An autopsy case of fulminant myocarditis after severe acute respiratory syndrome coronavirus 2 vaccine inoculation

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Abstract
A 61-year-old woman without significant medical history developed fever 3 days after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination and went into shock the next day. She was negative for SARS-CoV-2 mRNA in real-time polymerase chain reaction (PCR). Finally, she died 10 days after vaccination. At autopsy, the heart showed moderate dilatation of both ventricles, and the myocardium showed an uneven color change and decreased elasticity. Histologically, severe myocarditis with extensive myocytolysis was observed. The myocarditis showed severe inflammatory cell infiltration with T-lymphocyte and macrophage predominance, and in addition to the inflammatory cells described above, vast nuclear dust accompanying neutrophilic infiltration was observed. In the bone marrow and lymph nodes, hemophagocytosis was observed. In postmortem examination, nuclear acids of any cardiotropic viruses including SARS-CoV-2 were not detected using multivirus real-time PCR system. We discussed the relationship between the possible immune reaction after vaccination and the myocarditis observed in this case from immunopathological viewpoints. This mRNA vaccine is the first applied nucleic acid vaccine for humans, and its mechanism of efficacy and immune acquisition remain unclear. We hope the accumulation of more detailed analyses of the similar cases to reveal the mechanism of this kind of adverse reaction.

KEYWORDS
cytotoxic T-cells, hypercytokinemia, myocarditis, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination

INTRODUCTION
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in December 2019, causing a respiratory disease pandemic named coronavirus disease 2019 (COVID-19). The spread of this infection has become a global public health problem, and the spread of COVID-19 has triggered international efforts to accelerate vaccine development and vaccination on a global scale.¹

Abbreviations: COVID-19, coronavirus disease 2019; CTLs, cytotoxic T-cells; LNP, lipid nanoparticle; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
The need for vaccine development has led to the establishment of several SARS-CoV-2 vaccine candidates. One of the candidates is a messenger RNA vaccine, which encodes a SARS-CoV-2 spike glycoprotein and is expected to confer immunity. Initially, this vaccine was considered to be safe, but adverse events due to vaccine administration have been reported. Most of the adverse events were mild and transient reactions, such as fatigue, chills, headache, myalgia, and pain at the injection site. However, some cases of potentially serious adverse events were reported. Most vaccine was considered to be safe, but adverse events due to vaccine administration have been reported. Most of the adverse events were mild and transient reactions, such as myocarditis, have been reported.

The incidence of myocarditis after mRNA vaccination was higher in younger males, and the severity of myocarditis reported was mostly mild to moderate, and most of the patients were discharged with the improvement of symptoms. However, a fatal case has been reported, suggesting that it is an adverse event that should be recognized. At present, the accumulation of cases is small, and the association between vaccination and myocarditis is largely unknown.

Here we report an autopsy case of a 61-year-old woman who developed fulminant myocarditis and died after her first vaccination.

**CLINICAL SUMMARY**

The patient was a 61-year-old woman with no medical history of note. Three days after her first inoculation of the SARS-CoV-2 vaccine (Pfizer/BioNTech mRNA vaccine BNT162b2), she developed a fever of 39°C and general malaise and was admitted to a nearby hospital. She was negative for SARS-CoV-2 by real-time polymerase chain reaction (PCR) analysis using nasopharyngeal swab. Laboratory examinations revealed the elevation of brain natriuretic peptide (625.3 pg/mL; normal range < 100 pg/mL) and creatine kinase (CK) (603 IU/L; normal range < 197 IU/L). The day after her admission, CK further rose to 62,049 IU/L and CK-MB was 398 IU/L (normal range < 12 IU/L). She became pulseless and went into shock. Echocardiogram revealed the ejection fraction of left ventricle had decreased from 54% to 0% during one day after admission. An urgent assisted circulation using intra-aortic balloon pumping and percutaneous cardiopulmonary support and extracorporeal pacing were used to support her circulation, but her self-beating gradually disappeared, and she became dependent on extracorporeal pacing. Six days after inoculation, she went into shock again. Ten days after vaccination, she was transferred to Nagano Red Cross Hospital to receive further circulation assistance. Despite using a cardiac pump catheter for supplemental circulation, she was unable to maintain her blood pressure and died within the day. An autopsy was performed.

The investigation was conducted in accordance with the Declaration of Helsinki of 1975. This case report was exempt from the Institutional Ethics Review Board standard at Nagano Red Cross Hospital. Informed consent for the present study was obtained from the patient's bereaved family.

**PATHOLOGICAL FINDINGS**

The patient was a female corpse with severe subcutaneous edema, serous pleural effusion (left, 500 mL; right, 500 mL), ascites (400 mL), and pericardial effusion (150 mL). At autopsy, the heart weighed 450 g and was mildly enlarged.

