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Case Report

Adjunctive antibacterial agents as a salvage therapy in relapsed vascular pythiosis patients

Nuttapon Susaengrat[a,1], Pattama Torvorapanit[b,c,1], Rongpong Plongla[b,c], Nipat Chuleerarux[b], Kasama Manothummetha[b], Jarruprot Tuangsirisup[a], Navaporn Worasilchai[b], Ariya Chindamporn[b,c], Nitipong Permpalung[b,d,*]

[a] Khonkaen Hospital, Khonkaen, Thailand
[b] Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
[c] King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand
[d] Johns Hopkins University School of Medicine, Baltimore, MD, USA

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ABSTRACT

Human vascular pythiosis is a life-threatening condition caused by *Pythium insidiosum*. Patients with unresectable intra-abdominal artery involvement have not previously survived, despite being treated with antifungal agents and immunotherapy. We report two novel cases of intra-abdominal pythiosis in patients for whom surgery could not be performed, who were successfully treated with adjunctive antibacterial agents.

Introduction

Vascular pythiosis is a life-threatening disease with a mortality rate of 10–40% (Krajaejun et al., 2006; Permpalung et al., 2015; Worasilchai et al., 2018). Despite the absence of any established standard treatment, combination therapy with aggressive surgery, itraconazole, terbinafine, and immunotherapy has historically been recommended. According to previous studies, patients without complete surgical resection or patients with persistently elevated post-surgical (1,3)-β-D-glucan (BG) have not survived longer than 3 months, despite treatment with antifungal agents and immunotherapy (Worasilchai et al., 2018). The potential activity of antibacterial agents such as azithromycin, clarithromycin, and doxycycline against *Pythium insidiosum* has been demonstrated in in vitro studies (Loreto et al., 2011; Jesus et al., 2014) and animal models (Loreto et al., 2019; Jesus et al., 2015a), and more recently, successful therapy in ocular pythiosis has been reported (Bagga et al., 2018). Therefore, oral bacterial agents, in conjunction with antifungal agents, were administered in two patients with relapsed vascular pythiosis.

Case reports

Case 1

A 37-year-old, male Thai rice farmer with beta-thalassemia presented with a chronic necrotic wound and progressive claudication of the left leg for 7 months. Physical examination was notable for the absence of left popliteal and dorsalis pedis pulses, as well as circumferential dry gangrene from the left foot to the lower thigh, 40 cm in length. A computed tomography angiogram (CTA) showed total occlusion of the left superficial femoral artery. Serum BG was 489 pg/ml (Fungitell assay; Associates of Cape Cod, Inc., MA, USA) and testing for anti-*P. insidiosum* antibodies was positive (an in-house ELISA assay, King Chulalongkorn Memorial Hospital). The patient was presumptively diagnosed with vascular pythiosis. A left above-knee amputation with opened left external iliac artery ligation was performed. The pathological report showed hyphal elements from the left femoral artery with negative surgical margins at the left external iliac artery. *P. insidiosum* was isolated from the tissue culture. He was

* Corresponding author at: Carnegie Building Room 340, 601 N Wolfe Street, Baltimore, MD 21287, USA.
E-mail address: npermpa1@jhmi.edu (N. Permpalung).
[1] Nuttapon Susaengrat and Pattama Torvorapanit contributed equally to this publication.

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started on itraconazole and in-house immunotherapy, since terbinafine was not available. Immunotherapy was prepared according to the method described previously by Mendoza et al. (Mendoza et al., 2003); 1 ml of immunotherapy (2 mg/ml) was administered at time 0 and at 0.5, 1, 1.5, 3, and 6 months.

In vitro susceptibility testing by broth microdilution method based on the Clinical and Laboratory Standards Institute (CLSI, 2017) M38-A2 protocol was performed (Table 1). Briefly, antimicrobial solutions, prepared from commercial standard powder (Sigma-Aldrich, St. Louis, USA), were subjected to two-fold serial dilution in RPMI 1640 medium (Gibco, New York, USA) in the range of 0.125–32 μg/ml. In each reaction, an equal volume (100 μl) of antimicrobial solution and zoospores of *P. insidiosum* suspension (2×10^3 zoospores/ml) were co-incubated. All tests were performed in triplicate. The minimum inhibition concentration (MIC) of each agent was determined by 100% inhibition of mycelium growth after 48 h of incubation at 37 °C using visual observation. Combinations of two agents (itraconazole, doxycycline, clarithromycin, and azithromycin) were also tested for their activity against *P. insidiosum* according to the checkerboard technique (Argenta et al., 2008; Jesus et al., 2015b).

Twelve weeks post-diagnosis, the patient developed abdominal pain. CTA revealed a right internal iliac aneurysm with irregular aortic wall and peri-aortic soft tissue swelling. His serum BG was persistently elevated at 268 pg/ml. All data suggested ongoing *P. insidiosum* infection, and it was determined that his condition was inoperable. He was started on oral azithromycin 500 mg daily, increased itraconazole dosing from 200 mg twice daily to 200 mg three times daily, and was restarted on a course of immunotherapy with similar doses and intervals as above. His symptoms continued to improve and serum BG was first negative after 4 weeks of this salvage treatment (Figure 1).

