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Serum amyloid A-positive hepatocellular neoplasms in the resected livers from 3 patients with alcoholic cirrhosis

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Summary. Twelve hepatocellular nodules were characterized in the resected livers from 3 patients (2 men and a woman) with alcoholic cirrhosis. Imaging techniques suggested that the nodules were hypervascular and may be hepatocellular carcinoma. Five nodules (4-31 mm in diameter) were serum amyloid A-positive hepatocellular neoplasm, which shares features with inflammatory hepatocellular adenoma. The remaining 7 nodules (5-8 mm) were focal nodular hyperplasia-like nodules showing focal or no immunostaining for serum amyloid A. The serum amyloid A-positive hepatocellular neoplasms showed increased cellular density, inflammatory infiltrate, sinusoidal dilatation, and ductular reaction to various degrees. These histologic features tended to be less extensive in focal nodular hyperplasia-like nodules. Three of 4 serum amyloid A-positive hepatocellular neoplasms showed slight hypointensity in the hepatobiliary phase on the magnetic resonance (MR) imaging with gadolinium ethoxybenzyl diethylene-triaminepentaacetic acid (Gd-EOB-DTPA) enhancement. In contrast, 3 focal nodular hyperplasia-like nodules showed iso-intensity in the hepatobiliary phase. This study further confirms characteristics of serum amyloid A-positive hepatocellular neoplasm arising in alcoholic cirrhosis that share features with inflammatory hepatocellular adenomas. Serum amyloid A-positive hepatocellular neoplasms sometimes co-exist with focal nodular hyperplasia-like nodules and may show different findings on Gd-EOB-enhanced MR imaging.

Key words: Serum amyloid A-positive hepatocellular neoplasm, Inflammatory hepatocellular adenoma, Alcoholic cirrhosis, Focal nodular hyperplasia

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Introduction

Recent progress in hepatology disclosed that hepatocellular adenoma can be subclassified into several groups according to genotype and phenotype (Zucman-Rossi et al., 2006; Bioulac-Sage et al., 2007, 2009, 2010; Rebouissou et al., 2008). The subtypes include hepatocyte nuclear factor 1α-inactivated (35-50% of cases), ß-catenin-activated (15-18% of cases), and inflammatory hepatocellular adenomas (40-55% of cases), which could be identified by immunohisto-chemistry on paraffin-embedded material (Zucman-Rossi et al., 2006; Bioulac-Sage et al., 2007, 2009, 2010). The immunohistochemical markers such as liver fatty acid binding protein (LFABP), glutamine synthetase (GS), ß-catenin, and serum amyloid A provided us with new tools to reevaluate various hepatocellular nodular lesions such as focal nodular hyperplasia.

Several studies have demonstrated a hypervascular liver nodule showing similar imaging findings to hepatocellular carcinoma; so-called focal nodular hyperplasia-like nodules occur in severe alcoholic fibrosis or cirrhosis (Terada et al., 1993; Nakashima et

al., 2004; Kobayashi et al., 2007). We have recently disclosed that some of these nodules are serum amyloid A-positive hepatocellular neoplasm, which shares features with inflammatory hepatocellular adenoma arising in alcoholic cirrhosis (Sasaki et al., 2011, 2012). However, histological observation was limited in our previous report, since most serum amyloid A-positive hepatocellular neoplasms were diagnosed using needle biopsy materials (Sasaki et al., 2012). In this report, we demonstrated 12 hepatocellular nodular lesions, including 5 serum amyloid A-positive hepatocellular neoplasms and 7 focal nodular hyperplasia-like nodules in surgically-resected livers from 3 patients with alcoholic cirrhosis. Although one patient (case 3) was included in our previous study (Sasaki et al., 2012), other 2 patients have not been previously reported. This case report further confirmed characteristics of serum amyloid A-positive hepatocellular neoplasms arising in alcoholic cirrhosis.

