

## A case-series of six autopsy cases of COVID-19 including three cases of cytomegalovirus coinfection

To the Editor,

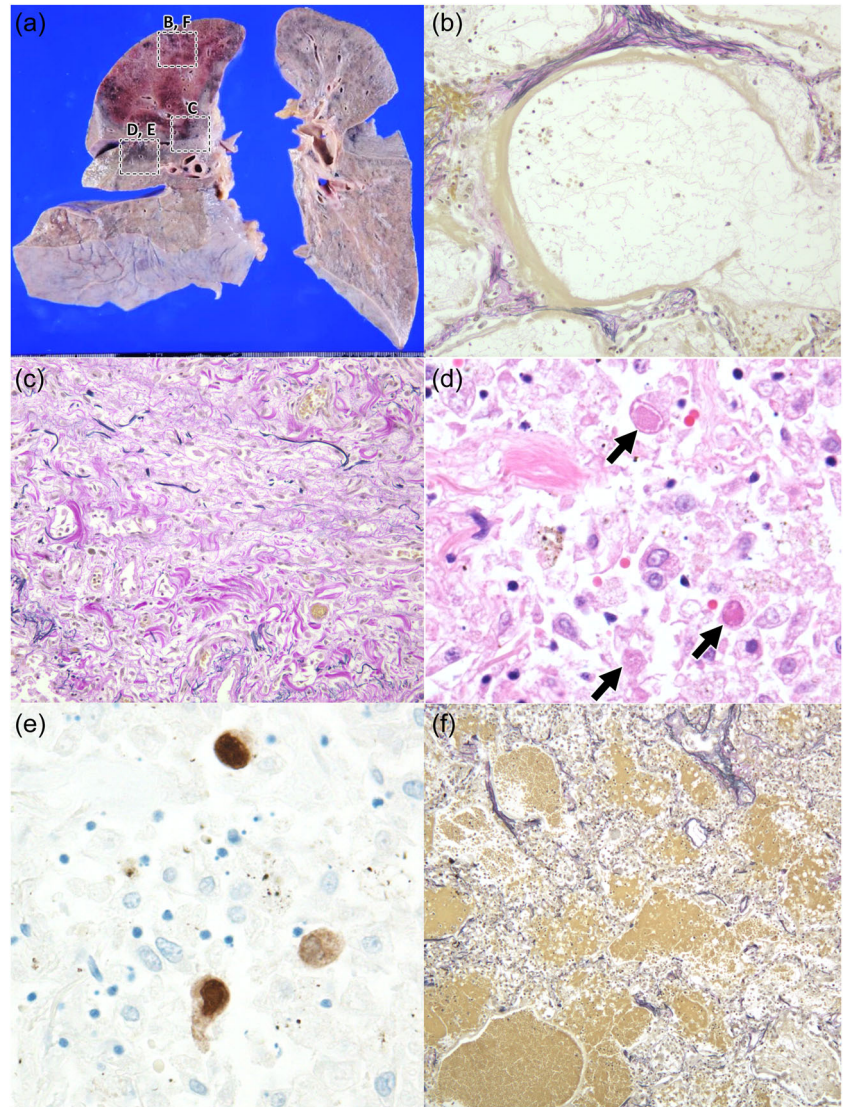
On March 11, 2020, the WHO declared coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), to be a global pandemic. As of March 29, 2022, over 480 million cases of COVID-19, including over 6.1 million deaths, have been confirmed globally. Previous COVID-19 autopsies revealed diffuse alveolar damage (DAD), chronic inflammation, and pulmonary edema to be the most common pathological findings. However, only few autopsy reports have discussed SARS-CoV-2 and cytomegalovirus (CMV) coinfection.<sup>1–3</sup> Herein, we reported six COVID-19 autopsy cases at our hospital, including three of SARS-CoV-2 and CMV coinfection.

