Pathology (April 2019) **51(3)**, pp. 316–343

CORRESPONDENCE

Diffuse inflammatory aneurysmal aortitis secondary to *Scedosporium apiospermum* complex in an immunocompetent individual

Sir,

Fungal aortitis complicated by aneurysm formation can rarely occur in immunocompetent patients. Occult infective aortitis should be carefully considered in cases of inflammatory aortitis, particularly if there is clinical progression and resistance to immunosuppressive therapy. We present a case of *Scedosporium apiospermum* complex aneurysmal aortitis, diagnosed post-mortem, in an immunocompetent man.

A 74-year-old previously well male presented with a 6-week history of lethargy, back and abdominal pain. A computed tomography (CT) angiogram revealed a diffuse abdominal aortitis with several small penetrating ulcers, and peri-aortic inflammatory change. Serum inflammatory markers were elevated [C-reactive protein (CRP) 120 mg/L, erythrocyte sedimentation rate (ESR) 89 mm/hr] in the absence of fever. A positron emission tomography-computed tomography (PET-CT) scan confirmed active inflammatory change in the aorta, extending from the diaphragmatic hiatus to the aortic bifurcation, in the absence of significant uptake elsewhere. Four sets of blood cultures were negative. Further microbiological and immunological screens (including treponemal, Q fever, hepatitis and HIV serologies plus antinuclear antibodies, anti-neutrophil cytoplasmic antibodies, serum immunoglobulins, IgG subclasses and cryoglobulins) did not reveal an aetiology for the aortitis.

In the absence of an evident infective cause in an immunocompetent individual (on the basis of history and normal routine investigations, including lymphocyte subsets), empiric prednisolone (60 mg daily) was commenced for an inflammatory aortitis. Initial improvement in symptoms, inflammatory markers and imaging allowed for patient discharge. However, difficulties with corticosteroid weaning necessitated re-admission and up-titration of prednisolone 2 weeks later. A further four sets of blood cultures were negative, and procalcitonin (PCT) remained normal, so a systemic infection was felt to be unlikely. Despite a pulse of methylprednisolone (cumulative corticosteroid dose: prednisolone 3115 mg, methyprednisolone 750 mg) and a single 1000 mg dose of intravenous cyclosphosphamide, the patient's abdominal symptoms worsened and inflammatory markers remained high (peak CRP 160 mg/L, ESR 53 mm/hr). A repeat PET-CT scan revealed progressive inflammatory changes and rapid aneurysmal expansion. The patient proceeded to surgical repair.

An endovascular approach was pursued to minimise perioperative morbidity. Given the inflammatory change and aneurysm progression, an aortic wall biopsy was planned post endovascular aneurysm exclusion (this could not be safely performed pre-repair). A four vessel (coeliac, superior mesenteric, right and left renal) thoraco-abdominal branched endovascular aortic aneurysm stent graft (Cook Medical, USA) was deployed in standard fashion. The patient recovered well in the immediate post-operative period. In view of the visceral stenting, dual anti-platelets (aspirin 100 mg daily and clopidogrel 75 mg daily) were commenced. The patient also received 40 mg subcutaneous enoxaparin for deep vein thrombosis prophylaxis as per routine care. Six days post-operatively, the patient had an unwitnessed collapse in hospital, and rapidly became comatose. A CT scan of his head revealed parenchymal and intraventricular haemorrhage, predominantly in the posterior fossa, with inferior tonsillar herniation. This was a non-survivable event.

