γ-glutamyl transpeptidase. Prothrombin activity, total protein and albumin were decreased. The mutation types were 851del4/IVS11 + 1G > A throughout most of late infancy, being more than 5 months of age in patients 27, 28, 29 and 30.

Histological findings

Histological findings of the 30 patients are shown in Table 3. The results of the fibrosis staging and inflammation grading are shown in Figure 1.

Fibrosis

Most specimens showed varying degrees of fibrosis; 35% of all specimens were classified as stage 0, while stages 1 and 2 together accounted for 50%. However, there was a wide spectrum of fibrosis: more advanced liver lesions with distorted lobular architecture (stage 3) (Fig. 2) and cirrhosis were observed in four and one specimens, respectively. One patient with cirrhosis developed hepatic failure. Therefore, this patient underwent a living-related liver transplant. One patient with cirrhosis developed at 10 months of age.[10]

Inflammatory reaction

The degree of inflammation varied with the specimens, where half showed moderate or severe inflammatory changes. Inflammatory cell infiltration in the portal tracts and piecemeal necrosis were observed (Fig. 3). Inflammatory cells present in the portal tracts were predominantly lymphocytes. Focal necrosis and acidophilic bodies in the parenchyma were seen in 23 (77%) and 12 (40%) specimens, respectively. The sinusoids showed the proliferation of mononuclear cells with scarce neutrophils and the activation of Kupffer cells.

Fat deposition in hepatocytes

Fat deposition in hepatocytes was observed in all specimens except one and severe fatty liver was noted for 20 (67%) specimens (Fig. 4a). Fat droplets deposited in the cytoplasm of hepatocytes varied in size, and fat-laden hepatocytes were classified as those with macrovesicular fat droplets, those with foamy, microvesicular fat droplets, and those with mixed macrovesicular and microvesicular fat droplets. Hepatocytes with microvesicular fat droplets had a centrally located nucleus. In 80% of 29 specimens with fat deposition including all 20 specimens which showed severe fatty livers, there was a mixture of macro- and microvesicular fat droplets (Fig. 4b,c). Macrovesicular and microvesicular fatty liver alone accounted for three (10%) and one (4%) specimens, respectively. A moderate and severe fatty liver with an inflammatory reaction and lipogranuloma were diagnosed as steatohepatitis, which accounted for 60% of the patients. The histopathological findings in this disease were different from those in non-alcoholic steatohepatitis. The clinical features of one patient who had no fat deposition in hepatocytes did not differ from that of other patients with such fat deposition.

Cholestasis

Cholestasis was observed in 77% of the specimens and was severe in 57%. The acinar arrangement of hepatocytes was prominent in specimens with severe cholestasis (Fig. 5) and multinucleated giant cell transformation was found in one case (Fig. 6).

Hemosiderin deposition

Hemosiderin deposition, mostly mild and localized in periportal hepatocytes and macrophages in portal areas (Fig. 4b), was observed in 57% of the specimens.

A combination of all five features, fatty liver, inflammatory cell infiltration, fibrosis, cholestasis and hemosiderin deposition was observed in the same liver biopsy specimen in 12 (40%) of the total specimens.

Relationship between the age and the histological findings

The mean score of each histological finding in each of groups A, B and C are summarized in Table 4. The degree of fibrosis, necroinflammatory reaction such as focal necrosis and acidophilic bodies, acinar arrangement of hepatocytes, cholestasis and steatohepatitis of infants more than 3 months old (groups B and C) were more accentuated than those of the early infants of group A. Conversely, hemosiderosis and extramedullary hematopoiesis in groups B and C were less pronounced than in group A. The staging score of fibrosis, grade of inflammation and steatohepatitis were significantly higher in the older than in the younger group in order of group A, B and C.

Histological findings of follow-up biopsy

Follow-up biopsies were conducted for patients 8, 9, 13 and 18, and the findings were as follows: patients 8, 9 and 13 showed histological deterioration of cholestasis and fatty change. Of note, patient 9 underwent a liver transplant at the age of 16 years because of hepatic failure. The findings for the explant liver were

Table 3 Histological findings of liver biopsy in the 30 patients with neonatal intrahepatic cholestasis caused by citrin deficiency

Patient no.	1	2	3	4	5	6	7	8	9	10
Stage of fibrosis	0	0	1	0	0	0	0	0	3	2
Grade of inflammation	1	2	2	1	1	1	2	1	1	1
Focal necrosis[a]	1	1	2	0	0	0	1	0	0	1
Acidophilic body[b]	0	1	0	2	0	1	0	1	0	0
Acinar arrangement[c]	0	1	3	3	0	1	0	1	2	1
Cholestasis[d]	0	3	3	3	1	0	1	2	3	1
Degree of fat deposit[e]	1	3	3	3	3	3	2	3	3	3
Type of fat deposit[f]	1	3	0	3	3	3	1	3	0	0
Steatohepatitis[g]	0	1	1	1	0	1	1	1	0	2
Hemosiderosis[h]	0	2	1	2	0	0	1	2	0	2
Extramedullary hematopoiesis[i]	0	2	0	3	2	1	0	2	0	0
Patient no.	11	12	13	14	15	16	17	18	19	20
Stage of fibrosis	0	2	2	1	0	0	3	2	1	1
Grade of inflammation	1	1	1	2	1	2	2	2	3	1
Focal necrosis	1	0	1	1	1	2	1	1	3	0
Acidophilic body	1	0	0	1	0	0	1	0	0	0
Acinar arrangement	2	0	0	2	2	1	1	1	2	1
Cholestasis	3	0	0	3	3	2	2	2	3	3
Degree of fat deposit	3	0	2	2	3	2	3	3	2	3
Type of fat deposit	3	0	2	3	3	3	3	3	3	3
Steatohepatitis	2	0	0	1	1	1	1	1	2	1
Hemosiderosis	2	0	1	0	2	1	1	0	2	1
Extramedullary hematopoiesis	0	0	0	3	2	0	1	0	0	0
Patient no.	21	22	23	24	25	26	27	28	29	30
Stage of fibrosis	2	2	0	2	2	3	1	3	3	4
Grade of inflammation	3	2	1	2	3	2	1	2	3	3
Focal necrosis	1	2	1	1	3	1	1	1	2	1
Acidophilic body	1	2	0	1	1	1	0	0	0	2
Acinar arrangement	3	2	0	2	2	1	2	1	3	2
Cholestasis	3	3	0	3	0	3	3	3	3	3
Degree of fat deposit	3	3	3	3	1	3	2	3	3	3
Type of fat deposit	3	3	3	3	1	3	3	3	3	3
Steatohepatitis	0	3	2	1	0	2	1	3	3	3
Hemosiderosis	3	1	1	1	0	1	1	0	0	0
Extramedullary hematopoiesis	1	0	1	1	2	0	0	0	1	0

[a]Focal necrosis was graded from 0–3 based on the number of counts per 10 fields at a magnification of ×40. A score of denotes 0 is none, 1 denotes 1–2; 2 denotes up to 4, and 3 denotes >4.
[b]Acidophilic bodies were counted and graded from 0–3, as similar to that for focal necrosis.
[c]The acinar arrangements of the hepatocytes were graded 0–3. A rating of 0 denotes none, 1 denotes involvement up to 30% of the hepatocytes, 2 denotes 30–60%, and 3 denotes >60%.
[d]Cholestasis was graded from 0–3. A score of 0 denotes none, 1 denotes cholestasis without a bile plug, 2 denotes scattered bile plugs, and 3 denotes frequent bile plugs.
[e]The degree of fat deposition in hepatocytes was graded from 0–3 based on the percentage of hepatocytes in the biopsy involved. A rating of 0 denotes none; 1 denotes up to 30%, 2 denotes 30–60%, and 3 denote >60%.
[f]The type of fat deposit was classified as being between 0–3. A score of 0 denotes no fatty change, 1 denotes predominantly macrovesicular fat droplets, 2 denotes predominantly microvesicular fat droplets, and 3 denotes mixed microvesicular and macrovesicular fat droplets.
[g]Steatohepatitis was graded from 0–3, where 0 denotes none, 1 denotes steatosis involving up to 60% and intra-acinar inflammation with no or mild portal inflammation, 2 denotes steatosis (>66%) with both acinar and portal inflammation, and 3 denotes panacinar steatosis with intra-acinar inflammation and piecemeal necrosis.
[h]Hepatocellular iron was graded between 0–3, where 0 denotes none, 1 denotes localized deposition in the portal and /or periportal area; 2 denotes iron deposition involving up to 60% of the parenchyma, and 3 denotes >60%.
[i]Extramedullary hematopoiesis was graded between 0–3, similar to that for focal necrosis.

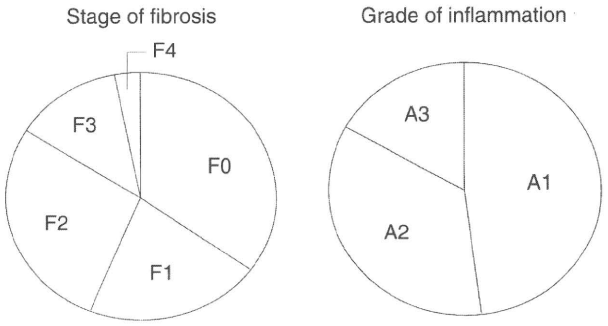

Figure 1 Results of fibrosis and the grade of necroinflammation.

more pronounced than those of the biopsy. Patient 8 showed progression of fibrosis from stage 1–3 and more pronounced portal inflammation. In contrast, patient 18 showed marked improvement of every

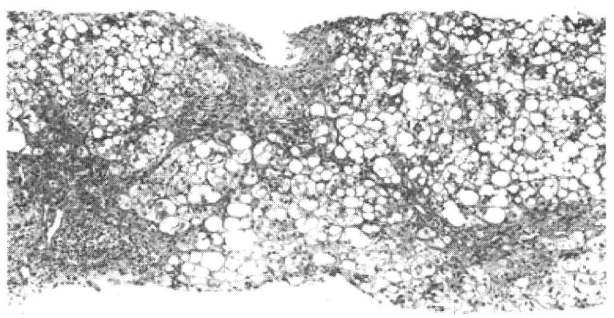

Figure 2 Severe fatty liver with distorted lobular architecture due to extensive fibrosis in stage 3 with portal inflammation (hematoxylin–eosin, original magnification ×50).

