Mucormycosis & pythiosis – new insights

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Hot topics in Asian medical mycology

Mucormycosis & Pythiosis – new insight

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Outline

• Introduction
• Recent Taxonomy
• Trend of incidence
• Epidemiology between developed and developing countries
• Pathogenesis: role of CotH receptor agents – agents causing infection
• Treatment
Taxonomy of Fungi Causing Mucormycosis and Entomophthoramycosis (Zygomycesis) and Nomenclature of the Disease: Molecular Mycologic Perspectives

Kyung J. Kwon-Chung
Molecular Microbiology Section, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

Figure 1. Old (A) and a proposed new (B) classification schemes of the kingdom Fungi.
Figure 3. Phylogenetic tree provided by T. Y. James at the University of Michigan, Ann Arbor, which was based on unpublished results from the AFTOL (Assembling the Fungal Tree of Life) project [10]. Results are similar to those reported by James et al [5], but additional basal taxa are included.
# Mucormycosis VS Entomophthoromycosis

<table>
<thead>
<tr>
<th></th>
<th>Mucormycosis</th>
<th>Entomophthoromycosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Synonym</strong></td>
<td>Phycomycosis, Zygomycosis</td>
<td>--</td>
</tr>
<tr>
<td><strong>Infection</strong></td>
<td><em>Host, mostly</em> Immunocompromised: HM, HSCT, SOT, Diabetic ketoacidosis</td>
<td>Immunocompetent</td>
</tr>
<tr>
<td><strong>Clin. Manifestation</strong></td>
<td>Sinus, Pulmonary, Cutaneous, GI, Acute thrombosis</td>
<td>Chronic &amp; Subcutaneous</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>AmphotericinB, posaconazole</td>
<td>Itraconazole</td>
</tr>
<tr>
<td><strong>Route of infection</strong></td>
<td>Inhalation, ingestion, or through direct inoculation via abraded skin</td>
<td>Abrasion</td>
</tr>
<tr>
<td><strong>Pathogenic form</strong></td>
<td>Aseptate hyphae 3-25 um, thin wall, non dichotomous branching</td>
<td>Aseptate hyphae surround by thick eosinophilic sleeves</td>
</tr>
<tr>
<td><strong>Habitat</strong></td>
<td>Decaying organic substrate</td>
<td>Amphibians, GI of Lizard, decayed plant</td>
</tr>
<tr>
<td><strong>Distribution</strong></td>
<td>World wide</td>
<td>Tropical &amp; Subtropical</td>
</tr>
<tr>
<td><strong>Pathogen</strong></td>
<td>Subphylum Mucoromycotina: Order Mucorales: <em>Rhizopus</em>, <em>Mucor</em>, <em>Lichtheimia</em> (Absidia), etc.</td>
<td>Subphylum Entomophthoromycotina: Order Entomophthorales: <em>Basidiobolus</em>, <em>Conidiobolus</em></td>
</tr>
</tbody>
</table>
Subcutaneous *Saksenaea vasiformis* infection presenting as disfiguring facial plagues

- 51 yr –old labourer
- 2 mo. painless mass, Normal nasal cavity & nasopharynxgeal mucosa
- History:
  - 4 mo. before: shallow abrasion
  - Abt & debridement – mass enlarged
  - No systemic symptom

AmB (1.2 mg/kg/d) & Itra (600 mg/d) 40 d.

Fig. Diffuse granulomas in the reticular dermis & subcu. tissue w necrobiotic collagen. H&E, x200

