SARS-CoV2: knowns and unknowns in this COVID-19 pandemic

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Conflicts of Interest: None

Disclaimer:
*Views are my own and should not be construed as an official statement from NCI/NIH.*
Goals of this webinar

• Why is this COVID-19 pandemic difficult to contain?

• What makes SARS-CoV2 different? What can we do about it?

• Questions for further research

“To know that we know what we know, and that we do not know what we do not know, that is true knowledge.”
-Copernicus
# Epidemiological Comparison of Respiratory Viral Infections

<table>
<thead>
<tr>
<th>Disease Causing Pathogen</th>
<th>Flu</th>
<th>COVID-19</th>
<th>SARS</th>
<th>MERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease</td>
<td></td>
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</tr>
<tr>
<td>Influenza virus</td>
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<tr>
<td>SARS-CoV-2</td>
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<tr>
<td>SARS-CoV</td>
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<tr>
<td>MERS-CoV</td>
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<table>
<thead>
<tr>
<th>R₀</th>
<th>Flu</th>
<th>COVID-19</th>
<th>SARS</th>
<th>MERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic Reproductive Number</td>
<td>1.3</td>
<td>2.0 - 2.5 *</td>
<td>3</td>
<td>0.3 - 0.8</td>
</tr>
<tr>
<td>CFR</td>
<td>0.05 - 0.1%</td>
<td>~3.4% *</td>
<td>9.6 - 11%</td>
<td>34.4%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Incubation Time</th>
<th>Flu</th>
<th>COVID-19</th>
<th>SARS</th>
<th>MERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 4 days</td>
<td></td>
<td>4 - 14 days *</td>
<td></td>
<td></td>
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<tr>
<td>2 - 7 days</td>
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<tr>
<td>6 days</td>
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</table>

<table>
<thead>
<tr>
<th>Hospitalization Rate</th>
<th>Flu</th>
<th>COVID-19</th>
<th>SARS</th>
<th>MERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>2%</td>
<td>~19% *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Most cases</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Community Attack Rate</th>
<th>Flu</th>
<th>COVID-19</th>
<th>SARS</th>
<th>MERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>10 - 20%</td>
<td>30 - 40% *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Most cases</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>4 - 13%</td>
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<td></td>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Annual Infected (global)</th>
<th>Flu</th>
<th>COVID-19</th>
<th>SARS</th>
<th>MERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>~ 1 billion</td>
<td></td>
<td>N/A (ongoing)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N/A (ongoing)</td>
<td></td>
<td></td>
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<tr>
<td>8098 (in 2003)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>420</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Annual Infected (US)</th>
<th>Flu</th>
<th>COVID-19</th>
<th>SARS</th>
<th>MERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 - 45 million</td>
<td></td>
<td>N/A (ongoing)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N/A (ongoing)</td>
<td></td>
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<tr>
<td>8 (in 2003)</td>
<td></td>
<td></td>
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<tr>
<td>2 (in 2014)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Annual Deaths (US)</th>
<th>Flu</th>
<th>COVID-19</th>
<th>SARS</th>
<th>MERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000 - 61,000</td>
<td></td>
<td>N/A (ongoing)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N/A (ongoing)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (since 2003)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (since 2014)</td>
<td></td>
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</tbody>
</table>

* COVID-19 data as of March 2020.

**SARS-CoV2: Severe Acute Respiratory Syndrome - Coronavirus 2**

4 other CoVs endemic to humans (common cold)
SARS-CoV2
single strand RNA virus
positive-sense

1. Binding and viral entry
2. Release of viral genome
3. Translation of viral polymerase
4. RNA replication and transcription
5. Translation of viral proteins
6. Structural proteins combine with nucleocapsid
7. Virion formation
8. Exocytosis/Viral shedding

- Replicated genomic RNA
- Subgenomic transcripts
  - Nucleocapsid (N)
  - Spike (S)
  - Membrane (M)
  - Envelope (E)
- Structural proteins
- Nucleocapsid
- Endoplasmic reticulum (ER)
Death of Type II pneumocytes during COVID-19
—> Loss of air exchange and fluid leakage into lungs

URT = upper respiratory tract (throat, nasopharynx)
LRT = lower respiratory tract (lungs)
Natural History

• From exposure to onset of symptoms: 4-14 days

• Illness duration
  • Mild cases: 2 weeks
  • Severe cases: 3-6 weeks
  • Fatalities: 2-8 weeks

• What is the viral load kinetics (replication, shedding) during the course of the illness?
Viral Load Kinetics during Mild COVID-19 Illness

Panels A to I correspond to cases #1, #2, #3, #4, #7, #8, #10, #14, and #16 in Böhmer et al.  
Dotted lines, limit of quantification. Experiments were performed in duplicate and the data presented are means of results obtained by two laboratories independently.

