CLINICAL DILEMMA: Post-acute COVID-19 Syndrome, or Tick-Borne Disease Reactivation?

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Presentation Outline

- COVID-19 basics
- Post-COVID clinical and pathological features
- Compare and contrast COVID-19 and chronic TBDs
- Lab testing- COVID-19
- Lab testing- TBDs
- Therapeutic challenge?
- Summary and conclusions

COVID – Basic Clinical Features

- Acute COVID-19: three phases are possible
  - Initial viral phase
  - Then inflammatory phase
  - Followed by immune compromise
- Acute COVID-19 pathology
  - Inflammatory and autoimmune features- cytokine, chemokine and complement cascades may activate
  - Vasculitis- endothelial cells carry ACE-2 receptors
  - Intravascular thromboses- arterial and venous; microscopic and macroscopic
    - Platelets and megakaryocytes get activated
  - Metabolic- damage to cellular and mitochondrial membranes; cell danger response
  - Residual fibrosis and scarring

Post COVID Syndrome

PROSPECTIVE STUDY: *JAMA Netw Open.* 2021;4(2):e210830. doi:10.1001/jamanetworkopen.2021.0830

- 32% of all people who got COVID-19 have symptoms lasting > 6 months
- 12.5% of those who did not have overt symptoms of COVID had “decreased quality of life”

ITALY, CHINA: 76% to 87% of severely ill COVID-19 patients have persisting symptoms 2 to 6 months post hospital discharge

CLINICAL:

- Fatigue, decreased stamina, headache, body aches, cognitive impairment, conjunctivitis, neuropathy, depression, sleep difficulty, upper and lower GI upset, difficulty breathing and loss of the sense of taste or smell
- Some develop new symptoms as well. These can vary widely person to person, and there are reports of everything from hair loss to rapid heart rates to anxiety

QUESTION: Could some Post-COVID cases in fact reflect reactivated Tick-Borne Diseases?

- Both are chronic
- Both are multi-system

Symptoms Common to Both

- Fatigue
- Headache
- Arthralgias, myalgias
- Cognitive deficits
- Sleep difficulties
- Palpitations, rapid pulse
- Sweats
- Chills
- Shortness of breath
- Cough
- Dizzy
- Conjunctivitis
- GI upset
- Anxiety, depression

SYMPTOM	BORRELIA	BABESIA	BARTONELLA	RICKETT/VIRUS
Fatigue	X	X	X	X
Headache	X	X	X	X
Arthralgia	X		X	
Myalgia			X	X
Cognitive deficits	X	X	X	X
Neuropathy	X		X	
Sleep disorder	X		X	
Palpitations	X	X		
Sweats		X	X	
Chills		X		
Shortness of breath		X		
Cough		X		
Dizzy	X	X		
Conjunctivitis			X	X
G.I. upset	X		X	
Anxiety, depression	X	X	X	

