INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

PAGE 224
2021 March; 27(2): 224-227
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

Cross-Reaction Antibody Test between SARS-CoV-2 and Dengue Hemorrhagic Fever in Indonesia

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ABSTRACT

Coronaviruses are a family of viruses that cause illness from the common cold to severe diseases such as Severe Acute Respiratory Syndrome (SARS-CoV). In December 2019, forty new cases of pneumonia of unknown etiology have been reported in Wuhan, China. The disease resembles Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and has been subsequently named the 2019-novel Coronavirus Disease (COVID-19). The antibody test is a blood test that provides quantitative and qualitative detection of IgG and IgM antibodies against the SARS-CoV-2. Reported a male, 43-year old suffering from DHF, but the results of an IgG and IgM rapid test were COVID-19 reactive. Also, reviewed rapid tests for COVID-19 and the results showed that only IgG was reactive. This explained that the patient already had SARS Cov-2 antibodies but was not suffering from the disease. The rapid test COVID-19 IgM result was deemed to be a false positive.

Keywords: COVID-19, dengue hemorrhagic fever, cross-reaction

INTRODUCTION

Coronaviruses are a family of viruses, these viruses can cause illness like common cold only or Severe Acute Respiratory Syndrome (SARS-CoV), or Middle East Respiratory Syndrome (MERS-CoV). In December 2019, Wuhan, China reported a new case of pneumonia with an unknown cause of pneumonia was reported. On January 7, 2020, the Chinese Center for Disease Control and Prevention (CDC) revealed a novel beta-Coronavirus from a throat swab sample of a patient through high-throughput sequencing. The disease resembled Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and has been subsequently named the 2019-novel Coronavirus Disease (COVID-19) by the World Health Organization (WHO).[1-3]

The antibody rapid test for COVID-19 is a test based on immunochromatography that provides qualitative detection of IgG and IgM antibodies to SARS-CoV-2. At the beginning of the disease, the IgM titer will increase, while the IgG will follow later. At the healing stage, IgG will increase while IgM decreases and eventually disappears. IgG will remain in the blood for a long time. SARS-CoV-2 IgM will be detected after three days of the onset of symptoms but sometimes these antibodies can be detected after the seventh day.[1,4] The clinical symptoms of COVID-19 are so similar that clinicians will find it difficult to distinguish it from Dengue Hemorrhagic Fever (DHF) because of the similarities in onset, clinical symptoms, and laboratory results. False-positive for rapid test COVID-19 is the condition that it is not a COVID-19 disease, but the results of the test are positive when antibodies are detected, while the actual cause is not COVID-19 (other viruses cause similar antibodies) so that they can cause cross-reactions, for example with dengue virus (dengue fever), or another Coronavirus infection.[4,5]

CASE

On June 27, 2020, a 43-years old male came to the Emergency Room in Bekasi, Indonesia with a fever for four days before. He had hypertension, and also said that his neighbor suffered from dengue fever. On March 18 2020 he was treated as COVID-19. Physical examination pulse 96 beats per minute, respiration rate 20 breaths per minute, body temperature 39.8^{o}C and blood pressure 132/87 mmHg. Bodyweight 70 kg. There was no sign of rhonchi or wheezing. Other general and systemic examinations revealed no abnormality. The working diagnosis on admission was dengue fever. The laboratory result of this patient can be seen in Table 1.

Table 1. Laboratory results

Lab. Results	Date						
	June 27	June 28 (09.30)	June 8 (20.00)	June 29 (07.00)	June 29 (19.00)	June 30 (06.00)	July 1
Hb (g/dL)	15	15.2	16.3	16.2	15.6	16.2	14.3
Leucocyte (uL)	4,050	3,170	2,990	6,250	14,400	16,690	11,310
Hematocrit (%)	42	42	45	44	43	42	40
Platelet (uL)	153,000	26,000	20,000	20,000	35,000	53,000	99,000
Diff	0/2/3/62/25/8						
HFLC (%)	0.5					10.7	5.7
C-RP (mg/L)	5.5						
Widal	Negative						
NS1	Positive						
Antibody SARS Cov-2	IgM and IgG reactive						IgM non-reactive; IgG reactive
Chest X-Ray	Bronchitis						
CT Scan Lung	No ground-glass opacity						
RT-PCR		Negative			Negative		

DISCUSSION AND CONCLUSIONS

Coronaviruses are a family of viruses, these viruses can cause illness like common cold only or SARS-CoV or MERS-CoV. In December 2019, the Chinese government reported a case of pneumonia with no known cause. This disease was so contagious that the Chinese government has implemented a lockdown in Wuhan. On January 7, 2020, the Chinese CDC revealed a novel beta-Coronavirus from the throat swab sample of a patient through high-throughput sequencing. The disease resembled severe SARS-CoV and has been subsequently named COVID-19 by the WHO.[1-4]

When a virus enters a person's body, the viral antigen will stimulate the immune system so that it will produce antibodies. In this situation, these antibodies can be detected in the patient's blood. The IgM antibodies appear early and are mostly positive after 3 days of onset and increase until day 28. Whereas IgG appears on day 10, increases until day 49, and can be found in plasma for a long time. IgG and IgM levels are higher in patients with severe clinical symptoms compared to mild clinical.

