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Bacillus coagulans-associated severe necrotizing scleral infection

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ABSTRACT

Purpose: We describe a case of severe scleritis possibly caused by *Bacillus coagulans*.
Observations: Conventional laboratory evaluation was inconclusive. The associated organism was identified with metagenomic RNA deep sequencing (MDS). The infection resolved with trimethoprim-sulfamethoxazole treatment.
Conclusions: This case demonstrates the utility of unbiased, high-throughput sequencing for infectious scleritis.

1. Case report

A 39-year-old man was referred with a one-month history of pain and redness in the left eye. He was initially seen by an outside provider and received treatment with oral prednisone, topical prednisolone, oral NSAIDs, and topical antibiotics, with no improvement of symptoms. The patient ran out of oral steroids one week prior to his initial presentation and noticed a worsening of symptoms during that period. The patient is a beekeeper and woodworker, though he did not recall any injury in or near the eye related to those activities. He had no past ocular or medical history, including cancer or HIV, and denied any systemic or infectious symptoms.

On examination, the patient was 20/20 in both eyes, with reactive pupils, normal pressure, and full ocular motility. Anterior segment examination of the left eye was notable for reactive ptosis with mild edema of the lids, diffusely dilated deep scleral and episcleral vessels, mild temporal chemosis, and small, multifocal infiltrates at the limbus circumferentially (Fig. 1A). Posterior segment examination was unremarkable. A provisional diagnosis of sclerokeratitis was made, with peripheral ulcerative keratitis and Mooren's ulcer placed on the differential. Extensive laboratory workup for suspected autoimmune and infectious etiologies was unrevealing (see Fig. 1, test results for January 2020). Given the inconclusive laboratory assessments, the patient was treated for idiopathic inflammatory sclerokeratitis. The patient was begun on prednisolone eye drops six times per day in the left eye, oral methotrexate and folic acid, 60mg of oral prednisone with a 10mg decremental taper every two weeks.

One month later, the patient presented with a scleral abscess just temporal to the limbus and increased deep scleral and episcleral injection (Fig. 1B). Given the worsening clinical picture on steroids and methotrexate, an infectious etiology was now suspected. The abscess was aspirated, and cytology demonstrated polymorphonuclear leukocytes, though gram stain, Giemsa stain, aerobic and anaerobic bacterial culture, fungal culture, and acid-fast bacillus (AFB) stain and culture were negative. The patient was treated with 800/160mg of oral trimethoprim-sulfamethoxazole twice daily, three subconjunctival injections of amikacin and ceftazidime spaced one week apart, and oral voriconazole 200 mg twice daily. Methotrexate was discontinued, and the dose of oral prednisone was reduced to 10mg daily. Five weeks passed without clinical improvement, and the patient underwent incision and drainage of the abscess and scleral biopsy. The aspirate was investigated with broad-range PCR for bacteria with reflex to next-generation sequencing (NGS) of the 16S ribosomal RNA (rRNA) gene, fungi with reflex to NGS of fungal 28S and Internal Transcribed Spacer sequence (ITS) DNA, and AFB (University of Washington Molecular Diagnosis Microbiology Section, Seattle, WA), all of which were negative. Pathological examination of scleral biopsy tissue demonstrated non-specific signs of inflammation. At this point, the decision was made to treat for both occult infectious and steroid-resistant inflammatory disease. Methotrexate was reintroduced at 20mg weekly, in addition to 60mg of oral prednisone daily with taper every two weeks. Antibiotics were transitioned to 100mg of oral doxycycline twice daily and

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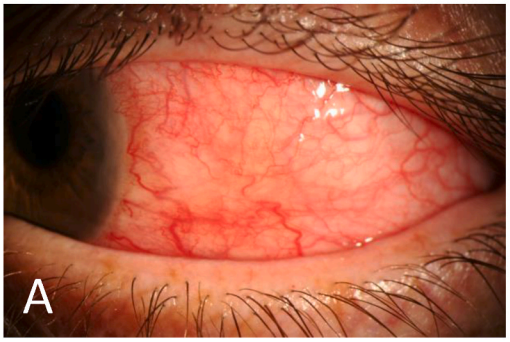

