Rapid detection of bacille Calmette-Guérin-associated mycotic aortic aneurysm using novel cell-free DNA assay

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ABSTRACT

Intravesical instillation of bacille Calmette-Guérin (BCG), an attenuated strain of *Mycobacterium bovis*, is an adjuvant immunotherapy for bladder carcinoma. Typical complications include fever, malaise, and dysuria. However, more severe complications have been reported, including granulomatous pneumonitis, BCG sepsis, and vascular infections. We present a case of an infrarenal abdominal aortic aneurysm complicated by iliopsoas abscess 2 years after BCG treatment and discuss a novel diagnostic tool for mycobacterial strain identification. (J Vasc Surg Cases and Innovative Techniques 2019;5:143-8.)

Keywords: Mycotic aortic aneurysm; *Mycobacterium*; BCG; Next-generation sequencing; Cell-free DNA

Bacille Calmette-Guérin (BCG) is a live attenuated strain of *Mycobacterium bovis* used for tuberculosis vaccination. It is also widely used as an immunotherapeutic adjuvant in the treatment of malignant neoplasms, particularly high-risk bladder carcinoma through intravesical instillation.[1,2] Common symptoms of BCG application include malaise, fever, and dysuria. More severe but uncommon complications include hematuria, granulomatous pneumonitis, and sepsis.[3] BCG-related vascular incidents are extremely rare, and reported cases typically involve mycotic aneurysms that develop predominantly in the aorta.[4]

We report a case of mycobacterial infection of an aortic graft with iliopsoas abscess secondary to intravesical BCG therapy for bladder carcinoma. We also discuss the Karius next-generation sequencing (NGS) test, which allowed us to determine the infectious agent within days using a single plasma sample. The patient consented to publication of case details and images.

CASE REPORT

A 68-year-old man with a past medical history of bladder carcinoma treated by intravesical instillation of BCG in 2016, abdominal aortic aneurysm status post endovascular repair in July 2017, and hypertension presented with back pain and lower abdominal tenderness. He had previously developed BCG-osis that was treated with a 3-month course of isoniazid and rifampin

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in July 2016. Before this admission in February 2018, the patient had a 2-week history of sweats, fever, and back pain. Computed tomography (CT) of the lumbar spine revealed a fluid collection surrounding the aortic graft suggestive of an aortic leak. On admission to the emergency department, vital signs were normal, and a complete metabolic panel and complete blood count revealed no significant abnormalities. C-reactive protein level was elevated to 37.3 mg/L. CT angiography of the abdomen and pelvis demonstrated an aneurysmal sac with a 5.4- × 6.0-cm fluid collection developing along the lower abdominal aorta and extending into proximal common iliac vessels (Fig), suggestive of a mycotic aneurysm. Initial blood culture was negative after 5 days.

We decided on open surgical excision involving exploration with débridement of the aorta and involved iliac arteries. The aorta was resected to the level of the renal and iliac arteries, including the bilateral common iliac arteries and proximal right external iliac artery with ligation of the right internal iliac artery. In situ reconstruction was performed with a rifampin-soaked bifurcated Dacron graft. Complete omental wrap was completed with the reconstruction. Intraoperative swab and tissue culture specimens were obtained.

Given the clinical history and the surgical findings, there was concern for mycotic aneurysm and iliopsoas abscess secondary to BCG treatment. Plasma was sent for the Karius test, a novel cell-free DNA NGS test performed by the Karius Clinical Laboratory Improvement Amendments-certified, College of American Pathologists-accredited laboratory (Redwood City, Calif).[5,6] Cell-free DNA was extracted from plasma, NGS libraries were prepared, and sequencing was performed by a NextSeq 500 sequencer (Illumina, San Diego, Calif). Sequencing reads identified as human were removed, and remaining sequences were aligned to a curated pathogen database. *Mycobacterium tuberculosis* complex was rapidly identified, and on further analysis, the causative organism was determined to be *M. bovis* (Supplementary Fig). These findings were confirmed by acid-fast bacilli smear and culture of the infected tissue. For more information, see Supplementary Methods.