Fig. Aseptate in multinucleated giant cells, PASx200

Diffuse erythematous infil. w skin thickening over forehead, both eyelids & nose

After AmB & Itra
Anamorph & Teleomorph Characters in Mucorales

VS Entomophthorales

Order Entomophthorales

Figure 4. Morphology of conidia and zygospores (scale bar, 20 µm). A, Reproduction of a conidium in Basidiobolus ranarum. A conidium discharged onto a Petri dish cover germinated and produced a conidiophore bearing a single conidium. B, Primary conidia of Conidiobolus incongruus germinated to produce long hyphae bearing subglobose conidia [38]. C, Secondary conidium formation by replication in Conidiobolus coronatus. D, Zygospore of Basidiobolus ranarum, with a characteristic beak, is produced by the fusion of 2 adjacent hyphal cells. E, Sporangial structure of Rhizopus species showing the sporangiophore (S), apophysis (A), columella (C), and sporangiospores (SP). F, Sporangium of Lichtheimia (Absidia) corymbifera. G, Electron microscopy of Zygospore (ZS) of Rhizopus species produced between 2 suspensors (S) originating from hyphae of 2 sexually compatible strains [courtesy of Dr S. L. Heggler]. Images in A and C–F are from Kwon-Chung and Bennett [33].
Annual number of published articles on mucormycosis since 1975 (SCOPUS, accessed July, 2013)
## Underlying Conditions in Mucormycosis patients in various studies

<table>
<thead>
<tr>
<th>Location</th>
<th>Period</th>
<th>Cases No.</th>
<th>DM</th>
<th>HM</th>
<th>SOM/SOT</th>
<th>DFO</th>
<th>HIV</th>
<th>AutoIm/Cortico</th>
<th>Trauma/no</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global</td>
<td>1885-2004</td>
<td>929</td>
<td>36.0</td>
<td>21.0</td>
<td>7.0</td>
<td>6.0</td>
<td>2.0</td>
<td>1.0</td>
<td>19.0</td>
<td>Roden et al. 2005</td>
</tr>
<tr>
<td>Italy</td>
<td>2004-2007</td>
<td>60</td>
<td>18</td>
<td>61.7</td>
<td>1.7</td>
<td>---</td>
<td>1.7</td>
<td>3.3</td>
<td>40.0</td>
<td>Pagano et al. 2009</td>
</tr>
<tr>
<td>Belgium</td>
<td>2000-2009</td>
<td>31</td>
<td>6.4</td>
<td>77.0</td>
<td>13.0</td>
<td>---</td>
<td>3.0</td>
<td>---</td>
<td>13.0</td>
<td>Saegeman et al. 2010</td>
</tr>
<tr>
<td>Europe</td>
<td>2005-2007</td>
<td>230(&gt;1-8%)</td>
<td>17.0</td>
<td>55.0</td>
<td>9.0</td>
<td>1.0</td>
<td>2.0</td>
<td>7.0</td>
<td>20.0</td>
<td>Skiada et al., 2011</td>
</tr>
<tr>
<td>India</td>
<td>2006-2007</td>
<td>178</td>
<td>73.6</td>
<td>1.1</td>
<td>0.6</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>19.1</td>
<td>Chakarbarti et al. 2006</td>
</tr>
<tr>
<td>Spain</td>
<td>2007-2015</td>
<td>19</td>
<td>0</td>
<td>52.6</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>52.6</td>
<td>Guinea et al. 2017</td>
</tr>
<tr>
<td>Mexico</td>
<td>1982-2016</td>
<td>418</td>
<td>72</td>
<td>18(5/770DM)</td>
<td>93 (HM)</td>
<td>52.6</td>
<td>9.3</td>
<td>---</td>
<td>---</td>
<td>Corzo-Leon et al. 2017</td>
</tr>
</tbody>
</table>
## Table 1. Demographic and clinical characteristics: differences between populations with diabetes and malignancy.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Diabetes</th>
<th>Malignancy*</th>
<th>Total population**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 302/418 (72%)</td>
<td>[77/418 (18%) Hematological 72/77 (93%)]</td>
<td>N = 418 (%)</td>
</tr>
<tr>
<td>Age, years (median, IIR)</td>
<td>50 (38–60)</td>
<td>26 (18–43)</td>
<td>42 (0–80)</td>
</tr>
<tr>
<td>Sex (Male)</td>
<td>96/187 (51)</td>
<td>11/27 (41)</td>
<td>22.5 (54)</td>
</tr>
<tr>
<td>Mortality rate</td>
<td>101/192 (53)</td>
<td>11/25 (44)</td>
<td>127/246 (52)</td>
</tr>
<tr>
<td>N = 246 (%)a</td>
<td></td>
<td>11/23 (48)</td>
<td></td>
</tr>
<tr>
<td>Type of infectionb</td>
<td>N = 181 (%)</td>
<td>N = 31 (%)</td>
<td>N = 418 (%)</td>
</tr>
<tr>
<td>Sinus (overall)</td>
<td>159 (88)</td>
<td>11 (35)</td>
<td>315 (75)</td>
</tr>
<tr>
<td>Palatine infection</td>
<td>39/159 (24)</td>
<td>2/11 (18)</td>
<td>45/315 (14)</td>
</tr>
<tr>
<td>Sinocerebral/cerebral</td>
<td>85/159 (53)</td>
<td>3/11 (27)</td>
<td>210/315 (66)</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>8 (4)</td>
<td>11 (35)</td>
<td>26 (6)</td>
</tr>
<tr>
<td>Cutaneous</td>
<td>9 (5)</td>
<td>2 (6)</td>
<td>28 (6.5)</td>
</tr>
<tr>
<td>Disseminated+</td>
<td>2 (1)</td>
<td>4 (13)</td>
<td>23 (5.5)</td>
</tr>
<tr>
<td>Unspecified***</td>
<td>1 (0.5)</td>
<td>0</td>
<td>19 (4.5)</td>
</tr>
<tr>
<td>Abdominal++</td>
<td>1 (0.5)</td>
<td>3 (10)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>Cerebral &amp;</td>
<td>1 (0.5)</td>
<td>0</td>
<td>2 (0.5)</td>
</tr>
</tbody>
</table>