Unfortunately, at 28 weeks post-diagnosis, the patient developed recurrent abdominal pain with a positive BG level >500 pg/ml. CTA showed a progression of the suprarenal aortic aneurysm. Doxycycline was added, and then the regimen was switched to a combination of doxycycline 100 mg twice daily and clarithromycin 500 mg twice daily because clarithromycin had a lower MIC than azithromycin and there was a synergistic effect between doxycycline and clarithromycin by checkerboard technique (Table 1). He became asymptomatic, and subsequent CTA showed stable aneurysms. Given the persistently elevated serum BG, the decision was made to continue itraconazole, doxycycline, and clarithromycin for suppression. The patient remained clinically well at 64 weeks after diagnosis.

Case 2

A 48-year-old, male Thai rice farmer with thalassemia major presented with progressive right leg claudication and chronic dry ulcer of the right foot, 10 cm in diameter, for 3 months. His right femoral pulse was absent and a CTA demonstrated complete occlusion of the right proximal common iliac artery. Serum BG was 446 pg/ml and testing for anti-*P. insidiosum* antibodies was positive (in-house ELISA assay). An above-knee amputation of the right leg with opened ligation of the right external iliac artery and the common iliac artery was performed. Hyphal elements morphologically compatible with *P. insidiosum* were seen on tissue pathology; however, the tissue culture was negative. Negative surgical margins at the common iliac artery were documented. He was started on oral itraconazole 200 mg twice daily and immunotherapy (similar preparation and dosing intervals as described above). A repeat CTA, obtained at 20 weeks post-diagnosis, showed a new infrarenal aortic aneurysm, and serum BG remained highly elevated. Hence, a left femoro-iliac bypass with opened ligation of the infrarenal aorta and left common iliac artery was performed in conjunction with an additional course of immunotherapy (similar preparation and dosing intervals) and adjunctive oral azithromycin 500 mg daily. However, the patient had ongoing abdominal pain with persistently elevated BG levels at 32 weeks post-diagnosis. Itraconazole was switched to voriconazole (for compassionate use), and doxycycline 100 mg twice daily was added given the concern for refractory infection. The voriconazole dosage was adjusted to achieve a trough level of 2–5.5

Table 1
In vitro susceptibility of the *Pythium insidiosum* isolate from case 1.

Antimicrobial agents	MIC (mg/l)	Antimicrobial agents	MICs (mg/l)
Tetracyclines		Quinolones	
Doxycycline	4	Ciprofloxacin	>32
Minocycline	2	Levofloxacin	>32
Tigecycline	2	Moxifloxacin	>32
Macrolides		Polymyxins	
Azithromycin	2	Colistin (polymyxin E)	>32
Clarithromycin	0.5	Polymyxin B	>32
Beta-lactams		Oxazolidinone	
Cefazolin	>32	Linezolid	8
Ceftriaxone	>32		
Ceftazidime	>32		
Carbapenems		Glycopeptides	
Meropenem	>32	Vancomycin	>32
Aminoglycosides		Antifungal agents	
Amikacin	>32	Amphotericin B	4
Gentamicin	>32	Fluconazole	2
Neomycin	>32	Itraconazole	4
Streptomycin	>32	Voriconazole	4
Tobramycin	>32	Anidulafungin	2
		Caspofungin	2
		Terbinafine	2

Drug combination	MICs (mg/l)	FICI[a]	Interpretation	Drug combination	MICs (mg/l)	FICI[a]	Interpretation
AZM/DOX	0.12/0.5	0.19	S	CLR/DOX	0.06/0.25	0.18	S
AZM/MIN	0.03/0.06	0.05	S	CLR/MIN	0.06/0.03	0.09	S

MICs, minimum inhibitory concentration; FICI, fractional inhibitory concentration index; AZM, azithromycin; DOX, doxycycline; MIN, minocycline; CLR, clarithromycin.
[a] The FICI was used to determine the drug interaction in the combination assay according to the following formula: FICI = (MIC of drug A in combination/MIC of drug A alone) + (MIC of drug B in combination/MIC of drug B alone). FICI interpretation: A = antagonism (FICI > 4); I: indifference (0.5 < FICI < 4); S:Lsynergism (FICI < 0.5).

