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Coexistence of Splenic Non-Hodgkin's Lymphoma With Hepatocellular Carcinoma in a Patient With Chronic Hepatitis C

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Chronic hepatitis C virus (HCV) infection is believed to play important roles in hepatocarcinogenesis (1), although there is no apparent evidence that HCV has direct oncogenic effects on hepatocytes. On the other hand, HCV involves many kinds of extrahepatic manifestations, including mixed cryoglobulinemia, Sjögren syndrome, interstitial pneumoniae, and chronic thyroiditis (2). Recently, strong association of HCV with non-Hodgkin's lymphoma (NHL) has been reported (3–9). Therefore, it has been suggested that chronic HCV infection may also contribute to the development of NHL. We report the case of a HCV-infected patient with coexistent splenic NHL with hepatocellular carcinoma (HCC). Fine needle splenic biopsy under the guidance of ultrasound imaging was a useful procedure for the diagnosis of the splenic lesion in the present case.

CASE REPORT

A 63-year-old man diagnosed with HCV infection, diabetes mellitus, and hypertension consulted a physician with a chief complaint of left hypochondric pain. He hid not have a past history of blood transfusion. Abdominal ultrasound scanning revealed a space-occupying lesion (SOL) in the spleen as well as another SOL in the liver. The levels of serum α-fetoprotein (AFP) were also elevated. He was referred to the Hospital of Kagawa University School of Medicine in June 2003.

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Physical examination on admission revealed no conjunctival anemia and no jaundice. He had no abnormal cardiopulmonary findings. The abdomen was soft and flat, and liver margin was palpated 2 fingerbreadths below the right costal margin. The spleen was also palpable for two fingerbreadths below the left costal margin. Palmar erythema was noted. Ascites or edema was not obvious. Abnormal neurologic findings were not observed. Superficial lymph nodes, including the cervical, axillary, and inguinal lymph nodes, were not enlarged.

Laboratory data on admission are presented in Table 1. Peripheral blood revealed a decrease in number of lymphocytes and mild anemia. Blood biochemistry revealed a slight decrease of serum albumin and cholinesterase and mild elevation of aspartate aminotransferase and lactate dehydrogenase. Fasting blood sugar and hemoglobin A1c were also slightly elevated. Serum AFP, AFP-L3 fraction, and plasma protein induced by vitamin K absence or antagonist-II (PIVKA-II) levels were markedly elevated. The level of soluble interleukin-2 receptor (sIL-2R) was also moderately increased. Antinuclear antibody was negative and cryoprecipitate was not tested in the patient. Hepatitis B virus surface antigen (HBsAg) was negative. HCV genotype and the viral load in the present case were 2a and 500 KIU/mL, respectively.

Dynamic computed tomography (CT) findings of the abdomen were as follows: Liver tumor of approximately 4 cm in diameter with ringed enhancement in the early phase was detected in the posterior segment of the right lobe. A mass composed of a low-density area and an isodensity areas without enhancement, measuring approximately 4 cm in diameter, was present in the enlarged spleen. Marked swelling of lymph nodes in the hilus of the spleen and behind the pancreatic head were also noted (Figure 1A). Tumor thrombosis was present in the portal vein trunk (Figure 1B).

Portography revealed complete occlusion in the portal vein and development of cavernous transformation (Figure 2A). Tumor stain in the area of the liver tumor was not very obvious with common hepatic artery angiography (Figure 2B). Splenic artery was compressed by the mass in the spleen (Figure 2C).

Positron emission tomography findings revealed the ringed dense uptake of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) in the spleen.

TABLE 1. LABORATORY FINDINGS ON ADMISSION

Peripheral Blood		*Blood Chemistry*		*Viral Marker*	
WBC	4,700/μl	TP	6.8 g/dl	HBs Ag	(−)
Segment	89.5%	Alb	3.1 g/dl	HCV-RNA	500 KIU/ml
Stab	0%	T-Bil	0.6 mg/dl		Genotype 2a
Lymphocyte	8.2%	AST	56 IU/l		
Monocyte	1.9%	ALT	35 IU/l	Tumor Marker	
Basophal	0.2%	LDH	533 IU/l (N: 230–460)	CEA 15 ng/ml ($N < 2.5$)	
Eosinophal	0.2%	ALP	279 IU/l (N: 100–280)	CA19-9	15 U/ml ($N < 37$)
Atypical lymphocyte	0%	γ-GTP	38 IU/l	AFP	13,100 ng/ml ($N < 10$)
RBC	$429 \times 10^4/\mu$l	T-Cho	162 mg/dl	AFP-L3	73.0%
Hb	12.6g/dl	ChE	184 IU/l (N: 200–420)	PIVKA-II	15,700 mA U/ml ($N < 10$)
Ht	39.3%	FBS	171 mg/dl	sIL-2R	1,616 U/ml (N: 135–433)
Plt	$21.0 \times 10^4/\mu$l	HbAlc	6.8%		

