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Histopathology of Appendiceal Specimens From Patients With Appendicitis and Concomitant COVID-19

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Abstract

Background: COVID-19 is a disease caused by SARS-CoV-2, which uses angiotensin-converting enzyme 2 (ACE2) receptors to infect cells. Research suggests that gastrointestinal (GI) manifestations of COVID-19 indicate the presence of ACE2 receptors throughout the GI system, including the appendix, and has identified SARS-CoV-2 nucleic acid in appendiceal specimens. Our study aimed to elucidate the relationship between appendicitis and COVID-19 through a histopathological analysis of appendixes from patients who underwent appendectomy with coexisting positive polymerase chain reaction (C+) results. We hypothesized that a strong presence of SARS-CoV-2 nucleic acid would be present in appendiceal samples of C+ patients.

Methods: We analyzed 34 appendiceal specimens from patients undergoing appendectomies from March 2020 to April 2022. Twenty-five specimens were from C+ patients, and nine were from C- patients; 5-µm sections of formalin-fixed paraffin-embedded tissue were subjected to C4d immunohistochemistry staining. SARS-CoV-2 nucleic acid was measured using RNAscope with a probe (V-nCoV2019-S; Advanced Cell Diagnostics, Newark, CA) against COVID-19, also on formalin-fixed paraffin-embedded tissue.

Results: Of the 25 C+ appendiceal specimens, three (two focally, one patchy) contained SARS-CoV-2 nucleic acid (3 C+ vs. 0 C-; p = 0.553). C4d staining was variable and had no difference in intensity (p = 0.553) or quantity (p = 0.188) between COVID-19 status. There was no difference in appendiceal wall thickness between C+ and C- patients (2.28 mm C+ vs. 2.11 mm C-; p = 0.656).

Conclusion: We found no differences in the presence of SARS-CoV-2 nucleic acid, C4d staining, or wall thickness between C+ and C- specimens. These findings indicate minimal histopathological differences between appendixes from C+ and C- patients, suggesting that SARS-CoV-2 was not heavily involved in the pathogenesis of appendicitis.

Categories: General Surgery, Pathology, Infectious Disease
Keywords: appendicitis, appendix histopathology, c4d, covid-19, pandemic

Introduction

COVID-19 is a respiratory disease caused by SARS-CoV-2. For invasion of respiratory epithelial cells, SARS-CoV-2 virions utilize a spike protein that binds to the angiotensin-converting enzyme 2 (ACE2) receptor, which allows for subsequent internalization of the virus and propagation of viral infection [1]. While the pathogenesis of SARS-CoV-2 in respiratory tissue has been extensively researched, there is evidence that SARS-CoV-2 can invade gastrointestinal (GI) tissue [2]. The presence of ACE2 receptors throughout the GI tract suggests that tissue invasion in the GI tract is similar to that of respiratory tissue. Its ability to invade GI tissue provides a possible explanation for some of the GI manifestations of COVID-19 [3].

There have been several case studies that suggest appendicitis or an appendicitis-like syndrome is a presentation of COVID-19 [4-8]. A case study from March 2020 demonstrated SARS-CoV-2 nucleic acid within the appendix of a patient who was COVID-19-positive at the time of their appendectomy, and the authors suggested that the appendicitis in that case was caused by COVID-19 [9]. Another study that assessed nine pediatric appendiceal samples taken from COVID-19-positive patients identified no SARS-CoV-2 nucleic acid in their samples [10].

The pathogenesis of appendicitis is not straightforward for as common a condition as appendicitis is. A 2026 study by Gelevski et al. posits that appendicitis is a disease impacted by etiological substrate, pathological severity, and patient systemic response [11]. Understanding this hybrid severity-etiology framework is key to reconciling inconsistencies observed from patient to patient, refining risk stratification strategies, and enabling more granular correlation between appendicitis and other diseases.

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In the present study, we sought to elucidate a possible relationship between appendicitis and COVID-19. To do so, we performed a histopathological analysis of appendiceal specimens taken from patients diagnosed with acute appendicitis who subsequently underwent appendectomy. We hypothesized that there would be a strong presence of SARS-CoV-2 nucleic acid in appendiceal tissue samples taken from those who were COVID-19-positive within 30 days of admission, supporting our hypothesis that SARS-CoV-2 plays a direct role in the pathogenesis of appendicitis in the cases studied.

