SARS and the Clinical Laboratory

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2005 APHL ID Conference “Emerging ID – Emerging Responses”
Canada put on SARS watchlist

Tourist entry ban "only as last resort"

Severe Acute Respiratory Syndrome
STILL WORRIED ABOUT TERRORISTS?

NO... CANADIANS

SARS SPREADS
Overview

• Overview of SARS
• Clinical Prediction Rules
• Diagnostics Tests
• Putting SARS into Context
Overview of SARS
SARS-associated Coronavirus (SARS-CoV)

- Enveloped
- Non-segmented, single-stranded, positive sense, RNA virus

Identification of a new human coronavirus

Lia van der Hoek¹, Krzysztof Pyrc¹, Maarten F Jebbink¹, Wilma Vermeulen-Oost², Ron J M Berkhout², Katja C Wolthers¹, Pauline M E Wertheim-van Dillen³, Jos Kaandorp⁴, Joke Spaargaren² & Ben Berkhout¹

Three human coronaviruses are known to exist: human coronavirus 229E (HCoV-229E), HCoV-OC43 and severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV). Here we report the identification of a fourth human coronavirus, HCoV-NL63, using a new method of virus discovery. The virus was isolated from a 7-month-old child suffering from bronchiolitis and conjunctivitis. The complete genome sequence indicates that this virus is not a recombinant, but rather a new group 1 coronavirus. The in vitro host cell range of HCoV-NL63 is notable because it replicates on tertiary monkey kidney cells and the monkey kidney LLC-MK2 cell line. The viral genome contains distinctive features, including a unique N-terminal fragment within the spike protein. Screening of clinical specimens from individuals suffering from respiratory illness identified seven additional HCoV-NL63-infected individuals, indicating that the virus was widely spread within the human population.
Characterization and Complete Genome Sequence of a Novel Coronavirus, Coronavirus HKU1, from Patients with Pneumonia

Patrick C. Y. Woo,1,2† Susanna K. P. Lau,1,2† Chung-ming Chu,3 Kwok-hung Chan,1 Hoi-wah Tsoi,1 Yi Huang,1 Beatrice H. L. Wong,1 Rosana W. S. Poon,1 James J. Cai,1 Wei-kwang Luk,4 Leo L. M. Poon,1,2 Samson S. Y. Wong,1,2 Yi Guan,1,2 J. S. Malik Peiris,1,2 and Kwok-yung Yuen1,2†*

Department of Microbiology1 and State Key Laboratory of Emerging Infectious Diseases,2 The University of Hong Kong, Division of Respiratory Medicine, Department of Medicine, United Christian Hospital,3 and Department of Microbiology, Tseung Kwan O Hospital,4 Hong Kong
SARS
“Severe Acute Respiratory Syndrome”

- acute onset respiratory syndrome
- typically moderate to severe
- asymptomatic and mild infection have also been detected
Schematic of Symptom Progression

fever/myalgia

cough / dyspnea

diarrhea

Figure created by R. Chow for Poutanen and Low. Chapter 36. SARS
In: Respiratory Infections (Eds Torres, Ewig, Mandell, Woodhead)
Schematic of Symptom Progression

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cough / dyspnea

diarrhea

Figure created by R. Chow for Poutanen and Low. Chapter 36. SARS
In: Respiratory Infections (Eds Torres, Ewig, Mandell, Woodhead)
Clinical Outcome

- 20% admitted to ICU
- 15% required mechanical ventilation
- ~10% died
- Increased risk of death or ICU admission if:
  * Increased age
  * Co-morbidity
  * High LDH
  * High neutrophil count

Tsui et al. EID 2003; 9: 1064-1069
Fowler et al. JAMA 2003; 290: 367-373
Lew et al. JAMA 2003; 290: 374-380
Chan et al. Thorax 2003;58:686-689
Choi et al. Ann Int Med 2003;139:715-72
Clinical Prediction Rules
Clinical Prediction Rules