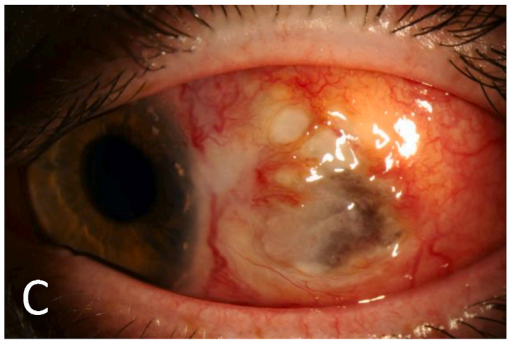

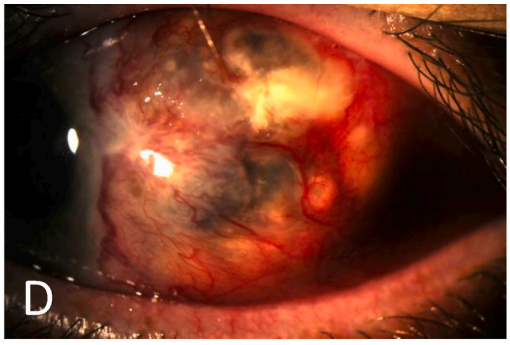

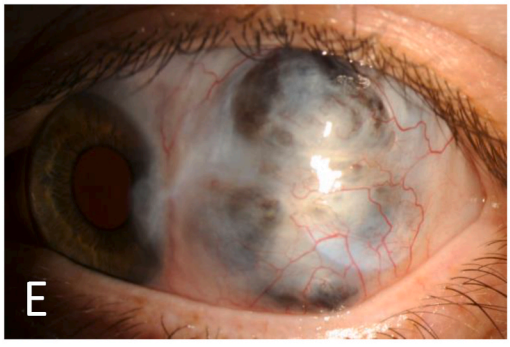

Date (Pictured)	Test Results	Treatment Ordered
January 2020 (A)	WNL: CBC, ANA, MPO/PR3 ANCA, RF, CCP, SS-A & SS-B Ab, ACE, lysozyme, C3, C4, C50, protein electrophoresis, RPR/FTA-Abs, interferon-gamma release assay, HBsAb, HBcAb, Hep C Ab CIA, HSV I & II IgG, Lyme serology, EBV nuclear Ag, CMV IgM, HIV (+): VZV IgG	Methotrexate 7.5mg PO QW Prednisone 60mg PO QD (10mg taper, Q2W) Prednisolone acetate 1% 6x/day OS
February 2020 (B)	Abscess aspirate → (+) cytology for PMNs (-) gram stain, Giemsa stain, fungal and bacterial culture	TMP/SMX 800/160mg PO BID Ceftazidime subconjunctival x3 Amikacin subconjunctival x3 Voriconazole 200mg PO BID Prednisone 10mg PO QD Prednisolone acetate 1% QID OS
April 2020	Abscess incision & drainage → (-) broad-range PCR for fungi, bacteria, mycobacterium Scleral biopsy pathology: Non-specific inflammation	Methotrexate 20mg PO QW Doxycycline 100mg PO BID Moxifloxacin 0.5% QID OS Prednisone 60mg PO QD (10mg taper Q2W) Prednisolone acetate 1% QID OS
May 2020 (C)		Methotrexate 25mg PO QW Doxycycline 100mg PO BID Moxifloxacin 0.5% QID OS Prednisone 60mg PO QD Prednisolone acetate 1% QID OS
June 2020 (D)	WNL: Chest x-ray, CBC, CMP, ESR, CRP, IgE, UA, IgG	Valacyclovir 1g PO TID Methotrexate 25mg PO QW Doxycycline 100mg PO BID Moxifloxacin 0.5% QID OS Prednisone 120 mg PO QD (x3 days) Prednisolone acetate 1% QID OS
July 2020	Subconjunctival infiltrate aspirate & swab → (-) bacterial culture, fungal stain, broad-range PCR; compromised sample sent for MDS	TMP/SMX 800/160mg 2 pills PO Q8H Voriconazole 300mg PO BID Prednisolone acetate 1% QID OS
October 2020	Subconjunctival swab, OU → (+) MDS for *Bacillus coagulans*	TMP/SMX 800/160mg 2 pills PO Q8H Prednisolone acetate 1% QID OS
November 2020		TMP/SMX 800/160mg 2 pills PO Q8H Polymyxin B/trimethoprim Q3H OS Moxifloxacin 0.5% QID OS Prednisolone acetate 1% QID OS
July 2021		Prednisolone acetate 1% QID OS
May 2022 (E)		Prednisolone acetate 1% QD OS Bromfenac 0.07% QD OS