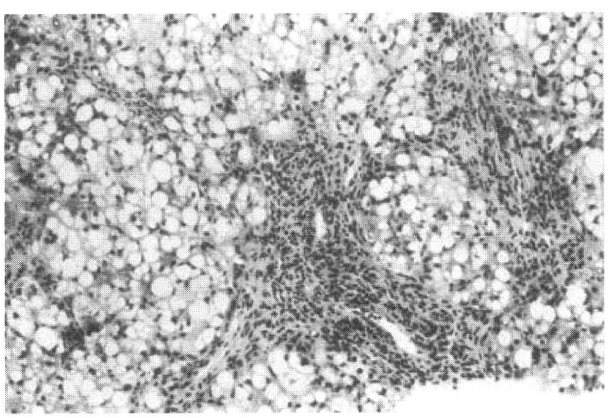

Figure 3 Fatty liver with moderate inflammatory cell infiltration in the portal tract and parenchyma, which is indicative of steatohepatitis (hematoxylin–eosin, original magnification ×100).

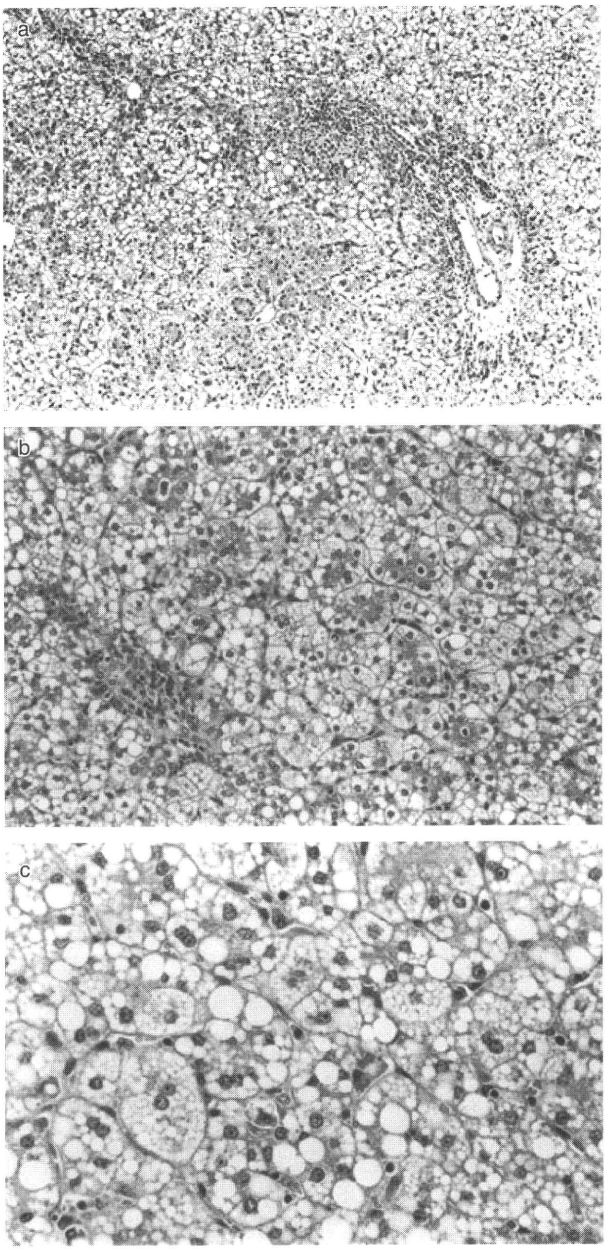

Figure 4 (a) Severe fatty liver with cholestasis. The portal tracts show mild inflammatory cell infiltration (hematoxylin–eosin [HE], original magnification ×50). (b) Pseudo-acinor transformation with bile plugs is observed. Hemosiderin-laden macrophages are present in a portal tract (HE, original magnification ×100). (c) Macro- and microvesicular-type fatty droplets. Some of the swollen hepatocytes have a foamy appearance and their cytoplasm packed with micro-fat droplets. Kupffer cells are activated (HE, original magnification ×200).

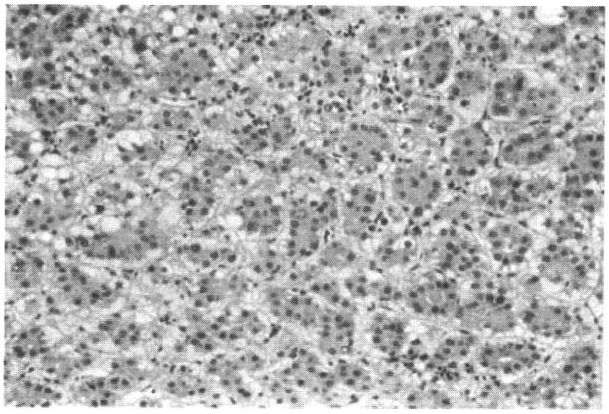

Figure 5 Striking pseudo-acinor transformation of the hepatic cords containing bile plugs. Small fatty droplets are present at the periphery of hepatocytes arranged in an acinar fashion (hematoxylin–eosin, original magnification ×100).

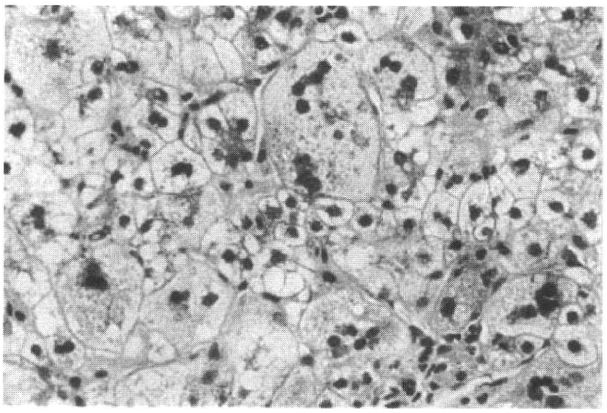

Figure 6 Giant cell hepatitis and cholestasis. Multinucleate giant cells contain several nuclei (hematoxylin–eosin, original magnification ×200).

histological finding, including decreased portal fibrosis and inflammation.

DISCUSSION

THE CAUSE OF liver dysfunctions such as fatty liver, hypoglycemia and galactosemia in this disease is as follows.[15] Citrin deficiency blocks the malate aspartate shuttle, which may increase the ratio of cytosolic nicotinamide adenine dinucleotide (NADH) to oxidized nicotinamide adenine dinucleotide ($NADH/NAD^+$), which in turn is associated with the inhibition of glycolysis and makes reduced alcohol metabolism. This may be why CTLN2 patients dislike carbohydrates and cannot drink alcohol, and why alcohol consumption often results in psychiatric symptoms. An increased $NADH/NAD^+$ ratio is also characteristic of the inhibition of gluconeogenesis involving reduced substrates.[19] This, together with the reduction in alanine metabolization to urea and glucose due to citrin deficiency may cause hypoglycemia in NICCD patients. Although NICCD patients suffer from galactosemia, which sometimes even leads to the development of cataracts, no abnormalities in the enzymes involved in galactose metabolism have been found.[20] Because uridine diphosphate-glucose epimerase which requires NAD as a cofactor is strongly inhibited by NADH,[21] galactosemia in NICCD may represent a high NADH level in the cytosol of the liver.

From the biochemical data of this study, 50% of the high level of total bilirubin was associated with direct bilirubin, but it was not always dominant. The levels of AST were increased to more than twice the levels of ALT. Low levels of total protein, albumin and prothrombin

Table 4 Relationship between age and histological changes

Pathological findings	Group A ($n = 16$) <2 months	Group B ($n = 9$) 3–4 months	Group C ($n = 5$) >5 months	*P*-value
Stage of fibrosis	0.69 ± 1.01	1.67 ± 0.87	2.80 ± 1.10	$P = 0.001$
Grade of inflammation	1.31 ± 0.48	2.11 ± 0.78	2.20 ± 0.84	$P = 0.004$
Focal necrosis	0.75 ± 0.68	1.44 ± 1.01	1.20 ± 0.45	$P = 0.063$
Acidophilic body	0.44 ± 0.63	0.67 ± 0.71	0.60 ± 0.89	$P = 0.523$
Acinar arrangement	1.19 ± 1.05	1.56 ± 0.88	1.80 ± 0.84	$P = 0.172$
Cholestasis	1.75 ± 1.29	2.11 ± 1.27	3.00 ± 0.00	$P = 0.059$
Degree of fat deposit	2.44 ± 0.89	2.67 ± 0.71	2.80 ± 0.45	$P = 0.333$
Steatohepatitis	0.81 ± 0.66	1.22 ± 0.97	2.40 ± 0.89	$P = 0.008$
Hemosiderosis	1.00 ± 0.89	1.11 ± 0.93	0.40 ± 0.55	$P = 0.356$
Extramedullary hematopoiesis	0.94 ± 1.18	0.67 ± 0.71	0.20 ± 0.45	$P = 0.297$

The data are expressed as means ± standard deviation.
P-values are by the Mantel–Haenszel linear trend test.

activity and high levels of citrulline, α-fetoprotein, ferritin and PSTI were observed as previously described in NICCD patients.[6–13] However, 11 patients showed high levels of ferritin, which were not observed in previous reports on NICCD patients. Therefore, the pediatric hepatologist should suspect NICCD when a neonatal cholestatic patient has high levels of AST of more than twice the ALT value, citrulline, α-fetoprotein and ferritin, and low levels of total protein and prothrombin activity.

The histological findings in this study such as a fatty liver, cholestasis, necroinflammatory reaction and iron deposition are not pathognomonic findings because they occur in various liver diseases.[22] However, the combination of mixed macrovesicular and microvesicular fatty hepatocytes and these histological findings are almost never observed in other liver diseases in infants and adults. Microvesicular fatty deposition was found in NICCD, this type of fatty change is a characteristic feature of Reye syndrome[23] and other rare conditions.[22] However, the histogenesis of the microvesicular fatty deposition in NICCD is unclear. It might reflect the acute impairment of β-oxidation of fatty acid due to mitochondrial dysfunction as in Reye syndrome.

Although our series of NICCD patients shared common liver histological findings as described above, there seemed a tendency that late infants of group C had more advanced fibrosis and more accentuated inflammation than those of early infants of group A. The duration of illness may be an aggravating factor of the progression of the disease in some cases. There was no difference between the liver histological findings and mutation type. Interestingly, however, the mutation type of patients with severe fibrosis who were 6 and 7 months of age was 851del4/IVS11 + 1G > A. Because evidence for this relationship between the mutation type and the progression of fibrosis is not clear, further investigation is needed. Moreover, in the follow-up liver biopsy patients, we observed improvements in their liver histological findings as the liver dysfunction was ameliorated. Therefore, we speculate that the correlations between the stage of the liver histological findings and the biochemical test data exist.

This study found that NICCD is a disease with characteristic hepatopathological features. If NICCD is suspected in the presence of cholestasis during infancy, a liver biopsy should be performed to screen for liver diseases. We believe that a liver biopsy is of high diagnostic value for NICCD, and is useful for accurately assessing inflammation and the degree of the progression of fibrosis.

