RT-PCR Negative COVID 19 – Akin to Smear Negative Pulmonary Tuberculosis? – A Case Report

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ABSTRACT

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We report a case of 34-year-old physician who presented with classical clinicoradiological features suggestive of COVID-19 but five sequential rRT-PCR (real time Reverse Transcription Polymerase chain reaction) tests were negative. After admission, he was hypoxic and febrile. He had clinical worsening and laboratory parameters suggestive of an impending cytokine storm and he was appropriately managed as per institution protocol. Meanwhile, his antibody test for COVID 19 came positive for both Ig G and Ig M with high IgM level. He had a dramatic recovery and was discharged within 10 days. It is difficult to distinguish COVID-19 pneumonia from other viral pneumonia based on RT-PCR and CT findings alone. In this context, this case report is being published to highlight this fact.

Keywords: False negative RT-PCR, COVID 19 antibody test, CO-RADS

Key Messages:

1. We emphasize the utility of chest HRCT in the evaluation of patients suspected of having COVID 19 infection.
2. Antibody testing is invaluable to diagnose COVID-19 pneumonia in special situations, especially in those with a high clinical suspicion but negative RT-PCR test.

*See End Note for complete author details

INTRODUCTION

The definite diagnosis of corona virus disease 2019 (COVID-19) is by viral isolation or by detection of nucleic acid/ viral antigen by polymerase chain reaction from nasal swab or throat swab or lower respiratory tract sample. However, the sensitivity to detect COVID-19 by real time rRT-PCR is reported to be low and hence they have a low negative predictive value.[1]

CASE REPORT

A 34-year-old medical professional, having hypertension as a comorbidity, presented to us with symptoms of sore throat, myalgia, and fever of one week duration. His complaints started as sore throat, a week back and the next day, he developed fever & myalgia. Fever lasted for two days and there after he became asymptomatic. Five days prior to onset of symptoms, he had high risk contact with many patients diagnosed to have COVID 19 infection. Before presenting to us, COVID 19 rRT- PCR was done thrice (one conventional rRT-PCR and two chip based rRT-PCR) elsewhere in which COVID 19 was not detected. He was started on treatment with Favipiravir, Piperacillin-Tazobactum, Azithromycin and Methylprednisolone. HRCT chest was done elsewhere which showed patchy areas of Ground Glass Opacities (GGOs) in left lower lobe. A week later, he started developing a recurrence of fever and hence came to us for further management. He was admitted in isolation room, repeat RT-PCR tests were done (both chip based and conventional rRT-PCR) but were negative. Laboratory investigations revealed elevated Neutrophil Lymphocyte Ratio (NLR) (7.27), normal total count 6100 cells/cu.mm (4000-11000 cells/cu.mm), CRP 5.7 mg/L (<5mg/L), D- dimer376ng/ml (<500ng/ml), Pro-calcitonin0.07ng/ml (<0.1 ng/ml). His Liver and renal function tests were within normal limits. His Ferritin level was elevated

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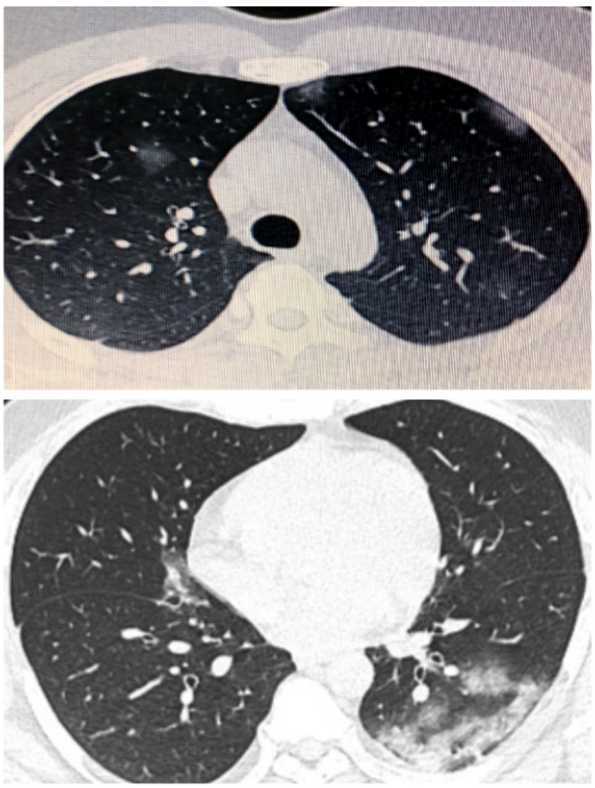

