RT-PCR NEGATIVE COVID-19

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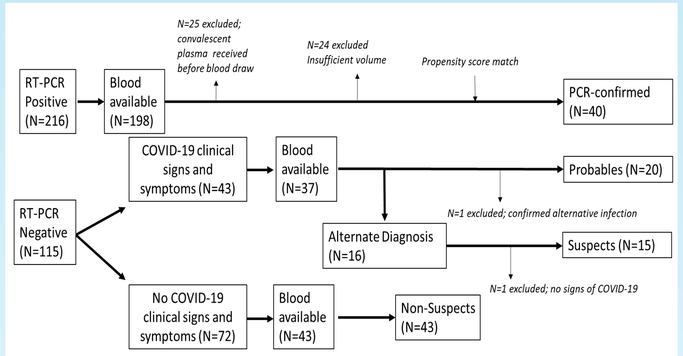
Background: *COVID-19 is a multi-system infection caused by the novel coronavirus SARS-CoV-2 that can manifest in a clinical spectrum from asymptomatic and mild upper respiratory infections to severe viral pneumonia with respiratory and multi-organ failure. Despite the progress in discovery and validation of effective therapeutic approaches across different disease stages, hospitals and clinical care systems have remained vulnerable to COVID-19 surges. Effective diagnostic approaches to ensure comprehensive detection and appropriate treatment of COVID-19 are critical to reducing COVID-19 related morbidity and mortality.*

The decline in SARS-CoV-2 shedding from upper respiratory tract specimens within the first week after symptom onset is associated with an increase in RT-PCR false negative rate of 2 to 29%. Thus, COVID-19 diagnosis may be missed in hospitalized patients who often present with more advanced disease. This could lead to delayed or missed interventions to reduce disease morbidity and mortality and unnecessary empiric therapies directed towards an inaccurate alternative diagnosis. Although there is no clear diagnostic reference standard for COVID-19 outside of RT-PCR, we reasoned that comparative serologic testing early in the COVID-19 epidemic could be used to confirm clinical suspicion of acute COVID-19 in RT-PCR negative patients, enabling the validation of RT-PCR negative COVID-19 as a clinical diagnosis.

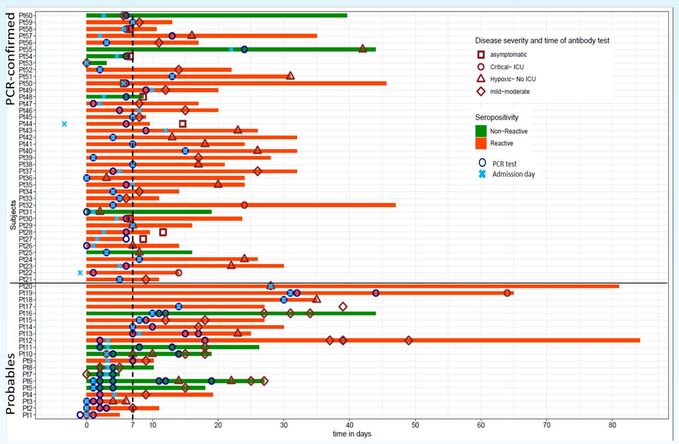
Study Outline:

Between April 2020 and October 2020, patients were identified patients by running a report for all University Hospital patients within the 6 days post visitation using the electronic medical records for one of four cohorts: (1) RT-PCR-confirmed COVID-19 ('PCR-confirmed') (N=40), (2) COVID-19 Probable ('Probables") (N=20), (3) COVID-19 Suspects ('Suspects') (N=15), (4) COVID-19 Non-suspects ('Non-suspects') (N=43). Probables and Suspects were identified with multiple negative RT-PCR tests in the past 4-21days with medical history, symptoms and radiographic findings consistent with COVID-19. Suspects, unlike Probables, had an alternative diagnosis but where COVID-19 could not be entirely ruled out on differential diagnosis scale. PCR-confirmed patients were hospitalized patients with at least one positive SARS-CoV-2 RT-PCR test result in the prior 4-21 days and were additionally propensity score matched to the Probables. Non-suspects were identified by selecting patients between September and October 2020 with at least one negative SARS-CoV-2 RT-PCR without clinical COVID-19 signs/symptoms. Non-suspects were also selected to match, on aggregate, by age, sex, body mass index, and co-morbidities to the Probable group. Pre-pandemic controls (N=55) were collected before 2019 as part of routine clinical diagnosis.

