Laboratory Diagnosis of HIV Co-Infection (and HIV Infection): What’s New?

AWACC 28th September, 2013
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Harvard Medical School
Massachusetts General Hospital
Summary

• Tuberculosis diagnostics
  – Gene Xpert MTB/RIF Assay
  – Urine LAM

• Cryptococcus diagnostics
  – Cryptococcal antigen lateral flow assay

• PCP diagnostics
  – 1-3-D-β-glucan

• HIV diagnostics
  – 4th generation HIV Ab/Ag rapid tests
  – POC CD4 testing
  – What’s in the pipeline
# Tuberculosis Diagnostics Summary

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Gene Xpert MTB/RIF Assay

• Operations
  – 2-hour run time
  – Machines with 1, 4, 16, or 48 modules
  – Requires minimally trained staff, electricity
  – $17,000 per machine in LMIC ($62,000 in UIC)
  – ~$15 per cartridge in LMIC ($120 in UIC)
Gene Xpert MTB/RIF Assay

• Functionality
  – Nucleic acid amplification (RT-PCR) of \( rpoB \) gene (RNA polymerase)
  – 5 probes encompassing 81-bp RNA pol gene
  – Amplification & detection of gene indicates TB
    • Positive control with \textit{Bacillus spp.} organism
  – Failed amplification of an intermediate probe indicates rifampin resistance
TB, RIF-susceptible

TB, RIF-resistant

Gene Xpert MTB/RIF Assay Accuracy

• Overall test performance for pulmonary TB
  – 90% overall sensitivity versus culture
  – 82% overall sensitivity among HIV+

• Smear positive versus negative disease
  – 98% sensitive in smear+/culture+ disease
  – 75% sensitive in smear-/culture+ disease
  • Not divided by HIV-infection

• Detection of RIF resistance
  – 94% sensitive to detect RIF resistance

## Gene Xpert MTB/RIF Assay Accuracy in Smear Negative-Culture Positive Disease

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<tr>
<th>Location</th>
<th>Patient Population</th>
<th>HIV prevalence</th>
<th>n (smear-, culture+ cases)</th>
<th>Sensitivity to detect smear-negative TB</th>
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<tr>
<td>Spain</td>
<td>TB suspects</td>
<td>Not reported</td>
<td>78</td>
<td>61/78 = 78%</td>
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<tr>
<td>Peru, Azerbaijan, South Africa, India</td>
<td>TB suspects</td>
<td>40%</td>
<td>174</td>
<td>121/174 = 73%</td>
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<td>South Africa</td>
<td>TB suspects</td>
<td>70%</td>
<td>18</td>
<td>11/18 = 61%</td>
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<td>72%</td>
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Gene Xpert MTB/RIF Assay in Non-Sputum Samples among HIV+

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<td>Stool</td>
<td>Germany</td>
<td>0%</td>
<td>2/2 = 100%</td>
<td>11/14 = 79%</td>
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<td>Urine</td>
<td>Germany</td>
<td>0%</td>
<td>5/5 = 100%</td>
<td>70/75 = 93%</td>
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<td>Gastric Fluid</td>
<td>Germany</td>
<td>0%</td>
<td>7/8 = 88%</td>
<td>19/19 = 100%</td>
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Gene Xpert Implementation

• Challenges to implementation
  • Study of 402 HIV/TB suspects in Durban with off-site, centralized Gene Xpert testing
  • Among 124 starting TB rx, 32 (26%) based on Gene Xpert vs. 39% based on clinical suspicion and 31% based on smear results

Gene Xpert Relevance

• POC test: results in 2-3 hours
  – 60-70% sensitive for smear -/culture + disease
  – Rifampicin resistance for rx decision
• Reference laboratory test at centralized labs
  – Early diagnosis (compared to culture)
  – Should not replace culture where culture exists
  – Requires reliable, efficient results reporting system to peripheral centers and ultimately to patients
Urine Lipoarabinomannan Assay

• Functionality
  – Lipoarabinomannan: MTB cell wall antigen
    • Also component of Actinomyces
  – Lateral flow antigen detection assay (Alere, Inc.)
  – Point-of-care urine test
  – Results in 25 minutes after sample collection
## Urinary Lipoarabinomannan Assay

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Urinary Lipoarabinomannan Assay

• LAM results dependent on immune status
  – Sensitivity varies by CD4 count
    • CD4<50: 12/18 = 67%
    • CD4<100 15/29 = 52%
    • CD4<200 23/59 = 39%
    • CD4>200 1/25 = 4%

• Highly immunogenic
  – LAM antibodies detected in TB patients
  – LAM-antibody complexes decrease urinary excretion of LAM