The patient was discharged on a 6-month regimen of rifampin, ethambutol, levofloxacin, and isoniazid, followed by
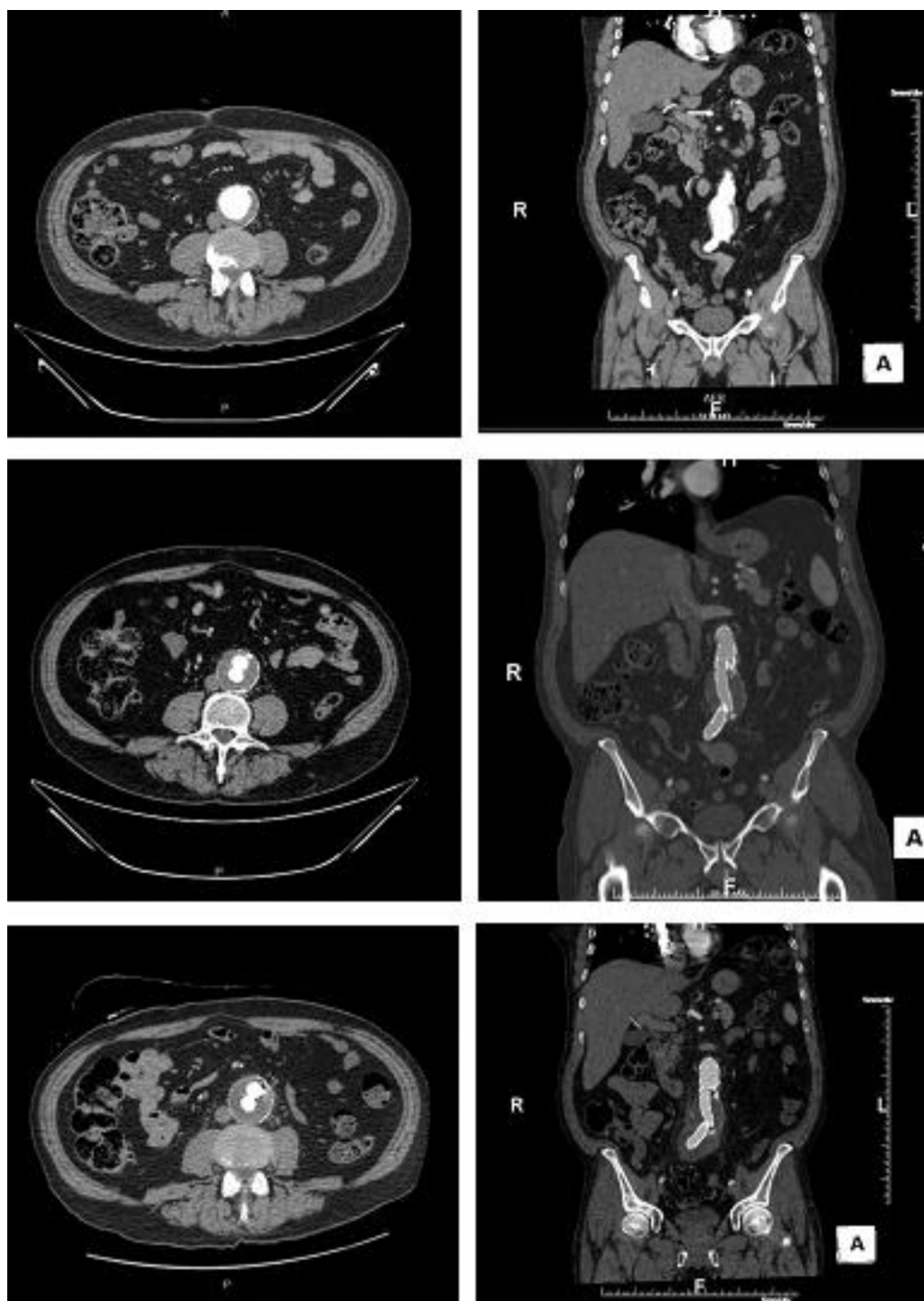
Fig. Axial and coronal computed tomography (CT) images demonstrating progression of aneurysm: *top*, before endovascular repair; *middle*, after endovascular repair; *bottom*, before in situ reconstruction.

dual therapy of rifampin and levofloxacin for a total of 18 months. In the postoperative phase, he visited the emergency department with idiopathic ascites that resolved spontaneously. In subsequent postoperative follow-up visits, he has returned to his clinical baseline. A CT scan taken 7 months postoperatively showed a widely patent reconstruction and no signs of recurrent infection.

