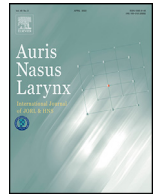




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Development of infectious mononucleosis as an unusual manifestation of COVID-19

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ABSTRACT

It has generally been reported that patients with COVID-19 show a fever, cough, and/or respiratory failure as the most common clinical symptoms but some have unusual symptoms, such as anosmia, diarrhea, and throat pain. We herein report a 26-year-old woman with chief complaints of lymphadenopathy and a fever. First, she underwent a laboratory examination, which showed a high proportion of atypical lymphocytes (19%) and an increase in hepatic enzyme activities, and was then hospitalized with a diagnosis of infectious mononucleosis (IM). However, the blood examination did not show any increase in anti-Epstein-Barr virus VCM-IgM. Subsequently, she developed tonsillar hypertrophy with purulent plugs. An additional examination for infection of other pathogens revealed positivity only for SARS-CoV-2 in a loop-mediated isothermal amplification (LAMP) test. The patient was transferred to the COVID-19-specific isolation ward, and none of the ward staff, patients, or either of the two otolaryngologists who had directly examined this patient showed positive signs for SARS-CoV-2 in a LAMP test. Consequently, this case suggests that even if patients show clinical symptoms and signs of common diseases for otolaryngologists, such as IM, we should keep in mind the possibility of COVID-19 without arbitrarily assuming that IM is caused by Epstein-Barr virus.

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1. Introduction

COVID-19, an infectious disease caused by a new type of coronavirus (SARS-CoV-2), first manifested in Wuhan, China, and rapidly spread as a global pandemic. COVID-19 patients are reported to present with a wide variety of symptoms, including a fever, cough, malaise, and respiratory failure as common manifestations and olfactory dysfunction, diarrhea, and abdominal pain as unusual ones. However, young COVID-19 patients reportedly tend to be asymptomatic [1]. Accordingly, clinicians should not rule out the possibility of

COVID-19, even if individuals show atypical symptoms rather than the common manifestation and no other symptoms.

Infectious mononucleosis (IM) is a common disease encountered by otolaryngologists, caused by the initial infection with various types of viruses. Generally, the main symptoms of IM are a fever, pharyngotonsillitis, cervical lymphadenopathy, and hepatosplenomegaly [2]. Of these symptoms, a fever is present in approximately 90% of IM patients and usually lasts for 1–2 weeks. Cervical lymphadenopathy and hepatosplenomegaly are respectively found in 90% and 10–50% [2]. In addition, a blood test shows an increase in both peripheral blood lymphocytes and atypical lymphocytes, and elevated activities of liver enzymes. Although the major

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causative virus of IM is Epstein-Barr virus (EBV, 60–90%), IM is sometimes reportedly induced by other types of virus, such as human herpesvirus 6 (HHV-6) (9%), cytomegalovirus (CMV), herpes simplex virus (HSV) (6%), and human immunodeficiency virus (HIV) (<2%) [3]. However, IM caused by SARS-CoV-2 has not been reported.

We herein present a case diagnosed with IM based on cervical lymphadenopathy and a marked increase in atypical lymphocytes and hepatic enzyme activities in a blood test in which subsequent serologic and genetic tests revealed negativity for the above-mentioned possible causal pathogens but positivity for SARS-CoV-2, suggesting that IM is developed as a manifestation of COVID-19.

2. Case report

The patient was a 26-year-old female office worker without any particular medical history. She presented to her primary care physician with swelling of the bilateral cervical lymph nodes and a slight fever of approximately 37 °C. She was diagnosed with acute lymphadenitis and started taking amoxicillin. After 4 days, she had a high temperature over 38 °C, and showed an increase in liver enzyme activities and the appearance of atypical lymphocytes in a blood test, suggesting a diagnosis of IM. At this point, she was referred to our hospital.

At her first visit to our hospital, she showed neither abnormal vital signs nor upper airway symptoms, such as a sore throat, cough, sputum, hoarseness and dysphagia, although she complained of a loss of appetite. In addition, visual and endoscopic examinations did not detect any abnormal laryngopharyngeal findings, including mucosal redness, mucosal swelling, or adenoid/tonsillar hypertrophy. Furthermore, no organic abnormalities were found in the ear, nose and oral cavity. On palpation, the bilateral upper deep cervical lymph nodes were swollen to a long diameter of approximately 30 mm, and a number of lymph nodes were palpable along the bilateral internal jugular veins. However, no skin abnormalities, such as a rash or erythema, were found. Laboratory data showed not only a slight increase in the count of white blood cells (WBCs; 8900 / μ L) and lymphocytes (3960 / μ L) but also a marked increase in the proportion of atypical lymphocytes (19%). The activities of liver enzymes, including AST, ALT, ALP, and γ -GTP, were markedly elevated, although the CRP level was only mildly elevated (1.77 mg/dL) (Table 1). Based on the above results, she was diagnosed with IM, recommended to receive complete bed rest, and consequently admitted to our hospital.

