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【 CASE REPORT 】

Hepatitis-associated Aplastic Anemia with Rapid Progression of Liver Fibrosis Due to Repeated Hepatitis

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Abstract:
Hepatitis-associated aplastic anemia (HAAA) is a variant of acquired aplastic anemia and characterized by bone marrow failure that follows the development of acute hepatitis. We herein report a rare case of HAAA with rapid progression of liver fibrosis due to repeated hepatitis. A pathological examination of liver specimens revealed liver fibrosis progression over a short period. Immunosuppressive therapy with cyclosporine effectively cured both the pancytopenia and hepatitis. Our case suggests that the pathological examination of the liver tissue is useful for determining a treatment plan and that immunosuppressive therapy is a promising treatment for both aplastic anemia and persistent hepatitis.

Key words: hepatitis-associated aplastic anemia, liver fibrosis

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Introduction

Aplastic anemia (AA) is a serious blood disorder resulting from failure of the bone marrow to produce blood cells. Although most cases of AA are classified as idiopathic, some patients develop symptoms after an episode of acute hepatitis. If the onset of AA is clinically related to acute liver injury, it is called hepatitis-associated aplastic anemia (HAAA). HAAA accounts for 4% to 10% of AA cases in East Asia (1), and the presumed causes of acute hepatitis is viral infection, such as by hepatitis A virus, hepatitis B virus, hepatitis C virus, parvovirus B19, cytomegalovirus, Epstein bar virus, and transfusion-transmitted virus (2-7), but the causative virus is not identified in most HAAA cases (8, 9). In general, pancytopenia appears two or three months after acute hepatitis attack (9, 10), and the development of AA can be fatal if not treated in a timely manner (8, 11).

We herein report a case of HAAA accompanied by rapid progression of liver fibrosis due to repeated hepatitis. In this case, immunosuppressive therapy was effective against the severe hepatitis as well as the AA.

Case Report

A 26-year-old man was referred to our institute for repeated hepatitis and a blood cell disorder and admitted. One year before admission to our hospital, he developed acute hepatitis with elevated levels of aspartate transaminase (AST, 1,750 U/L), alanine transaminase (ALT, 3,110 U/L), and total bilirubin (T-bil, 8.9 mg/dL) after receiving a flu shot. Although a further examination, including a liver biopsy, was performed, the cause of the liver dysfunction remained unclear, and the laboratory data improved without treatment (Fig. 1A). Six months after the initial episode, the patient was admitted to another hospital due to recurrence of the liver dysfunction with elevated AST (957 U/L) and ALT (1,970 U/L) levels, as well as leukocytopenia and thrombocytopenia. The cause of the blood disorder, however, was still unclear; the results of tests for typical viruses, such as hepatitis A, B, C, and E, and cytomegalovirus, and for various autoantibodies were negative. His liver dysfunction again improved without treatment, but the leukocytopenia

