A false positive serology test of SARS-CoV-2 in a patient with Waldenström's macroglobulinemia: A case report

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Abstract. The serology test of SARS-CoV-2 is one of the critical assays to make a diagnosis of SARS-CoV-2 infection. The gold immunochromatography assay (GICA) is a common measure to test SARS-CoV-2 specific IgG and IgM. The sensitivity and specificity of the assay are ~>80%. It has been reported that the result of GICA could be compromised in various situations, such as auto-immune diseases, Kawasaki disease, pregnancy or other conditions. However, following the European Hematology Association's consensus statement on the management of Waldenström's Macroglobulinemia (WM) patients, serological tests for SARS-CoV-2 specific IgM should not be affected by the total IgM or paraprotein levels. The present study reports a patient with duplicate positive serology tests of SARS-CoV-2 which is hypothesized to be due to monoclonal IgM caused by WM.

Introduction

During the pandemic of COVID-19, the test of virus specific antibody and RNA is an effective way to control the outbreak of the virus. The test not only helps physicians to diagnose a patient with SARS-CoV-2 infection and to assess whether the patient is cured, but also to evaluate the patient's immunity status to the virus. Generally, two types virus specific antibodies will be evaluated in a fast serology test of SARS-CoV-2: Immunoglobulin G(IgG) and immunoglobulin M(IgM). A positive result of IgG indicates that an individual has been exposed to the virus or has undergone SARS-CoV-2 vaccination and acquired an immunity to the virus. A positive result of IgM indicates an individual might be infected recently and further tests including SARS-CoV-2 RNA should been performed to verify the result.

Waldenström's Macroglobulinemia (WM) is a rare B cell lymphoma, which accounts for <2% of all non-Hodgkin lymphomas. The manifestations of WM include: Lymphadenopathy, hepatosplenomegaly, anemia, neurologic symptoms and infiltration of organs in some cases. Hyperglobulinemia of monoclonal IgM protein is the hallmark of the disease. Recently, somatic mutation of the MYD88 gene has been reported in the majority of patients with WM (1). Notably, the response to SARS-CoV-2 vaccine attenuates in patients with WM (2,3).

The present study reports a patient with duplicate positive serology tests of SARS-CoV-2 which is hypothesized to be due to monoclonal IgM caused by WM.

Case report

An 86-year-old Chinese woman was admitted to Taizhou Central Hospital (Taizhou University Hospital) in December 2020 as she had suffered lumbago for years. She denied contact with SARS-CoV-2 patients or SARS-CoV-2 vaccination history or travel to SARS-CoV-2 outbreak areas. Although not suffering from a fever or cough, she was isolated in a separate ward in the infectious diseases department after her SARS-CoV-2 spike protein and nucleocapsid specific IgM proved to be positive. Her thoracic spinal MRI scan confirmed a fracture of 10th thoracic vertebra which contributed to her lumbago. Her chest computerized tomography (CT) scan showed mild exudation in the basal segment of bilateral inferior lobes (Fig. 1). Repeated serology and RNA tests for SARS-CoV-2 were performed. However, only SARS-CoV-2 specific IgM was confirmed positive while SARS-CoV-2 specific IgG and RNA were negative (Table I).

Further studies on her immunoglobulin showed a IgG of 11.10 g/l, IgA of 0.81 g/l, IgM of 19.40 g/l, κ light chain of 18.70 g/l and λ light chain of 2.67 g/l. The light chain in her urine was abnormal as well, with a κ light chain of 1,340.00 mg/l and λ light chain <50.00 mg/l. Serum immunofixation electrophoresis proved the existence of monoclonal IgM and κ light