The left ventricles were slightly dilated, and the wall elasticity was decreased. The myocardium showed heterogeneous color tone, indicating mild fibrosis in the antero-septal area (Figure 1a). Coronary arteries exhibited slight atherosclerosis without apparent stenosis. Histologically, extensive myocyte lysis was observed, with severe inflammatory cell infiltration and edema (Figure 1c). The inflammatory infiltrates adjacent to the damaged cardiomyocytes were predominantly lymphocytes and macrophages (Figure 1c). Contraction band necrosis was absent. Fibrotic change corresponding to the whitish color change in macroscopic findings was not confirmed in the antero-septal area. On the other hand, there was slight fibrosis predominantly in the perivascular area (Figure 1d), which might suggest presence of certain cardiac risk factor. Eosinophils were rarely observed and giant cells were not observed in myocarditis lesion (Figure 1e). Microthrombi were absent. The lesion exhibited the focal, geographical, and transmural distribution, in part, and affected throughout the myocardial layer (Figure 1b). The myocarditis lesion also involved conduction system, however, the infiltration of inflammatory cells was a little milder. Immunohistochemistry showed diffuse infiltration of PG-M1-positive cells (Figure 2a), with CD3-positive cells (Figure 2b) predominating over CD20-positive cells (results not shown). There were many more CD8-positive cells than CD4-positive cells (Figure 2c,d). In addition to lymphocytes and macrophages, vast nuclear dust accompanying neutrophilic infiltration was observed (Figure 1f). Mild fibrosis and slight infiltration of lymphocytes and macrophages were observed in the epicardium, confirming the diagnosis of pericarditis (results not shown).

In the subpleural region of the lungs, hemorrhagic infarcts were observed (Figure 3a), and small fibrin thrombi were visible in the lumen of the pulmonary arteries (Figure 3b). Centrilobular necrosis of the liver (Figure 3c), acute tubular necrosis of the kidney (results not shown), and severe pulmonary congestion...
(Figure 3a) were observed, and there were consistent findings in various organs of the body as changes associated with biventricular heart failure due to myocarditis. There was no evidence of diffuse alveolar damage in the lung. Erythrophagocytosis was seen in the bone marrow (Figure 3d) and lymph nodes (results not shown).

DISCUSSION

The current case is an autopsy case of a 61-year-old woman who had a rapid course of illness to death after her first inoculation of the SARS-CoV-2 vaccine. Published information of heart pathology of the illness of after SARS-CoV-2 vaccination is small. The infiltration of eosinophils in addition to T-cells and macrophages is noted in two case of myocarditis after SARS-CoV-2 vaccination, although infiltrating eosinophils were very few in our case. In two adolescent autopsy cases following the second SARS-CoV-2 mRNA vaccine dose, the histopathology revealed the myocardial injury resembling a catecholamine-inducing injury which exhibits contraction band necrosis and/or myocytolysis with interstitial and perivascular infiltration of inflammatory cells consisting of predominantly neutrophils and macrophages and distributing separately from damaged cardiomyocytes. However, in our case, the infiltrating
**FIGURE 2** Immunohistochemistry of infiltrating inflammatory cells of the heart (a, PG-M1; b, CD3; c, CD4; d, CD8). Infiltrating cells are predominantly PG-M1-positive or CD3-positive cells, with a predominance of CD8-positive cells (a–d, scale bar is 100 μm).

**FIGURE 3** Histological findings of the other organs (a and b, lung; c, liver; d, bone marrow). (a) Hematoxylin and eosin staining. Scattered hemorrhagic infarction in the lung and severe pulmonary congestion are visible. (b) Hematoxylin and eosin staining. Tiny fibrin thrombi are visible in the pulmonary artery lumen. (c) Elastica-Goldner staining. Centrilobular necrosis is visible. (d) Hematoxylin and eosin staining. Hemophagocytosis (arrowheads) is visible (a, scale bar is 1000 μm; b, scale bar is 50 μm; c, scale bar is 200 μm; d, scale bar is 50 μm).
inflammatory cells were predominantly T-cells and macrophages and showed diffuse infiltration with adjacent damaged cardiomyocytes. Therefore, the histopathology and also the mechanisms in myocardial injury after SARS-CoV-2 vaccination seem to be heterogeneous.