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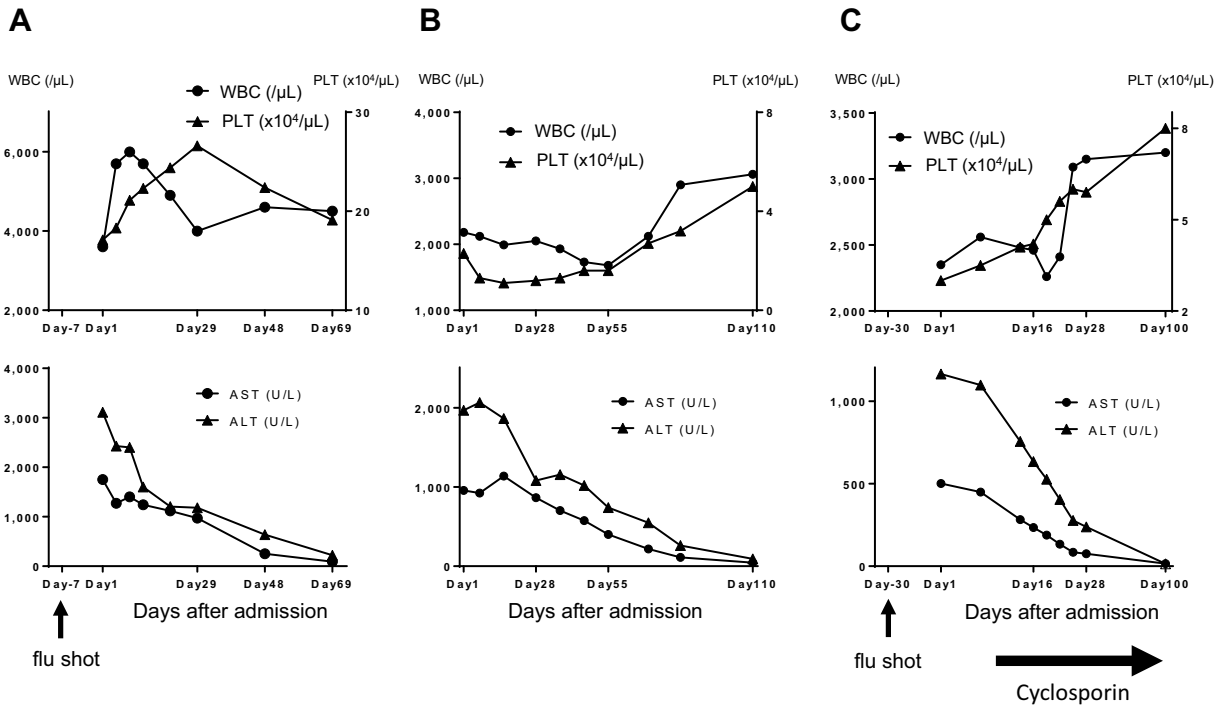

Figure 1. **Changes in laboratory values in the course of hepatitis. (A) The first episode. (B) The second episode. (C) The third episode. WBC: white blood cell count, PLT: platelet, AST: aspartate transaminase, ALT: alanine transaminase**

and thrombocytopenia persisted even after discharge from the hospital (Fig. 1B). Seven months after the second episode, he presented to his primary care physician with general fatigue and was diagnosed with recurring liver dysfunction (AST 434 U/L and ALT 1,017 U/L). He was referred to our institute for a re-examination of his liver dysfunction.

He had no remarkable medical history other than the repeated liver dysfunction and persistent leukocytopenia and thrombocytopenia. He had received a flu shot one month before visiting our hospital. All vital signs were normal, and there was no evidence of jaundice. His abdomen was flat, and neither hepatomegaly nor ascites were observed. The laboratory evaluation revealed abnormalities in the liver panel results (AST 501 U/L, ALT 1,165 U/L, alkaline phosphatase 429 U/L, and γ-glutamyl transpeptidase 103 U/L). The T-bil, albumin, and prothrombin time values were within the normal ranges. A complete blood count revealed the following values: white blood cell (WBC) 2.35×10^3/μL, neutrophils 0.94×10^3/μL, hemoglobin 14.0 g/dL, reticulocyte 8.0×10^4/μL, and platelets 3×10^4/μL. The serologic test results were negative for hepatitis B surface antigen, hepatitis C virus IgG, cytomegalovirus IgM, and Epstein-Barr virus IgM antibodies, as well as for antinucleic and antimitochondrial antibodies. The values of the representative laboratory examinations are summarized in the Table. Abdominal ultrasonography and contrast-enhanced computed tomography revealed mild splenomegaly but no specific findings in the liver (Fig. 2).

To evaluate the pathologic features of the liver tissue, we performed a transjugular liver biopsy and compared the sample with the liver biopsy specimen that had been collected at the previous hospital when he had had liver dysfunction one year before. While lymphocyte infiltration was prominent in the previous sample (Fig. 3), most of the lymphocytes had disappeared and been replaced by histiocytes (Fig. 4A, B). Interestingly, bridging hepatic necrosis of the portal area and progression of the fibrosis in the centrilobule were observed in the second biopsy sample, whereas fibrotic changes were not observed in the first sample (Fig. 4C, D). These findings suggest that the repeated severe hepatic inflammation led to the rapid progression of liver fibrosis.