DISCUSSION

Aneurysms infected by *M. bovis* due to BCG instillation are extremely rare, with roughly 29 reported cases in the literature.[7] The most frequent symptoms of BCG-induced aneurysm are abdominal or back pain, malaise, and fever.[8] Proposed mechanisms include spread of tuberculous bacilli by invasion of adventitia through the

vasa vasorum, direct arterial wall invasion, and direct extension from a bacterial focus in adjacent lymph nodes or a psoas abscess as in this case.[9-11]

BCG-induced aneurysms have typically been diagnosed by culture of acid-fast bacilli, clinical presentation, or pathologic examination at autopsy. The most definitive test is direct culture. However, mycobacterial cultures often require 6 to 8 weeks for growth. Furthermore, positive results require 10,000 organisms per gram of tissue[10]; if the sample load is not high enough, the culture could yield a false-negative result. Some cases use immunochromatographic assays that detect specific mycobacterial proteins, but these tests have significant false-negative rates compared with rapid polymerase chain reaction-based diagnostics.[12] In this case, NGS of cell-free DNA by the Karius test yielded definitive identification of the pathogen in only 2 days, thus expediting diagnosis and treatment. This test uses cell-free DNA NGS to search for a pathogenic match in Karius' proprietary reference database, that is, significant levels of a microorganism's presence.[13] The Karius test is a College of American Pathologists-accredited, Clinical Laboratory Improvement Amendments-certified NGS assay capable of identifying >1300 pathogens including bacteria, viruses, fungi, and other eukaryotes based on the cell-free DNA they liberate in the plasma. It is widely available as a send-out test (performed at the Karius facility in Redwood City, Calif); results in most cases are returned the next day from sample receipt. In a study of adults presenting to the emergency department with sepsis, it has a sensitivity of 92.9%; moreover, the assay was able to identify a probable cause of sepsis in 48.6% of patients compared with 18.1% identified by blood culture.[13] In this case, it enabled a rapid, noninvasive means of preoperative diagnosis.

Treatment of mycotic aortic aneurysms due to *M. bovis* may involve surgery, which includes débridement along with either in situ repair with graft or extra-anatomic bypass. Some graft cases reported soaking the graft in rifampin before placement, although there is no specific evidence to support this approach.[9] Endovascular repair can be considered if open surgery is determined to be too risky.[7] However, endovascular results in the literature have been mixed, with many cases ending in recurrence or death.[14,15] Sorelius et al[16] reviewed 11 cases of mycotic aneurysms that underwent endovascular repair. Of these, four patients died of aortoesophageal fistula, ischemia, and sepsis, whereas two had septic recurrences. Endovascular repair may be effective with a longer postoperative or preoperative antituberculosis medication regimen,[16,17] but there are currently few data to support this idea. Cases treated with concurrent antituberculosis medications have reported no recurrences. Few cases reported success with surgery alone, and medical management alone typically succeeded for smaller aneurysms.[8] In our patient, in situ repair with a postoperative antituberculosis regimen was considered the best approach as the patient had an iliopsoas abscess and there were no absolute contraindications to surgery. In general, surgical repair with follow-up medication seems to be the optimal approach, but there is no standard treatment algorithm currently.

CONCLUSIONS

A mycotic aneurysm is an extremely rare but dangerous complication of BCG therapy. In our case, the aneurysm was identified as a result of prompt evaluation for abdominal and back pain. Some patients described in the literature have fever and malaise, which highlights the importance of follow-up evaluation in the setting of BCG treatment. In this patient, successful treatment involved in situ repair with a rifampin-soaked graft, postoperative medical therapy, and rapid identification of the causative agent by a novel plasma-based NGS test.

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SUPPLEMENTARY METHODS

Plasma next-generation sequencing (NGS) for pathogen detection. Plasma NGS testing was performed at Karius, Inc (Redwood City, Calif), a reference laboratory with Clinical Laboratory Improvement Amendments certification and College of American Pathologists accreditation. This broad plasma NGS test was validated to detect >1000 microorganisms including bacteria, DNA viruses, and eukaryotic pathogens (yeasts, mold, and protozoa). For plasma NGS, whole blood (minimum of 4 mL) was collected in a plasma preparation tube by a peripheral blood draw. The sample was centrifuged at 1100 rcf for 10 minutes within 6 hours of collection to separate the plasma. The processed specimen was then shipped at ambient temperature to Karius, Inc.

Sequencing. The plasma sample was centrifuged at 16,000 rcf for 10 minutes at room temperature and spiked with a known concentration of synthetic DNA molecules for quality control purposes. Cell-free DNA was extracted from 0.5 mL of plasma by a magnetic bead-based method (Omega Bio-Tek, Norcross, Ga). DNA libraries for sequencing were constructed with a modified Ovation Ultralow System V2 library preparation kit (NuGEN, San Carlos, Calif). Negative controls (buffer only instead of plasma) and positive controls (healthy human plasma spiked with a known mixture of microbial DNA fragments) were processed alongside the sample. The sample was then multiplexed and sequenced on the NextSeq 500 (Illumina, San Diego, Calif).