A hospital post-mortem confirmed intracerebral haemorrhage as the immediate cause of death. Significantly, a large burden of invasive fungal elements were found within the aortic wall, with associated inflammation and necrosis (Fig. 1A,B). Microbiology revealed abundant growth of *Scedosporium apiospermum* complex on all plates [Brain Heart Infusion Agar plus chloramphenicol (BHIA+) and Sabouraud Dextrose Agar plus Chloramphenicol (SABC) at 35°C and 30°C] with scanty growth of *Candida albicans* on two of three plates (the significance of which was uncertain). The *Scedosporium* species was confirmed by DNA sequencing [polymerase chain reaction (PCR) of the internal transcribed spacer (ITS) region, sequenced and compared with the GenBank database using a BLASTn search]. There were no abnormalities in the cerebral vasculature, specifically, no evidence of aneurysmal disease; and no fungal elements were detected on periodic acid-Schiff diastase (PAS-D) staining. Given this and the pre-mortem clinical suspicion of an aortic vasculitis, brain tissue/cerebral vessels were not submitted for culture. Consequently, the intracerebral haemorrhage was surmised to have been spontaneous in the context of dual anti-platelet therapy and mild atherosclerotic disease. There was no fungal growth from lung tissue, which was also tested. Antibodies to interferon-γ (IFNγ) and granulocyte-macrophage colony-stimulating factor (GM-CSF) were negative in serum, when tested retrospectively.

The differential diagnosis for an inflammatory aortitis encompasses a broad range of infectious and non-infectious pathologies.[1] Diagnostic work-up requires a combined clinical, radiological, laboratory, and where possible, histopathological approach. Infectious aortitis is most commonly due to bacterial organisms; however, mycobacterium, syphilis, and rarely fungi, are also implicated.[1]

Scedosporium apiospermum complex represents one of the major human pathogenic *Scedosporium* species.[2] *Scedosporium* species are ubiquitous in soil and polluted water, and have a global distribution including North and South America, Australia and Europe.[2] Human infection usually results from inhalation of spores or through direct cutaneous inoculation.[2] In retrospect, our patient was an avid gardener and presumably acquired the infection in this way. He had no significant sinopulmonary disease and no clinically evident skin abrasions. *Scedosporium* species are well recognised to cause invasive infection in immunocompromised individuals, but are also increasingly reported in immunocompetent individuals, particularly following major trauma or near drowning.[3,4] With hindsight, the immunosuppressive therapy may have accelerated the rapid aneurysmal expansion in our patient.

Print ISSN 0031-3025/Online ISSN 1465-3931

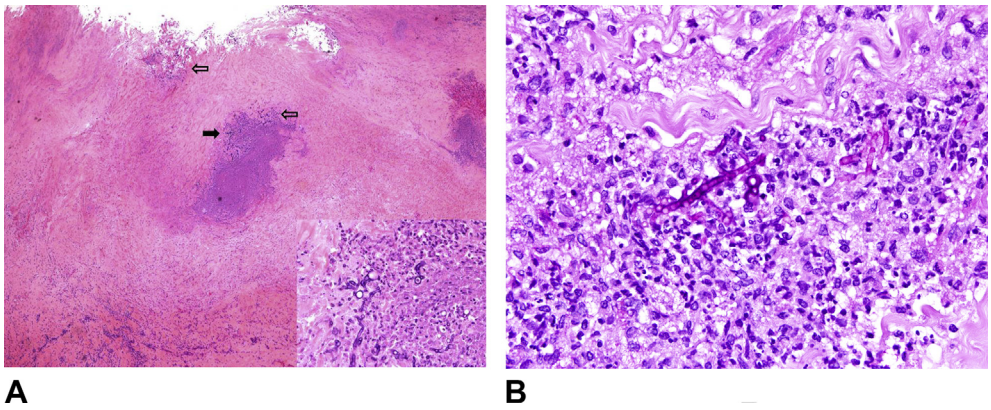

Fig. 1 (A) Aortic wall with intimal disruption and mural necrosis and sites of fungal infection (indicated by arrows); section marked by solid arrow is seen at higher power in insert and shows intermixed fungal elements and hyphae. (B) Aortic wall containing fungal elements and hyphae surrounded by necrotic debris (PAS-D). Methenamine silver (MAG) stain not shown. (Images courtesy of Dr Celia Green.)