^{18}F-FDG was also absorbed in the lymph nodes of the splenic hilus and behind the pancreatic head; there were no abnormal hot areas in the liver (Figure 3).

The liver tumor was suspected to be HCC owing to extremely high levels of AFP and PIVKA-II. The CT and angiographic findings in this patient were, however, different from those typical of HCC. Ultrasonically guided percutaneous liver biopsy was carried out to make the histologic diagnosis for the liver tumor. The needle specimen from the tumor revealed sheets arrangements of neoplastic hepatocytes which nuclear/cytoplasmic ratio was generally increased. The tumor cells exhibited eosinophilic cytoplasms and round nuclei with prominent nucleoli (Figure 4A). Most of the tumor cells in the specimen were positive for cytokeratin 18 staining. These findings described resulted in the pathologic diagnosis of moderately differentiated HCC. On the other hand, chronic active hepatitis with severe activity of necroinflammatory reaction and bridging fibrosis was present in the nontumorous tissue of the liver (Figure 4B).

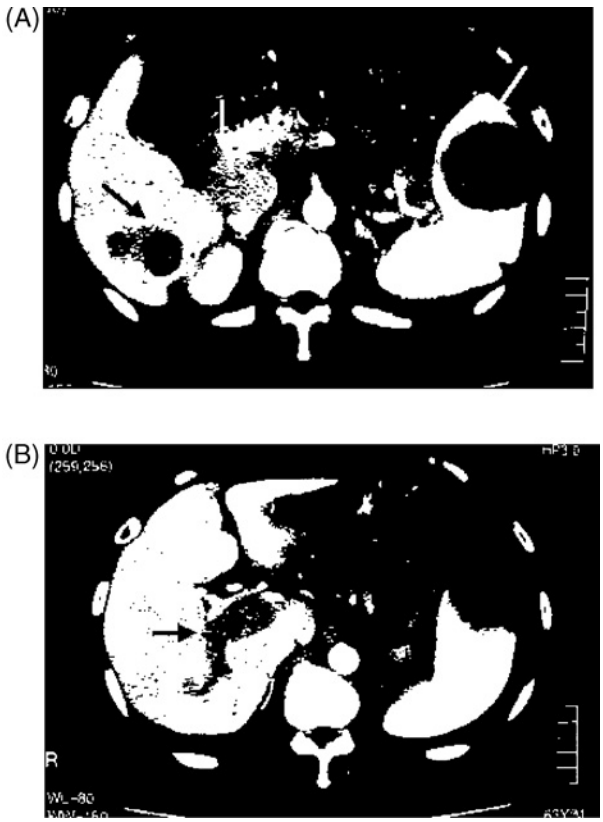

Fig 1. Dynamic CT findings of abdomen showing (A) ringed enhancement of the liver tumor in the early phase (*arrow*), tessellated mass in the spleen (*arrow*), and enlarged lymph nodes in the splenic hilum (*arrows*) and behind the pancreatic head (*arrow*) with splenomegaly. (B) Portal vein trunk was obstructed by tumor thrombosis (*arrow*).

Therefore, we needed to make differential diagnoses for the splenic lesion. One of the differential diagnoses for the lesion was the extrahepatic metastasis of HCC into the spleen. Another was involvement of malignant lymphoma owing to moderate increase in the level of sIL-2R and swelling of lymph nodes in the splenic hilum and behind pancreatic head with splenomegaly. To make the definite diagnosis of the splenic lesion, ultrasonically guided aspiration biopsy was performed, using a 21-gauge needle. Medium- to large-sized atypical lymphoid cells with pleomorphic nuclei were extensively involved in the tissue section. These tumor cells were accompanied by prominent nucleoli and narrow rim of cytoplasm (Figure 5A). Immunohistochemical examination revealed that most of these tumor cells in the spleen were stained with anti-CD20 antibody, which confirmed B-cell origin (Figure 5B); these tumor cells were negative for CD3 and cytokeratin 18 staining (data not shown). Therefore, the diagnosis of NHL, diffuse large B-cell type was made from the splenic specimen. With percutaneous aspiration biopsy against the splenic lesion, there were no severe complications such as intraperitoneal hemorrhage.