IIR, Interquartile interval range.

* Mortality rate estimated with the available information of 246 individuals.

b Type of infection was estimated depending on the number of individuals with available information: 181 with diabetes mellitus and 31 with malignancy. Although an overall estimation was possible for some variables among the 418 cases, in some reports only the site of infection was reported without mention of the underlying disease.

* Five individuals with malignancy had diabetes mellitus as comorbidity

** Includes cases without underlying condition or without diabetes and without malignancy (N = 39, 9.3%). This group had mortality rate in 52% (15/29). Ten individuals with no underlying condition (19/39, 49%) did not have information available and eight were reported as previously healthy. Prior trauma was present in eight of 39 (20%), of these five individuals had no other associated condition. Five individuals had autoimmune disease (5/39, 13%), three with HIV (human immunodeficiency virus) infection (3/39, 8%), other prior conditions were drug toxicity (2/39, 5%), post-surgery (2/39, 5%). Drug toxicities consisted in agranulocytosis or neutropenia due to drugs. Numbers reported in populations with diabetes and malignancy vary due to the availability of the information.

*** Unspecified: refers to information of the site/type of infection was unavailable

+2 or more sites affected.

++ Only abdominal infection. These were gastric, renal, hepatic, splenic, and intestinal presentation.

& Only due to trauma and postsurgical process, no sinus infection.
Literature review in Mexico 1982-2015

Performance of Diagnostic Testing

<table>
<thead>
<tr>
<th>Diag tool</th>
<th>nonspecialized center</th>
<th>specialized center</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos. Direct smear/cytology</td>
<td>73/76 (95%)</td>
<td>158/158 (100%)</td>
<td>231/234 (98%)</td>
</tr>
<tr>
<td>Pos. Culture</td>
<td>120/211 (57%)</td>
<td>142/158 (90%)</td>
<td>262/369 (71%)</td>
</tr>
</tbody>
</table>

- 158/369 (41%) cases were reported by a specialized center,
- 211 cases by non specialized center

Figure 3. Clinical presentations of mucormycosis and entomophthoromycosis in Mexico. Pie charts showing the clinical presentations by underlying condition using proportions