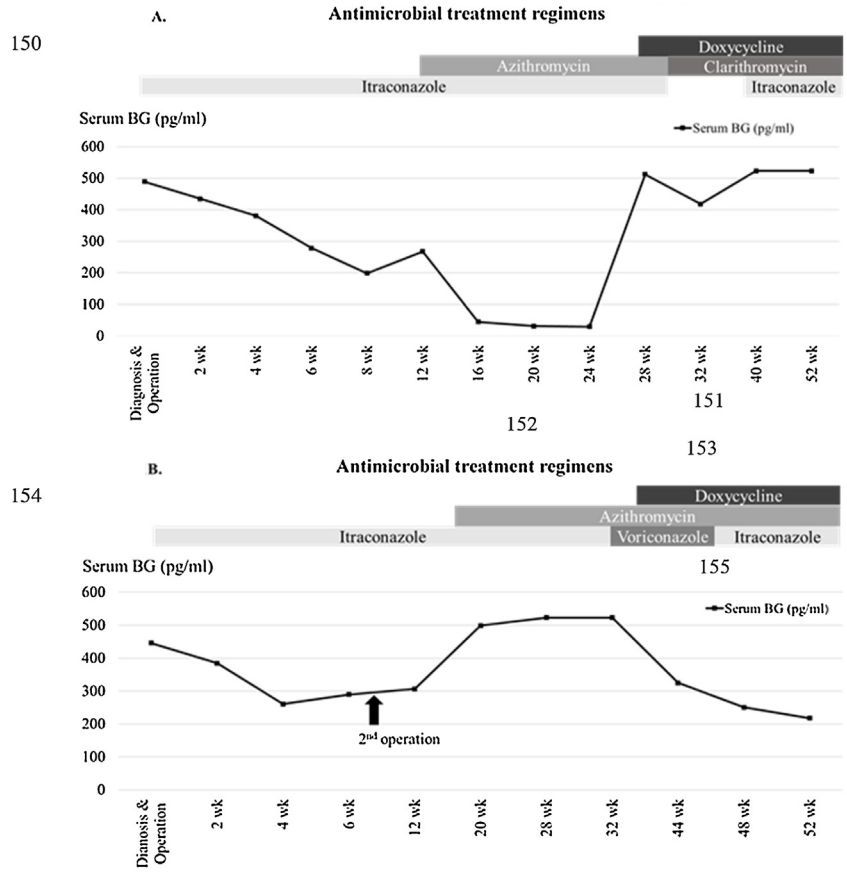

Figure 1. Serum β-D-glucan (BG) levels and antimicrobial agents across the treatment course: (A) case 1, (B) case 2.

mg/l. Subsequently, serum BG slowly declined, and voriconazole was transitioned back to itraconazole after 12 weeks of treatment (at 44 weeks post-diagnosis). Itraconazole, azithromycin, and doxycycline were continued for suppression. The patient remained clinically well at 64 weeks post-diagnosis.

Discussion

In Thailand, vascular pythiosis has been treated with aggressive surgery, itraconazole, terbinafine, and immunotherapy (Krajaejun et al., 2006; Permpalung et al., 2015; Worasilchai et al., 2018). The combination of itraconazole and terbinafine was implemented as a result of a single case report of a patient with *P. insidiosum* deep tissue infection (Shenep et al., 1998). However, in vitro susceptibility testing of Thai *P. insidiosum* isolates did not reveal a synergistic effect of this combination, and terbinafine is generally not available in rural areas of Thailand (Permpalung et al., 2015; Permpalung et al., 2019; Worasilchai et al., 2018).

In this report, voriconazole, azithromycin, doxycycline, and clarithromycin were administered as salvage therapy for relapsed vascular pythiosis. Although standard MIC interpretation for *P. insidiosum* has not been established, the *P. insidiosum* isolate from case 1 had favorable azithromycin, clarithromycin, and doxycycline MICs (Table 1), consistent with previous reports published in the literature (Loreto et al., 2011; Jesus et al., 2014; Jesus et al., 2015a). Azithromycin was the first agent that was considered when the patients had relapsed disease, since it has more clinical data in pythiosis and there is less drug–drug interaction with itraconazole compared to clarithromycin. Subsequently, doxycycline was added to the regimen because a synergistic effect was observed among the combinations of azithromycin/doxycycline and clarithromycin/doxycycline.

Voriconazole was substituted for itraconazole in case 2, because only 12.5% of Thai *P. insidiosum* strains have had a voriconazole MIC > 4 mg/l, whereas approximately 70–80% of isolates have had an itraconazole MIC > 1 mg/l (Permpalung et al., 2015; Permpalung et al., 2019; Worasilchai et al., 2018). Despite the lack of standard interpretation of the MICs, voriconazole is more likely to achieve therapeutic serum levels above the MIC than itraconazole. However, the cost of voriconazole in Thailand remains prohibitive.

Based on previous studies, vascular pythiosis patients with residual disease, particularly intra-abdominal artery involvement, do not survive beyond 12 weeks post diagnosis (Worasilchai et al., 2018). However, the two patients reported here were clinically well at 64 weeks post diagnosis. The combination of antibacterial and antifungal agents could potentially be a new strategy to combat this life-threatening disease and deserves more attention for further study. It is also noteworthy that both patients had negative surgical margins after the surgery but developed relapsed disease later. It has historically been believed that negative surgical margins indicate complete removal of the infected tissues (Permpalung et al., 2015; Sermsathanasawadi et al., 2016). This emphasizes the importance of utilizing serum BG to monitor disease activity after surgery (Worasilchai et al., 2018).

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Ethical approval

Ethical approval is not required for case reports.

Conflict of interest

All authors report no potential conflicts.

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