Next, we examined his bone marrow function to elucidate the cause of the leukocytopenia and thrombocytopenia. Scintigraphy imaging showed hypoplastic bone marrow with a diffuse reduced uptake of ^{111}In-tracer in the whole body (Fig. 5A). A bone marrow biopsy study showed severe hypocellularity with a nucleated cell density of 10% and fatty replacement (Fig. 5B). In addition, small populations of paroxysmal nocturnal hemoglobinuria (PNH)-type cells were identified among granulocytes and red blood cells in the peripheral blood by flow cytometry. Taken together, these findings led us to diagnose the patient with HAAA of unknown origin.

Given the rapid progression of the liver fibrosis and the presence of PNH-type cells, treatment with cyclosporin was introduced on day 20 of hospitalization. The liver enzyme levels promptly decreased to the normal range, and the WBC and platelet counts increased after beginning cyclosporin treatment. After discharge on day 29 of hospitalization, he continued treatment with cyclosporin without re-

Table. **Laboratory Findings on Admission.**

Complete blood cell count			
WBC	2,350 /μL	Serology	
Neutro	940 /μL	CRP	0.1> mg/dL
RBC	4.39×10^6 /μL	IgG	1,169 mg/dL
Hb	14.0 g/dL	IgA	117 mg/dL
Plt	3.0×10^4 /μL	IgM	32 mg/dL
Reti	8.0×10^4 /μL		
		Immunology	
Coagulation		Antinuclear antibody	< 1:40
PT-INR	0.96	Antimitochondrial antibody	< 1.5
APTT	30.9 sec	Anti-LKM-1 antibody	< 5.0
Biochemistry		Viral markers	
AST	501 U/L	HBsAg	(-)
ALT	1,165 U/L	HBsAb	(+)
LDH	309 U/L	HBcAb	(-)
ALP	429 U/L	HCVAb	(-)
gGTP	103 U/L	EBV-VCA-IgG	(+)
T-bil	1.0 mg/dL	EBV-VCA-IgM	(-)
TP	7.1 g/dL	EBNA	(+)
Alb	4.5 g/dL	EBV-DNA	(-)
		CMV-IgG	(+)
		CMV-IgM	(-)
		CMV antigenemia	(-)

WBC: white blood cell, Neutro: neutrophil, RBC: red blood cell, Plt: platelet, PT: prothrombin time, INR: international normalized ratio, APTT: activated partial thromboplastin time, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, ggTP: gamma glutamyl transpeptidase, T-bil: total bilirubin, TP: total protein, Alb: albumin, CRP: C-reactive protein, HBsAg: hepatitis B surface antigen, HBsAb: hepatitis B surface antibody, HBcAb: hepatitis B core antibody, HCVAb: hepatitis C virus antibody, EBV: Epstein-Bar virus, VCA: viral-capsid antigen, EBNA: Epstein-Bar nuclear antigen, CMV: cytomegalovirus

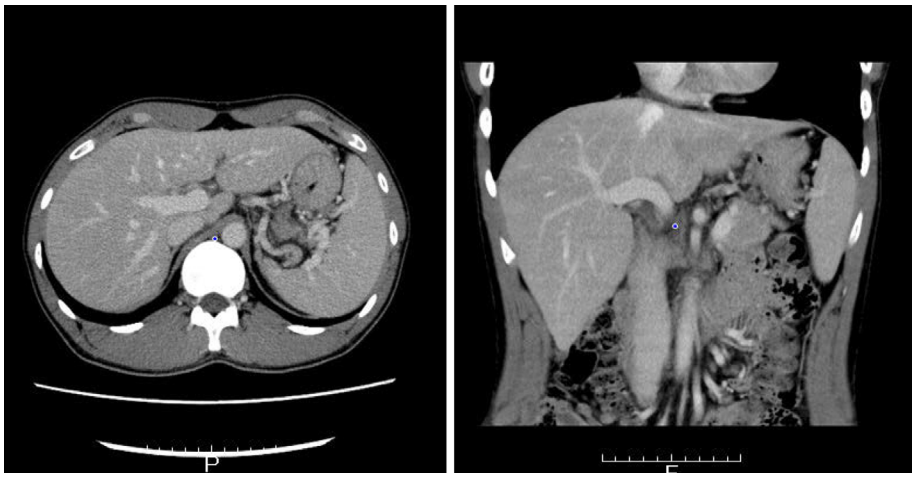

Figure 2. **Images of abdominal contrast-enhanced computed tomography obtained on the day of hospitalization are shown. The left and right panels show the axial and coronal views of the abdomen, respectively.**