Although we were not able to elucidate the natural history of the disease, we previously found that despite a benign course in the majority of the patients, it leads to the development of liver cirrhosis in some patients with CTLN2.[5,10] This suggests that it involves the risk of progressive fibrosis and eventually leads to the development of cirrhosis. This possibility is suggested by the above histopathological findings characteristic of NICCD in the patients who progressed to stage 3 chronic hepatitis and cirrhosis. Although the process responsible for the progression of liver lesions is not clear, some patients with steatohepatitis including nonalcoholic steatohepatitis (NASH) progress to cirrhosis.[24] In this study, steatohepatitis was found in 60% of the specimens. It is likely that, in NICCD, steatohepatitis repeatedly deteriorates, persists and progresses.

In conclusion, if NICCD is suspected in the presence of cholestasis during infancy, a liver biopsy should be performed to screen for other liver diseases. NICCD is a disease with characteristic hepatopathological features, such as the combination of mixed macrovesicular and microvesicular fatty hepatocytes, cholestasis, necroinflammatory reaction and iron deposition. Therefore, it is possible to diagnose NICCD based on histological liver findings in most cases. However, when cirrhosis with fat deposition is detected in hepatocytes in liver specimens, the patient should be carefully observed, because the prognosis of NICCD patients is not always fair, with some developing progressive liver failure by 1 year of age. Finally, we could not infer the development of CTLN2 from the histological findings of the patients with NICCD who were examined in this study.

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SPECIAL REPORT

GENERAL RULES FOR RECORDING ENDOSCOPIC FINDINGS OF ESOPHAGOGASTRIC VARICES (2ND EDITION)

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General rules for recording endoscopic findings of esophageal varices were initially proposed in 1980 and revised in 1991. These rules have widely been used in Japan and other countries. Recently, portal hypertensive gastropathy has been recognized as a distinct histological and functional entity. Endoscopic ultrasonography can clearly depict vascular structures around the esophageal wall in patients with portal hypertension. Owing to progress in medicine, we have updated and slightly modified the former rules. The revised rules are simpler and more straightforward than the former rules and include newly recognized findings of portal hypertensive gastropathy and a new classification for endoscopic ultrasonographic findings.

Key words: endoscopic findings, esophagogastric varices, portal hypertension.

INTRODUCTION

Bleeding from esophagogastric varices is a catastrophic complication of chronic liver disease. A precise system for the systematic evaluation and recording of esophagogastric varices is essential for the management of portal hypertension. In 1980, the Japanese Research Society for Portal Hypertension proposed a new system called 'The General Rules for Recording Endoscopic Findings on Esophageal Varices'.[1] In 1991, the revised rules were proposed as 'General Rules for Recording Endoscopic Findings of Esophagogastric Varices (1991).[2,3] These rules have gradually been accepted and are now used in many countries. Recently, portal hypertensive gastropathy (PHG) has been recognized as a distinct histological and functional entity. Endoscopic ultrasonography (EUS) can now clearly depict vascular structures around the esophageal wall in patients with portal hypertension. Owing to such progress in medicine, we slightly revised the former rules. The revised rules are simpler and more straightforward than the former rules, and include newly recognized findings of PHG and a new classification for endoscopic ultrasonographic findings.[4]

OUTLINES OF THE NEW RULES

The revised rules are titled 'General Rules for Recording Endoscopic Findings of Esophagogastric Varices (2nd Edition)'.[4] These new general rules incorporate recommendations for recording newly recognized findings of PHG and a new classification for findings on EUS. Similar to the former rules, the revised rules comprise six main categories: location (L), form (F), color (C), red color signs (RC), bleeding signs, and mucosal findings (Table 1). In principle, the endoscopic diagnosis is based on endoscopic findings assessed with the naked eye. Findings of gastric varices, which were included with esophageal varices in the former rules, are now listed separately.

Endoscopy

Esophageal varices

Location. There is no change in the rules for location (L). The longitudinal location of esophageal varices (EV) of different caliber is classified into three distinct regions: (i) locus superior (Ls) varices are located in the upper part of the esophagus; (ii) locus medialis (Lm) varices are located in the middle part of the esophagus; and (iii) locus inferior (Li) varices are located in the lower part of the esophagus.

Form. The rules for form (F) also remain the same. EV are classified into four groups according to their shape and size: (i) F_0 lesions lack a varicose appearance. This classification is useful for documenting the disappearance of EV in response to treatment, even if red veins or blue veins are present (Fig. 1a,b); (ii) F_1 lesions are straight, small-caliber varices. Small venous dilatations that disappear on insufflation of the esophagus are not included in this subgroup (Fig. 1c); (iii) F_2 lesions are moderately enlarged, beady varices (Fig. 1d); and (iv) F_3 lesions are markedly enlarged, nodular or tumor-shaped varices (Fig. 1e).

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Table 1. General rules for recording endoscopic findings of esophagogastric varices (2004)

Category	Code subcategory
Location (L)	Ls: Locus superior
	Lm: Locus medialis
	Li: Locus inferior
	Lg-c: Adjacent to the cardiac orifice
	Lg-cf: Extension from the cardiac orifice to the fornix
	Lg-f: Isolated in the fornix
	Lg-b: Located in the gastric body
	Lg-a: Located in the gastric antrum
Form (F)	F_0: No varicose appearance
	F_1: Straight, small-caliber varices
	F_2: Moderately enlarged, beady varices
	F_3: Markedly enlarged, nodular or tumor-shaped varices
Color (C)	Cw: White varices
	Cb: Blue varices
	Cw-Th: Thrombosed white varices
	Cb-Th: Thrombosed blue varices
Red color signs (RC)	RWM: Red wale markings
	CRS: Cherry red spots
	HCS: Hematocystic spots
	Esophageal varices: RC_0, RC_1, RC_2, RC_3
	Gastric varices: RC_0, RC_1
	Te: Telangiectasia
Bleeding signs	Gushing bleeding
	Spurting bleeding
	Oozing bleeding
	Red plug
	White plug
Mucosal findings	E: Erosion
	Ul: Ulcer
	S: Scar

Color. The color of EV was referred to as 'fundamental color' in the former rules. In the revised rules, the 'color' of EV is classified into two categories: (i) white varices (Cw) are whitish and look like large folds of the esophageal mucosa (Fig. 2a); (ii) blue varices (Cb) are bluish-white or cyanotic, and distended by blood (Fig. 2b). The esophageal mucosa overlying varices of this category appears very thin. High-risk blue varices are characterized by a fully expanded appearance with a glossy surface, similar to that of an over-inflated balloon. Blue varices that have become purple or violet because of increased variceal pressure are designated as such by adding a 'v' (violet) to their color (Cbv) in the revised rules. Thrombosed varices are indicated by adding Th (thrombosis) to their color (i.e. Cb-Th [Fig. 2c] or Cw-Th [Fig. 2d]).

Red color signs. Red color signs (RC) refer to reddish changes seen immediately beneath the submucosa. RC are known to be reliable predictors of the risk of variceal bleeding[5] and are classified into the following three categories: (i) red wale markings (RWM) are dilated venules oriented longitudinally on the mucosal surface, somewhat like wale or whip marks (Fig. 3a); (ii) cherry-red spots (CRS) are small red spots on the mucosal surface (Fig. 3b); and (iii) hematocystic spots (HCS) are large, round, crimson-red projections that look like blood blisters (Fig. 3c). RC are the most important predictor of variceal bleeding.

RC are graded as 0, 1, 2, or 3 according to their density and distribution: (i) RC_0 = absent; (ii) RC_1 = small in number and localized (Fig. 3d); (iii) RC_2 = intermediate between RC_1 and RC_3 (Fig. 3e); and (iv) RC_3 = large in number and circumferential (Fig. 3f). The RC category (RWM, CRS and/or HCS) is stated in parentheses after the RC grade. RC in patients presenting with F_0 is recorded as F_0, $RC_{1\text{-}3}$. If telangiectasia (Te) is noted, its presence is recorded as Te.

Bleeding signs. Bleeding signs are divided into those found during bleeding and those found after hemostasis. In the former rules, bleeding was classified as spurting or oozing. In the revised rules, bleeding is classified as gushing (Fig. 4a), spurting (Fig. 4b), or oozing. Findings after hemostasis are classified as red plug (Fig. 4c) or white plug (Fig. 4d), similar to the former rules.

Mucosal findings. There is no change in the rules for recording mucosal findings. Mucosal findings are classified into three categories: (i) erosion (E) (Fig. 5a); (ii) ulcer (Ul) (Fig. 5b); and (iii) scar (S) (Fig. 5c). The presence of these findings is recorded as E, Ul, and S.

Evaluation of the effectiveness of treatment

'Eradication' means the disappearance of varices after treatment, including thrombosed varices (F_0, RC_0). 'Residue' means residual varices with F or RC after treatment. 'Recurrence' means the reappearance of eradicated varices (F_0, RC_0) with F and/or RC. 'Relapse' means the worsening of residual varices with F and/or RC.

Note. Findings of EV should be recorded in the order of the six main categories (L, F, C, RC, bleeding signs, and mucosal findings) as shown in the following examples.

1. EV with RWM and CRS: Ls, F_3, Cb, RC_3 (RWM, CRS).
2. Spurting bleeding from EV: Lm, F_2, Cb, RC_1 (CRS), spurting bleeding.
3. Thrombosed blue varices treated by endoscopic injection sclerotherapy: Lm, F_2, Cb-Th, RC_0, Ul.
4. Completely eradicated EV: F_0, RC_0.
5. Recurrent EV with RWM: Li, F_1, RC_1 (RWM).

Gastric varices

Location. Gastric varices (GV) are classified into three main groups based on their relation to the cardiac orifice. GV are classified as Lg-c if they are adjacent to the cardiac orifice (Fig. 6a), Lg-cf if they extend from the cardiac orifice to the fornix (Fig. 6b), and Lg-f if they are localized to the fornix (Fig. 6c). In addition, GV located in the body of the stomach are classified as Lg-b, and those located in the antrum are classified as Lg-a. This classification is based on the relation between the location and the blood supply route of GV.[6]

Red color signs. RC are graded as 0 or 1: (i) RC_0 = absent; (ii) RC_1 = GV with RWM, CRS, and/or HCS. All other codes used to describe EV are also used for GV.