Corzo-Leon DE., et al. Med Mycol.2017, 0, 16
Etiologic agents of Zygomycosis (Mucormycosis)

The data shown are from studies by Chakrabarti et al., Mohapatra (India), Skiada (Europe), Lanterniaer et al. (France), and Pagano et al. (Italy)
Laboratory Diagnosis

• Collect specimens: pus, bloody tissue, debris
• Transportation: Not on ice
• Processing:
  o Cut into small pieces in sterile plate
  o Direct examination: KOH preparation, KOH calcofluor stain - REPORT
  o Other common stain in Microbiol lab.: Gram stain, AFB stain - REPORT
  o Histopathology: tissue reaction (H&E, PAS); shape (GMS)
  o Culture: SDA, SDA+abt, SDB, Blood agar 2-3 days, 30&35C
  o Identification: classical (colony & sporulation)/ PCR/ MALDI-TOF
• Serology: Negative GM & BG
Sizes
- 3-11um
- >10 um

- IR: Steriod: impair ϕ migration, ingestion. Phagolysosome fusion
- Hemat. Malignacies: Neutropenia: impair chemotaxis & diminish fungicidal mechanisms
- DKA: weak neutrophil, low pH, higher glucose level, free iron
- Mucorales is able to extract iron from desferrioxamine.
Isavuconazole:

- Broad spectrum 2\textsuperscript{nd} gen. triazole
- Inhibit CYP enzyme laosterol 14-alpha-demethylase (CYP51) – blocking synthesis of ergosterol
- Metabolites via CYP3A4 & CYP3A5 which may alter the plasma concentrations
- a moderate inhibitor of CYP3A4, and a mild inhibitor of P-glycoprotein (P-gp), and organic cation transporter 2 (OCT2)
Isavuconazole: good \textit{in vitro} activity against \textit{Rhizopus} & \textit{Rhizomucor}

- However, its activity is limited for other Mucolares especially \textit{Mucor circinelloides}
- Overall, MIC of Mucorales higher than \textit{Aspergillus} group

\textit{Shirley et al. 2016}
• A 57 year-old Thai man with β-thalassemia disease was admitted (day 0) due to low grade fever and swelling Rt arm & forearm (deep and superficial soft tissue) with rapid progression lesions for ~3 months.

• History of planting in a swampy area before lesion occurred.

• **KOH & PAS & GMS**: Non-septate hyphae

What?? and How should we do next ??

• **CTA**: Rt upper extremity: Occluded distal Rt. Brachial artery above the bifurcation.

• **Pythium insidiosum antibody** by ELISA: Pos

• **Dx**: Pythiosis

• **Tx**: Debridement + oral terbinafine & itraconazole + Immunotherapy

After 1st debridement (+6 days)  
After 2nd debridement (+15 days)  
Before discharge (+24 days)
Human pythiosis: *Pythium insidiosum* (fungus-like organism)


**Macroscopic:** Mycelium like fungi, rapid growing, submerged, white to colorless colony, 35C, 24h

**Microscopic:** Sparsely rare septate hyphae
Recent Taxonomy

Kingdom **Straminipila**
Class **Oomycetes**
Order **Pythiales**
Family **Pythiaceae**
Genus **Pythium**
Species **insidiosum**

**Phylogenetic tree of** *P. insidiosum* **based on**
- **ITS** region *(Schurko et al. Mycol Res, 2003)*
- **IGS** region *(Frank N et al. Mycologia, 2003)*
- **Cox 2** gene *(Kammarnjessadakul et al., Med Mycol, 2011)*
- **Exo-1,3-beta glucanase** *(Ribeiro TC et al. Infection, Genetics and Evolution, 2017)*
Recent Taxonomy & Epidemiology

Kingdom **Straminipila**  
Class **Oomycetes**  
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Phylogenetic tree of *P. insidiosum* based on:

- **ITS region**  
- **IGS region**  
  (Frank N et al. Mycologia, 2003)
- **Cox 2 gene**  
  (Kammarnjessadakul et al., Med Mycol, 2011)
- **Exo-1,3-beta glucanase**  
  (Ribeiro TC et al. Infection, Genetics and Evolution, 2017)
Natural habitat

- Tropical & Sub tropical regions
- Moist soil / stagnant water *i.e.* rice field
- Also has been isolated from irrigation water and reservoir in northern part of Thailand *(Supabandhu, Med Mycol, 2008)*

Environmental form
- Hyphae&zoospore form

Infected Stage
- Zoospore form

Infected Host
- Hyphae form
Clinical manifestations

Vascular pythiosis

Sudjaritruk T, Inf Dis, 2011

Cerebral pythiosis (cerebral hemisphere)

Narkwiboonwong T, J Infect Dis Microb Agents, 2011

Keratitis pythiosis

Hong H et al. Am J Case rep, 2016

(sub) Cutaneous form

Bosco S et al, EID 2005

Orbital and Facial Infection

Kirzhner M, J Ped Inf Dis, 2014
Human Pythiosis

- Human pythiosis was first described in Thailand, in 1987.
- So far Thailand has been ranked as the highest incidence of human pythiosis in the world.

- Based on the immunotherapy requested from Mycology unit, KCMH, the increasing trend was presented.
# Human Pythiosis

**Pubmed Search** (search on 29th Nov 2017)

<table>
<thead>
<tr>
<th>Keywords</th>
<th>Before 2006</th>
<th>Number of publications</th>
<th>2006-present (12 years approx.)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human pythiosis</td>
<td>36</td>
<td>87</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td><em>Pythium</em> in human</td>
<td>75</td>
<td>120</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>Human pythiosis case report</td>
<td>10</td>
<td>22</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Human vascular pythiosis</td>
<td>6</td>
<td>14</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Human keratitis pythiosis</td>
<td>6</td>
<td>10</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Human ocular pythiosis</td>
<td>2</td>
<td>12</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Thai human pythiosis</td>
<td>3</td>
<td>11</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>
Human Pythiosis

- Not only in Thailand, some human pythiosis cases were also reported from other countries around the world except Europe.

<table>
<thead>
<tr>
<th>Regions</th>
<th>Country</th>
<th>Pythiosis Cases</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td>Malaysia (Kuala Lumpur)</td>
<td><em>Pythium</em> keratitis disposable contact lens wear, and swimming in the Kelang River</td>
<td>Badenoch et al., 2001</td>
</tr>
<tr>
<td></td>
<td>India (Telangana)</td>
<td>13 <em>Pythium</em> keratitis cases during 2010-2012</td>
<td>Sharma S et al. 2015</td>
</tr>
<tr>
<td></td>
<td>China (Hainan)</td>
<td><em>Pythium</em> keratitis in a boy who was scraped by twigs while climbing a tree</td>
<td>Hong H et al. 2016</td>
</tr>
<tr>
<td></td>
<td>Israel</td>
<td>Contact lens-related <em>Pythium</em> keratitis</td>
<td>Tanhehco TY et al. 2011</td>
</tr>
<tr>
<td></td>
<td>Australia</td>
<td><strong>New Zealand (Auckland)</strong></td>
<td>Keratitis pythiosis in a man who played ball in hot pool</td>
</tr>
<tr>
<td></td>
<td>Australia (Darwin)</td>
<td>Keratitis pythiosis in a child who swam in public and backyard swimming pools</td>
<td>Badenoch PR et al. 2009</td>
</tr>
<tr>
<td></td>
<td>Australia</td>
<td>2 cutaneous pythiosis cases with the history of exposure to either swampy water or horses.</td>
<td>Triscott JA et al. 1993</td>
</tr>
<tr>
<td></td>
<td>America</td>
<td><strong>Brazil</strong></td>
<td>Subcutaneous pythiosis in a police after spend his vacation with water-associated leisure activities</td>
</tr>
<tr>
<td></td>
<td>USA (Iowa, Florida, Texas)</td>
<td>5 Orbital pythiosis in young children</td>
<td>Mendoza L et al. 2004</td>
</tr>
</tbody>
</table>
### Symptoms & Diagnosis and Treatment