Characteristics that *increased* the likelihood of SARS:
- Previous contact with a patient with SARS
- Fever, myalgia, malaise
- AbN CXR, abN lymphocyte and low platelet counts

Characteristics that *decreased* the likelihood of SARS:
- Age $\geq 65$ years or $< 18$ years
- Productive sputum
- Sore throat, rhinorrhea
- Abdominal pain
- High neutrophil count

Clinical Prediction Rules

• Score translates to low or high risk group
• Hong Kong cohort
  – Sensitivity 92%
  – Specificity 63%
• Taiwan cohort
  – Sensitivity 99%
  – Specificity 52%

Diagnostic Tests
RT-PCR Positivity:

Symptoms:

Transmissibility:

Sero-positivity:

Figure created by R. Chow for Poutanen and Low. Chapter 36. SARS In: Respiratory Infections (Eds Torres, Ewig, Mandell, Woodhead)
Viral Detection
Viral Load:

Figure 4: Sequential quantitative RT-PCR for SARS-associated coronavirus in nasopharyngeal aspirates of 14 SARS patients

Peiris et al. Lancet 2003; 361: 1767-72
RT-PCR Positivity:

Chan et al.  EID 2004;10(2):294-299
RT-PCR Positivity of Plasma:

Grant et al. NEJM 2003;349:2468
RT-PCR Positivity of Plasma:

79% positivity

Grant et al. NEJM 2003;349:2468
Schematic of RT-PCR Positivity

Figure created by R. Chow for Poutanen and Low. Chapter 36. SARS In: Respiratory Infections (Eds Torres, Ewig, Mandell, Woodhead)
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Viral Detection - Specimens

- Week 1: plasma/serum, respiratory specimens*
- Week 2: respiratory specimens*, stool
- Week 3: stool

* Multiple specimens over time

* LRT higher yield compared to URT samples
## Respiratory Specimens

<table>
<thead>
<tr>
<th>Days after onset</th>
<th>Nasopharyngeal aspirates* (n=615)</th>
<th>Other upper respiratory tract*† (n=368)</th>
<th>Lower respiratory tract specimens‡ (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>N</td>
<td>Positive</td>
</tr>
<tr>
<td>0–2</td>
<td>66</td>
<td>(35%)</td>
<td>32</td>
</tr>
<tr>
<td>3–5</td>
<td>140</td>
<td>(45%)</td>
<td>34</td>
</tr>
<tr>
<td>6–8</td>
<td>87</td>
<td>(58%)</td>
<td>21</td>
</tr>
<tr>
<td>9–11</td>
<td>37</td>
<td>(60%)</td>
<td>6</td>
</tr>
<tr>
<td>12–14</td>
<td>13</td>
<td>(42%)</td>
<td>4</td>
</tr>
<tr>
<td>15–17</td>
<td>9</td>
<td>(39%)</td>
<td>4</td>
</tr>
<tr>
<td>18–20</td>
<td>1</td>
<td>(13%)</td>
<td>6</td>
</tr>
<tr>
<td>21–23</td>
<td>1</td>
<td>(20%)</td>
<td>1</td>
</tr>
<tr>
<td>&gt;23</td>
<td>1</td>
<td>(10%)</td>
<td>8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>355</td>
<td>(45%)</td>
<td>116</td>
</tr>
</tbody>
</table>