On Day 1 of admission, she became able to eat, although she complained of a loss of appetite. After consultation with gastroenterologists, an abdominal ultrasonography showed splenomegaly but not hepatomegaly (Fig. 2A). In a serologic test for EBV infection, all of the titers of anti-EBV antigen-antibodies, including anti-EBV viral capsid antigen (VCA)-IgM, showed low values of <10-fold. Accordingly, retesting was scheduled for two days later.

On Day 3, her body temperature decreased to about 37 °C, but a blood test showed an increase in the atypical lymphocyte

proportion up to 26% and a further increase in liver enzyme activities (Table 1 and Fig. 1). In contrast, the second examination of the anti-EBV-VCA-IgM showed just only 20-fold, which did not suggest EBV infection. Therefore, to investigate the possibility of initial infection with other viruses capable causing IM, we tested the titers of IgM antibodies against antigens of HHV-6 and HSV and those of IgG antibodies against antigens of CMV, HIV, HBV, and HCV. In addition, antibody tests against Toxoplasma, a parasite capable of inducing IM, and anti-nuclear antibody for the diagnosis of autoimmune hepatitis were conducted. However, later days, these tests later showed all negative results (Table 1).

On Day 5, she continuously had a slightly high temperature of about 37 °C, and started to complain of a sore throat. On a visual examination of the oral cavity and pharynx, hypertrophy, redness, and caseous coating were found in the bilateral tonsils. Cervical computed tomography (CT) examination showed bilateral tonsillar hypertrophy without any marked changes in the cervical lymph nodes (Fig. 3A).

On Day 7, the activities of AST and ALT further increased to 500 IU/mL and 632 IU/mL, respectively. The tonsillar enlargement and pus coating were continuously seen. We finally suspected the possibility of COVID-19, collected her saliva, and ordered a genetic test using the Loop-Mediated Isothermal Amplification (LAMP) method for SARS-CoV-2, which consequently showed positive results. She was therefore promptly transferred to another isolation ward dedicated to COVID-19. Chest CT scan showed no lesions suggestive of pneumonia (Fig. 2B). After the circumstances were explained to all of the patients hospitalized in the previous ward, all the areas of the ward were disinfected, and all of the inpatients as well as the ward staff, including nurses and two otolaryngologists, which had directly examined this patient, were tested using the LAMP method, with negative results fortunately obtained from all examinees.

On Day 9, the AST and ALT activities were peaked at 337 IU/mL and 528 IU/mL, respectively (Fig. 1). The symptoms of a sore throat gradually improved. On Day 13, since the AST and ALT activity decreased to 42 IU/mL and 129 IU/mL, respectively (Fig. 1), and all of the subjective symptoms disappeared, the patient was discharged. According to the instructions of the health center of Osaka City, the patient remained home for two weeks after discharge to quarantine.

One week after the discharge, the subjective symptoms were still gone, and the pharyngeal abnormalities, including the mucosal redness, tonsillar hypertrophy, and pus coating had disappeared (Fig. 3B). In addition, the AST and ALT activity had further decreased to 56 IU/mL and 68 IU/mL, respectively.

3. Discussion

IM is characterized by the main symptoms of a fever, pharyngeal tonsillitis, cervical lymphadenopathy, and hepatosplenomegaly, as well as typical findings of blood tests, that include an increase in peripheral blood lymphocytes, atypical lymphocytes and hepatic enzyme activities. IM is diagnosed based on these symptoms and laboratory findings. In

Table 1. Results of the blood test at the initial visit and subsequent serological tests for infectious pathogens.