The final clinical diagnosis was coexistent NHL of B-cell type with HCC in the course of chronic HCV infection. Combination therapy of percutaneous ethanol injection with radiofrequency ablation (10–14) was done for HCC in the posterior segment of right lobe. NHL was treated with 3 courses of chemotherapy including cyclophosphamide, adriamycin, vincristine, and prednisolone (CHOP therapy). The liver tumor was almost eradicated by the local treatment, and CHOP therapy showed moderate effects for the NHL lesions with reduction of enlarged spleen and lymph node in the splenic hilus.

DISCUSSION

Helicobacter pylori (15) or various kinds of viruses such as Epstein-Bar virus (16), human herpes virus (17),

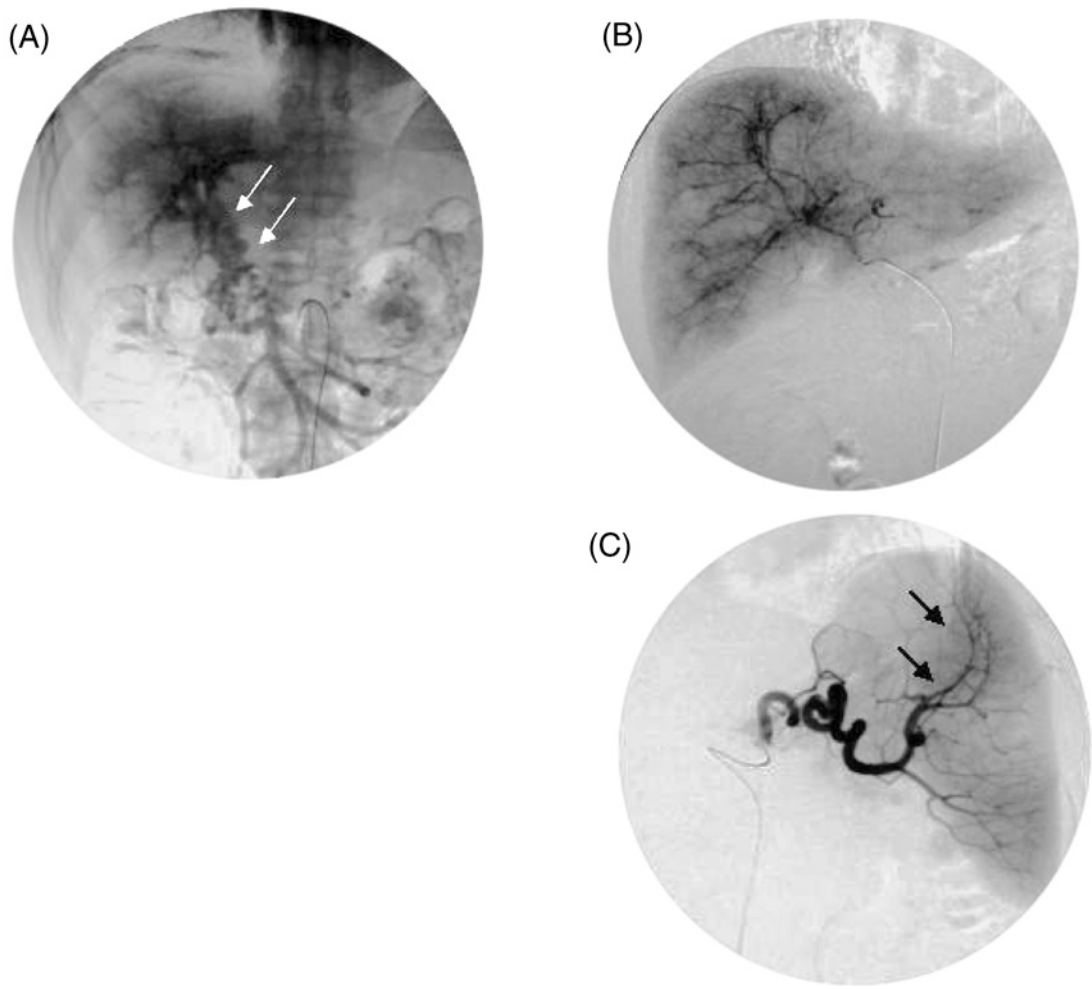

Fig 2. Abdominal angiographic findings showing (A) complete occlusion of the portal vein trunk, developing cavernous transformation (*arrows*). (B) Vascularity of the liver tumor was not obviously high. (C) The splenic artery was compressed by the splenic mass (*arrows*).