Materials and methods

Case reports

Case 1. A 42 year-old man was admitted to our hospital for closer examination of type 2 diabetes and chronic hepatitis C. He was obese (BMI 35.4) and had drunk alcohol (60 g/day) for 20 years. Hepatitis B markers were negative, but anti-HCV antibody and HCV-RNA were positive. Alpha-fetoprotein (AFP) was slightly increased (12.54 ng/ml), while PIVKA-II was within normal range. Imaging studies disclosed a hepatocellular nodule (31 mm in diameter) in S3 segment and hepatocellular carcinoma (HCC) was suggested. The nodular lesion showed early enhancement on dynamic contrast-enhanced computed tomography (CT) images and slight hypointensity with a hyperintensity rim in the hepatobiliary phase on magnetic resonance (MR) imaging with gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid (Gd-EOB-DTPA) enhancement (Fig. 1) (Table 1). Partial hepatectomy was performed.

Case 2. A 54-year old man was admitted to our hospital because of hematoemesis due to ruptured gastric varices. He had drunk alcohol (200 g/day) for 30 years. Hepatitis B and C viral markers were negative and AFP and PIVKA-II were not increased. Imaging studies disclosed multiple hepatocellular nodules and HCC was suggested. Four nodular lesions showed early enhancement on dynamic contrast-enhanced CT images (Table 1). Three nodular lesions showed slight hypointensity in the hepatobiliary phase on the Gd-EOB-DTPA-enhanced MR imaging (Table 1). Partial hepatectomy was performed.

Case 3. A 41 year-old woman was admitted to our hospital because of ascites and jaundice. She had drunk alcohol (100-200 g/day) for 20 years and had been followed up for alcoholic cirrhosis. Hepatitis B and C markers were negative and AFP and PIVKA-II were not increased. Imaging study revealed multiple hypervascular hepatocellular nodules. These nodular lesions showed early enhancement on dynamic contrast-enhanced CT images (Table 1). CT during arterial portography showed portal perfusion defects. Dual-phase CT during hepatic arteriography showed early enhancement and corona-like enhancement on late-phase images. These nodules were diagnosed as HCC and chemotherapy using intraarterial perfusion was performed. After that, liver transplantation was performed.

Immunohistochemical and special stains

Liver tissue samples were fixed in 10% neutral-

Table 1. Imaging characteristics of nodular lesions in 3 patients.

			CT			MRI		Gd-EOB-DTPA		
		Size (mm)	Arterial phase	Portal phase	Equilibrium phase	T1WI	T2WI	Arterial phase	Equilibrium phase	Hepatobiliary phase
Case 1	#1	31	high	high	iso	hyper	iso	hyper	slight hyper	hypo (rim-hyper)
Case 2	#1	15	high	high	iso	hyper	hyper	hyper	slight hyper	hypo
	#2	8	high	iso	iso	hyper	hyper	hyper	slight hyper	slight hypo
	#3	8	high	iso	iso	hyper	iso	hyper	slight hyper	hypo
	#4	8	iso	iso	iso	iso	iso	hyper	slight hyper	iso
	#5	8	iso	iso	iso	iso	iso	hyper	slight hyper	iso
	#6	5	high	iso	iso	hyper	hyper	hyper	slight hyper	iso
	#7	8	nd	nd	nd	nd	nd	nd	nd	nd
Case 3	#1	4	nd	nd	nd	nd	nd	nd	nd	nd
	#2	7	high	high	iso	hyper	hypo-iso	nd	nd	nd
	#3	12	high	high	iso	hyper	hypo-iso	nd	nd	nd
	#4	8	high	high	iso	hyper	hypo-iso	nd	nd	nd

CT, computed tomography; MRI, magnetic resonance; Gd-EOB-DTPA, gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid. nd, not determined

