Anti-nuclear Antibody and a Granuloma Could be Biomarkers for iCIs-related Hepatitis by Anti- PD-1 Treatment

Yasuteru Kondo (yasuteru@ebony.plala.or.jp)
Sendai Kousei Hospital
Junichi Akahira
Sendai Kousei Hospital
Tatsuki Morosawa
Sendai Kousei Hospital
Yukihiro Toi
Sendai Kousei Hospital
Akashi Endo
Sendai Kousei Hospital
Hiroaki Satio
Sendai Kousei Hospital
Mareyuki Endo
Sendai Kousei Hospital
Shunichi Sugawara
Sendai Kousei Hospital
Yasuhito Tanaka
Kumamoto University

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Abstract

Background and Aims

It has been reported that various kinds of immune checkpoint inhibitors (iCIs) could induce immune-related liver damage. We should focus on the programmed cell death-receptor-1 (PD-1) antibody and non-small cell lung cancer (NSCLC) to analyze the characteristics of hepatitis related to iCIs and find the factors that could be useful biomarkers for the diagnosis.

Methods

A single-center retrospective study of 252 NSCLC patients who received PD-1 antibody (nivolumab or pembrolizumab). Some of the biochemical markers and immunological markers were analyzed during PD-1-antibody treatment with or without ALT elevation. Histopathological features were reviewed by a single expert of hepatic pathology focusing on the following features: fibrosis, portal inflammation, lobular inflammation, lobular necrosis. The formation of macro- and micro- granulomas was also evaluated.

Results

The frequency of liver damage induced by nivolumab including grade 1 to 4 (ALT) was 41.9% (78/186 patients). The positive rate of anti-nuclear antibody in the nivolumab group with iCIs-related hepatitis was significantly higher than that in the nivolumab group without iCIs-related hepatitis (*p<0.0001*). Granulomatous changes were significantly increased in patients with iCIs-related hepatitis compared with DILI and AIH patients (*p<0.05*). The ratios of inflammatory cells CD4/CD8, and CD138/CD3 in ICIs-related hepatitis were significantly lower than in those of AIH or DILI patients (*p<0.05*).

Conclusions

We demonstrated that the pre-existing ANA and characteristic liver histology including $CD8^+$ cells dominancy and granulomatous hepatitis could be biomarkers for the diagnosis of iCIs-related hepatitis in the NSCLC with anti-PD-1 therapy.

Introduction

Various kinds of Immune-check point inhibitors (iCIs) that target immune-suppressive molecules to enhance cytotoxic T lymphocytes (CTLs) could improve the survival in patients with advanced non-small cell lung cancer. Among them, anti-programmed cell death protein 1 (anti-PD-1) is widely used. However, it has been reported that various kinds of immune-related adverse events (irAEs) might be induced by dysregulating the immune systems. The Department of Pulmonary Medicine in our institute has reported various kinds of irAEs in advanced non-small cell lung cancers patients treated with anti-PD-1[1].

It has been reported that the most common phenotype of iCIs-related liver damage was iCIs-related hepatitis[2]. However, the detailed characteristics of iCIs-related hepatitis induced by treatment with anti-PD-1 have not been clarified. Some groups described that a characteristic liver histology could be observed in patients with liver injury during the treatment with iCIs[3–5]. An analysis of immune cell subsets such as CD8 positive cells and CD4 positive cells was carried out in iCIs-related liver injury[6,7]. However, the characteristics of liver histopathology and subsets of immune cells from iCIs-related hepatitis have not been clarified due to the limited number of samples.

