A COVID-19 patient with repeatedly undetectable SARS-CoV-2 antibodies

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Keywords: coronavirus disease -19 (COVID-19), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), antibody, chronic lymphocytic leukemia, immunodeficiency

Case Description

A 49-year-old male presented to the emergency department with 7 days of subjective fevers, headaches, muscle aches, increasing shortness-of-breath and nonproductive cough. One of his family members had been tested positive for SARS-CoV-2 three days ago. A review of the patient's past medical history revealed he had chronic lymphocytic leukemia (CLL) Rai stage IV, Binet stage C, with complex cytogenetics on daily venetoclax of an obinutuzumab/venetoclax regimen. His past medical history was also notable for uncontrolled insulin dependent diabetes mellitus type 2 for which he had recently stopped taking his insulin medication.

On physical examination, the patient was afebrile with a heart rate 93 beats per minute, blood pressure 125/86 mm/Hg, oxygen saturation 95% on 4 liters nasal cannula. He was alert with significant findings of diminished breath sounds at lung bases with some mild crackles. A chest radiograph showed patchy peripheral airspace opacities in the lungs bilaterally consistent with a nonspecific pneumonia with atypical features (Fig 1). The laboratory findings on admission showed leukopenia with a white blood cell count of 2,700/mm^3 (reference range: 4,000-10,400) with a decreased absolute lymphocyte count of 380/mm^3 (reference range: 1,000-3,400) and thrombocytopenia with platelet count 144,000/mm^3 (reference range: 150,000-350,000). His basic metabolic panel showed an elevated anion gap at 19 mmol/L (reference range: 5-14) and glucose of 20.6 mmol/L (reference range: 4.1-6.1). Routine electrolytes and renal function tests results were within the normal ranges. Alanine aminotransferase was mildly elevated at 46 U/L (reference range: <41). His procalcitonin and C-reactive protein were both

elevated at 0.21 µg/L (reference range: < 0.08) and 62.9 mg/L (normal range: <5), respectively. His erythrocyte sedimentation rate was higher than normal at 67 mm/Hr (normal range: <25 mm/Hr). Serum immunoglobulin G (IgG) concentration was 7.73 g/L, which was near the lower limit of the normal range (7.00-16.00). The antigen test results for influenza A and B and respiratory syncytial virus were negative. The patient was admitted and a nasopharyngeal swab test for SARS-CoV-2 RNA by real-time reverse transcription polymerase chain reaction (RT-PCR) with Simplexa COVID-19 Direct assay (DiaSorin Molecular, Cypress, CA, USA) was found to be positive. The patient was started on hydroxycholoroquine, doxycycline, and insulin, and continuously monitored.

On day 2 of hospitalization the patient experienced desaturation of oxygen to 88% on 4 liters of oxygen and cardiac arrhythmias, at which point hydroxychloroquine and the patient's venetoclax were discontinued. On day 5 of hospitalization, the patient experienced increased difficulty breathing and could not maintain oxygen saturation above 93% on 6 liters of oxygen. A repeat chest radiograph showed worsening features with oxygen saturation of 70% and the patient was transferred to the intensive care unit the following day for non-invasive ventilation. His oxygen requirements progressively increased and eventually he was sedated and intubated.

The patient's clinical course was complicated by acute kidney injury, fevers, progressive worsening of SARS-CoV-2 infection with involvement of the upper lobes, subarachnoid hemorrhages and failure to wean off of intubation.

Repeat SARS-CoV-2 testing by RT-PCR remained positive throughout his hospital admission. His longitudinal remnant blood specimens after routine laboratory testing were used for clinical verification of SARS-CoV-2 antibody assays. The antibody was negative in the sample collected one week after COVID-19 symptom onset. His subsequent weekly antibody testing remained negative 44 days post symptom onset. The antibody test was initially performed with the Roche Elecsys Anti-SARS-CoV-2 total antibody assay on the Cobas e602 analyzer (Roche Diagnostics, Indianapolis, IN, USA) followed by the confirmation with the Abbott SARS-CoV-2 IgG assay on the ARCHITECT i1000 analyzer (Abbott Laboratories Diagnostics Division, Abbott Park, IL, USA) and the DiaSorin SARS-CoV-2 S1/S2 IgG assay on the LIAISON XL analyzer (DiaSorin, Stillwater, MN, USA).

Case Discussion

Current evidence indicates SARS-CoV-2 antibodies begin to develop approximately 6 to 10 days after the infection [(1)]. IgM appears to peak approximately 12 days after infection and persists in sufficient quantities for as long as 35 days, after which the quantity declines rapidly. IgG has been observed in patients 2 weeks after symptom onset [(2)] and it peaks approximately 17 days after infection and persist for at least 49 days [(3)]. Our patient's SARS-CoV-2 antibody testing was negative from one week to more than 6 weeks post COVID-19 symptom onset, so the negative results were not due to inappropriate timing for the antibody testing.

In addition to the time post infection, antigens used in the anti-SARS-CoV-2 assays and different assay formats may affect the detection of the antibody because of variable

sensitivity [4]. To rule out this possibility, we used 3 assays with different antigens and formats to test the patient. The Roche assay uses recombinant protein representing the nucleocapsid (N) antigen as both capture and tracer molecules for the determination of total antibodies. While the N protein also serves as the capture antigen in the Abbott assay, the tracer molecule is anti-human IgG, so this assay detects the IgG antibody. The format of the DiaSorin assay is similar to the Abbott assay which also detects the IgG antibody, but the tracer molecule is SARS-CoV-2 spike (S) protein including subunit 1 (S1) and 2 (S2). All 3 assays showed repeatedly negative antibody in the patient. Therefore, the negative antibody tests in this case did not result from the failure of a particular assay. However, we have not tested this patient's samples using other commercially available assays such as the Siemens SARS-CoV-2 total antibody assay.