Viral Detection – Post-Mortem Samples

- Lung
- Bowel
- Lymph node
- Spleen
- Liver
# Viral Detection – Post-Mortem

<table>
<thead>
<tr>
<th>Organ</th>
<th>Maximum viral load, copies/g of tissue</th>
<th>Median viral load, copies/g of tissue</th>
<th>Patients with virus detected in organ, % (no./total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>$1.0 \times 10^{10}$</td>
<td>$3.6 \times 10^{5}$</td>
<td>100 (19/19)</td>
</tr>
<tr>
<td>Small bowel</td>
<td>$2.7 \times 10^{9}$</td>
<td>$2.7 \times 10^{5}$</td>
<td>73 (11/15)</td>
</tr>
<tr>
<td>Large bowel</td>
<td>$3.7 \times 10^{8}$</td>
<td>$1.3 \times 10^{5}$</td>
<td>73 (11/15)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>$8.9 \times 10^{8}$</td>
<td>$7.1 \times 10^{4}$</td>
<td>69 (9/13)</td>
</tr>
<tr>
<td>Spleen</td>
<td>$7.2 \times 10^{5}$</td>
<td>$4.8 \times 10^{4}$</td>
<td>53 (9/17)</td>
</tr>
<tr>
<td>Liver</td>
<td>$1.6 \times 10^{6}$</td>
<td>$1.8 \times 10^{4}$</td>
<td>41 (7/17)</td>
</tr>
<tr>
<td>Heart</td>
<td>$2.8 \times 10^{7}$</td>
<td>$3.2 \times 10^{4}$</td>
<td>40 (7/18)</td>
</tr>
<tr>
<td>Kidney</td>
<td>$7.4 \times 10^{5}$</td>
<td>$4.8 \times 10^{4}$</td>
<td>38 (6/16)</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>$2.8 \times 10^{4}$</td>
<td>$2.8 \times 10^{4}$</td>
<td>12 (2/17)</td>
</tr>
</tbody>
</table>

Farcas et al. JID 2005;191:193-197
Viral Detection - Methods

• Culture
• Nucleic Acid Amplification Testing (NAAT)
• Other (e.g. for tissue analysis)
  – Electron microscopy, immunohistocytochemistry, in situ hybridization
Culture

- Requires level 3 laboratory
- Not as sensitive as NAAT
  - more readily isolated from resp tract than stool; most successful during the first 2 weeks of illness

Chan et al. EID 2004;10(2):294-299
Nucleic Acid Amplification Testing

• RT-PCR
• Multiple targets
  – polymerase, nucleocapsid, spike genes
• Variations
  – Traditional vs. real-time platform
  – “In-house” vs. commercial
  – Microarray-based
  – Bead-based
  – Extraction methodology
## WHO EQA Study

<table>
<thead>
<tr>
<th>Factor</th>
<th># Labs</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qiagen viral RNA extraction kit</td>
<td>38</td>
<td>0.9</td>
</tr>
<tr>
<td>Roche MagnaPure/HighPure extraction kit</td>
<td>7</td>
<td>0.2</td>
</tr>
<tr>
<td>Silica particle-based extraction method (Boom)</td>
<td>9</td>
<td>0.9</td>
</tr>
<tr>
<td>Primers originally developed in own laboratory</td>
<td>16</td>
<td>0.5</td>
</tr>
<tr>
<td>Any nested PCR assay</td>
<td>25</td>
<td>0.9</td>
</tr>
<tr>
<td>Any real-time PCR assay</td>
<td>37</td>
<td>0.7</td>
</tr>
<tr>
<td>Any commercial test kit</td>
<td>14</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Drosten et al.  EID 2004;10(12):2200-2203
Viral Detection - WHO

Reverse transcription polymerase chain reaction (RT-PCR), positive for SARS-CoV using a validated method from:

1. At least two different clinical specimens (e.g. nasopharyngeal and stool)
   OR
2. The same clinical specimen collected on two or more occasions during the course of the illness (e.g. sequential nasopharyngeal aspirates)
   OR
3. Two different assays or repeat RT-PCR using a new RNA extract from the original clinical sample on each occasion of testing.
Detection of SARS-CoV RNA by RT-PCR validated by CDC, with confirmation in a reference laboratory, from:

- Two clinical specimens from different sources, or
- Two clinical specimens collected from the same source on two different days
Sero logic Tests
Schematic of Sero-Positivity

Figure created by R. Chow for Poutanen and Low. Chapter 36. SARS In: Respiratory Infections (Eds Torres, Ewig, Mandell, Woodhead)
SARS-CoV IgG Kinetics