currence of liver dysfunction (Fig. 1C).

Discussion

Since 1955, when Lorenz et al. (12) reported the first two

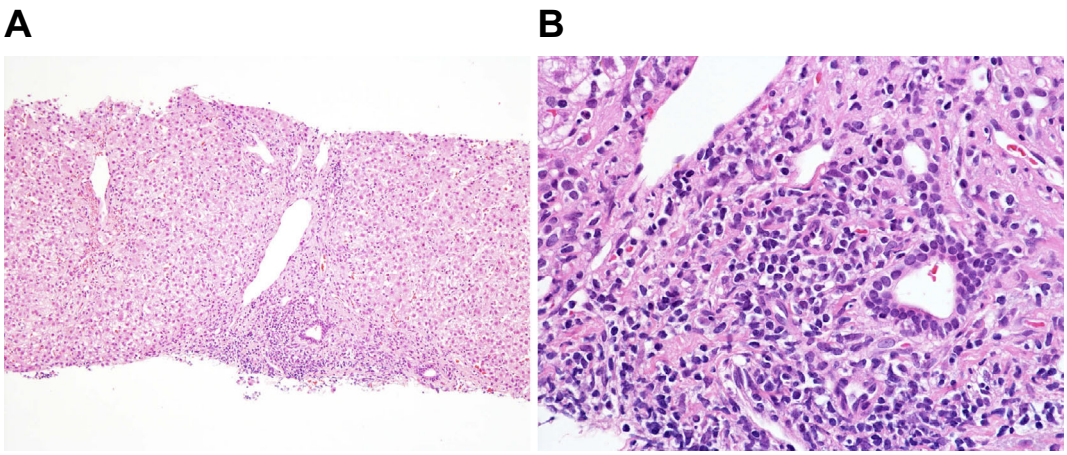

Figure 3. **Images of Hematoxylin and Eosin staining liver biopsy specimen collected at the previous hospital [original magnification, (A) ×100, (B) ×400].**