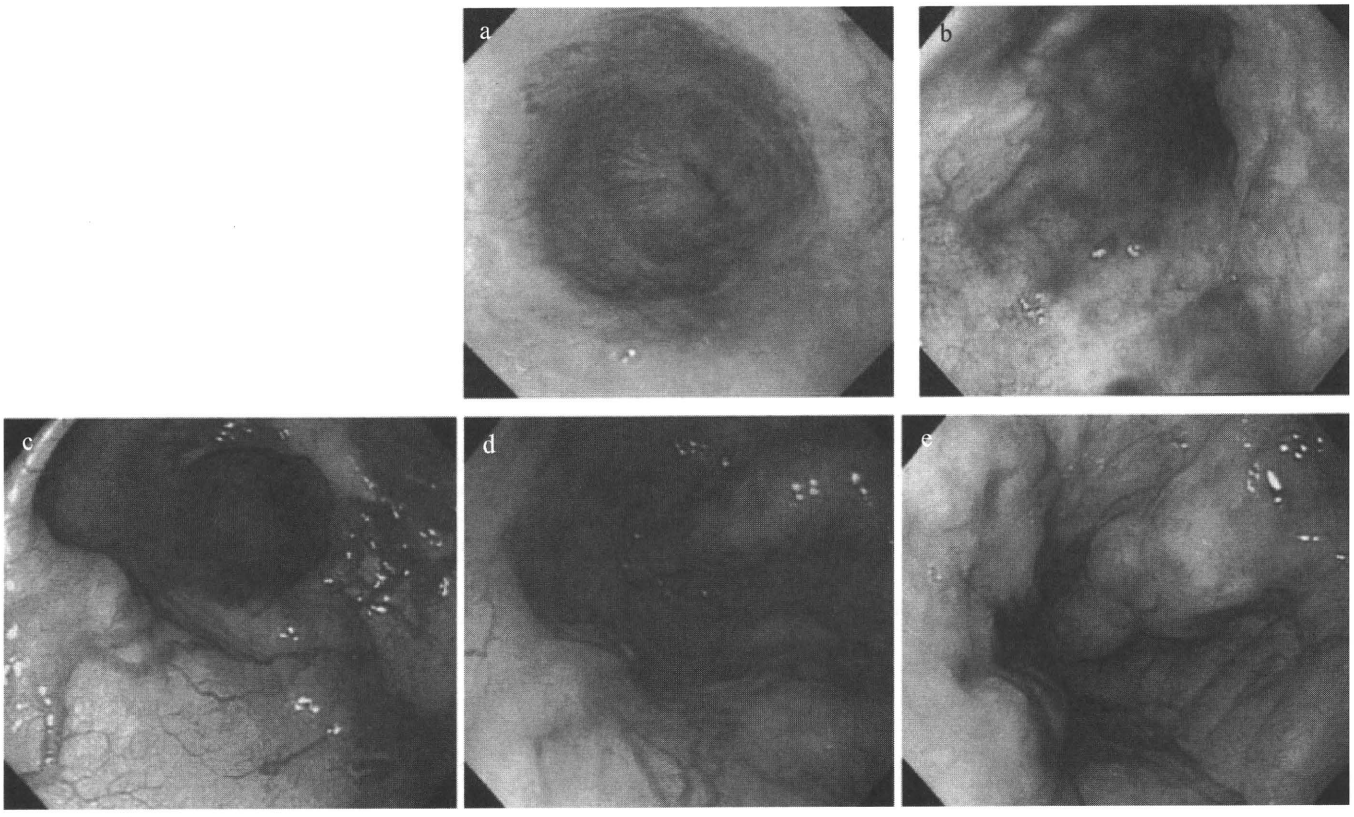

Fig. 1. F_0 lesions lack a varicose appearance. (a) F_0, RC_0, S. (b) F_0, RC_1. (c) F_1 lesions are straight, small-caliber varices. (d) F_2 lesions are moderately enlarged. (e) F_3 lesions are markedly enlarged, nodular or tumor-shaped varices.

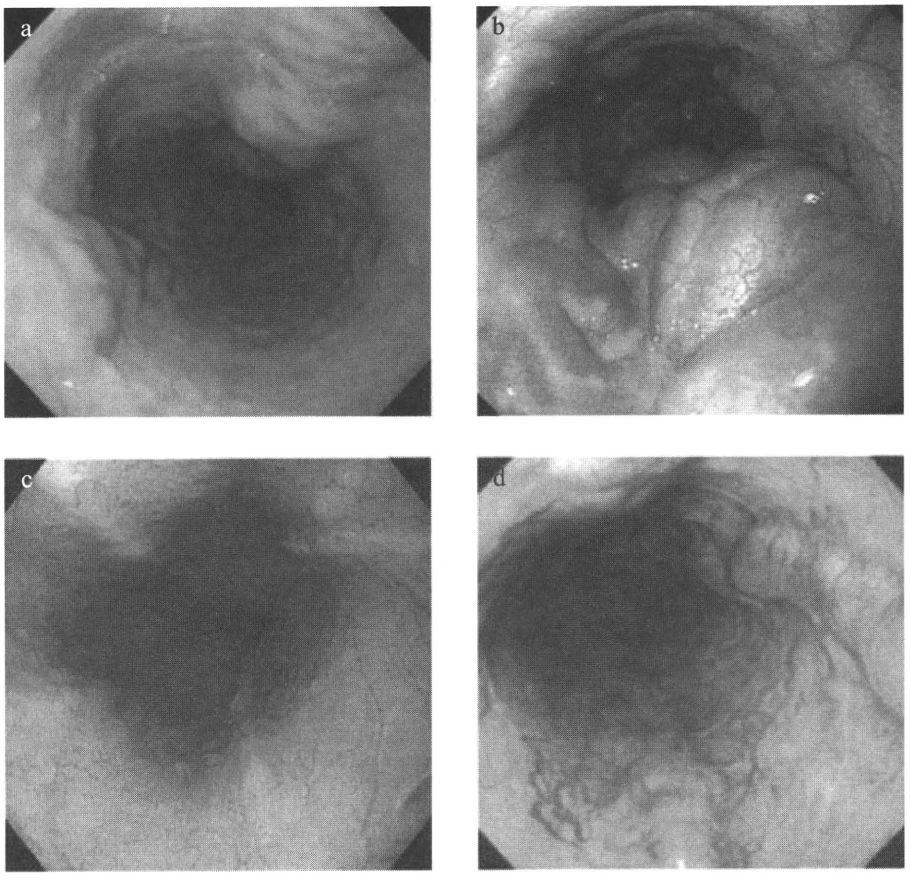

Fig. 2. (a) White varices (Cw), (b) blue varices (Cb), (c) thrombosed white varices (Cw-Th), (d) thrombosed blue varices (Cb-Th).

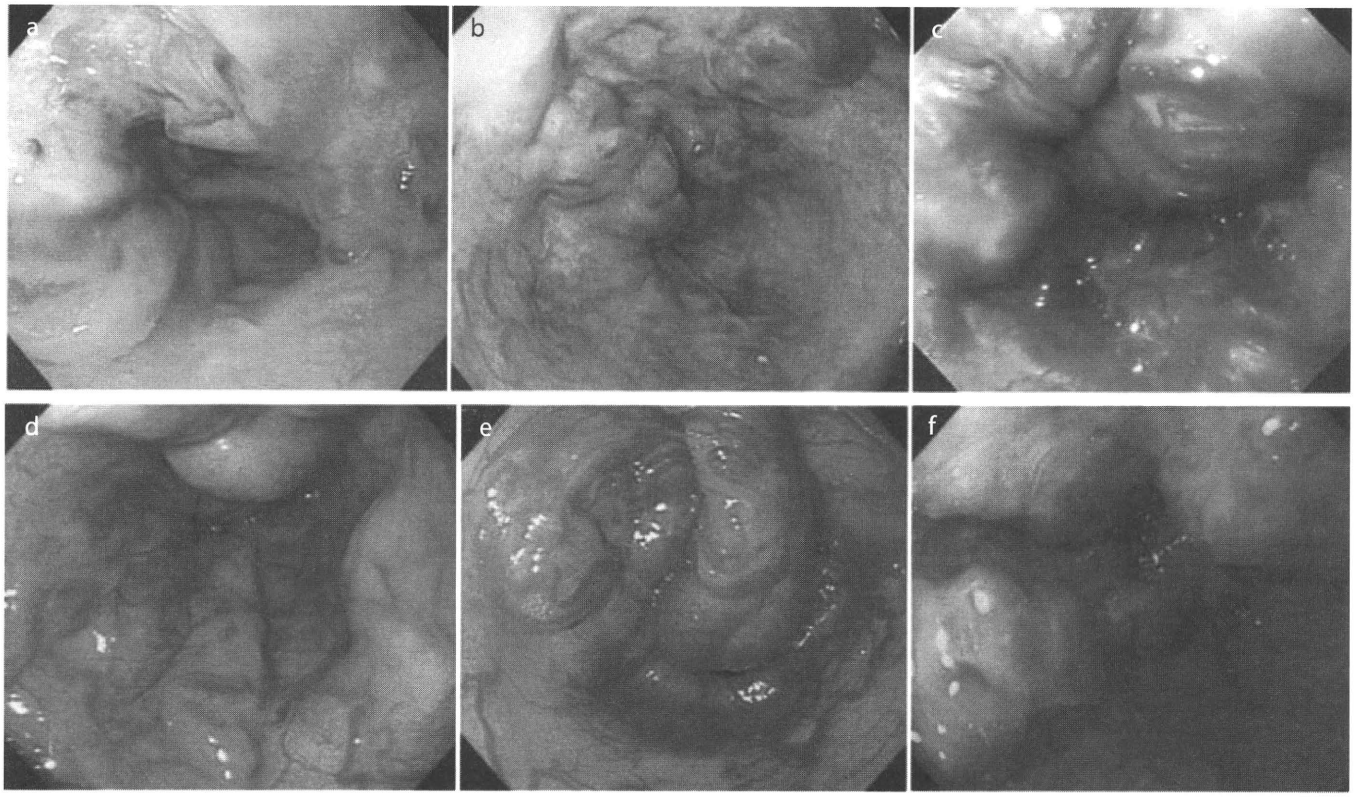

Fig. 3. Red color signs. (a) Red wale markings (RWM), (b) cherry-red spots (CRS), (c) hematocytic spots (HCS), (d) RC_1, (e) RC_2, (f) RC_3.