and human immunodeficiency virus (18) were considered to be candidates for causative agents of malignant lymphoma besides HCV. Ferri *et al.* (3) reported significantly higher frequency of HCV infection in patients with NHL of B-cell origin than in those with Hodgkin's lymphoma and in the general population. The prevailing hypothesis that HCV plays a pathogenic role in the involvement of B cell NHL may explain the direct or indirect contribution of HCV (19). HCV has a direct oncogenic effect on B cells. HCV is known to infect B cells and trigger their clonal expansion (20). On the other hand, HCV indirectly induces the malignant transformation of B lymphocytes. Persistent infection with HCV stimulates B lymphocytes and subsequently results in polyclonal, and later monoclonal, expansion of B cells in malignant transformation (20, 21).

To our knowledge, all 5 reported patients with HCV-related chronic liver disease who had coexistent splenic NHL with HCC (including the present case) had B-cell type (22–25). It was also noted that 4 of these 5 patients (including our case) had the diffuse large-cell type of B-cell NHL, which has been reported to be associated with HCV infection (5, 7, 26, 27). De Vita *et al.* (7) found the following features of B-cell NHL in HCV-infected patients: 1) an extranodal localization (*i.e.*, liver and major salivary glands) being significantly overrepresented; 2) a diffuse large-cell histotype without any prior history of low-grade B-cell malignancy or bone marrow involvement; and 3) a weak association with a full-blown predisposing autoimmune disease, although serum autoimmune features were common and cryoglobulins were always present.

