Interpretation of multistage PCR to detect respiratory viruses in patients with respiratory failure

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Conflicts of Interest

Nothing to declare related to this presentation
Acute infections

75%

Acute respiratory disease (ARD)

80%

Viral infections

Upper respiratory tract infections (UTRIs)

Acute viral respiratory tract infection is the leading cause of hospitalization for infants and young children in developed countries and is a major cause of death in developing countries.
Discovery of respiratory viruses

• The diagnostics of respiratory viral infections began in 1933 by the discovery of influenza A virus
• In the 1990s, a new era began in viral diagnostics due to the development of PCR techniques.
Respiratory virus pathogens

“Classical” respiratory viruses

- Influenza virus A, B, C,
- Rhinoviruses groups A & B (>100 serotypes),
- Respiratory Syncytial virus, (RSV) A and B,
- Parainfluenza virus (PIV) 1, 2, 3
- Adenoviruses (> 50 serotypes),
- Coronaviruses (initial viruses: 229E & OC43),
- Enteroviruses (~10 serotypes)

New respiratory viruses

- Parainfluenza virus 4,
- human Metapneumovirus (hMPV),
- Rhinoviruses groups C & D
- Torque Teno virus (TTV)
- Coronaviruses
  - NL63 and HKU1
  - SARS CoV
  - Middle East Respiratory syndrome coronavirus (MERS-CoV) 2012
- Bocavirus
- Polyomavirus KI & MU
- Influenza virus H1N1 2009
- Avian influenza A H7N9 2013
- H10N8 etch

Emerging viral respiratory pathogens

Figure: Geographical distribution of human cases of emerging respiratory viruses
Respiratory viral infections: is laboratory diagnosis necessary?

- RVI are largely benign and self limited but

- Newer studies have highlighted the Importance of respiratory viruses as pathogens in children and adults pneumonia

Pathogens in bold are thought to be the most common etiologies. hMPV, human metapneumovirus; PIV, parainfluenza virus 1, 2, 3; RSV, respiratory syncytial virus.
Respiratory viral infections: is laboratory diagnosis necessary?

- RVI are largely benign and self-limited but...

- Newer studies have highlighted the importance of respiratory viruses as pathogens in adults and children pneumonia

- RVs can exacerbate underlying chronic cardiopulmonary diseases

- Possibly fatal complications among patients at the extremes of age with underlying diseases or those with immunodeficiency, these illnesses can be associated with

- Unnecessary prescriptions of antibiotics (most important in the outpatient setting)

- Implications in isolating infected patients in hospitals or in long-term care settings to prevent transmission of disease
Laboratory diagnosis

Direct methods
- Electron microscopy
- Culture in cell lines
  - conventional
  - shell vial centrifugation culture

Indirect methods
- Serology

Non-culture-based methods
- Antigen detection
  - Immunofluorescence
  - Enzyme immunoassay
  - Immunochromatography

Molecular methods
- DNA/RNA detection
Laboratory diagnosis

Serological tests are seldom useful for general diagnostic purposes

- Test is not available or not used for lab diagnosis of infection
- Requirement for paired sera
  - Acute and convalescent-phase serum samples collected at least 10 days apart are needed to detect a significant (fourfold or greater) increase in serum antibody levels

Indirect methods

Serology

Antibodies detection with
- Enzyme immunoassay (EIA)
- Immunofluorescent assay (IFA)

Epidemiological studies

Retrospective diagnosis (influenza viruses)
Laboratory diagnosis

Direct examination

Specimen collection, transport and processing
• Collection during the first days of illness (maximum viral load)
• Technique
  • nasopharyngeal swab (older children and adults)
  • nasopharyngeal aspirates (infants and young children)
  • Oropharyngeal swab
  • Nasal wash
  • Throat swab (not traditionally accepted)
  • BAL fluid
• Transport to the lab ASAP and storage at
  • 4°C for < 48h (DIF, cell culture)
  • -70°C (viability), -20°C (EIA and PCR)
Laboratory diagnosis

Direct methods

Viral detection direct in specimen

Antigen detection

Influenza A & B
Parainfluenza 1, 2, 3, 4
RSV
Adenovirus
hMTV

Advantages:
Sensitive and specific method
Rapid method (2-4 h to perform)
Sample quality can be determined

Disadvantages:
Need for specialized equipment
Impact of technician expertise on assay performance

FA assay identify viral Ag present on, or in, infected exfoliated epithelial cells present in specimen

The fluorescent staining appears predominantly in the cytoplasm of the cells
Laboratory diagnosis

