Prevention of cryptococcosis and other AIDS-related fungal infections: Strategies for the Asia Pacific region

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Prevention of Cryptococcosis and Talaromycosis

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Challenges in Opportunistic Fungal Infections

- Invasive fungal infections are a leading cause of AIDS-related deaths, with case fatality rates of 20-70%.

- Cryptococcosis, PCP, histoplasmosis, and talaromycosis contribute to substantial 25-35% AIDS-related mortality
  - Stable numbers entering care with advanced HIV (30-40% with CD4 <200);
  - Increasing numbers of ART defaulters;
  - Increasing numbers with virologic failure;
  - Rise in use of immunosuppressive medicines in non-HIV populations

- Cryptococcal Antigen (CrAg) screening recommended in CD4<200 as preemptive fluconazole therapy in CrAg+ results in a survival benefit.

- Reality Trial demonstrated benefit for fluconazole 100mg prophylaxis for 12 wks in Africans with CD4<100 (& cotrimoxazole, INH, azithromycin)\(^1\)
  - 14.4% mortality reduced to 11.0%
  - Concern over driving widespread fungal resistance
  - Less relevant in Asia due to *Talaromyces*

\(^1\) Hakim J, et al. NEJM 2017
Agenda: Screening to Prevent Fungal OIs

1. Cryptococcosis
   - CrAg Lateral Flow Assay
   - CrAg Prevalence
   - CrAg+ Outcomes
   - ART Timing in CrAg+ persons

2. Talaromycosis
   - Diagnostics
   - *Talaromyces* Antigen Prevalence
   - Role for Screening
Cryptococcosis
Unmasking of Cryptococcosis

- Sub-clinical period prior to meningitis
- 2-fold higher mortality when ART started in 2 weeks prior to meningitis diagnosis.\(^1\)
- Cryptococcal antigen (CRAG) detectable in blood \(\geq 3\) weeks prior to headache onset.\(^2\)
- Caused 18\% of attributable mortality in a Ugandan cohort after starting ART.\(^3\)

1 Rhein J, et al. Open Forum ID 2018
2 French N, et al. AIDS 2002
CrAg Lateral Flow assay

- Immunochromatographic lateral flow assay (LFA)
- First CrAg LFA U.S. FDA approved July 2011.
  - 5 manufacturers: Immy, Biosynex, Liming Bio, Dynamiker, Era Biology,
  - Only 1 US FDA approved (Immy)
  - 2 CE marked in Europe (Immy, Biosynex)
  - 2 have large clinical validation studies (Immy, Liming Bio)

1. Add 1 drop of specimen diluent
2. Add 40 μL of specimen (1 drop)
3. Insert strip
4. Incubate 10 min
5. 1 line = negative, 2 lines = positive
# Diagnostic Performance in Cryptococcal Meningitis

<table>
<thead>
<tr>
<th>Diagnostic Test</th>
<th>n</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRAG LFA</td>
<td>666</td>
<td>99.3%</td>
<td>99.1%</td>
<td>99.5%</td>
<td>98.7%</td>
</tr>
<tr>
<td>CSF Culture †</td>
<td>806</td>
<td>90.0%</td>
<td>100%</td>
<td>100%</td>
<td>85.3%</td>
</tr>
<tr>
<td>100 μL CSF volume</td>
<td>524</td>
<td>94.2%</td>
<td>100%</td>
<td>100%</td>
<td>91.2%</td>
</tr>
<tr>
<td>10 μL CSF volume</td>
<td>282</td>
<td>82.4%</td>
<td>100%</td>
<td>100%</td>
<td>75.8%</td>
</tr>
<tr>
<td>India Ink</td>
<td>805</td>
<td>86.1%</td>
<td>97.3%</td>
<td>98.2%</td>
<td>80.2%</td>
</tr>
<tr>
<td>CRAG-latex (Meridian)</td>
<td>279</td>
<td>97.8%</td>
<td>85.9%</td>
<td>92.6%</td>
<td>95.5%</td>
</tr>
<tr>
<td>CRAG-latex (Immy)</td>
<td>749</td>
<td>97.0%</td>
<td>100%</td>
<td>100%</td>
<td>95.3%</td>
</tr>
</tbody>
</table>

* All CSF CRAG lateral flow assay (LFA) “false positives” did not have any other pathogen identified, but did not have a serum or plasma specimen available for testing of cryptococcal antigenemia to determine if this is enhanced detection.

