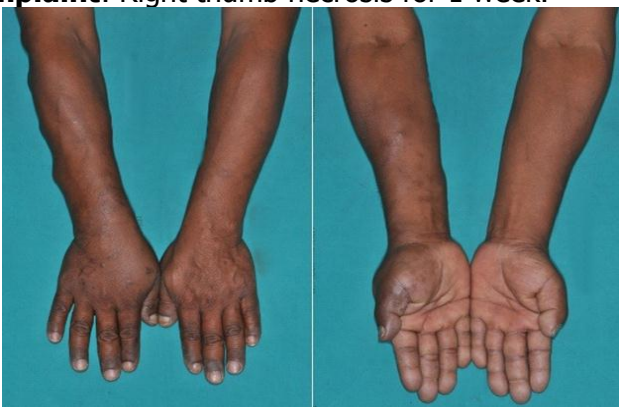
Case 3
A 54-year-old Thai male farmer from Petchaboon.
Chief complaint: Right thumb necrosis for 1 week.



Present illness:

The patient had intermittent pain on the right hand and arm when working 6 months ago. About 2 months later, he noticed slightly painful lumps with slowly enlarging on the right arm, but the lumps did not cause him trouble.

Until 5 weeks before the admission, he developed pain and swelling on the right thumb. A line of multiple subcutaneous nodules on the lateral side of the right arm, and swelling on the right thumb were found. Skin biopsy of the subcutaneous nodule was done.

1 week before the admission, the symptoms got worse and he noticed the darkening of the distal part of the right thumb. He went to the same hospital and another skin biopsy was performed. Giant cell arteritis with arterial occlusion was suspected, so he was referred to Ramathibodi hospital for the proper management under the primary care of vascular surgeons.

Past history: Unknown underlying diseases

Personal history:

- He is a farmer and raises pigs and dogs.
- He smokes a cigarette a day for 35 years, and drinks a can of beer a day for 30 years.

Physical examination:

V/S: BT 37°c, BP 130/70 mmHg, PR 90/min, RR 20/min
HEENT: pale conjunctiva, icteric sclera, no lymphadenopathy
Heart & Lung: normal
Abdomen: distension, soft, not tender, no hepatosplenomegaly, positive fluid thrill and shifting dullness tests
Extremities: gangrene at the distal part of the right thumb
bluish discoloration of the 2^{nd}, 4^{th}, 5^{th} right fingers
swelling on the right thenar area with fluctuation
right radial and digit pulses cannot be palpated

Laboratory investigation:

CBC: Hct 28.5%(NCNC), WBC 11,750/µl(N71%, L17%, M8%), plt 121,000/µl
BUN 28 mg/dl, Cr 0.7 mg/dl
LFT: ALP 130 U/L, AST 110 U/L, ALT 59 U/L, alb 19.5 g/L, TB 4 mg/dL, DB 3.1 mg/dL
PT 19.5 sec, PTT 32.3 sec, INR 1.59
Urinalysis: normal
ESR 23 mm/hr
Serum iron 40 µg/dL, TIBC 168 µg/dL, ferritin 920 ng/mL
Hb typing: Hb Constant Spring trait
Anti-HIV: negative, VDRL: non-reactive, TPHA: reactive
HBsAg: negative, anti-HBs: positive, anti-HCV: positive
ANA: negative, c-ANCA and p-ANCA; negative
Cryoglobulin <1%
Lupus anticoagulant,antithrombin III, $\beta_2$glycoprotein: negative
Protein C and protein S level: normal

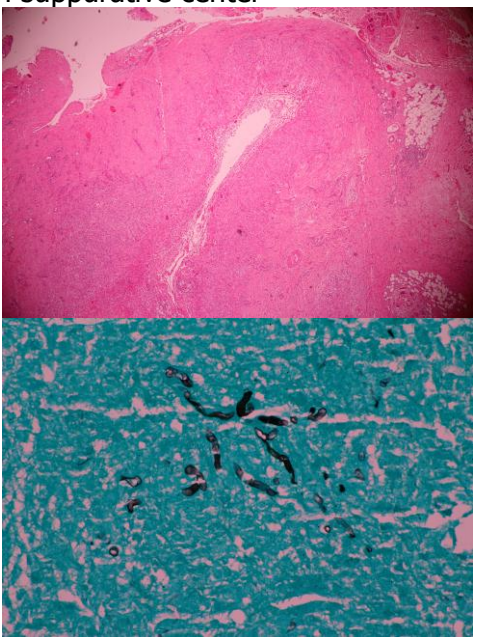
Imaging study

Right arm angiogram: total occlusion of radial and ulnar arteries
Chest x-rays: normal study
USG abdomen: liver cirrhosis with splenomegaly
and moderate amount of ascites
MRI aorta: normal study

