Understanding COVID-19
Testing for CV Specialists: Small Words with Big Pictures

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Speakers

- Edward Fry, MD, FACC, Session Moderator
- Emily Hyle, MD
- Bernard Macatangay, MD
- Steven Woloshin, MD
Presenter Disclosure Information

- Edward Fry, MD, FACC, Session Moderator
  - Nothing to disclose
- Emily Hyle, MD
  - I am a co-author at UpToDate.com on an unrelated topic.
- Bernard Macatangay, MD
  - Nothing to disclose
- Steven Woloshin, MD
  - Nothing to disclose
SARS CO-V BASICS: THE VIRUS AND THE ANTIBODY RESPONSE

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Division of Infectious Diseases
University of Pittsburgh School of Medicine
Coronaviruses and SARS CoV-2

- Enveloped RNA viruses
- With spiked glycoprotein embedded in envelope
- Inter- and intra-species transmission plus genetic recombination contribute to emergence of new coronavirus strains

Table 1. Human coronaviruses.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Genus</th>
<th>Disease</th>
<th>Discovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoV-229E</td>
<td>Alpha</td>
<td>Mild respiratory tract infection</td>
<td>1967</td>
</tr>
<tr>
<td>CoV-NL-63</td>
<td>Alpha</td>
<td>Mild respiratory tract infection</td>
<td>1965</td>
</tr>
<tr>
<td>CoV-HKU-1</td>
<td>Beta</td>
<td>Mild respiratory tract infection; pneumonia</td>
<td>2005</td>
</tr>
<tr>
<td>CoV-OC43</td>
<td>Beta</td>
<td>Mild respiratory tract infection</td>
<td>2004</td>
</tr>
<tr>
<td>SARS-CoV</td>
<td>Beta</td>
<td>Human severe acute respiratory syndrome, 10% mortality rate</td>
<td>2003</td>
</tr>
<tr>
<td>MERS-CoV</td>
<td>Beta</td>
<td>Human severe acute respiratory syndrome, 37% mortality rate</td>
<td>2012</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>Beta</td>
<td>Severe acute respiratory infections, &lt;2% mortality rate</td>
<td>2019</td>
</tr>
</tbody>
</table>
• Although there is a lot of variability in viral shedding, likelihood of recovering replication competent virus declines after onset of symptoms.
• Epidemiologic and virologic evidence support transmission from people who are “pre-symptomatic” and asymptomatic.
• 95% of specimens without replication competent virus 15d after symptom onset.
Immune Response to SARS CoV-2

- Innate immune response (sensing of viral RNA – first line of defense)
- Adaptive/Cellular: T cell response
- Adaptive/Humoral: B cell response → antibodies
Neutralizing Abs recognize epitopes that can eliminate infective virus vs non-neutralizing Abs which binds to the pathogen but does not affect infectivity.
Ab responses in COVID-19

• Ab <40% within 1-week of symptom onset (Zhao et al.)
• Increased to 100% (IgM 94.3%) and (IgG 79.8%) by day 15 (Zhao et al.)
• Median duration of IgM/IgA detection was 5d at 85.4% and 92.7% positive rate
• Median for IgG was 14d at 77.9% positive rate (Guo et al.)
Ab responses in COVID-19

- 100% for IgG within 19d of onset of symptoms
- Seroconversion of IgM and IgG occurred simultaneously or sequentially
- Titers plateaued within 6d of seroconversion
COVID-19

SARS CoV2 and Ab response

Azkur et al. 2020
Allergy
Take Home Points

- Viral shedding occurs prior to onset of symptoms
- Detection of replication competent virus declines with onset with most specimens without replication competent virus by d15 from onset
- Detection of antibodies dependent on timing of specimen collection:
  - Low likelihood of detecting antibodies within 7 days of symptom onset
  - Nearly 100% of cases with detectable Abs after day 15 from symptom onset
Panel Discussion
The Promise and Pitfalls of Antibody Testing for SARS-CoV-2

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Division of Infectious Diseases
Massachusetts General Hospital
Assistant Professor of Medicine
Harvard Medical School
Key Takeaways

• Antibody testing can have a limited role in diagnosis of COVID-19 but should be used with caution

• More data are needed regarding whether detectable antibodies confer immunity to SARS-CoV-2

• Risks of false positive and false negative antibody tests remain unacceptably high for clinical decision-making when seroprevalence is low
Outline

• Antibodies for diagnosis
  • As a marker of recent or past infection

• Antibodies to identify immunity
  • As evidence to change clinical recommendations

• Antibody test characteristics
  • Sensitivity, specificity, population seroprevalence, $R_0$
Introduction

• SARS-CoV-2 has prompted an unprecedented era of innovation and scale-up of a variety of diagnostic tests