† Different quantitative CSF culture methods were used in 2006-09 with 10 μL input volume vs. 2010-12 with 100 μL CSF volume.
Clinical Relevance of CrAg+ in Blood

- In a 2009 South African retrospective cohort (n=707), 7% (46/707) CrAg+.
- CrAg+ was 100% sensitive and 96% specific for developing cryptococcal meningitis within 1 year.
- 28% CrAg+ developed meningitis and ~50% died.
  - Fluconazole use was unknown in this cohort.

- Ugandan prospective cohort (n=609), 8.2% CRAG+.
  - Fluconazole + ART => 76% (19/25) alive at 2.5 years.
  - ART only => 6 of 8 died, all 5 with CD4<100.

Jarvis JN. Clinical Infectious Dis, 2009.
Meya DB. Clinical Infectious Dis, 2010.
Survival with Asymptomatic Cryptococcal Antigenemia in Uganda

Fluconazole
200-400mg
2-4 weeks

Fluconazole use P=0.002
CD4 count P=0.029

N=33
All CD4 CRAG+
5-year Survival of Cryptococcosis in Uganda

CrAg Prevalence in Blood

Vietnam

LMIC Average ~6%

Indonesia

Cambodia

Thailand

Thailand

Rajasingham et al. Lancet ID 2017
Does CrAg Screening work to reduce all cause mortality?
in the real world
CRAG Screening + Adherence Support
Tanzania & Zambia, in CD4<200

Fluconazole 800mg x 2 weeks, 400mg x 8 weeks
~35% of CrAg positive died before ~4 months

Proportion Died

Follow up (months)

Number at risk
CRAG screening: 1001 899 869 854 842
Standard of care: 998 869 834 811 794

Mfinanga et al. Lancet 2015
Reality Trial

- Fluconazole 100mg prophylaxis for all with CD4<100 (and TMP/SMZ, INH, Azithromycin)
- Reduced all-cause mortality 11.0% vs. 14.4%
- CrAg Prevalence of ~7%
- Cryptococcal meningitis 1.0% vs. 2.6%
  - Unclear how diagnosed. (Not microbiologic confirmed)
- Pharmacokinetic (PK) modeling of fluconazole suggests 40% sub-therapeutic below Crypto MIC.

Hakim J et al. NEJM 2017
Pett SL et al. CROI Abstract 2018
CrAg Titers in REALITY Trial

- CrAg Titers not fully equal by arm (P=0.06)
- Low titers may only need prompt ART

CrAg+ No Treatment
- 11.5% died;
- 20.3% developed meningitis

CrAg+ fluconazole 100mg x 12 week
- 4.7% died;
- 7.8% Meningitis

Unexpected “Low mortality” may be related to the timing of the testing
- Tested at time of starting ART
- Other studies tested when entering into care at time of CD4

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Pett CROI Abstract 2018

**Baselinel titre**

<table>
<thead>
<tr>
<th>Baseline titre</th>
<th>≤80</th>
<th>≥160</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45 (4.7%) P+x+</td>
<td>22 (2.5%) P+x+</td>
</tr>
<tr>
<td></td>
<td>37 (4.2%) Std</td>
<td>32 (3.6%) Std</td>
</tr>
</tbody>
</table>

Number of participants

- 0
- 5
- 10
- 15
- 20
- 25
CRAG Screening Algorithm

Entry into HIV care
Perform CD4 count

If CD4 ≤100, and ART naïve, perform CRAG screening*

CRAG Positive

Return to physician for evaluation

CRAG Negative

Routine medical care
Initiate ART at 2 weeks

Signs or symptoms of CNS infection

Diagnostic lumbar puncture

Asymptomatic

Treat with fluconazole 800mg x2 weeks, then 400mg x8 weeks

Refer to Hospital

Treat for cryptococcal meningitis per WHO guidelines

* Lateral Flow Assay (LFA) can be performed as a reflex test on plasma samples collected for CD4 testing (or serum samples). Latex agglutination can be performed on serum samples only.
ORCAS Trial
Operational Research in CrAg Screening

• Stepped Wedge Design Randomized Clinical Trial
  – Prospective Observational Phase, then
  – CRAG screening rolled out over 2 years

• ~8% CrAg prevalence

• Trial was confounded by doubling of clinic sizes, ~9% worse rate of return to starting ART.

