Urology Case Reports 51 (2023) 102575

Contents lists available at ScienceDirect

Urology Case Reports

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

Inflammation and infection

Fibrin microthrombi in bladder urothelium after SARS-CoV-2 infection: Case report

Ly Hoang Roberts [a], Bernadette M.M. Zwaans [a,b,*], Kausar Jabbar [c], Sarah N. Bartolone [a], Priya Padmanabhan [a,b], Kenneth M. Peters [a,b]

[a] Department of Urology, Corewell Health, Royal Oak, MI, USA
[b] Oakland University William Beaumont School of Medicine, Rochester, MI, USA
[c] Department of Pathology, Beaumont Health, Royal Oak, MI, USA

A B S T R A C T

A 45-year-old male with diabetes, hypertension and hyperlipidemia was referred to urology due to persistent symptoms of urinary frequency, urgency, nocturia, erectile dysfunction, and constant pain localized to the bladder, pelvis, and perineal area, 3–4 months after SARS-CoV-2 infection. A bladder biopsy showed urothelial mucosa and submucosa with hemorrhage and fibrin microthrombi in blood vessels. Hydrodistention of the bladder and pelvic floor physical therapy resolved symptoms, though bladder and pain symptoms returned upon reinfection with SARS-CoV-2. Urinalysis revealed elevated urinary interleukin-8, which may indicate localized bladder inflammation.

1. Introduction

Infection with SARS-CoV-2, resulting in COVID-19, not only impacts the body in the acute phase, but also long term. Up to 80% of those infected continue to suffer from one or more long lasting symptoms.[1] Consistent with other subspecies in the coronavirus family, SARS-CoV-2 typically infects the upper respiratory system first. However, it may then progress and affect other organs such as the heart, brain, and kidney, leading to over 50 possible long-term side effects and even death from multi-organ failure.[2,3] One explanation for the systemic nature of the disease can be attributed to the cytokine storm and release of pro-inflammatory markers that occurs, especially in severe cases. Mulchandani and colleagues demonstrated in a recent meta-analysis that IL-6, IL-8, IL-10, IL-2R, and TNF-α are significantly higher in severe cases, compared to those with moderate symptoms, while other immunological agents such T lymphocytes are lower.[4] Severe COVID-19 infection can also lead to a hypercoagulable state resulting in microvascular thrombi to form in heart, lungs, liver, brain, and skin.[5] In a clinic-histopathologic study of 69 patients who died of COVID-19, Brener et al. reported that the most common abnormality in the cardiac tissue was microthrombi (n = 48, 70%) with increased presence of fibroblasts and extracellular matrix.,[5] and not focal inflammatory infiltrate (n = 25, 36%) or cardiomyocyte necrosis (n-25, 36%). This led the authors to highlight the role of fibroblasts leading to microthrombi and subsequent cardiac death.

Within the realm of urological symptoms, there have been clinical observational studies which report increased urinary frequency, urgency, and nocturia after COVID-19 infection. In a case series by Dhar and colleagues, 39 patients were reported to have developed significant de novo urinary frequency and nocturia after their hospitalization with COVID-19.[6] Eighty-two percent were male (n = 32) and 18% female (n = 7). 100% of patients reported urge incontinence, and frequency, with the majority reporting 13 or more voiding episodes/24 h (84.6%) and nocturia with 4 or more episodes/night (87.2%). They termed this condition COVID-associate cystitis (CAC).[6] Recent work has also supported this novel condition and reported an incidence of 36% amongst a large cohort (n = 1895) of hospital employees, 22% of which had de novo urological symptoms.[7] The pathophysiology of CAC is yet to be determined though several mechanisms have been hypothesized, including the direct binding of SARS-CoV-2 to angiotensin-converting enzyme II (ACE2), or the upregulation of cytokines similar to adenovirus and BK virus in causing hemorrhagic cystitis.[8–10]

The aim of this paper is to report on a case of microvascular thrombi discovered in bladder urothelium of a COVID-19 patient.