Aneurysmal formation has been attributed to *Scedosporium* species infection in just 15 cases in the international literature.[4–7] Ong *et al*. outlined 12 published cases, of which seven involved intra-cerebral vessels, two involved the aorta, and three involved other sites.[4] Within this cohort six patients were immunosuppressed (organ transplantation, haematological malignancy or diabetes mellitus); four had survived near drownings; one had suffered a major trauma with soil exposure; and the final patient developed a basilar artery aneurysm in the context of underlying purulent sinusitis. Three further cases have been described:[5–7] one Canadian patient was immunosuppressed following a lung transplant for cystic fibrosis;[5] one developed aortitis due to contiguous spread from spondylodiscitis in the context of intravenous drug use;[6] and the third was an 85-year-old patient with underlying sinus disease who developed an intracranial aneurysm.[7]

Culture remains the mainstay of detection.[2,8,9] Our patient had multiple negative blood cultures in BD BACTEC Plus Aerobic/F and Anaerobic Lytic 10 (Becton Dickinson, USA) blood culture bottles. The yield might have been enhanced by the utilisation of Myco/F Lytic blood culture bottles and prolonged incubation (beyond the standard 5 days); however, fungal culture results may be limited by false negatives, slow growth and contamination from other microorganisms.[2] Histopathological visualisation is sensitive, but not very specific, and fungal species identification (now largely via DNA sequencing) is important because susceptibility to antifungal agents varies considerably.[9] Susceptibility testing was not performed in our case. *Candida albicans* is not uncommonly identified in the post-mortem setting and it was not possible to definitively exclude a *Candida albicans* infection or co-infection in our patient. However, this was considered unlikely given the absence of blood culture positivity, fungal appearance on microscopy and scant growth as compared to the abundant *Scedosporium apiospermum* complex.

Scedosporium species are inherently resistant to many antifungal agents and clinical responsiveness to treatment of systemic infection is often poor (of the cases described above, nine were treated with anti-fungal therapy and only three survived). Innate immune responses, in particular the number and function of polymorphoneuclear leukocytes (PMNs), are important in the containment and clearance of fungal pathogens. IFNγ and GM-CSF enhance PMN function; specifically, their addition has been shown to augment PMN activity against *Scedosporium* species *in vitro*.[10] Furthermore, there are case reports of GM-CSF +/− IFNγ being utilised as effective adjuncts to anti-fungal treatment regimes.[11,12]

We describe the case of an immunocompetent individual who developed an extensive, rapidly expanding aortic aneurysm, which was subsequently recognised to be a *Scedosporium* species infection on post-mortem analysis. Patients rarely provide a history suspicious of a *Scedosporium* species infection. Consequently, consideration should be given to such infections in patients presenting with an inflammatory aortitis in the absence of another identifiable cause, and when there is resistance to immunosuppressive therapy. Negative blood cultures do not exclude fungal infection and microbiology/infectious diseases input is essential in the management of these patients.

Acknowledgements: Dr Celia Green and Dr Marais Combrinck, Department of Anatomical Pathology, PathWest, Sir Charles Gairdner Hospital, Nedlands, Western Australia; Dr Gar-Hing Lee and Dr Jonathan Chambers, Department of Microbiology, PathWest, Sir Charles Gairdner Hospital, Nedlands, Western Australia.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

Elizabeth Mary Klinken[1], Brittany Rose Stevenson[1], Chi Ho Ricky Kwok[2], Joseph Allan Hockley[2], Michaela Lucas[1,3]

[1]Department of Immunology, PathWest, Sir Charles Gairdner Hospital, Nedlands, WA, Australia; [2]Department of Vascular and Endovascular Surgery, Sir Charles Gairdner Hospital, Nedlands, WA, Australia; [3]Medical School, University of Western Australia, Nedlands, WA, Australia

Contact Dr Michaela Lucas.
E-mail: michaela.lucas@health.wa.gov.au

1. Stone RJ, Bruneval P, Angelini A, *et al*. Consensus statement on surgical pathology of the aorta from the Society for cardiovascular pathology and association for European cardiovascular Pathology: I inflammatory diseases. *Cardiovasc Pathol* 2015; 24: 267–78.
2. Shinohara MM, George E. Scedosporium apiospermum: an emerging opportunistic pathogen that must be distinguished from Aspergillus and other hyalohyphomycetes. *J Cutan Pathol* 2009; 36 (Suppl): 39–41.