Serology testing for SARS-Cov2 is of interest because of the relatively short time to diagnose and the ability to test for an active immune response against the virus. Research has demonstrated that the nucleocapsid (N) and spike (S) proteins are the primary viral antigens against, which antibodies are raised. All of these antigens are commonly used in rapid tests. IgA antibodies may also increase during infection, and are typically found in mucous (such as saliva).[1] The results of total antibodies, IgM, and IgG were reported in 173 patients. Usually, antibodies start forming in the first week of infection. At this stage, only 40% of antibodies can be detected and will increase rapidly in the second week. This is inversely proportional to viruses. The PCR instrument could detect SARS-CoV-2 RNA about 66.7% in the first week and will decrease to 45.5% after the second week.[6]

COVID-19 and dengue fever are difficult to distinguish because they have shared similar laboratory and clinical features. The meaning of false positive for the COVID-19 rapid test is that the patient does not have the COVID-19 disease, but the results of the test are positive where antibodies are detected, but the actual cause is not COVID-19 (other viruses cause similar antibodies) so that they can cause cross-reactions, for example with dengue virus (dengue fever), or another Coronavirus infection.[4,5]

From the literature, it is known that two patients in Singapore detected a false positive fast test result of dengue fever, which later turned out to have COVID-19 and not dengue fever. In the first case, on February 9, 2020, a male, 57 years old without a clear history of infection, experienced DHF-like symptoms, namely coughing and fever for three days. The laboratory results found thrombocytopenia (platelet count 140,000/uL) and normal chest X-ray. He was discharged after a negative rapid test for dengue IgG, IgM, and NS1. However, when the patient came to the hospital again, the second dengue rapid test was positive for dengue IgG and IgM. It turned out that

based on the results of the nasopharyngeal swab samples tested by RT-PCR gave positive results. Doctors also repeated rapid tests for dengue IgG and IgM and the results were not reactive. From the results, doctors suspected that the results of the IgG and IgM rapid tests for dengue fever were false positives.[5]

On February 13, a second case was found, a 57-year old female who came to a hospital in Singapore with an unclear clinical history. There was no history of contact with DHF sufferers. She suffered from cough, fever, and muscle aches for 4 days. The laboratory result: positive for dengue IgM. Laboratory results showed lymphocytopenia and thrombocytopenia (65,000/uL). The test results with the SARS-CoV-2 RT-PCR were positive. Repeated rapid dengue fever tests were negative, RT-PCR for dengue fever was also negative. The initial dengue IgM result was deemed to be false positive.[5]

The WHO divides diseases caused by the dengue virus into mild undifferentiated febrile illness, Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF), and Dengue Shock Syndrome (DSS). Dengue virus has 4 serotype variants, namely DEN-1, DEN-2, DEN-3, and DEN-4. Dengue fever is an acute infectious disease caused by the dengue virus and characterized by symptoms of biphasic fever, headache, myalgia, rash, pain in some parts of the body, lymphadenopathy, leukopenia, and thrombocytopenia.[7]

Clinical criteria for DHF were based on the WHO, such as an increase in hematocrit as much as more than 20% (hemoconcentration) or plasma leakage. Dengue hemorrhagic fever criteria established by WHO is when at least two clinical criteria plus one laboratory criteria are met (at least there is an increase in blood hematocrit). Fever that occurs is usually sudden (2-7 days) and sometimes biphasic, and spontaneous bleeding, such as petechiae, ecchymosis, or purpura can be found and, thrombocytopenia (<100,000/uL).

Dengue virus can trigger the cellular and humoral immune system so that after the third day of fever, the anti-dengue IgM serological examination can become reactive, which is then followed by IgG. The changes in the humoral immune system can be detected through the formation of IgM and IgG antibodies that can be identified by rapid/serological examination.[4,6,7]

The changes in the cellular immune system can be assessed by finding atypical lymphocytes that are typical for dengue fever infection, namely, blue plasma lymphocytes are considered as useful markers for early diagnosis with high sensitivity and specificity. These atypical lymphocytes can be evaluated on peripheral blood smear with Wright's or Giemsa stain. Budi *et al.* reported that an increase in atypical lymphocytes can be detected with hematology analyzer and the results are presented as High-performance Lymphocyte Count (HFLC). In cases of DHF, there is an increase in HFLC (normal HFLC is < 1.4%).[5,7,8]

Non-Structural Protein 1 (NS1) is a non-structural protein NS1 from the dengue virus. If infected with DHF, this protein is detected in the patient's blood from the first day of fever and will disappear after the seventh day of fever. This NS1 test can be used to diagnose dengue viral infection.[9]

In March 2020 patient recovered from COVID-19, but in June 2020 he came again to the Emergency Room with a fever four days before. Laboratory results were in leukopenia, normal platelets, HFLC, and C-RP, no lymphocytopenia, Widal test was negative, NS1 positive, antibody SARS-CoV-2 IgM and IgG were reactive. Chest X-ray: bronchitis pattern, no obvious signs of infiltrates. High-resolution CT lung: no visible picture of infiltrates/ground-glass opacity, which is typical for active COVID-19 infection.

