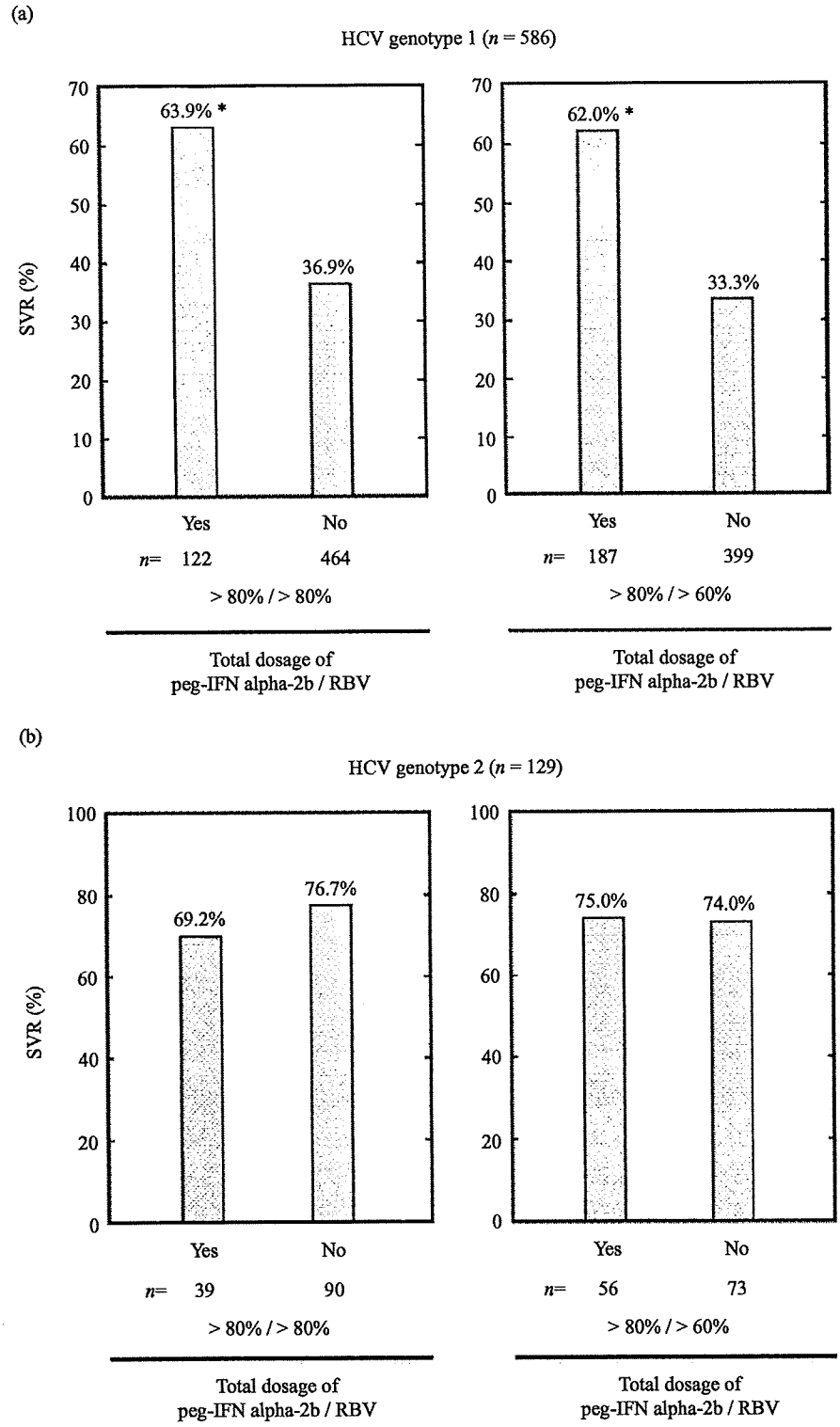

Figure 4 Sustained virological response (SVR) rates classified by percentage of total combined dosage of pegylated interferon alpha-2b (peg-IFN alpha-2b) plus ribavirin (RBV) treatment and by hepatitis C virus (HCV) genotype: An intention-to-treat analysis (Fig. 4a for genotype 1 and Fig. 4b for genotype 2). *indicates a significant difference between the groups.

significant difference. These findings suggest that the target dosage can be reduced for genotype 2 patients to avoid the adverse effects such as general fatigue, depression, and anemia and that the 24-week combination treatment can still be successfully completed.

An EVR, a virological clearance by antiviral treatment in the initial 12 weeks, is significantly related with sustained response.[27] The present study also showed that the first 12-week combined dosage was significantly related with EVR in both genotype 1 and 2 patients, leading to the attainment of an SVR.

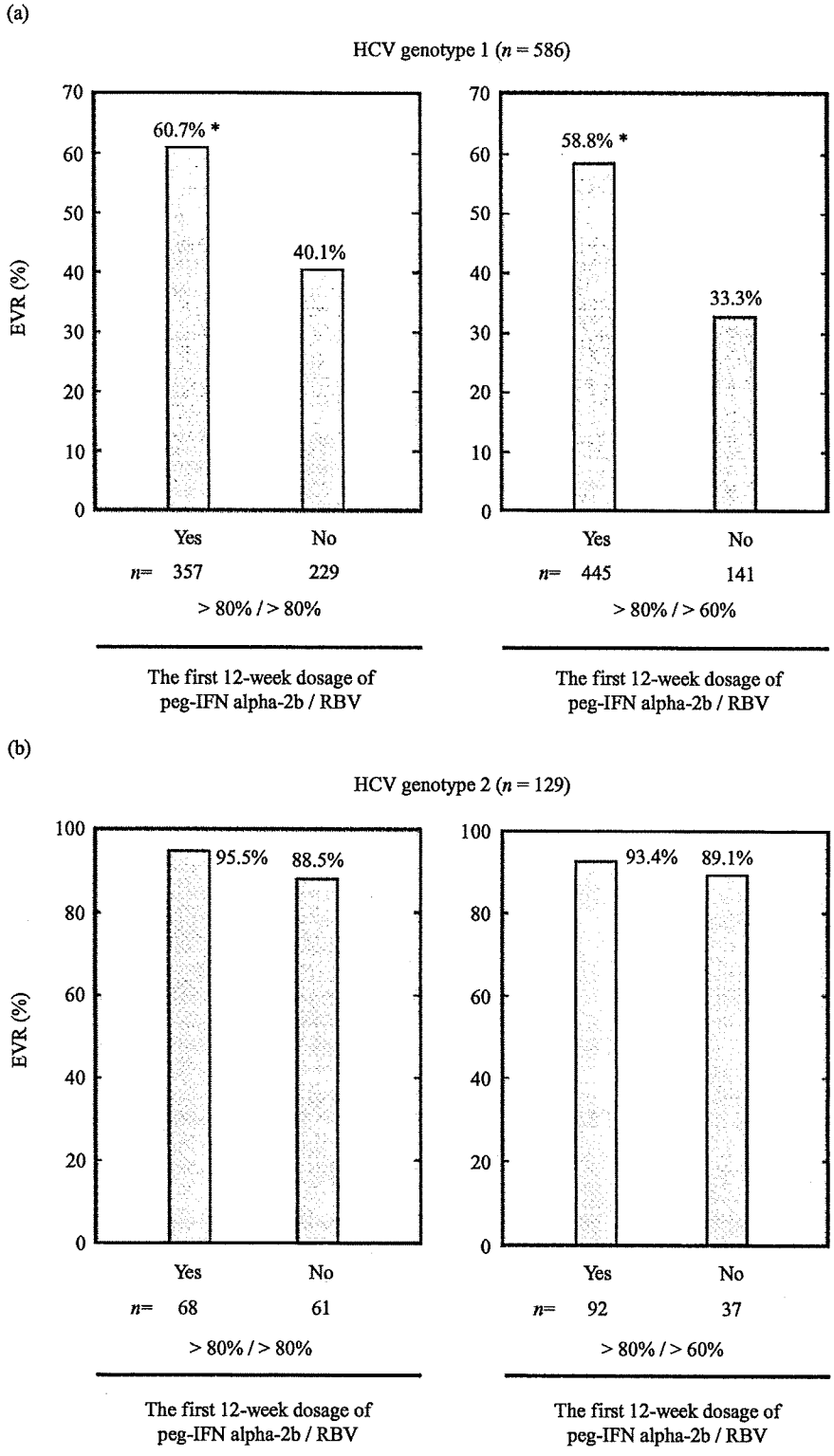

Figure 5 Early virological response (EVR) rates classified by percentage of the first 12-week combined dosage of pegylated interferon alpha-2b (peg-IFN alpha-2b) plus ribavirin (RBV) treatment and by hepatitis C virus (HCV) genotype: An intention-to-treat analysis (Fig. 5a for genotype 1 and Fig. 5b for genotype 2). *indicates a significant difference between the groups.

Because of the impact of medical adherence during the first 12-week dosage on EVR, it is important to continue the dosage from the early stage to the target period in peg-IFN alpha-2b plus RBV treatment.