Figure 1. **HRCT chest, axial section, lung window showing multifocal bilateral peripheral predominant rounded GGO with consolidation on the left lower lobe, consistent with CO-RADS 5.**

475 ng/ml (30-400ng/ml). He was hemodynamically stable, with SpO2 95% in Room air. HRCT chest was repeated and on comparison with previous HRCT chest, there was increase in GGOs bilaterally. HRCT chest showed bilateral peripheral predominant rounded GGOs, reverse halo sign with consolidation in left lower lobe **(Figure 1).** CT findings were suggestive of CO- RADS 5.

Hence, the patient was managed with Dexamethasone, Enoxaparin prophylaxis, broad spectrum antibiotics. Nasopharyngeal swab for Multiplex PCR -respiratory panel was sent which did not detect any pathogen. His blood culture did not grow any organism. Despite repetitive negative rRT-PCR for COVID-19, in view of strong clinical and radiological suspicion, the patient was managed in isolation room. He had ongoing fever spikes. After two days of admission, he developed shortness of breath and desaturation. Oxygen supplementation was initated. Chest x ray revealed increase in infiltrates bilaterally **(Figure 2).** Bronchial lavage was contemplated but deferred because patient was hypoxic. It was decided to do lavage if he progresses to invasive mechanical ventilation. Favipiravir was stopped and he was initiated on Remdesivir after a multidisciplinary discussion and as per advice of state medical board, after obtaining a detailed informed

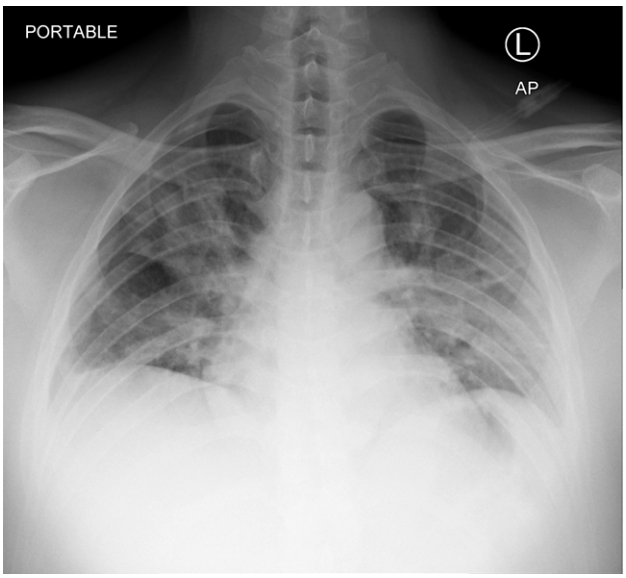

Figure 2. **Portable chest x ray- AP film showing bilateral infiltrates**

consent for the same. Enoxaparin was switched to therapeutic dosage. His CRP (50.6mg/L), D-Dimer (524ng/ml), Ferritin(1382ng/ml) were increasing. His LDH was very high 309 U/L (133-225 U/L), IL-6 was 34pg/ml (<7pg/ml). Echocardiography was done, it showed good biventricular function. He was initiated on oxygen via High Flow Nasal Cannula (HFNC) with 40 L/min flow and 50% FiO_2. In view of clinical, radiological, biochemical worsening, elevated IL-6 levels, possibility of cytokine storm was considered, and patient was initiated on Tocilizumab. He responded well, there was no further fever spike and there was partial radiological clearance in chest x ray. His IL-6 levels were repeated the next day and it was 313pg/ml but he was improving clinically. Two days after the first dose of Tocilizumab, patient had shortness of breath & radiological worsening in chest x ray and hence a second dose of Tocilizumab was given. His repeat IL-6 value was 136.4pg/ml.