Direct methods

Viral detection direct in specimen

Antigen detection

Enzyme immunoassay

Immunochromatography
• cart, tube, stick
• results within 15-30min
• some can be used as POC tests

Immunofluorescence
• Direct & indirect fluorescent-antibody (FA) staining

Influenza A & B
Parainfluenza 1, 2, 3, 4
RSV
Adenovirus
hMTV
Laboratory diagnosis

Direct methods

Viral detection direct in specimen

Antigen detection

Immunofluorescence
• Direct & indirect fluorescent-antibody (FA) staining

EIA

Rapid tests

Rapid Ag Detection: PPV is low when prevalence is low

Viral isolation

Culture in cell lines
- conventional cell culture
- shell vial centrifugation culture

Disadvantages:
- Time consuming method (5-21 days)
- Culture is not available for all RVs
- Need for more than one cell lines

Virus isolation in cell culture and related techniques may remain important in special situations, for example in order to identify unknown infectious agents, where identification of the SARS corona virus is an important example.
Laboratory diagnosis

Direct methods

Viral detection direct in specimen

Molecular methods

DNA/RNA detection

- Detection of all viruses
- More sensitive and specific for the detection of
  - RSV
  - Influenza virus
  - PIV
  - Adenovirus
- Sensitivity in the detection of low viral load
- Detection of more than 20 different agents in the same sample.
PCR methods used in laboratory diagnosis of respiratory viruses.


<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional PCR</td>
<td>Usually evaluated by colorometry or agarose gel electrophoresis</td>
<td>No quantitation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High risk of cross-contamination, especially when using nested format</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Absolute</td>
</tr>
<tr>
<td>Real-time PCR</td>
<td>PCR products evaluated in real-time during each PCR cycle</td>
<td>Suitable for limited multiplexing</td>
</tr>
<tr>
<td></td>
<td>Evaluation either by colorometry or by specific, light-emitting probes</td>
<td>Permits quantification or semiquantitation of virus</td>
</tr>
<tr>
<td></td>
<td>(e.g., hydrolysis of FRET probes)</td>
<td>Highly specific provided that specific probes are used</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Very low risk for cross-contamination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Excellent for in-house tests, resulting in an affordable analysis for the clinic</td>
</tr>
<tr>
<td>Multiplex PCR linked with liquid array (Luminex®; Luminex Corporation); commercial systems: X-TAG® RVP (Abbott) ResPlex® (Qiagen) MultiCode®-PLx (Edana Biosciences)</td>
<td>Traditional PCR designed to affix amplicons to specific beads in a highly multiplexed format</td>
<td>Excellent multiplexing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Several commercial tests available</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No quantification option</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exogenous handling of amplified materials results in risks for cross-contamination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Need for commercial systems not cost-compatible</td>
</tr>
<tr>
<td>NASBA; LAMP</td>
<td>Isothermal cyclic amplification involving DNA (LAMP) or RNA/DNA (NASBA) intermediates</td>
<td>No need for thermal cycler</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No reverse transcriptase step for RNA viruses†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High sensitivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Suitable for in-house tests, resulting in an affordable analysis for the clinic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quantitation cumbersome</td>
</tr>
<tr>
<td>Rapid diagnostic PCR instruments:</td>
<td>Integrated extraction and PCR run in preloaded reaction cassettes, requiring a minimum of hands-on time</td>
<td>No specimen batching needed</td>
</tr>
<tr>
<td>Xpert® (Cepheid)</td>
<td></td>
<td>Suitable for point-of-care testing</td>
</tr>
<tr>
<td>FilmArray® (Idaho)</td>
<td></td>
<td>Locked to commercial assays linked to the instrument</td>
</tr>
<tr>
<td>Jaguar® (BD diagnostics)</td>
<td></td>
<td>Relatively expensive</td>
</tr>
<tr>
<td>Infiniti® (AutoGenomics)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

†Nucleic acid sequence-based amplification only.

For references, see section ‘Procedures for molecular diagnosis of respiratory viruses’ in text.

FRET: Fluorescence resonance energy transfer; LAMP: Loop-mediated isothermal amplification; NASBA: Nucleic acid sequence-based amplification.
There are a wide array of emerging technologies for the detection and quantification of respiratory pathogens directly from clinical specimens. Some of these technologies have potential for high-throughput testing, and others will allow rapid near patient testing.