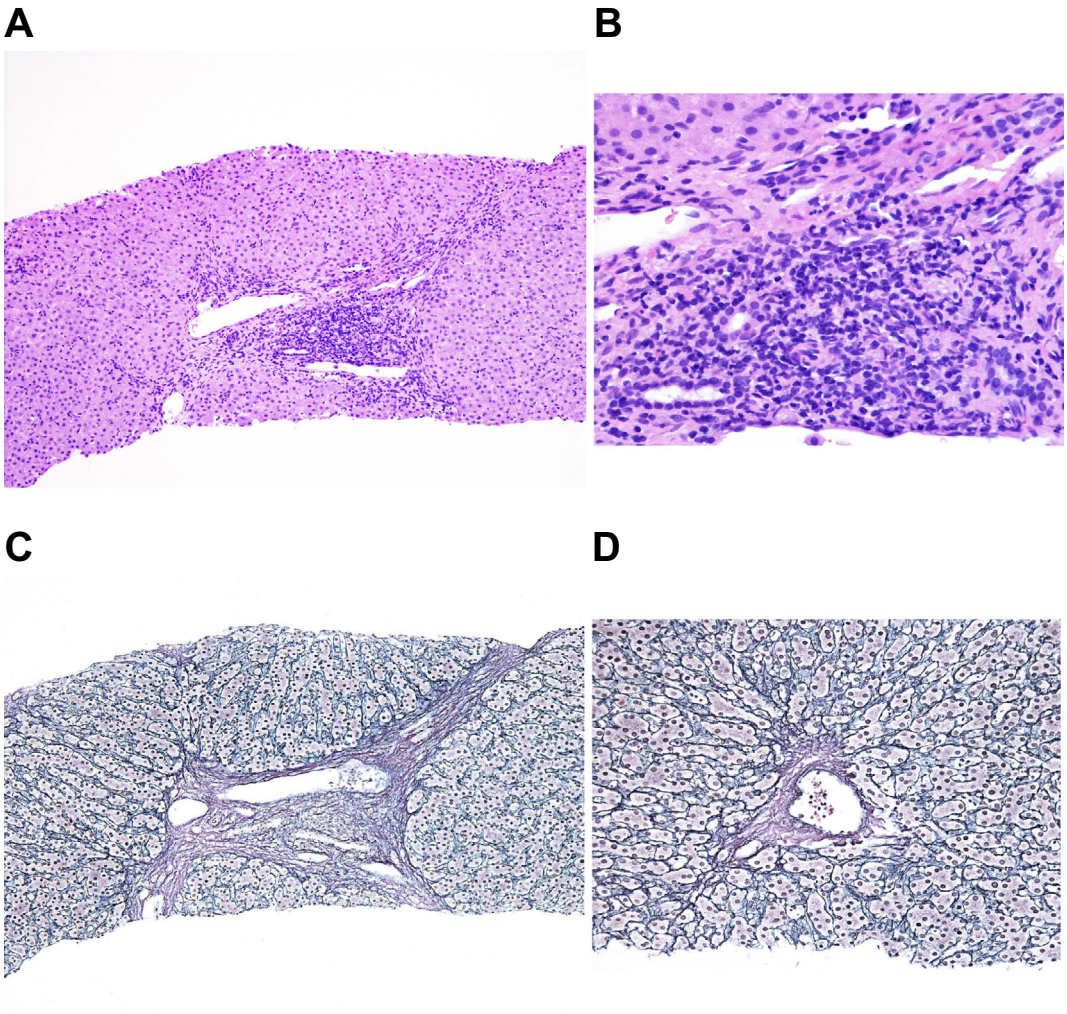

Figure 4. **Images of liver biopsy specimens at the second relapse. (A) (B) Hematoxylin and Eosin staining sections. (C) (D) Silver impregnation-stained section [Original magnification, (A) (C) ×100, (B) (D) ×400].**

cases, HAAA has been established as one of the main clinical forms of AA, and usually occurs several months after an episode of acute hepatitis (9, 10). The assumed etiology of the preceding hepatitis is viral infection, but the causal virus is usually not identified. In the present case, the patient had received a flu vaccine one month before the onset of hepatitis. Sasaki et al. reported two cases of autoimmune hepatitis that developed after receiving a flu shot (13). This finding, along with the first episode of acute hepatitis appearing after a flu shot, suggested that an excessive immune response to