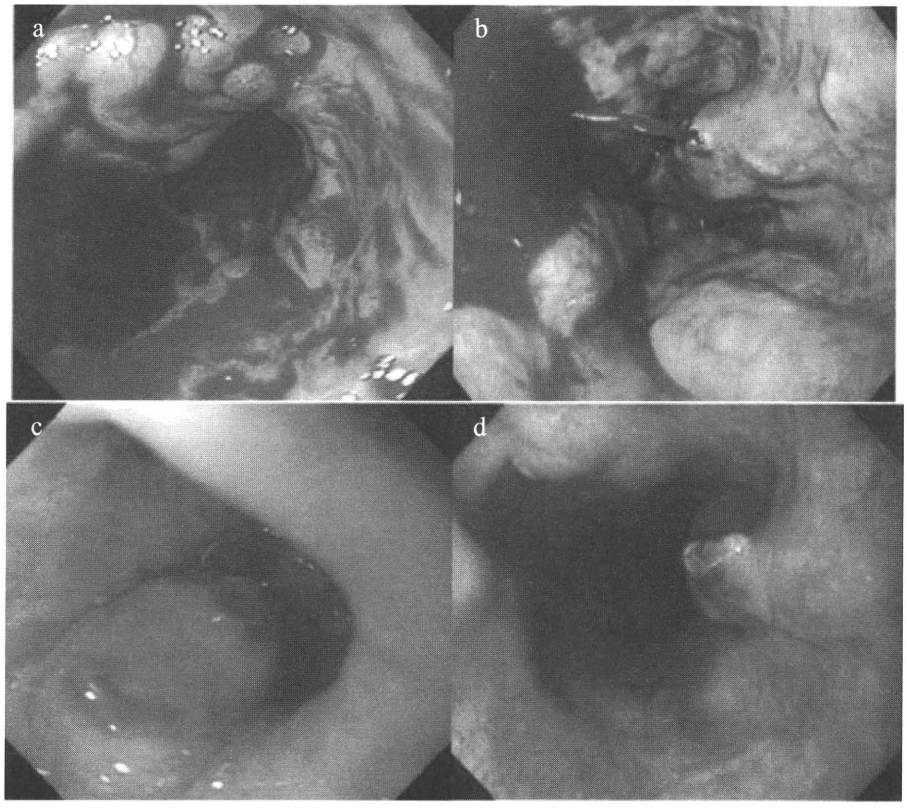

Fig. 4. Bleeding is classified as (a) gushing, (b) spurting, or oozing. Signs appearing after hemostasis are classified as (c) red plug or (d) white plug.

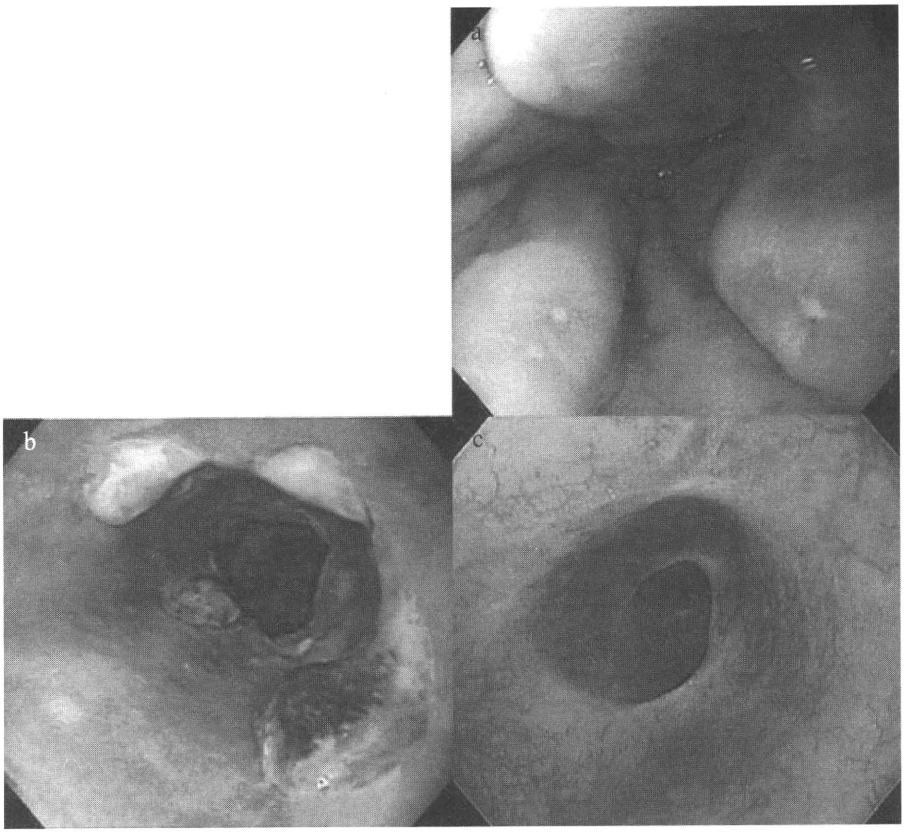

Fig. 5. Mucosal findings are classified as (a) erosion (E), (b) ulcer (Ul), or (c) scar (S).

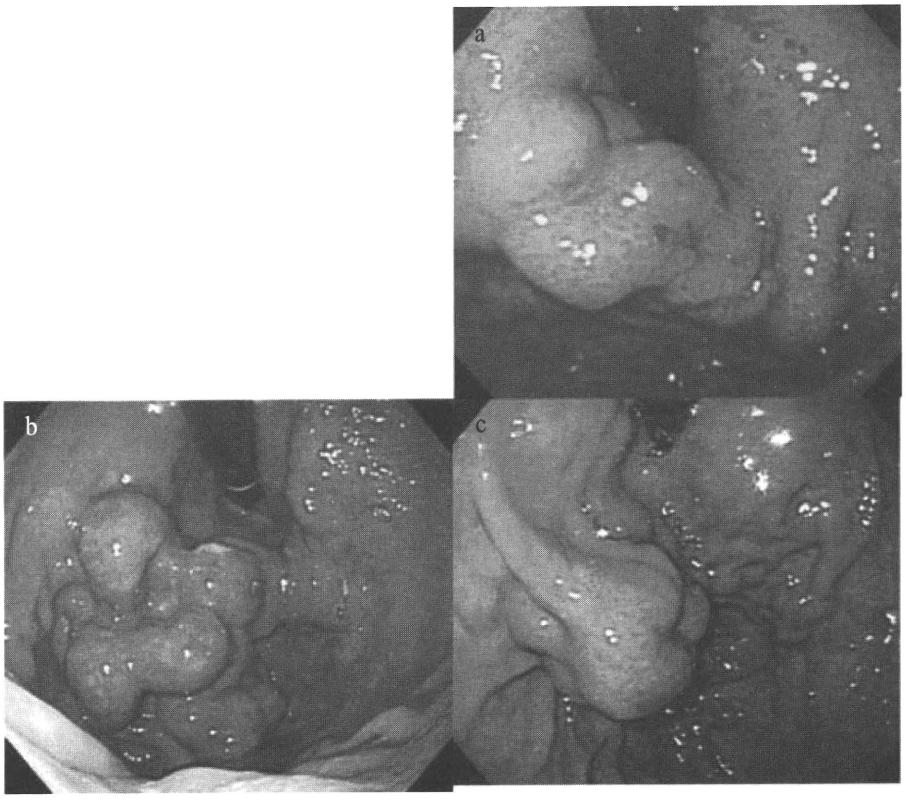

Fig. 6. Gastric varices are classified as (a) Lg-c if they are adjacent to the cardiac orifice, (b) Lg-cf if they extend from the cardiac orifice to the fornix, and (c) Lg-f if they are distant from the cardiac orifice.

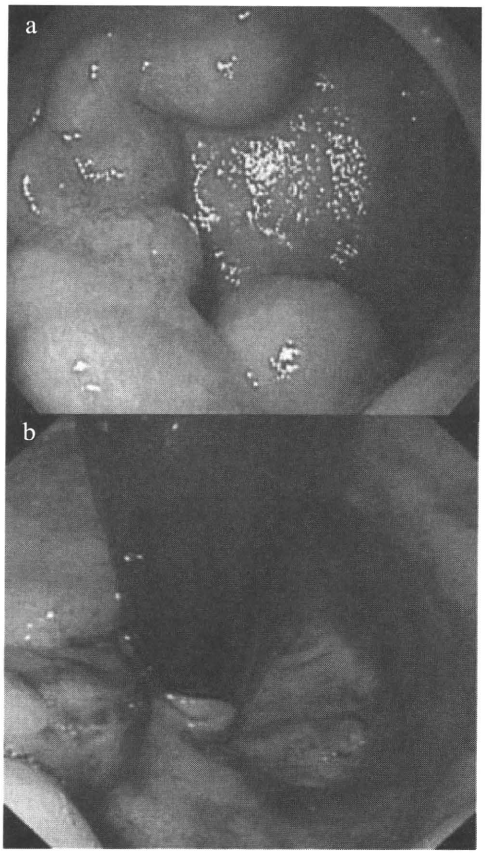

Fig. 7. Ectopic varices. (a) Duodenal varices, (b) rectal varices.

Note. Findings of GV should be recorded in the order of the six main categories (L, F, C, RC, bleeding signs, and mucosal findings) as shown in the following examples.

1. EV and fundic varices: Ls, F_3, Cb, RC_3 (RWM, CRS), Te, Lg-f, F_2, RC_0.
2. Spurting bleeding from GV extending from the cardiac orifice to the fornix: Lg-cf, F_3, spurting bleeding.
3. Thrombosed blue fundic varices: Lg-f, F_2, Cb-Th, RC_0.
4. Recurrent fundic varices: Lg-f, F_1, RC_1.

Ectopic varices

Ectopic varices are defined as gastrointestinal varices other than esophagogastric varices. All codes for EV are used to describe ectopic varices, such as duodenal varices (Fig. 7a), jejunoileal varices, colonic varices, and rectal varices (Fig. 7b).

Portal hypertensive gastropathy

In patients with portal hypertension, bleeding can be associated with gastric mucosal lesions such as hemorrhagic gastritis, acute gastric erosions, bleeding gastritis, or acute erosive gastritis. Recently, mucosal lesions associated with portal hypertension have been referred to as PHG.[7] Findings associated with PHG are classified into three categories: (i) grade 1, erythematous flecks or maculae; (ii) grade 2, red spots and/or diffuse redness; and (iii) grade 3, intramucosal or luminal hemorrhage. A snakeskin (mosaic) appearance can be associated with all three grades of PHG (Fig. 8a–f).

Endoscopic ultrasonography

Esophageal varices

On EUS images, EV appear as an echo-free or hypoechoic lumen in the esophageal submucosa (Figs 9, 10a,b).

Diameter. The maximum minor-axis diameter (mm) of EV should be recorded. The absence of an echo-free lumen and hypoechoic lumen after treatment is recorded as D(0).

Perforating veins. The presence or absence of perforating veins (Pv) should be recorded as Pv(+) and Pv(–), respectively. If Pv is present, its maximum diameter (mm) should be recorded.

Peri-esophageal veins. Peri-esophageal veins (Peri-v) refer to a group of small vessels adjacent to the muscularis externa of the esophagus or partly invading the muscular wall of the esophagus. The presence or absence of Peri-v should be recorded as Peri-v(+) and Peri-v(–), respectively.

Para-esophageal veins. Para-esophageal veins (Para-v) refer to a group of rather large vessels distal to the muscularis externa of the esophagus. The presence or absence should be recorded as Para-v(+) and Para-v(–), respectively.

Note. EUS findings on EV should be recorded in the order of the four main categories (D, Pv, Peri-v, Para-v) as shown in the following examples.

1. EV (EUS): D (3), Pv(–), Peri-v(–), Para-v(–).
2. EV (EUS): D (4), Pv(+) (3), Peri-v(+), Para-v(–).

Post-treatment EUS findings. Esophageal wall thickness (mm) should be recorded as an index of hypertrophy (Hy).

