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Case Report

Case Report: Discordant RT-PCR Results in COVID-19 Patient Challenges Clinical Decisions

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ABSTRACT

We report a case study of a COVID-19 patient whose PCR results fluctuated from detected to not detected over a period of 3 weeks depending on the PCR assay used and the challenge this presented to clinicians. This is the first report of the longitudinal assessment of a COVID-19 patient in Ireland who was followed up for 45 days since the first positive result. Our findings highlight the difficulty for clinicians in making a decision to move patients to a different ward or for discharge from hospital whilst adhering to initial recommendations published by the WHO to confirm clearance of the virus which was that the patient required to be clinically recovered and to have two negative RT-PCR results on sequential samples taken at least 24 hours apart. Furthermore, researchers have stressed the need for caution in interpreting any negative results of RT-PCR diagnostic tests as many factors can play a role in the accuracy of the results.

Key clinical message: This case report highlights that although in general PCR assays are able to detect SARS- CoV-2, the detection limits and the ability to differentiate between true negatives and positives at low RNA concentrations are variable between assays.

Keywords: SARS-CoV-2; RT-PCR; Immunity

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has spread rapidly throughout the world since the first cases of Coronavirus Disease 2019 (COVID-19) were observed in December 2019 in Wuhan, China [1]. On 11th March 2020 due to the alarming levels of spread and severity of disease as well as the number of affected countries, the World Health Organisation declared COVID-19 a pandemic. In response, several real time Reverse Transcription Polymerase Chain Reaction (RT-PCR) commercial assays were developed for diagnostic testing for the presence of SARS CoV-2 virus in nasopharyngeal and throat swabs. In St Vincent's University Hospital in Dublin, the Altona SARS CoV-2 RT PCR assay was used in the initial testing of patient samples however due to supply chain rationalisation, the Genesig SARS CoV-2 RT PCR assay was verified for future use. This is the first report of the longitudinal assessment of a COVID-19 patient in Ireland who was followed up for 45 days since the first positive result. Our findings highlight the difficulty for clinicians in making a decision to move patients to a different ward or for discharge from hospital whilst adhering to initial recommendations published by the WHO on 12 January 2020 [2].

CASE STUDY

An 81 year old lady presented to the emergency department of St. Vincent's University Hospital on 14th March 2020 with shortness of breath and a temperature of 38.7oC. She had been diagnosed with multiple myeloma in 2019 and was on treatment with Lenalidomide and Dexamethasone. The day before presenting to the emergency department she had suffered from 2 falls when trying to get up from the sitting position because 'her legs gave way'. On presentation, the emergency department doctor noted crackles over the right base, her lactate was 1.1 U/L, pH 7.44 and her oxygen saturation was 94% on 4 litres of oxygen via nasal prongs. The peripheral white cell count was 4.8 × 109/l with a decreased lymphocyte count of 0.6 × 109/l and a C-reactive protein of 211 mg/L. Her chest X-ray showed consolidation in the left mid and lower zone and a smaller inflammatory infiltrate in the right base Figure 1A. Her nasopharyngeal swab did not detect Influenza A, B or RSV but detected SARS-CoV-2 giving a Ct value of 18.06 using the Altona RT-PCR assay. She was started on empiric Piperacillin/Tazobactam to cover her for possible concomitant bacterial pneumonia and admitted to the COVID-19 ward.

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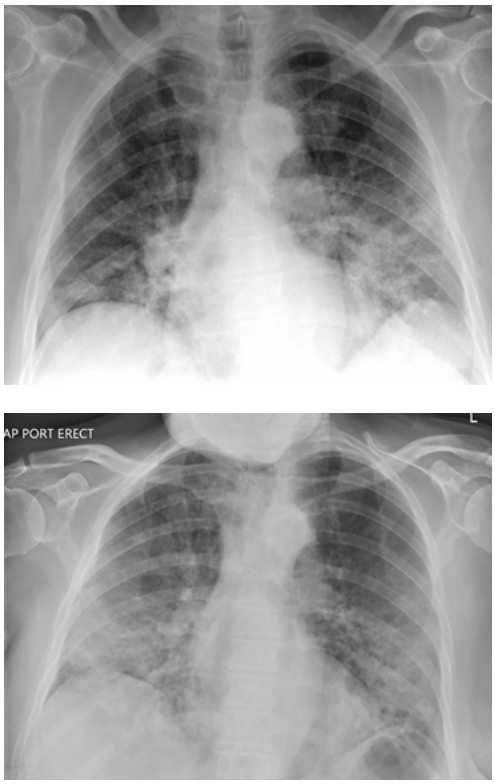

Figure 1: Chest X-rays taken on the day of admission, 14 March (A) shows consolidation of the left mid and lower zone. Minor inflammatory infiltrate right base was also noted. (B) Chest X-ray taken on 24 March shows worsening air space opacity in mid and lower zones bilaterally.