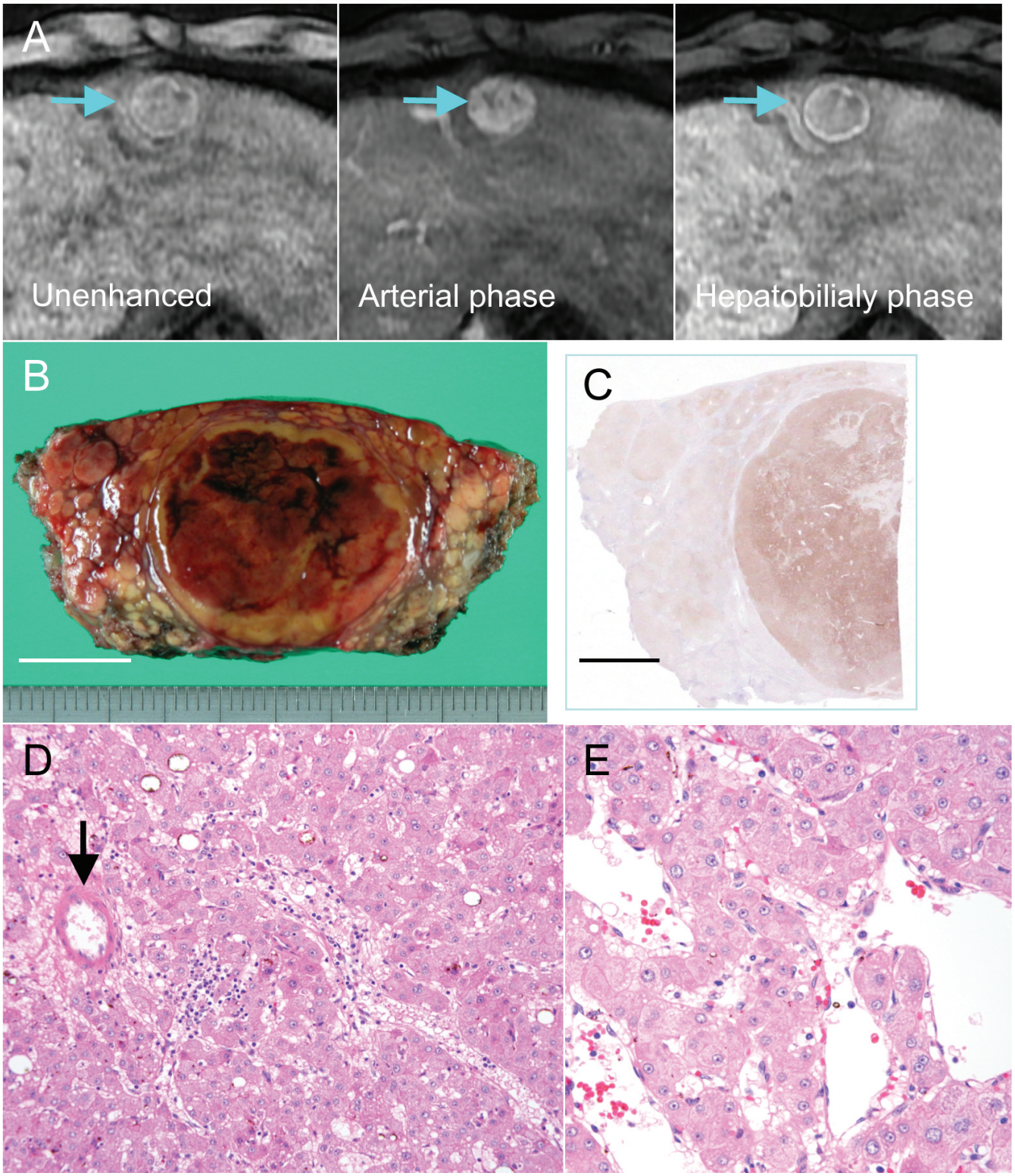

Fig. 1. A. Transverse T1-weighted image of arterial phase Gd-EOB-DTPA-enhanced MR imaging shows well-attenuated hepatic nodular lesion, suggesting abundant blood supply (middle, arrow). Transverse T1-weighted image of hepatobiliary phase Gd-EOB-DTPA-enhanced MR imaging shows hypointense nodule with a rim of slight-hyperintensity (right, arrow). **B.** Cut section of the tumor in the liver. A hepatic nodule (31 mm in diameter) had well-defined borders and an area of congestion. Bar indicates 1cm. **C.** The nodular lesion showed a strong immunoreactivity for serum amyloid A in contrast to the background liver. Immunostaining for serum amyloid A and hematoxylin. Bar indicates 1cm. **D.** Portal tract like structures with mild lymphocytic infiltration, abnormal thickened wall artery are seen in the nodular lesion. HE. **E.** Sinusoidal dilatation is seen focally in the nodular lesion. Hypatocytes show few cellular atypia. HE. D, x 200; E, x 400

