Recent Advances in the Pathology of Pulmonary Infections

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Current Advances in the Pathology of Lung Infections
Overview - Viral Pneumonias

- Viruses which are identifiable on H&E
- Current Common Diagnostic Tests
  - Direct Fluorescent Assay
  - PCR-based Assays
- Development of the Virochip
  - SARS epidemic
  - Diagnostic uses

Identifiable Viruses

- Viruses can be identified by:
  - Presence of inclusions.
  - Location of inclusions.
  - Cellular changes
    - Multinucleation
    - Cytomegaly

Viral Pneumonias - Histology

- Patterns of Disease
  - Diffuse alveolar damage
  - Fibrinous organizing pneumonia
  - Necrotizing pneumonia
  - Necrotizing bronchiolitis
  - Organizing pneumonia
  - Pulmonary edema
  - Cellular bronchiolitis
  - Cellular interstitial pneumonia (including perivascular)
  - Pulmonary alveolar proteinosis
For Example

• 65-year-old man status post lung transplant
  – 3 months earlier for severe emphysema
• Now with ground glass changes on CT
• Transbronchial and endobronchial biopsies

Viral Pneumonias - Histology

• Patterns of Disease
  – Diffuse alveolar damage
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  – Pulmonary edema
  – Cellular bronchiolitis
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  – Pulmonary alveolar proteinosis
Case 1 - Continued

• While an airway infection is favored, the possibility of rejection was considered.
• Cultures negative.
• Viral tests performed
  – Direct Fluorescent Antibody Assay

Traditional Diagnostic Methods

• Viral isolation and culture
  – 3 or 4 cell lines and embryonated hen eggs
  – 8 to 10 days for culture
• Shell vial cultures in 1990s
  – Combined with monoclonal antibodies
  – 1 to 2 days for identification
• Serologic studies
  – Testing paired acute and convalescent sera

DFA for Viruses

• Lavage cells sent for examination
  – Often performed on nasopharyngeal samples
• Typically tests for eight viruses
  – Respiratory syncytial virus
  – Influenza A and B
  – Parainfluenza types 1, 2, 3
  – Adenovirus
  – Human metapneumovirus
Case 1 - Continued

• While an airway infection is favored, the possibility of rejection was considered.
• Cultures negative.
• Viral tests performed
  — Direct Fluorescent Assay
    • NEGATIVE
  — PCR-based Viral Test

• PCR positive for rhinovirus
• The common cold can be uncommonly troubling for the transplant patient
  — In the current patient, the infection lasted four months.

PCR-Based Viral Test

• Also performed on BAL material
• Uses several primers for each virus (or random hexamers) paired with capture material.
• Typically tests for similar as DFA (plus rhinovirus)
  — Respiratory syncytial virus A and B
  — Influenza A (H1, H3) and B
  — Parainfluenza types 1, 2, 3
  — Adenovirus
  — Human metapneumovirus
  — Rhinovirus

fig. 1. the swine flow.
Severe Acute Respiratory Syndrome

- Nov. 2002 Initial cases in Guangdong province
- Feb. 2003 American businessman dies
- March 2003 WHO issues alert

- 8,273 cases with 775 deaths, 9.4% fatality
  - (2009 Swine Flu 13,000 cases with 100 deaths, 0.7%)

SARS

- Flu-like illness
  - Temperature greater than 38 degrees C
  - Myalgias
  - Lethargy
  - Cough
  - Sore throat
  - Later dyspnea
  - Occasional progression to ARDS

2003 SARS outbreak

- Images of people wearing masks and looking at signs.
Rewind Three Years

- Joe DeRisi
- Was there a way we could test for every virus using a single test?
- Balance between rapid viral evolution and ultraconservation
Original Virochip

- DeRisi and David Wang
- Based on genomes of viruses from known human pathogens.
- Used highly conserved sequences.
  - ~1,600 oligos
  - ~140 viruses

Rapid SARS coronavirus identification

- Feb 2003 ~150 cases and 5 deaths in Guangdong Province, China
- March 12 – Suspected cases in 7 countries; WHO issues SARS global alert
- March 14 – CDC activates emergency operations, joins WHO consortium
- March 22 – DeRisi, with some begging, receives samples from the CDC

Virochip detects oligos from two virus families

<table>
<thead>
<tr>
<th>Oligo ID</th>
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<th>Family</th>
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<tr>
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<td>Bovine coronavirus</td>
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<tr>
<td>1217545_728</td>
<td>Human coronavirus 229E</td>
<td>Corona</td>
</tr>
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Methodology

- Isolate RNA and reverse transcribe into cDNA.
- Amplify by Random PCR.
- Couple fluorescent dyes to DNA.
- Mix fluorescent DNA and hybridize to the microarray.
- Data Analysis.
Five oligos detect the same sequence

Conserved RNA motif shared by 3’ UTRs of astroviruses and several coronaviruses (Jonassen et. al, 1998)

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Virochip suggests a divergent coronavirus

Rapid SARS coronavirus identification

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- March 12 – Suspected cases in 7 countries; WHO issues SARS global alert
- March 14 – CDC activates emergency operations, joins WHO consortium
- March 22 – DeRisi Lab receives samples from the CDC
- March 23 – Virochip results suggest novel coronavirus
- March 24 – CDC: “…a previously unrecognized virus from the coronavirus family is the leading hypothesis…”
Viral sequence recovery

Virochip growth

- Virochip (2001) – David Wang
  - Based on genomes of viruses from families with known human pathogens
  - Most conserved sequences
  - ~1,600 oligos (140 viruses)

- MegaViro (2002)
  - Based on every Reference viral genome in GenBank (human, animal, plant, bacteriophages)
  - ~12,000 oligos (~950 viruses)

