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Coronavirus Pandemic

Delayed RT-PCR Time-To-Positivity in an adult with SARS-CoV-2 Infection

Nur Chandra Bunawan[1], Md Ikhsan Mokoagow[2], Aryan Yohanes Djojo[2], Natalia Wistriany[1], Hardijatmo Muljo Nugroho[1], Annisa Dian Harlivasari[1], Setioningsih Diponegoro[1]

[1] Coronavirus Disease 2019 (COVID-19) Task Force, Kramat Jati Regional General Hospital, Jakarta, Indonesia
[2] Department of Internal Medicine, Fatmawati General Hospital, Jakarta, Indonesia

Abstract

Early diagnosis is among the crucial measures to control the spread of SARS-CoV-2 infection. To date, reverse transcription polymerase chain reaction (RT-PCR) is the gold standard for COVID-19 testing, but various factors can affect its performance leading to false negative results. Hereby we present a patient with a high clinical suspicion for COVID-19 and had multiple negative RT-PCR results over 5 days. A 22-year-old woman presented with fever, dry cough, nausea, myalgia, headache, and mild dyspnea. Eleven days before, she was in close contact with her father who had tested positive for COVID-19. RT-PCR on nasopharyngeal and oropharyngeal swabs were performed on day 8, 9, and 12 of illness which all came back negative even after she started having a worsening dyspnea and showing an increased lung opacity from radiographic findings on day 11 of illness. Interestingly, her rapid antibody test (VivaDiag™ COVID-19 IgM/IgG rapid test by VivaChek Biotech (HangZhou,China) was positive for anti-SARS-CoV-2 Ig M and Ig G. Due to the worsening condition, she was referred to a tertiary hospital where her RT PCR result was positive on day 13 of illness. After 28 days from her first symptom, she was discharged from the hospital with improved symptoms and chest X-ray. As conclusions, in patients with high suspicion of COVID-19, repeat swab tests are mandatory if previous tests were negative. The diagnosis and treatment plan of COVID-19 should not solely be based on RT-PCR, but also consider the patient's history, symptoms, laboratory result, and radiographic findings.

Key words: RT-PCR; time-to-positivity; SARS-CoV-2; false negative.

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Introduction

Since its first appearance in China, numerous efforts have been made to control the spread of Coronavirus Disease 2019 (COVID-19) [1,2]. Early reporting systems, case identification, large-scale surveillance, city lockdown, and preparing healthcare facilities have been implemented by the Chinese government and have successfully reduced the epidemic in China generally [2]. However, until December 2020, the number of cases has grown exponentially outside China, reaching a cumulative number of 65.8 million cases with 1.5 million deaths globally [3]. In a setting where ongoing community transmission happens, the suppression strategy through early case detection is critical. The earlier a case gets detected, the sooner the isolation can be performed, preventing further spread of the disease [4]. Several diagnostic methods have been used to detect and diagnose COVID-19, namely, protein testing (antigen or antibody), Computed Tomography scanning (CT scan), and nucleic acid testing [5]. So far, nucleic acid testing, especially Reverse Transcription Polymerase Chain Reaction (RT-PCR), has been the gold standard for diagnosing Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [6,7]. Although considered a gold standard method, RT-PCR has some limitations such as the requirement of sophisticated resources and trained staff, expensive cost, time-consuming result, and lack of sensitivity [8]. The sensitivity of RT-PCR test on nasopharyngeal specimen, which ranges from 60-85%, relies on sample collection and viral load. This result varies depending on the days after disease onset. The high false-negative rate is higher when the test is performed too soon [5,9-11], making the interpretation more challenging and could lead to a catastrophic decision. This report aimed to describe clinical and radiographic features of a COVID-19 patient with initial multiple negative RT-PCR result which turned positive after five days from the initial test.