Since the introduction of peg-IFN alpha plus RBV combination regimen, the treatment of chronic hepatitis C has dramatically improved over the past decade and can cure a significant proportion of the patients.[5,6] However, the combination treatment has its

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limitations, especially for HCV genotype 1 patients. Although the limited efficacy and adverse effects necessitate the development of new therapeutics approaches, we must acknowledge the current situation in which many older Japanese patients with chronic hepatitis C are candidates for antiviral treatment. Therefore, a key to solving the problem is managing antiviral treatment for these older patients. Recent analysis suggests that using erythropoietic agents (epoetin and dartbepoetin) for the reduction of anemia may not be cost-effective for the majority of patients.[28] A new RBV analog, viramidine, is reported to be associated with a lower incidence of anemia than RBV (4% *vs* 27%),[29] and, if proven effective, may eventually be substituted for RBV in combination with peg-IFN alpha for patients with chronic hepatitis C.

In conclusion, in peg-IFN alpha-2b plus RBV treatment for chronic hepatitis C, it is important to complete the target duration and reach the target dosage to achieve virological efficacy, especially for genotype 1 patients.

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References

1 Hayashi J, Kishihara Y, Yamaji K *et al.* Hepatitis C viral quasispecies and liver damage in patients with chronic hepatitis C virus infection. *Hepatology* 1997; **25**: 697–701.
2 Hayashi J, Furusyo N, Ariyama I, Sawayama Y, Etoh Y, Kashiwagi S. A relationship between the evolution of hepatitis C virus variants, liver damage, and hepatocellular carcinoma in patients with hepatitis C viremia. *J. Infect. Dis.* 2000; **181**: 1523–7.
3 Niederau C, Lange S, Heintges T *et al.* Prognosis of chronic hepatitis C: results of a large, prospective cohort study. *Hepatology* 1998; **28**: 1687–95.
4 Lindsay KL, Trepo C, Heintges T et al., Hepatitis Interventional Therapy Group. A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. *Hepatology* 2001; **34**: 395–403.
5 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958–65.
6 Wong JB, Davis GL, McHutchison JG, Manns MP, Albrecht JK, International Hepatitis Interventional Therapy Group. Economic and clinical effects of evaluating rapid viral response to peginterferon alfa-2b plus ribavirin for the initial treatment of chronic hepatitis C. *Am. J. Gastroenterol.* 2003; **98**: 2354–62.
7 Nomura H, Sou S, Tanimoto H *et al.* Short-term interferon-alfa therapy for acute hepatitis C: a randomized controlled trial. *Hepatology* 2004; **39**: 1213–19.
8 Hayashi J, Kishihara Y, Ueno K *et al.* Age-related response to interferon alfa treatment in women vs. men with chronic hepatitis C virus infection. *Arch. Intern. Med.* 1998; **158**: 177–81.
9 Furusyo N, Hayashi J, Ueno K *et al.* Human lymphoblastoid interferon treatment for patients with hepatitis C virus-related cirrhosis. *Clin. Ther.* 1997; **19**: 1352–67.
10 Furusyo N, Kubo N, Toyoda K *et al.* Helper T cell cytokine response to ribavirin priming before combined treatment with interferon alpha and ribavirin for patients with chronic hepatitis C. *Antiviral Res.* 2005; **67**: 46–54.
11 Furusyo N, Hayashi J, Ohmiya M *et al.* Differences between interferon-alpha and beta treatment for patients with chronic hepatitis C virus infection. *Dig. Dis. Sci.* 1999; **44**: 608–17.
12 Furusyo N, Hayashi J, Kashiwagi K *et al.* Hepatitis C virus (HCV) RNA level determined by second-generation branched-DNA probe assay as predictor of response to interferon treatment in patients with chronic HCV viremia *Dig. Dis. Sci.* 2002; **47**: 535–42.
13 Poynard T, Marcellin P, Lee SS *et al.* Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet* 1998; **352**: 1426–32.
14 McHutchison JG, Gordon SC, Schiff ER *et al.* Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N. Engl. J. Med.* 1998; **339**: 1485–92.
15 Muir AJ, Bornstein JD, Killenberg PG, Atlantic Coast Hepatitis Treatment Group. Peginterferon alfa-2b and ribavirin for the treatment of chronic hepatitis C in blacks and non-Hispanic whites. *N. Engl. J. Med.* 2004; **350**: 2265–71.
16 Missiha S, Heathcote J, Arenovich T *et al.* Impact of Asian race on response to combination therapy peginterferon alfa-2a and ribavirin in chronic hepatitis C. *Am. J. Gastroenterol.* 2007; **102**: 1–8.
17 Furusyo N, Takeoka H, Toyoda K *et al.* Long-term lamivudine treatment for chronic hepatitis B in Japanese patients: a project of Kyushu University Liver Disease Study. *World J. Gastroenterol.* 2006; **12**: 561–7.
18 Furusyo N, Katoh M, Tanabe Y *et al.* Interferon alpha plus ribavirin combination treatment of Japanese chronic hepatitis C patients with HCV genotype 2: a project of the Kyushu University Liver Disease Study Group. *World J. Gastroenterol.* 2006; **12**: 784–90.
19 Nakamuta M, Kotoh K, Tanabe Y *et al.* Body surface area is an independent factor contributing to the effects of lamivudine treatment. *Hepatol. Res.* 2005; **31**: 13–17.
20 Nakamuta M, Morizono S, Tanabe Y *et al.* Relationship between body surface area and ALT normalization after long-term lamivudine treatment. *World J. Gastroenterol.* 2005; **11**: 6948–53.
21 Nakamuta M, Kotoh K, Enjoji M *et al.* Effects of lamivudine on serum albumin levels correlate with pretreatment HBV-DNA levels in cirrhotic patients. *Comp. Hepatol.* 2007; **6**: 3.
22 Hayashi J, Kishihara Y, Yamaji K *et al.* Transmission of hepatitis C virus by health care workers in a rural area of Japan. *Am. J. Gastroenterol.* 1995; **90**: 794–9.
23 Simmonds P, Holmes EC, Cha TA *et al.* Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J. Gen. Virol.* 1993; **74**: 2391–9.
24 Nomura H, Tanimoto H, Kajiwara E *et al.* Factors contributing to ribavirin-induced anemia. *J. Gastroenterol. Hepatol.* 2004; **19**: 1312–17.
25 Shiffman ML, Ghany MG, Morgan TR *et al.* Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* 2007; **132**: 103–12.
26 Hadziyannis SJ, Sette H Jr, Morgan TR *et al.* Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann. Intern. Med.* 2004; **40**: 346–55.
27 Davis GL, Wong JB, McHutchison JG *et al.* Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 645–52.
28 McHutchison JG, Manns MP, Brown RS Jr, Reddy KR, Shiffman ML, Wong JB. *et al.* Strategies for managing anemia in

hepatitis C patients undergoing antiviral therapy. *Am. J. Gastroenterol.* 2007; **102**: 880–9.
29 Gish RG. Treating HCV with ribavirin analogues and ribavirin-like molecules. *J. Antimicrob. Chemother.* 2006; **57**: 8–13.

Appendix I

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Review Article

Transient elastography: Applications and limitations

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Transient elastgraphy with use of FibroScan is one of most accurate methods for assessment of liver fibrosis. FibroScan can be readily used with an operator with a short training. In many different studies, liver stiffness measured by transient elastgraphy correlates well with fibrosis stages, and cutoff values of liver stiffness for fibrosis staging are similar even among different diseases. However there is wide variation of stiffness values in the same fibrosis stage, and some overlap between the adjacent stages. In addition, inflammatory activity and size of nodule of cirrhosis affect the liver stiffness values. The reproducibility may be reduced by age, obesity, steatosis, narrow intercostal space and lower degrees of hepatic fibrosis in patients. Thus the estimation of fibrosis stages from liver stiffness should be cautiously done. To improve the accuracy of liver fibrosis staging, the combination of transient elastography with other noninvasive methods such as FibroTest should be required.

Key words: cirrhosis, FibroScan, fibrosis, FibroTest, inflammation, liver stiffness

The search for a noninvasive method to assess liver fibrosis has encouraged the development of a number of new approaches. The prognosis and treatment of chronic liver diseases depend on the stage of liver fibrosis. In chronic viral hepatitis, the presence of significant fibrosis ($F \geq 2$) requires the use of antiviral therapies, and the response to therapy is assessed by the alleviation of fibrosis. In liver cirrhosis, the risk of hepatocellular carcinoma (HCC) or bleeding from esophageal varices is high. Thus, in the patients with liver cirrhosis, the frequent screening for HCC by serum tumor markers and ultrasound sonography and endoscopic evaluation of varices is required. Liver biopsy, the gold standard of assessment of liver fibrosis, is an invasive and expensive procedure, the accuracy of which is sometimes questionable due to sampling errors, inadequate specimens, and subjective observer diagnosis. Recently transient elastography for the noninvasive measurement of liver stiffness was developed – employing the new apparatus, FibroScan.[1] This article reviews the applications and limitations of transient elastography.

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PRINCIPLE AND PROCEDURE OF TRANSIENT ELASTOGRAPHY

FIBROSCAN (ECHOSENS, FRANCE) is equipped with a probe including an ultrasonic transducer and a vibrator.[1] A vibration of mild amplitude and low frequency is transmitted from the vibrator placed on body surface toward the liver through the intercostal space. The vibration induces an elastic shear wave that propagates through the liver tissue. The pulse-echo ultrasound acquisitions follow the propagation of the shear wave and determine its velocity. The velocity is directly related to tissue stiffness; the harder the tissue, the faster the shear wave propagates. The liver stiffness is calculated from velocity and expressed in kilopascal (kPa).