1.1. Clinical presentation

SA is a 45-year-old male with a past medical history of hypertension, poorly controlled Type 2 diabetes mellitus, and hyperlipidemia who developed shortness of breath with coughing at the end of September

* Corresponding author. Department of Urology, Corewell Health William Beaumont University Hospital, Royal Oak, MI, USA.
E-mail addresses: bernadette.zwaans@corewellhealth.org (B.M.M. Zwaans), Kenneth.Peters@corewellhealth.org (K.M. Peters).

https://doi.org/10.1016/j.eucr.2023.102575
Received 7 September 2023; Accepted 24 September 2023
Available online 25 September 2023
2214-4420/© 2023 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

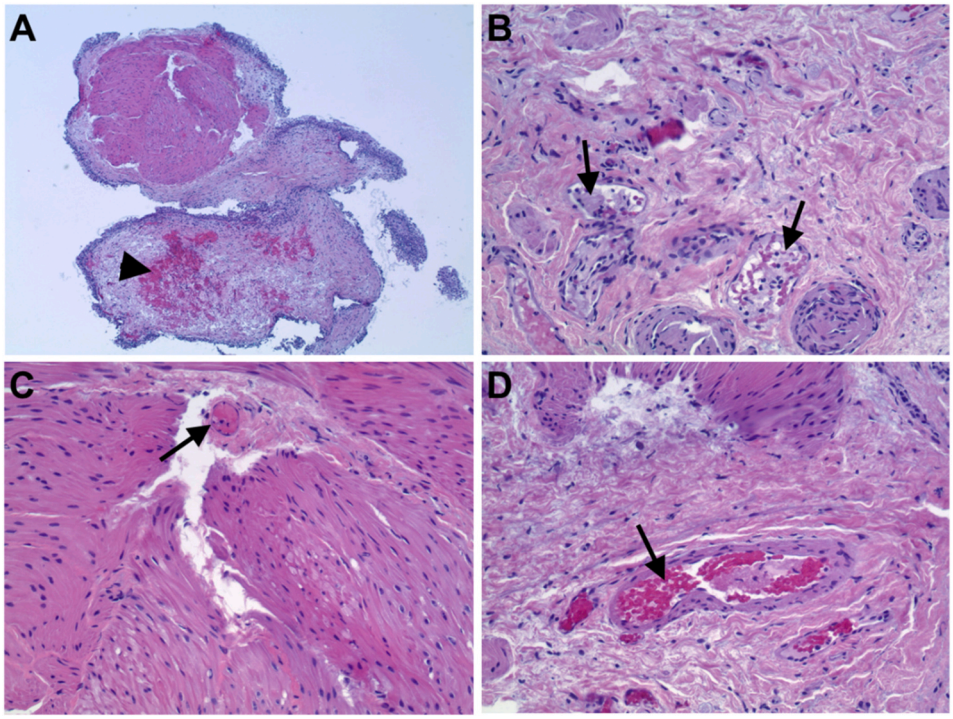

Fig. 1. Histological detection of microthrombi in bladder tissue. (A) Low power (4x) view of H&E-stained urothelial mucosa and submucosa with hemorrhaging (arrowhead). High power (20x) view of microthrombi (arrows) within a submucosal blood vessel (B), within blood vessel in muscularis propria (C), and within submucosal blood vessel (D).