Case presentation

A 22-year-old woman presented to Kramat Jati Regional General Hospital, a primary hospital in

Indonesia, with medical symptoms of fever, nausea, malaise, headache, and myalgia since 8 days prior to admission. Her fever was subside, but she started to have dry cough and mild shortness of breath since 2 days prior to admission. No sore throat, runny nose, diarrhea, loss of smell, or loss of taste were experienced by the patient. Her past medical history was unremarkable. Patient was unemployed, living with her parents, and had been staying at home for 2 months. Eleven days prior to admission, she took care of her sick father who had tested positive for COVID-19 in our hospital. Upon admission, her blood pressure was 120/70 mmHg, with a temperature of 37.1°C, a heart rate of 132 beats per minute, and a respiratory rate of 24 times per minute. In the respiratory system examinations, no crackles, wheezing, or rales were detected. There was mild epigastric tenderness from abdominal examination. The laboratory test result is shown in Table 1. At admission, her chest X-ray showed right peripheral opacity (Figure 1A). The patient was hospitalized on May 25, 2020 (day 8 of illness) and treated as a COVID-19 suspect case. Intravenous fluid, Ceftriaxone 2 gram OD, Zinc 20 mg bid, Vitamin C 500 mg OD, N-acetyl cysteine 200 mg tid, and Azithromycine 500 mg OD were administered. RT-PCR on nasopharyngeal and oropharyngeal swabs were performed on May 25 and May 26, 2020 (day 8 and day 9 of illness, respectively) which came back negative (Cycle-threshold value threshold: 40). On May 28, 2020 (day 11 of illness) her cough and dyspnea worsened. A repeat chest X-ray showed progressive worsening of right pneumonia (Figure 1B). On May 29, 2020 (day 12 of illness) her rapid antibody test (VivaDiag™ COVID-19 IgM/IgG rapid test by VivaChek Biotech [HangZhou] Co Ltd [China]) showed positive for anti-SARS-CoV-2 Ig M and Ig G, which was not aligned with the negative RT-PCR (Cycle-threshold value threshold: 40) performed subsequently on the same day. RT-PCR on bronchoalveolar lavage was not conducted in our hospital due to the limited availability of these resources. Given the worsening of clinical and radiographic features, the patient was referred to Fatmawati General Hospital, a tertiary hospital in Indonesia, at which she was given oseltamivir 75 mg bid in addition to previous medication.

On the next day (May 30, 2020, day 13 of illness), RT-PCR test on nasopharyngeal and oropharyngeal swabs were repeated in Fatmawati General Hospital. The result was positive (Cycle-threshold value threshold: 31.5). The patient's clinical condition improved during the care. On June 6, 2020 (day 20 of illness), repeated chest X-rays showed an improvement (Figure 1C). The patient was discharged from the hospital on June 14, 2020 (day 28 of illness).

Discussion

Our patient represents a classical clinical picture of COVID-19. Her symptoms started eight days before admission, three days after she was in close contact with her father who was positive for SARS-CoV-2 infection. This is in accordance with early literature from China which reveals that the incubation period of COVID-19 ranged between 2-13 days (average 4-5 days) [12,13]. Fever, dry cough, and malaise are the three most common symptoms found in COVID-19 patients. Other constitutional symptoms could be found were headache, myalgia, nausea/vomiting, and shortness of breath [12]. Our patient's laboratory result showed elevated lactate, mild liver injury marked by slightly elevated AST, ALT, and LDH level. According to a study by Gholizadeh *et al.*, 14.7% of patients with COVID-19 have increased ALT. This study also found that patients with COVID-19 had an elevated LDH

Table 1. Laboratory Examination result of the Patient during Hospitalization.

Laboratory exam	25/05/2020	31/05/2020	02/06/2020	04/06/2020	06/06/2020	13/06/2020
Hb (g/dL)	14.3	12.1	13.2	13.5	13.1	12.7
Leukocytes count (/uL)	6,600	4,700	5,100	4,100	4,500	4,700
Platelet count (/uL)	144,000	292,000	325,000	283,000	226,000	203,000
NLR	2.25	1.7	1.5	1.5	2.1	1.4
ALC (/uL)	1,848	1,551	1,785	1,435	1,215	1,739
AST (U/L)	41	42	38		37	
ALT (U/L)	22	53	46		58	
LDH (u/l)		311	316			
Lactate (mmol/l)		4.4	2.8			
CRP (mg/dl)		< 0.4	< 0.4	< 0.4	< 0.4	
Procalcitonin (ng/ml)		< 0.07				
Ferritin (ng/ml)			207			