A broad diagnostic panel, preferably consisting of 15 or more agents, offers an additional diagnostic value:
1. a negative result is more valid if many agents have been targeted,
2. the quantitative component of the test improves the interpretation of a positive result, not least if several agents are detected and the Ct values of these agents can then be compared.
### Run Summary

<table>
<thead>
<tr>
<th>Sample ID:</th>
<th>14725</th>
</tr>
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<tbody>
<tr>
<td>Detected:</td>
<td>None</td>
</tr>
<tr>
<td>Equivocal:</td>
<td>None</td>
</tr>
</tbody>
</table>

| Run Date: | 06 Feb 2015 7:46 AM |
| Controls: | Passed |

### Result Details

<table>
<thead>
<tr>
<th>Result</th>
<th>Interpretation</th>
<th>Call</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Detected</td>
<td>Adenovirus</td>
<td>Negative</td>
<td>Adeno</td>
</tr>
<tr>
<td>Not Detected</td>
<td>Coronavirus 229E</td>
<td>Negative</td>
<td>CoV-229E</td>
</tr>
<tr>
<td>Not Detected</td>
<td>Coronavirus HKU1</td>
<td>Negative</td>
<td>CoV-HKU</td>
</tr>
<tr>
<td>Not Detected</td>
<td>Coronavirus NL63</td>
<td>Negative</td>
<td>CoV-NL63</td>
</tr>
<tr>
<td>Not Detected</td>
<td>Coronavirus OC43</td>
<td>Negative</td>
<td>CoV-OC43</td>
</tr>
<tr>
<td>Not Detected</td>
<td>Human Metapneumovirus</td>
<td>Negative</td>
<td>MPV</td>
</tr>
<tr>
<td>Not Detected</td>
<td>Human Rhinovirus/Enterovirus</td>
<td>Negative</td>
<td>Entero1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Entero2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>HRV1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>HRV2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>HRV3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>HRV4</td>
</tr>
<tr>
<td>Not Detected</td>
<td>Influenza A</td>
<td>Negative</td>
<td>FluA-H1-2009</td>
</tr>
<tr>
<td>Not Detected</td>
<td>Influenza B</td>
<td>Negative</td>
<td>FluB</td>
</tr>
<tr>
<td>Not Detected</td>
<td>Parainfluenza Virus 1</td>
<td>Negative</td>
<td>PIV1</td>
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<tr>
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<td>PIV3</td>
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<tr>
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<td>Parainfluenza Virus 4</td>
<td>Negative</td>
<td>PIV4</td>
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<tr>
<td>Not Detected</td>
<td>Respiratory Syncytial Virus</td>
<td>Negative</td>
<td>RSV</td>
</tr>
<tr>
<td>Not Detected</td>
<td>Bordetella pertussis</td>
<td>Negative</td>
<td>Bper</td>
</tr>
<tr>
<td>Not Detected</td>
<td>Chlamydia pneumoniae</td>
<td>Negative</td>
<td>Cpne</td>
</tr>
<tr>
<td>Not Detected</td>
<td>Mycoplasma pneumoniae</td>
<td>Negative</td>
<td>Mpne</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Result</th>
<th>Control</th>
<th>Call</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pass</td>
<td>PCR2 Control</td>
<td>Positive</td>
<td>PCR2</td>
</tr>
<tr>
<td>Pass</td>
<td>RNA Process Control</td>
<td>Positive</td>
<td>yeastRNA</td>
</tr>
</tbody>
</table>
Practical aspects on molecular diagnosis of infections with respiratory viruses

- **Disease episodes & virus shedding**
  - The detection frequency for enveloped respiratory viruses decreased from more than 51 to 30% for sampling on days 0–6 versus 7–14 from symptom onset
  - Some respiratory viruses, such as bocaviruses, may be shed for several months

- **Significance of a positive test result**
  - An obvious risk with a sensitive assay is that a positive result may reflect a clinically irrelevant *carriership* rather than a symptomatic infection
  - HRV may remain detectable by PCR methods for 2 weeks or more after the onset of symptoms
  - Immunocompromised individuals may shed detectable amounts of respiratory viruses
  - Quantitative methods, such as real-time PCR, offer additional diagnostic value over nonquantitative methods, because the quantity of the agent detected may aid in distinguishing a true etiological agent from an innocent bystander
Sampling and manufacturing obstacles

• Getting specimens from the site of infection that are not contaminated with upper respiratory tract flora is a constant problem.
• Endotracheal aspirates from ventilated patients are often of better quality than that of expectorated sputum from patients with HAP but may still be contaminated with upper respiratory tract flora.
• BALs and protected brush samples are more likely to yield samples from the site of infection but require significantly more effort to obtain and thus offer a much smaller market for a new molecular test.
How to exploit PCR results into clinical practice?
Right Cohorting of patients