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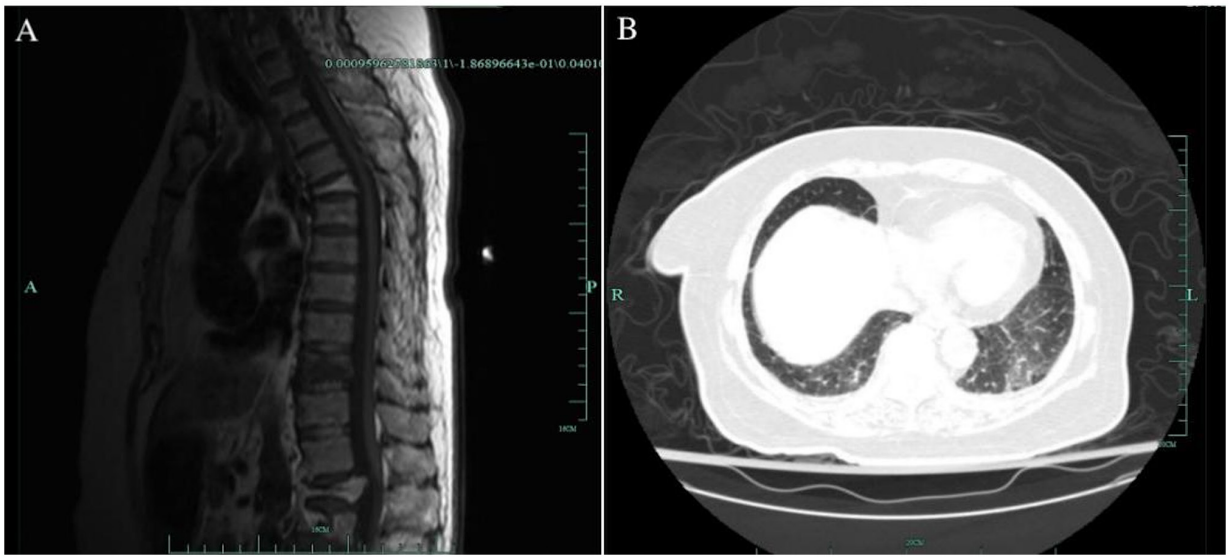

Figure 1. Patient scans. (A) Thoracic spinal MRI showed flattening of the 10th thoracic vertebra with abnormal signals suggesting subacute compression fractures, and wedge-shaped changes in the 5th thoracic vertebra and 1-3 lumbar vertebrae suggests old fractures. (B) Chest CT scan showed mild exudation in the basal segment of bilateral inferior lobes. MRI, magnetic resonance imaging; CT, computerized tomography.

chain. Tests for rheumatism including antinuclear antibody, rheumatoid factor, anti-double stranded DNA antibody and anti SSA and SSB antibodies were all negative. Serum tumor markers were also negative.

A bone marrow biopsy was then performed. The bone marrow biopsy showed lymphocytes accounted for 32% of the total nucleated cells and plasma cells 2%. Flow cytometric analysis indicated a population of monoclonal B lymphocytes accounting for 17.4% of total nucleated cells in bone marrow. These B lymphocytes were CD5 negative and CD10 dim in terms of immunophenotype (Fig. 2). Droplet digital PCR indicated a MYD88 L25P gene mutation with a mutation proportion of 17.9%. Taken together, she was diagnosed WM and transferred to hematology department. She refused a vertebroplasty. Celecoxib was given 200 mg twice a day to ameliorate her lumbago and alendronate sodium 70 mg once a week to inhibit the bone destruction. Her lumbago was alleviated after 2 weeks treatment and she was discharged from hospital.

The present study was conducted according to the guidelines of the Declaration of Helsinki and approved by the ethics committee of Taizhou Central Hospital for publishing the case details. Written informed consent was provided by the participant in the study.