Myocarditis with transmural myocardial necrosis was consistent with the cause of cardiogenic shock and death. One of the causes of myocarditis is a viral infection. Unfortunately, the antibodies to the viruses which might cause myocarditis were not evaluated in the current case. Histologically, viral myocarditis has been reported to have an inflammatory picture with the infiltration of macrophages and cytotoxic T-cells (CTLs). In the current case, infiltration of PG-M1-positive cells and CD8-positive cells was noticeable. In addition, there were hemophagocytic images in the bone marrow and lymph nodes, suggesting the coexistence of hypercytokinemia. Based on these histological findings, the possibility of viral myocarditis was initially considered. However, nucleic acids of any cardiotropic viruses including SARS-CoV-2 were not detected in formalin-fixed and paraffin-embedded and fresh-frozen samples of the heart using multivirus real-time PCR system. Furthermore, the following clinicopathological features would not be consistent with viral myocarditis.

First, the current case fell into a shock four days after vaccination. If the same pathologic change observed at autopsy is responsible for the patient's shock at 4 days after vaccination, the infiltration of CD8-positive CTLs would be a little earlier histological change compared with the standard time course of the primary immune response. It takes about 5 days for naive T-cells to differentiate into CTLs. Borrow et al. reported that in immune responses to lymphocytic choriomeningitis virus in mice, the number of CD8-positive cells reaches a peak about one week after infection. The patient's condition began to worsen 3 days after the first inoculation of the vaccine, and over the next few days, the course of the disease became fatal. On the third day after vaccination, organ damage appeared, and the patient died without improvement in her general condition. The autopsy showed findings of myocarditis with extensive infiltration of CTLs. This clinical course suggests that CTL-mediated myocarditis had already presented at her first attack of shock condition. Therefore, we considered the possibility of cross-immunity or immune memory as a mechanism for the early expansion of CTLs. Multiple independent assays have shown that anti-SARS-CoV-2 antibodies are also present in coronavirus-uninfected individuals. Mateus et al. found that a fraction of memory CD4-positive T-cells cross-react with both SARS-CoV-2 antigens and coronavirus antigens, with which people are routinely infected. These results suggest that memory helper T-cells to conventional coronavirus antigens may have triggered a rapid immune response at the time of vaccination, and the infiltration of CTLs may have been conspicuous for the time course. However, none of the reports currently examine the antigenic similarity between the conventional coronavirus and the spike protein produced by vaccination, and we had not analyzed this aspect.

Second, the conspicuous presence of vast nuclear debris accompanying neutrophilic infiltration in the myocardium is atypical for viral myocarditis. Vast nuclear debris suggests the activation of neutrophils, due to such as antineutrophil cytoplasmic antibodies, however, these antibodies were negative with less than sensitivity (data not shown). Although we could not rule out the possibility that the myocardial ischemia due to low output syndrome causes neutrophilic infiltration and subsequent nuclear debris formation, we also considered the possibility that the lipid nanoparticle (LNP) in the vaccine caused neutrophilic infiltration and nuclear dust formation. LNP is a substance that is added to the vaccine during SARS-CoV-2 vaccination to ensure that mRNA is efficiently introduced into the body. There is an experimental report in mice that innate immunity, including neutrophils, was elicited when LNP was administered alone. These effects of LNP may explain the reaction of neutrophilic infiltration and activation observed in the histology slides of the heart. However, there have been no reports on the pharmacokinetics of LNP in humans. Therefore, it is challenging to clarify the relationship between LNP and neutrophil infiltration in the current case.

Therefore, it may be possible that the myocarditis observed in this case was developed through a condition different from viral infection. Although SARS-CoV-2 vaccination has an extremely favorable risk ratio for myocarditis and should be recommended in adolescent and adult populations, the SARS-CoV-2 vaccine is the first mRNA vaccine and there remain many unanswered questions.

In this case, thrombus formation was observed in the arterial lumen of the peripheral lung tissue, and pulmonary infarction was observed. It has been reported that the spike protein produced after SARS-CoV-2 vaccination may induce endothelial damage and thrombus formation through the ACE2 receptor. Based on this report, micro thrombosis in the lumen of the pulmonary artery and pulmonary infarction can be explained as changes due to endothelial damage caused by spike proteins.

The clinicopathological findings of the current case might shed light on the pathogenesis of myocarditis possibly induced by inoculation of SARS-CoV-2 mRNA vaccine, and give suggestions in the direction of further research. We hope the accumulation of detailed immunological and histopathological analyses of similar cases in the near future.
AUTHOR CONTRIBUTIONS
Hidetoshi Satomi performed the autopsy and histological evaluation and drafted the manuscript and figures. Harutaka Katano performed nucleic acid analysis and review of the manuscript; Hiroyuki Kanno, Mikiko Kobayashi, and Ichiro Ito performed histological evaluation and review of the manuscript. Yukari Ohkuma, Naoto Hashidume, Tatsuya Usui, and Shunichi Tsukada performed the evaluation of clinical data and review of the manuscript.

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CONFLICT OF INTEREST
None declared.

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