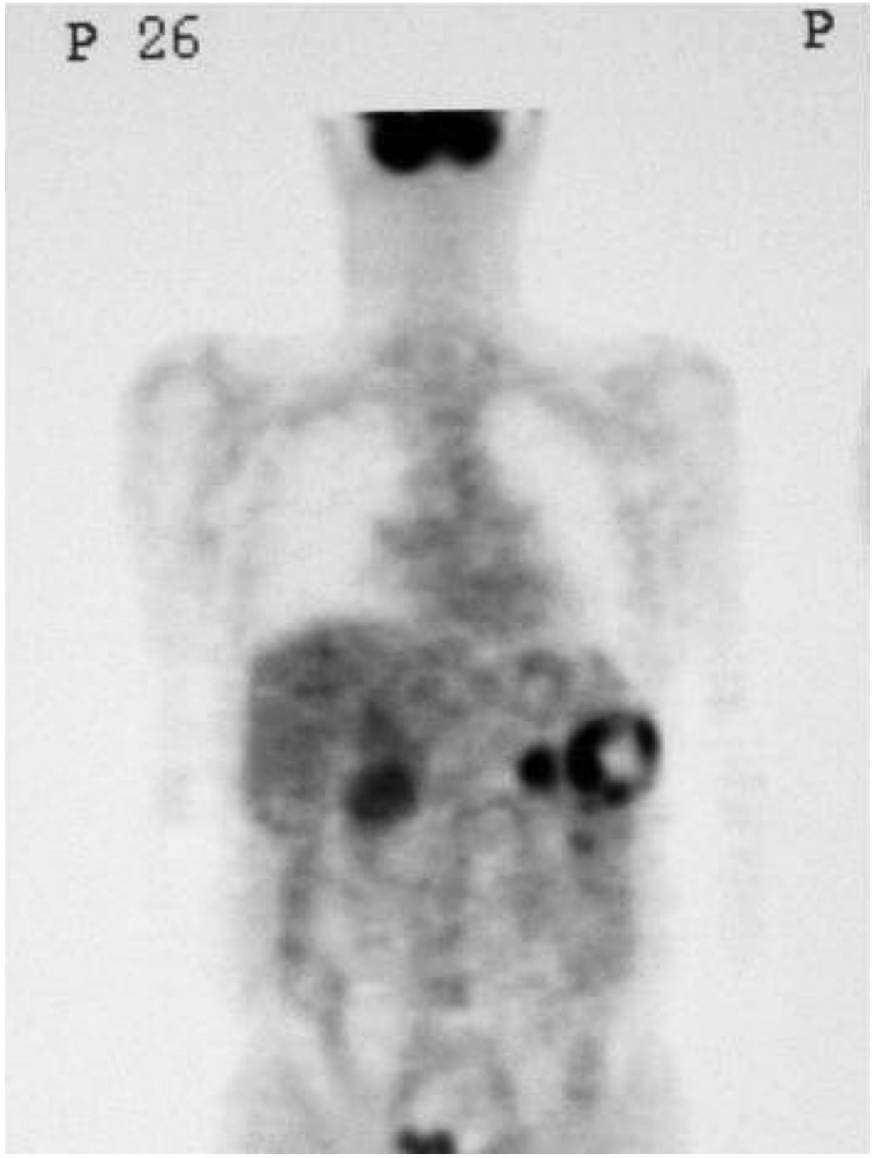

Fig 3. PET findings showing ringed uptake in the splenic lesion and homogeneous uptake in the mesenteric lymph node.

Genotype 2ac of HCV was reported to be associated with NHL in Italy (28), which is consistent with our present case. On the other hand, there are some reports that the genotype of HCV which caused co-occurrence of NHL and HCC was 1b (29, 30). The involvement of B NHL in HCV-related patients is considered to be independent of HCV genotype at present (26, 27).

The prognosis for the HCV-infected patients who have simultaneous co-occurrence of NHL and HCC is generally poor (30). The histologic type of NHL in the present case was diffuse large cell, which was considered to be high-grade malignancy (23, 31, 32). Interestingly, Goerg *et al.* (33) showed that high-grade malignancy of NHL exhibited focal lesion larger than 3 cm in diameter in the spleen.

Spleen is one of the organs abundant in blood flow and covered with thin capsules. Intraperitoneal hemorrhage, dissemination of tumor cells, and peritonitis are regarded as serious complications caused by fine needle aspiration biopsy. Previous reports, however, elucidated that the incidence of complication by fine needle aspiration biopsy of the spleen was 0–0.5% (34, 35). Clinical diagnosis of the splenic lesion in the present case was not made by radiologic imaging or laboratory findings. As mentioned, we distinguished between metastasis of HCC to the spleen

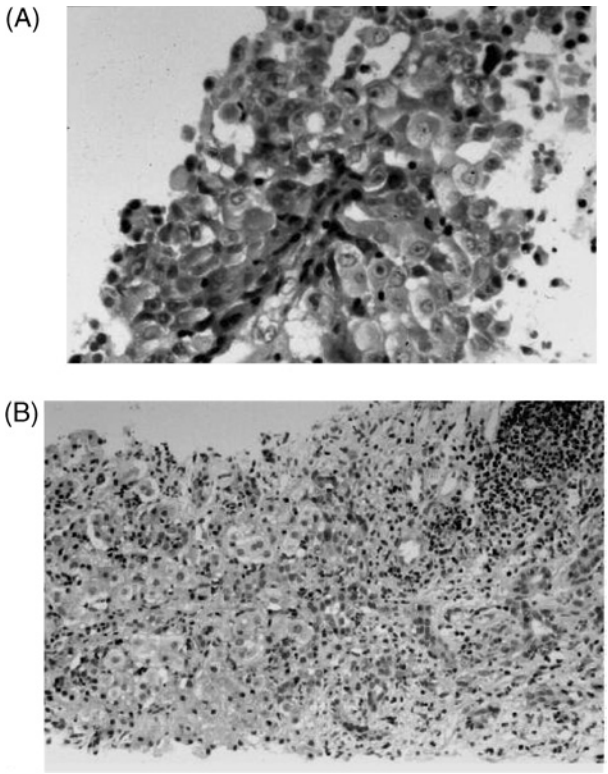

Fig 4. Percutaneous liver biopsy specimen from the liver tumor showing (A) moderately differentiated HCC (hematoxylin and eosin staining, original magnification 200×) and liver tissue obtained from nontumorous lesion demonstrating (B) chronic active hepatitis (hematoxylin and eosin staining, original magnification 100×).