Combining LAM with Smear for POC Diagnosis

• Prospective study of 208 TB suspects in Uganda
  – 101 confirmed TB, 107 without TB
    • Median CD4: 60
  – Sensitivities: Xpert 77%, LAM 50%, Smear 31%
  – Smear + LAM: 68% (similar to Xpert)
  – Xpert + LAM: 87% (superior to either test alone and similar to culture)

Cryptococcal Disease: The New Prevention Paradigm

- CRAG in blood & urine predates meningitis by weeks to months, enabling screening and pre-emptive treatment
- WHO and South African Clinicians’ Society now recommend blood CRAG screening for all HIV+ with CD4<100
  - South Africa, Kenya, Rwanda established screening programs
  - Many other countries in development
- Screening and pre-emptive therapy are highly cost effective (estimates: $77-266/life saved; $2-20/DALY saved)

Rajasingham et al, JAIDS, 2012
Meya et al, CID 2010
French et al, AIDS, 2002
Cryptococcal Meningitis Prevention: A New Paradigm

- Cryptococcal antigen (CRAG) positive prevalence 5-20% in SSA and SE Asia among those with advanced disease

Rajasingham et al, JAIDS, 2012
CRAG Lateral Flow Assay

• Lateral flow CRAG assay recently developed
  – Similar method to TB LAM (Immy, Inc.)
  – Detects F12D2 and 339 antigens of the cryptococcal polysaccharide capsule glucuronyxylomannan
  – Results in <15 minutes after sample collection
  – Valid for whole blood, serum, urine, cerebrospinal fluid specimens
  – $2/test in resource limited settings
  – Titers can be performed for disease burden, prognostication
### CRAG Lateral Flow Assay

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## CRAG Lateral Flow Assay

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<td>South Africa</td>
<td>Hospitalized, Cryptococcal Meningitis</td>
<td>Serum, Plasma, and Urine</td>
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<td>Thailand</td>
<td>Hospitalized, respiratory illness</td>
<td>Serum</td>
<td>CRAG EIA in serum</td>
<td>87/92 (95%)</td>
<td>371/373 (99%)</td>
</tr>
<tr>
<td>“</td>
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<td>Urine</td>
<td>CRAG EIA in blood</td>
<td>52/74 (70%)</td>
<td>N/R</td>
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CRAG Lateral Flow Assay

• Evidence for superiority of the LFA
  – In paired testing, ~5x higher titers than standard latex agglutination
  – Potentially positive earlier than culture in CSF
    • One study 2/112 specimens LFA+/culture negative, were culture+ 14 days later

Jarvis et al, *CID* 2011
Kabanda et al, *CID*, 2013
**Pneumocystis jirovecii Pneumonia:**

- How common is PCP in sub-Saharan Africa?

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<tr>
<td>Senegal</td>
<td>2008</td>
<td>62</td>
<td>19%</td>
<td>3/37 (8%)</td>
<td>PCP immunostain</td>
</tr>
<tr>
<td>Central African Republic</td>
<td>2008</td>
<td>199</td>
<td>40%</td>
<td>2/48 (4%)</td>
<td>PCP immunostain</td>
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<tr>
<td>Uganda</td>
<td>2012</td>
<td>74</td>
<td>60%</td>
<td>5/132 (3%)</td>
<td>PCR/Giemsa stain</td>
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PCP Diagnosis

• Lactate dehydrogenase
  – Highly sensitive (>90%) but lacks specificity in ill patients

• Microbiology (Geimsa stain, immunostain, PCR)
  – All with high sensitivity and specificity
  – Specimen (normal sputum vs induced sputum vs bronchoscopy) and reader dependent
1-3-D-β-Glucan

• Fungal cell wall component (not of cryptococcus)
• Serologic blood test (colorimetric test involving a cleaved enzyme), interpreted by spectrophotometry
1-3-D-β-Glucan

• Accuracy in HIV+ patients with suspected OIs (n=252):
  – Sensitivity 92%, Specificity 65% (threshold >80 pg/ml)
  – PPV 85%, NPV 80% (PCP prevalence 69%)

• Accuracy in HIV+ patients with respiratory symptoms (n=159):
  – Sensitivity 93%, Specificity 75% (threshold >80 pg/ml)
  – PPV 96%, NPV 60% (PCP prevalence 87%)
  – If prevalence 10%: 30% PPV, 99% NPV
HIV Diagnosis

• WHO Guidelines
  – HIV 1/2 serology-based testing algorithm
• Prior US guidelines
  – HIV 1/2 serology
  – Confirmatory test
    • Western blot (≥2 bands)
    • Immunofluorescence
    • P24 Ag (Genprobe)
HIV Diagnosis

N: Nucleic acid amplification test; Numbers refer to generation of HIV antibody testing

Nucleic acid (RNA)
- IgG/IgM + p24 Ag
- IgG/IgM (HIV 1 and 2)
- IgG Recombinant virus
- IgG Viral lysates