(caption on next page)

Fig. 1. Clinical images of the left eye and clinical course of the patient. Abbreviations: ANA = anti-nuclear antibody; MPO/PR3 ANCA = myeloperoxidase and proteinase 3 antineutrophil cytoplasmic antibody; RF = rheumatoid factor; CCP = cyclic citrullinated peptide; SS-A & SS-B Ab = anti-Sjogren's syndrome A and anti-Sjogren's syndrome B antibody; ACE = angiotensin converting enzyme; C3 and C4 = complement components 3 and 4; C50 = total complement activity; HBsAb = hepatitis B serum antibody; Hep C Ab CIA = hepatitis C antibody by chemiluminescence immunoassay; HSV I & II IgG = herpes simplex virus I and II immunoglobulin G; EBV nuclear Ag = Epstein Barr virus nuclear antigen; CMV IgM = cytomegalovirus immunoglobulin M; RPR/FTA-Abs = rapid plasma reagin and fluorescent treponemal antibody absorption; VZV IgG = varicella zoster virus immunoglobulin G; PMN = polymorphonuclear neutrophil; PCR = polymerase chain reaction; MDS = metagenomic deep sequencing.

moxifloxacin eye drops four times per day in the left eye, with discontinuation of oral trimethoprim-sulfamethoxazole and voriconazole. At six-week follow-up, the patient reported symptomatic improvement, with less pain and photophobia, and appeared to have less redness on examination. Infection was suspected, and steroid dose was reduced accordingly. Two weeks later, the patient presented with scleral necrosis temporal to the previous abscess, surrounding avascular areas, and punctate corneal infiltrates at the limbus (Fig. 1C). Since clinical progression occurred midway through a prednisone taper, it was presumed that he was not immunosuppressed sufficiently and hence the decision was made to increase prednisone to 60mg daily and methotrexate to 25mg daily. Despite these increases in immunosuppressive therapy, scleral necrosis continued to progress over the next month, with previously avascular areas thinning to expose more visible choroid with a transillumination defect (Fig. 1D). Posterior extension of scleral inflammation was evident on fundus examination, which revealed a yellow area of subretinal necrosis superotemporally, with ultrasonography confirming the presence of fluid in sub-Tenon's space and Spectralis OCT of the macula demonstrating cystoid macular edema (Fig. 2).