buffered formalin and embedded in paraffin. Immunohistochemistry was performed as described previously (Sasaki et al., 2006), using the primary antibodies; LFABP (rabbit polyclonal; Abcam, Cambridge, UK, 1:50 dilution), GS (mouse monoclonal, clone GS-6; Millipore, Billerica, MA, 1:200 dilution), ß-catenin (mouse monoclonal, clone 14/Beta-Catenin; BD Transduction Labs, CA, 1:100 dilution), serum amyloid A (mouse monoclonal; clone mc1, Dako, Glustrup, Denmark, 1:100 dilution) and glypican-3 (Di Tommaso et al., 2009) (mouse monoclonal, clone 1G12; Nichirei, Tokyo, Japan, pre-diluted). In brief, after pretreatment using a microwave with citrate buffer (pH6), 95 degrees, for 20 min, blocking endogenous peroxidase, sections were incubated with the primary antibody at 4 degrees overnight. The Envision+ solution for mouse and rabbit (Dako) was then applied for 30 min at room temperature. The reaction products were visualized using 3-3'-diaminobenizidine tetrahydrochloride (Sigma Chemical, Co., St. Louis, MO) and H_2O_2. The sections were then lightly counterstained with hematoxylin. Similar dilution of the control mouse or rabbit Immunoglobulin G (Dako) was applied instead of the primary antibody as a negative control. Positive and negative controls were routinely included.

Results

Case 1. The resected S3 segment contained a nodular lesion measuring 31x30mm in diameter (Fig.1). The nodule was demarcated by thin fibrous capsule and showed congestion. Histological findings are summarized in Table 2. Microscopically, most hepatocytes lacked atypia, but showed mild nuclear crowding (Fig. 1). Acinar formation was focally seen. There was no reduction of reticulum fiber along hepatic