- Nosocomial viral infections impose a substantial burden particularly in children's hospitals and pose a particular risk to immunocompromised children.
- Early identification allows effective cohorting and isolation.
- The practice of cohorting children by syndrome without a viral diagnosis exposes them to substantial risk of acquiring a new virus during hospitalization.
- Virus-specific cohorting should reduce this risk.
Transplant recipients

- For transplant recipients with respiratory infection, the ability to detect the full range of viral pathogens is critical.
- The known respiratory viral pathogens should be sought in addition to cytomegalovirus, Epstein Barr virus, human herpesvirus type 6, and herpes simplex virus.
- Highly sensitive multiplex platforms offer obvious advantages over the combination of direct fluorescent antibody testing (DFA), culture, and individual PCR assays.
How to interpret the presence of specific viral pathogens
The role of respiratory viruses in severe pneumonia

• Respiratory viruses are a very important cause of severe pneumonia and respiratory failure in immunocompromised patients, particularly hematopoietic stem cell transplant recipients

Kim YJ, Semin Respir Crit Care Med. 2007;28:222-42.
The role of respiratory viruses in CAP (and how can we interpret a positive NAAT)

- can be the sole cause of a viral pneumonia (often referred to as primary viral pneumonia),
- can be present as a co-infection (virus-bacteria or virus-virus),
- can act as a predisposing factor to facilitate or worsen bacterial pneumonia
- detection of some viruses in the upper respiratory tract of asymptomatic patients is relatively common and therefore may indicate convalescent shedding or asymptomatic infection

Jartti T et al 2008
Viruses associated with pneumonia

**Common**
- Respiratory syncytial virus (RSV)
- Influenza virus A and B
- Human metapneumovirus*
- Adenovirus
- Parainfluenza virus 1, 2, 3, and 4*
- Human Coronavirus types 229e, OC43, HKU1*, NL-63*
- Rhinovirus*
- Bocavirus*

**Less common or predominantly in specific hosts or settings**
- Measles
- Cytomegalovirus
- Varicella zoster virus
- Herpes simplex virus
- Epstein Barr virus
- Hantavirus
- Enterovirus
- Parechovirus*
- SARS coronavirus

* Recently described and/or of unknown significance
How can we determine if the detection of a virus in the airway in a patient with CAP indicates a causal role?

- A problem common in interpreting prospective studies and tests in individual patients.
- More difficult for rhinovirus and coronaviruses that have been detected in 2-45% and 0-6% of asymptomatic subjects respectively by PCR than for influenza and hMPV, which are rarely detected in the absence of symptoms.
- Some studies have shown higher viral loads in patients with pneumonia, suggesting that quantitative assays might increase the specificity.

DeVincenzo JP, J Infect Dis 2005
Martin ET, Diagn Microbiol Infect Dis. 2008
Jartti T, Paed infect Dis J 2008
Influenza virus

- Pneumonia was recognized as a complication of influenza during the pandemic of 1918-1919, long before the virus was identified.
- Among patients hospitalized with influenza, radiographic pneumonia has been reported in 16-55%.
- Patients admitted with influenza who have pneumonia are at risk to be admitted to ICU or die.

Lee N, J Infect Dis 2011
Pavia A, Infect Dis Clin N Am 2013
Morens DM, J Infect Dis 2008
Therapeutic implications of a “positive” multiplex PCR

The Influenza model

• Studies in both seasonal and pandemic 2009 H1N1 influenza showed that hospitalized patients treated with neuraminidase inhibitors had decreased ICU admissions and mortality.
• Benefits were independently demonstrated among children, pregnant women, and critically ill patients.
• Earlier initiation of therapy is associated with the greatest benefit, but among hospitalized patients, benefits were observed when oseltamivir was started as late as 5 days after symptom onset compared to no therapy.

Kumar A, JAMA 2009
Louie JK, Clin Infect Dis 2012
Siston AM, JAMA 2010
Lee N Thorax 2010
Jain S NEJM 2009
Yu H Clin Infect Dis 2011
Adenovirus

- Adenoviruses are non-enveloped DNA viruses
- More than 50 serotypes have been described since 1953
- Historically, adenovirus pneumonia has been primarily documented among children, immunocompromised adults, and outbreaks in hospitalized patients and healthy adults in closed settings such as military recruits
- Adenoviruses cause a wide variety of infections including
  - conjunctivitis, epidemic keratoconjunctivitis, pharyngitis, URI, pneumonia, meningitis, hepatitis and gastroenteritis.
- Severe respiratory disease is associated with serotypes 5, 7, 14, and 21
  - In 2005, a new variant of serotype 14 emerged as a cause of severe lower respiratory tract disease in immunocompetent adults in the community and in the military
- The genetic diversity of adenoviruses has limited the sensitivity of culture and PCR-based diagnostics