Gastric varices

On EUS images, GV appear as an echo-free or hypoechoic lumen in the gastric submucosa (Fig. 10c,d).

Diameter. The maximum minor-axis diameter of GV (mm) should be recorded. The absence of an echo-free lumen and hypoechoic lumen after treatment is recorded as D(0).

Perforating veins. The presence or absence of Pv should be recorded as Pv(+) and Pv (–), respectively. If Pv is present, its maximum diameter (mm) should be recorded.

Peri-gastric veins. Peri-gastric veins (Peri-v) refer to a group of small vessels adjacent to the gastric wall or partly invading the muscular wall of the stomach. The presence or absence of Peri-v should be recorded as Peri-v(+) and Peri-v(–), respectively.

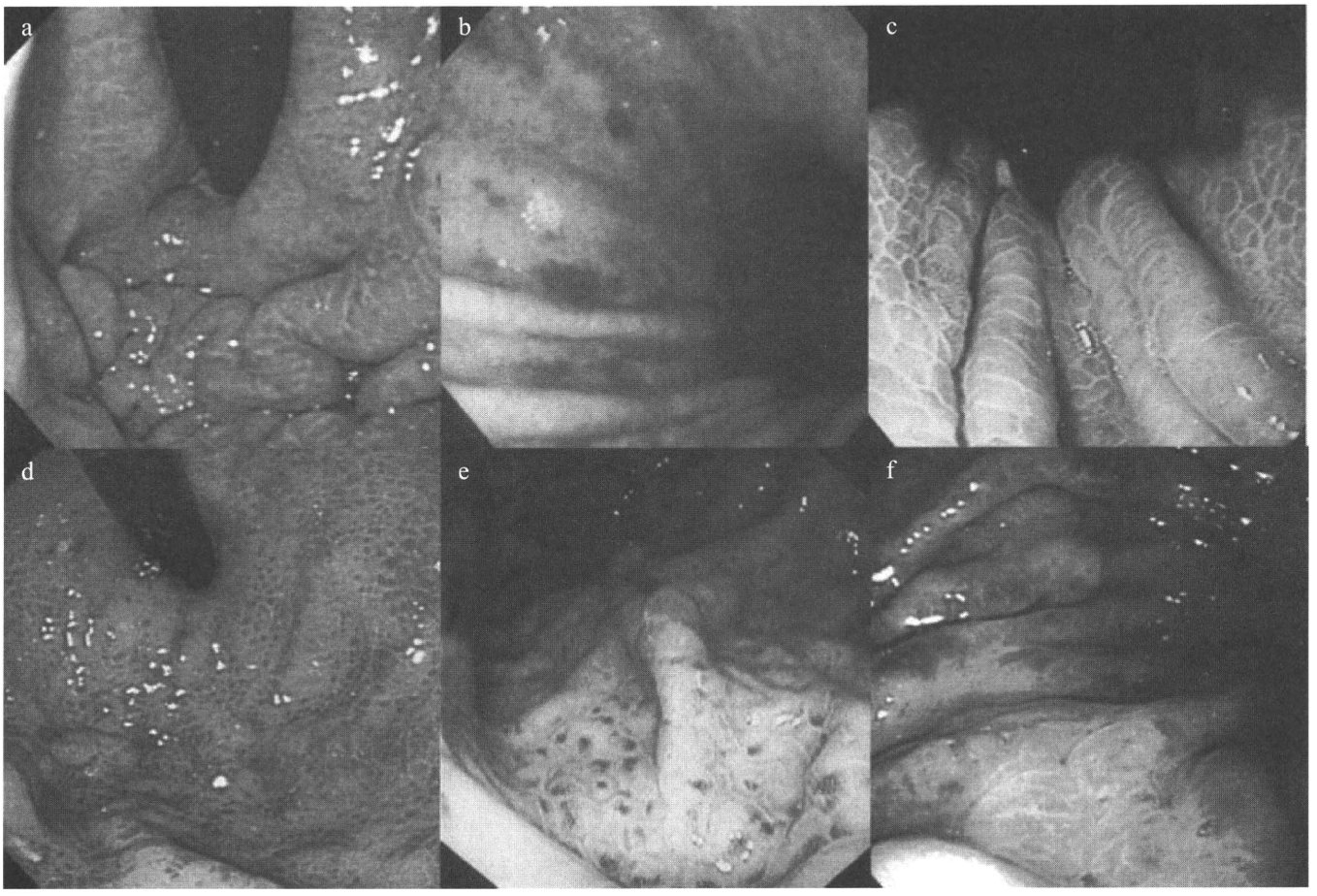

Fig. 8. (a) Fine pink speckling, (b) superficial reddening, (c) snakeskin (mosaic) appearance, (d) diffuse redness, (e) cherry red spots, (f) intramucosal hemorrhage.

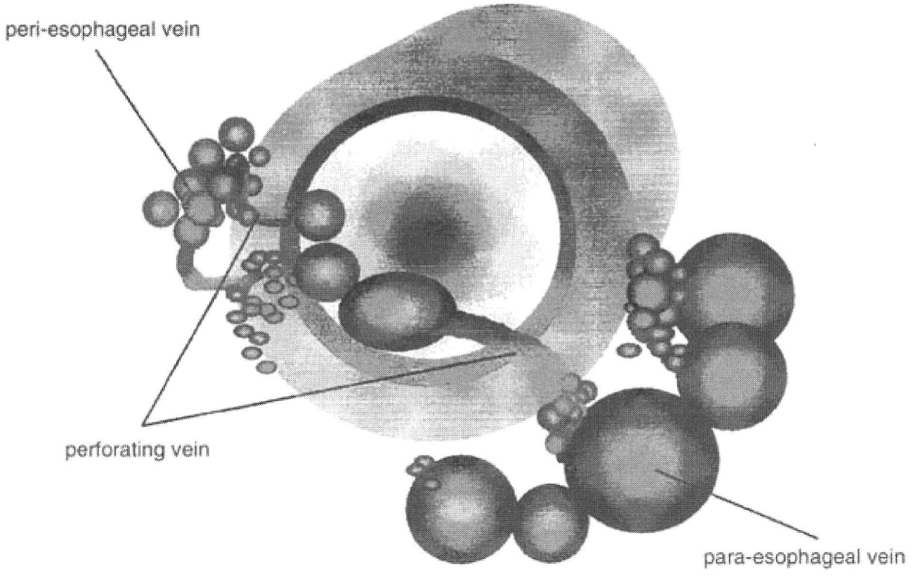

Fig. 9. Schematic drawing of the vascular structure inside and outside the esophageal wall.

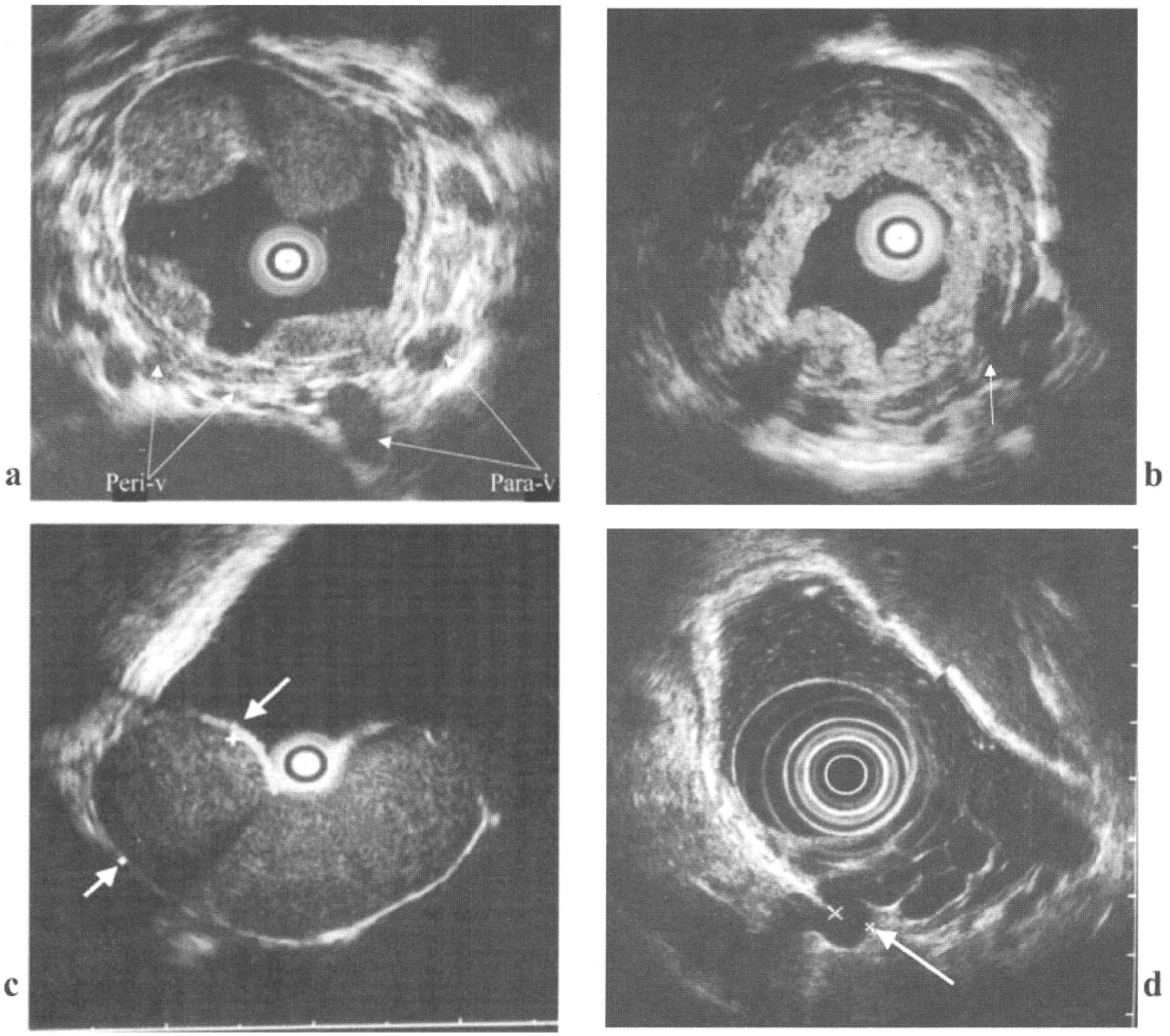

Fig. 10. Endoscopic ultrasonography (EUS) images of esophageal varices: (a) peri-esophageal veins (Peri-v), para-esophageal veins (Para-v); (b) perforating vein (Pv) (arrow). EUS images of gastric varices: (c) using a 20-MHz probe (arrow: diameter of gastric varices), (d) using a 7.5-MHz probe (arrow: perforating vein).