Rheumatology was consulted, though no further clinical or historical

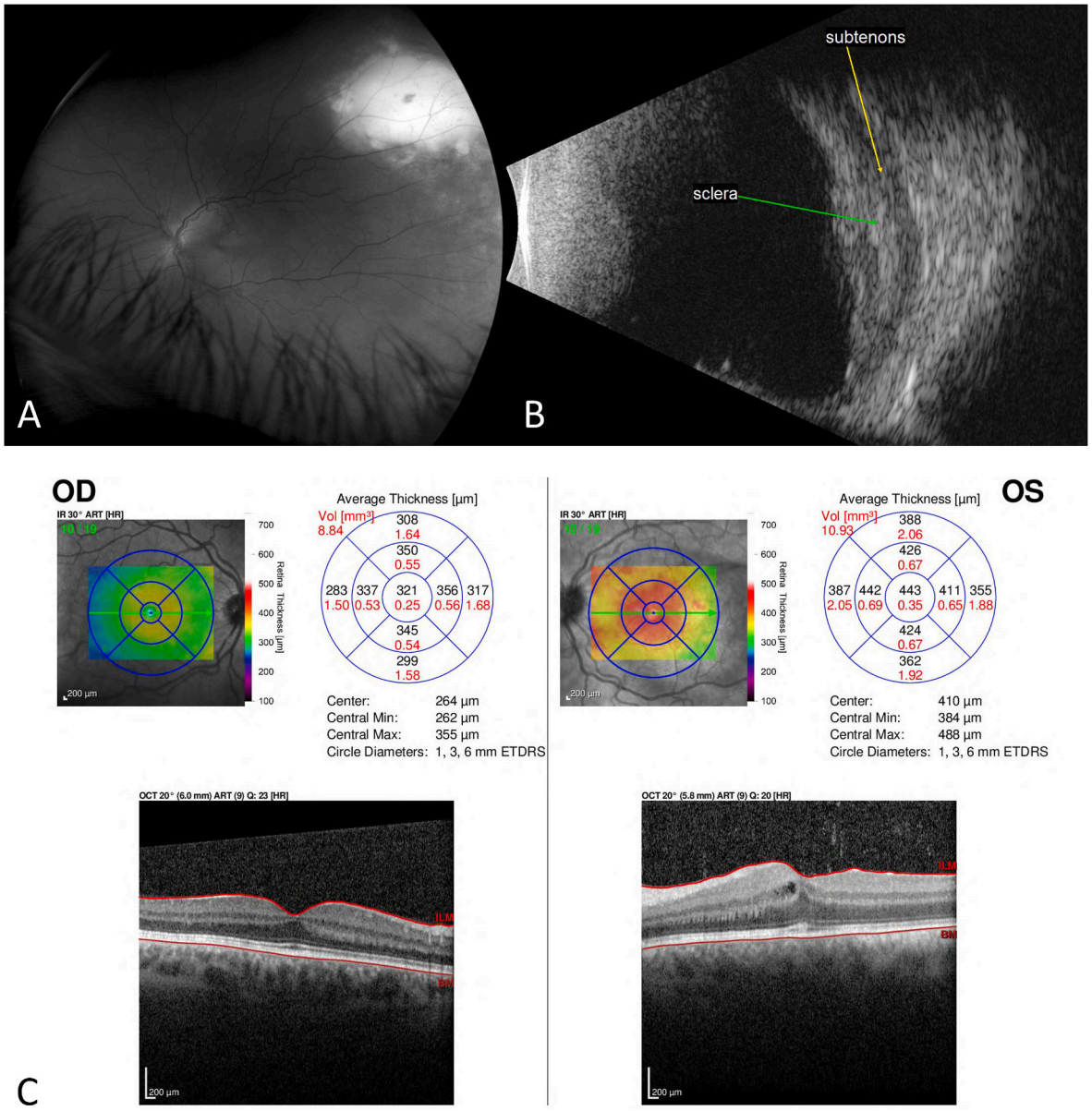

Fig. 2. Characterization of posterior extension of disease by fundus photography, ultrasonography, and optical coherence tomography (OCT) of the macula. Imaging acquired in June of 2020 demonstrates subretinal necrosis superotemporally on fundus photograph (A), hypoechoic fluid accumulation in sub-Tenon's space on ultrasound b-scan (B), and intraretinal fluid and macular edema on OCT of the macula (C).