- Viro3 (2004) – Kael Fischer
  - Based on every viral sequence in GenBank
  - Most new oligos – genus and species conservation
  - ~20,000 oligos (~1800 viruses)

Rapid SARS coronavirus identification

- Feb 2003 – ~150 cases and 5 deaths in Guandong Province, China
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- March 14 – CDC activates emergency operations, joins WHO consortium
- March 22 – We receive samples from the CDC
- March 23 – Virochip results suggest novel coronavirus
- March 24 – CDC: “…a previously unrecognized virus from the coronavirus family is the leading hypothesis…”
- April 1 – Recover ~2 kb of viral genome, sent to CDC
- April 14 – SARS coronavirus genome sequenced by BCCA and CDC
- April 17 – Evidence for causality: infection of monkeys and recovery of virus

E-Predict Development

- Designed as a method of “auto-diagnosis” necessitated by the number of viral oligos.
- Uses algorithm for interpreting Virochip hybridization patterns to identify viruses.
- Theoretical hybridization energy profiles circumvent the need for positive controls.
- Mixed viral infections can be detected.
E-Predict Development

Virochip Diagnosis Case 1

- Previously healthy young woman with 10 day history of fever, cough, night sweats, bloody sputum, and muscle pain.
- 3 days prior, treated with antibiotics.
- 3 days after admission progresses to respiratory failure.

(Chiu, CY, Clinical Infectious Diseases, 2006)

Diagnostic Tests:
- blood, urine, and sputum viral, bacterial, and fungal cultures
- urine Legionella antigen
- Serum rheumatoid factor and anti-nuclear antibody
- Coccidioidomycosis, histoplasmosis, Mycoplasma, Chlamydia Ab titers
- HIV serum antibody
- Bordetella pertussis DFA and PCR
- Immunofluorescence test for Pneumocystis jiroveci
- Serology tests for blastomycosis, tularemia, sporotrichosis, Q fever, and leptospirosis

Viral Assays:
- shell vial assay for cytomegalovirus
- DFA tests for respiratory syncytial virus, adenovirus, influenza A/B, parainfluenza virus types 1, 2, and 3
- Metapneumovirus PCR
- SARS coronavirus PCR
- ELISA for hantavirus (Sin Nombre)

All tests returned negative.

ViroChip Analysis:
Endotracheal aspirate from hospital day 4.
Virochip Diagnosis Case 1

• Identified Parainfluenza Virus 4
  — Not even on the DFA panel
  — Thought to not cause severe disease
  — Made it into the medical lore
• Virochip identifies a pathogen previously unrecognized as clinically significant.

Virochip Diagnosis Case 2

• 65 year old man with CLL
• Recent cruise to Greece and Turkey
• Worsening respiratory status despite Abx
• Progressive dyspnea – intubated on HD3


Diagnostic Tests:
• Blood, urine, and sputum viral, bacterial, acid-fast bacilli, and fungal cultures
• Urine Legionella antigen
• Serum rheumatoid factor and anti-nuclear antibody
• Serum Cryptococcus antigen
• Histoplasma urine antigen and histoplasma buffy coat
• Coccioidiomycosis, histoplasmosis, Mycoplasma, Chlamydia Ab titers
• HIV serum antibody
• Bordetella pertussis DFA and PCR
• Immunofluorescence test for Pneumocystis jiroveci
• Serology tests for blastomycosis, tularemia, sporotrichosis, Q fever, and leptospirosis
• Cytology of bronchoalveolar lavage fluid: (-) malignancy, mod acute inflammation, (-) viral inclusions
• Peripheral blood for flow cytometry: CLL without evidence of transformation
• Bone marrow biopsy: 5-10% prolymphocytes in bone marrow
• Stool O&P, fecal leukocytes, C. difficile cytotoxin assay
• Induced sputum x 3 for acid-fast bacilli

Viral Assays:
• Shell vial assay and quantitative PCR for cytomegalovirus
• Influenza A/B rapid test
• Membrane immunochromatographic assay for RSV
• DFA tests for RSV, adenovirus, influenza A/B, parainfluenza virus types 1, 2, and 3 (x 2)
• Metapneumovirus PCR
• ELISA for hantavirus (Sin Nombre)
Virochip Diagnosis Case 2

E-Predict: Human Metapneumovirus

Diagnostic Tests:
- Blood, urine, and sputum viral, bacterial, acid-fast bacilli, and fungal cultures
- Urine Legionella antigen
- Serum rheumatoid factor and anti-nuclear antibody
- Serum Cryptococcal antigen
- Histoplasma urine antigen and histoplasma buffy coat
- Coccidioidomycosis, histoplasmosis, Mycoplasma, Chlamydia Ab titers
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Metapneumovirus PCR

- Identification of human metapneumovirus.
- Missed by PCR due to divergent gene sequences at primer regions (therefore not amplified).
Future Directions in Viral Diagnosis

• Recent case (published yesterday) of hemorrhagic fever.
  – Patient dies.
  – Paramedic dies.
  – Nurse in ICU dies.
  – Worker who cleaned room after patient died dies.
  – Nurse who cared for paramedic survived (with ribavirin).

Lujo Virus

• Unbiased, high-throughput pyrosequencing.
• RNA samples from post-mortem liver and serum.
• 87,500 to 106,500 sequence reads produced.
• Alignment of unique singleton and contiguous sequences to the GenBank database.
• Aligned to an arenavirus scaffold.
• Filled in rest of sequence by PCR.
• 72 hrs from receipt of sample to sequencing.


Conclusions

• Viral infections are common respiratory illnesses with occasionally specific histology.
• Nonspecific histologies can be supported by use of laboratory tests including DFA, PCR, and (perhaps) Virochip.
• When you have a disease which looks like a possible viral syndrome, the Infection Control Division can activate Public Health protocols.