Para-gastric veins. Para-gastric veins (Para-v) refer to a group of rather large vessels distal to the gastric wall. The presence or absence of Para-v should be recorded as Para-v(+) and Para-v(–), respectively.

Note. EUS findings on GV should be recorded in the order of the four main categories (D, Pv, Peri-v, Para-v) as shown in the following examples.

1. GV (EUS): D (8), Pv(+) (7), Peri-v(+), Para-v(+).
2. GV (EUS): D (0), Pv(–), Peri-v(–), Para-v(+).

Post-treatment EUS findings. Gastric wall thickness (mm) should be recorded as an index of hypertrophy (Hy).

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ORIGINAL ARTICLE

Efficacy and resistance of entecavir following 3 years of treatment of Japanese patients with lamivudine-refractory chronic hepatitis B

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Abstract

Purpose Lamivudine treatment of chronic hepatitis B (CHB) is associated with frequent resistance and loss of clinical benefit. We present outcomes of lamivudine-refractory Japanese patients treated with entecavir for 3 years.

Methods Eighty-two patients refractory to lamivudine therapy received entecavir 0.5 or 1 mg daily for 52 weeks in phase II study ETV-052, directly entered rollover study ETV-060, and received entecavir 1 mg daily. Responses were evaluated among patients with available samples.

Results After 96 weeks in ETV-060 (148 weeks total entecavir treatment time), 55% (36/65) of patients had hepatitis B virus (HBV) DNA of >400 copies/mL, 85% (52/61) had alanine aminotransferase (ALT) of $\geq 1 \times$ upper limit of normal (ULN), and 14.6% (7/48) achieved HBe seroconversion. A subset of 42 patients received entecavir 1 mg from phase II baseline through 148 weeks: 54% (19/35) had HBV DNA of >400 copies/mL, 84% (27/32) had ALT of $\geq 1 \times$ ULN, and 15% (4/27) achieved HBe seroconversion. Sixteen patients in the 1-mg subset had baseline and week 148 evaluable biopsy pairs: 81% (13/16) showed histologic improvement and 38% (6/16) showed

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improvement in fibrosis. Genotypic resistance to entecavir emerged in 31 patients for a 3-year cumulative resistance probability of 35.9%. Entecavir was generally well tolerated during ETV-060, with no on-treatment ALT flares.

Conclusions Long-term entecavir treatment of lamivudine-refractory CHB resulted in virologic suppression, ALT normalization, and improvements in liver histology. Resistance was consistent with that observed in worldwide studies.

Keywords Japanese · Chronic hepatitis B · Entecavir · Lamivudine refractory · Lamivudine resistant

Introduction

Chronic hepatitis B (CHB) infection is a global public health problem that is estimated to cause between 500,000 and 1.2 million deaths annually [1–3]. Three-quarters of all chronically infected individuals live in the Asia–Pacific region, where hepatitis B virus (HBV) is the leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [4]. In Japan, the prevalence of HBV infection was estimated to be 0.8% in 2000, and the vast majority of individuals are infected with HBV genotype C [4–6]. Genotype C virus has been associated with high rates of progression to the complications of CHB, including cirrhosis and HCC [7–11]. In addition to genotype, the level of HBV DNA in the serum is strongly associated with liver disease progression [12, 13]. Persistently detectable and elevated viral loads predict the highest risk of progression to cirrhosis and HCC [12–14]. Suppression of HBV replication with antiviral therapy may reduce the risk of complications and improve the long-term outcomes of CHB patients [15].

Lamivudine has been widely used for the treatment of CHB since its development and initial approval 10 years ago [16, 17]. Lamivudine has demonstrated efficacy and long-term safety and was shown to result in histologic improvement when administered for up to 3 years [16, 18, 19]. However, resistance to lamivudine emerges at a rate of approximately 20% per year and is found in approximately 70% of patients after 4 years of therapy [20, 21]. The emergence of lamivudine resistance may be associated with increases in HBV DNA and alanine aminotransferase (ALT) levels, and loss of histologic response [16, 18, 22]. In patients with cirrhosis, lamivudine resistance may lead to hepatic decompensation and HCC [15, 23, 24]. Recently published CHB treatment guidelines no longer recommend lamivudine as first-line therapy for treatment-naive patients because of the problems that resistance introduces in the management of individual patients and the negative impact that lamivudine resistance has on the subsequent use of other antivirals [25].

Entecavir is a guanosine nucleoside analog that has demonstrated efficacy against nucleoside-naive and lamivudine-refractory CHB [26–29]. In global clinical studies, patients with lamivudine-refractory CHB treated with entecavir 1 mg daily for 48 weeks experienced reduction in HBV DNA levels of more than 5 log copies/mL and improvements in hepatic necroinflammation and fibrosis [28, 29]. Treatment for up to 96 weeks resulted in continued improvement of virologic, biochemical, and serologic end points [30]. In contrast to the nucleoside-naive population, emergence of resistance to entecavir occurred more frequently in the lamivudine-refractory population [30, 31]. To date, there are limited data on the efficacy of entecavir treatment beyond 96 weeks in the lamivudine-refractory patient population. A phase II study in Japan (ETV-052) demonstrated the efficacy and safety of entecavir in Japanese patients who were refractory to lamivudine therapy [32]. Immediately following completion of treatment in study ETV-052, patients were eligible to enroll in rollover study ETV-060 and receive entecavir 1 mg daily for up to 96 weeks. We present efficacy, safety, and resistance results for all patients treated in ETV-052 who rolled over into study ETV-060 for a total entecavir treatment time of up to 3 years (148 weeks). A subset of this cohort received the recommended dose of entecavir (1 mg daily) continuously from ETV-052 baseline, and results for this subset are also reported.

Materials and methods

Study design

Study ETV-060 was a long-term rollover study designed to provide open-label entecavir to lamivudine-refractory patients who completed treatment in the phase II study ETV-052 in Japan. In study ETV-052, 84 patients were randomized 1:1 to entecavir 0.5 mg ($n = 41$) or 1 mg ($n = 43$) daily for 52 weeks [32]. At baseline in this study, all patients had detectable lamivudine-resistance substitutions. Patients who completed 52 weeks of dosing in ETV-052 could enroll in ETV-060 and receive entecavir 1.0 mg daily in an open-label fashion. After completing 96 weeks of treatment in study ETV-060, patients could discontinue therapy or were eligible to receive commercially available entecavir that was approved by Japanese health authorities while ETV-060 was ongoing. The current analysis reports results for patients who completed ETV-052 and were subsequently treated in ETV-060 ($n = 82$) for a total entecavir treatment time (ETV-052 plus ETV-060) of up to 148 weeks. This cohort is termed the *lamivudine-refractory, long-term treatment cohort* (Fig. 1).

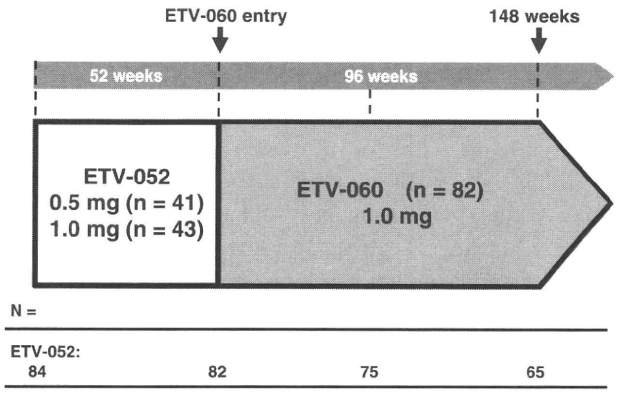

Fig. 1 Lamivudine-refractory, long-term treatment cohort. Eighty-two patients completed 52 weeks of treatment in study ETV-052 and entered rollover study ETV-060, with no interruption or gap in treatment. Sixty-five patients remained on treatment (entecavir 1.0 mg daily) through 96 weeks in study ETV-060, for a total entecavir treatment time of 148 weeks

During study ETV-060, clinical and laboratory measurements (serum chemistries, hematology, prothrombin time/international normalized ratio, and urinalysis) were assessed at baseline, weeks 2 and 4, and every 4 weeks thereafter throughout the dosing period. HBV DNA by PCR and HBV serologies were assayed at baseline, weeks 12 and 24, and subsequently every 24 weeks until week 96 or end of dosing. Liver biopsy specimens were obtained and scored for all patients at baseline and end (48 weeks) of study ETV-052, and repeat biopsy specimens were obtained at week 96 of study ETV-060 (148 weeks total entecavir treatment time) for patients who consented. Biopsy specimens were evaluated using the Knodell necroinflammatory and fibrosis scores and the corresponding New Inuyama classifications [33, 34].

Written informed consent was obtained from all patients, and the study was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice Guidelines, and Articles/Notifications of the Ministry of Health and Labor in Japan.

Patients

The inclusion criteria for study ETV-052 have been fully described elsewhere [32]. Eligible patients were adults with CHB infection and either evidence of active viral replication (HBV DNA $\geq 10^5$ copies/mL) despite at least 24 weeks of lamivudine therapy that was ongoing at the time of randomization or documented evidence of infection with HBV expressing lamivudine-resistance mutations. Patients could be hepatitis B e antigen (HBeAg)-positive or -negative and were required to have elevated levels of ALT [(1.3–10) × upper limit of normal (ULN)] and compensated liver disease. Exclusion criteria included coinfection with hepatitis C virus, hepatitis D virus, or human immunodeficiency virus; other forms of liver disease; therapy with any anti-HBV medication other than lamivudine within 24 weeks prior to randomization; and more than 12 weeks of therapy with a nucleoside or nucleotide analog (other than lamivudine) with activity against HBV. Pregnant and breast-feeding women were also excluded. All patients who completed 52 weeks of dosing in study ETV-052 were eligible to enroll in study ETV-060.

Efficacy and safety end points

Efficacy end points included the proportion of patients who achieved undetectable HBV DNA by PCR assay (<400 copies/mL), the proportion achieving ALT normalization (ALT $\leq$ 1.0 × ULN) among those with abnormal ALT at baseline, and the proportion with HBeAg loss and HBe seroconversion among those who were HBeAg-positive at baseline. Histologic results are presented for the cohort of patients who received entecavir 1 mg daily from phase II baseline and had evaluable liver biopsy pairs. Histologic improvement was defined as a $\geq$2-point decrease in the Knodell necroinflammatory score and no worsening of fibrosis (worsening: $\geq$1-point increase in the Knodell fibrosis score). Improvement in fibrosis was defined as a $\geq$1-point decrease in the Knodell fibrosis score. Histologic results were also assessed by the New Inuyama classification [34].

Safety analyses included the incidence of adverse events, serious adverse events, laboratory abnormalities, and discontinuations due to adverse events of treatment during study ETV-060, including results for patients treated beyond 96 weeks. ALT flare was defined as an on-treatment ALT measurement of more than 2 × baseline and more than 10 × ULN.

