State of the Art and Unmet Diagnostics Needs for Respiratory Diseases

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Presentation Outline

- Why respiratory diseases?
- Diagnostics for viral respiratory infections
- Non-etiologic advances in diagnostics for pneumonia
  - Improvements in measurement of respiratory rate
  - Differential diagnosis of bacterial vs. viral infections
  - Use of ultrasound as an alternative to chest X-ray for diagnosis of pneumonia
- Etiologic advances in diagnostics for pneumococcal pneumonia
  - Molecular methods
  - Urine antigen
- Conclusions
Acute respiratory infections – the leading infectious cause of death

Estimates for adults 2002; under 5's 2008; World Health Report 2004/10
## Definitions & Terms – Acute Respiratory Tract Infections

### Upper Respiratory Tract Infections
- **Involves:** everything above vocal cords
- **Viruses** - rhinovirus, parainfluenza virus, coronavirus, adenovirus, respiratory syncytial virus, coxsackievirus, and influenza virus
- **Bacteria** – Group A streptococci, *M. catarrhalis*, non-typable hemophilis

### Lower Respiratory Tract Infections
- **Involves:** portion of trachea, bronchi
- Lungs. Results in:
  - Pneumonia
  - Bronchitis
  - Lung abscess
- **Viral infections** – influenza, RSV
- **Bacteria** – *S. pneumoniae*, *Hemophilus influenzae*, *M. catarrhalis*
Diagnosing Respiratory Diseases

Blood culture positive in <10% of cases

More invasive lung aspirates
Viruses, bacteria and fungi detected in 1003 NPA samples from South Africa using Mass-Tag PCR

- 64% of patients are infected with multiple bacteria
- 34% co-infected with multiple viral infections

Lipkin 2011, unpublished data
Diagnosis of Viral Respiratory Infections
Nucleic acid based amplification tests (NATs) for respiratory viruses form the backbone of clinical virology laboratory testing around the world.

Other Diagnostic Approaches

- Viral isolation in cell culture
- Shell vial culture
- Antigen detection by direct fluorescent antibody staining
- Rapid Enzyme Immunoassay Assays

NATs for respiratory viruses

Advantages

- Improved sensitivity over other diagnostic approaches
- Significant multiplexing capabilities - multiplex PCR assays introduced in the last five years detect up to 19 different viruses in a single test.
- Demonstrated viruses play significant role in pneumonia disease burden
- Detection of viral RNA in asymptomatic patients

Disadvantages

Future Expansion

The addition of allelic discrimination and detection of single nucleotide polymorphisms associated with antiviral resistance together with viral load assays will be the future expansion of these assays.

Pneumonia Diagnosis

- Non-etiologic advances in diagnosis
- Etiologic advances in diagnosis
Estimated number of episodes of severe ALRI worldwide in children <5 years in 2000-13.1 (10.1-19.6) million (Rudan et al., 2008)

Estimated number of episodes of severe ALRI worldwide in children <5 years in 2010-19.2 (15.6-23.8) million

Burden on hospital in-patient services

- Number of cases that reach hospitals 11.9 (10.3-13.9) million (62%)
- Case-fatality ratio in hospitalized cases 2.1 (1.4 - 3.1)%
- Number of in-hospital deaths 0.27 (0.16-0.45) million (19%)

Burden not accessing hospital in-patient services

- Number of cases that do not reach hospitals 7.3 (5.3-9.9) million (38%)
- Case-fatality ratio in communities 15.4 (10.4-22.5)%
- Number of deaths outside hospitals 1.13 (1.03-1.19) million (81%)

Estimated (severe) ALRI deaths in children <5 years in 2010-1.40 (1.19-1.64) million (Liu et al., 2012)

Source: Nair et al., 2013
Measuring Respiratory Rate & Oxygen Saturation

Challenges of ARI Timer & Counting Beads
- Timer ticks for 60 seconds, but beeps at half way mark, often startling child & parent
- Short battery life
- Both ARI timer & counting beads require the child to remain still
- Visual determination of counts is required with both methods
- Child’s caretaker often don’t believe result, if antibiotics are not indicated.