Signs Common to Both

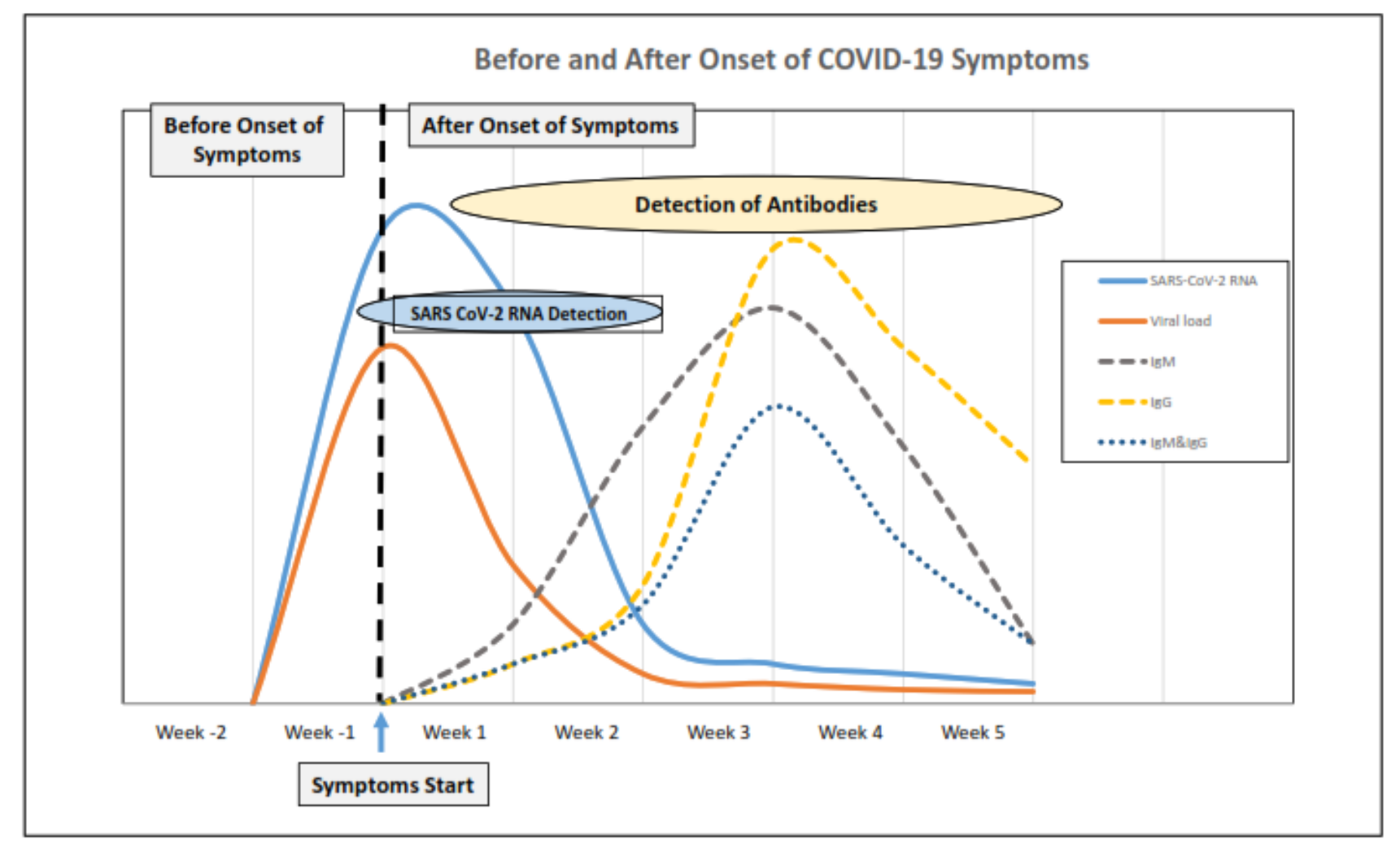
THESE MAY BE SEEN IN SOME BUT NOT EVERY PATIENT….

- Fever
- False positive Anti Nuclear Antibody
- False positive Rheumatoid Factor
- Anti-phospholipid antibodies
- Activated cytokines
- Abnormal brain scans (MRI, SPECT)
- Possible cardiac and pericardial involvement

Differentiating Late TBD from Late COVID

- Chronic Lyme
  - Prior history of Lyme or tick exposure
  - Symptoms are migratory and cyclic
  - Positive or suggestive tests for TBDs
- Chronic COVID
  - History of prior COVID
  - Symptoms do not migrate or cycle
  - End-organ damage- renal failure, hypoxia, abnormal chest x-ray, intravascular clots
- BUT- it is possible to have both!!

Time Course in COVID-19



Laboratory Testing for Late Stage COVID-19

TESTS FOR COVID AND FOR SPECIFIC DAMAGE ASSOCIATED WITH IT

- SARS CoV-2 lab tests-
  - Serology- immunoblot is preferred
  - Consider PCR- but yield is low
- Arterial oxygen level, chest x-ray, renal function, ferritin
- Consider pulmonary stress test- look for oxygen desaturation
- Cardiac echo- low ejection fraction (can be seen in TBDs)
- Cardiac MRI- used to document myocarditis and pericarditis and to confirm echo findings (can be seen in TBDs)

PCR Testing in COVID-19

- RT-PCR is highly insensitive and time-dependent
  - 100% false negative on day 1 of infection
  - 67% false negative on day 4 (the day before symptom onset)
  - 38% false negative on day of symptom onset (day 5)
  - 20% false negative on day 8
  - 21% false negative on day 9
  - 66% false negative on day 21
- "If clinical suspicion is high, infection should not be ruled out on the basis of RT-PCR alone"

  Ann Intern Med. doi:10.7326/M20-1495; 13 May 2020

Antibody Testing in COVID-19

One week after symptom onset (2 weeks after exposure), IgM appears and persists for 6+ weeks

- IgG appears 1 to 2 weeks after IgM and persists for at least 4 months; declines earlier in the elderly
- False positives- because almost half of common colds are due to a mild coronavirus- cross reactivity?
- False negatives- need to turn down test sensitivity to decrease these false positives.