2020. On October 10, 2020, he tested positive for SARS-CoV-2 via a nasopharyngeal swab and, because of persistent symptoms, was retested and still found positive on October 23, 2020. Three to four months later, he developed de novo low volume urinary frequency (every 3–4 hours), urgency, nocturia (1x/night), erectile dysfunction, and a constant pain (7/10 pain score) localized to the bladder, pelvis, and perineal area, that was relieved with urination. He denied dysuria or hematuria, his urinalysis was unremarkable and he tested negative for sexually transmitted diseases. CT imaging of the abdomen and pelvis did not show any abnormalities. He started daily antibiotics of ciprofloxacin for presumed prostatitis and was encouraged to make behavioral modifications such as decreasing spicy food intake, caffeine, and processed oils. Due to persistent symptoms, he was subsequently referred to the urology clinic in October 2021. Based on physical exam, he was found to have right pelvic floor tenderness with banding at the right levator ani muscles and a 30–40 gm nontender, non-indurated prostate. Given the acute onset of de novo severe frequency, urgency, and pelvic pain after COVID diagnosis, it was recommended he undergo cystoscopy and hydrodistension to assess his anesthetic bladder capacity and rule out Hunner's Lesions. In addition, he was started on pelvic floor physical therapy to manage his pelvic floor dysfunction that was noted on exam. Intraoperatively, no bladder masses, lesions, or distinct Hunner's ulcers were seen. After distending his bladder to 80 cm H_20 for 2 minutes, only sparse petechial hemorrhages were observed, and his anesthetic bladder capacity was 650 ml (normal 1000 ml). A bladder biopsy was performed, and pathology showed fragments of urothelial mucosa and submucosa with hemorrhage and fibrin microthrombi in blood vessels (Fig. 1). Tissue was negative for dysplasia or malignancy, without signs of fibroblast or macrophage infiltration. His bladder and pelvic pain symptoms improved after the hydrodistension and pelvic floor physical therapy. Yet, approximately 1 month later, January 2022, he developed Covid again, despite vaccination, with return of his bladder and pain symptoms. At follow up on February 2022, he scored a 7 out of 16 on the ICIQ-OAB with significant frequency (3/4, "11 to 12 times a day") and nocturia (2/4, "two times a night"). He only had urgency "sometimes" (2/4) though all three symptoms were bothersome (8/10). He denied any urinary incontinence. At this visit, urine was collected for urinalysis. He was then started on hydroxyzine 50 mg at night and, by June 2022 (4 months later), he reported his symptoms to be markedly improved; his urinary frequency was mostly resolved, pain was minimal, and his erectile dysfunction was successfully managed with daily tadalafil.

Table 1
Urinalysis of CAC urine sample.

Analyte	Reading
Glucose	**500 mg/dL**
Bilirubin	Negative
Ketones	Negative
Specific gravity	≥ 1.030
Blood	Negative
pH	5.5
Protein	Negative
Urobilinogen	0.2 E.U./dL
Nitrite	Negative
Leukocytes	Negative

*Abnormal readings are marked in red

1.2. Urinalysis

A urine sample of the case was obtained on February 21, 2022, for urinalysis. The urine sample was yellow in color and had clear clarity. Urinalysis was performed using Multistix 10 SG urine test strips (Siemens). Urinalysis of the sample was unremarkable other than high levels of glucose, which can be explained by the patients' Type 2 diabetes mellitus status (Table 1). Upon approval from the institutional IRB (#2022-055) consent was obtained from the patient through an information sheet to perform additional testing on this urine sample. Based on previous work from our group that demonstrated elevated urinary levels of IL-6 and IL-8 in patients with CAC, we performed Multiplex