Peripheral blood			Biochemistry			Pathogen-specific antibodies/antigens			Auto-immune			Coagulation		
WBCs	<u>8900</u>	/μL	T-Bil	0.7	mg/dL	VCA-IgM	≤10	times	ANA	≤40	times	PT	109	%
Neut	31	%	<u>AST</u>	<u>413</u>	IU/L	VCA-IgG	20	times				APTT	36.5	sec.
Lymph	44.5	%	<u>ALT</u>	<u>462</u>	IU/L	EA	≤10	times						
Mono	5	%	<u>ALP</u>	<u>1179</u>	IU/L	EBNA	≤10	times						
Eos	0	%	<u>GGT</u>	<u>168</u>	IU/L	HSV-IgM	≤0.8	times						
Baso	0.5	%	<u>LDH</u>	<u>745</u>	IU/L	CMV-IgG	≤2.0	times						
Aty. Lymph	<u>19</u>	%	TP	6.1	g/dL	CMV-IgG	≤0.8	times						
RBCs	448	×10 ⁴ /μL	A lb	3.4	g/dL	Toxoplasma-IgM	≤0.8	times						
Hb	13.4	g/dL	BUN	3	mg/dL	HBs Ag	Negative							
Ht	39.1	%	Cre	0.5	mg/dL	HBs Ab	Negative							
Plt	17.3	×10 ⁴ /μL	G lu	81	mg/dL	HBc Ab	Negative							
			CRP	1.77	mg/dL	HCV Ab	Negative							
						HIV Ag/Ab	Negative							

The abnormal values are underlined. The counts of WBCs and atypical lymphocytes were slightly and remarkably high, respectively. The activities of liver enzymes, including AST, ALT, ALP, GGT, and LDH, were increased. The VCA-IgM value was negative.

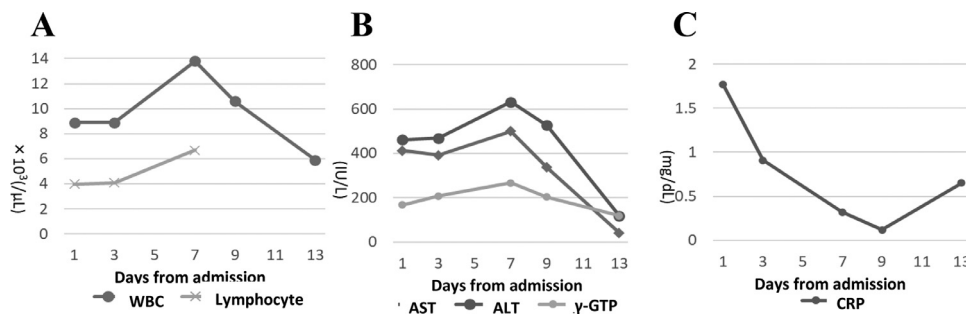


Fig. 1. Changes in the laboratory data since admission. The WBC and lymphocyte count, (B) liver enzyme activities, and (C) CRP level. The activities of AST, ALT, and γ -GTP once increased and subsequently peaked one week after the admission. The mildly increased CRP level gradually decreased during hospitalization.

addition, although the most common causative virus of IM is EBV, IM has been reported to result from the initial infection of HHV-6, CMV, HSV, HIV, and Toxoplasma [3–5].

Regarding the treatment for IM, only symptomatic treatment, such as the use of acetaminophen against a fever and throat pain, is performed, since there are no therapeutic drugs specific for IM at present. Generally, the prognosis of IM is good, as the symptoms generally resolves spontaneously in two or three weeks. However, IM patients occasionally suffer fatal complications, such as splenic rupture (0.1–0.2%), encephalitis (<1%) and autoimmune anemia (0.5–3%) [2]. Splenic rupture is particularly serious, with a reported mortality rate of 9% [2]. Accordingly, when splenomegaly related to IM is found, hospitalization and bed rest are required.

The initial chief complaint of the present case was lymphadenopathy, followed by splenomegaly and tonsillitis, in that order. In addition, the laboratory examination showed a mild increase in peripheral blood lymphocytes, a marked increase in atypical lymphocytes, and an increase in hepatic enzyme activities. The presence of these characteristic symptoms and laboratory findings helped us diagnose the patients with IM but led to the delayed diagnosis of COVID-19.

In the present case, the serologic test did not show any marked increase in IgM/IgG antibodies against specific antigens of either of EBV or other major causative viruses of IM, such as HHV-6, CMV, HSV, and HIV, as well as toxoplasma,

a parasite capable of inducing IM. Particularly at the initial infection with EBV, generally, the titer of anti-VCA-IgM generally increases first, followed by an increase in anti-VCA-IgG in the second week. In addition, the incubation period from EBV infection to the onset of IM is reportedly approximately four to six weeks [6]. Accordingly, the marginal increase in the anti-VCA-IgM and anti-EBNA-IgG found in the present case indicated not only that the IM symptoms had not been caused by EBV infection but also that she was not infected with EBV at all.