• Accurate and timely diagnosis of COVID-19 is essential to ensure appropriate isolation for patients and targeted use of personal protective equipment for staff and providers

• Antibodies are another tool in the armamentarium; they show promise but have pitfalls
Antibodies for diagnosis
Adapted from Sethuraman et al. JAMA. 2020.
Antibodies for diagnosis

- **Acute COVID-19:**
  - Never useful early in illness
  - If antibody-positive after at least 8d of illness, can suggest COVID-19
  - If antibody-negative, cannot rule out COVID-19

- **Past COVID-19:**
  - Cross-reactivity with other coronaviruses appears to be low
  - Older or immunosuppressed less likely to make detectable antibodies
  - Data available for only 35 days after infection; long-term duration and significance of detectable antibodies remains unknown

Antibodies and immunity
Do positive antibodies = immunity?

- We don’t know for certain
  - Based on other viral diseases, the presence of antibodies likely signals immunity to reinfection

- Many remaining questions
  - Does a certain level of antibodies need to be attained ("titer")?
  - If immune, how long will immunity persist? And can positive antibodies serve as a proxy for immunity over time?
  - If mild disease, will antibodies and immunity wane more rapidly?

Why do we want to know about immunity?

• Safely reduce adherence to social distancing guidelines
  • Wear masks?
  • Travel?
  • Attend in person work?
  • Visit vulnerable family and friends?

• Implications for “immune patients” in healthcare settings
  • Reduce COVID-19 testing?
  • Conserve PPE?

• Consequences are serious for false positive test results
Antibody test characteristics
## Test characteristics: the 2x2 table

<table>
<thead>
<tr>
<th></th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>
If +antibodies, will the patient have a + test?

<table>
<thead>
<tr>
<th>Test</th>
<th>SARS-CoV-2 Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+ TP</td>
</tr>
<tr>
<td>-</td>
<td>- FP</td>
</tr>
<tr>
<td></td>
<td>- FN</td>
</tr>
<tr>
<td></td>
<td>+ TN</td>
</tr>
</tbody>
</table>

Sensitivity
Without antibodies, will the patient have a test?

<table>
<thead>
<tr>
<th>SARS-CoV-2 Antibodies</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>TP</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>FP</td>
</tr>
<tr>
<td>-</td>
<td>FN</td>
</tr>
<tr>
<td></td>
<td>TN</td>
</tr>
</tbody>
</table>

Specificity
Current challenges with antibody tests

• A wide range of reported sensitivity and specificity

• Tested mostly among COVID-19 patients confirmed by RT-PCR

• Insufficient testing among non-hospitalized COVID-19 patients (ie, people with less severe disease or who are asymptomatic)

If test+, does the patient have antibodies?

<table>
<thead>
<tr>
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<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>TP</td>
</tr>
<tr>
<td>-</td>
<td>FP</td>
</tr>
<tr>
<td>+</td>
<td>TN</td>
</tr>
<tr>
<td>-</td>
<td>FN</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Positive Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>TP</td>
</tr>
<tr>
<td>-</td>
<td>FN</td>
</tr>
<tr>
<td>+</td>
<td>TN</td>
</tr>
<tr>
<td>-</td>
<td>FP</td>
</tr>
</tbody>
</table>
If test-, does the patient have no antibodies?

<table>
<thead>
<tr>
<th>SARS-CoV-2 Antibodies</th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>TP</td>
<td>FP</td>
</tr>
<tr>
<td>-</td>
<td>FN</td>
<td>TN</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Predictive Value</td>
<td></td>
</tr>
</tbody>
</table>

+ TP (True Positive)  
  - FP (False Positive)  
  - FN (False Negative)  
  - TN (True Negative)
Seroprevalence remains low in the US

Havers et al. JAMA IM. 2020.
If seroprevalence is 5%, then

- True positive
- False negative
- False positive
- True negative

Se: 92%
Sp: 98%

Perfect tests do not exist

- We use imperfect tests all the time in health care
  - Rapid flu tests (sensitivity: 50-70%; specificity: >90%)
  - BNP (sensitivity: >95%; specificity: >98%)

- Let’s not wait for a “perfect” antibody test but rather think critically about the criteria at which the test would be useful
  - Specificity and sensitivity
  - Population seroprevalence
  - $R_0$ – the average number of transmissions resulting from one infection

Key Takeaways

• Antibody testing can have a limited role in diagnosis of COVID-19 but should be used with caution

• More data are needed regarding whether detectable antibodies confer immunity to SARS-CoV-2

• Risks of false positive and false negative antibody tests remain unacceptably high for clinical decision-making when seroprevalence is low
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Patients, providers, and staff at Massachusetts General Hospital
If seroprevalence is 20%, then

- True positive
- False negative
- False positive
- True negative

Se: 92%
Sp: 98%
