Cryptococcal Antigen Screening: Perspectives from Uganda

Dr. David Meya, MMed
Dr. David Boulware, MD MPH
ASLM, Capetown 2012
Introduction

• Globally, an estimated 957,900 cases of cryptococcal meningitis (CM) occur annually, with 624,700 deaths within 3 months of infection.¹

• Highest burden in Sub-Saharan Africa (SSA) with annual incidence of 720,000 cases with mortality of 20-50% ²,³ even with ART availability ⁴,⁵

• In Uganda, mortality is between 20-39% despite access to ART ⁴,⁶

ART coverage

Number of people receiving antiretroviral therapy, 2002-2011
Introduction

• CM complicated by Immune Reconstitution Inflammatory Syndrome (IRIS) after initiating ART

• Incidence between 10-25% with mortality of 20-36% \(^8,^9\)

• High incidence of CM in patients who are cryptococcal antigen (CRAG\(^+\)) but not treated with fluconazole \(^10\)

• Utility of serum CRAG testing in asymptomatic cryptococcal infection & clinical impact of treatment not clearly defined

\(^8\) Monika Müller et al *Lancet Infect Dis* 2010; \(^9\) Boulware et al *PLOS Medicine*, 2010; \(^10\) Castelnuovo B et al *CID*, 2009
Asymptomatic Cryptococcal Antigenemia

- Cryptococcal antigen (CrAg) can be detected in blood a median of 22 days prior to development of meningitis symptoms.\(^\text{11}\)

- Majority of people (>95%) with asymptomatic cryptococcal antigenemia have a CD4<100 cells/\(\mu\)L

Prevalence of CrAg+

Rhein J. *Neurobehavioral HIV Med* 2012
Meya DB et al. *Clin Infect Dis* 2010
## Recent Prevalence Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Screened (N)</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wajanga et al, <em>JIAS</em>, 2011 (Tanzania)</td>
<td>In-patient population</td>
<td>333</td>
<td>5.1%</td>
</tr>
<tr>
<td>Oyella J et al, <em>JIAS</em>, 2011 (Uganda)</td>
<td>Hospital population with CD4&lt;100 cells/μL</td>
<td>367</td>
<td>19%</td>
</tr>
<tr>
<td>Patel S, et al 2012 (London,UK)</td>
<td>UK HIV cohort with CD4&lt;100 cells/μL</td>
<td>157</td>
<td>5%</td>
</tr>
<tr>
<td>Osazuwa OF, et al 2012 (Nigeria)</td>
<td>Out patient HIV clinic with CD4&lt;200 cells/μL</td>
<td>150</td>
<td>12.7%</td>
</tr>
<tr>
<td>Etolu W, et al (Unpublished data) (Uganda)</td>
<td>Hospital in-patients with CD4&lt;100 cells/μL</td>
<td>240</td>
<td>21%</td>
</tr>
<tr>
<td>Pfizer Study (Uganda) (Unpublished data)</td>
<td>Out patient HIV clinic</td>
<td>171</td>
<td>7.6%</td>
</tr>
</tbody>
</table>
Clinical relevance of CrAg+

- In a S. African study of 707 HIV-infected patients, 7% (46/707) were CRAG+\(^{12}\)

- CrAg\(^+\) was 100% sensitive and 96% specific for developing CM within 1 year

- 28% of CrAg\(^+\) developed CM within 1 year

- CrAg\(^+\) is an independent predictor of mortality

Survival of People with Asymptomatic Cryptococcal Antigenemia starting HIV Therapy

13Meya DB et al, *Clin Infect Dis.* 2010
Cost-Benefit in Uganda

• With 8.8% prevalence in CD4<100,
  – To detect 1 case  NNT = 11.3
  – To prevent 1 death NNT=15.9  = $266 by CrAg latex

• CrAg-latex cost $16.75 in Kampala in 2010

• CrAg LFA cost is $2.00 in Africa

Meya Clin Infect Dis 2010; 51:448-453
Targeted Screening & Pre-emptive Therapy

- Fluconazole treatment of CRAG\(^+\) can prevent overt cryptococcal meningitis, avoid hospitalizations, improve survival, and reduce costs
- ART alone is not sufficient for CRAG\(^+\) persons
- Cost effective
  - cost of detecting 1 person with asymptomatic antigenemia with the LFA would be $28.37 while cost of saving one life - $39.73  \(^{15}\)

\(^{15}\) Rajasingham R et al, JAIDS, 2012
CrAg Screening in Kenya

- 6.2% (108/1726) screened persons were CrAg+16

- Treated with fluconazole 1200mg x2 weeks, then 800mg x8 weeks

- Median survival of 2.5 weeks without ART or fluconazole

- Both ART and fluconazole significantly associated with improved survival

Lateral Flow Assay

Point-of-Care Urine Antigen Screening Tests for Tuberculosis and Cryptococcosis: Potential for Mortality Reduction in Antiretroviral Treatment Programs in Africa