Bioinformatics pipeline analysis. Primary sequencing output files were processed using bcl2fastq (v2.17.1.14) to generate the demultiplexed sequencing reads files. Reads were filtered on the basis of sequencing quality and trimmed on the basis of partial or full adapter sequence. The bowtie2 (version 2.2.4) method was used to align the remaining reads against human and synthetic molecule references. Sequencing reads exhibiting strong alignment against the human references or the synthetic molecule references were collected and filtered out from further analysis. Remaining reads were aligned against Karius' proprietary microorganism reference database using National Center for Biotechnology Information BLAST (version 2.2.30). Only taxa present at abundances above a predetermined statistical threshold were reported, and the entire process from DNA extraction through analysis was completed within 48 hours.

Simulations to differentiate *Mycobacterium bovis* from *Mycobacterium tuberculosis*. Of the 1883 reads, there were 5 that aligned only to *M. bovis* and 0 that aligned only to *M. tuberculosis* (Supplementary Fig).

However, the remaining reads aligned to both organisms and with high percentage identity (>80% of reads aligned with 100% identity to both species). Therefore, given the homology between *M. tuberculosis* and *M. bovis*, we used single-nucleotide variants as additional evidence confirming that the sample contains *M. bovis*.

For each read in the sample that aligned to *M. bovis* and *M. tuberculosis*, the number of mutations away from its best alignment to *M. bovis* and the number of mutations from its best alignment to *M. tuberculosis* were calculated. We found that of the 1878 reads that aligned to both species, 1652 aligned perfectly to both species, 31 aligned perfectly to *M. bovis* but had one mutation from any *M. tuberculosis* assembly, 2 aligned perfectly to a tuberculosis assembly and had one mutation from any *M. bovis* assembly, 2 had one mutation from *M. bovis* but two or three mutations from *M. tuberculosis*, 159 had one mutation from both species, and 32 had more than one mutation from both species. The 159 reads that were one mutation away from both species are likely to be the result of sequencing errors, and the reads that were more than one mutation away are equally distant from both species and are likely due to either sequencing error or potential pathogen cross-reactivity (ie, originating from a different microbe but mapping to *M. bovis* or *M. tuberculosis* because of homology).

To confirm that the distribution of reads with different amounts of variation from the reference matches that which is expected from an *M. bovis* assembly and not an *M. tuberculosis* assembly, we used simulated data. Namely, we created in silico samples from 11 *M. tuberculosis* and 6 *M. bovis* assemblies. From each assembly, we randomly sampled 1900 75-base pair fragments, without introducing de novo mutations. Then we ran each simulated sample through our analytical pipeline while blinding the algorithm to the references used to generate the simulated reads. Using blinding, we emulated real samples, as the strain in the sample is assumed to be diverged from the reference assembly that exists in our database. As these simulations do not include sequencing error, we do not expect as many reads that are one or two mutations away from both species. After running each simulation, we analyzed the alignments as we had done for the clinical sample and found that the data in our sample most closely matched the pattern of divergence found in *M. bovis* assemblies.
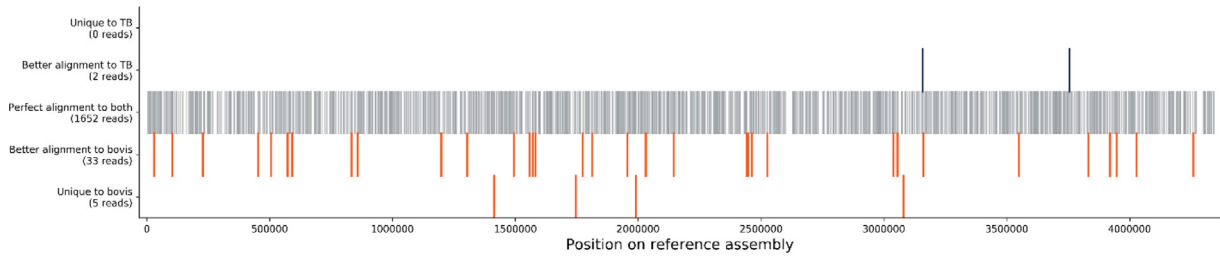
Supplementary Fig. Coverage across the *Mycobacterium bovis* genome. Each row contains the start site of reads that map to *M. bovis* (there were no reads that mapped only to *M. tuberculosis* [TB]), separated by reads that map uniquely to *M. bovis* (5), reads that map to both species but with higher percentage identity to *M. bovis* (33), and reads that map to both species with 100% identity (1652).