Discussion

Currently in China, it is a standard procedure to perform SARS-CoV-2 screening before admitting patients into the inpatient department. The screening strategy varies among different hospitals in China. At Taizhou Central Hospital (Taizhou University Hospital), it is mandatory to test SARS-CoV-2 specific IgG, IgM and RNA simultaneously before a patient is hospitalized. Previous studies reported that SARS-CoV-2 specific IgM could be detected in patients at 1 week after onset. The level of the IgM peaks at the second week, and begins to decline at the third or fourth week. However, SARS-CoV-2 specific IgG emerges later than IgM. The medium time of SARS-CoV-2 specific IgG to be detected is 12 days to 14 days. The level of the IgG rises fast and peaks at the third or fourth week (4-9). In general, IgG antibody could maintain in peripheral blood for a long time. However, Long *et al* (10) reported that the level of SARS-CoV-2 specific IgG declined by 70% at the second month after SARS-CoV-2 infection in >90% infected patients, which is different to the change of SARS specific IgG. This indicates that SARS-CoV-2 specific IgG might not protect patients against the second infection.

The present study adopted the gold immunochromatography assay (GICA) to test SARS-CoV-2 specific IgG and IgM. The sensitivity and specificity of the assay for anti-SARS-CoV-2 IgG are 0.85 and 0.99 respectively, and 0.74, 0.99 for IgM respectively. Pan *et al* (11) used GICA to analyze 86 samples of 67 SARS-CoV-2 patients who were confirm by reverse transcription (RT) PCR and found that the IgM positive rate increased from 11.1% in early stage (1-7 days after onset) to 78.6 and 74.2% in intermediate stage (8-14 days after onset) and late stage (>15 days), respectively. The IgG positive rate increased from 3.6% in early stage, 57.1% in intermediate stage to 96.8% in late stage, respectively. Notably, IgM and IgG combinatorial detection significantly increased the sensitivity of GICA, especially at the intermediate stage. Nevertheless, several studies and case reports showed that the result of GICA could be interfered with by auto-immune diseases, Kawasaki disease, pregnancy or other conditions. A high level of rheumatoid factor brings a marked false positiveness of SARS-CoV-2 specific antibodies (12-15). According to European Hematology Association's consensus statement on the management of WM patients during the SARS-CoV-2 pandemic, serological lab tests for SARS-CoV-2 specific IgM should not be affected by the total IgM or paraprotein levels (16). However, in the present case, the patient had no history of contacting any SARS-CoV-2 patients or traveling to a SARS-CoV-2 outbreak area. Neither had she any signs of rheumatoid diseases or malignancies. In addition, the CT scan and the dynamic assays of her SARS-CoV-2 specific IgM, IgG and RNA did not fulfill the criteria of SARS-CoV-2 pneumonia.

Table I. Serology and RNA tests for SARS-CoV-2.

Days since admission	1	2	3	4	5
IgM	+	+	+	+	+
IgG	-	-	-	-	-
RNA	-	-	-	-	-

+ positive; - negative.