The patients are asked to lie in the dorsal decubitus position with the right arm in maximal abduction. The tip of the probe is placed on the body surface between the ribs. The operator, assisted by ultrasound time-motion and A-mode images, places the probe upon the liver that is at least 6 cm thick and free of large vascular structures. The operator presses the button, the vibration starts toward the liver, and an acquisition of the propagation of the shear wave made by vibration follows. The

measurement depth is between 25 and 45 mm. Ten successful acquisitions were performed on each patient. The median value is adopted as representative of the liver stiffness.

LIVER STIFFNESS AND THE NUMERICAL SYSTEM ASSESSMENT OF FIBROSIS IN LIVER BIOPSY SPECIMENS

IN LIVER BIOPSY specimens, fibrosis has been measured with numerical systems of Scheuer,[2] the Metavir group,[3] Ishak,[3] Knodell[4] or the new Inuyama criteria using stages that range from 0–4 for Scheuer, Metavir, Knodell and new Inuyama criteria or 0–6 for Ishak. The numbers, while intended to be semiquantitative, actually represent categories of increasing severity based on a combination of location and extent of fibrosis, and whether the fibrous tissue forms septa, bridges, or nodules. Direct measurement of the amount of fibrosis in the biopsy specimen by computer-assisted morphometric image analysis has also been reported, where the mean morphometric collagen content was 0.0552 in stage 4, 0.0856 in stage 5, and 0.1163 in stage 6 of Ishak scores.[5]

Many studies have reported the correlation of liver stiffness with the numerical system of assessment of fibrosis in liver biopsy specimens (Table 1).[1,6–20]

In patients infected with hepatitis C virus (HCV), optimal stiffness cutoff values for Metavir score F ≥ 2, F ≥ 3 and F = 4 were 7.0–8.8, 9.5–9.75 and 12.1–14.5 kPa, respectively.[1,6,8,9,15] In HCV patients with normal alanine aminotransferase (ALT), an optimal stiffness cutoff value for Metavir score F ≥ 2 was 8.74 kPa,[10] which was similar to that in the patients with abnormal ALT. However, in the patients in biochemical remission (either spontaneous or after antiviral therapy), liver stiffness was reported to be lower than in patients with an identical fibrosis stage with elevated ALT, which indicates that liver stiffness correlates not only with fibrosis but also with necroinflammatory activity.[21]

In HCV patients coinfected with human immunodeficiency virus (HIV), optimal stiffness cutoff values for fibrosis scores were similar to those in the patients with HCV single infection.[11,17]

In patients with various liver diseases, optimal stiffness cutoff values for Metavir score F ≥ 2, F ≥ 3 and F = 4 were 7.2–7.9, 8.85–12.5 and 11.9–17.6 kPa, respectively.[12,13,18,19] There was wide variation of stiffness values in the same fibrosis stage, and some overlap between the adjacent stages in the reports, which may be explained by the mixed study population with various liver diseases.

In patients with primary biliary cirrhosis (PBC) or primary sclerosing chorangitis (PSC), optimal stiffness cutoff values for Metavir score F ≥ 2, F ≥ 3 and F = 4 were 7.3, 9.8 and 17.3 kPa, respectively.[14] In patients with nonalcoholic fatty liver disease (NAFLD), optimal stiffness cutoff values for Brunt score F ≥ 1, F ≥ 2, F ≥ 3 and F = 4 were 5.6, 6.65, 8 and 17 kPa, respectively.[20] In both studies, optimal stiffness cutoff values for F = 4 was rather high compared with those in patients infected with HCV.

Barreiro *et al.* suggested the interpretation of liver stiffness according to Metavir score based on the study of Castera *et al.* as follows: F0-F1 if < 7 kPa, F2 if 7.1–9.4 kPa, F3 if 9.5–12.5 kPa, and F4 if > 12.5 kPa.[22] Cutoff values for fibrosis stages are similar among different studies on different diseases. However there is wide variation of stiffness values in the same fibrosis stage, and some overlap between the adjacent stages. Thus the estimation of fibrosis score from liver stiffness should be cautiously done.

OTHER NONINVASIVE METHODS FOR ASSESSMENT OF LIVER FIBROSIS AND LIVER STIFFNESS

SEVERAL ROUTINE LABORATORY tests have been used to assess the liver fibrosis, such as prothrombin time, albumin level, and platelet count. Direct fibrosis markers are also used for assessing fibrosis, such as hyaluronic acid, collagen IV, collagen VI, laminin, aminoterminal peptide of procollagen III, tissue inhibitor of metalloproteinase 1 (TIMP-1), and matrix metalloproteinase 2. An array of laboratory tests have been developed and have been demonstrated to be useful, including PGA (prothrombin index, gamma-glutamyl transpeptidase (GGT), and apolipoprotein),[23] PGAA (prothrombin index, GGT, apolipoprotein, and alpha-2-macroglobulin),[24] Bonacini index (Platelet count, ALT/aspartate aminotransferase (AST) ratio, and prothrombin time-international normalized ratio),[25] APRI index (AST/platelet ratio),[26] Forns fibrosis index (age, platelet count, GGT, and cholesterol),[27] FibroTest (alpha-2-macroglobulin, haptoglobin, gamma globulin, apolipoptotein, and bilirubin),[28] HepaScore (bilirubin, GGT, hyaluronic acid, alpha-2-macroglobulin, age, and sex)[29] and FibroSpect (hyaluronic acid, TIMP-1, and alpha-2-macroglobulin).[30]

Table 1 Summary of investigation of transient elastography for assessment of liver fibrosis

Author (year)	Disease	Number of patients (*n*)	System of fibrosis staging	Stage of fibrosis									
				F = 0	F ≥ 1		F ≥ 2		F ≥ 3		F = 4		
					Cutoff value (kPa)	AUROC	Cutoff value (kPa)	AUROC	Cutoff value (kPa)	AUROC	Cutoff value (kPa)	AUROC
Nitta (2005)[6]	HCV	165	New Inuyama		5.55	0.77	7	0.88	9.75	0.9	12.1	0.9
Sandrin (2003)[1]	HCV	106	Metavir			0.9	7.6	0.88		0.91		0.99
Saito (2004)[7]	HCV	75	New Inuyama		6.25 for F1 (median)		7.8 for F2 (median)		13.7 for F3 (median)		34.0 for F4 (median)	
Castera (2005)[8]	HCV	183	Metavir				7.1	0.83	9.5	0.9	12.5	0.95
Ziol (2005)[9]	HCV	327	Metavir				8.8	0.79	9.6	0.91	14.6	0.97
Colletta (2005)[10]	HCV with normal ALT	40	Metavir				8.74					
Ledinghen (2006)[11]	HIV/HCV	72	Metavir					0.72		0.91	11.8–14.5	0.97
Ganne-Carrie (2006)[12]	Various diseases	1007	Metavir								14.6	0.95
Foucher (2006)[13]	Various diseases	711	Metavir				7.2	0.8	12.5	0.9	17.6	0.96
Corpechot (2006)[14]	PBC and PSC	101	Metavir				7.3	0.92	9.8	0.95	17.3	0.96
Shaheen (2007)[15]	HCV	546 (meta-analyses)	Metavir				8	0.83				0.95
Ogawa (2007)[16]	HCV	161	Metavir	6.3 (median)	6.7 for F1 (median)		9.1 for F2 (median)		13.7 for F3 (median)		26.4 for F4 (median)	
Ogawa (2007)[16]	HBV	68	Metavir	3.5 (median)	6.4 for F1 (median)		9.5 for F2 (median)		11.4 for F3 (median)		15.4 for F4 (median)	
Vergara (2007)[17]	HIV/HCV	169	Scheuer				7.2	0.87			14.6–17.6	0.95
Kim (2007)[18]	Various diseases	47	Metavir				7.35		8.85		15.1	
Kim (2007)[18]	Potential liver donors	80	Metavir	Fibrosis and steatosis were not correlated with liver stiffness.								
Franquelli (2007)[19]	Various diseases	200	Metavir				7.9	0.86	10.3	0.87	11.9	0.9
Yoneda (2007)[20]	NAFLD	67	Brunt		5.6	0.881	6.65	0.876	8	0.914	17	0.997

ALT, alanine aminotransferase; AUROC, area under the receive operating characteristic curve; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficienty virus; kPa, kilopascal; NAFLD, nonalcoholic fatty liver disease; PBC, primary biliary cirrhosis; PSC, primary sclerosing chorangitis.

The comparison of liver stiffness with some of these serum fibrosis markers has been reported. In a study of HCV patients by Castera *et al.*, the area under the receiver operating characteristic curve (AUROC) of FibroScan, FibroTest, and APRI values were of the same order for the prediction of either $F \geq 2$, $F \geq 3$, or $F = 4$.[8] Castera *et al.* demonstrated that the combined use of FibroTest and FibroScan evaluates fibrosis more efficiently. Where FibroScan and FibroTest results agreed, liver biopsy confirmed the findings in 84% of cases for $F \geq 2$, in 95% for $F \geq 3$, and in 94% for $F = 4$. In the meta-analysis of HCV patients by Shaheen *et al.*, the sensitivity and specificity for the prediction of F2-F4 fibrosis of the FibroTest at threshold of 0.58–0.60 were 47% and 90%, while those of FibroScan at threshold of 7.1–8.8 kPa were 64% and 87%, respectively.[15] Thus the accuracy of the two methods was equally less dependent, while the identification of cirrhosis of the two was excellent. Colletta *et al.* demonstrated that, among HCV carrier with normal ALT, FiroScan is superior to the FibroTest in the identification of fibrosis.[10]

Some other methods such as FibroTest showed the same order of accuracy of for assessment of liver fibrosis as FibroScan. The combined use of these methods could improve the efficiency of fibrosis staging.