Days from start of illness ("post symptom onset")
Swab (yellow) = URT
Sputum (orange) = LRT

Wolfel et al. Nature 2020
Mild COVID-19 Illness
(Wolfel et al. Nature 2020)

- RT-PCR (reverse transcription and polymerase reaction)
  - All swabs day 1-5 were positive
  - After day 5, ~40% detection rate
  - Last positive swab @ day 28
  - None of urine and serum samples were positive

- Isolation of infectious virus (can grow on cells): no virus isolated after day 7

- Majority of patients are beyond shedding peak in URT at time of 1st testing

- Seroconversion in 50% of patients by day 7, all by day 14

- All patients showed neutralizing Ab; titer did not correlate with clinical course

- Neutralizing Ab - cross-reactivity with 4 endemic CoVs
Viral Load Kinetics during Moderate COVID-19 Illness

A

Viral RNA load, copies/mL

Detection limit

ND

High viral RNA load from LRT
Needs oxygen support

Patients 1 and 2

URT specimen
LRT specimen

Sx onset
Adm
Lopinavir/ritonavir

Fever/chill/myalgia
Nasal congestion
Dyspnea
Cough
Sputum
Loose stool, mild

Room air
O₂ 3L
O₂ 6L
O₂ 10L
O₂ 6L
O₃
Room air

Bilateral multiple GGO on chest CT
Progressive CXR
Improving CXR

Viral shedding

• Patient is spreading virus that can still grow

  ▶ in contrast to detecting bits of virus that have been cleared/non-viable ex. RNA genome

• Mild/moderate: 7-12 days (day 7 for mild, Wolfel paper)

• Severe: > 2 weeks
<table>
<thead>
<tr>
<th>Sites of replication</th>
<th>SARS-CoV2</th>
<th>SARS-CoV (2003)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak viral RNA</td>
<td>before day 5</td>
<td>day 7-10</td>
</tr>
<tr>
<td>Copy #</td>
<td>~$7 \times 10^8$ copies/swab</td>
<td>$5 \times 10^5$ copies</td>
</tr>
<tr>
<td></td>
<td>Throat, Lung</td>
<td>Lung</td>
</tr>
</tbody>
</table>
Asymptomatic & Presymptomatic Viral Shedding

- True asymptomatic infection rate can only be known if serology is done in population

- Spreading virus 2-8 days before onset of symptoms
Asymptomatic & Presymptomatic Viral Shedding

- “Cluster F: A woman aged 58 years (patient F1) attended a singing class on February 27, where she was exposed to a patient with confirmed COVID-19. She attended a church service on March 1, where she likely infected a woman aged 26 years (patient F2) and a man aged 29 years (patient F3), both of whom sat one row behind her. Patient F1 developed symptoms on March 3, and patients F2 and F3 developed symptoms on March 3 and March 5, respectively.”

**CDC MMWR, “Presymptomatic Transmission of SARS-CoV-2 — Singapore, January 23–March 16, 2020” , published April 1, 2020**

<table>
<thead>
<tr>
<th>Cluster F</th>
<th>Dates of likely transmission, symptom onset, and other exposure</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feb 27 28 29</td>
<td>Mar 1 2 3 4 5</td>
</tr>
<tr>
<td>Patient F1</td>
<td>★</td>
<td></td>
</tr>
<tr>
<td>Patient F2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient F3</td>
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</tbody>
</table>

**Day of exposure**
## COVID-19 in Children

<table>
<thead>
<tr>
<th></th>
<th>Age &lt;18</th>
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</thead>
<tbody>
<tr>
<td>Total population of USA</td>
<td>22%</td>
</tr>
<tr>
<td>COVID-19 cases</td>
<td>1.7%</td>
</tr>
<tr>
<td>Feb 12-Apr 2, 2020</td>
<td></td>
</tr>
</tbody>
</table>

- Milder symptoms
- Less hospitalizations /ICU
- Except: Infants, children with underlying conditions (asthma, etc.)
The conundrum of pediatric patients

- Do recent immunizations protect against COVID-19?
- Children and the common cold - does recent infection with CoVs causing colds have a protective effect?
- Exception: Children born prematurely have worse outcomes (lung development?)
Why is COVID-19 so deadly in some patients?