Histopathology

(review on histopathology of subcutaneous nodule)

- Large well-circumscribed palisading infiltrate of histiocytes, multinucleated giant cells and central suppurative foci with neutrophils and cell debris
- Dense fibrous capsule around the inflammatory nodule with scattered small tuberculoid granuloma
- PAS and GMS stains showing broad branching hyphae embedded in suppurative center



Tissue from debridement
- GMS staining: broad, ribbon-like non-septate hyphae
- Bacterial and Fungal cultures: *Pythium insidiosum*

Serological assay: *P. insidiosum* antibody is weakly positive

Diagnosis
- Vascular pythiosis of the right arm
- HCV liver cirrhosis, Child C
- Hemoglobin Constant Spring trait

Treatment
- Above elbow amputation of the right arm
- Immunotherapy (pythium vaccine)
- Combination of oral SSKI, itraconazole and terbinafine

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Discussion

'Pythiosis' is a rare, non-transmissible infectious disease, caused by Pythium species, mainly occurring in tropical regions. Pythium species are oomycetes, fungus-like microorganisms living in the water.

Oomycetes belong to the kingdom Stramenopila, another living kingdom distinct from animal, plant and fungi kingdoms. Although oomycetes develop hyphae like fungus, they have many characteristic differences from fungus, particularly the difference in compositions of cell wall and cell membrane. Unlike fungus, cell membrane of oomycetes is lack of ergosterol resulting in poor response to conventional antifungal drugs, which are esgosterol-targeted agents.[1]

Among Pythium species, *Pythium insidiosum* had been considered to be the only pathogen causing disease in humans. Until recently a case of human pythiosis by other Pythium species was reported.[2]

Human pythiosis was first described in 1985 from Thailand[3], since then, several cases have been reported from the countries mainly in tropical regions. Interestingly, 80% of reported cases are from Thailand, which is documented to be an endemic area.

Four clinical presentations are described: (sub)cutaneous pythiosis, ocular pythiosis, vascular pythiosis and pythiosis of unusual sites. In Thailand, the majorities of cases are vascular form, and associated with agricultural occupation, male sex, and hematological underlying diseases, in particular thalassemia. Contrastly, pythiosis cases reported outside Thailand appear to occur in healthy hosts, and are related to water-associated leisure activities. In addition, almost entire patients presented with (sub)cutaneous and ocular forms.

Susceptible hosts are become infected after contacting the water contaminated with motile zoospores, infective units of *P. insidiosum*, through the injured tissue in an exposure area. In (sub)cutaneous, ocular and vascular pythiosis, direct contact with the pathogen to the broken skin is likely an initial step of the infection, whereas in unusual forms presenting with different clinical features, such as GI infection and rhinosinusitis, different routes of infection are suspected.

Clinically, patients with (sub)cutaneous pythiosis, the infection confined to the cutaneous or subcutaneous tissue, usually present with chronic swelling, a painful subcutaneous lump, a chronic ulcer or necrotizing cellulitis. The extremities, especially lower extremities, are the most common location, following by the periorbital area. Extensive debridement is needed, and the treatment with a single agent of conventional antifungal drugs is generally ineffective. However, the combination therapy of dual

antifungal agents has been reported to be successful in few cases[4], and the most commonly used regimen has been the combination of itraconazole and terbinafine. SSKI also shows some benefits in limited cases.

Unlike other forms of pythiosis, ocular pythiosis tends to occur in healthy hosts. It is usually presented with a corneal ulcer or keratitis, and frequently to have a history of eye trauma. Radical surgery is eventually preformed to remove the infected tissue as the treatment with topical or systemic antifungal agents is not successful. Although all patients are survived, about 80% of cases lost their eyes, and the rate of morbidity does not depend on the duration of symptoms before seeking the treatment.