Immunoblotting may be the answer- more sensitive and more specific!

ImmunoBlotting in COVID-19

IMMUNOBLOT FOR SARS COV-2 IS AN IGENEX EXCLUSIVE

- Uses recombinant antigens to drastically increase specificity
  - Far less concern for unwanted cross-reactivity with other coronaviruses
- Sensitivity also far better thanks to the recombinant antigens
- Results of validated testing in confirmed cases and controls:
  - Sensitivity: IgM 72.2%; IgG 91.7%; Overall 97.2%
  - Specificity: IgM 99.2%; IgG 98.9%; Overall 98.1%

Laboratory Testing for Late-Stage TBDs

Basic points:

- Be aware of the wide variety of TBDs that may be present in chronic Lyme-need to test for them
- Very late in the infection, with chronic illness
  - often immune response is compromised
  - however, pathogen load may also be low
  - therefore one often needs to do both indirect as well as direct tests
    - Example: In an internal lab study of Babesiosis, adding serology to direct tests increased positive yield by 12.4%
- Biofilms are most likely present
  - May affect test choice and test sensitivity

Bartonella Testing

INDIRECT TESTS

- IFA- old technology; designed to detect only *B. henselae*.
- Western Blot: *An Igenex exclusive*- Designed to detect multiple species
- Is more sensitive than the IFA and replaces it
- Need 2 or more bands to be reported as positive
- T-cell response assay- for those with impaired B-cell function

DIRECT TESTS

- FISH- is genus-specific thus offers extended species coverage
- Another advantage is that it can detect organisms embedded in biofilms thus is the most sensitive direct test currently available
- Culture-enhanced PCR- still low yield and requires multiple samples
- Droplet Digital ePCR- PCR using novel technology to increase sensitivity and specificity. Three sequential blood samples needed. Unclear if can detect organisms trapped in biofilms

Bartonella

RECOMMENDATIONS

Due to low test sensitivity, recommend combinations of tests to increase yield- *direct* + *indirect*

- Bartonella FISH + Western Blot
  - Maximizes sensitivity
  - Maximizes species coverage
  - PCR may substitute for the FISH
  - If IgG deficient, do T-cell response test in place of the western blot

Babesia – Multiple Species

The two dominant species are *B. microti* and *B. duncani* (formerly known as WA-1). Others may occasionally infect humans

- *B. duncani* is found across the entire USA- not just the west coast
- Therefore broadly sensitive tests are needed to detect multiple species

Testing limitations

- Not everyone has detectable parasitemia
- Not everyone will become seropositive
- Not all labs can detect multiple species
- Therefore false negatives are common but false positives are rare

Babesia Testing

- Stained blood smear- only useful within first two weeks of infection so not worth doing in late-stage disease
- FISH- Genus-specific, therefore offers broad species coverage
  - Far more sensitive than smear and can be useful in chronic disease
  - IGeneX FISH scored 100% on NYS validation testing.
- Serology (IFA)-
  - The IGeneX IFA is broadly reactive and can detect multiple species
- PCR-
  - The IGeneX PCR is broadly reactive and can detect multiple species

RECOMMENDATION: FISH+PCR+IFA

Borrelia – Highly Diverse Group

- Multiple species
- Over ¼ of clinically diagnosed Lyme patients had TBRF instead

SERIES 1: Thirty-six Lyme cases

- 10 *B. mayonii*
- 6 *B. spielmanii*
- 6 *B. californiensis*
- 5 *B. garinii*
- 2 *B. afzelii*
- 1 *B. valaisiana*
- 1 *B. burgdorferi* strain B31
- 5 cases could not be speciated