Over the following days the patient's condition deteriorated with increasing oxygen requirements. With 15 litres of supplemental oxygen delivered by Airvo, she was able to maintain oxygen saturations of 92%. But as the patient found it impossible to tolerate the Airvo mask, she requested to remove it. Her oxygen saturations dropped to 54% at room air and the Airvo was replaced with intermittent delivery of supplemental oxygen by nasal prongs. Her early warning score rose intermittently up to 8 and after discussion with herself and her family the decision was made that she would not be admitted to the intensive care unit for resuscitation in case of cardiac arrest. Despite her hypoxia the patient remained comfortable and refused any pain medication. She declined physiotherapy. A follow up chest X-ray on the 24th of March showed worsening air space opacity in mid and lower zones bilaterally (Figure 1B). Piperacillin/Tazobactam was discontinued after 10 days.

Two weeks after admission to hospital, the patient was saturating up to 97% on 15 litres of oxygen via wide bore nasal prongs. She was able to speak full sentence and her condition had stabilised. Due to an increase in inflammatory markers and biochemical parameters (Figure 2) and persistent dense consolidation in mid and lower zones bilaterally, Piperacillin/ Tazobactam was restarted two days later. Her oxygen saturation improved (97% on 2l). Daily COVID-19 testing was initiated to determine whether she could be discharged to the non- COVID ward. She was discharged to the non-COVID ward on the 5th of April with a persistent cough after two swabs taken on the 2nd and 3rd of April did not detect SARS-CoV-2 using the Altona and Genesig PCR assays respectively. Retrospective testing 6 on the sample of 2nd April using the Genesig PCR assay revealed detectable viral load with Ct 20.6. Table 1 shows the comparison of the PCR results upto 45 days after the patient first tested positive for SARS CoV-2.

Table 1: Longitudinal analysis of RT-PCR for SARS CoV-2 in a patient with severe COVID-19. The Altona SARS-CoV-2 RT-PCR Kit is based on the detection of the lineage B- betacoronavirus (B-βCoV targeting the E gene) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 targeting the S gene which encodes the Spike protein) specific RNA. The Genesig assay is based on the detection of SARS-CoV-2 using primers selected from the orf1 ab gene region which encodes the RdRP protein.

Date	Days	Altona		Genesig	
		Ct	Interpretation	Ct	Interpretation
16/03/2020	0	18.06	Detected	NT	
22/03/2020	6	-	Not detected	NT	
24/03/2020	8	-*	Indeterminate	35.62	Detected
25/03/2020	9	-	Not detected	35.81	Detected
27/03/2020	11	26.66	Detected	31.56	Detected
29/03/2020	13	29.3	Indeterminate	37.41	Indeterminate
30/03/2020	14	-	Not detected	37.85	Indeterminate
31/03/2020	15	26.18	Detected	33.17	Detected
02/04/2020	17	-	Not detected	20.6	Detected
03/04/2020	18	NT		-	Not detected
06/04/2020	21	NT		36.24	Detected
16/04/2020	32	NT		38.98	Indeterminate
29/04/2020	45	NT		-	Not detected

NT = Not tested; *Only E gene detected.

