Recognizing the Burden of Illness and Diagnostic Challenges of Mucormycosis in Canada

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Conflict of Interest and Disclosures

- Advisory Boards
  - AVIR Pharma Inc.
- Grants
  - Amplyx Pharmaceuticals
  - Merck Canada
- Speaker Travel Expenses
  - AVIR Pharma Inc.
Learning Objectives

- Review the current body of evidence characterizing mucormycosis based on underlying medical condition, clinical presentation, aetiology, and patient geography

- Compare and contrast the current state and future state of laboratory detection of mucormycosis
Mucormycosis is an uncommon opportunistic fungal infection caused by Mucorales (Zygomycetes) moulds.

Rapid progression and angioinvasion that primarily affects patients with diabetes mellitus or compromised immune systems.

Rarity of disease prevents clinical trial study and epidemiology relies on case series and registries.

Diagnosis is challenging and the burden of disease is very likely underestimated.

Earlier diagnosis leads to improved survival.

Understanding the Burden of Illness

- Developed countries
  - Infrequent but 2nd most common mould cause of invasive disease in haematological malignancy, HSCT, and SOT patients

- Developing countries
  - Prevalent in patients with uncontrolled diabetes
  - Increasingly recognized in trauma patients

Understanding the Burden of Illness

- Prevalence and incidence estimations and considerations
  - Increasing susceptible population
    - Underlying malignancy, HSCT, SOT
  - Increased physician awareness and education
    - Haematology-Oncology services
  - Lack of standardized diagnostic strategies
    - Imaging and microbiological tools
  - No harmonized denominators
    - Cases/1,000 patient days, cases/patient group, cases/million population, cases/10,000 discharges

# Contemporary Review of 851 Cases of Mucormycosis: Epidemiology

<table>
<thead>
<tr>
<th>Underlying Condition</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td>340 (40)</td>
</tr>
<tr>
<td>Diabetic ketoacidosis *</td>
<td>71 (21)</td>
</tr>
<tr>
<td>Haematological malignancy</td>
<td>275 (33)</td>
</tr>
<tr>
<td>AML</td>
<td>116 (42)</td>
</tr>
<tr>
<td>Solid organ transplant</td>
<td>116 (14)</td>
</tr>
<tr>
<td>Kidney</td>
<td>67 (58)</td>
</tr>
<tr>
<td>Haematopoietic stem cell transplant</td>
<td>90 (11)</td>
</tr>
</tbody>
</table>

* status unknown for 248 patients

Jeong et al. CMI. 2019;25:26-34.
Contemporary Review of 851 Cases of Mucormycosis: Genera Distribution

<table>
<thead>
<tr>
<th>Mucorales Identified *</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rhizopus</strong></td>
<td>213 (48)</td>
</tr>
<tr>
<td><strong>Mucor</strong></td>
<td>63 (14)</td>
</tr>
<tr>
<td><strong>Lichtheimia</strong></td>
<td>60 (13)</td>
</tr>
<tr>
<td><strong>Cunninghamella</strong></td>
<td>30 (7)</td>
</tr>
<tr>
<td><strong>Apophysomyces</strong></td>
<td>34 (8)</td>
</tr>
<tr>
<td><strong>Rhizomucor</strong></td>
<td>28 (6)</td>
</tr>
<tr>
<td><strong>Saksenaea complex</strong></td>
<td>12 (3)</td>
</tr>
<tr>
<td>Others (<em>Syncephalastrum, Cokeromyces</em>)</td>
<td>7 (2)</td>
</tr>
</tbody>
</table>

* Culture and/or molecular identification to genus/species level available for 447 (53%) cases, despite 79% of cases culture-positive for Mucorales

Jeong et al. CMI. 2019;25:26-34.
Contemporary Review of 851 Cases of Mucormycosis: Causative Genera by Disease

Jeong et al. CMI. 2019;25:26-34.
Contemporary Review of 851 Cases of Mucormycosis: Causative Genera by Geography

Jeong et al. CMI. 2019;25:26-34.
## Paediatric Mucormycosis – Registry Data from Europe