Materials And Methods

After Institutional Review Board approval, we compared appendiceal samples from patients aged 5 years or older who tested positive for COVID-19 by polymerase chain reaction (PCR) with those who tested negative. These COVID-19-negative samples served as our control group. Thirty-four appendiceal specimens from patients admitted with an acute appendicitis diagnosis from March 2020 to April 2022 at our community hospital underwent histopathological analysis. Three specimens were from patients with perforated appendicitis. These patients were identified from a previous study by Harshaw et al., which examined the severity of appendicitis before and during the COVID-19 pandemic [12]. Specimens were identified from the study's COVID-19-era, defined as patients presenting at our institution from March 11, 2020, to April 1, 2022. Twenty-five of these appendiceal specimens were from COVID-19-positive patients (defined as PCR positivity within 14 days of admission), and nine appendiceal specimens were randomly selected from COVID-19-negative patients as controls. There were 841 COVID-19-negative patients during the COVID-19-era identified by Harshaw et al. Every 84th negative patient was considered a negative control for this study. Random selection was used to obtain a diverse sample of controls in an effort to control for confounding variables. Because the COVID-19-positive patient specimens were identified from the COVID-19-positive patients in Harshaw et al., they were a sample of convenience.

We used the COVID-19 (V-nCoV2019-S) probe (ACD 848568; Advanced Cell Diagnostics, Newark, CA) to detect SARS-CoV-2 nucleic acid. RNAscope on 5-µm sections of formalin-fixed paraffin-embedded tissue was performed using a probe against COVID-19, along with positive (PPIB Leica RS7755) and negative (Dapb Leica RS7756) control probes for each case. Staining was done on a Leica Bond-IIITM instrument (Leica Biosystems Melbourne Pty Ltd., Australia) using the Bond RNAscope. For detection, a Brown System (Leica Microsystems DS9790; Leica Biosystems Melbourne Pty Ltd) was used. Heat-induced epitope retrieval was done for 20 minutes with ER2 solution (Leica Microsystems AR9640).

All the experiments were done at room temperature. The slides were washed three times between each step with bond wash buffer or water. Each sample was subjected to complement 4d (C4d) immunohistochemistry staining, and appendiceal wall thickness was measured. Samples were then reviewed by two different pathologists, both of whom were blinded to the COVID-19 PCR status of each of the samples.

Characteristics and demographics of COVID-19-positive and COVID-19-negative samples were then described by study personnel. We assessed the normality of continuous variables via a Shapiro-Wilk test. We then performed independent-samples t-tests for continuous variables and reported the data as mean (standard deviation; 95% confidence interval). For categorical variables, we conducted Fisher's exact tests because of our limited sample size. These data were reported as n (percent of population). While we conducted these analyses to examine a potential relationship between COVID-19 and appendicitis, the descriptive statistics became more informative due to the small sample size. Analyses were performed in Stata/IC 16.0 (College Station, TX, USA). A p value of <0.05 was considered statistically significant. We followed the STrengthening the Reporting of OBservational Studies in Epidemiology guidelines in structuring this article [13].

Results

Histopathological analysis of these specimens showed no significant differences in appendiceal wall thickness, SARS-CoV-2 nucleic acid staining, or C4d staining characteristics between appendiceal samples from COVID-19-positive and COVID-19-negative patients. Results are shown in Table *1*.

Variables	COVID-19 negative (n = 9)	COVID-19 positive (n = 25)	p value
Wall thickness (mm), mean (standard deviation)	2.11 (0.93)	2.28 (0.98)	0.656
Nucleic acid stain, n (%)			
Negative	9 (100%)	22 (88%)	
Focal	0 (0%)	2 (8%)	0.553
Patchy	0 (0%)	1 (4%)	
C4d intensity, n (%)			
Variable	0 (0%)	1 (4%)	
Moderate	0 (0%)	2 (8%)	0.553
Strong	9 (100%)	22 (88%)	
C4d quantity, n (%)			
Focal	1 (11.11%)	1 (4%)	
Patchy	3 (33.33%)	17 (68%)	0.188
Diffuse	5 (55.56%)	7 (28%)	
C4d percentage, n (%)			
0%-20%	2 (22.22%)	4 (16%)	
21%-60%	2 (22.22%)	14 (56%)	0.205
61%-100%	5 (55.56%)	7 (28%)	