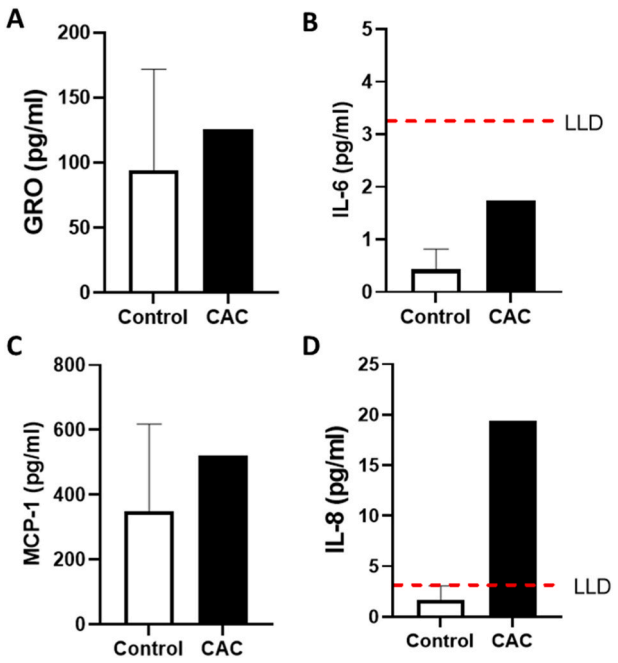

Fig. 2. Cytokine levels in urine of CAC versus healthy control samples. Urinary protein levels of the cytokines (A) GRO (CXCL1), (B) IL-6, (C) MCP-1 (CCL2) and (D) IL-8 were measured in the urine samples of 5 healthy controls and the CAC case using Multiplex Luminex assay. LLD = Lower Level of Detection.

immunoassay (Millipore), to assess urinary cytokine levels of IL-6, IL-8, MCP-1 (CCL2) and GRO (CXCL1).[11] Multiplex immunoassay was performed according to manufacturer's instructions. Urine samples of 5 healthy age-matched male controls were included. Of the 4 chemokines tested, IL-8 was higher in CAC sample (19.38 pg/ml) versus healthy control samples (1.69 pg/ml ± 1.36) (Fig. 2). IL-8 levels in healthy control levels were below the lower limit of detection (LLD) of the assay. IL-6 appeared to be increased in the CAC case, however measurements for CAC case and healthy controls were all below the LLD of the assay (1.74 pg/ml and 0.43 pg/ml ± 0.39 respectively).

2. Discussion

This manuscript reports the first case of microthrombi in the bladder of a patient with de novo or worsening urinary urgency, frequency, and nocturia after SARS-CoV-2 infection (CAC). In this case report, our patient developed significant urinary symptoms 3–4 months after COVID infection. Symptoms improved with treatment but returned upon repeat COVID infection, despite vaccination. Pelvic pain from pelvic floor muscle dysfunction was also present, which may be secondary to the bladder changes, and which resolved with appropriate physical therapy.[12]

It is unsure how SARS-CoV-2 affects the bladder. Microthrombi in response to SARS-CoV-2 infection have been reported in other organ systems including heart, lung, liver, brain, and skin.[5] Interestingly, this did not lead to signs of ischemic necrosis, as is the case in COVID-19 mediated microthrombi in cardiac tissue.[5,13] Though there was no evidence of increased fibroblast or macrophage activity as seen in other COVID-affected organs, urinary biomarkers showed elevated cytokine levels (Fig. 2). SARS-CoV-2 has been reported to induce endothelial dysfunction, resulting in secretion of pro-inflammatory cytokines including IL-6 and IL-8.[14] In our CAC case, IL-8 was measured at 19.38 pg/ml in comparison to 1.69 pg/ml (±1.36) in age-matched male healthy controls, an over 10-fold increase in IL-8 in CAC. IL-8 is secreted by macrophages, epithelial and endothelial cells to attract and activate neutrophils in areas of inflammation.[15] In a study by Kaiser et al., IL-8 has been reported to drive a pro-thrombotic phenotype in patients with severe COVID-19.[16] We did not observe a pathological influx of macrophages in the bladder tissue, thus the source of IL-8 may either be due to higher expression in the resident macrophages, and/or from the damaged endothelial cells. IL-6 levels in this study were below the lower level of detection of the assay and thus we cannot reliably conclude that IL-6 was elevated in the CAC case. An alternate proteomic assay may be able to detect lower levels of IL-6.