▼**Figure 1.** Flowchart of patient and samples included in the analysis.



▼**Figure 2:** Swim plot illustrating hospital admission(skyblue cross), RT-PCR test timeline (darkblue circles), antibody test and disease severity at each blood collection time-point (brown square, brown circle, brown triangle, brown diamond) from symptom onset among Probables (Pt1to Pt20,N=20) and matched PCR-confirmed (Pt21 to Pt40;N=40). Sample with (red bars=Reactive) and without (green bars= Non-Reactive) COVID-19 specific IgG or IgM antibodies at any point of blood draw.

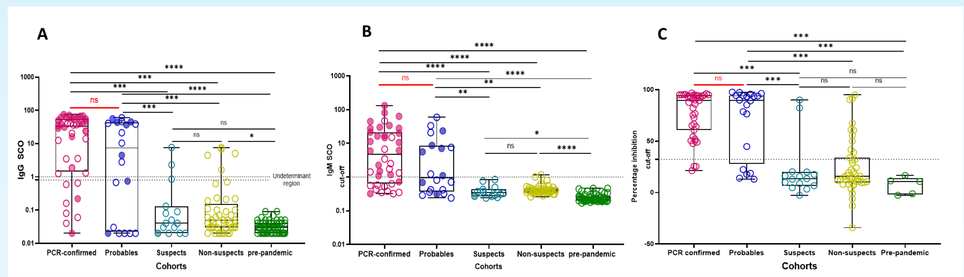


Methods:

For serological analysis, we used FDA-EUA approved IgG and IgM immunological assay (Beckman-Coulter, Brea,CA) and SARS-COV-2 surrogate virus neutralization assay (Genscript, Piscataway, NJ).

Results:

▼**Figure 3:** Signal to cut-off ratio of (A) IgG and (B) IgM and (C) Neutralization assay among samples collected before 2019 (pre-pandemic), RT-PCR and clinically negative (COVID-Non Suspects), RT-PCR negative with high clinical suspicion of COVID-19, with potential alternate diagnosis (COVID-possible) and no alternate diagnosis (COVID-Suspect) and matched RT-PCR confirmed for COVID-19 (PCR confirmed). Boxplot indicates the interquartile range as the box and the minimum and maximum values as whiskers. Dashed line indicates cut-off values for call for reactivity. Filled circles are individuals who received COVID-19 directed therapies. Comparison between groups were by two-sided Wilcoxon signed rank test. P1> p-value between PCR-confirmed and Probables, P2> p-value between Probables and Suspects, P3> p-value between Probables and Non-suspects and P4> p-value between Probables and Pre-pandemic.



		PCR confirmed	Probables	Suspects	Non-suspects	Pre-pandemic	P1	P2	P3	P4
Serological assays	IgG SCO Median (IQR)	33.0 (1.7- 7.0)	7.4 (0.03- 41.5)	0.04 (0.02-0.1)	0.1 (0.03-0.2)	0.03 (0.02-0 0.04)	0.096	0.025	0.007	< 0.001
	IgM SCO Median (IQR)	4.8 (0.7- 20.3)	1.0 (0.4- 20.4)	0.4 (0.3-0.4)	0.4 (0.3- 0.4)	0.2 (0.2- 0.3)	0.092	0.003	0.003	< 0.001
	IgG Reactivity	32/40 (80.0%)	12/20 (60.0%)	2/15 (13.3%)	5/43 (11.1%)	0/5 (0.0%)	0.126	0.008	< 0.001	< 0.001
	IgM Reactivity	29/40 (72.5%)	10/20 (50.0%)	0/15 (0.0%)	2/43 (4.4%)	0/5 (0.0%)	0.096	0.001	< 0.001	< 0.001
Neutralization assay	Positivity	37/40 (92.5%)	15/20 (75.0%)	2/15 (13.3%)	13/43 (30.2%)	0/5 (0.0%)	0.036	< 0.001	< 0.001	< 0.001
	Percent inhibition Median (IQR)	89.7 [63.3-94.7]	89.5 [39.2-94.3]	13.6 [7.8-18.8]	15.6 [10.2-33.0]	-1 [-2.5-11.4]	0.689	< 0.001	< 0.001	< 0.001