• No effect on survival.
  – But was not worse despite 9% more not returning to start ART during interventional arm.

Meya DB, et al. JAIDS 2019
CRAG⁺ Survival by initial CRAG titer

Meya DB, et al. JAIDS 2019
Plasma CrAg titer vs. Outcome

Pooled cohorts from Uganda, Tanzania, Ethiopia, and South Africa (n=287)

Fluconazole Daily Dose

- 800, 400 mg
- ± 200mg in one cohort

Pooled cohorts from Uganda, Tanzania, Ethiopia, and South Africa (n=287)

Plasma CrAg Titer vs. CSF Positivity

Asymptomatic CrAg+

Symptomatic (Headache) CrAg+

CrAg titers at 1:160 and 1:640 are most useful (i.e. could do two titers only)
CrAg LFA Titers below <=1:80 unlikely to have CNS involvement if asymptomatic;
CrAg LFA Titers above >=1:640 assume CNS involvement regardless of symptoms
CrAg Titers vs. Outcome by ± Headache

A. Asymptomatic CrAg+ Outcomes by Titer

B. Symptomatic CrAg+ Outcomes by Titer
When to Lumbar Puncture (LP)

• Guidelines recommend LP always if CrAg+
  – Not always feasible
• CrAg LFA Titers \(\leq 1:80\) unlikely to have CNS involvement if asymptomatic;
• CrAg LFA Titers \(\geq 1:640\) assume CNS involvement regardless of symptoms
• Diagnostic utility of LPs a titers 1:160 to 1:320
• Therapeutic utility to lower ↑intracranial pressure
  – Thus, use manometer, IV tubing, or remove ~20mL
Symptomatic Cryptococcal Antigenemia in Suspected Meningitis

- Suspected Meningitis: n=1201
- Blood Fingerstick CRAG Positive: n=725
- CSF CRAG Positive: n=671
- CSF Culture Positive: n=590

N=54 (4.5% overall) with:
- Symptomatic Antigenemia
- Blood CrAg-positive
- CSF CrAg-negative
- 34% in-hospital mortality with fluconazole therapy

Culture sensitivity 88%
CrAg LFA sensitivity 99.6%
Timing of ART in CrAg+

- Early ART harmful in cryptococcal meningitis.¹
- No Data on asymptomatic CrAg+ persons
- WHO guidelines recommend a delay of ART for 2 weeks of fluconazole therapy.
- “Test and Treat” is happening
  - Where ART is being started before fluconazole
  - Is this harmful?

¹ Boulware DR, et al NEJM 2014
ART Timing in CrAg+ Uganda Data (Prelim)

Log-Rank P-Value = 0.009
CrAg Summary

• Pre-HIV therapy CrAg Screening
  – WHO recommended in CD4<200 cells/μL.
  – Or in all entering into care (or hospital) if no CD4
• Preemptive Therapy for CrAg+
  – 800mg x 10 weeks, 200mg prophylaxis to 26 weeks
• Assume CNS involvement if CrAg titer ≥1:640
• ART Initiation at ~2 weeks
Talaromycosis

Most common AIDS-defining Opportunistic Infection in parts of SE Asia
Talaromycosis

Most common AIDS-defining Opportunistic Infection in parts of SE Asia

Nga, TVT and Le, T et al. *Transactions of the Royal Society of Tropical Medicine & Hygiene* 2012.
Talaromycosis

- Subacute illness
- Median symptom duration 15 days (IQR 7 to 30)
- Antigen is detectable at time of illness
- Can one screen for subclinical infection and preemptively treat, such as in *Cryptococcus*?