3. Slavin M, van Hal S, Sorrell TC, *et al*. Invasive infections due to filamentous fungi other than Aspergillus: epidemiology and determinants of mortality. *Clin Microbiol Infect* 2015; 21. 490.e1−10.
4. Ong A, Blyth CC, Bency R, *et al*. Fatal mycotic aneurysms due to *Scedosporium* and *Pseudallecheria* infection. *J Clin Microbiol* 2011; 49: 2067−71.
5. Thomson S, Alibhai K, Winkelaar G, *et al*. Case report of vertebral osteomyelitis and mycotic abdominal aortic aneurysm caused by *Scedosporium apiospermum* in a lung transplant patient with cystic fibrosis. *Transplant Proc* 2015; 47: 204−9.
6. Blasco-Lucas A, Reyes-Juarez JL, Nazarena M, *et al*. Aortic arch mycotic aneurysm due to *Scedosporium Apiospermum* reconstructed with homografts. *Ann Thorac Surg* 2015; 99: 2218−20.
7. Ogawa Y, Sato M, Tashiro M, *et al*. Rapid development of a mycotic aneurysm of the intracranial artery secondary to Scedosporium apiospermum sinusitis. *Med Mycol Case Rep* 2016; 14: 30−2.
8. Rodriguez-Tudela JL, Berenguer J, Guarro J, *et al*. Epidemiology and outcome of Scedosporium prolificans infection, a review of 162 cases. *Med Mycol* 2009; 47: 359−70.
9. Douglas AP, Chen SCA, Slavin MA. Emerging infections caused by non-Aspergillus filamentous fungi. *Clin Microbiol Infect* 2016; 22: 670−80.
10. Gil-Lamaignere C, Winn RM, Simitsopoulou M, *et al*. Interferon gamma and granulocyte-macrophage colony-stimulating factor augment the antifungal activity of human polymorphonuclear leukocytes against Scedosoporium spp.: comparison with Aspergillus spp. *Med Mycol* 2005; 43: 253−60.
11. Goldman C, Akiyama MJ, Torres J, *et al*. *Scedosporium apiospermum* infections and the role of combination antifungal therapy and GM-CSF: a case report and review of the literature. *Med Mycol Case Rep* 2016; 11: 40−3.
12. Abzug MJ, Walsh TJ. Interferon-gamma and colony-stimulating factors as adjuvant therapy for refractory fungal infections in children. *Pediatr Infect Dis J* 2004; 23: 769−73.

DOI: https://doi.org/10.1016/j.pathol.2018.10.021

Vancomycin dependent *Enterococcus*: an unusual mutant?

Sir,

Vancomycin resistant enterococci (VRE) are increasingly important healthcare associated pathogens. The incidence of infection and colonisation with these organisms has risen significantly both in Australia[1] and around the world. Interestingly, there have been rare reports describing isolates of *Enterococcus* that both require and are dependent on vancomycin for growth. Herein, we report two such cases, where we isolated this vancomycin-dependent *Enterococcus* (VDE) from rectal surveillance swabs. Both patients in question had received prolonged oral vancomycin therapy for the treatment of recurrent *Clostridioides difficile* infection.