<table>
<thead>
<tr>
<th>Underlying Condition</th>
<th>Cases (%)</th>
<th>Disseminated</th>
<th>Pulmonary</th>
<th>Skin + soft tissue</th>
<th>Sinus + sinoorbital</th>
<th>Rhinocerebral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematological malignancy</td>
<td>29</td>
<td>12</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>HSCT</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SOT</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other Malignancy</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Trauma/surgery</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>None</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total cases</strong></td>
<td><strong>63</strong></td>
<td><strong>24</strong></td>
<td><strong>12</strong></td>
<td><strong>12</strong></td>
<td><strong>10</strong></td>
<td><strong>5</strong></td>
</tr>
</tbody>
</table>

Paediatric Mucormycosis – Registry Data from Europe

<table>
<thead>
<tr>
<th>Mucorales Identified</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhizopus</em></td>
<td>26</td>
</tr>
<tr>
<td><em>Lichtheimia</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Mucor</em></td>
<td>8</td>
</tr>
<tr>
<td><em>Cunninghamella</em></td>
<td>4</td>
</tr>
<tr>
<td>Unidentified</td>
<td>16</td>
</tr>
</tbody>
</table>
Prevalence of Mucormycosis-related Hospitalizations in the US: 2005-2014

- 555 cases from ~47M inpatient encounters
  - 177 hospitals, majority urban teaching facilities
  - Diabetes (52%), haematological malignancy (40%)
- Prevalence of 0.12 per 10,000 discharges

Incidence of Invasive Fungal Infection per 100,000 Patients from 2006-2016.

<table>
<thead>
<tr>
<th></th>
<th>06</th>
<th>07</th>
<th>08</th>
<th>09</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
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</thead>
<tbody>
<tr>
<td>MM</td>
<td>0.5</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.5</td>
<td>0.9</td>
<td>0.2</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>IA</td>
<td>2.4</td>
<td>2.8</td>
<td>1.4</td>
<td>1.8</td>
<td>1.9</td>
<td>2.4</td>
<td>3.3</td>
<td>2.7</td>
<td>2.9</td>
<td>2.5</td>
<td>2.4</td>
</tr>
<tr>
<td>All IFI</td>
<td>30.2</td>
<td>27.2</td>
<td>23.2</td>
<td>25.4</td>
<td>24.3</td>
<td>26.7</td>
<td>28.7</td>
<td>28.1</td>
<td>26.1</td>
<td>31.8</td>
<td>27.2</td>
</tr>
</tbody>
</table>

MM, mucormycosis; IA, invasive aspergillosis; IFI, invasive fungal infection

- 36 cases
- Diabetes (36%), 19% Haematological malignancy (19%), HSCT (11%)
- Sinus (39%) and Lung (28%)
- Breakthrough infection (33%)

Webb et al. OFID. 2018.
Mucormycosis in Uncontrolled Diabetic Patients

- Healthcare access to diabetes management
  - <40% of diabetics worldwide achieve good glycemic control

- 388 proven/probable MM patients in India
  - 56.8% patients from 4 tertiary care centres had uncontrolled diabetes
  - 1.6 cases / 1000 diabetic patients
  - 65.7% ROC patients had uncontrolled diabetes

Mucormycosis in Uncontrolled Diabetic Patients

Breakthrough Invasive Mould Infection in the Setting of Azole Prophylaxis

Understanding Diagnostic Challenges

- Tissue histopathology and direct microscopy are relatively specific but not routinely performed.

- Culture is poorly sensitive, ‘slow’, and requires expertise for genus and species identification.

- Importance of knowing whether it’s mucormycosis or aspergillosis:
  - Clinical presentations and patient risks overlap
  - Antifungal therapy is different
  - Increased mortality associated with delayed therapy
    - Amphotericin B >6 days after diagnosis increased mortality rate from 48.6% to 82.9%

Chamilos et al. 2008. CID
Imaging Mucormycosis

- In neutropenic patients with suspected pulmonary mucormycosis, CT scan may be beneficial if:
  - Multiple nodules (>10)
  - Pleural effusion
  - Vessel occlusion sign
  - Reverse halo sign

- In diabetic patients with symptoms consistent with rhino-orbito-cerebral disease, MRI or cranial CT may be beneficial

- **Lack of specificity** for Mucorales vs *Aspergillus* warrants comprehensive laboratory testing

Histopathology

- Reference standard for microbiological diagnosis
- Fresh or FFPE tissue
- Broad, aseptate, ribbon-like hyaline hyphae, with irregular branching
- Imperfect science and morphology can be mistaken for *Aspergillus*
- Confirmation by culture or molecular method required
What’s That Fungus?!?