Days After HIV Infection

HIV Diagnosis

- Updated 2013 CDC HIV Diagnosis Algorithm
HIV Diagnosis

• Implications of 4\textsuperscript{th} gen HIV testing in resource limited settings
  – Feasibility depends on viral load testing capacity and strong linkage to care systems
  – If no viral load testing available, difficult to interpret positive Ab/Ag test with discordant negative confirmatory immunoassay
  – If linkage systems are poor, communication of viral load result and need for HIV care challenging
  – Management of acute HIV infection remains highly debated
Point of Care CD4 Testing

• PIMA Test (Alere, Inc) point-of-care CD4

• Operations
  – Finger stick or venous blood
  – 20 minute time to result
  – $5000 machine and $6/test cartridge
Point of Care CD4 Testing

• PIMA POC accuracy in resource limited settings
  – Thai validation study in venous specimens
    • n=203, median CD4 500 (range 4–2000)
    • High overall correlation with FACS, $r^2 = 0.95$
    • Systematic negative bias with PIMA
      – PIMA CD4 counts averaged ~50 cells less than FACS scanners
      – Bias was less (~20 cells) when restricted to CD4 0-350
  – Senegal validation study in fingerstick specimens
    • n=95, median CD4 364, IQR 212-550
    • Pearson’s correlation=0.89
    • Average negative of bias of 39 cells with PIMA vs FACS
    • 91% sensitivity, 97% specificity to detect CD4 < 200
    • 91% sensitivity, 79% specificity to detect CD4 < 350
Point of Care CD4 Testing

• Role in linkage to care
  – Observational cohort study of 932 HIV-infected patients enrolling in care in Mozambique
  – Improvements in major programmatic indicators
    • LTFU decreased from 57% to 21%
    • Time from enrollment to ART initiation: 48 to 20 days
    • Primarily decrease in time to staging: 32 to 3 days

Technology Pipeline – Viral Load

- Liat
- WAVE 80
- EOSCAPE
- Micronics
- ALL
- Alere
- SAMBA VL
- Cavidi AMP
- Gene Xpert
- Biohelix
- NWGHF VL
- Lumora
- 2012
- 2013
- 2014
- 2015
- 2016

*Estimated - timeline and sequence may change
Point of Care Resistance Testing?

• Multiplex PCR-like technology
  – “Gene Xpert” for HIV

• Complex problem
  – Regimen specific
    – Although some classes (e.g. NNRTIs/INSTIs) have highly predictive patterns, others (e.g. protease inhibitors, NRTIs) are complex
  – Other classes (e.g. entry inhibitors) do not rely on genetic mutations to determine resistance
Summary

• TB diagnosis: Gene Xpert MTB/RIF
  – Detects 65% of smear-/culture+ in HIV+ as POC test
    • Accurate RIF-susceptibility testing
  – Depends on communication system to augment culture as a centralized laboratory assay
  – Accuracy in non-sputum specimens to be determined

• TB diagnosis: Urine LAM
  – POC care, $3-4, no lab infrastructure required
  – 30-60% sensitive versus culture for pulmonary TB in advanced HIV disease
  – Highest utility in hospitalized patients or CD4<50
  – Sensitivity decreases with increasing CD4 count (approaches 0% with CD4 > 200)
Summary

• Cryptococcal CRAG assay
  – $2/test, <15 minutes, no infrastructure required
  – Near 100% sensitivity in blood, urine, CSF for meningitis
    • Titers predict mortality
  – ~95% sensitive in blood, ~70-80% sensitive in urine for cryptococcemia/earlier stages of disease

• β-glucan for diagnosis of PCP
  – Blood spectrophotometry assay (requires lab infrastructure)
  – Usefulness limited in low-prevalence settings
Summary

• HIV diagnosis
  – 4th generation HIV tests detect both IgM/IgG antibodies and p24 antigen
  – Detect HIV infection at ~20 days
  – Discordant results challenging to interpret in absence of VL testing

• PIMA Point-of-Care CD4 testing
  – Rapid, low-cost test ($6/specimen)
  – Good overall correlation, with systematic bias towards lower CD4 counts (mostly at higher CD4 counts)
  – Implementation can improve linkage to care
Thank you!

• Conference Organizers
  – Raj Gandhi, Henry Sunpath, Yunus Moosa Karen Moodley

• Clinical Mentors
  – Raj Gandhi, Nesli Basgoz, Cameron Ashbaugh, Paul Sax

• Research Mentors
  – David Bangsberg, Alexander Tsai, Jessica Haberer, Jeffrey Klausner

• Life Mentors
  – My wife and daughter
Questions?
References: HIV/TB Diagnosis


References: MTB/RIF Gene Xpert


References: Urine LAM TB Test


References: CRAG LFA


References: PCP Diagnosis and Epidemiology in SSA


References: CD4 POC Testing