Cumulative % of patients with seroconversion

Time after onset of symptoms

Peiris et al. Lancet 2003; 361: 1767-72
SARS-CoV IgM and IgG Kinetics

Cumulative % of patients with probable SARS CoV infection positive for SARS CoV antibody over time after onset of fever (days)

- SARS CoV IgG
- SARS CoV IgM

Chen et al.
JID
2004;189:11
58-63
SARS-CoV IgM and IgG Kinetics

Shi et al.
J Clin Virology
3004;31:66-68
Serologic Testing - Specimens

- Acute serum
- Convalescent serum
  - defined as >28 days post symptom onset
Serologic Testing - Methods

- Immunofluorescent assays
- Enzyme immunoassays
- Western Blot
- Immunodot Assay
- Neutralization assay
Serologic Testing - WHO

- Negative antibody test on acute state serum followed by positive antibody test on convalescent phase serum tested in parallel.

  OR

- Fourfold or greater rise in antibody titre between acute and convalescent phase sera tested in parallel.
Detection of any of the following by a validated test, with confirmation in a reference laboratory:

- Serum antibodies to SARS-CoV in a single serum specimen, or
- A four-fold or greater increase in SARS-CoV antibody titer between acute- and convalescent-phase serum specimens tested in parallel, or
- Negative SARS-CoV antibody test result on acute-phase serum and positive SARS-CoV antibody test result on convalescent-phase serum tested in parallel; or
Other Diagnostic Tests
Antigen Capture EIA

- Antigen capture EIA based on monoclonal antibodies against nucleocapsid protein
  - Sensitivity 94% using serum first 5 days
  - Specificity 99.9%

SELDI-TOF

- Surface-enhanced laser desorption/ionization time-of-flight
  - Diagnostic patterns
  - Prognostic patterns

Putting SARS into Context
Will SARS Return?

How SARS might return:
• Humans
• Animals
• Research Laboratories
V. Laboratory Testing for SARS-CoV

Laboratory testing for SARS-CoV is now available at many state public health laboratories. Available tests include antibody testing using an enzyme immunoassay (EIA) and reverse transcription polymerase chain reaction (RT-PCR) tests for respiratory, blood, and stool specimens. In the absence of person-to-person transmission of SARS-CoV, the positive predictive value of a diagnostic test is extremely low. False-positive test results may generate tremendous anxiety and concern and expend valuable public health resources. Therefore, **SARS-CoV testing should be performed judiciously, and preferably only in consultation with the local or state health department.** SARS-CoV testing should be considered if no alternative diagnosis is identified 72 hours after initiation of the clinical evaluation and the patient is thought to be at high risk for SARS-CoV disease (e.g., is part of a cluster of unexplained pneumonia cases).
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“Testing should be performed only... at high risk for SARS-CoV disease”

CDC. SARS Laboratory Guidance. Jan 2004
Rule out Testing

- Cannot be used to rule out SARS
  - Co-infections with hMPV, influenza virus
- May help identify cause of clusters

Chan et al. EID 2003;9;1058-63
Schrag et al. EID 2004;10(2);185-195
Rule out Testing

Blood culture

Sputum for C&S

Nasopharyngeal swab in viral transport for:
  • virus culture (influenza, parainfluenza, RSV, adenovirus)
  • direct antigen testing

Nasopharyngeal swab in transport medium for:
  • Chlamydia pneumoniae PCR or culture
  • Mycoplasma pneumoniae PCR or culture

Serology for Mycoplasma pneumoniae

Other diagnoses to consider may include:
  • Tuberculosis: sputum, lower respiratory tract specimen such as BAL if available
  • Legionella: urine, sputum, lower respiratory tract specimen such as BAL if available, acute and convalescent serum
Add-ons

- hMPV
- ?non-SARS coronaviruses
- ?rhinoviruses
- ?others