Despite repetitive negative RT-PCR for COVID 19, in view of high clinical & radiological suspicion of COVID 19, antibody test for COVID 19 (CLIA method, Target antigen: Spike S1 and Nucleocapsid protein) was sent on the 14th day of illness and it came positive for both Ig M 2391.46 AU/ml (<10 AU/ml) and Ig G 34.96 AU/ml (<10 AU/ml) thus finally confirming COVID-19. Remdesivir, Methyl Prednisolone and Enoxaparin were continued. The patient showed gradual clinical improvement, and there was good radiological clearance in chest x ray **(Figure 3),** his NLR became less than 3, Ferritin, D-dimer, LDH, CRP decreased. HFNC support was gradually tapered and stopped. He continued to be hemodynamically stable,

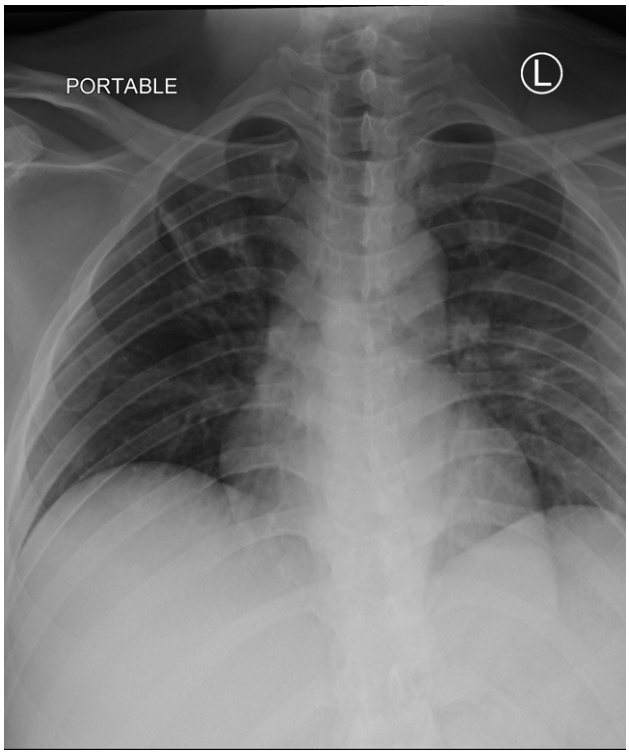

Figure 3. **Portable chest x ray – AP film follow up image taken five days later showing significant clearance of infiltrates.**

he was mobilized. He became clinically stable, he was off oxygen support for more than 3 days, and he was discharged with Apixaban (5mg twice daily, planned for 2 weeks duration).

DISCUSSION

The diagnosis of COVID-19 is made primarily by direct detection of SARS-CoV-2 RNA by nucleic acid amplification tests (NAATs), most commonly reverse-transcription polymerase chain reaction (RT-PCR) from the upper respiratory tract specimen. Various RT-PCR assays are used around the world and different assays amplify and detect different regions of the SARS-CoV-2 genome. Some target two or more genes, including the nucleocapsid (N), envelope (E), and spike (S) genes, and regions in the first open reading frame, including the RNA-dependent RNA polymerase (RdRp) gene.[2] Overall sensitivity of rRT-PCR for COVID-19 is about 71%.[3]