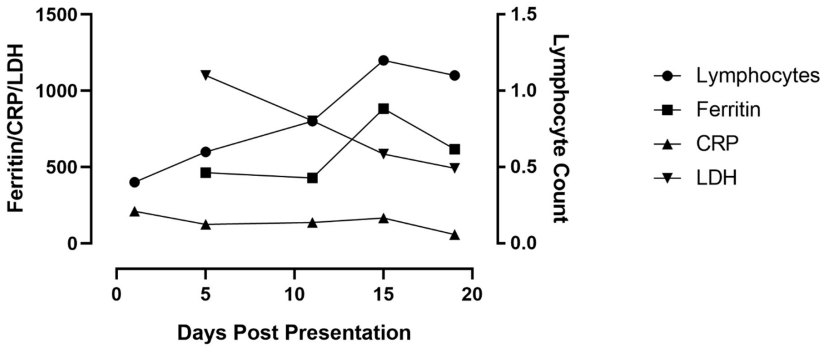

Figure 2: Longitudinal laboratory assessment of biochemical markers including lymphocyte count (cells × 109/L), ferritin (ug/L), CRP (mg/L) and LDH (U/L) Normal Ranges: Lymphocyte cells 0.9-2.9 × 109/L; Ferritin 13-150 ug/L; CRP 0-5 mg/L; LDH 240-480 U/L

As this was the first COVID-19 positive patient being discharged from the COVID-19 ward but requiring further hospital care the receiving ward decided to repeat SARS-CoV-2 testing. Follow up testing on the 6th of April was detected at a Ct 36.24, indeterminate on 16th April and not detected on 29th April using the Genesig PCR assay. The patient was continued to be cared for with enhanced droplet precautions till April 10th. She continued to slowly improve and was discharged to convalescence on May 4th.

DISCUSSION

The accurate diagnosis of people infected with the SARS CoV-2 virus is essential to curb the global spread of COVID-19. This case study highlights the detection of SARS CoV-2 using RT-PCR upto 45 days post infection in an elderly patient with multiple myeloma who required a hospital stay of 7 weeks. The initial recommendation by the WHO of 2 negative RT-PCR tests at least 24 hours apart put intense pressure on laboratory scientists, clinicians and hospital capacity [2]. Although the patient had a persistent cough at Day 17, the results of the RT-PCR tests found no detectable virus on two consecutive days and the patient was moved to a non-COVID ward. The recent scientific brief from the WHO on the criteria for releasing COVID-19 patients from isolation on 17 June 2020,[3] states that the amount of detectable virus is substantially reduced over time and has been shown to correlate with reduced infectivity [4,5]. The report however does state that after resolution of symptoms, the risk of transmission is low however it cannot be completely ruled out [3]. It was noted in our patient that the chest X-rays showed a worsening of the lung air space opacity (Figure 1) despite the viral load decrease over this time period (Table 1). Our findings raise questions regarding the value of a detectable viral load on nasopharygeal swabs in the context of infectivity and the implications for infection control. In our case patient, it was 20 days post infection that laboratory markers such as lymphocyte count, CRP and LDH returned to within the normal ranges (Figure 2).

The findings in our patient are in agreement with a study which reported that in five patients who received glucocorticoid treatment, the duration of viral RNA detection from oropharyngeal swabs was found to be 15 days which was significantly longer than that in the non-glucocorticoid treatment group which was 8 days [6]. Furthermore, a recent study showed a reduction in symptoms in about 30% of severe cases [7]. However, in our patient, dexamethasone was stopped when COVID was diagnosed.

This case report highlights that although in general RT-PCR assays are able to detect SARS- CoV-2, the detection limits and the ability to differentiate between true negatives and positives at low RNA concentrations are variable between assays and therefore careful evaluation to determine Ct value cut-offs to differentiate between positives and negatives is required in individual laboratories. Furthermore, the viral loads should not be used as the sole basis for patient management decisions but must be analysed in combination with clinical observations, patient history, and epidemiological information to inform appropriate clinical decisions.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Dr Smith: Made substantial contributions to acquisition of data, has been involved in drafting and revising the manuscript, and has given final approval of the version to be published.

Dr Smyth: Made substantial contributions to the clinical management of the patient, acquisition of data and has given final approval of the version to be published.

Dr Feeney: Made substantial contributions to design, clinical management of the patient, acquisition of data and has given final approval of the version to be published.

Dr Schaffer: Made substantial contributions to conception and design and acquisition of data; clinical management of the patient, has been involved in drafting and revising the manuscript critically for important intellectual content and has given final approval of the version to be published.

Dr Hassan: Made contributions to acquisition of data, has been involved in drafting and revising the manuscript critically for important intellectual content and has given final approval of the version to be published.

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