<table>
<thead>
<tr>
<th>Vascular form</th>
<th>Ocular form</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Symptoms</strong></td>
<td></td>
</tr>
<tr>
<td>• Presents as granulomatous cutaneous and subcutaneous lesions</td>
<td>• pain and redness</td>
</tr>
<tr>
<td>• Intermittent claudication</td>
<td>• Less vision</td>
</tr>
<tr>
<td>• Arterial obstruction / aneurysm resulting ischemia / gangrene</td>
<td>• Ulcerative keratitis which may progress to endophthalmitis</td>
</tr>
<tr>
<td>• Other signs of arterial insufficiency</td>
<td></td>
</tr>
<tr>
<td><strong>Underlying Dis. &amp; History</strong></td>
<td></td>
</tr>
<tr>
<td>• Underlying hemoglobinopathy (thalassemia, PNH)</td>
<td>• No</td>
</tr>
<tr>
<td>• Agriculture-related occupations ie. farmer or history of water exposure</td>
<td>• Water spilled</td>
</tr>
</tbody>
</table>
## Symptoms & Diagnosis and Treatment

<table>
<thead>
<tr>
<th>Vascular form</th>
<th>Ocular form</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnosis</strong></td>
<td><strong>Confocal microscope</strong></td>
</tr>
<tr>
<td>• Arterial occlusion by CT angiogram</td>
<td>Beaded string-like with mean branching angles at 78.6 degrees. The diameter of the hyphae varied from 1.5 to 7.5 mm. (95% sens.)</td>
</tr>
</tbody>
</table>

### Positive Arterial occlusion by CT angiogram

- **Fusarium spp.**
- **Aspergillus spp.**
- **Candida albicans**
- **Penicillium spp.**

- **P. insidiosum**
  - Beaded string-like hyperreflective branching structures
  - Thin hyperreflective long lines
### Symptoms & Diagnosis and Treatment

<table>
<thead>
<tr>
<th>Vascular form</th>
<th>Ocular form</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnosis</strong></td>
<td></td>
</tr>
<tr>
<td>• Arterial occlusion by angiogram</td>
<td>• Positive for <em>P. insidiosum</em> isolation &amp; zoospore production</td>
</tr>
<tr>
<td>• <em>P. insidiosum</em> specific antibody (ID / ELISA / WB / Lateral flow / HA)</td>
<td>• Molecular approach for identification both culture and specimens</td>
</tr>
<tr>
<td>• <em>P. insidiosum</em> isolation &amp; zoospore production</td>
<td>• Molecular approach for identification both culture and specimens</td>
</tr>
<tr>
<td>• Molecular approach for identification both culture and specimens</td>
<td>• Histopathology</td>
</tr>
<tr>
<td>• Histopathology</td>
<td>• Histopathology</td>
</tr>
</tbody>
</table>

PAS (5 mins); *Pythium* spp. >> 1+ to 2+  
PAS (5 mins); Other fungal infection >> 4+

Cellulose: a weaker reaction than chitin and pectin, so it needs a longer exposure time to PAS for complete oxidation to aldehydes, which then reacts with Schiff reagent to give a magenta color (purplish-red).
IKI-H$_2$SO$_4$ staining

- Corneal tissue
  - Pythium spp.
  - Other fungi

- Destained tissue of
  - KOH
  - Gram stain

- Pure isolate

Mittal et al., Basic Invest, 2017
# Symptoms & Diagnosis and Treatment

## Vascular form

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ocular form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AK amputation or debridement</td>
<td>Corneal grafting or enucleation</td>
</tr>
<tr>
<td>Antifungal agent: Itraconazole + Terbinafine</td>
<td>Amphotericin B /terbinafine/Azoles</td>
</tr>
<tr>
<td>Immunotherapy by PIA</td>
<td>Immunotherapy by PIA</td>
</tr>
</tbody>
</table>