FAILURE OF MEASUREMENT OF LIVER STIFFNESS BY TRANSIENT ELASTOGRAPHY

KETTANEH *ET AL.* showed that success rate of shots decreased with age and obesity of the patients, and increased with an operator with more than 50 prior exams.[31] Fraquelli *et al.* analyzed the intraoperator and interoperator agreement using the intraclass correlation coefficient (ICC) and correlated with different patient-related and liver disease-related covariates.[19] The overall rate of indeterminate results was 2.4%, which was due to high body mass index (BMI; >28 kg/m^2) in four patients and narrow intercostal space in one patient. The interoperator agreement ICC was 0.98, which indicated that FibroScan is highly reproducible and operator-friendly apparatus. The reproducibility of FibroScan was significantly reduced in patients with steatosis, increased BMI and lower degrees of hepatic fibrosis.

FibroScan can be readily used with an operator with a short training. However, the reproducibility may be reduced by age, obesity, steatosis, narrow intercostal space and lower degrees of hepatic fibrosis in patients.

FACTORS AFFECTING LIVER STIFFNESS OTHER THAN FIBROSIS

RECENTLY SEVERAL REPORTS questioned the generally accepted supposition that liver stiffness is determined entirely by the stage of hepatic fibrosis. As mentioned in the former section, Franquelli *et al.* cautioned that the clinical use of FbroScan as a surrogate for liver biopsy because of the significant reduction of reproducibility of transient elastography in patients with steatosis, increased BMI and lower degrees of hepatic fibrosis.

The association between liver stiffness and necroinflammatory activity has been reported. Coco *et al.* reported that, in the patients with biochemical remission either spontaneous or after antiviral therapy, liver stiffness was lower than in patients with identical fibrosis stage, but elevated ALT.[21] In the study of Sagir *et al.*, 15 of 20 patients with acute liver damage of different etiologies showed high values of liver stiffness suggestive of liver cirrhosis, while none of them had any other signs of liver cirrhosis, and six of them who were followed up showed the decrease of liver stiffness values below the cutoff value of liver cirrhosis.[32] Arena *et al.* also reported that 18 patients with acute liver damage showed high liver stiffness values over the cutoff value of liver cirrhosis and then the progressive decrease of liver stiffness values in the follow-up period.[33] They also described the significant correlation between aminotransferases and liver stiffness at the onset of acute viral hepatitis. Thus liver stiffness measurement is not always a reliable means by which to detect liver cirrhosis when patients are suffering from acute hepatitis.

Ganne-Carrie *et al.* showed that FibroScan is reliable method for the diagnosis of cirrhosis in patients with chronic liver diseases, better at excluding than at predicting cirrhosis using threshold of 14.6 kPa and that most of false-negative diagnoses of cirrhosis by FibroScan are attributable to inactive or macronudular cirrhosis.[12]

So far, no well-controlled studies of transient elastography in patients with NAFLD have been done. Kim *et al.* reported that hepatic steatosis does not affect liver stiffness.[18] However it is likely that different cutoff values will be required for patients with nonalcoholic steatohepatitis.

Inflammatory activity and size of nodule of cirrhosis affect the liver stiffness values. Thus caution is needed in the clinical use of FibroScan as a surrogate for liver biopsy.

OTHER METHODS FOR MEASUREMENT OF LIVER STIFFNESS

REAL-TIME ELASTOGRAPHY is also a new noninvasive method for the assessment of liver fibrosis. Real-time elastography is done with conventional ultrasound probes; the Hitachi EUB-8500 and EUB-900 machines (Hitachi, Japan).[34] The examined tissue is divided in up to 30 000 finite elements before compression. During compression, the displacement of each element is measured. In hard tissue, the amount of displacement is low, whereas in soft tissue, the amount of displacement is high. The calculation of tissue elasticity distribution is performed in real time, and the examination results are presented as color-coded images with conventional B-mode image in the background. In the study of patients with HCV or hepatitis B virus and healthy volunteers, the cutoff elasticity scores and AUROC for Metavir score $F \geq 2$, $F \geq 3$ and $F = 4$ were 100.1, 102.5 and 111.75, and 0.75, 0.73, and 0.69, respectively. Since the AUROC of APRI for Metavir score $F \geq 2$, $F \geq 3$ and $F = 4$ were 0.87, 0.88 and 0.88 in the same study, the diagnostic efficiency of APRI was superior to that of real-time elastography. The new method of elastography using heart beats instead of manual compression for displacement also has been developed.

Novel techniques including magnetic resonance (MR) spectroscopy, diffusion weighted MR, and MR elastography have also developed for detecting liver fibrosis.[35]

CONCLUSIONS

A NUMBER OF noninvasive methods for assessment of liver fibrosis are now available, and many which are reasonably dependable. Transient elastgraphy is one of most accurate methods available. However, there are many limitations in transient elastography. The reproducibility of transient elastography is significantly reduced in patients with steatosis, increased BMI and lower degrees of hepatic fibrosis. Inflammatory activity affects liver stiffness values. To improve the accuracy of liver fibrosis staging, the combination of transient elastography with other noninvasive methods should be employed.

REFERENCES

1 Sandrin L, Fourquet B, Hasquenoph JM *et al.* Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; 29: 1705–13.
2 Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. *J Hepatol* 1991; 13: 372–4.
3 Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group. *Hepatology* 1994; 20: 15–20.
4 Knodell RG, Ishak KG, Black WC *et al.* Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; 1: 431–5.
5 Goodman ZD, Becker RL Jr, Pockros, PJ, Afdhal NH. Progression of fibrosis in advanced chronic hepatitis C: evaluation by morphometric image analysis. *Hepatology* 2007; 45: 886–94.
6 Nitta Y, Yoshioka K, Sakamoto K *et al.* Clinical usefulness of Fibroscan. *Acta Hepatologica Japonica* 2005; 46: A438.
7 Saito H, Tada S, Nakamoto N *et al.* Efficacy of non-invasive elastometry on staging of hepatic fibrosis. *Hepatol Res* 2004; 29: 97–103.
8 Castera L, Vergniol J, Foucher J *et al.* Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; 128: 343–50.
9 Ziol M, Handra-Luca A, Kettaneh A *et al.* Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; 41: 48–54.
10 Colletta C, Smirne C, Fabris C *et al.* Value of two noninvasive methods to detect progression of fibrosis among HCV carriers with normal aminotransferases. *Hepatology* 2005; 42: 838–45.
11 de Ledinghen V, Douvin C, Kettaneh A *et al.* Diagnosis of hepatic fibrosis and cirrhosis by transient elastography in HIV/hepatitis C virus-coinfected patients. *J Acquir Immune Defic Syndr* 2006; 41: 175–9.
12 Ganne-Carrie N, Ziol M, de Ledinghen V *et al.* Accuracy of liver stiffness measurement for the diagnosis of cirrhosis in patients with chronic liver diseases. *Hepatology* 2006; 44: 1511–17.
13 Foucher J, Chanteloup E, Vergniol J *et al.* Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. *Gut* 2006; 55: 403–8.
14 Corpechot C, El Naggar A, Poujol-Robert A *et al.* Assessment of biliary fibrosis by transient elastography in patients with PBC and PSC. *Hepatology* 2006; 43: 1118–24.
15 Shaheen AA, Wan AF, Myers RP. FibroTest and FibroScan for the prediction of hepatitis C-related fibrosis: a systematic review of diagnostic test accuracy. *Am J Gastroenterol* 2007; 102: 2589–600.
16 Ogawa E, Furusyo N, Toyoda K *et al.* Transient elastography for patients with chronic hepatitis B and C virus infection: non-invasive, quantitative assessment of liver fibrosis. *Hepatol Res* 2007.
17 Vergara S, Macias J, Rivero A *et al.* The use of transient elastometry for assessing liver fibrosis in patients with HIV