Key Findings:

This study confirms a high probability of acute COVID-19 among RT-PCR negative with typical signs/symptoms and no alternative diagnosis. Despite matched disease severity, these RT-PCR negative patients were half as likely to receive treatment as PCR-confirmed COVID-19.

▼**Table 1: Clinical characteristics of all patients in four cohorts:**

		COVID-19 patient cohorts				P1	P2	P3
		PCR confirmed	Probables	Suspects	Non-Suspects			
	N=	40	20	15	43			
Median (IQR)	Age (years)	60.0 (43.5- 65.3)	53.0 (40.5-64.5)	57.0 (47.5-62.5)	53.0 (46.5- 61.0)	0.742	0.740	0.941
Median (IQR)	BMI	26.35 (24.0- 32.5)	30.8 (26.0-35.0)	25.9 (24.2-32.9)	28.10 (25.0- 31.4)	0.101	0.217	0.111
N (%)	Male	28.0 (70.0%)	13.0 (65.0%)	8.0 (53.3%)	31.0 (68.9%)	0.772	0.510	0.570
Median (IQR)	PCR test after symptom onset (in days)	6.0 (3.8-7.0)	2.5 (1.0-8.5)	1.0 (0.0-4.5)	NA	0.676	0.154	NA
Median (IQR)	Ab test after symptom onset (in days)	11.3 (7.0-17.3)	14.0 (3.8-24.0)	14.0 (4.0-16.0)	NA	0.655	0.688	NA
	Race/Ethnicity							
N (%)	Black or African American	19 (47.5%)	8 (40.0%)	8 (53.3%)	26 (60.4%0	0.735	0.809	0.318
N (%)	Hispanic or Latino	13 (32.5%)	9 (45.0%)	5 (33.3%)	12 (27.0%)			
N (%)	Caucasian	6 (15.0%)	3 (15.0%)	2 (13.3%)	4 (9.3%)			
N (%)	Others	2 (5.0%)	0 (0.0%)	0 (0.0%)	1 (2.3%)			
N (%)	**Chest imaging findings consistent with COVID**	26 (65.0%)	18 (90.0%)	9 (60.0%)	NA	0.061	0.051	NA
N (%)	**Symptoms**							
N (%)	Fever	14 (35.0%)	6 (30.0%)	3 (20.0%)	0 (0.0%)	0.778	0.700	<0.001
N (%)	Coughing	12 (30.0%)	5 (25.0%)	4 (26.7%)	0 (0.0%)	0.768	1.000	0.002
N (%)	Dyspnea	19 (47.5%)	13 (65.0%)	8 (53.3%)	0 (0.0%)	0.274	0.511	<0.001
N (%)	Chills	6 (15.0%)	2 (10.0%)	1 (6.7%)	0 (0.0%)	0.706	1.000	0.097
N (%)	Sore throat	1 (2.5%)	1 (5.0%)	0 (0.0%)	0 (0.0%)	1.000	1.000	0.317
N (%)	Diarrhea	4 (10.0%)	3 (15.0%)	2 (13.3%)	0 (0.0%)	0.676	1.000	0.028
N (%)	Altered Mental Status	3 (7.5%)	2 (10.0%)	1 (6.7%)	0 (0.0%)	1.000	1.000	0.090
	Disease severity at admission							
N (%)	asymptomatic	10 (25.0%)	2 (10.0%)	5 (33.3%)	NA	0.513	0.279	NA
N (%)	mild-moderate	11 (27.5%)	5 (25%)	5 (33.3%)	NA			
N (%)	hypoxic-No ICU	18 (45.0%)	11 (55.0%)	5 (33.3%)	NA			