Six COVID-19 autopsies were performed between 2020 and 2021. In all the cases, the presence of SARS-CoV-2 was confirmed by a pre-mortem reverse transcriptase polymerase chain reaction (RT-PCR) analysis of nasopharyngeal swab specimens. All the patients had a comorbidity, such as diabetes mellitus, obesity, and hypertension. In five cases, a bacterial or fungal infection was confirmed by pre-mortem blood cultures. Four patients had received steroid therapy, and CMV coinfection had been detected in three of four patients during COVID-19 treatment (Cases 3, 4, and 6 in Table S1). In Case 3, a CMV infection was confirmed in a lung autopsy using an anti-CMV antibody test (Anti-CMV blend, 8B1.2, 1G5.2&2D4.2; Roche). Notably, the patient in Case 4 was thought to be immunocompromised at the time of admission. The patient had had a history of diabetes mellitus and had received chemotherapy for lymphoma. The CMV infection was detected by a pre-mortem CMV antigenemia assay. In all the cases, gross examination found pleural effusions; the lungs were heavy and edematous, and the cut surfaces showed an unevenly distributed, whitish area (Figure 1a). Microscopy of the lungs found mixed phases of diffuse alveolar damage (DAD) in Cases 1, 2, 4, and 5 (Table S2; Figure 1b,c), where the alveolar spaces were filled with degenerated pneumocytes, macrophages, and chronic inflammatory cells. Hyaline membranes were observed throughout the alveolar lumen, and focally, the alveolar walls were thickened by loose connective tissue. In Cases 1 and 5, fibrotic

lesions were also observed, and in Case 6, organizing pneumonia was observed. Bacterial pneumonia was seen in Cases 1 and 3, but lung abscess was only observed in the latter. In Cases 3, 4, and 6, nuclear inclusion bodies and swollen eosinophilic cytoplasm, indicative of CMV infection, were observed (Figure 1d). In Case 4, an anti-CMV antibody test of the nucleus of the degenerated cells returned positive for CMV (Figure 1e), and CMV-positive cells were detected throughout the lungs. In Case 6, CMV infection was observed in the small intestine and colon. Although immunohistochemical analysis for SARS-CoV-2 nucleocapsid and RT-PCR analysis using formalin-fixed paraffin-embedded samples were performed in Cases 1 and 4, the results returned negative for both, possibly because of the prolonged period of formalin fixation. Acute alveolar hemorrhage was focally present in all the CMV-positive cases and Case 2 (Figure 1f). Thrombi were detected only in Case 1. Acute myocardial infarction was detected in Case 6. The spleen in several cases showed white pulp depletion, but it was difficult to distinguish these findings from post-mortem changes. No remarkable changes were observed in the other organs. These findings suggested that the patients' death was mainly caused by either DAD associated with SARS-CoV-2 or CMV or both or fungemia or viremia.

Diffuse alveolar damage is a well-known, post-mortem pulmonary finding associated with COVID-19. Satturwar et al. reported that post-mortem histopathological findings of DAD were seen in 80.9% of patients with COVID-19.<sup>1</sup> DAD is also known to occur in CMV-related pneumonia. Thus, SARS-CoV-2 and CMV-related pneumonia have similar pathological findings and exhibit degeneration and viral inclusions in infected pneumocytes. Therefore, if CMV coinfection had not been clinically considered in patients with COVID-19, it may be worthwhile to assess for CMV coinfection by post-mortem tissue analysis to investigate the factors of lethality. Our three CMV-positive cases showed no DAD or an earlier DAD phase whereas our three, CMV-negative cases showed a greater degree of the fibrotic DAD phase. The duration from onset to death tended to be longer in the CMV-positive patients than CMV-

**FIGURE 1** (a) Macroscopic appearance of the bilateral lungs. Whitish, hemorrhagic areas were observed. Each square indicates an area of (b)–(f). (b–f) Microscopic features of the lungs. Both the exudative and proliferative phases of diffuse alveolar damage (DAD) were observed (elastica van Gieson's stain [EVG]). (b) Hyaline membranes lined the alveolar ducts (EVG). (c) The interstitium of the alveolar walls was thickened by loose connective tissue (EVG). (d) Nuclear inclusion bodies were observed in the degenerated cells (arrows) (H & E). (e) Immunohistochemically, the nucleus was positive for cytomegalovirus (CMV) (CMV stain). (f) Acute alveolar hemorrhage (EVG).