From the results data that confused because was NS1 showed positive with reactive IgM and IgG COVID-19 but the CT scan and chest X-ray did not support COVID-19. He also had suffered COVID-19 before. After conducting RT-PCR, which gave negative results, concluded that a cross-reaction had occurred in the rapid test COVID-19. Concluded that the patient was suffering from dengue fever although the platelet count was still normal.[4,5]

From further data (June 28 to July 1, 2020) supporting DHF, for example, thrombocytopenia, increased HCT and HFLC, leukopenia, lymphocytosis. Many studies report that COVID-19 often occurs in lymphocytopenia. Based on these data, lymphocytes were increased and did not support COVID-19.[10-12]

The platelets decreased from 153,000/uL (June 27) to 20,000/uL (June 29) and increased again on June 30 and Jul y 1, 2020, with no fever in 3 days. All these data supported DHF.[8] Also reviewed rapid tests for COVID-19 and the results showed that only IgG was reactive. The initial COVID-19 IgM result was deemed to be false-positive because the patient already had SARS-CoV-2 antibodies but was not suffering from the disease.

REFERENCES

1. To KKW, Tsang OTY, Leung WS, Tam AR, Wu TC, *et al.* Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: An observational cohort study. Lancet Infect Dis, 2020; 20: 565–74. Available from: https://doi.org/10.1016/S1473-3099(20)30196 (accessed 3 July, 2020).
2. Hoffman T, Nissen K, Krambrich J, Rönnberg G, Akaberi D, *et al*. Evaluation of a COVID-19 IgM and IgG rapid test; An efficient tool for assessment of past exposure to SARS-CoV-2. Published by Informa UK Limited, trading as Taylor & Francis Group. Available from: https://doi.org/10.1080/20008686.2020.1754538 (accessed 4 July, 2020).
3. Tufan A. COVID-19, immune system response, hyper inflammation and repurposing antirheumatic drugs. Turk J Med Sci, 2020; 50: 620-632.
4. Hadi WS. Pemeriksaan laboratorium pada COVID-19, RSUP Soeradji Tirtonegoro. Available from: https://rsupsoeradji.id/pemeriksaan-laboratorium-pada-covid-19/ (accessed 4 July, 2020).
5. Yan G, Lee CK, Lam LTM, Yan B, Chua YX, *et al*. Covert COVID-19 and false-positive dengue serology in Singapore. The Lancet Infectious Diseases. Available from:https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(20)30158-4/fulltext (accessed 4 July, 2020).
6. Wu KC, Leddin D. Antibody testing for SARS-CoV-2: Role in management of the disease. Available from: ttps://www.worldgastroenterology.org/UserFiles/file/COVID19/AntibodytestingtoCOVIDv10.pdf?utm_source=WGO%20Website&utm_medium=Website&utm_campaign=COVID19Informationwebpage_COVID19_Antibodies (accessed 7 July, 2020).
7. Rahardjo B. High fluorescent lymphocyte count examination in dengue hemorrhagic patients with Sysmex XN-1000 hematology analyzer. Indonesian Journal of Clinical Pathology and Medical laboratory, 2019; 25(2): 207-10. Available from: https://indonesianjournalofclinicalpathology.org/index.php/patologi/article/view/1443 (accessed 15 July, 2020).
8. Larantika H. Sensitivity and specificity of atipical lymphocyte for diagnosis of dengue virus at Mataram Hospital, West Nusa Tenggara. The 6th International Conference on Public Health, October 23-24, 2019. Available from:https://doi.org/10.26911/the6thicph-FP.05.05.http://theicph.com/id_ID/2020/01/20/sensitivity-and-specificity-of-atypical-lymphocyte-for-diagnosis-of-dengue-virus-infection-at-mataram-hospital-west-nusa-tenggara-2/5-larantika_r1/ (accessed 8 February, 2021).
9. Maimunah S. Pemeriksaan antigen non-structural 1 sebagai deteksi dini infeksi akut virus. Essence of Scientific Medical Journal, 2020; 17(2): 40-43.
10. Tan Li, Wang Qi, Zhang D, Ding J, Huang Q, *et al*. Lymphopenia predicts disease severity of COVID-19: A descriptive and predictive study. Signal Transduction and Targeted Therapy, 2020; 5: 33. Available from: https://www.g/content/10.1101/2medrxiv.or020.03.01.20029074v1.full-text, https://doi.org/10.1038/s41392-020- 0148-4 (accessed 7 July, 2020).
11. Erlina B. Pneumonia, COVID-19 diagnosis dan penatalaksanaan di Indonesia. Jakarta, Perhimpunan Dokter Paru Indonesia (PDPI), 2020; 21-22. Available from https://covid19.idionline.org/wp-content/uploads/2020/04/5.-Buku-PDPI-.pdf (accessed 9 July, 2020).
12. Yang AP, Liu JP, Tao WQ, Li HM. The diagnostic and predictive role of NLR, d-NLR, and PLR in COVID-19 patients. International Immunopharmacology, 2020; 84: 106504.