- Host response to virus
Transcriptome analysis of infected lung cells \textit{in vitro} and \textit{in vivo} (ferrets)

Also done on NHBE cells
(normal human bronchial epithelial cells from 79 yo Caucasian female)

RSV = respiratory syncytial virus
IAV = influenza A virus

Blanco-Melo D, et al. (tenOever Lab, Mt. Sinai, NY), preprint
https://www.biorxiv.org/content/10.1101/2020.03.24.004655v1
Transcriptome analysis of infected lung cells *in vitro* and *in vivo* (ferrets)

Trachea samples from ferrets
For RNA-Seq

Also induction of the following cytokines

- EDN1 (Endothelin 1)
  - Also increased in children with asthma
  - Increased during cigarette smoking
- TNFSF15

*Muted immune response, including absence of induction of Type I and III interferons in SARS-CoV2*

Blanco-Melo D, et al. (tenOever Lab, Mt. Sinai, NY), preprint
https://www.biorxiv.org/content/10.1101/2020.03.24.004655v1
Interferons activate signaling cascades to mount an antiviral response
Experimental therapies and Clinical Trials

- Hydroxychloroquine + Azithromycin
  - Anecdotes of efficacy
  - Mixed results in small trials
  - Need large, randomized, controlled trials

- Remdesivir (Gilead and NIAID/NIH)
  - Promising preclinical data (Baric Lab, Denison Lab)

- Lopinavir-ritonavir
  - Not effective?

- Anti-IL6 (Tocilizumab)

- Convalescent Plasma
  - From patients who have developed immunity after illness

- Etc.

[www.ClinicalTrials.gov]
[WHO Solidarity Trial]
e. Novavax starting Phase I in mid-May, results by end of June 2020
f. Moderna and NIAID/NIH started Phase I in March 2020
Why is SARS-CoV2 difficult to contain?  
It is highly transmissible and replicates efficiently.

- Clues:
  - Efficient viral replication in throat (1,000x more than SARS-CoV) and lungs, then expelled via droplets through sneezing, coughing, talking, singing…); evidence for airborne
  - Peak shedding prior to day 5 (including 2-8 days presymptomatic); also asymptomatic spreaders
  - Persistence of viral particles in air, surfaces, etc. (3 hours half-life)
  - Insertion of poly basic furin-type cleavage site -> faster entry into cell (?)
  - No/weak interferon response to virus -> host unable to clear virus
Why is SARS-CoV2 difficult to contain? It is highly transmissible and efficient.

- Implications:
  - Diagnostic testing within first 3 days of symptoms (peak of shedding)
  - PPE for everyone caring for COVID+ patients; mask for patients
  - After discharge, patients need to continue self-isolation (moderately ill patients may continue to shed virus)
  - Disinfection of hospitals, nursing facilities, etc.
  - Assume everyone is COVID+ (asymptomatic)
    - wear mask, social distancing (6 ft vs. 25 ft.)
So many questions...

- Why do children have milder COVID-19?
  - Endothelin 1 gene expression in children?

- What conditions or co-morbidities predispose host to weak interferon response? (elderly, hypertension, diabetes, obesity/metabolic syndrome?)
  - Ability to mount antiviral response

- What factors lead to COVID-19 complications? (lung damage, encephalitis, myocarditis?)
  - Inability to clear the virus?
  - Destructive inflammation?

- What is the intermediate host? Can we vaccinate them? (ex. in MERS, camels were vaccinated to mitigate transmission to humans)

- Animal models for continuing research to understand how virus works (ferrets? mouse model?)

- How can we end this pandemic? How do we break the transmission chain?
  - Dr. Jomar Rabajante’s webinar from last week
What can we do about COVID-19?

- More diagnostic testing at earlier time point
- Support the front-liners (more PPE, please!)
- Accelerated pace of research: clinical trials, vaccine studies
  - Pivot one’s research/expertise to SARS-CoV2
  - Volunteer (study participant, etc.)
- Open access to reliable, reproducible, validated data
“No way of thinking or doing, however ancient, can be trusted without proof”

–Henry David Thoreau, “Walden”