Recently, cases with a co-infection of both SARS-CoV-2 and EBV have been reported [7,8]. In a case report by García-Martínez, et al. [7], the patient was diagnosed for a primary EBV infection by the reverse transcriptase polymerase chain reaction (RT-PCR). In addition, Chen et al. [8] examined EBV infections in COVID-19 patients by detecting anti-EBV-VCA-IgM, and reported that EBV-positive COVID-19 patients tended to have a higher risk of fever and a higher level of liver enzyme activities than EBV-negative COVID-19 patients. The present case, which was judged to be negative for EBV infection based on a marginal increase in anti-VCA-IgM and anti-EBNA-IgG, had a slight fever and showed an increase in AST/ALT to at most 500/632 IU/mL.

In addition, the symptoms and laboratory abnormalities observed in the present case, such as cervical lymphadenopathy, tonsillitis, tonsillar hypertrophy, elevated hepatic enzymes,

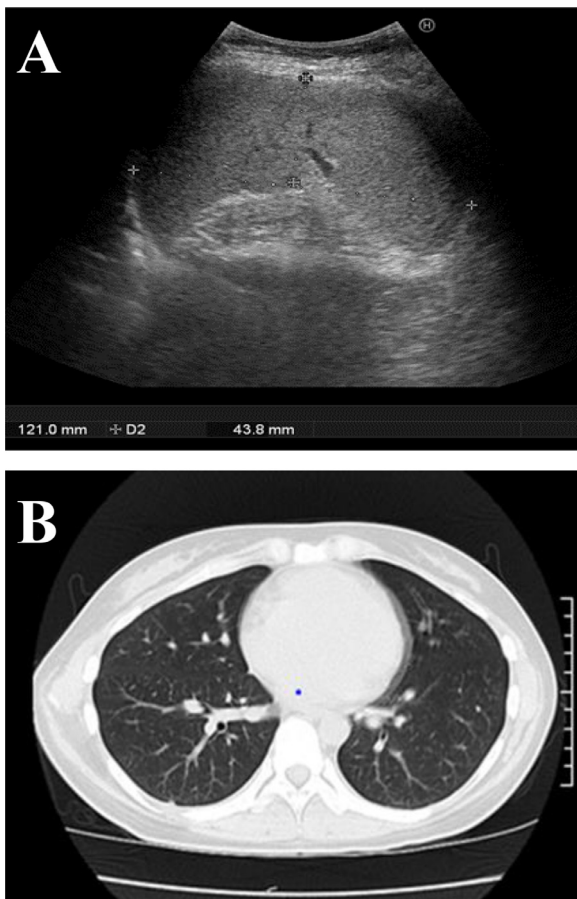


Fig. 2. Results of the ultrasonic and computed tomographic examinations. An ultrasonic image of the abdomen. A hypertrophied spleen was observed. A CT image of the chest region. No abnormalities were found in the bilateral lungs.

and an increased atypical lymphocyte count were typical for IM but unusual for COVID-19. Immediately after the diagnosis of SARS-CoV-2, we found literature reporting that some COVID-19 cases showed IM-like symptoms and laboratory abnormalities as discussed below.

Wu et al. [9] have reported that the proportion of COVID-19 patients showing an increased activity of AST, ALT, γ -GTP, and ALP was 21.8%, 20.4%, 35.8%, and 4.7%, respectively, indicating that liver dysfunction is not rare in COVID-19 cases. In particular, increased AST and ALT activities are considered to result from the destruction of hepatocytes and subsequent leakage of intracellular enzymes into the blood. Regarding the mechanisms underlying the injury of hepatocytes by SARS-CoV-2 infection, several hypotheses have been proposed: (1) SARS-CoV-2 directly infects hepatocytes via angiotensin-converting enzyme 2 (ACE2) and injures hepatocytes; (2) some drugs, such as acetaminophen used for fevers, injure hepatocytes; and (3) cytokine storm induced by SARS-CoV-2 infection activates macrophages and cytotoxic T cells, leading to systemic inflammatory response syndrome (SIRS) [10].