and hepatitis C virus coinfection. *Clin Infect Dis* 2007; 45: 969–74.
18 Kim KM, Choi WB, Park SH *et al.* Diagnosis of hepatic steatosis and fibrosis by transient elastography in asymptomatic healthy individuals: a prospective study of living related potential liver donors. *J Gastroenterol* 2007; 42: 382–8.
19 Fraquelli M, Rigamonti C, Casazza G *et al.* Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. *Gut* 2007; 56: 968–73.
20 Yoneda M, Yoneda M, Fujita K *et al.* Transient elastography in patients with non-alcoholic fatty liver disease (NAFLD). *Gut* 2007; 56: 1330–1.
21 Coco B, Oliveri F, Maina AM *et al.* Transient elastography: a new surrogate marker of liver fibrosis influenced by major changes of transaminases. *J Viral Hepat* 2007; 14: 360–9.
22 Barreiro P, Rodriguez-Novoa S, Labarga P *et al.* Influence of liver fibrosis stage on plasma levels of antiretroviral drugs in HIV-infected patients with chronic hepatitis C. *J Infect Dis* 2007; 195: 973–9.
23 Poynard T, Aubert A, Bedossa P *et al.* A simple biological index for detection of alcoholic liver disease in drinkers. *Gastroenterology* 1991; 100: 1397–402.
24 Naveau S, Poynard T, Benattar C, Bedossa P, Chaput JC. Alpha-2-macroglobulin and hepatic fibrosis. Diagnostic interest. *Dig Dis Sci* 1994; 39: 2426–32.
25 Bonacini M, Hadi G, Govindarajan S, Lindsay KL. Utility of a discriminant score for diagnosing advanced fibrosis or cirrhosis in patients with chronic hepatitis C virus infection. *Am J Gastroenterol* 1997; 92: 1302–4.
26 Wai CT, Greenson JK, Fontana RJ *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; 38: 518–26.
27 Forns X, Ampurdanes S, Llovet JM *et al.* Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; 36: 986–92.
28 Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; 357: 1069–75.
29 Adams LA, Bulsara M, Rossi E *et al.* Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. *Clin Chem* 2005; 51: 1867–73.
30 Patel K, Gordon SC, Jacobson I *et al.* Evaluation of a panel of non-invasive serum markers to differentiate mild from moderate-to-advanced liver fibrosis in chronic hepatitis C patients. *J Hepatol* 2004; 41: 935–42.
31 Kettaneh A, Marcellin P, Douvin C *et al.* Features associated with success rate and performance of FibroScan measurements for the diagnosis of cirrhosis in HCV patients: a prospective study of 935 patients. *J Hepatol* 2007; 46: 628–34.
32 Sagir A, Erhardt A, Schmitt M, Haussinger D. Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. *Hepatology* 2008; 47: 592–5.
33 Arena U, Vizzutti F, Corti G *et al.* Acute viral hepatitis increases liver stiffness values measured by transient elastography. *Hepatology* 2008; 47: 380–4.
34 Friedrich-Rust M, Ong MF, Herrmann E *et al.* Real-time elastography for noninvasive assessment of liver fibrosis in chronic viral hepatitis. *AJR Am J Roentgenol* 2007; 188: 758–64.
35 Talwalkar JA, Yin M, Fidler JL, Sanderson SO, Kamath PS, Ehman RL. Magnetic resonance imaging of hepatic fibrosis: emerging clinical applications. *Hepatology* 2008; 47: 332–42.

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Association of hepatitis B virus subgenotypes and basal core promoter/precore region variants with the clinical features of patients with acute hepatitis

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Background. In endemic areas, including Japan, basal core promoter (BCP) and precore (PC) variants of hepatitis B virus (HBV) have been reported to be associated with the clinical outcome of acute hepatitis B patients. However, the associations of BCP/PC variants with clinical outcomes have not been observed in non-endemic areas. HBV subgenotypes, which show geographic variations in prevalence, may underlie this discrepancy in clinical outcomes. Little is known about the differences in the clinical and virological features of HBV subgenotypes and BCP/PC variants. The aim of this study was to investigate the distributions of subgenotypes and BCP/PC variants to identify clinical differences in acute hepatitis B patients. ***Methods.*** One hundred thirty-nine patients with acute hepatitis were enrolled. Nested polymerase chain reaction was used to amplify the pre-S region of HBV for genotyping and the BCP/PC regions for variant screening. ***Results.*** HBV subgenotypes A1 ($n = 3$), A2 ($n = 28$), B1 ($n = 3$), B2 ($n = 9$), C1 ($n = 5$), C2 ($n = 84$), C variant ($n = 1$), D2 ($n = 3$), and H ($n = 3$) were detected. BCP/PC variants were not associated with progression to chronic hepatitis. Patients infected with subgenotype C2 who progressed to fulminant hepatic failure frequently carried variants at nucleotides non-T1753 and non-T1754 and T1762, A1764, and A1896. ***Conclusions.*** BCP/PC variants would be associated with progression to fulminant hepatitis in subgenotype C2. Knowledge of HBV subgenotypes and BCP/PC variants is useful for developing strategies to treat acute hepatitis B patients.

Key words: hepatitis B virus, fulminant hepatic failure, subgenotypes, basal core promoter/precore region variants

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Introduction

Approximately 350 million people worldwide are infected with hepatitis B virus (HBV).[1] HBV infection has a variety of clinical courses, including self-limited acute hepatitis, fulminant hepatic failure, chronic hepatitis, and progression to cirrhosis and hepatocellular carcinoma.[2] Therefore, HBV infection is a significant global health problem. HBV has been classified into eight major genotypes on the basis of divergence of 8% of the full-length nucleotide sequence, and the prevalence of each genotype differs by region.[3,4] Each genotype shows different responses to antiviral treatments and different virological characteristics;[5–7] therefore, HBV genotype information may be useful for developing strategies to treat HBV-related liver disease. Moreover, HBV genotypes have been subdivided into subgenotypes that differ in their geographic distribution.[4] Therefore, HBV subgenotypes can be used to study geographic distributions in greater detail than can simple genotypes. Recently, the prevalence and geographic distribution of HBV subgenotypes in Japanese HBV carriers, including patients with acute hepatitis, were reported.[8,9] However, the effects of HBV subgenotypes on the clinical course of acute hepatitis have not been well documented. Several studies have reported that variants of the basal core promoter (BCP) and precore (PC) regions may be associated with progression to fulminant hepatic failure.[10–12] However, the roles of BCP and PC variants in acute hepatitis are controversial.[13,14] The prevalences of BCP and PC region variants depend on genotype, and some researchers have proposed that genotype differences influence clinical outcome.[15–17] Therefore, one reason that may explain the discrepant results for the roles of BCP and PC variants in acute hepatitis may be HBV genotype and subgenotype differences. Therefore, the relationships of BCP and PC region variants with clinical features need to be considered with respect to the HBV subgeno-

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(海外での最近の知見)

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Key words
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テノホビル,
TDF

要　約

フマル酸テノホビル ジソプロキシル（TDF）のB型肝炎ウイルス（HBV）単独感染患者に対する2つの第III相試験の72週の結果が海外にて報告された。HBe抗原陰性およびHBe抗原陽性のHBV単独感染患者において，TDFは，アデホビル（ADV）に比較して優れた抗HBV作用を示した。また，HBe抗原陰性およびHBe抗原陽性のHBV単独感染患者にADVを投与し，48週目以降，ADVからTDFに切り替えることによって，さらに強いHBV DNA抑制作用が認められた。TDFはADVと同様に忍容性や安全性が良好であった。

海外においては，これまで，HBVに対する核酸アナログは4剤が使用可能であったが，これらの結果を受けて，今年，TDFがヨーロッパおよび米国において承認に至っている。

はじめに

現在，選択可能なB型慢性肝炎の治療戦略としては，①HBV DNAの複製を直接，阻害する「核酸アナログ」および②ウイルス複製の抑制に加え，免疫増強作用を有する「インターフェロンα（標準およびペグ化）」が主なものであるが，「long-term treatment」としては経口薬で副作用も少ない核酸アナログが有望である。これまで，海外においては，ラミブジン（LAM），アデホビル（ADV），エンテカビル（ETV）およびテルビブジン（L-dT）の4つの核酸アナログが承認されていたが，今年，新たに，フマル酸テノホビル ジソプロキシル（TDF）が承認された（2008年4月：ヨーロッパ，2008年8月：米国）。これらの薬剤の短期的なベネフィットは認められているが，長期的な有効性を検証するデータはまだ十分ではない。TDFについては，第III相試験が進行中であり，5年間（240週間）の観察が計画されているが，2008年4月に72週までの結果が報告された。今回，これらの内容を概説する。

TDFは開環（acyclic）した糖鎖をもつアデニン誘導体のヌクレオチド系逆転写酵素阻害剤であり，本邦では2004年3月にHIV-1感染症を適応として承認され，鳥居薬品株式会社より「ビリアード®錠」として販売されている。

1．HBe抗原陰性のHBV単独感染患者におけるTDFの抗HBV作用（Study GS-US-174-0102；102試験）[1,2]

本試験は，未治療のHBV単独感染患者（HBe抗原陰性：pre-core変異を有する患者）375例を対象に，TDF 300mgの1日1回投与（以下，TDF群。N=250）とADV 10mgの1日1回投与（以下，ADV群。N=125）を比較した多施設二重盲検の無作為化コントロール試験である。主要評価項目としては，肝線維化の悪

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表1　ベースライン時の患者背景（102試験）

CHARACTERISTIC	TDF (N=250)	ADV (N=125)
Mean Age (years)	44	43
Race Caucasian Asian	 64% 25%	 65% 24%
Male	77%	78%
Prior Lamivudine Experience	17%	18%
Mean HBV DNA ($\log_{10}$ copies/mL)	6.86	6.98
Mean ALT (U/L)	128	164
Mean Knodell Necroinflammatory Score Mean Knodell Fibrosis Score	7.8 2.3	7.8 2.4
Knodell Fibrosis Score=4 (Cirrhosis)	19%	20%
Viral Genotype A B C D	 12% 9% 12% 64%	 11% 14% 10% 63%

化を伴わず，投与後48週に完全寛解（血清HBV DNA＜400 copies/mL，かつ組織学的改善：Knodell壊死炎症スコア2ポイント以上の低下）に至った症例の比率である。48週以降，TDF群はTDFを継続投与（TDF-TDF群），ADV群はTDFに切り替えて（ADV-TDF群），さらに4年間（計5年間=240週間）の観察を行うが，72週までの結果が得られている。

ベースライン時の患者背景は，両群ともに同様であった（表1）。平均HBV DNA量はTDF群6.86 $\log_{10}$ copies/mL，ADV群 6.98 $\log_{10}$ copies/mLであった。平均ALT値はTDF群 128 U/L，ADV群 164 U/Lであり，Knodell壊死炎症スコアは両群ともに7.8，Knodell線維化スコアはTDF群 2.3，ADV群 2.4であり，両群ともに約20%が肝硬変を有していた。