CLL is the commonest type of leukemia in adults. CLL is associated with a profound immunodeficiency highlighted by the presence of recurrent infections and failure of antitumor immune responses. This immunodeficiency is caused by a combination of impaired T-cell and natural killer cell function, and increased numbers of regulatory T cells and nurse-like cells. Many of the standard treatments for CLL have significant effects on the immune system [5]. The immunodeficiency in CLL patients chiefly manifests as hypogammaglobulinemia but involves all elements of the immune system including abnormal T-cell function, phagocytic defects and defects of the complement system [6]. It is caused by the interpolation of tumor cells among immunological cells and mediated by bi-directional cell contact and secretion of cytokines, which both sustain and invigorate the tumor and suppress immunity. CLL treatments generally exacerbate

the existing immunodeficiency, which can result in severe infective complications [5]. This renders patients susceptible to infections which is the usual cause of morbidity and mortality in these patients. The patient in this case report was treated with obinutuzumab/venetoclax regimen. Obinutuzumab is a humanized anti-CD 20 monoclonal antibody. It binds to CD20 on B cells to destroy B cell by activating intracellular apoptosis pathway and the complement system. Venetoclax blocks the anti-apoptotic B-cell lymphoma-2 (Bcl-2) protein, leading to programmed cell death of CLL cells. Both drugs can cause immunodeficiency. Although this patient's CLL treatment was held upon being found positive for COVID-19, on admission, he was already immunocompromised.

The etiology of hypogammaglobulinemia in CLL is unclear. Numerous factors have been implicated in the pathogenesis of hypogammaglobulinemia including functional abnormalities of T cells and inhibition of normal CD5 negative B cells [7]. CLL cells can suppress the CD40 ligand-mediated helper signal necessary for normal lymphocyte differentiation and development. CLL cells also secrete TGF-β, which inhibits B-cell proliferation and produce high levels of circulating IL-2 receptor. This can downregulate T-helper cell function and remove endogenous IL-2. This interruption of T-cell/B-cell interaction may be a mechanism of immune deficiencies of patients with CLL, including humoral defects. In this patient's case, his total IgG concentration was near the lower limit of normal due to immunodeficiency.

In terms of phagocytic defects, disease progression and treatment with chemotherapy contribute to its development. Neutropenia due to bone marrow infiltration or cytotoxic therapy tends to present as a late feature of CLL. Our patient had both severe leukocytopenia and lymphopenia.

Considering his compromised immune system due to CLL and related treatment, the persistently negative SARS-CoV-2 antibody results 44 days after COVID-19 symptom onset were most likely because of the patient's failure in developing specific antibodies against SARS-CoV-2 or developing very low level of the antibodies that were undetectable by all three methods. It is listed as a limitation of the assay in the package insert of the Abbott assay that immunocompromised patients who have COVID-19 may have a delayed antibody response and produce levels of antibody, which may not be detected as positive by the assay. Therefore, negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Testing with a molecular diagnostic assay should be considered to evaluate for active infection in these individuals.

To our knowledge, this is the first reported case that the COVID-19 patient failed to develop SARS-CoV-2 antibody in a CLL patient most likely due to immunodeficiency. This phenomenon has been well reported in the literature. For example, Abravanel et al [8] used two immunoassays to detect anti-hepatitis E IgM antibody in 30 immunocompetent and 30 immunocompromised patients and found that the sensitivities were 90% (95% CI: 72.1-100%) and 96.6% (95% CI: 78.77-100%) in immunocompetent

patients, but only 73.3% (95% CI: 55.4-91.2%) and 83.3% (95% CI: 65.44-100%) in immunocompromised patients. Similarly, Lok et al [9] reported that diminution or loss of reactivity to hepatitis C virus antigens was observed after kidney and bone marrow transplantation in patients with hepatitis C virus infection. Landry et al [10] reported a case with disseminated adenovirus infection in an immunocompromised transplant patient with negative antibody against adenovirus and pointed out that a diagnostic rise in antibody titer may never develop in immunosuppressed patients despite serious infection. Thus, the common practice of attempting to diagnose viral illnesses by serum antibody titers alone is of limited value in immunocompromised patients.

Takeaways

1. Normally human anti-SARS-CoV-2 IgM and IgG antibodies can be detected in patient blood 7 and 14 days post the infection, respectively. Negative results can occur if samples are collected too early, sensitivity of the assay is poor, or patients are immunocompromised.
2. CLL patients are usually immunocompromised because of the disease and related therapies. They are at high risk of various infections and often fail to develop sufficient antibodies against the pathogens.
3. Antibody testing in patients with immunodeficiency may have limited clinical value. For those patients, nucleic acids and antigens test should be utilized.

Acknowledgment

We thank Edward C. Pederson, Monica L. Straub, Terri L. Neibauer, Sofia C. Scott (Penn State Hershey Medical Center) and Christopher Hamilton, Kimberly Robyak, Pamela McGhee, Teresa Speicher (Penn State University College of Medicine) for excellent technical support.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

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Figure Legend

Figure 1. Chest Radiograph on Presentation

The patient's chest radiograph shows patchy peripheral airspace opacities in the lungs bilaterally consistent with a nonspecific pneumonia with atypical features.