SERIES 2: Forty-eight TBRF cases

- 5 *B. hermsii*
- 8 *B. miyamotoi*
- 7 *B. turicatae*
- 2 *B. turcica-like*
- 26 cases could not be speciated

Borrelia Testing must be Broadly Inclusive

- **NONE** of the Lyme IFAs, ELISAs, western blots, PCRs, urine tests or T-cell tests have been validated for Bb sl
- **NONE** of the IFAs, ELISAs or PCRs for TBRF have been validated for all these species
- Therefore must use tests that offer broad, multispecies coverage

Exclusive testing options at IGeneX

- ImmunoBlots- use multiple antigens for multiple species
  - Available for Lyme and TBRF
  - Usually can identify species
  - Replaces western blot
- Broad Coverage Assay- replaces ELISA
- A broadly inclusive PCR- IGX PCR is genus-specific so able to detect multiple species
  - At IGeneX, FOUR separate PCRs are done on every sample- serum, WB; genomic, plasmid

RECOMMENDATIONS: Lyme + TBRF ImmunoBlots + PCR

Therapeutic Challenge as an Aid to Diagnosis

Antivirals and COVID-19

- Anecdotal reports that some patients with Post-COVID will respond to a course of ivermectin
- However, ivermectin may have antibacterial qualities as well, so a positive response does not conclusively exclude TBDs as contributing to the syndrome

Therapeutic Challenge as an Aid to Diagnosis

TBD Antibiotics and COVID

- Many antibiotics used for *Borrelia* are also antiviral- tetracyclines, macrolides and azoles
- Most treatments for Babesia are antiviral- those that are based on quinine or artemesia, plus the macrolides that commonly accompany them
- Azole antifungals are also antiviral
- Many complementary treatments used in Lyme are antiviral and/or help the immune system
- So a positive response to these likewise cannot exclude persistence of SARS CoV-2

Therapeutic Challenge as an Aid to Diagnosis

However, some clinicians will administer an antibiotic challenge PRIOR TO testing for TBDs-

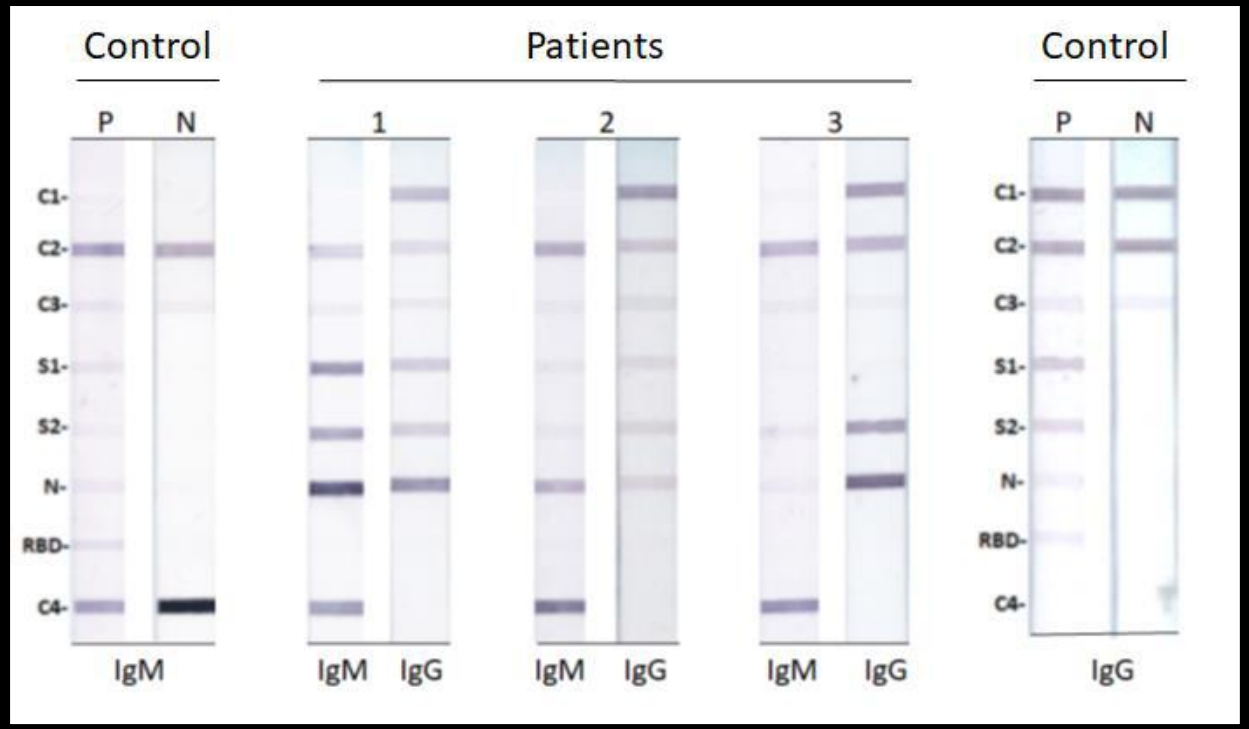
- A course of intracellular-acting antibiotics prior to PCR, presumably to release intracellular organisms
  - This has NOT been validated
- Several days of antibiotics prior to collecting urine for Lyme antigen assay (Lyme Dot-Blot).
  - A common practice
- Six or more weeks of antibiotics can cause a seronegative Lyme patient to revert to seropositive due to breakup of immune complexes and release of free antibody
  - Clinically validated