投与後48週における主要評価項目および副次的評価項目のまとめを図1に示した。完全寛解に至った症例の比率は，TDF群は71%であり，ADV群の49%に比較して，有意に高率であった（$p<0.001$）。また，HBV DNA＜400 copies/mLに至った症例の比率は，TDF群が93%であり，ADV群の63%に比較して，有意に高率であった（$p<0.001$，図1）。一方，組織学的改善においては，TDF群が72%，ADV群が69%であり，両群間で明らかな差は認められなかった（図1）。

投与後48週以降の結果を図2～4に示す。図2は，HBV DNA＜400 copies/mLに至った症例の比率の推移であるが，48週時ではADV群は63%とTDF群（93%）に比べ，有意に低い値であった。しかし，48週以降にADVからTDFに切り替えたことによって，72週時にはADV-TDF群の88%がHBV DNA＜400 copies/mLに至った（TDF-TDF群：91%，$p=0.315$，図2）。図3は，平均HBV DNA量の推移であるが，投与後48週時ではTDF群の方が有意に減少が大きく（$p<0.001$），ADV群では投与後48週までに検出限界未満に至らなかった。TDFに切り替えた後，ADV-TDF群は56週以降，検出限界未満に至った（図3）。また，ALTの正常化は，ADVからTDFに切り替えた後も77%で維持されていた（TDF-TDF群：79%，図4）。TDF-TDF群において，HBV DNA＜400 copis/mLであった2例が，投与後72週までに，ウイルス量のリバウンド（≧400 copies/mL）を示した。2例ともアドヒアランス不良で

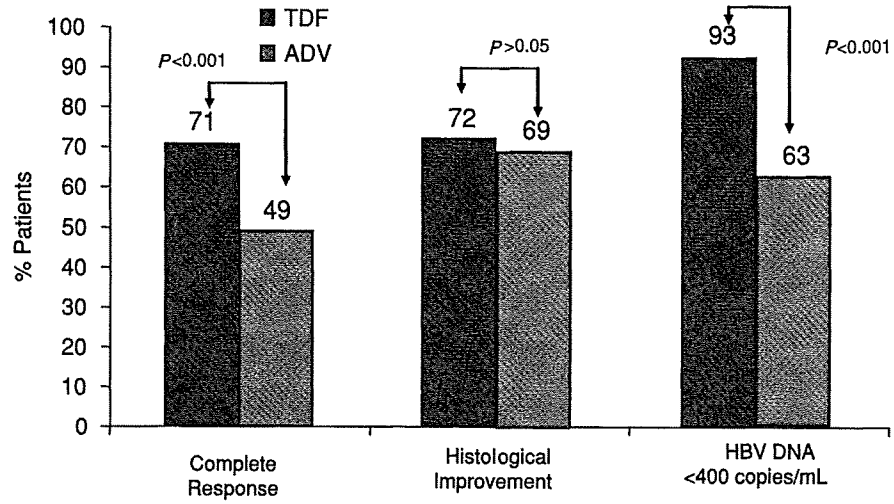

図1　102試験　主要評価項目および副次的評価項目

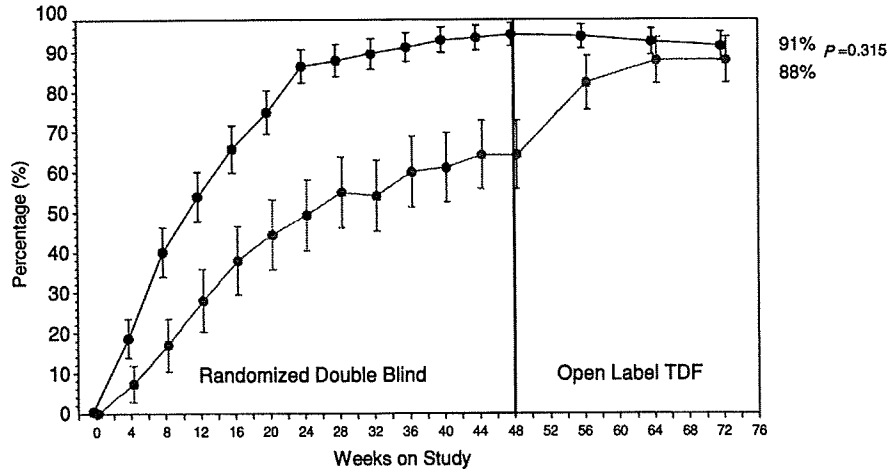

図2　HBV DNA＜400 copies/mLに至った症例比率（Missing=Failure，102試験）

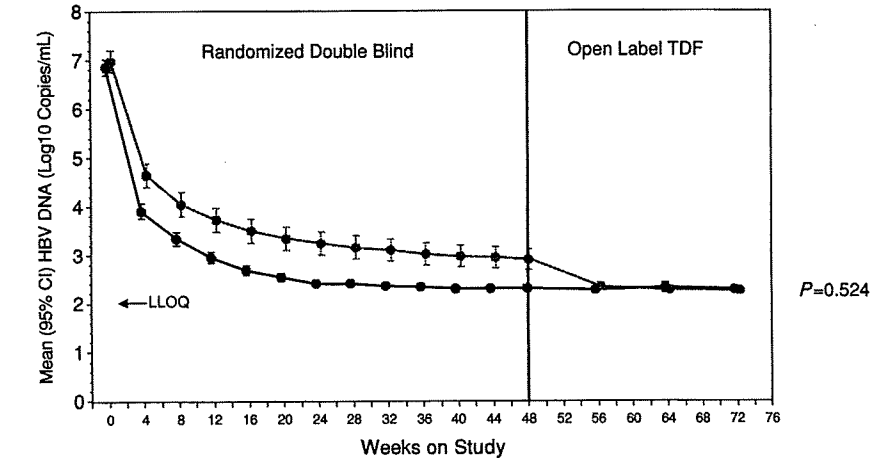

図3　平均HBV DNA量の推移（102試験）

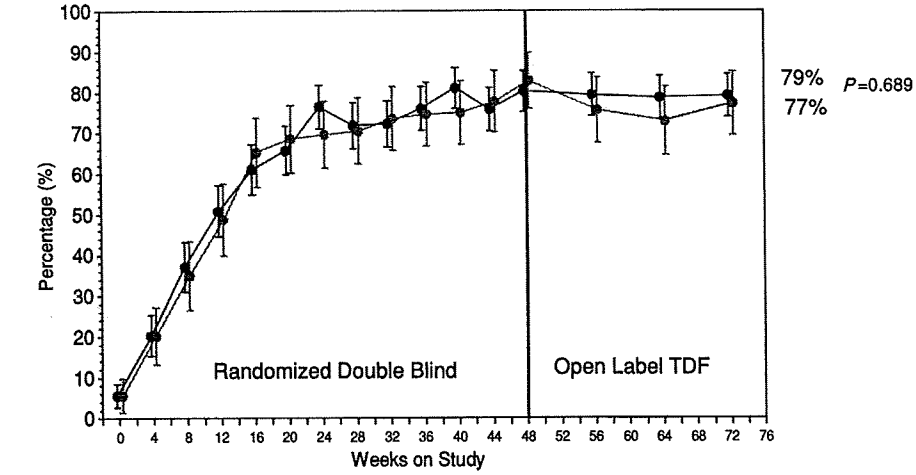

図4　ALTの正常化に至った症例比率（Missing=Failure，102試験）

表2　ADV-TDF群におけるTDF切り替えに対するHBV DNAの反応性（102試験）

Week 48 ADV Viral Response	Week 72 TDF Complete Viral Response
Complete Response (<400 copies/mL)	76 (100%)
Incomplete Response (≥400 copies/mL)	33 (94%)

表3　TDF-TDF群における24週時ウイルス不応例に対する転帰（102試験）

Week 24 TDF Viral Response	W48 Complete Response (<400 copies/mL)	W72 Complete Response (<400 copies/mL)
Complete Response (<400 copies/mL)	205 (99%)	197 (99%)
Incomplete Response (≥400 copies/mL)	24 (86%)	23 (92%)

あり，耐性検査を実施したが，TDF耐性にかかわる変異は認められなかった。ADV-TDF群において，TDFへの切り替えに対する反応性をみたところ，ADV投与後48週時にHBV DNA＜400 copies/mLであった症例は，TDFに切り替えても全例がHBV DNA＜400 copies/mLを維持していた（表2）。また，ADV投与後48週時にHBV DNA≧400 copies/mLであった症例の94%は，TDFに切り替えたことによって，ウイルスが速やかに抑制された（表2）。TDF-TDF群において，TDF投与後24週時にHBV DNA＜400 copies/mLであった症例は，その後も99%の症例がHBV DNA＜400 copies/mLを維持していた（表3）。また，TDF投与後24週時にHBV DNA≧400 copies/mLであった症例は，その後のTDF投与継続により，48週時には86%，72週時には92%がHBV DNA＜400 copies/mLに至った（表3）。

本試験における安全性については，投与後72週までの薬剤に起因する重篤な有害事象およびグレード3/4の臨床検査値異常については，両群間で同様であった（表4）。また，両群とも，クレアチニン0.5 mg/dL以上の増加やクレアチニンクリアランス＜50 mL/minを示す症例は認められず，腎関連の有害事象による投与中止例も認められなかった。

以上より，HBe抗原陰性のHBV単独感染患者において，TDFは効果が強力であり，HBV DNAの抑制に関しては，TDFの方が，ADVより優れていることが示された。また，TDFは，ADVと同様に忍容性や安全性が良好であることが示された。