TABLE 1: Histopathological comparison between COVID-19-positive and COVID-19-negative specimens

C4d: complement 4d

Figures *1A*, *1B*, *2A*, *2B* are histopathological images from one COVID-19-positive patient. C4d intensity refers to the staining density of the signal. C4d quantity refers to the distribution of the signal throughout the specimen. C4d percentage refers to the approximate percentage of the sample that contains the stain. Only three out of 25 COVID-19-positive samples contained SARS-CoV-2 nucleic acid. For the COVID-19-positive group, C4d intensity was mostly strong, the quantity was mostly patchy, and the C4d percentage was mostly in the 21%-60% range. For the COVID-19-negative group, C4d intensity was always strong, mostly diffuse, and mostly in the 61%-100% range.

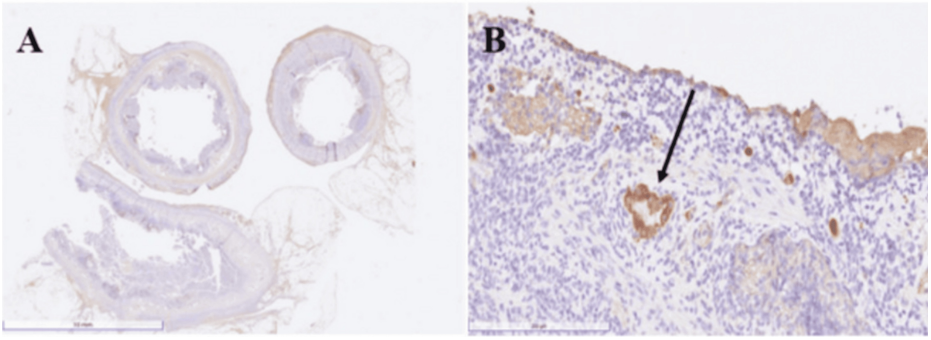


FIGURE 1: Appendiceal specimen 1

(A,B) Strong C4d staining in the vessels of the appendiceal specimen (100% C4d percentage). (B) High-power view of Image A

C4d: complement 4d

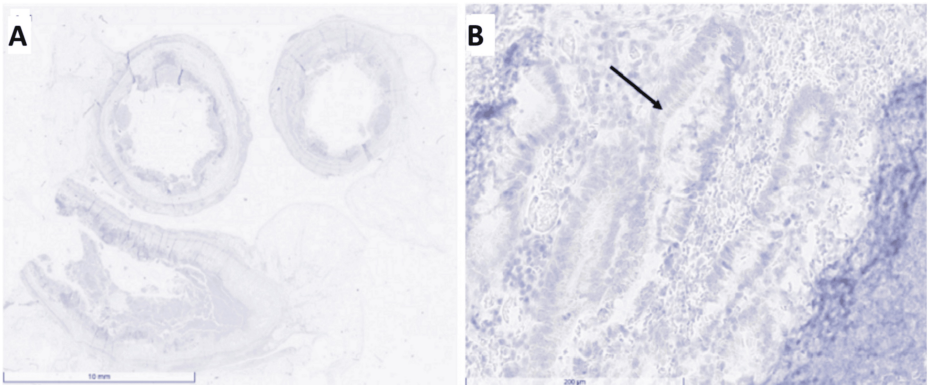


FIGURE 2: Appendiceal specimen 2

(A,B) RNAscope staining for SARS-CoV-2 nucleic acid, which demonstrates patchy, weak cytoplasmic positivity in epithelial cells. (B) High-power view of Image A

Figures *1A*, *1B*, *2A*, *2B* are of the same specimen chosen to show the contrast between C4d and RNAscope staining. This specimen showed the most robust evidence of staining. The arrows in Figures *1B*, *2B* indicate areas of the appendiceal specimen that picked up the most staining in the vessels and epithelial cells.