Summary

- Contemporary clinicians are faced with managing an unprecedented mix of serious, potentially chronic illnesses
- Have to use all of our clinical skills because it is not always clear whether a symptomatic individual has COVID-19, TBDs, or both
- Need to take advantage of the most reliable tests available to help us and our patients
- Therapeutic trials have been suggested, but there is no data to support this; informed consent is essential
- As always, recording and sharing detailed records and data assessments are essential

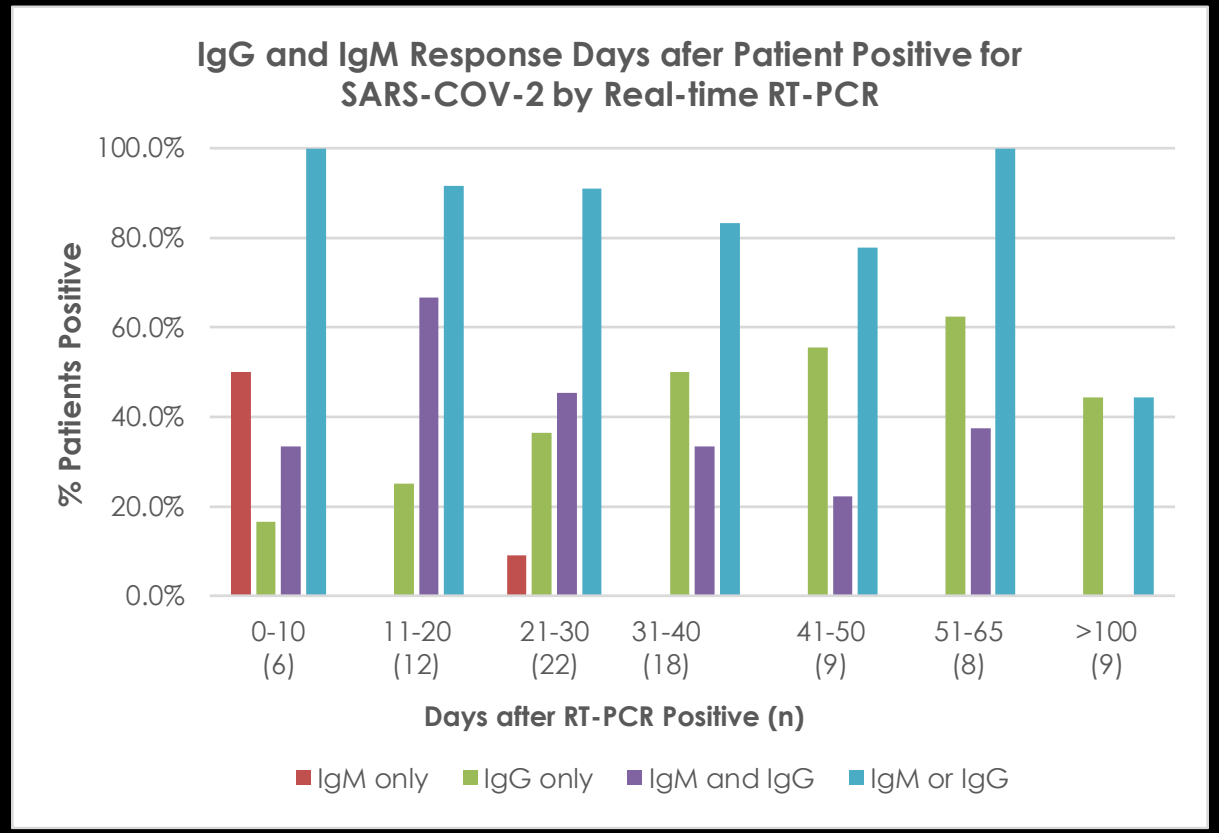
COVID-19 Tests

Photographs of COVID-19 IgG and IgM IBs with sera from three patients (1, 2 and 3) and positive (P) and negative (N) control human sera.



The positive control (P) was a pool of sera from SARS-CoV-2 RT-qPCR positive patients that reacted with S1, S2, N and RBD proteins in both COVID-19 IgG and IgM IBs. The negative control (N) was pooled human sera from the pre-pandemic period and did not react with the four SARS-CoV-2 antigens. C1: purified IgG; C2: Protein L; C3: internal calibrator; C4: purified IgM. The positions of S1, S2, N and RBD proteins in the membrane strips are also indicated.

IgG and IgM Response



The graph shows the percentage of sera positive for IgM and IgG antibodies to SARS-COV-2 by covid-19 ImmunoBlots between 0 -125 days after positive for SARS-COV-2 BY RT-PCR. The number of sera tested for each time interval are shown in parentheses in the abscissa. Reactivity with the IgM only, IgG only, IgG, IgM and IgG and IgM or IgG shown in different colors. The blue bars show percentage of sera scored as positive overall for IgM and IgG antibodies in COVID-19 IBs.

COVID-19 ImmunoBlots – Sensitivity and Specificity (37 patients – 84 Samples)

		Estimated	95% Confidence Interval	
		Value	Lower Limit	Upper Limit