N (%)	critical- ICU	1 (2.5%)	5 (25.0%)	0 (0.0%)	NA			
	Disease severity at collection							
N (%)	asymptomatic	8 (20.0%)	0 (0.0%)	3 (20.0%)	NA	0.079	0.151	NA
N (%)	mild-moderate	14 (35.0%)	12 (60.0%)	7 (46.7%)	NA			
N (%)	hypoxic-No ICU	16 (40.0%)	7 (35.0%)	3 (20.0%)	NA			
N (%)	critical- ICU	2 (5.0%)	1 (5.0%)	2 (13.3%)	NA			
	Disease severity at peak							
N (%)	asymptomatic	8 (20.0%)	0 (0.0%)	3 (20.0%)	NA	0.039	0.209	NA
N (%)	mild-moderate	8 (20.0%)	4 (20.0%)	4 (26.7%)	NA			
N (%)	hypoxic-No ICU	22 (55.0%)	12 (60.0%)	6 (40.0%)	NA			
N (%)	critical- ICU	2 (5.0%)	4 (20.0%)	2 (13.3%)	NA			
	Chronic Medical conditions							
N (%)	Hypertension	21 (52.5%)	11 (55.0%)	7 (46.7%)	25 (55.6%)	1.000	0.738	1.000
N (%)	Heart Disease	5 (12.5%)	3 (15.0%)	4 (26.7%)	10 (22.2%)	1.000	0.430	0.520
N (%)	Diabetes	13 (32.5%)	7 (35.0%)	5 (33.3%)	10 (22.2%)	1.000	1.000	0.370
N (%)	Liver disease	2 (5.0%)	3 (15.0%)	6 (40.0%)	2 (4.4%)	0.322	0.129	0.315
N (%)	Lung disease	7 (17.5%)	6 (30.0%)	5 (33.3%)	5 (11.1%)	0.326	1.000	0.060
N (%)	Kidney disease	6 (13.3%)	4 (20.0%)	5 (33.3%)	7 (15.6%)	1.000	0.246	1.000
	Microbiology							
N (%)	Positive respiratory pathogen panel	0 (0.0%)	1 (5.0%)	0 (0.0%)	NA	0.154	1.000	NA
N (%)	Positive sputum culture	4 (10.0%)	4 (20.0%)	2 (13.3%)	NA	0.283	0.680	
N (%)	Positive blood culture	5 (12.5%)	2 (10.0%)	2 (13.3%)	NA	0.776	1.000	
N (%)	Positive unrine culture	8 (20.0%)	6 (30.0%)	6 (40.0%)	NA	0.388	0.721	
	Treatments#							
N (%)	COVID directed (any)	23 (71.8%)	7 (35.0%)	1 (6.7%)	0	**0.008**	0.100	NA
	Length hospital days							
Median (IQR)		14 (7.8- 19.3)	16 (7.0-23.0)	15 (5.5-22.5)	NA	0.813	0.676	NA
	Outcomes							
N (%)	Uninfected	NA	NA	NA	43 (100.0%)			NA
N (%)	Survived	36 (90.0%)	19 (95.0%)	14 (93.3%)	NA	0.656	1.000	

NA > Not available; IQR> inter quantile range; PCR> polymerase chain reaction, ICU> Intensive Care Unit; BMI> Basal Metabolic Index

P1= p-value between Probables and PCR-confirmed, P2= p-value between Probables and Suspects, P3= p-value between Probables and Non-suspects

only symptomatic PCR confirmed patients

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