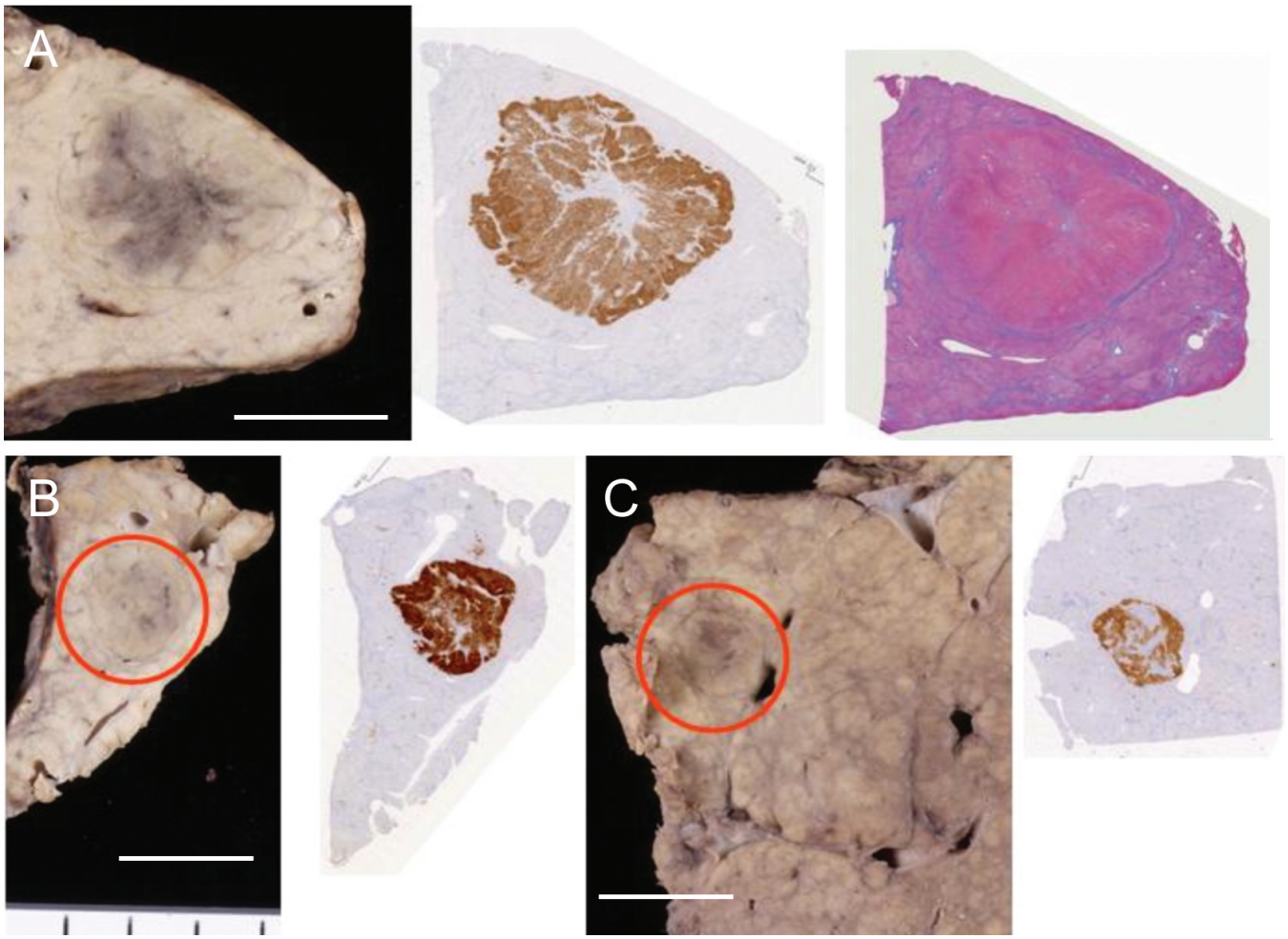

Fig. 2. Case 2. Cut section of the nodules in the liver and immunostaining for serum amyloid A. **A-C.** Show the nodules of #1, #2 and #3, respectively. The nodular lesions showed a strong immunoreactivity for serum amyloid A in contrast to the background liver. Immunostaining for serum amyloid A and hematoxylin. Bar: 1 cm

columns. There were several portal tract-like fibrous areas with mild lymphocytic infiltration in the nodules (Fig. 1). Sinusoidal dilatation was observed focally in the nodule (Fig. 1). Immunohistochemical study revealed that this nodule was strongly and diffusely positive for serum amyloid A, irrespective of negative immunostaining in the background livers (Fig. 1). The other markers for hepatocellular adenoma (LFABP and beta-catenin) did not show any significant findings. Peripheral area of the nodule was positive for GS, but most part was negative for GS. The background liver showed micronodular cirrhosis.

Case 2. Seven hepatocellular nodules were identified macroscopically (Table 2). Histologic findings showed benign hepatocellular nodules without cellular atypia and stromal invasion. Immunohistochemical study disclosed that 3 nodules (15, 8 and 8 mm in diameter) were strongly and diffusely positive for serum amyloid A, suggesting serum amyloid A-positive hepatocellular neoplasm (Fig. 2). Two nodules (both 8 mm in diameter) showed focal immunoreactivity for serum amyloid A and the remaining 2 nodules (8 and 5 mm in diameter) were negative for serum amyloid A. The latter 4 nodules were regarded as focal nodular hyperplasia-like nodules. The serum amyloid A-positive hepatocellular neoplasms showed increased cellular density, inflammatory infiltrate, sinusoidal dilatation, and ductular reaction to

Table 2. Histologic and immunohistochemical characteristics of nodular lesions in 3 patients.

			Histology						Immunostaining
		Size (mm)	Steatosis	Sinusoidal dilatation	Ductular reaction	Abnormal thick arteries	Inflammatory reaction	Cellular atypia	Serum amyloid A
Case 1	#1	31	-/+	+	-	+	+	-	++, diffuse
Case 2	#1	15	-	+	++	+	++	-	++, diffuse
	#2	8	-	+	+	-/+	++	-	++, diffuse
	#3	8	-	+	++	-/+	++	-	++, diffuse
	#4	8	-	-	+	-/+	-/+	-	+, focal
	#5	8	-	-	+	-/+	-/+	-	+, focal
	#6	5	-	+	+	-/+	+	-	-
	#7	8	-	-	+	-/+	+	-	-
Case 3	#1	4	-	+	-/+	-	-/+	-	++, diffuse
	#2	7	-	+	-/+	-/+	-/+	-	+, focal
	#3	12	-	+	+	-	-/+	-	+, focal
	#4	8	-	+	-	-/+	-	-	+, focal