negative ones and therefore carry an attendant, higher risk of opportunistic infections (Tables S1 and S2). Additionally, multiple cases of bacterial and fungal coinfection were observed among the CMV-positive cases, indicating the possibility that immature lesions may represent the immunosuppressed status of the patients. Notably, all the cases of CMV coinfection were associated with pulmonary hemorrhage. While the etiology of the pulmonary hemorrhage was unclear, past reports have suggested that severe COVID-19-associated pneumonia may involve microvascular injury.<sup>1</sup> The direct cause of death in cases of coinfection is often difficult to identify, but CMV may be a risk factor of poor clinical outcomes in COVID-19.<sup>2–4</sup>

Cytomegalovirus belongs to the herpes virus family and infects most people at some point in their lives. CMV reactivation occurs in about 30% of patients in intensive care units and increases their risk of mortality.<sup>3</sup> Niitsu et al. reported that six of 26 patients with COVID-19 acquired a CMV infection during

mechanical ventilation; these patients required a longer duration of mechanical ventilation and had a higher mortality rate than a non-CMV group.<sup>3</sup> Shrock et al.'s deep serological profiling of patients with COVID-19 demonstrated that a hospitalized group had a higher seroprevalence rate for CMV than a nonhospitalized group (83/101 vs. 49/131). They concluded that the effects of CMV infection on the immune system potentially influenced COVID-19 outcomes.<sup>2</sup> CMV decreases the naive T cell pool, hindering adaptation to novel pathogens, such as SARS-CoV-2. Naive T cells are recruited from the thymus and decline in number with age. CMV infection is estimated to accelerate the attrition of the naive T cell pool by about 20 years. In addition, tumor necrosis factor  $\alpha$  is known to be elevated in COVID-19 and can stimulate CMV immediate-early promoter and CMV reactivation. SARS-CoV-2-activated macrophages also promote reactivation of latent CMV, causing further inflammation.<sup>4</sup>

We have experienced six COVID-19 autopsies to date and have detected a CMV coinfection in three cases. CMV coinfection was not confirmed before the patient's death in one case. In general, CMV reactivation and coinfection may have been overlooked in other cases of severe COVID-19.

A murine model demonstrated that at least 6 months of antiviral therapy were required to improve the immune response.<sup>5</sup> Although CMV-specific drugs may not be able to reverse human immunosenescence rapidly, confirmation and treatment of a CMV infection may improve COVID-19 outcomes. More research is needed to investigate the effects of, and establish a treatment for, SARS-CoV-2 and CMV coinfection.

In summary, we reported six autopsy cases of COVID-19 patients. The possibility of CMV coinfection needs to be considered in COVID-19 cases.

### AUTHOR CONTRIBUTIONS


Jotaro Nakashima wrote the initial draft of the manuscript. Jotaro Nakashima, Takahiro Kiri, Shingo Itagaki, Yuichiro Kadomatsu, and Toshinori Otani performed the autopsy. Jotaro Nakashima, Takahiro Kiri, Shingo Itagaki, Yuichiro Kadomatsu, Toshinori Otani, and HO contributed to the analysis of the clinical course and histopathological findings. Yuichiro Kadomatsu, Atsumi Matsunaga, Saya Munakata, and Haruka Okada assisted with the preparation and critical review of the manuscript, approved the final version, and agreed to be accountable for all aspects of the work by ensuring that questions related to the accuracy or integrity of a part of the work were appropriately investigated and resolved.

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### DISCLOSURE STATEMENT

The authors declare no conflicts of interest.

Jotaro Nakashima   
Yuichiro Kadomatsu  
Shingo Itagaki  
Toshinori Otani  
Takahiro Kiri  
Atsumi Matsunaga  
Saya Munakata  
Haruka Okada

*Department of Pathology,  
Tokyo Metropolitan Tama Medical Center, Tokyo,  
Japan*

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