In most individuals with symptomatic COVID-19 infection, viral RNA in the nasopharyngeal swab as measured by the cycle threshold (Ct) becomes detectable as early as day 1 of symptoms and peaks within the first week of symptom onset. The Ct is the number of replication cycles required to produce a fluorescent signal, with lower Ct values representing higher viral RNA loads. A Ct value less than 40 is clinically reported as PCR positive. This positivity starts to decline by week 3 and subsequently becomes undetectable. However, in severe hospitalized cases, PCR positivity may persist beyond 3 weeks after illness onset but a "positive" PCR result reflects only the detection of viral RNA and does not necessarily indicate presence of viable virus.[3] The timeline of PCR positivity is different in specimens other than nasopharyngeal swab. PCR positivity declines more slowly in sputum and may still be positive after nasopharyngeal swabs are negative.[3]

The accuracy and predictive values of SARS-CoV-2 NAATs have not been systematically evaluated. Their clinical performance is more variable. Sensitivity of these test likely depends on the following: 1.Type and quality of the specimen obtained (BAL-95%, sputum-72%, oropharyngeal swab – 32%, nasopharyngeal swab – 66%),[4] 2.Duration of illness at the time of testing, 3. Specific assay (Point Of Care NAAT assays have lesser sensitivity).

Possible causes of false negative RT-PCR include: Analytical causes and Pre analytical causes. Analytical causes include intrinsic limitations and presence of inhibitory substances. Pre analytical causes includes faulty sample collection or transportation, inadequacy of the sample, inappropriate site of sampling for the stage of the disease. In a systematic analysis, pooled estimation of false negative RT-PCR was found to be 8.5%.[5] In this case, patient had started Favipiravir on day 1 of illness, perhaps that may be the reason for repeated negative rRT-PCR.

The most sensitive and earliest serological marker is total antibodies, which begin to increase from the second week of symptom onset. Although IgM and IgG ELISA have been found to be positive even as early as the fourth day after symptom onset, higher levels occur in the second and third week of illness. IgM and IgG seroconversion occur between the third and fourth week of clinical illness onset. Thereafter, IgM begins to decline and reaches lower levels by week 5 and almost disappears by week 7 whereas IgG persists beyond 7 weeks.[6]

Combined sensitivity of PCR and IgM ELISA directed at nucleocapsid (NC) antigen is 98.6% vs 51.9% with a single PCR test. IgM ELISA has a higher positivity rate after day 5.5 of illness.[3]

CT chest has higher sensitivity than RT-PCR in COVID 19 diagnosis.[7] The 7th Chinese Novel Coronavirus Pneumonia Diagnosis and Treatment Plan incorporates CT imaging into the clinical definition criteria

of COVID-19.[8] The Fleischner Society suggests a role for CT scanning, as a major tool if symptoms worsen. Hence, CT chest is critical for early detection and improvement of diagnostic confidence for patient with COVID-19 amid possible negative RT-PCR.

Typical CT findings include Ground-glass regions with unsharp demarcation (half) rounded shape or sharp demarcation outlining the shape of multiple adjacent secondary pulmonary lobules, Crazy paving Patterns compatible with organizing pneumonia, thickened vessels within parenchymal abnormalities, spider web sign and reverse halo sign.[8] The pooled sensitivity and specificity is 94% and 37% respectively, for chest CT.[7] CO-RADS (COVID 19 Reporting And Data System) scoring is used to determine the level of suspicion of COVID-19 in CT chest.[8]

The role of CT chest in COVID-19 is constantly evolving with modest scientific evidence but there are substantial differences in opinion on when and how the technique should be used for clinical workup or treatment decisions. Hence, patients with severe COVID-19 should be screened for hyperinflammation using laboratory trends (e.g., increasing ferritin, decreasing platelet counts, LDH, or erythrocyte sedimentation rate)to identify the subgroup of patients for whom immunosuppression could improve mortality.[9] Also, rising lab parameters alone should not be the sole reason to initiate immunosuppression. They should always be corelated with clinical assessment for decision making.

This case is being published because of its uniqueness in terms of the atypical presentation, diagnostic challenges it posed and the timely and appropriate therapeutic decision making which was the turning point in the course of the disease.

END NOTE

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