Potter RN, Emerg Infect Dis 2012
Pavia A, Infect Dis Clinics N Am 2013
Respiratory Syncytial virus

- Paramyxovirus that causes URI, bronchiolitis and CAP in children (3 to 31% of children hospitalized with CAP)
- The incidence and severity varies with age; mostly affects younger children
  - Studies in the mid 1990’s demonstrated that RSV affects also adults (4-7% of adults with CAP)
- RSV-associated CAP more common and severe among older adults.
  - During the 1990s, RSV was associated with an average of more than than 11,000 deaths each year in the United States, the majority of these deaths in persons over 65

Falsey JR, J Infect Dis 1995
Dowell SF, J Infect Dis 1996
Jennings LC, Thorax 2008

Thomson WW JAMA 2005
Falsey JR, NEJM 2005
Johansson N Clin Infect Dis 2010
Human metapneumovirus (hMPV)

- hMPV is a paramyxovirus in the sub-family pneumovirineae (as RSV)
- First described by Dutch researchers first described in 2001 in children with bronchiolitis
- An important cause of acute respiratory infections in children and adults, with a worldwide distribution and a wide array of manifestations from asymptomatic infections to respiratory failure
- Pneumonia is most commonly seen among younger children, older adults, and those with underlying medical conditions
- In prospective studies of adults hospitalized in US incidence of hospitalization for hMPV among persons >65 years old was 220/100,000 compared to 254/100,000 and 123/100,000 for RSV and influenza virus respectively

References:

Williams JV, NEJM 2004
Widmer J, J Infect Dis 2012
Walsh EE Arch Intern Med 2008
Schildgen V, Clin Microbiol Rev 2011
Parainfluenza virus

- Parainfluenza viruses (PIV) are paramyxoviruses that are antigenically divided into 4 serotypes PIV1-4.
- They are common causes of acute respiratory infections including URI, croup, bronchiolitis, and pneumonia.
- Seasonal outbreaks occur in the fall and spring.
- Most pneumonia associated with parainfluenza viruses occurs in infants, young children, and immunocompromised hosts.
- Parainfluenza virus (particularly type 3) has been detected in 0-8% of adults with community acquired pneumonia.

Henrickson KJ. Clin Microbiol Rev 2003
Templeton KE, Clin Infect Dis 2005
Bocavirus

- Human bocavirus is a recently described parvovirus that has been frequently detected in respiratory secretions of children with respiratory tract infection.
- Its role in CAP remains unclear.
- Interpretation is complicated by common detection in asymptomatic children and prolonged detection after infection.
- At least 2 studies showed that isolation of bocavirus alone was more common in ill children than in control subjects.
- Most bocavirus-infected children had a co-infection, but viral loads were higher in the mono-infected children.
- Bocavirus loads were significantly higher in patients with only bocavirus than in those with coinfection.

Fry AM, J Infect Dis. 2007;195:1038-45
Non-SARS Coronaviruses

- Human coronaviruses (HCoV) 229E and OC43 have been long recognized as causes of viral URI and were linked to pneumonia in children and immunocompromised adults.
- Two novel human coronaviruses, NL63 and HKU1 were identified in the past decade. All four human coronaviruses show distinct winter seasonality and affect all age groups.
- An etiologic role in hospitalized CAP was demonstrated in a prospective study from Scotland but not in another from Thailand.
- In a prospective study of patients with severe pneumonia undergoing bronchoalveolar lavage about half of whom were transplant recipients, coronaviruses were detected in 5.8%.
- The role of coronaviruses in pneumonia has not been completely clarified.

Dare RK, J Infect Dis 2007
Garbino J, Clin Infect Dis 2006
Van der Hoek L, Nat Med 2004
Van der Hoek L, J Clin Virol 2010
The recognition of rhinoviruses as important LRTI pathogens

- Recent studies demonstrated that rhinovirus can replicate at higher temperatures and infect the lower respiratory tract

Papadopoulos NG, J Med Virol 1999
Papadopoulos NG, J Infect Dis 2000
Rhinoviruses

- The use of PCR and sequencing has greatly enhanced detection of rhinoviruses in severely ill patients and led to the recognition of a third rhinovirus species, genogroup C
- Many rhinovirus PCR assays also detect other picornaviruses, particularly enterovirus which complicates the literature.