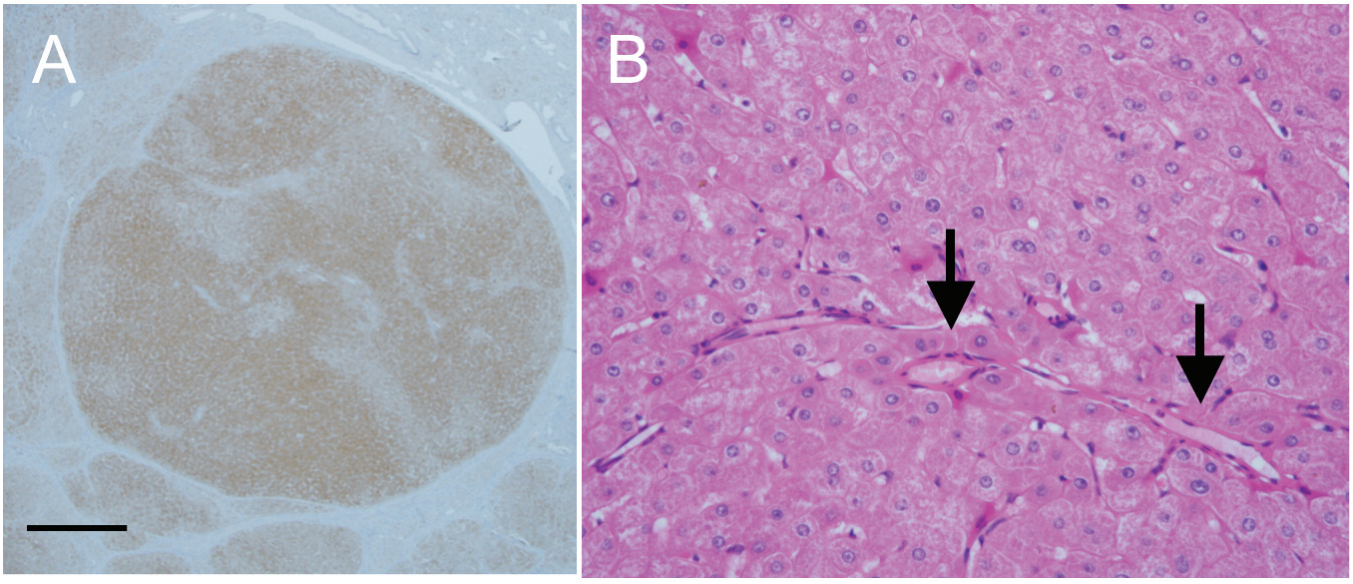

Fig. 3. Case 3. **A.** The nodule (4 mm in diameter) showed a strong immunoreactivity for serum amyloid A in contrast to the background liver. Immunostaining for serum amyloid A and hematoxylin. **B.** Several abnormal vessels (arrows) are seen in the nodule. Hypatocytes show few cellular atypia. HE. A, Bar: 1 mm; B, x 400

various degrees. These histologic features tended to be less extensive in focal nodular hyperplasia-like nodules. The other markers for hepatocellular adenoma (LFABP, GS, beta-catenin) did not show any significant findings. The background liver showed micronodular cirrhosis, which is consistent with alcoholic cirrhosis.

Case 3. Three hepatocellular nodules detected by imaging showed mildly increased cellular density, mild sinusoidal dilatation and ductular reaction. These hepatic nodules showed focal immunoreactivity for serum amyloid A and were regarded as focal nodular hyperplasia-like nodules. GS pattern of these nodules were map-like. In addition, a small hepatic nodule (4 mm in diameter) was histologically identified and this nodule showed a strong and diffuse immunoreactivity for serum amyloid A (Fig. 3). Several abnormal arteries, Mild sinusoidal dilatation, ductular reaction and inflammation were seen in this nodule (Fig. 3). Cellular density was mildly increased, but cellular atypia and reduction of reticulum fiber were not observed. This nodule showed faint immunoreactivity for GS.