IgM antibodies only	Sensitivity	70%	53%	84%
	Specificity	99%	97%	100%
	PPV	93%	75%	99%
	NPV	95%	92%	98%
IgG antibodies only	Sensitivity	92%	77%	98%
	Specificity	99%	97%	100%
	PPV	94%	80%	99%
	NPV	99%	96%	100%
Either IgG or IgM antibodies	Sensitivity	97%	84%	100%
	Specificity	98%	95%	99%
	PPV	90%	75%	97%
	NPV	100%	97%	100%

COVID-19 IgXSpot

COV-19 IgX Spot Test (1 family -3 members)

Weeks after SARS COV-2 PCR (+)	1	2	3	4	5	6	7	9	10	11	12	13	14
Patient 1 (F)	Pos	Pos	Neg	Neg	Neg	N/A	Neg	N/A	N/A	N/A	N/A	N/A	N/A
Patient 2 (F)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Pos	Pos
Patient 3 (M)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Neg	Neg	NA

Note: 30 controls were tested. All were negative. In addtion, 30 adults before vaccination were tested. All were negative. All 30 are being followed weekly after vaccination. Results have to analysed

Summary

Patient 1: COVID-19 IgX Spot test positive for 2 weeks after being positive for SARS-COV-2 by RT-PCR

Patient 1: COVID-19 IgX Spot test positive for 10 weeks after being positive for SARS-COV-2 by RT-PCR; Reactivation at 13th week

Patient 1: COVID-19 IgX Spot test positive for 9 weeks after being positive for SARS-COV-2 by RT-PCR

Conclusion

Based on the data presented:

- The COVID-19 ImmunoBlots detect both SARS-COV-2 specific IgM and IgG antibodies during the full spectrum of the diseases. 36/37 patients that were SARS-COV-2 positive by RT-PCR for SARS-COV-2 were positive by the COVID-19 ImmunoBlots. Since multiple antigens are used in the ImmunoBlot, SARS-COV-2 specific antibodies in patients infected with mutants too should be detected.

- COVID-19 IgX Spot test specifically detects T-Cell response to SARS-COV-2. Since multiple antigens are used in the assay, patients infected with mutants too should be detected.

Thank You!

Stay safe, and best wishes