evidence of systemic disease was uncovered, and chest x-ray was negative for nodules or signs of vasculitis or hilar lymphadenopathy. Rituximab was recommended for treatment of suspected idiopathic scleritis. For immediate control of inflammation, the dose of oral prednisone was increased to 120mg for a three-day trial, with the addition of 1g of valacyclovir three times per day as prophylaxis for potential VZV reactivation given known VZV seropositivity, and rituximab initiation was deferred with the intent to introduce more aggressive immunosuppression if high-dose prednisone should fail. Instead, the patient responded rapidly, with reduced scleral injection and subretinal fluid at follow-up; as a result, the patient was maintained on 120mg of prednisone for another week. At the end of the additional week, however, necrosis continued to progress, with new subconjunctival infiltrates noted. The infectious disease department was consulted, and because the clinical presentation was inconsistent with acute bacterial infection, *Nocardia* and other aerobic actinomycetes, fungi, and acid-fast bacilli were among the atypical infectious etiologies suspected. It was noted that, although the patient had received trimethoprim-sulfamethoxazole, prior dosage was insufficient to treat scleral *Nocardia* infection.[1] In addition, though the patient had previously been treated with voriconazole, he had only received a two-week course at a relatively low dose for his weight. Before starting a much higher dose of trimethoprim-sulfamethoxazole at two 800/160 mg pills every 8 hours, restarting voriconazole at a higher dose of 300 mg twice daily, and reducing the prednisone dose to 60mg daily, the subconjunctival infiltrate was swabbed and aspirated, with samples sent for bacterial culture and fungal stain (of note, samples were not sent for AFB stain and culture), a second round of broad-range PCR for bacteria, fungi, and AFB, and metagenomic RNA deep sequencing (MDS), an unbiased and sensitive test with the ability to detect bacterial, viral, fungal, and parasitical pathogens not on the differential. Furthermore, we surmised that even a negative result from MDS might be helpful as it will allow us to confidently escalate his immunosuppression. MDS was carried out at the Proctor Foundation at UCSF under a research protocol previously described by Doan et al.[2] Culture, fungal stain, and PCR were negative. Unfortunately, the sample for MDS was delivered to the wrong location initially. Eventually, the sample made its way to the Proctor Foundation but by this time, the sample had been at room temperature for days and thus the sample's integrity was questionable. The patient was maintained stably on this treatment regimen for three months, with a dose reduction of prednisone to 40mg five days after the initiation of trimethoprim-sulfamethoxazole and voriconazole and 5mg taper every two weeks thereafter, while new samples of purulent subconjunctival material were obtained and investigated by an additional round of MDS and a repeated third round of broad-range PCR for bacteria, fungi, and mycobacteria from the University of Washington Molecular Diagnosis Microbiology Section.

MDS detected *Bacillus coagulans* in the subconjunctival samples of the affected eye and the conjunctival sample of the unaffected contralateral eye (Supplemental Fig. 1). Over 1000 matched read pairs of *B. coagulans* per million sequencing reads present in the purulent tissue, an order of magnitude more abundant than the next most abundant organism detected in the affected eye (Supplemental Table 1). Repeat broad-range PCR was, once again, negative for bacteria, fungi, and mycobacteria. The patient was continued on the same dose of trimethoprim-sulfamethoxazole, which, coincidentally, covers most *Bacillus* species including *B. coagulans*,[3] along with topical steroids, and voriconazole was discontinued. A month and a half later, a new corneal infiltrate was observed in clinic. The patient was treated with topical polymyxin B/trimethoprim every 3 h and moxifloxacin four times per day, with resolution of the infiltrate two weeks later; polymyxin B/trimethoprim was discontinued five months later, and moxifloxacin was discontinued eight months later. Over the next year and a half following resolution of the corneal infiltrate, signs of infection improved with stable retinal atrophy and scleral ectasia (Fig. 1E), but no progression of necrosis, and trimethoprim-sulfamethoxazole was stopped after one year of high-dose treatment. The patient continued to be maintained on a once daily topical steroid dose and is 20/200 eccentrically in the left eye, partly attributable to cystoid macular edema and posterior capsular cataract.