Arden KE, Rev Med Virol 2010
Rhinoviruses

- Studies using PCR consistently identify rhinoviruses in nasopharyngeal or pharyngeal specimens from children and adults with lower respiratory tract infections
- Rhinovirus has also been detected in 4-45% of children and 2-17% of adults with CAP

Louie JK, Ped Infect Dis J 2009
Falsey AR J Infect Dis 2002
Charles PG, Clin Infect Dis 2008
Johnstone J, Chest 2008

Cevey Macherel M, Eur J Ped 2009
Tsolia MN Clin Infect Dis 2004
Templeton KE Clin Infect Dis 2005
Lieberman D, Chest 2010
Rhinoviruses: a causal role in CAP?

- Particularly problematic to assume
- High rate of co-detection of rhinovirus with other viruses and bacteria
- Detection of rhinovirus in asymptomatic patients, representing convalescent shedding or asymptomatic infection.
- Shedding generally does not persist beyond 2-3 weeks, but only few studies have done careful molecular subtyping, to distinguish prolonged shedding from re-infection
Rhinoviruses

- Recent studies have demonstrated an emerging role of rhinoviruses in SARI and CAP.
- Are higher viral loads in the nasopharynx better predictors of lower respiratory tract infection and rhinovirus pneumonia?
- Does co-infection with rhinovirus facilitate infection with a second viral or bacterial pathogen and does rhinovirus co-infection increase the severity?
Severe CAP or HCAP

- This study demonstrated that viral infection is common in adult patients with severe pneumonia.
- About one-third of patients with severe CAP or HCAP had viral infections, and the mortality from viral infection and bacterial infection were comparable.

Choi SH, AJRCCM 2012
**Rhinoviruses as stand-alone cause of SARI**

**TABLE E3. THE NUMBER OF VIRUS TEST-POSITIVE SPECIMENS FOR EACH VIRAL PATHOGEN**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Total (n=72)</th>
<th>BAL fluid only (n=25)</th>
<th>Both BAL fluid and nasopharyngeal specimen (n=15)</th>
<th>Nasopharyngeal specimen only (n=32)</th>
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<tr>
<td>Rhinovirus</td>
<td>17 (23.6)</td>
<td>3</td>
<td>5</td>
<td>4</td>
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<tr>
<td>Parainfluenza virus</td>
<td>15 (20.8)</td>
<td>5</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Type 3</td>
<td>8 (11.1)</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Type 1</td>
<td>5 (6.9)</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Type 2</td>
<td>1 (1.4)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Type 4</td>
<td>1 (1.4)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>13 (18.1)</td>
<td>0</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Influenza virus</td>
<td>12 (16.7)</td>
<td>3</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Influenza A</td>
<td>11 (15.3)</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Influenza B</td>
<td>1 (1.4)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>10 (13.9)</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Respiratory syncytial virus A</td>
<td>4 (5.6)</td>
<td>2</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Respiratory syncytial virus B</td>
<td>6 (8.3)</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>8 (11.1)</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Human coronavirus-OC43</td>
<td>4 (5.6)</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>1 (1.4)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>1 (1.4)</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Data are presented as the number (percentage) of patients.

*Two viruses were identified in 9 patients.*

Choi SE et al, AJRCCM 2012
Rhinoviruses as stand-alone cause of SARI

- Among 17 patients with rhinovirus infection, 13 samples (76.5%) were isolated from bronchoscopic BAL fluid.
- Ten patients (58.8%) had rhinovirus as the only identified pathogen, five (29.4%) had coinfection with other viruses, and two (11.8%) had coinfection with bacteria.
- These results suggest that rhinovirus should be considered as an independent cause of severe pneumonia.
- Furthermore, among the respiratory viruses, rhinovirus infection was associated with the highest mortality (52.9%, 9 of 17).
- Of the 17 patients with rhinovirus infection, the majority of those patients had a severe underlying disease or condition.

Choi SH, AJRCCM 2012
Recognition of true co-infections
Two commercial multiplex RT-PCR were used subsequently: ProFlu plus (ProFlu+, Prodesse, Inc. Gen-Probe, Waukesha, WI, USA), which detects influenza A virus, influenza B virus and respiratory syncytial virus (RSV), and ProFlu influenza A subtyping (ProFlu-ST, Prodesse, Inc. Gen-Probe), which detects seasonal H1, seasonal H3 and new H1 variant subtypes of the influenza A virus.
Evolution of clinical picture during the 1\textsuperscript{st} post pandemic year