In addition to the histopathology of iCIs-related hepatitis, we should consider the possible biomarkers to predict hepatitis after using iCIs. It has been reported that auto-immune disorders such as paraneoplastic syndromes could be potential biomarkers to predict immune-related liver injury induced by treatment with iCIs[8]. Immune related liver diseases such as auto-immune hepatitis (AIH) and primary biliary cholangitis (PBC) could be worsened by using iCIs. Therefore, an analysis of potential biomarkers to predict the onset of AIH or PBC such as anti-nuclear antibody (ANA) and anti-mitochondria antibody (AMA) might be useful to predict liver injury induced by iCIs. Some groups including ours have reported that preexisting antibodies and rheumatoid factor were common among patients who developed irAEs[1,9]. However, the relation between preexisting antibodies and immune-related liver injury induced by iCIs has not been clarified. In this study, we tried to identify possible biomarkers to detect iCIs-related hepatitis induced by PD-1 antibody in lung cancer patients by analyzing the histopathology of liver and serum markers.

Materials And Methods

Study design and inclusion criteria

This study was approved by the Ethics Committee of Sendai Kousei Hospital following ethical guidelines of the 1975 Declaration of Helsinki, and written informed consent was obtained from each individual.. The patients with advanced non-small cell lung cancer (NSCLC) who were treated with anti-PD-1 (nivolumab (3mg/kg every 2 weeks) (186 patients) or pembrolizumab (200mg every 3 weeks) (66 patients)) monotherapy at Sendai Kousei Hospital between January 2016 and June 2018 were enrolled in this study. We focused on the patients treated with monotherapy of PD-1 antibody (nivolumab or pembrolizumab) to exclude other factors potentially inducing liver damage such as molecular targeting agents, other iCIs and cell-killing anticancer drugs. In addition to the regimen of treatment, we focused on NSCLC patients to exclude the effects of primary cancer influencing the immune reactions.

Biochemical analysis and clinical factors analysis

Patients with iCIs-related hepatitis were diagnosed by the following criteria. Alanine aminotransferase (ALT) elevations were consistent at least two weeks after using PD-1 antibody. The ALT elevations were dominant compared with aspartate aminotransferase (AST) and alkaline phosphatase (ALP) elevations. The patients were excluded if they had liver metastasis, disseminated intravascular coagulation, multiple

organ failure, alcohol drinking (>30g / day), chronic hepatitis C. All HBsAg positive patients (3 patients) were inactive carriers. Age, sex and tissue type of lung cancer were compared between patients with iCIs-related hepatitis and those without iCIs-related hepatitis. The grade of liver damage was analyzed by using common terminology criteria for adverse events (CTCAE) v5.0. We evaluated iCIs-related hepatitis together with ALT elevation using the grading of CTCAE v5.0. Some of biochemical markers and immunological markers were analyzed during PD-1-antibody treatment with or without iCIs-related hepatitis.

Histological evaluation

Liver biopsies were carried out immediately after consulting the Department of Hepatology about liver damage in some patients treated with PD-1 antibody. The selection of patients who should be carried out liver biopsy were evaluated by two hepatologists to exclude the risk of bleeding and the progression of lung cancer. All biopsy specimens were fixed in formalin and paraffin embedded (FFPE). Sections (3μm) were then stained with hematoxylin and eosin. Histopathological features were reviewed by a single expert of hepatic pathology focusing on the following features: fibrosis according to Brunt's criteria (G1-G4)[10], portal inflammation (0-3), lobular inflammation (0-3), lobular necrosis (0-3). The formation of macro- and micro- granulomas was also evaluated.

Immunohistochemical evaluation

Immunohistochemical staining for T-cell markers (CD3, CD4, CD8), B-cell marker (CD20), and plasma cell marker (CD138) were performed by an automatic immunostainer (Ventana BenchMark GX, Roche). The antibodies and sources were as follows: CD3 (mouse monoclonal, Leica, Germany), CD4 (mouse monoclonal, Nichirei, Japan), CD8 (mouse monoclonal, DAKO, Denmark), CD20 (mouse monoclonal, DAKO, Denmark) and CD138 (mouse monoclonal, Nichirei, Japan). Immunohistochemical analyses were performed in all of the patients and compared to those with typical auto-immune hepatitis (AIH) (n=3) and classical drug-induced liver injury (DILI) (n=3).