Images property of J. Fuller
Culture

- Genus/species identification and susceptibility testing
  - Importantly, Mucorales vs *Aspergillus*
  - Epidemiological value has limited relevance currently to clinical management
- Culture-negative in up to 50% of histopathology-positive specimens
- Tissue grinding impacts viability of hyphae
- In lab, mucorales are ‘rapid’ growers (3-7 days)
  - require 30°C and 37°C incubation

Molecular Methods for Direct Detection

- Offers faster time to result and genus/species identification
- Clinical utility from fresh or FFPE tissue has been demonstrated
- rRNA locus is a common target for fungal identification
  - DNA encoding 18S, 5.8S, 28S rRNA subunits;
  - Internal transcribed spacer region ITS1 and ITS2
- ITS 1-2 and 5’ end of 28S (D1-D2) most often cited
- 28S (12F/13R) recently shown to be an improved target for Mucorales

Molecular Methods for Direct Detection

Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi

Conrad L. Schoch¹, Keith A. Seifert¹, Sabine Huhndorf⁵, Vincent Robert⁴, John L. Spouge⁶, C. André Levesque⁵, Wen Chen², and Fungal Barcoding Consortium²

¹National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 20892; ²Biodiversity (Mycology and Microbiology), Agriculture and Agri-Food Canada, Ottawa, ON, Canada K1A 0C6; ³Department of Botany, The Field Museum, Chicago, IL 60605; and ⁴Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre (CBS-KNAW), 3508 AD, Utrecht, The Netherlands

Edited* by Daniel H. Janzen, University of Pennsylvania, Philadelphia, PA, and approved February 24, 2012 (received for review October 18, 2011)

- Highest probability of successful identification for the broadest range of fungi
Canadian Services for Direct PCR Detection

Personal communication with Site Leaders.

Linda Hoang, BCCDC
Tanis Dingle, APL
Julianne Kus, PHOL
Philippe Dufresne, LSPQ
Aaron Campigotto, HSC
Alternative PCR Approach for FFPE Mucorales

- DNA fragmentation in FFPE tissue hinders ITS, D1/D2, and other primers targeting large amplicons (~800 bp)

- ITS or D1/D2 for Mucorales have limitations because of large introns and heterogenic regions

- CDC recently evaluated 12F/13R (28S) target (~200 bp)
  - Direct sequence of unambiguous reads from amplicons
  - Genus level discrimination

- Useful Mucorales target, especially when ITS PCR fails

- Public domain sequence databases lack robust 28S identifications

Improved Patient Care?

- Reliance on specialized services (clinical and laboratory)
- 1 to 2 week algorithm
Progression Towards Near-Patient Testing

- *Aspergillus* PCR technical standards and clinical utility published
- EAPCRI Working Group plans to develop similar standards for Mucorales PCR
- In-house assays demonstrate utility of serum and BAL fluid for direct PCR detection of Mucorales
- Likely that pre-emptive surveillance strategies will complement existing diagnostics with improved and earlier mucormycosis detection

Quantitative PCR Detection of Mucorales in Serum

- Retrospective, multi-centre study from a prospective French IFI surveillance program (RESSIF)
- Serum from proven (n=25) and probable (n=19) patients with mucormycosis
  - 34 HM, 3 DM, 2 SOT
  - 17 pulmonary, 14 disseminated, 8 rhinocerebral
- Assay: *Rhizomucor, Rhizopus/Mucor, and Lichtheimia*
- qPCR 81% SN (92% when insufficient sera excluded)
- First positive serum was median 9 days earlier than first mycological sign (culture or histopath) in 36 pts

Quantitative PCR Detection of Mucorales in Serum

- Serum-direct Mucorales assay shows promising results
- Suggests 90% sensitive in high-risk patients
- Earlier detection than tissue histopathology or culture
  - Mucorales-specific result (genus)
  - Potential as a screening tool with serum GM
- Potential for monitoring response to antifungal therapy

Quantitative PCR Detection of Mucorales in Serum

- Important observations
  - Sera had high concentration of DNA (1-10 fg/ul)
  - 0.1 fg/ul is typical for invasive aspergillosis