- Influenza A (H1N1) 2009 and RSV epidemics peaked simultaneously, affecting a vulnerable population.
- Primary viral pneumonia still predominated among ICU admissions for influenza, whereas cases of severe acute respiratory infection without chest X-ray opacities increased.
- Clinical presentation as severe bronchiolitis and co-infection with RSV in 42.8\% of children <2 years of age in the September 2010-January 2011 season was an observation reported for the first time.
- Such coexistence could not be demonstrated in adults.
Advancing our understanding of respiratory syndromes with multiplex PCR
The Influenza model with other viral co-infections

- Bacterial co-infections doubled (21.7% vs 10%, p >0.20) and the total rate of co-infections (either viral or bacterial) increased almost four-fold (39.1% vs 10%, OR 5.8, 95% CI 1.3-24.8, p 0.01) during September 2010-January 2011
Therapeutic implications of a “positive” multiplex PCR beyond influenza

- Limited therapeutic options for the treatment of other viruses
- Ribavirin has broad antiviral activity in vitro that includes RSV, hMPV, parainfluenza virus and influenza
- Inhaled ribavirin for RSV
  - limited benefits among severely immunocompromised patients
  - Among other populations, the benefits are questionable and the costs and risks limit the use of inhaled ribavirin
- Intravenous ribavirin may have a potential role for overwhelming viral pneumonia in severely immunocompromised patients

Boeckh M, Clin Infect Dis. 2007
Ventre K, Cochrane Database Syst Rev. 2007
Therapeutic implications of a “positive” multiplex PCR beyond influenza

- Palivizumab, a monoclonal antibody directed against the fusion glycoprotein of RSV is recommended for the prevention of RSV hospitalization in specific subgroups of premature infants and infants with some types of congenital heart disease or chronic lung disease.
- It has not demonstrated any value in the treatment of RSV disease

Shachor-Meyouhas Y, Pediatr Blood Cancer. 2011
Therapeutic implications of a “positive” multiplex PCR beyond influenza

- Cidofovir has potent activity against adenovirus and case reports suggest clinical benefit in immunocompromised patients.
- It should be considered for patients with overwhelming adenovirus pneumonia including adenovirus type 14.
- Because of the toxicity and difficulty with administration, cidofovir is not appropriate for CAP in immunocompetent hosts.
- An orally available prodrug of cidofovir, CMX001 is in advanced development.

*Doan ML, J Heart Lung Transplant. 2007*  
*Darr S, Clin Infect Dis. 2008*  
*Toth K, Proc Natl Acad Sci U S A. 2008*
Therapeutic implications of a “positive” multiplex PCR beyond influenza

• Pleconaril is active against picornaviruses including rhinovirus (inhibits viral uncoating)
  ▫ A clinical trial showed reduction in the duration of symptoms for naturally occurring colds
• Most pools of intravenous immunoglobulin (IVIG) have significant titers of antibody, including neutralizing antibody against common respiratory viruses
  ▫ IVIG should be considered for hypogammaglobulinemic and severely immunocompromised patients with viral pneumonia

Hayden FG Clin Infect Dis 2003
Research for influenza

- Combination antivirals
  - iv oseltamivir, iv zanamivir
  - iv peramivir, non inferior to oseltamivir
  - inh Laninamivir, non inferior to oseltamivir
  - iv ribavirin ?

- HAI, siRNA, Pol Inhibitors - favipiravir, sphingosine analog, Μονοκλωνικά Ab, nitazoxanide, DAS 181, AVI-7100

- statins

Clin Infect Dis. 2010;51(10):1167
AAC 2011;55(11):5267-76
Hayden FG. (2012) Influenza & Other Respiratory Viruses, 63-75.
Which patients with CAP should be tested for viral infections and how should it alter clinical care?

- The use of sensitive and specific influenza tests is appropriate for children and adults with CAP during influenza season, and is recommended in recent guidelines.
- In younger children with moderate to severe CAP and immunocompromised patients viral pneumonia is common and testing for an array of viral causes of pneumonia can direct therapy and improve infection control.
- Additional studies are needed to determine the impact of viral testing in other groups.

Bradley JS, Clin Infect Dis 2011
Mandell LA, Clin Infect Dis 2007
Can we distinguish between viral and bacterial pneumonia by use of multiplex PCR?