Statistical analysis

χ2 test or *independent t test* was employed to compare the differences between the patients treated by nivolumab without liver damage and those treated by nivolumab with liver damage using JMP Pro 15.0 (Table 1). We also compared the clinical factors of irAEs induced by pembrolizumab with liver damage or without liver damage in the same way. *Independent t test* was employed to compare the frequencies of immune cell subsets between the patients treated with PD-1 antibody and control subjects (Table 3).

Results

Characteristics of iCIs related hepatitis induced by anti-PD-1 antibody

The median onset time points of iCIs-related hepatitis induced by the administration of nivolumab and pembrolizumab were 119 days and 114 days, respectively (Figure 1A and 1B). However, the iCIs-related hepatitis induced by the administration of PD-1 antibody occurred at various time points. The peak total bilirubin, ALT and ALP levels with liver damage induced by PD-1 antibody (nivolumab and pembrolizumab) administration were shown in Table 1 and 2. The frequency of liver damage induced by nibolumab including grade 1 to 4 (ALT) was 41.9% (78/186 patients). The numbers of patients with grade 1/2/3/4 liver damage (ALT) were 54/12/10/2 patients, respectively. A HBsAg positive patient (inactive carrier) had grade 1 ALT elevation. However, the significant decline of HBsAg had not been detect in these patients. The frequencies of iCIs-related hepatitis induced by pembrolizumab including grade 1 to 4 (ALT) were 37.9% (25/66). The numbers of patients with grade 1/2/3/4 liver damage (ALT) were 17/3/2/3 patients, respectively.

Most of the patients with iCIs-related hepatitis induced by PD-1 antibody were recovered by observation or Strong neo minophagen C® (SNMC) consisting of monoammonium glycyrrhizinate, glycine, aminoacetic acid and L-Cystein hydrochloride hydrate administration. However, some of the patients needed steroid or steroid with azathioprine. We could not determine the cell type of lung cancer that could easily induce liver damage by the administration of PD-1 antibody (Table 1 or 2).

Possible serum biomarkers for iCIs-related hepatitis induced by anti-PD-1 antibody

We analyzed several immune-related markers (anti-nuclear antibody, anti-mitochondrial antibody and the amount of immunoglobulin G and M) to predict the susceptibility to liver damage induced by PD-1 antibody (Table 1 and 2). The positive rate of anti-nuclear antibody in the nivolumab with iCIs-related hepatitis group was significantly higher than that in the Nivolumab without iCIs-related hepatitis group ($p<0.0001$). Moroever, we could determine a significant difference in the positive rate of anti-nuclear antibody between Pembrolizumab with iCIs-related hepatitis and that without iCIs-related hepatitis ($p=0.00012$).

Liver histology of hepatitis induced by PD-1 antibody

Ten and 8 patients received liver biopsy after or during Nivolumab and Pembrolizumab treatment, respectively. The results of morphological and immunohistochemical analysis are summarized in Table 3. The degree of portal and lobular inflammation, fibrosis, and necrotic changes were significantly increased in patients with Grade3/4 patients compared to Grade1/2 patients ($p<0.05$). Granulomatous changes, especially large granulomatous change with necrosis (Figure 2A and B), were significantly

increased in patients with ICIs patients compared with DILI and AIH patients (*p<0.05*). The ratios of inflammatory cells CD4/CD8, and CD138/CD3 in ICIs (0.63 and 0.044) were significantly lower than those in AIH (1.89 and 0.21) or DILI (1.08 and 0.39) patients (*p<0.05*, respectively)(Figure 3). Steatosis, ballooning, and Mallory's bodies were rarely identified in these patients.