## Ocular form
Retrospective study in 18 pythiosis cases in KCMH from 2003 to 2013

Vascular pythiosis; n=9

- 44% of vascular cases died
- Definitive surgery with adequate surgical margins

Ocular pythiosis; n=9

- 55% of ocular pythiosis underwent enucleation.
- Age might be one prognostic factor, significantly younger of non-enucleated cases than those who underwent enucleation.
- Higher non-enucleated cases (45%) was found in our center than others (12-21%), might have been due to the routine administration of PIA in our center.

RAPID and DEFINITE diagnosis + treatment !!
are significant for the patients survived (vascular) and saved globe (ocular).
Retrospective study in 22 vascular cases: 10-years period (2004-2014) in Maharaj Nakorn Chiang Mai, Chiang Mai University Hospital.

- Successful management of vascular pythiosis requires early recognition
- 4 classic clinical presentations need to be concerned:
  1. underlying thalassemia
  2. no atherosclerotic risk
  3. history of previous leg wound
  4. presentation with acute or chronic limb ischemia

  **Important to note that “serum antibody for Pythium should be tested in all suspected cases before treatment”**

- Survival rate was around 63.6%.
- The only effective treatment was complete excision of the infected tissue, which was done mainly by major amputation.
- This report raises awareness of this disease, which needs preemptive diagnosis and appropriate treatment.
One more novel technology can help for pythiosis diagnosis

**tHDA-RFLP**

- Thermophillic helicase DNA Amplification (tHDA) using *P. insidiosum* specific primer
  - Isothermal DNA amplification, no need PCR machine
  - Rapid & accurate, species-specific identification
- Can differentiate *P. insidiosum* from closely related pathogenic fungi by CviKI-1 digestion.
- Limit of Detection:
  - 100 pg (1.74 x 10^2 copies) for 1-step protocol
  - 100 fg (1.74 x 10^-1 copies) for 2-step protocol – add denature step
- Directly amplification in clinical samples was also evaluated.

Worasilchai et al., Med Mycol, 2017
One more novel technology….for clade pythiosis diagnosis

High Resolution Melting Analysis: A Novel Approach for Clade Differentiation in *Pythium insidiosum* and Pythiosis.

Navaporn Worasilchai, Nitipong Permpalung, Ariya Chindamporn

- Real-time polymerase chain reaction (qPCR) with subsequent High Resolution Melting (HRM) using *P. insidiosum* specific primer
  - No need sequencing step
  - Rapid & accurate, clade-specific identification
- Limit of Detection:
  - 100% specificity
  - 1 pg limit of detection

Normalized graph (A) and difference graph (B): comparison among Clade A<sub>TH</sub>, Clade B<sub>TH</sub>, and C<sub>TH</sub> *P. insidiosum* (—) and other related fungi (---) which were amplified COX2 gene:

*L. albertoi* (1); *P. aphanidermatum* (2); *P. catenulatum* (3); *C. coronatus* (4); *Phy. sojae* (5); *B. meristosporus* (6); *Phy. parasitica* (7)

Worasilchai et al., Med Mycol, 2017
Mucormycosis

- Underlying dis. in developed country: DM; developing country: HM
- Seems like a clear sky of treatment in future: 2nd triazole
- From translational research of pathogenesis: CotH member

Guideline for pythiosis diagnosis (Thai patients, experience)

Vascular pythiosis

- Underlying hemoglobinopathy ie. thalassemia, PNH etc.
- Agricultural related occupations ie. farmer or history of water exposure
- Present acute or chronic ischemia with rapid progression
- No atherosclerotic risk & no response to any antifungal agents

Ocular pythiosis

- History of water spilled to the eye
- Present ulcerative keratitis / endophthalmitis with rapid progression
- No response to any antifungal agents

RAPID and DEFINITE diagnosis + treatment !!
are significant for the patients survived (vascular) and saved globe (ocular).