- Lab only parameters do not seem to effectively differentiate between the two entities
- Epidemiology/season
- Age (extreme ages: prone to viral)
- Clinical features (chest pain, high temperature, rigors/wheezeing)
- Xray (alveolar vs interstitial infiltrates)
- CRP and PCT values
- The combined use of biologic markers and viral testing holds the promise of correctly identifying patients who whom antibiotic exposure can be safely limited

Ruuskanen O, Lancet 2011
Virkki R, Thorax 2001
Gilbert DN, Clin Infect Dis 2011
# Etiology of community acquired pneumonia in hospitalized adults and role of viruses in 6 recent studies

<table>
<thead>
<tr>
<th></th>
<th>Charles (N = 885)</th>
<th>Johansson (N = 184)</th>
<th>Johnstone (N = 193)</th>
<th>Lieberman (N = 183)</th>
<th>Jennings (N = 225)</th>
<th>Templeton (N = 105)</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td>Not stated</td>
<td>Mean 63</td>
<td>Median 71</td>
<td>Not stated</td>
<td>Median 70</td>
<td>Median 60</td>
</tr>
<tr>
<td>Any pathogen</td>
<td>46%</td>
<td>67%</td>
<td>39%</td>
<td>Not stated</td>
<td>58%</td>
<td>76%</td>
</tr>
<tr>
<td>Any bacteria</td>
<td>38%</td>
<td>58%</td>
<td>20%</td>
<td>Not stated</td>
<td>48%</td>
<td>46%</td>
</tr>
<tr>
<td>Any virus</td>
<td>15%</td>
<td>29%</td>
<td>15%</td>
<td>32%</td>
<td>34%</td>
<td>54%</td>
</tr>
<tr>
<td>Co-infection</td>
<td>9%</td>
<td>23%</td>
<td>4%</td>
<td>Not stated</td>
<td>30%</td>
<td>27%</td>
</tr>
<tr>
<td>RSV</td>
<td>2%</td>
<td>4%</td>
<td>3%</td>
<td>7%</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>Influenza virus</td>
<td>8%</td>
<td>8%</td>
<td>4%</td>
<td>4%</td>
<td>12%</td>
<td>10%</td>
</tr>
<tr>
<td>hMPV</td>
<td>NS</td>
<td>2%</td>
<td>4%</td>
<td>1%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>&lt;1%</td>
<td>2%</td>
<td>1%</td>
<td>2%</td>
<td>4%</td>
<td>4%</td>
</tr>
<tr>
<td>Parainfluenza virus</td>
<td>&lt;1%</td>
<td>4%</td>
<td>2%</td>
<td>0</td>
<td>1%</td>
<td>8%</td>
</tr>
<tr>
<td>Rhinovirus/enterovirus</td>
<td>5%</td>
<td>7%</td>
<td>2%</td>
<td>5%</td>
<td>13%</td>
<td>17%</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>NS</td>
<td>2%</td>
<td>2%</td>
<td>13%</td>
<td>2%</td>
<td>13%</td>
</tr>
</tbody>
</table>
Etiology of community acquired pneumonia in hospitalized adults and role of viruses in 6 recent studies

- At least one virus was detected in 15-54%.
- In the 5 studies that reported bacterial etiologies, bacterial pathogens were detected in 20-58% and co-infection was detected in 4-30%.
- Viral infections are generally a more prominent cause of CAP among older adults.
What is the role of mixed infections in CAP?

- Clinical evidence from prospective studies on the role of co-infection on severity is somewhat conflicting.
- There is a suggestion that viral-bacterial co-infection is associated with more severe disease among adults.
- Johansson found that compared to those with only bacterial infections, adults with co-infection were much more likely to have pneumonia severity index (PSI) scores of IV or V and had longer length of stay.
- Templeton reported that age older than 60, rhinovirus in mixed infection, and mixed infection were all associated with PSI score classes IV and V.
- Jennings reported that rhinovirus infection with pneumococcal infection was independently associated with more severe disease by either PSI or CURBAge criteria.
- In contrast, Charles reported similar 30-day mortality among those with co-infection and single infection.
What is the role of mixed infections in CAP?

- The clinical interaction between influenza and *S. pneumoniae* and *S. aureus* has long been appreciated as a major contributor to influenza mortality.
- Similar interactions may occur with other respiratory viruses, including RSV, hMPV and possibly rhinovirus and parainfluenza virus.
Conclusions

Future research on new methodologies for the diagnosis of viral respiratory tract infections should focus on the development:

- of sensitive, rapid and cost-effective test systems allowing the screening for multiple probable causative agents with ability to detect viral load and ideally resistance
- Varying testing protocols for summer and winter months based on epidemiologic data
- Improve current caveats of the nucleic acid based assays: high costs and limited standardization
Conclusions

PCR-based testing has contributed to:

- The detection of newer viral agents
- The identification of new therapeutic targets
- The improvement of our ability to detect “old” viral infections such as influenza virus and rhinovirus
- The improvement of our understanding of respiratory syndromes and their diagnostic and therapeutic challenges

Better ways to diagnose viral CAP and to integrate detection into management are urgently needed.
Thank you for your attention