Discussion

The liver damage induced by treatment with iCIs has been widely recognized throughout the world. There are various phenotypes of liver damage induced by treatment with iCIs[11–13]. Most of the patients with iCIs-related liver damage revealed the phenotype of hepatocyte injury. We called this phenotype iCIs-related hepatitis. It is difficult to diagnose iCIs-related hepatitis by clinical symptom. Therefore, we diagnosed iCIs-related hepatitis by ALT level to avoid the selection bias. This selection method might increase the frequency of iCIs-related hepatitis by PD-1 antibody. Some groups reported that iCIs treatment could induce cholangiocyte injury in a small number of patients[2,11]. It is difficult to confirm the representative phenotypes of liver damage induced by iCI treatment since various kinds of iCIs and the origins of the tumor could affect the pathogenesis of iCIs-related liver damage. Therefore, we focused on the single agent anti-PD-1 therapy with nivolumab or pembrolizumab for NSCLC to analyze the characteristics of hepatocyte injury. In our data, the median onset time points of iCIs-related hepatitis induced by the administration of nivolumab and pembrolizumab were 117 days and 114 days, respectively. Previously, another group reported treatment-related autoimmunity could occur within 5–15 weeks from the start of iCI treatment[14]. We found a positive rate of ANA in the nivolumab treatment group with hepatocyte injury that was significantly higher than that without hepatocyte injury. Moreover, we could detect significant differences among the pembrolizumab treatment groups even in the small number of patients. In our institute, it was reported that preexisting antibodies and rheumatoid factors were more common among patients with irAEs[1]. In this study, AMA, IgG and IgM did not affect the incidence of liver damage. The pre-existence of some host factors including ANA might influence the induction of iCIs-related hepatitis.

In addition to the topic of host factors that might induce iCIs-related hepatitis, the characteristics of the liver histology were important for the management of iCIs-related hepatitis[3–5,15–17]. Granulomatous changes, especially large granulomatous changes with necrosis, were significantly increased in patients with ICIs-related hepatitis compared with DILI and AIH patients in this study. Moreover, we found that the ratios of inflammatory cells CD4/CD8, and CD138/CD3 in ICIs were significantly lower than those in AIH or DILI patients. These data indicated that iCIs-related hepatitis might be induced by $CD8^+$ T cells. Previously, another group reported that granulomatous hepatitis was observed in liver with grade >/=3 hepatitis caused by anti-PD-1/PD-L1 or CTLA-4 immunotherapies[15]. We were able to detect these characteristics of the liver histology when we carried out liver biopsy at the time of the G1 ALT elevation (5 patients / 7 patients). This finding could be helpful to manage patients with anti-PD1 treatment. It is difficult to decide the discontinuation of PD-1 antibody and use of steroid since such decisions might affect the survival of the patients.

ICIs-related hepatitis could be critical for patients of hepatocellular carcinoma (HCC) since many HCC patients could have limited liver function due to the liver cirrhosis. Previously, we reported that various kinds of factors including hepatitis B virus, hepatitis C virus, and the HCC microenvironment might affect the immune pathogenesis of liver diseases[18–24]. In this study, we included 3 HBsAg positive inactive carrier. Recently, it has been reported that iCIs treatment might induce hepatitis in the HBsAg positive patients[25–27]. Therefore, it has been difficult to determine the characteristics of iCIs-related hepatitis by analyzing HCC patients treated with iCIs, so far. We should analyze HCC patients with various background diseases case by case in the near future.

In conclusion, we demonstrated that pre-existing ANA and a characteristic liver histology including $CD8^+$ cells dominancy and granulomatous hepatitis could be biomarkers for the diagnosis of iCIs-related hepatitis in NSCLC with anti-PD-1 therapy. We should analyze the characteristics of iCIs-related hepatitis in various kinds of malignancies, and iCIs and combination therapy separately to understand the precise pathogenesis of iCIs-related hepatitis induced by specific treatment regimens of iCIs.