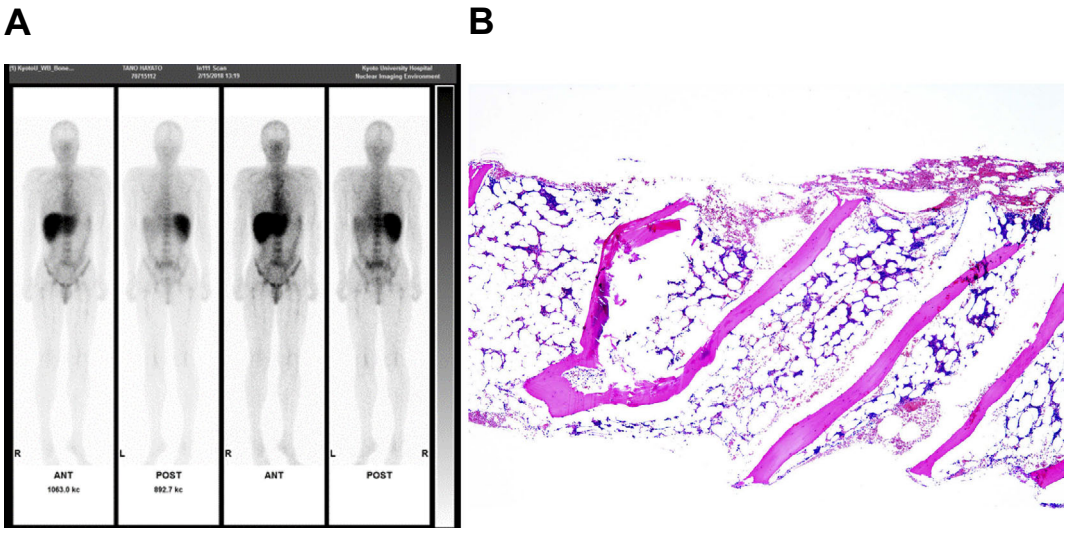

Figure 5. **An examination of the bone marrow function. (A) Bone scintigraphy with ^{111}In-tracer. (B) Representative image of a bone marrow biopsy specimen stained with Hematoxylin and Eosin staining (original magnification, ×100).**

the vaccination might have caused severe hepatitis. The trigger of the second episode of hepatitis with leukocytopenia and thrombocytopenia, however, was still unclear.

Autoimmune hepatitis was a differential diagnosis of hepatitis in this case because rapid progression of fibrosis was observed and the hepatitis onset was suspected to have been triggered by a flu shot. However, an antinucleic antibody test was negative, and the serum immunoglobulin G level was within the normal range. According to the International Autoimmune Hepatitis Group Criteria, his pretreatment score was 3 points (ALP/ALT ratio +2, hepatitis viral markers +3, drug history +1, alcohol intake +2, liver histology -5). HAAA was thus more likely than autoimmune hepatitis in the present case.

As a case of HAAA with repeated acute hepatitis has never, to our knowledge, been reported, we performed a second liver biopsy after a one-year interval to reevaluate the hepatic inflammation and fibrosis. Although no typical histologic features of hepatitis related to HAAA have yet been proposed (14), we observed two important differences between the two biopsy timepoints. First, the lymphocytes infiltrating at the portal regions observed at the first biopsy had disappeared and been replaced by histiocytes. These findings imply that the first liver biopsy represented the initial stage of hepatitis, while the second liver biopsy represented the chronic stage. Second, while no fibrotic changes were observed in the first sample, clear bridging fibrosis was detected in the second sample. This finding suggests that repeated hepatic inflammation caused the rapid progression of liver fibrosis in just one year.

Treatment for AA depends on the patient's stage. Although the evidence for treating patients with mild AA is lacking, watchful observation is proposed because some patients improve without any therapeutic intervention. For patients with severe AA, bone marrow transplantation is a representative curative therapy with a favorable prognosis, but immunosuppressive therapy using cyclosporin and/or antithymocyte globulin is considered instead for patients older than 40 years old or those without human leukocyte antigen (HLA)-identical siblings (15). Immunosuppressive therapy is especially effective for AA patients with PNH-type cells, which lack the expression of glycosylphosphatidylinositol anchor membrane proteins, such as CD55 and CD 59 (16, 17). However, immunosuppressive therapy was also reported to be effective in several HAAA cases, suggesting that immune-mediated pathogenesis is associated with the onset of HAAA (7, 18). In the present case, cyclosporine was administered as first-line therapy, because his AA was classified as mild but rapidly progressive. As his pancytopenia rapidly recovered after that, we did not administer further treatment, such as antithymocyte globulin. Notably, his hepatic inflammation was simultaneously ameliorated, suggesting that the hepatic inflammation had been induced by the same immunoreactive mechanism as the bone marrow dysfunction.

In conclusion, pathologic examinations of the liver tissue at multiple time-points were useful for determining a treatment plan in this case of HAAA with repeated liver dysfunction. Because hepatitis observed in the patients with HAAA occurs through immunogenic mechanisms, immunosuppressive therapy may be effective not only for AA but also for persistent or repeated hepatic inflammation, especially in PNH-type cell-positive cases.

The authors state that they have no Conflict of Interest (COI).

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