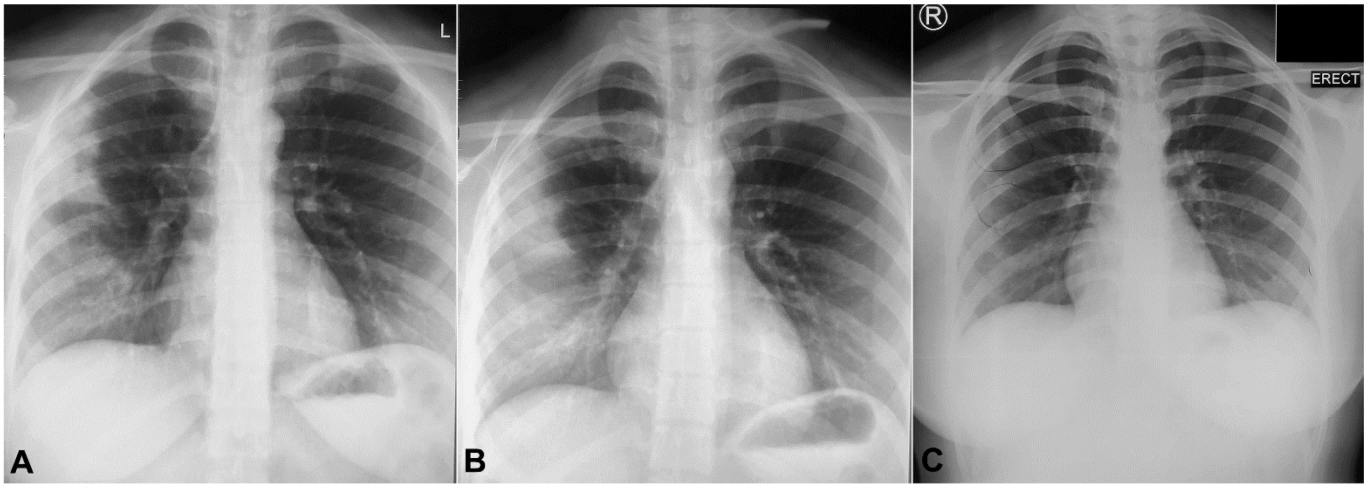
Hb: Hemoglobin; NLR: Neutrophil-Lymphocyte Ratio; ALC: Absolute Lymphocyte Count; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; LDH: Lactate Dehidrogenase; CRP: C-Reactive Protein.

level, especially in patient with increased ALT [14]. Other findings associated with COVID-19 infection are leukopenia (21.3%) and lymphopenia (83.2%) [12]. Although our patient's leukocyte level was not below normal, the trend tended to decline during hospitalization.

Her clinical presentation along with radiographic evidence met the criteria for a suspect case of COVID-19 [15]. A nasopharyngeal swab and an oropharyngeal swab were obtained on day-1 and day-2 of admission for RT-PCR analysis and came back negative. However, the result was presumed to be false-negative as she demonstrated high clinical suspicion of COVID-19. False-negative happens when an individual with a true infection shows a negative result [16]. It occurs with a ratio of around 1 in 5 COVID-19 patients [10]. In an article by Yang *et al.,* which reported positivity rate of RT-PCR from several specimens, specimens taken within seven days after illness onset have false-negative rate around 27.3-46.8% (oropharyngeal specimen), 14.7-37.9% (nasopharyngeal specimen), and 12.5-17.4% (sputum specimen) respectively. This study also showed specimen from Bronchoalveolar Lavage yielded 86% of positivity rate if taken within fourteen days after illness onset [11]. However, due to resource limitation, Bronchoalveolar Lavage was not performed in our hospital. Another study about RT-PCR false negative rate by Li *et al*., revealed that almost 25% of SARS-CoV-2 positive patients were negative at initial testing [17]. Moreover, an early study in Wuhan showed that 21.4% of a total of 70 patients were tested positive after two consecutive negative results [18]. They explained that this result may be caused by a false negative of RT-PCR test along with prolonged nucleic acid conversion.