Declarations

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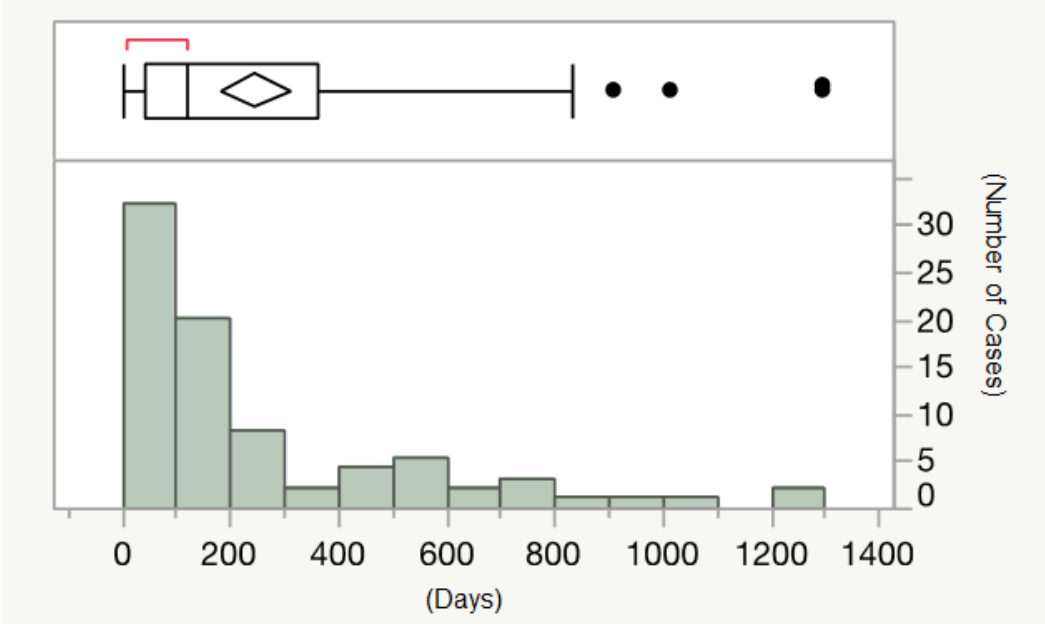
Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures

Figure 1

A.



B.

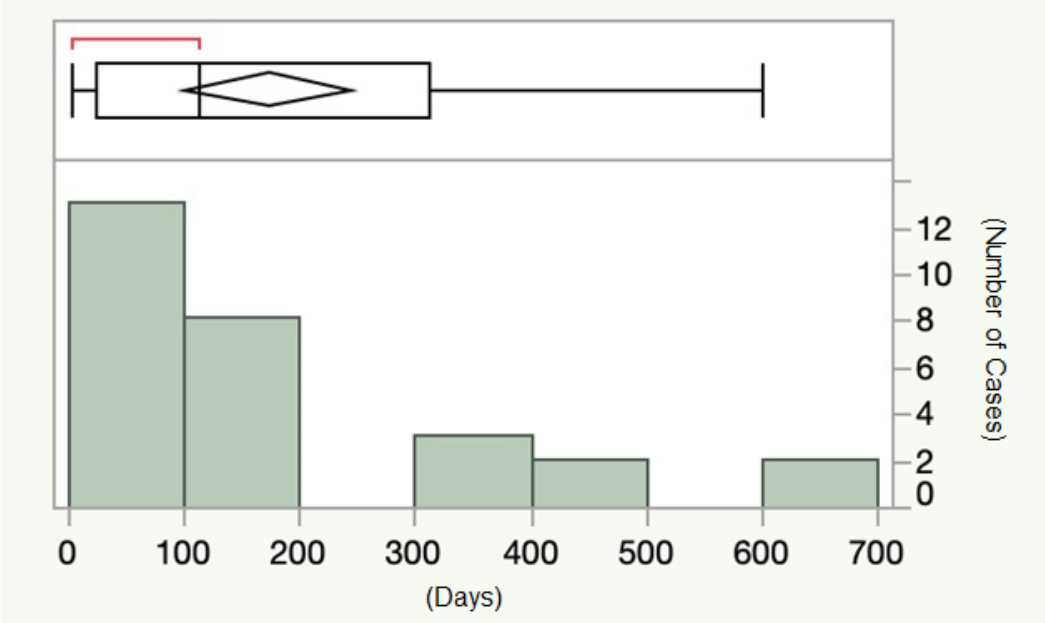
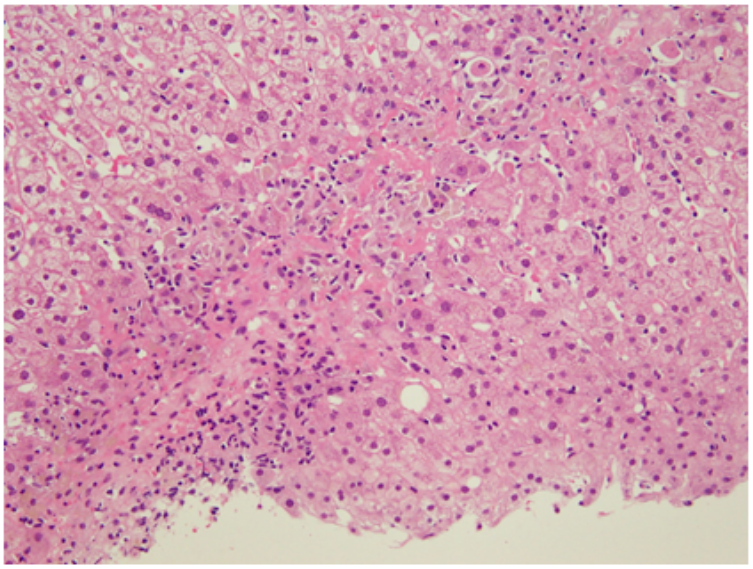