- Most likely due to degree of Mucorales angioinvasion
  - May prove to be a valuable diagnostic attribute

- Could correlate to late stage disease in study patients
  - >80% of patients had culture-positive tissues

- In a related study of 23 leukemia patients with pulmonary mucormycosis, positive qPCR was strongly associated with reverse halo sign on CT

Retrospective study applied Millon assay to BAL fluid
Mucorales culture from BAL is ~20%; similar for IA

Mucorales DNA detectable in BAL with radiological evidence of IFI
- 10/10 proven/probable mucormycosis positive
- 7/9 companion sera were also positive

May prove complementary to serum qPCR

Scherer et al. JCM.2018;56:e00289-18.
Progression Towards Near-Patient Testing

- Benefits of serum and BAL PCR for mucormycosis
  - Earlier diagnosis and potential mortality benefit
  - Specimen acquisition simpler than biopsy
  - Assay is relatively simple to set up
  - Run PCR in parallel with *Aspergillus* PCR

- Prospective study initiated (completed) to assess SP and verify SN, benefit of early diagnosis, and value of [DNA] for treatment monitoring (NCT02845934)
Progression Towards Near-Patient Testing…?

- PCR Electrospray-Ionization Mass Spectrometry (ESI-MS)
- PCR (16-plex) unfixed tissue with Mucorales hyphae
- ESI-MS for MW and base composition of amplicon
- Compared to culture, serum qPCR, ITS, & 18S PCR
- Acceptable, rapid ID from microscopy-positive tissue
- Species-level ID limited only by depth of database
  - Ex. Cunninghamamella and Saksenaea
- Limited access to technology
- Expensive test ($200 USD)

# Challenges for MALDI-TOF

<table>
<thead>
<tr>
<th>Organism (no. of isolates)</th>
<th>No. (%) of isolates identified at the genus or species level by log(score) value&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bruker library</td>
<td>Bruker library plus BMU database</td>
</tr>
<tr>
<td></td>
<td>≥2.0</td>
<td>≥1.7</td>
</tr>
<tr>
<td>R. arrhizus (20)</td>
<td>19 (95)</td>
<td>20 (100)</td>
</tr>
<tr>
<td>R. microsporus (27)</td>
<td>24 (88.9)</td>
<td>27 (100)</td>
</tr>
<tr>
<td>R. stolonifer (1)</td>
<td>0 (0)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>R. pusillus (4)</td>
<td>4 (100)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>S. racemosum (2)</td>
<td>2 (100)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>L. corymbifera (4)</td>
<td>4 (100)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>L. ramosa (6)</td>
<td>0 (0)</td>
<td>3 (50)</td>
</tr>
<tr>
<td>L. omata (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>M. circinelloides (9)</td>
<td>2 (22.2)</td>
<td>8 (66.7)</td>
</tr>
<tr>
<td>M. irregularis (23)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>M. hiemalis (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>M. racemosus (4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>C. bertholletiae (3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>C. phaeospora (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>C. echinulata (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total (111)</td>
<td>55 (49.5)</td>
<td>67 (60.4)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mis-ID, misidentification. Symbols: *, misidentified as *Lichtheimia corymbifera*; †, misidentified as *Lichtheimia corymbifera*; ‡, misidentified as *Mucor ramosissimus*; §, misidentified at the species level but correctly identified at the genus level.

Shao et al. JCM. 2018; 56: e01886-17.
Challenges for Antifungal Susceptibility Testing

- The only clinical breakpoint available for any mould was just approved January 2019 (CLSI)
  - *A. fumigatus* and voriconazole
- CLSI M38 – technical standard for BMD testing approved for Mucorales
- Paucity of available MIC data
- 1st need wild-type distributions
- CLSI call out for data
- Species ID will become more relevant

Summary of Mucorales Epidemiology and Diagnostics

- Epidemiological data supports mucormycosis as a rare IFD with no clear emergence.
- Clinico-diagnostic practices are likely under-representing true epidemiology.
- Underlying predisposing conditions will continue to perpetuate infection.
- Laboratory diagnostics for mucorales are insufficient to contribute to appropriate patient care needs.
- Serum-based molecular approaches show practice-changing promise.