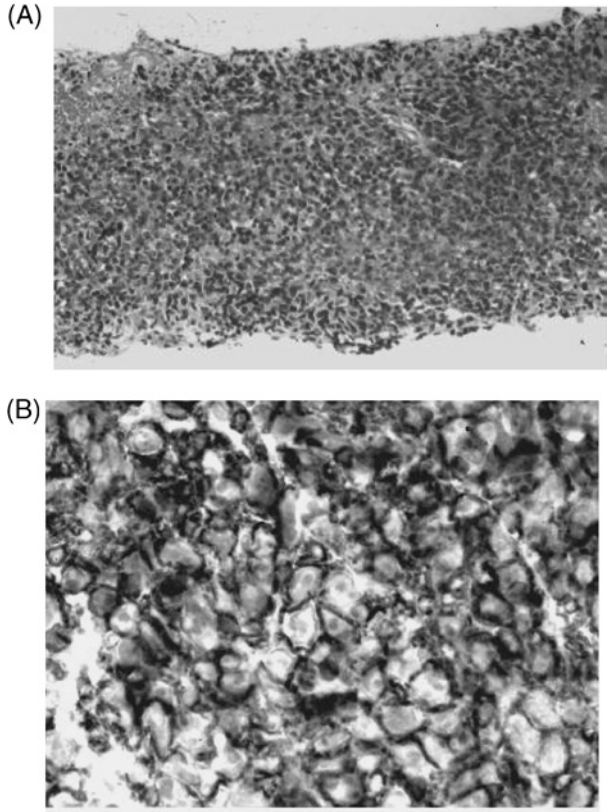

Fig 5. Percutaneous splenic biopsy specimen showing (A) NHL of the diffuse large-cell type (hematoxylin and eosin staining, original magnification 200×). (B) Immunohistochemical examination of tumor cells exhibited positive staining with anti-CD20 antibody (original magnification 400×).

and malignant lymphoma in the spleen. Several reports disclosed that splenic metastasis from HCC showed no enhancement in the dynamic CT (36, 37). The present case did not undertake histologic examination of the superficial lymph nodes because there was no involvement of the superficial lymph nodes. Therefore, we performed percutaneous fine needle aspiration biopsy against the splenic lesion. The specimen obtained by fine needle aspiration biopsy resulted in the pathologic diagnosis, although a lot of splenic specimens by aspiration biopsy did not because of insufficient or necrotic tissues (22, 31, 35). Percutaneous splenic aspiration biopsy was extremely useful in this patient. As described, several reports presented coexistent splenic NHL with HCC in HCV-related patients (22–25). However, to our knowledge, this is the first report that histologic diagnosis of the splenic lesion was made by ultrasonically guided aspiration biopsy in patients with HCV-related disease, with NHL and HCC simultaneously. In contrast, the diagnosis was made by splenectomy (23, 24) or biopsy of the superficial lymph nodes (25) in the previous reports on coexistence of splenic NHL with HCC.

The frequency of primary lymphoma of the spleen is relatively rare (32, 38, 39), although malignant lymphoma commonly involves the spleen. Gupta *et al.* (40) proposed the following diagnostic criteria for primary lymphoma of the spleen: (I) splenomegaly; (II) clinical, laboratory, and roentgenographic examination reveals no other lesions; (III) on laparotomy, no lymphoma is detected in mesenteric or para-aortic lymph nodes and liver biopsy shows no infiltration; and (IV) there is an interval of at least 6 months between the diagnosis of the splenic lymphoma and subsequent diagnosis of other lymphomatous lesions. This patient did not fulfill the criteria because an enlarged mesenteric lymph node was found. Another criteria for primary lymphoma of the spleen has been defined by Spier *et al.* (41): clinically, splenomegaly without peripheral lymphadenopathy, and pathologically, involvement of the spleen with or without involvement of regional lymph nodes, bone marrow or liver, but without peripheral blood involvement. According to Spier's criteria, our patient was diagnosed with primary lymphoma

of the spleen, although he did not undergo bone marrow aspiration.

The standard treatment for HCV-related NHL has not been established yet, although most patients with HCV-related NHL underwent splenectomy and/or chemotherapy (CHOP therapy). This patient was also treated with CHOP therapy and the treatment was effective for NHL. Interestingly, Silvestri *et al.* reported the treatment with interferon-α alone or in combination with ribavirin was effective against the HCV-related patients with B-cell NHL (19, 42, 43). The efficacy of rituximab, monoclonal anti-CD20 antibody, in patients with mixed cryoglobulinemia and HCV infection refractory to interferon-α as also documented (44).

In summary, we report a case of simultaneous splenic NHL and HCC in the course of chronic HCV infection. Fine needle aspiration biopsy under ultrasound guidance was a useful and safe procedure for the diagnosis of splenic lesion.

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