Evaluation of a Novel Point-of-Care Cryptococcal Antigen Test on Serum, Plasma, and Urine From Patients With HIV-Associated Cryptococcal Meningitis

Evaluation of a Newly Developed Lateral Flow Immunoassay for the Diagnosis of Cryptococcosis
Cost per Life Saved by CrAg LFA Screening vs. Prevalence

<table>
<thead>
<tr>
<th>Prevalence of CRAg+</th>
<th>Cost per life saved</th>
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</thead>
<tbody>
<tr>
<td>0%</td>
<td>$140</td>
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<tr>
<td>5%</td>
<td>$70</td>
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<td>10%</td>
<td>$46.5</td>
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<td>15%</td>
<td>$35</td>
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<td>20%</td>
<td>$35</td>
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<td>25%</td>
<td>$14</td>
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</table>

$2.50 CrAg LFA total lab test cost
CRAG Screening in Uganda

• The CDC-sponsored ORCAS Study - Operational Research for Cryptococcal Antigen Screening

• Implementing CRAG screening at 9 urban clinics to improve survival and reduce CM incidence
  – Stepped Wedge Design to Roll out over ~8 months
ORCAS Objectives

• **Primary:**
  – To evaluate survival benefit of CRAG screening and preemptive treatment using fluconazole

• **Secondary:**
  – To determine cryptococcal meningitis-free survival among those with asymptomatic antigenemia
  – To evaluate how best to implement CRAG screening and treatment
  – To determine compliance and tolerability of fluconazole preemptive therapy
CRAG Screening Algorithm

Entry into HIV care
Perform CD4 count

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

CRAG Positive

Return to physician for evaluation

Signs or symptoms of CNS infection

Diagnostic lumbar puncture

- LP

+ LP

Treat for cryptococcal meningitis per WHO guidelines*31

CRAG Negative

Routine medical care
Initiate ART at 2 weeks

Asymptomatic

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

* 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.

Figure 3: Algorithm for CRAG screening upon entry into HIV care

Rajasingham JAIDS 2012; 59:85-91
## Screen 2500, Enroll 150

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Mo 0</th>
<th>Mo 2</th>
<th>Mo 4</th>
<th>Mo 6</th>
<th>Mo 8</th>
<th>Mo10</th>
<th>Mo12</th>
<th>Mo14</th>
<th>Mo16</th>
<th>Mo18</th>
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<td>KCC 3/4</td>
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<td>KCC 7/8</td>
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<td>Site A/B</td>
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<td>Site C/D</td>
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<td>Site E/F</td>
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<td>Site G/H</td>
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**Data Collection, Pre-intervention**

**Implementation of WHO CrAg Recommendation**
This patient has a positive Cryptococcal antigen (CRAG). CRAG screening is routinely being done on patients with a CD4<100cells/μL.
Those who are CRAG+, without symptoms are very high risk for developing cryptococcal meningitis, and subsequent mortality.

As the patient’s medical provider, it is recommended:

1. To rule out symptoms or signs of meningitis, such as:
   - Fever
   - Headache
   - Neck pain
   - Photophobia
   - Confusion
   - Seizures
   - Stiff neck

If the patient has any of the above features or in your medical judgment you are concerned for a diagnosis of meningitis, consider referral for further evaluation such as lumbar puncture.

2. If the patient is without symptoms, treatment with high dose fluconazole is recommended. The following regimen is recommended:

   - Fluconazole 400mg twice daily for 2 weeks, then 400mg daily for 8 weeks.

Contraindications to fluconazole include: Pregnancy, breastfeeding, current use of protease inhibitors, Creatinine twice the upper limit of normal, and known liver disease. Patients on fluconazole and AZT concurrently should be monitored for AZT-related side effects. An Efavirenz-based ART regimen is preferred because fluconazole increases nevirapine concentrations. If fluconazole and nevirapine are administered together, the patient should be closely monitored for nevirapine-related side effects.
## Follow up of patients