Discussion

The histopathologic analysis of COVID-19-positive and COVID-19-negative appendiceal specimens yielded no significant differences in appendiceal wall thickness, SARS-CoV-2 nucleic acid in appendiceal tissue, or C4d staining. This is consistent with the evidence found in the study by Jiang et al., in which all nine of their pediatric appendiceal specimens taken from COVID-19-positive patients displayed no SARS-CoV-2 nucleic acid. This refutes our hypothesis and the proposed mechanism of SARS-CoV-2 infection playing a direct role in the appendicitis cases we studied. Alternative explanations for the relationship between COVID-19 and appendicitis exist. These primarily point toward the general inflammatory state generated by COVID-19 as providing the opportunity for appendicitis to occur, but not necessarily as a causative agent [14].

Our motivation to study appendiceal specimens and COVID-19 came after a case study that was done at our institution regarding the role of SARS-CoV-2 in second-trimester patients who experienced intrauterine fetal demise. Briguglio found that there was a strong concentration of C4d complement staining in the syncytiotrophoblastic layer of the placental villi in their samples. The complement activation was associated with histopathologic findings of extensive fibrin deposition and villous infarction, suggesting a role of immune-mediated vascular injury in adverse fetal outcomes. They suggested that because previous research has demonstrated that pregnant women have higher levels of ACE2 receptors in the placenta, this provides the entry point for SARS-CoV-2 [15]. This case study highlights the vascular insult SARS-CoV-2 can inflict on ACE2-abundant tissues. This case study, in conjunction with the previously aforementioned studies that investigated the relationship between COVID-19 and the appendix, provided much of the inspiration for our study.

Similar vascular pathology has been studied in the GI tract, with an association between ischemic colitis and COVID-19. In a case study performed by Uhlenhopp et al., two patients developed left-sided colitis after being diagnosed with COVID-19. Both imaging and endoscopy confirmed nonocclusive ischemia and thrombotic events, suggesting that the underlying pathophysiology was secondary to the hypercoagulable state induced by the viral infection [16]. In this study, we had no significant presence of viral nucleic acid (only three of 25 specimens) in appendiceal tissue, and C4d staining showed no significant difference between COVID-19-positive and COVID-19-negative patients. These results reinforce the idea that SARS-CoV-2 may not directly affect GI tissue in all cases but may exacerbate underlying vulnerabilities in vascular integrity through complement activation and endothelial injury. This aligns with the broader observation that COVID-19 is a multisystem inflammatory disease, which is capable of triggering pathology through immune-mediated responses rather than direct virus-induced cell damage.

Limitations of our study include our relatively small sample size and small control group. Including more samples from multiple centers could grant a more powered analysis through which more definitive and generalizable findings could be drawn. Also, the samples were taken from March 2020 to April 2022. This time frame contained numerous variants of SARS-CoV-2 with different levels of virulence, creating the possibility for variable effects on the GI tract. While C4d staining was used to identify the degree of host-mediated damage caused by the SARS-CoV-2 infection, there are also limitations to using the C4d stain. It is a recognized marker for complement activation and has diagnostic utility in transplant pathology in identifying antibody-mediated rejection, but it is not the most specific tool for evaluating COVID-19. C4d staining has limitations as a biomarker due to its lack of specificity, as accumulation of the C4d protein can occur in a wide range of conditions. The presence of C4d indicates that the complement pathway has been activated but does not clarify the underlying cause or distinguish between different inflammatory triggers [17].

Conclusions

In this exploratory and descriptive analysis, histopathological differences between appendiceal specimens from COVID-19-positive and COVID-19-negative patients with acute appendicitis were minimal, suggesting that direct SARS-CoV-2 infection of appendix specimen may not be heavily involved with the pathogenesis of appendicitis in the patients studied. Because of the small sample size in this study, significant associations were not established; however, this does not necessarily indicate an absence of biological relevance, especially when considering descriptive findings. From a clinical standpoint, these results should be considered with caution, and more studies should be done to further investigate this work.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Lindsey L. Perea, Nathaniel Harshaw, Isabella Filippone, Samantha LaRosa, Katherine Moore

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Disclosures

Human subjects: Informed consent for treatment and open access publication was obtained or waived by all participants in this study. Lancaster General Hospital Institutional Review Board issued approval 2023-48. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** This research was funded by the Louise Von Hess Medical Research Institute at Penn Medicine Lancaster General Health. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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