2. HBe抗原陽性のHBV単独感染患者におけるTDFの抗HBV作用（Study GS-US-174-0103；103試験）[3, 4]

本試験は，未治療のHBV単独感染患者（HBe抗原陽性）266例を対象に，TDF 300mgの1日1回投与（以下，TDF群。N=176）とADV 10mgの1日1回投与（以下，ADV群。N=90）を比較した多施設二重盲検の無作為化コントロール試験である。主要評価項目としては，102試験と同様に，肝線維化の悪化を伴わず，投与後48週に完全寛解（血清HBV DNA＜400 copies/mL，かつ組織学的改善：Knodell壊死炎症スコア2ポイント以上の低下）に至った症例の比率である。48週以降，102試験と同様に，TDF群はTDFを継続投与（TDF-TDF群），ADV群はTDFに切り替えて（ADV-TDF群），さらに4年間（計5年間=240週間）の観察を行うが，72週までの結果が得られている。

表4　HBe抗原陰性のHBV単独感染患者における72週までの安全性データ（102試験）

	TDF-TDF (N=250)	ADV-TDF (N=125)
Study Drug-Related SAE	0.4%	0.8%
G3 Laboratory G4 Laboratory	14% 5%	13% 2%
Confirmed ↓phosphorus < 2mg/dL	2%	0
Confirmed 0.5 mg/dL↑ in creatinine	0	0
Confirmed creatinine clearance <50 mL/min	0	0

表5　ベースライン時の患者背景（103試験）

	TDF (N=176)	ADV (N=90)
Mean Age	34	34
Race Caucasian Asian	 52% 36%	 51% 36%
Male	68%	71%
Mean HBV (log10 copies/mL)	8.64	8.88
Mean ALT (U/mL)	142	155
Mean Knodell Necroinflammatory Score	8.3	8.5
Knodell Fibrosis (Score = 4)	20%	21%
Viral Genotype A B C D	 24% 15% 25% 32%	 21% 11% 30% 35%

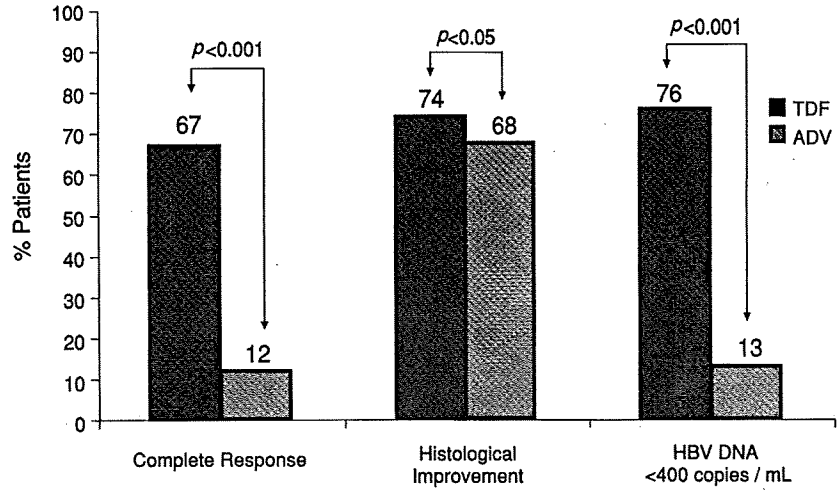

図5　103試験　主要評価項目および副次的評価項目

ベースライン時の患者背景は，両群ともに同様であった（表5）。平均HBV DNA量はTDF群8.64 $\log_{10}$ copies/mL，ADV群 8.88 $\log_{10}$ copies/mLであり，平均ALT値はTDF群 142 U/L，ADV群 155 U/Lであった。Knodell壊死炎症スコアはTDF群 8.3，ADV群 8.5であり，両群ともに約20%が肝硬変を有していた。

投与後48週において完全寛解に至った症例の比率は，TDF群は67%であり，ADV群の12%に比較して，有意に高率であった（$p<0.001$，図5）。また，HBV DNA＜400 copies/mLに至った症例比率は，TDF群が76%であり，ADV群の13%に比較して，有意に高率であった（$p<0.001$，図5）。一方，組織学的改善においては，両群で明らかな差は認められなかった（74% vs 68%, $p>0.05$，図5）。

投与後48週以降の結果を図6～10に示す。図6は，HBV DNA＜400 copies/mLに至った症例の比率の推移であるが，48週時ではADV群は13%とTDF群（76%）に比べ，有意に低い値であった。48週以降のADVからTDFへの切り替えによって，72週時には76%となった（TDF-TDF群：79%，$p=0.617$，図6）。図7は，平均HBV DNA量の減少の推移であるが，投与後48週時では，TDF群の方がADV群に比べ，有意に減少の程度は大きかった（$p<0.001$）。TDFに切り替えた後，ADV群のプラトー状態であった平均HBV DNA

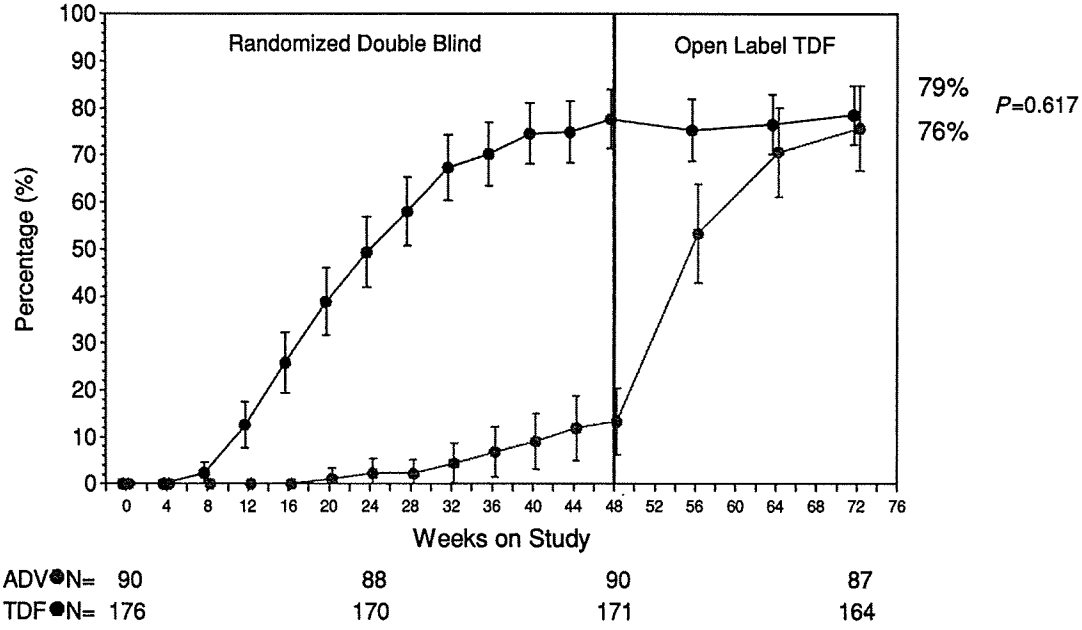

図6　HBV DNA＜400 copies/mLに至った症例比率（Missing=Failure，103試験）

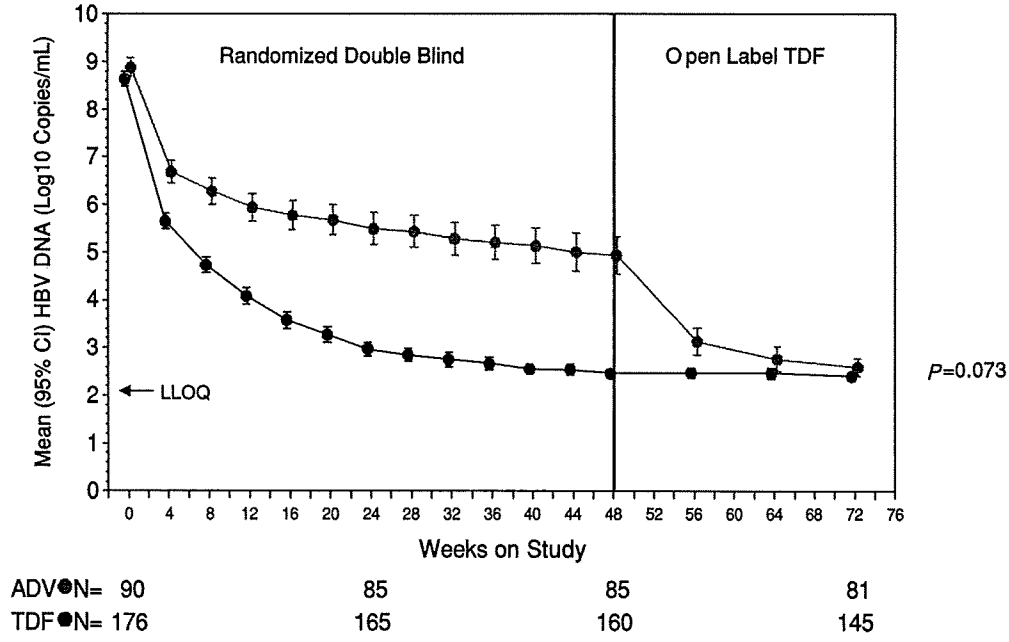

図7　平均HBV DNA量の推移（103試験）

量は，さらに減少を認め，72週時にはTDF-TDF群と同程度までに至った（$p = 0.073$）。ALTの正常化を示した症例の比率は，TDF-TDF群では72週時，77%であったが，ADV-TDF群では依然，72週時，TDFに切り替え以降も61%と有意に低率であった（$p = 0.014$，図8）。ADV-TDF群において，TDFへの切り替えに対する反応性をみたところ，ADV投与後48週時にHBV DNA＜400 copies/mLであった症例は，TDFに切り替えても全例がHBV DNA＜400 copies/mLを維持しており，また，ADV投与後48週時にHBV DNA≧400