<table>
<thead>
<tr>
<th>Location</th>
<th>Procedures</th>
<th>Screen</th>
<th>Enrollment</th>
<th>2 week</th>
<th>6 week</th>
<th>10 week</th>
<th>26 week</th>
<th>Early Termination</th>
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<tbody>
<tr>
<td>Clinical Laboratory</td>
<td>CRAG Screening</td>
<td>X¹</td>
<td>1</td>
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<tr>
<td></td>
<td>CRAG+ Information sheet</td>
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<td>CD4 Measurement</td>
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<td>X</td>
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<td></td>
<td>Urine Pregnancy test (♀)</td>
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<td>Before</td>
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<tr>
<td></td>
<td>Plasma Collection</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>Informed Consent</td>
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<tr>
<td>Medical Provider</td>
<td>CM Symptom Screen</td>
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<td></td>
<td>Fluconazole</td>
<td>Initiate</td>
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<td>Stop</td>
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<td>ART counseling</td>
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<td>X</td>
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<td></td>
<td>Initiate ART</td>
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<td>Research coordinator</td>
<td>Assess for survival</td>
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<td>X</td>
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<td></td>
<td>Assess CM episodes</td>
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<td>X</td>
<td>X</td>
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<td></td>
<td>Assess pharmacy records</td>
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<td>X</td>
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</tbody>
</table>
Results of CrAg Screening at Infectious Disease Institute

- 241 (CD4<100 cells/μL) screened over 9 months
- Represent 23% of total new patients (n=1028)
- Overall CrAg⁺ = 6.6% (16/241)
- Median (IQR) CD4 count: 45 cells/μL (19, 80)
- 60% Male
Results of CrAg Screening at Infectious Disease Institute

• One third concurrently on TB treatment
  – Fluconazole dose adjusted (↑50%)

• Of 8 CrAg+ with 6 month outcome, 6 alive and in care, 1 had died, while outcome for 1 unknown

• Median (IQR) time to ART start: 47(14, 56) days
# Diagnostic Experience

in Uganda (2006-2012) and Cape Town (2011-2012)

<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>India Ink</td>
<td>616</td>
<td>84.6% (341/403)</td>
<td>96.2% (205/213)</td>
<td>97.7% (341/349)</td>
<td>76.8% (205/267)</td>
</tr>
<tr>
<td>CSF Culture*</td>
<td>619</td>
<td>89.4% (363/406)</td>
<td>100% (213/213)</td>
<td>100% (363/363)</td>
<td>83.2% (213/256)</td>
</tr>
<tr>
<td>100 µL CSF volume</td>
<td>337</td>
<td>95.1% (213/224)</td>
<td>100% (113/113)</td>
<td>100% (213/213)</td>
<td>91.1% (113/124)</td>
</tr>
<tr>
<td>10 µL CSF volume</td>
<td>282</td>
<td>82.4% (150/182)</td>
<td>100% (100/100)</td>
<td>100% (150/150)</td>
<td>75.8% (100/132)</td>
</tr>
<tr>
<td>CRAG LFA</td>
<td>477</td>
<td>98.8% (329/333)</td>
<td>98.6%§ (142/144)</td>
<td>99.4% (329/331)</td>
<td>97.3% (142/146)</td>
</tr>
<tr>
<td>CRAG latex (Meridian)</td>
<td>279</td>
<td>97.2% (141/145)</td>
<td>85.9% (85/99)</td>
<td>91.0% (141/155)</td>
<td>95.5% (85/89)</td>
</tr>
<tr>
<td>CRAG latex (Immy)</td>
<td>525</td>
<td>96.3% (337/350)</td>
<td>100% (175/175)</td>
<td>100% (337/337)</td>
<td>93.1% (175/188)</td>
</tr>
</tbody>
</table>

- India ink missed 1 in 6.6 cryptococcal diagnoses
- Poor sensitivity (52%) at low CSF fungal burdens (<1000 CFU/mL of CSF)
  - 10-fold more likely to be missed. (RR=9.6, 95% CI: 5.5–16.7, P<.001)
- Most common diagnosis (23%) among India ink negative was Cryptococcus
- Number Needed to Test with CRAG LFA per additional diagnosis = 10.2
- LFA Assay Cost per additional diagnosis is $20 (95% CI: $16 to $26).
  - $2 per Quality-adjusted life year (QALY) saved

Boulware, Meya Unpublished
Implementation challenges- Lab Perspective

• Integration of CD4 testing and CRAG testing
  – Currently, 8 clinics have CD4 testing in one location

• Reflex testing vs Clinician driven
  – Tendency of clinicians to forget
  – Target group to test, ART-naive

• PIMA testing vs FACS calibur
  – Daily volume
  – Electricity supply
  – Reagents/servicing

• Reporting
  – Integrated System
Ugandan Perspective: Challenges

• 1 CRAG negative subject developed CM 2 weeks post ART initiation
  – Repeat CRAG testing if ART start delayed?
  – How often?
  – Long return dates following screening

• Referral to higher level center if Lumbar Puncture not possible
Ugandan Perspective : Challenges

• Staff training
  – not to consider CRAG screening, documentation & follow up as extra work

• Loss to follow up post-registration, with subsequent death
  – mechanism of tracking patients required

• Acceptability of treatment
  – Treating asymptomatic patients

• 800mg fluconazole tablet formulation?
Acknowledgement

• ORCAS team

• IDI

• Kampala Capital City Authority

• CDC/PEPFAR