2. Discussion

Bacillus coagulans is a gram positive, catalase positive, spore forming, facultative anaerobe found in soil, with clinical and food industry use as a probiotic for promotion of healthy gut microbiota.[4–6] Reports of *B. coagulans* causing opportunistic infections are rare,[7,8] and the addition of *B. coagulans* to foods and supplements is recognized as safe by the United States Food and Drug Administration (FDA) and European Union Food Safety Authority (EFSA), with inclusion on the Generally Recognized As Safe (GRAS) and Qualified Presumption of Safety (QPS) lists.[9] The *B. coagulans* GBI-30, 6086 strain was investigated with genomics-based assessments and phenotypic assays and found to contain no virulence factors, transferrable antibiotic resistance-related genes, or enzymes producing harmful metabolites.[10] While a literature review on April 22, 2024 utilizing PubMed and Google Scholar using the key words "bacillus coagulans scleritis" did not yield any prior reports of *B. coagulans* causing scleritis in the literature, a case report by Vanbijsterveld et al. describes corneal abscess formation with *B. coagulans* following perforating trauma inflicted by explosive debris. In the same article, conjunctival swabs from forty eyes from healthy horse stable workers were cultured on agar plates, with positive *Bacillus* cultures in seven eyes including one culture positive for *B. coagulans*.[11] It is possible that our patient was colonized with *B. coagulans* through woodworking or beekeeping activities, with possible subsequent introduction into the scleral tissue through incidental microtrauma in the left eye. Without trauma to the right eye, the low virulence *B. coagulans* may be unable to penetrate the deeper immune privileged tissues of the eye and thus not pathologic to the contralateral eye.

Broad-range PCR and MDS are molecular microbiological approaches with the potential to contribute to the detection of culture-negative, occult infections. Broad-range PCR utilizes DNA primers to target highly conserved regions of the bacterial 16s ribosomal RNA (rRNA) subunit. Subsequent PCR amplification, DNA sequencing, and comparison to known sequences in genomics databases allow for the identification of bacteria present in the sample, with species-level resolution dependent upon targeting and sequencing of variable regions in the 16S rRNA unit. MDS is an unbiased technique involving the extraction of RNA from a sample, reverse transcription of RNA, next-generation sequencing of the resultant cDNA, and bioinformatic analysis to identify all organisms and viruses with RNA present in the sample. The advantages of analyzing RNA rather than DNA in diagnostic samples include the ability to detect RNA viruses and information about expressed genes that may be implicated in disease pathogenesis. Limitations of MDS and broad-range PCR include cost, accessibility, and clinical interpretability. The identification of a pathogen using molecular diagnostic testing does not indicate causation and clinical correlation remains crucial. In the case of our patient, MDS yielded a clear signal and the dramatic clinical response to the proper antimicrobial medication and dose therapeutic to the target organism appeared to be consistent with the presumed diagnosis. Despite lacking known virulence factors, the persistence of *B. coagulans* on the ocular surface could have triggered an immune response insufficient to eradicate the offending organism, though damaging to infected tissue. It is important to consider an alternative diagnosis of fungal sclerokeratitis given the clinical picture of satellite corneal lesions, multiple negative cultures and PCR tests, worsening with steroids, failure to respond to conventional antimicrobial coverage, and interim improvement in infectious signs following combination voriconazole and trimethoprim/sulfamethoxazole treatment. In addition to other infectious etiologies, it is possible that resolution or remission of an unknown inflammatory cause could have coincided with changes in treatment and the eradication of

B. coagulans.

In summary, this was a challenging case of progressive necrotizing scleritis associated with *Bacillus*. The presumptive pathogen was initially identified with unbiased RNA-seq, indicating the potential usefulness of MDS to diagnose infectious scleritis.

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Patient consent

The subject has given his written informed consent to publish his case, including publication of images.

Authorship

All listed authors meet the International Committee of Medical Journal Editors criteria.

CRediT authorship contribution statement

Beau Sylvester: Writing – review & editing, Writing – original draft, Conceptualization. **Spencer D. Fuller:** Writing – review & editing, Writing – original draft, Conceptualization. **Laura Certain:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Akbar Shakoor:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Thuy Doan:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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