3. Conclusion

Many questions remain to be answered about CAC, but our case report highlights the new finding of bladder microthrombi exhibited after COVID infection. The findings of this study suggest that increased levels of IL-8 in the bladder may drive microthrombi in the bladder vasculature and that urinary IL-8 levels might provide a means of detecting if a patient is at risk for developing symptoms from these microthrombi after SARS-CoV-2 infection.

Funding

No funding source was used for this study.

Ethics approval and informed consent

All work was conducted in accordance with the Declaration of Helsinki. Experiments beyond standard of care were carried out with full approval of the institutional Interval Review Board (#2022-055) and with patient's written consent and understanding.

Declaration of competing interest

The authors declare no financial competing interests with this study.

References

1. Raveendran AV, Jayadevan R, Sashidharan S. Long COVID: an overview. *Diabetes Metabol Syndr*. 2021;15(3):869–875.
2. Carfi A, Bernabei R, Landi F, Gemelli Against C-P-ACSG. Persistent symptoms in patients after acute COVID-19. *JAMA*. 2020;324(6):603–605.
3. Greenhalgh T, Knight M, A'Court C, Buxton M, Husain L. Management of post-acute covid-19 in primary care. *Bmj*. Aug 11 2020;370:m3026.
4. Mulchandani R, Lyngdoh T, Kakkar AK. Deciphering the COVID-19 cytokine storm: systematic review and meta-analysis. *Eur J Clin Invest*. 2021;51(1), e13429.
5. Brener MI, Hulke ML, Fukuma N, et al. Clinico-histopathologic and single-nuclei RNA-sequencing insights into cardiac injury and microthrombi in critical COVID-19. *JCI insight*. Jan 25 2022;7(2).
6. Dhar N, Dhar S, Timar R, Lucas S, Lamb LE, Chancellor MB. De novo urinary symptoms associated with COVID-19: COVID-19-Associated cystitis. *J Clin Med Res*. 2020;12(10):681–682.
7. Roberts L, Zwaans B, Chancellor M, Peters K, Padmanabhan P. The incidence of new or worsening OAB symptoms in patients with prior SARS CoV-2 infection. *American Urol Assoc*. 2022. annual meeting; May 14, 2022, 2022; New Orleans, LA.
8. Jhang JF, Hsu YH, Peng CW, Jiang YH, Ho HC, Kuo HC. Epstein-barr virus as a potential etiology of persistent bladder inflammation in human interstitial cystitis/bladder pain syndrome. *J Urol*. 2018;200(3):590–596.
9. Pinto M, Dobson S. BK and JC virus: a review. *J Infect*. 2014;68(Suppl 1):S2–S8.
10. Zou X, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front Med*. Apr 2020;14(2):185–192.
11. Lamb LE, Dhar N, Timar R, Wills M, Dhar S, Chancellor MB. COVID-19 inflammation results in urine cytokine elevation and causes COVID-19 associated cystitis (CAC). *Med Hypotheses*. 2020;145, 110375.
12. Peters KM, Carrico DJ, Kalinowski SE, Ibrahim IA, Diokno AC. Prevalence of pelvic floor dysfunction in patients with interstitial cystitis. *Urology*. 2007;70(1):16–18.
13. Pellegrini D, Kawakami R, Guagliumi G, et al. Microthrombi as a major cause of cardiac injury in COVID-19: a pathologic study. *Circulation*. 2021;143(10):1031–1042.

14. Pons S, Fodil S, Azoulay E, Zafrani L. The vascular endothelium: the cornerstone of organ dysfunction in severe SARS-CoV-2 infection. *Crit Care*. Jun 16 2020;24(1):353.
15. Bickel M. The role of interleukin-8 in inflammation and mechanisms of regulation. *J Periodontol*. May 1993;64(5 Suppl):456–460.
16. Kaiser R, Leunig A, Pekayvaz K, et al. Self-sustaining IL-8 loops drive a prothrombotic neutrophil phenotype in severe COVID-19. *JCI insight*. Sep 22 2021;6(18).