Figure 1

The median onset time point of iCIs-related hepatitis induced by the administration of nivolumab and pembrolizumab are shown (A: nivolumab and B: pembrolizumab). X-axis shows the onset time point of iCIs-related hepatitis. Y-axis shows the number of patients with iCIs-related hepatitis.

Figure 2

A.



B.

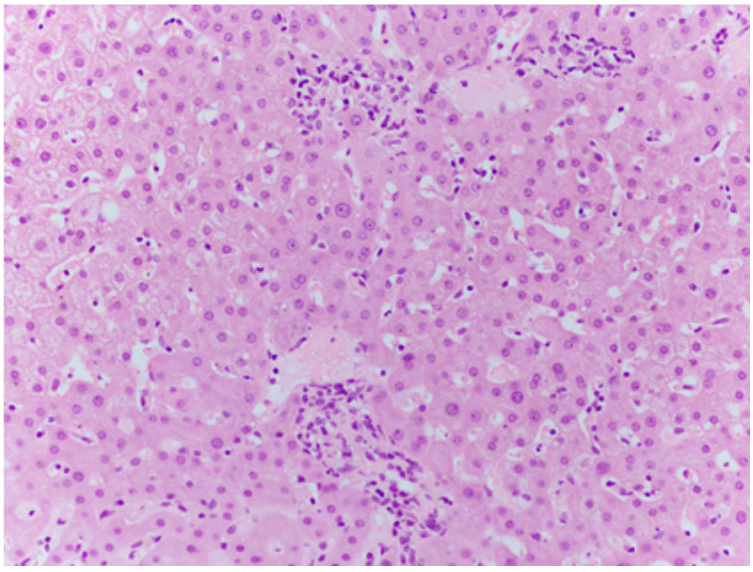


Figure 2

Hematoxylin and eosin staining of liver biopsy in case 17 (A). Panlobular hepatitis was seen in this patient. Macrogranulomatous changes were easily identified and were composed of massive necrosis with inflammatory infiltrates comprising activated lymphocytes and histiocytes. Severe liver cell injuries and regenerative changes were also seen(A). Hematoxylin and eosin staining of liver biopsy in case 13 (B). Mild lobular hepatitis with microgranulomas was seen in this patient. Microgranulomatous changes

were composed of foci of inflammatory infiltrates comprising activated lymphocytes and histiocytes. Mild liver cell injuries and regenerative changes were also seen(B).