The first patient was a 54-year-old woman with relapsed acute myeloid leukaemia on palliative chemotherapy. She had suffered from multiple recurrences of *C. difficile* colitis and was on a prolonged treatment course of oral vancomycin. She was known to be colonised with *vanB* VRE *faecium*, identified on a rectal swab, predating her vancomycin treatment course. A rectal surveillance swab several weeks after commencement of vancomycin, isolated three phenotypically different colony types on chrom ID VRE (bioMérieux, France). All three isolates were identified by the matrix assisted laser desorption ionisation time-of-flight (MALDI-TOF; Bruker Daltronics, USA) assay as *Enterococcus faecium*. Following standard laboratory procedures, the three colonies were subcultured onto horse blood agar (HBA) with a 30 μg vancomycin disc (Oxoid, UK) and incubated in 5% CO_2 at 35°C for 48 h. One of the *E. faecium* colonies only exhibited growth adjacent to the vancomycin disc (Fig. 1). Further investigation using a vancomycin Etest (bioMérieux) on Mueller-Hinton agar (MHA) confirmed this unusual vancomycin dependence with the density of growth of the organism increasing with higher concentrations of vancomycin on the Etest strip (Fig. 1). Susceptibility testing using the VITEK2 GP card (bioMérieux) was unsuccessful as the instrument terminated due to insufficient growth of the organism. Molecular testing using the Roche LightCycler VRE detection platform (Roche Diagnostics, Germany) detected a *vanB* gene within this VDE isolate.

The second patient was a 68-year-old man with a history of paroxysmal nocturnal haemoglobinuria and mesothelioma who was admitted to the intensive care unit (ICU) with septic shock due to pneumonia. He had a history of recurrent episodes of debilitating *C. difficile* colitis and had been treated on multiple occasions with short courses and subsequently a prolonged weaning regimen of oral vancomycin. Previous surveillance swabs had isolated both *vanB* and *vanA* VRE *faecium*. Similar to the first patient, ICU admission VRE rectal surveillance swabs identified two phenotypically different colonies of *E. faecium* which demonstrated growth most apparent around the vancomycin disc on subculture on HBA. One of the VDE isolates also demonstrated 'partial teicoplanin dependence' (i.e., no growth in the absence of teicoplanin, with growth in the presence of low concentrations of teicoplanin and inhibition at higher teicoplanin concentrations) (Fig. 2).

Since first described in 1993, there have been a very small number of VDE cases worldwide. The first case described by Fraimow *et al.* was a vancomycin-dependent *E. faecalis* isolated in the urine of a patient on a course of intravenous vancomycin for >100 days.[2] Subsequent case series have described the detection of vancomycin-dependence in *E. faecalis*, *E. faecium* and *E. avium* from a variety of clinical specimens.[2−10] These included both stool or rectal surveillance cultures and more invasive specimens such as blood, urine and intra-abdominal fluid. Dever *et al.* describe a similar case to ours, where they isolated a vancomycin-dependent *E. faecium* in stool of a patient 10 days after treatment with oral vancomycin for *C. difficile* infection.[3] Likewise, both our patients were also found to be carriers of VDE with no clinically significant disease.

The development of resistance to glycopeptides in enterococci is thought to be due to acquisition of genetic elements carrying *vanA* and *vanB* that result in the production of peptidoglycan cell wall precursors that confer resistance to vancomycin. Normal bacterial cell walls are composed of the dipeptidoglycan d-alanyl d-alanine ('d-ala d-ala'). When exposed to vancomycin, organisms with acquired resistance are able to synthesise an alternative d-alanyl d-lactate ('d-ala d-lac') cell wall precursor that binds to vancomycin with significantly lower affinity. In the absence of vancomycin, VRE still retain their ability to make the original d-ala d-ala. Conversely, due to potential amino acid substitutions or deletions in the *ddl* gene, which encodes d-ala d-ala ligases, vancomycin-dependent organisms are unable to produce d-ala d-ala, and are only able to produce d-ala d-lac cell wall precursors.[2,4,5,8] Therefore their growth is dependent on the continued presence of vancomycin. These VDE strains can spontaneously revert to vancomycin-independent growth either by restoration of d-