Discussion

This case report clearly demonstrated 5 serum amyloid A-positive hepatocellular neoplasms in the resected livers from 3 patients with alcoholic cirrhosis. These serum amyloid A-positive hepatocellular neoplasms showed strong immunoreactiviy for serum amyloid A in contrast to background livers and share histological features with inflammatory hepatocellular adenomas, as we have recently reported (Sasaki et al., 2011, 2012). If this nodule were a regenerative nodule, it would show similar immunoreactivity for SAA to the background liver. Therefore, it is conceivable that serum amyloid A-positive hepatocellular neoplasms may be a unique type of hepatic neoplasm arising in alcoholic cirrhosis. However, it is needed to examine monoclonality and genetic alterations in this tumor to conclude this is a true neoplasm/adenoma.

Interestingly, serum amyloid A-positive hepatocellular neoplasms sometimes co-existed with focal nodular hyperplasia-like nodules. Both serum amyloid A-positive hepatocellular neoplasms and focal nodular hyperplasia-like nodules are detected as hypervascular hepatic nodules showing similar imaging findings to HCC in severe alcoholic fibrosis or cirrhosis (Terada et al., 1993; Nakashima et al., 2004; Kobayashi et al., 2007; Sasaki et al., 2011, 2012). In the present study, serum amyloid A-positive hepatocellular neoplasms tended to show histological features resembling inflammatory hepatocellular adenoma, such as inflammatory infiltrate, sinusoidal dilatation, and ductular reaction more evidently, compared to focal nodular hyperplasia-like nodules. However, it seems to be difficult to differentiate serum amyloid A-positive hepatocellular neoplasms from focal nodular hyperplasia-like nodules by histologic findings only. Therefore, the immunoreactivity for serum amyloid A may be critical for differential diagnosis. Differentiation of these 2 types of hepatic nodules may be important because focal nodular hyperplasia generally does not require treatment and follow-up, whereas hepatocellular adenoma does. Given that the prevalence of malignancy in hepatocellular adenoma was 10 times more frequent in men than in women (Farges et al., 2011), it might be necessary to follow up carefully serum amyloid A-positive hepatocellular neoplasms, a possible inflammatory hepatocellular adenoma, in male patients. Since follow-up data are limited regarding serum amyloid A-positive hepatocellular neoplasms, the natural history of this type of nodules remains to be clarified.

Serum amyloid A-positive hepatocellular neoplasms and focal nodular hyperplasia-like nodules showed different findings in the hepatobiliary phase on Gd-EOB-DTPA-enhanced MR imaging. That is, serum amyloid A-positive hepatocellular neoplasms showed low intensity, whereas focal nodular hyperplasia-like nodules showed iso-intensity in the hepatobiliary phase. The expression of SLCO1B3, a transporter of bile acid and bilirubin, is reportedly related to imaging in the hepatobiliary phase on MR imaging with Gd-EOB-DTPA (Kitao et al., 2011). It is reported that SLCO1B3 expression is regulated by ß-catenin signaling and is closely related to the status of ß-catenin mutation in HCC (Sekine et al., 2011). In our previous study, serum amyloid A-positive hepatocellular neoplasms did not show overexpression of glutamine synthetase, indicating activated ß-catenin signaling (Sasaki et al., 2012). In contrast, focal nodular hyperplasia-like nodules showed a typical map-like staining pattern of glutamine synthetase (Sasaki et al., 2011, 2012). Taken together, the different activation status of ß-catenin signaling may be related to the different imaging on MR imaging with Gd-EOB-DTPA in serum amyloid A-positive hepatocellular neoplasms and focal nodular hyperplasia-like nodules. Since the number of nodules examined is so limited in this study, further studies are mandatory to confirm this speculation.

In summary, this study further confirmed the characteristics of serum amyloid A-positive hepatocellular neoplasm that share features with inflammatory hepatocellular adenomas in patients with alcoholic cirrhosis. Serum amyloid A-positive hepatocellular neoplasm may be sometimes co-existent with focal nodular hyperplasia-like nodules and show different findings on MR imaging with Gd-EOB-DTPA from focal nodular hyperplasia-like nodule.

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Conflict of interest. There are no conflicts of interest.

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