Figure 3

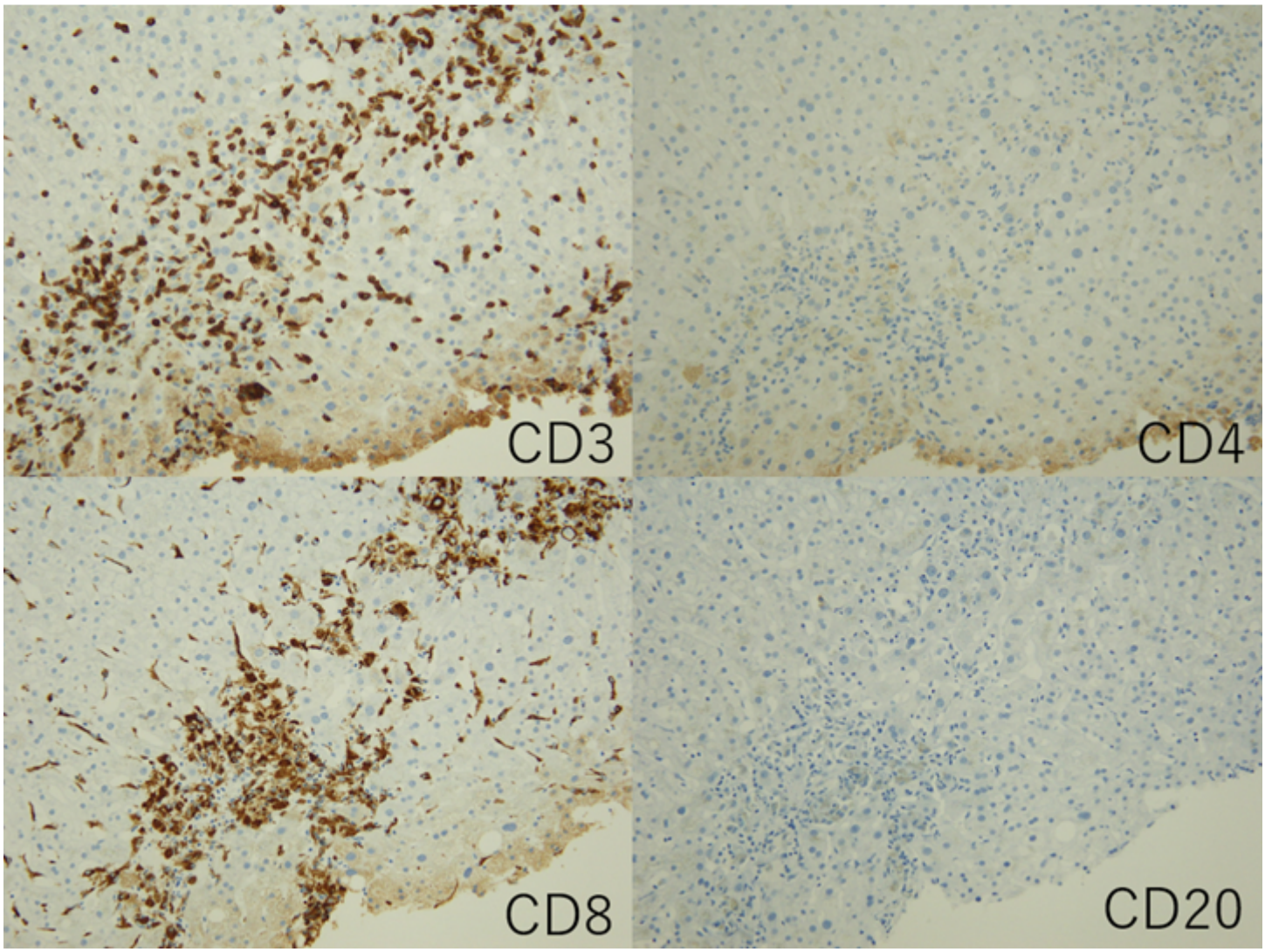


Figure 3

Immunohistochemical staining of liver biopsy in case 17. Almost all infiltrating lymphocytes were CD3+/CD8+ cytotoxic T-cells. CD4+ T-cells, CD20+ B-cells and CD138+ plasma cells were rarely identified.

Supplementary Files

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