Besides the disseminated infection, vascular pythiosis carries the worst prognosis of all. The infection is found to be confined to the arterial tissue and tends to affect medium-to-large sized arteries. The most common presentation is chronic arterial insufficiency syndrome, which manifests ranging from intermittent claudication to a gangrenous ulcer. Other presentations include an absent pulse, aortic and femoral aneurysm. The infection is far more common to occur on the lower extremities rather than upper extremities. Moreover, soft tissue swelling can be found with the arterial insuffiency syndrome, demonstrating at presentation, later or preceding. The removal of infected arteries is the principle of treatment. While many surgical procedures to remove the infected arteries can be performed, thromboembolectomy is strongly not recommended as the infection can be spread through the way of the clot being removed out. Importantly, normal good-looking arteries are not enough, microscopic demonstration of surgical-free margin is needed. Neither treatment with systemic antifungal agents nor SSKI is effective. Furthermore, immunotherapy has been used with some success as an adjunctive therapy, particularly in the cases that are not able to undergo the surgery. The most frequent causes of death in vascular pythiosis are ruptured aneurysm and

sepsis, and the overall mortality rate is about 40%. Only the cases that underwent amputation are cured.[5]

Regarding the relationship between hematological underlying diseases and pythiosis, thalassemia and paroxysmal nocturnal hemoglobinuria are the main underlying diseases in pythiosis patients. Both these conditions share the feature of chronic hemolysis in common, so the status of iron overload is hypothesized to be susceptible for *P. insidiosum* infection. Moreover, the gene encoding ferrochelatase, the enzyme needed in the process of adding ferrous ions to synthesize the heme, is discovered in *P. insidiosum*. The growth of *P. insidiosum* is also restricted in the low-iron environment. Thus, all these evidences support the idea that 'iron-hungry pythium' tends to affect the people with 'iron-overload' conditions.[6]

Human pythiosis is an emerging disease, whereas some authors consider it as a misdiagnosed or under-diagnosed disease as the way to be successful culture is challenging. *P. insidiosum* develops hyphae in the infected tissue with the feature of sparsely septate, ribbon-like broad hyphae, which cannot be distinguished from Zygometes. Demonstrating both a compatible colony and sporangia containing zoospores is considered to be positive culture for *P. insidiosum*. *P. insidiosum* is fast growing, within 24 to 48 hours, and grows well on varies media in the temperature of 34-36ºc, such as when it is incubated. Its colony is white to colorless with an irregular radiate pattern, and appears to be flatter than fungal colonies. As zoospores are only developed in the water cultures with the presence of plant materials, animal hairs or animal tissue to be chemical attractants, induction step is needed next to the suspected colony. When a compatible colony is suspected, another media plate with chemical attractants is inoculated. Next, the colony on the attracted materials is moved to the water media and seen under the microscope to identify the sporangia and zoospores.

Apart from culturing, *P. insidiosum* infection can be diagnosed in two other ways: detection of anti-*P. insidiosum* antibodies using serological assays, and detection of *P. insidiosum* DNA in the infected tissue by PCR and DNA sequencing. Serological testing is related to immune response of hosts. The antibodies may be low in the early infection or in the cases with poor immunity, and false negative results are so common in ocular pythiosis. Many testing methods are developed for serological assays, including immunodiffusion(ID), ELISA, immunochromatography(ICT), and hemagglutination(HA). A comparison study shows that ELISA and ICT methods provide 100% sensitivity and specificity in detecting *P.insidiosum* antibodies.[7] Additionally, DNA detection can be done in both fresh tissue and fixed tissue.

Human pythiosis is associated with high rates of morbidity and mortality. Early diagnosis is crucial for a successful treatment and improves the prognosis. Removal of infected tissue is the most important management in pythiosis patients. Conventional antifungal therapies, particularly single-agent therapies, are generally ineffective. As cell wall of *P.insidiosum* is consisted of cellulose and β-glucan, new antifungal agents which inhibit glucan synthesis, such as caspofungin, are expected to be effective. In vitro study, caspofungin shows some benefits[8, 9], which need to be further evaluated in the clinical study.

Our patient presented with chronic arterial insufficiency syndrome of the right hand and arm, starting from the intermittent claudication to developing digital gangrene and absent pulses, in association with several subcutaneous nodules on the same site of arterial insufficiency syndrome. He was a farmer and had not known about his underlying diseases. Vascular pythiosis was eventually diagnosed, and his underlying diseases of decompensated HCV liver cirrhosis and Hemoglobulin Constant Spring trait were discovered. He underwent above elbow amputation, and was treated with immunotherapy. The

combination of SSKI, terbinafine and itraconazole was also given. His condition was much better after the amputation. Unfortunately, he lost to follow-up after he was discharged from the hospital.

References

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