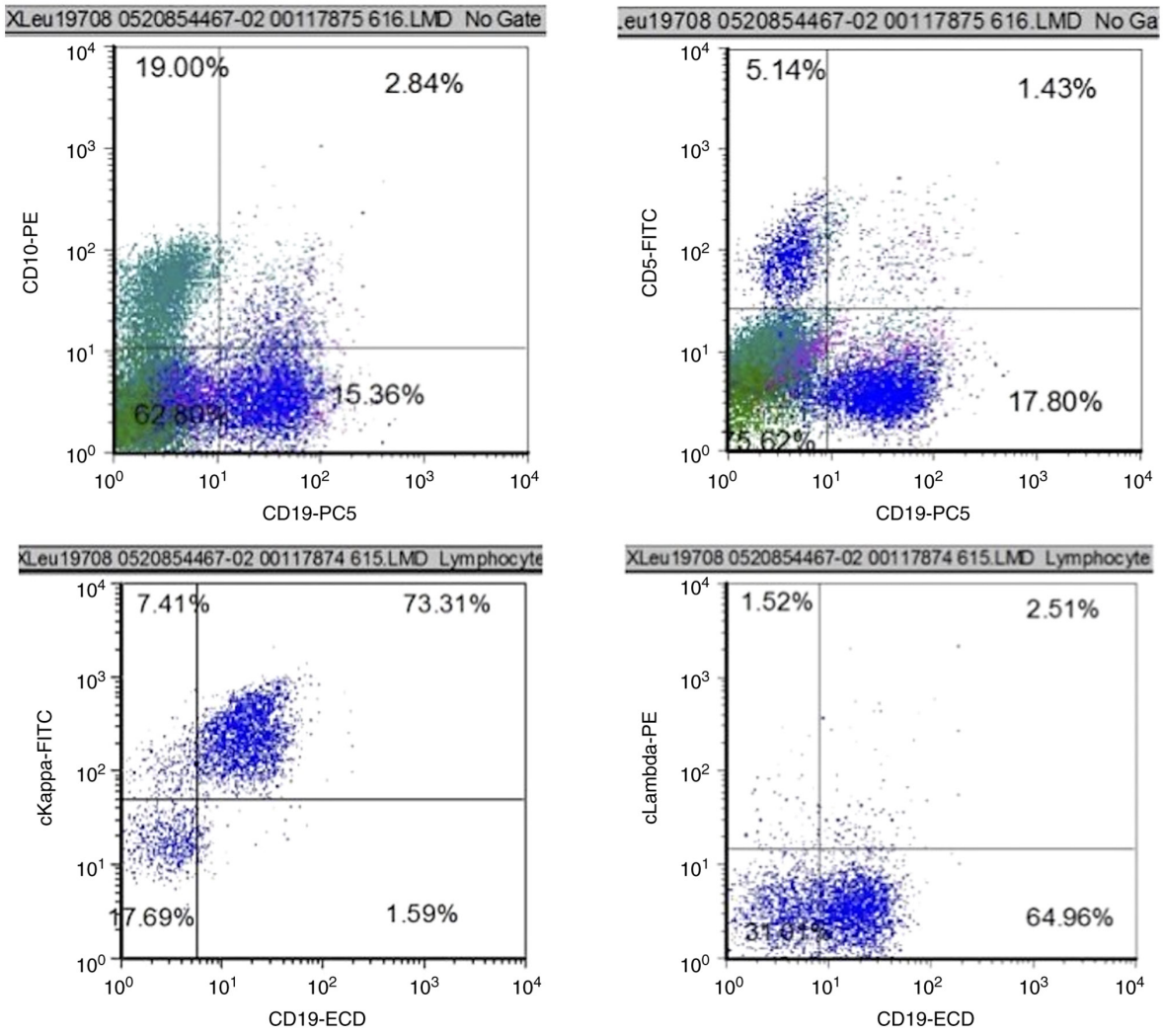

Figure 2. Flow cytometry of bone marrow. The blue events represented monoclonal B lymphocytes which were CD5 negative, CD10 dim, CD19 and cytoplasm κ light chain positive.

As a result, her SARS-CoV-2 specific IgM is considered to be false positive due to an immune cross-reactivity between SARS-CoV-2 and WM paraprotein. It should be noted that the patient's titer of the IgM was not measured.

Several conditions could attribute to false positive result of SARS-CoV-2 specific antibodies with GICA. The GICA result should be interpreted in the context of patients' clinical manifestation and RT-PCR test, together with a consistent radiological picture. In spite of the European Hematology Association's consensus statement, SARS-CoV-2 specific IgM test by GICA could be affected by monoclonal IgM. Further study is warranted.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YQZ and HRS conceived the present study. LZL and XYY were responsible for the methodology. LLX, NNL and HRS were responsible for the investigation. LZL and BYS checked assays results, the raw data and validated the authenticity of the data. LLX and NNL wrote the original draft. HRS and NNL were responsible for review and editing. LLX was responsible for funding acquisition. LZL and BYS confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted according to the guidelines of the Declaration of Helsinki and approved by the ethics committee of Taizhou Central Hospital for publishing the case details. Written informed consent was provided by the participant in the study.

Patient consent for publication

The patient provided the consent for publication.

Competing interests

The authors declare that they have no competing interests.

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