Resistance assessment

Genotypic analysis was performed on serum samples from all patients at baseline of study ETV-052 for evidence of the lamivudine-resistance substitution M204V/I in the HBV polymerase/reverse transcriptase. During study ETV-052, genotypic analysis to detect substitutions associated with entecavir resistance (at residues L180, T184, S202, M204, or M250 in the HBV polymerase/reverse transcriptase) was performed for patients with virologic breakthrough, defined as an increase in HBV DNA of ≥ 1 $\log_{10}$ copies/mL from nadir in two consecutive measurements or the last on-treatment measurement. During study ETV-060, serum samples were subjected to genotypic analysis to detect substitutions associated with entecavir resistance for patients who had HBV DNA of more than 400 copies/mL at week 100 or 148 (from study

ETV-052 baseline), or at the end of treatment (for patients who discontinued prior to week 148), and for patients who experienced virologic breakthrough.

Assay methods

All clinical laboratory tests, including HBV DNA levels, HBV serologies, and genotypic analyses, were performed at a central laboratory designated by the sponsor (SRL, Inc., Tokyo, Japan). Serum HBV DNA levels were determined by the Roche Amplicor™ PCR assay (limit of quantification = 400 copies/mL; Roche Diagnostics K.K., Tokyo, Japan). Lamivudine-resistance substitutions were identified using a PCR enzyme-linked minisequence assay (Medical & Biological Laboratories Co., Ltd., Aichi, Japan). On-treatment resistance testing was carried out by extraction of HBV DNA followed by PCR amplification and sequencing of codons 1–344 of the reverse transcriptase encoding region.

Statistical analysis

Descriptive summaries were performed. Analyses of efficacy and safety end points were based on patients who received at least one dose of study medication in study ETV-060. For binary end points, patients with missing on-treatment measurements were treated as missing (non-completer = missing analysis). Parameters represented by continuous variables were summarized by means and standard errors. Analyses of HBV DNA as a continuous parameter were applied after $\log_{10}$ transformation.

Results

Study population

Eighty-four patients were treated with entecavir in phase II study ETV-052, and 82 patients entered ETV-060, constituting the lamivudine-refractory, long-term treatment cohort (Fig. 1). Seventeen patients discontinued treatment during ETV-060 for the following reasons: adverse event ($n = 8$), protocol violation ($n = 1$), loss to follow-up ($n = 1$), and insufficient effect in the judgment of the investigator ($n = 7$). Sixty-five patients completed 96 weeks of treatment in ETV-060 for a total of 148 weeks of entecavir from ETV-052 baseline through ETV-060 (Fig. 1). Baseline (pretreatment) demographics and disease characteristics of this cohort ($n = 82$) are shown in Table 1. Eighty-seven percent (71/82) of patients were men, and mean age was 44 years. Mean HBV DNA level was 7.69 $\log_{10}$ copies/mL, mean ALT level was 135 IU/L, and 76% (62/82) of patients were HBeAg positive. All patients had documented lamivudine-resistance substitutions at M204. Ninety-four percent (77/82) of patients were infected with HBV genotype C.

Table 1 Pretreatment baseline demographics and disease characteristics of the lamivudine-refractory long-term treatment cohort ($n = 82$)

Characteristic	ETV-060 Etecavir 1.0 mg, $n = 82$
Male, *n* (%)	71 (86.6)
Age, years, mean	43
Weight, kg, mean (±SD)	66.81 (10.58)
HBV DNA, mean $\log_{10}$ copies/mL (±SD)	7.69 (0.91)
HBeAg-positive, *n* (%)	62 (75.6)
ALT, IU/L, mean (±SD)	134.7 (111.3)
ALT > 1.0 × ULN, *n* (%)	78 (95.1)
M204V/I mutation present, *n* (%)	82 (100)
HBV genotype, *n* (%)	
A	1 (1.22)
B	2 (2.44)
C	77 (94)
Others	2 (2.44)

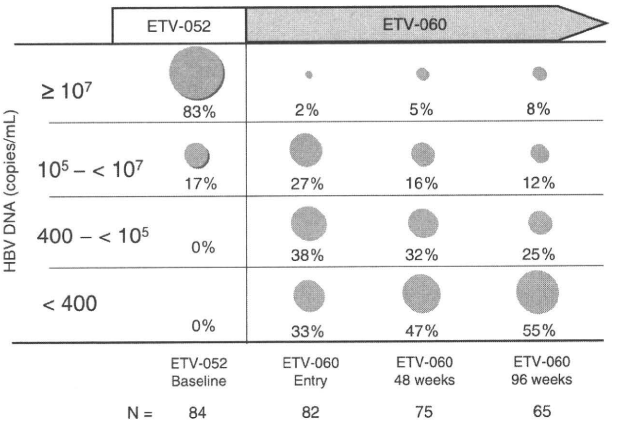

Fig. 2 Distribution of HBV DNA over time in the lamivudine-refractory, long-term treatment cohort. The proportion of patients with HBV DNA of >400 copies/mL increased through ETV-060 week 96 (148 weeks of total entecavir treatment time)

Virologic response

HBV DNA was suppressed and decreased rapidly during phase II study ETV-052 [32]. For the 82 patients who entered ETV-060 after completing ETV-052, mean HBV DNA level decreased from 7.69 $\log_{10}$ copies/mL at pretreatment baseline to 3.99 $\log_{10}$ copies/mL at ETV-060 entry (after 52 weeks of entecavir treatment). HBV DNA was further suppressed during 96 weeks of treatment in ETV-060. At baseline of study ETV-060, 33% of patients (27/82) had HBV DNA of >400 copies/mL (Fig. 2), and

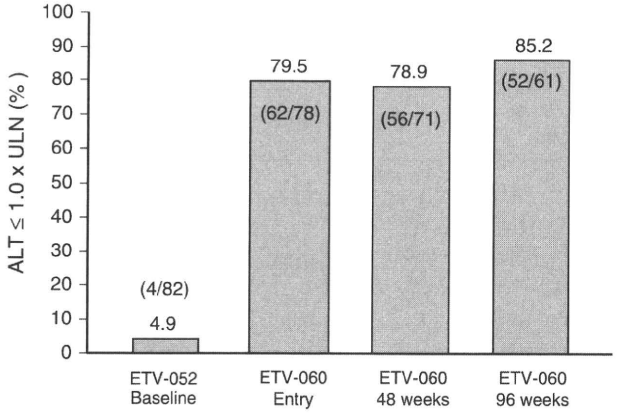

Fig. 3 Proportions of patients with normal ALT (ALT $\leq$ 1.0 $\times$ ULN) over time in the lamivudine-refractory, long-term treatment cohort. Seventy-eight patients had abnormal ALT (ALT $>$ 1.0 $\times$ ULN) at pretreatment baseline. At week 96 of study ETV-060, patients had received a total of 148 weeks of entecavir therapy

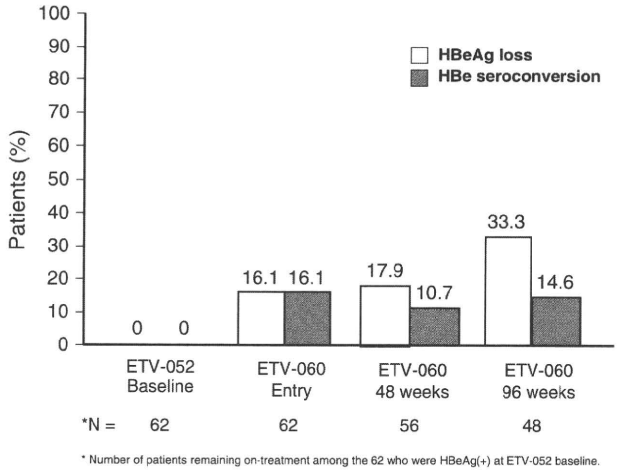

Fig. 4 Proportions of patients with HBeAg loss and HBe seroconversion over time in the lamivudine-refractory, long-term treatment cohort. Sixty-two patients were HBeAg positive at pretreatment baseline. At week 96 of study ETV-060, patients had received a total of 148 weeks of entecavir therapy

this proportion increased to 55% (36/65) by week 96 of ETV-060 (148 weeks total entecavir treatment time). Of the 17 patients who discontinued treatment during ETV-060, one patient had HBV DNA of >400 copies/mL at the last on-treatment measurement.

Biochemical response

At pretreatment baseline, 95.1% (78/82) of patients had abnormal ALT (ALT $>$ 1.0 $\times$ ULN; Table 1; Fig. 3). After 52 weeks of treatment in ETV-052, 79.5% (62/78) of patients had normalized ALT. After 96 weeks of further treatment in ETV-060 (148 weeks total entecavir treatment time), ALT had normalized in 85.2% (52/61) of patients.

Serologic response

Sixty-two patients (76%) were HBeAg-positive at pretreatment baseline (Table 1; Fig. 4). At ETV-060 entry, 16.1% (10/62) of these patients had achieved HBe seroconversion and the same number had lost HBeAg (Fig. 4). After 96 weeks in ETV-060 (148 weeks total entecavir treatment time), 33.3% of patients (16/48) had lost HBeAg and 14.6% (7/48) had undergone HBe seroconversion.

Resistance analysis

No substitutions associated with entecavir resistance emerged during study ETV-052 [32]. Eighty-one of 82 patients were monitored for resistance from ETV-052 baseline through to the end of treatment in ETV-060 (1 patient refused consent for resistance testing). Thirty-one patients developed genotypic resistance to entecavir during the second or third year of treatment, of whom 21 experienced virologic breakthrough. The 3-year cumulative probability of resistance was 35.9% [35].

Safety

Mean exposure to entecavir during study ETV-060 was 101.3 weeks (range 7.1–148). All patients experienced at least one adverse event, and 11% (9/82) experienced serious adverse events (Table 2). One patient was diagnosed with HCC at week 57 of ETV-060. Eight patients (9.8%) discontinued treatment during ETV-060 because of adverse events, such as increased ALT, virologic breakthrough, and genotypic resistance emergence. Five of these eight patients had received entecavir 0.5 mg daily during phase II study ETV-052, and three received entecavir 1 mg from phase II baseline. There were no ALT flares during ETV-060, and no deaths were reported during the study.

Entecavir 1-mg cohort

A subset of 42 patients (42/82) received the recommended 1-mg dose of entecavir for lamivudine-refractory CHB from phase II baseline through to the end of treatment in study ETV-060. In this subset, among patients with available samples, 54% (19/35) had HBV DNA of >400 copies/mL, 84% (27/32) had ALT of $\geq$1 $\times$ ULN, and 15% (4/27) achieved HBe seroconversion after 3 years of continuous treatment with entecavir 1 mg daily. Genotypic resistance emerged in 13 patients in this cohort, and 9 of 13 patients experienced virologic breakthrough. The cumulative 3-year probability of resistance was 30.4%.