RT-PCR false-negative results can be caused by several conditions (i.e., genetic diversity, sampling errors, inappropriate sample type, viral load, and optimal time). Genetic diversity creates variation in viral sequences leading to mismatches between target regions and primers; Sampling errors occur during sample collection, transportation, and handling; Inappropriate sample type is defined as specimen collection from the wrong anatomical site of the body at the wrong time. For reference, sputum, nasopharyngeal, and oropharyngeal specimen are best collected in the early stage of the disease; Viral load depends on when the patient reaches the peak SARS-CoV-2 concentration after symptom onset [16]; The optimal time for RT-PCR according to Kucirka *et al*., was on day 8 of infection (3 days after symptoms onset) with 20% rate of false negativity [10]. Taking samples before and after this time frame will increase the likelihood of false-negative result. The patient's clinical condition deteriorated on day 11 of illness as the non-productive cough became more frequent and dyspnea worsened. The clinical spectrum of COVID-19 starts from the early minimal symptoms (stage I), moderate pulmonary symptoms (stage II), to severe systemic inflammation (stage III). Our patient belonged to stage II of the disease where viral multiplication and localized inflammation in the lung appeared. In this stage, the host inflammatory response starts to take over the viral response [19]. A repeat chest X-ray showed worsening of the lung lesions. Xu *et al*., explained that at least one out of three following criteria is required to confirm COVID-19 infection in suspected cases: 1) positive RT-

Figure 1. Patient's Chest X-Ray Images.



A: Chest x-ray on 25/05/2020 (day 8 of illness), B: Chest x-ray on 28/05/2020 (day 11 of illness), C: Chest x-ray on 06/06/2020 (day 20 of illness).

PCR for SARS-CoV-2 nucleic acid; 2) high SARS-CoV-2 homology from gene sequencing; 3) serological criteria (positive serum specific Ig M and Ig G antibodies for SARS-CoV-2 infection, negative to positive changes of serum specific Ig G antibodies, and 4-fold increase of antibody level in convalescent stage) [20]. Due to laboratory insufficiency, we were only able to perform a rapid antibody test on our patient initially, in which her Ig M and Ig G were shown to be positive. The antibody test is helpful to confirm SARS-CoV-2 infection in people with symptoms and negative RT-PCR test or in the area where RT-PCR is not available. However, careful consideration of when to use this test is crucial to yield accurate results [21]. The patient was finally tested positive on day 13 of illness. To our knowledge, this is one of a few reports describing persistent negative RT-PCR results (day 8, day 9, and day 12 of illness) before eventually turning positive. A previous case report from Indonesia also described a patient with a clinical picture of COVID-19 whose RT-PCR test was negative on day 10 of illness but turned positive on day 14 of illness [22]. One case series from the United States of America (USA) described three patients with suspected COVID-19 who had initial negative RT-PCR results from nasopharyngeal samples. All of them had deterioration in clinical picture and developed respiratory failure during hospitalization. Repeated RT-PCR test was positive from endotracheal sputum sample, endotracheal sample, and nasopharyngeal sample respectively in those three cases [23]. The interval between the initial negative to positive RT-PCR in our case was 5 days. This is similar to a study conducted by Ai *et al*., which found that the mean interval between the initial negative to positive RT-PCR results was 5.1 days ± 1.5 [24].

Conclusions

Given the high false-negative rate of RT-PCR, health care professionals must be vigilant in interpreting the result. Repeat testing is mandatory in patients with high clinical suspicion but multiple negative RT-PCR results. Diagnosis, treatment, and discharge criteria should not only depend on RT-PCR. Other clinical manifestations, laboratory profile, and radiographic findings must also be taken into consideration.

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Corresponding author

Nur Chandra Bunawan, MD
Department of Internal Medicine, Kramat Jati Regional General Hospital,
Jalan raya inpres, No.48, 13540, Jakarta, Indonesia
Phone: +6282153852892
Email: nur.chandra86@gmail.com

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