State of the Art
- Fully automated devices, with on board data processing capabilities to determine respiratory rate
  - Cell phone based “apps”
  - Accelerometry
  - Other technical approaches – extrapolate respiratory rate from lung sounds or the plethysmographic wave of pulse oximetry
Differential Diagnosis of Bacterial vs. Viral Infection

- Rapid, simple to use test to indicate if antibiotic therapy should be administered
- Measures levels of host response biomarker in patient sample
  - fingerstick blood
- Will use inexpensive diagnostic platform, such as lateral flow
  - Could be combined with malaria RDT

Progress to Date

- Several biomarkers identified & demonstrated encouraging data (> 90% sensitivity; >95% specificity) with samples from Tanzanian Cohort
- Additional validations planned for early 2014
Lung ultrasound: Abnormal causes artifacts

- Lung disease is a disruption of the air/tissue ratio: fluid, pus, blood, fibrosis
Preliminary results in children show high concordance with radiographically confirmed pneumonia

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>AUC</th>
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</thead>
<tbody>
<tr>
<td>All pneumonia</td>
<td>92 (86-95)</td>
<td>98 (92-99)</td>
<td></td>
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<tr>
<td>Lobar pneumonia</td>
<td>95 (89-98)</td>
<td>98 (92-99)</td>
<td>0.96 (0.94-0.99)</td>
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<tr>
<td>Interstitial pneumonia</td>
<td>84 (71-92)</td>
<td>98 (92-99)</td>
<td>0.91 (0.86-0.96)</td>
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Courtesy Will Checkley, JHSPH - final results pending
Etiological Role of the Pneumococcus in Pneumonia

- Our tools to identify the pneumococcus as a cause of pneumonia are insensitive.
- Blood culture identifies less than 10% of presumed pneumococcal pneumonia.
- Lung puncture, BAL and protected specimen brush techniques are rarely done and are overly invasive.
- Serological tests have been confounded by lack of specificity.
- Urine antigen is promising in adults but is confounded by NP carriage in children; serotype specific assays are in development.
- PCR of blood was no better than culture – quantitative RT-PCR may be more promising in blood; very useful in empyema.
- Proteomic and pneumococcal gene expression studies are in the experimental stage.
rtPCR in blood
Improved Detection of IPD in Blood by qPCR Using *lytA* Gene

rtPCR in NP Swabs
NP colonization densities in HIV–infected patients with pneumococcal community-acquired pneumonia (CAP) and HIV-infected asymptomatic controls

Urine Antigen
Luminex monoclonal Ab based serotype specific UAD is highly specific and a significant advance in the detection of non–bacteremic pneumococcal pneumonia in adults

**Reported Performance of Assay**

- 1,095 CAP patients - *S. pneumoniae* identified in 257 patients (23%), using conventional diagnostic methods; in 357 (32.6%) when UAD was added.
- 48/49 bacteraemic episodes caused by the 13 serotypes covered by the UAD were detected - **specificity 100%**.
- 0/77 CAP episodes with a ‘non-UAD’ had a positive UAD result - **specificity 100%**.
- HIV infected adults and controls in South Africa
  - UAD positive in 6/7 (85.7%) patients with bacteremia due to a PCV13 serotype
  - UAD detected significantly more cases than the **composite** 106/235 (45.1%) vs 71/235 (30.2%); p<0.001
- Of 615 blood cultures only 12 (2.0%) had positive blood cultures for *S. pneumoniae*. **11 (91.7%) had positive UAD results**, and 5 (41.7%) had positive BinaxNOW® results.

**Test Validations**

- **Netherlands**
- **South Africa**
- **United States**

Data from evaluation of the assay in children will be available at the end of 2013
Conclusions

- Molecular methods are the state of the art to make etiological diagnosis of viral respiratory infections

- Serotype specific urine antigen detection shows promise and data from evaluation in children will be available by end of year

- Mobile phone technology and new medical devices are the new state of art being applied to the non-etiologic diagnosis of pneumonia
Thank you!

Melinda French Gates visits Mbagathi Hospital, Kenya to better understand the challenges associated with management of severe pneumonia.