomatic.²⁰ Consideration should therefore extend beyond immediate infectivity of the patient as determined by test-based or time-based strategies and include overall risk to patient outcome. The results demonstrated in this study reflect the need for timely, agile, and multidisciplinary interpretation in the face of constantly evolving understanding when faced with the threats posed at the outset of a novel pandemic. As long as the future of the pandemic remains uncertain, we encourage continuous assessment of similar preprocedural testing protocols to determine the real-world impacts of such a process.

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CONFLICT OF INTEREST STATEMENT

None declared.

AUTHOR CONTRIBUTIONS

Study concept and design: P.A.L. and R.C.C. Acquisition of data: J.L., C.B., and A.S. Analysis and interpretation of data: C.B., A.S., and P.A.L. Drafting of the manuscript: C.B., P.A.L., and A.S. Critical revision of the manuscript: C.B., P.A.L., and A.S. Literary review: C.B. and A.S. Administrative, technical, and material support: J.L. and R.C.C. Study supervision: P.A.L. R.C.C.

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