Acknowledgements to Dr. Thuy Le
Diagnosis of Talaromycosis

**Gold standard:**
- Histopathology → invasive
- Culture → incubation time (≤14 days)
  - Blood 60-80% sensitivity
  - Skin ~70% present

**Alternatives:**
- Molecular: PCR → sensitivity (70%)
- Antigen detection → ELISA exists
  - future Lateral Flow Assays (LFA)
**Talaromyces PCR History**

1994
- Lobuglio: Conventional PCR Amplify ITS region

1998
- Vanittanakom: Conventional PCR/hybridization isolate samples

2002
- Prariyachatigul: One-tube semi-nested PCR 2 clinical samples

2003
- Vanittanakom: Nested PCR 34 serum samples, sensitivity 68% (24/34)

2009
- Pornpraset: Real-time PCR 20 whole blood samples sensitivity 60% (12/20)

**PCR Sensitivity ~67% (95%CI, 59%-75%) in Talaromycosis Disease.**¹

**PCR likely not a viable screening test**

Talaromyces Serologic Diagnostic History

Ab = Antibody; Ag = Antigen

**Immuno diffusion**

*Viviani*, detect Ab

*L. Kaufman*, detect Ag + Ab

1993

**Indirect immunofluorescent**

*K. Yuen*, detect Ag

1994

**Mp1p Polyclonal Ab ELISA**

*Cao*, detect Ab

1996

**Mp1p Monoclonal Ab ELISA**

*Cao*, detect Ag

1997

1999

**Whole cell PAb ELISA**

*Desakorn*, urine Ag

Sens = 97.5% (n=53)
 Spec = 92.5% (n=331)

2002

**Pooled MAb ELISA**

*Chaiyaroij*, detect Ag

1 Sensitivity 80% (355/445) calculated from:

But not all antigens are created equal

Antigen pooled Sensitivity 80% (95%CI, 76% to 83%)\(^1\)
**Talaromyces Mannoprotein Mp1p ELISA Antigenemia in Hospitalized Patients**

Sensitivity: 86.4 - 89.3%; Specificity: 98%

Acknowledgements to Dr. Thuy Le
Talaromyces Mannoprotein Mp1p ELISA Antigenemia in Outpatient Clinic Patients

Acknowledgements to Dr. Thuy Le
Talaromyces Antigen Mp1p Prevalence in Vietnam

In a Vietnamese cohort of 1081 HIV+ adults with CD4<100 across 10 provinces:
• 74% men
• Median Age = 35 (IQR, 30-41) years
• Median CD4 = 25 (IQR, 11-50) cells/μL

<table>
<thead>
<tr>
<th>Vietnam Region</th>
<th>N</th>
<th>Mp1p positive</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>526</td>
<td>40</td>
<td>7.6%</td>
</tr>
<tr>
<td>Southern</td>
<td>589</td>
<td>13</td>
<td>2.2%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1081</strong></td>
<td><strong>53</strong></td>
<td><strong>4.7%</strong></td>
</tr>
</tbody>
</table>

Talaromyces Ag+ = 3.2-fold higher 6-month mortality

Acknowledgements to Dr. Thuy Le
Antigen Prevalence in Guangzhou, China: adults entering care with CD4 <100 cells/μL

- Talaromyces Mp1p Ag+
  - 26.2% in CD4 <50
  - 4.7% in CD4 >50
- 98% (43/44) Mp1p Ag+ developed culture-confirmed Talaromycosis
- Detected 88% of Talaromycosis cases

Ling-hau Li et al. CROI Abstract 2019
Talaromyces LFAs?

- Any ELISA can be developed into a lateral flow assay (LFA)

- Challenges:
  - LFAs have decreased analytical sensitivity
  - Need high quantity Monoclonal Antibody
  - High Quality Manufacturing

Chiang Mai Talaromyces LFA

3 mcg limit of detection
Talaromyces Antigen Screening

• Commercial ELISA exists in China
  – Beijing Wantai Biological Pharmacy Enterprise

• Preemptive therapy to be defined, but
• Itraconazole therapy for active disease is:
  – 600mg daily x 3 days
  – 400mg daily x 10 weeks
  – 200mg daily to 6 months

? Any role of amphotericin for high antigen loads