In addition, Yip et al. [11] and Chong et al. [12] have respectively reported that an increased atypical lymphocyte count was observed in 14 of 15 cases (93%) and in 23 of

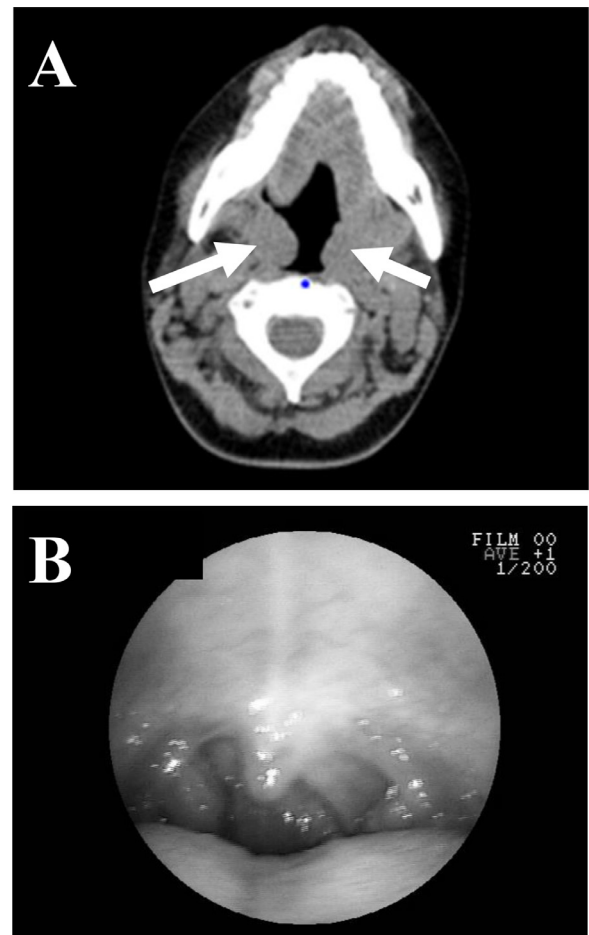


Fig. 3. Changes in the tonsillar size. (A) Cervical CT image. Bilateral tonsillar hypertrophy was shown (arrow). (B) An endoscopic image of the oropharyngeal area when the patient revisited as an outpatient. The bilateral tonsils had shrunk.

32 cases (72%). These results indicate that the appearance of atypical lymphocytes is a common finding of COVID-19. Atypical lymphocytes at the initial EBV infection are commonly understood to be CD8-positive cytotoxic T lymphocytes (CTLs) activated by cytokines produced from CD4-positive T cells and natural killer (NK) cells that respond to B lymphocytes, which become infected by EBV and proliferate. Downey and McKinlay [13] classified atypical lymphocytes into three types of Type I (monocyte-like), Type II (plasma cell-like), and Type III (lymphoblast-like) and reported that EBV-induced atypical lymphocytes are often type II [14]. In addition, Weinberg et al. [15] assessed the types of atypical lymphocytes in COVID-19 and reported the proportion of the type II atypical lymphocytes to be low among COVID-19 patients. In this case, we were unable to investigate the type of atypical lymphocytes in detail, as the microscopic observation of atypical lymphocytes was prohibited in the COVID-19 dedicated ward according to the rules of our hospital.

Guan et al. [16] reported that 2.1% of COVID-19 patients showed tonsillar hypertrophy. In IM caused by an initial EBV infection, massive proliferation of T lymphocytes activated by EBV-infected B cells is considered to cause the enlargement of the tonsils and cervical lymph nodes. Roldán-

Santiago et al. [17] reported that SARS-CoV-2 was detected in the cervical lymph nodes by fine-needle aspiration cytology in three COVID-19 cases showing lymphadenopathy. SARS-CoV-2 has been reported to infect various types of cells expressing ACE2 via the viral S protein, since ACE2 is ubiquitously expressed in almost all cells. Indeed, several studies [18,19] have reported that SARS-CoV-2 was detected in lymphoid cells, such as monocytes, B cells, and helper T cells [18,19]. SARS-CoV-2 presumably invades the oral cavity and directly infects the lymphoid cells of the tonsils, leading to the onset of IM, similarly to how EBV infects tonsillar B cells and subsequently causes IM. However, we have found no reports regarding the onset of tonsillitis, such as tonsillar redness and pus coating, in COVID-19 patients, except for a single case report of co-infection with both EBV and COVID-19 [15]. In addition, if both fine-needle aspiration cytology and a subsequent LAMP examination had been conducted on the specimens from the swollen cervical lymph nodes in this case similarly as Roldán-Santiago et al. [17] reported, then the cause-effect relationship between the IM and SARS-CoV-2 might have been confirmed.

4. Conclusion

Taken together, the present findings suggest us that even when encountering patients who show typical symptoms of common diseases, such as IM, we should not determine the diagnosis at first glance but instead make an appropriate diagnosis or treatment while keeping the possibility of COVID-19 in mind.

Disclosure statement

The authors have no conflicts of interest to declare.

Informed consent

Written informed consent was obtained from the patient for the publication of this case report.

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