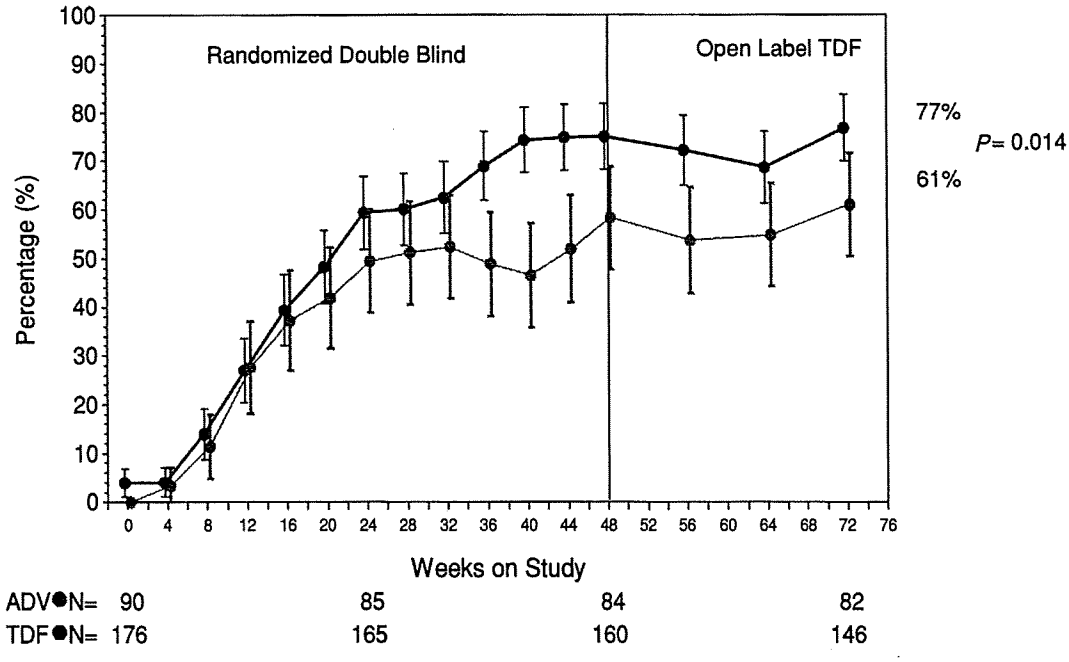

図8　ALTの正常化に至った症例比率（Missing=Failure，103試験）

表6　ADV-TDF群におけるTDF切り替えに対するHBV DNAの反応性（103試験）

Week 48 ADV Viral Response	Week 72 TDF complete Viral Response
Complete Response (＜400 copies/mL)	12 (100%)
Incomplete Response (≥400 copies/mL)	54 (78%)

表7　TDF-TDF群における24週時ウイルス不応例に対する転帰（103試験）

Week 24 Viral Response	W48 Complete Viral Response (＜400 copies/mL)	W72 Complete Viral Response (＜400 copies/mL)
Complete Response (＜400 copies/mL)	80 (100%)	71 (100%)
Incomplete Response (≥400 copies/mL)	50 (66%)	56 (79%)

copies/mLであった症例の78%が，TDFに切り替えたことによって，HBV DNA＜400 copies/mLに至った（表6）。TDF-TDF群において，TDF投与後24週時にHBV DNA＜400 copies/mLであった症例は，その後も100%の症例がHBV DNA＜400 copies/mLを維持していた（表7）。また，TDF投与後24週時にウイルスがコントロールされていなかった症例は，その後のTDF投与継続により，48週時には66%，72週時には79%がHBV DNA＜400 copies/mLに至った（表7）。図9に投与後48週および64週（ADV群ではTDFに切り替え後24週）のHBeセロコンバージョンの比率を示した。64週時のTDF-TDF群におけるHBeセロコンバージョン率は26%であり，ADV-TDF群の21%と比較して，明らかな差はなかった（p = NS）。一方，HBsセロコンバージョン（図10）については，「HBs抗原の消失」に至った症例は，64週時，ADV-TDF群では0%であったが，TDF-TDF群では48週以降，3%から5%に増加し，有意差が認められた（p = 0.004）。一方，「HBs抗体の出現」まで至った症例は，ADV-TDF群では64週時に0%であったが，TDF-TDF群では48週以降，1%から2%に増加していた（p=NS）。

本試験における安全性については，投与後72週までの薬剤に起因する重篤な有害事象およびグレード3/4の臨床検査値異常については，両群間で顕著な差は認められなかった（表8）。また，ADV-TDF群で1%にクレアチニン0.5 mg/dL以上の増加が認められたが，これ以外には，両群においてクレアチニンクリアランス＜50 mL/minを示す症例は認められず，腎関連の有害事象による投与中止例も認められなかった。

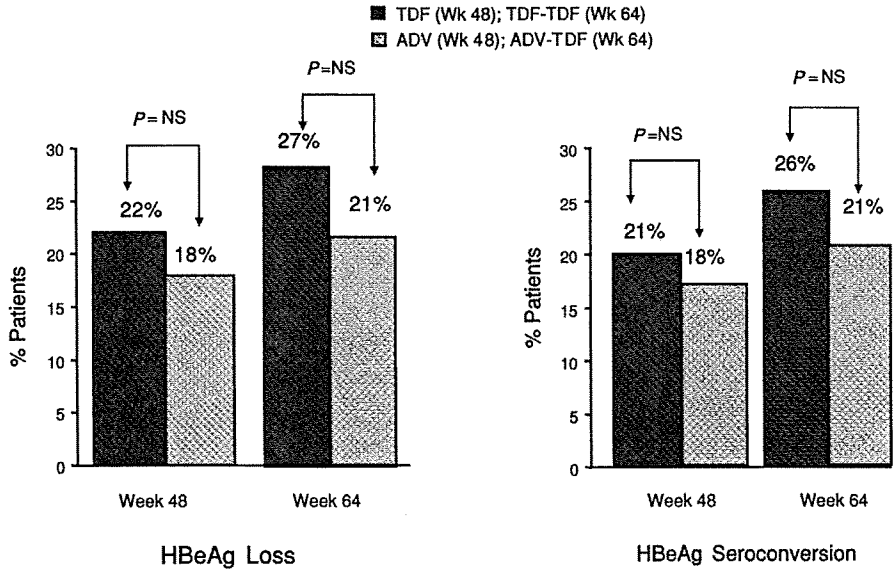

図9　投与後48週および64週におけるHBeセロコンバーション（103試験）

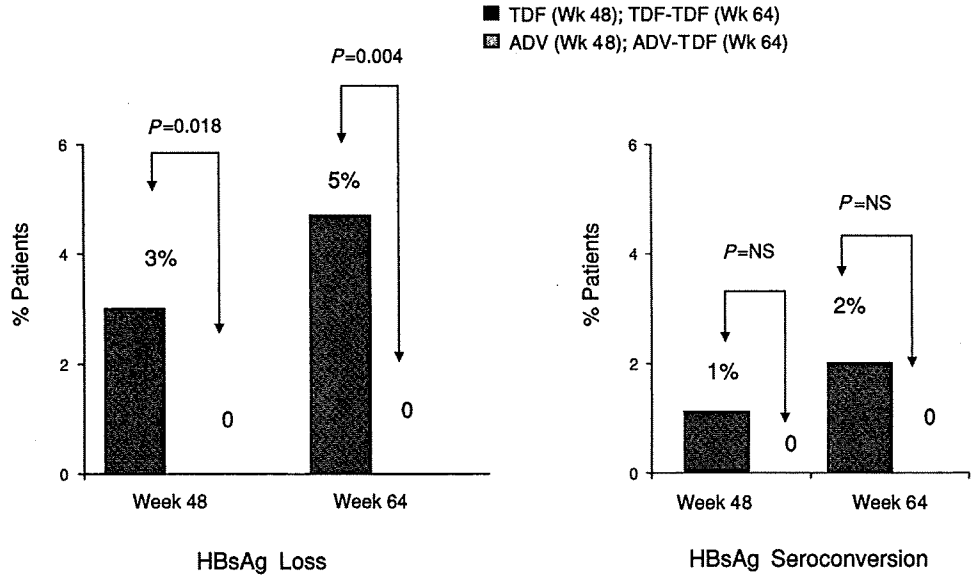

図10　投与後48週および64週におけるHBsセロコンバージョン（103試験）

以上より，HBe抗原陽性のHBV単独感染患者において，TDFは効果が強力であり，HBV DNAの抑制に関しては，TDFの方が，ADVより優れていることが示された。また，TDFは，ADVと同様に忍容性や安全性が良好であることが示された。さらに，102試験および103試験の結果より，TDF投与後24週時にウイルスが検出限界未満に至っていないということは，TDFの治療失敗を予知させるものではなく，TDFを継続投与することにより，ウイルスが検出限界未満にコントロールされるというベネフィットがある。

3. TDFの安全性

概説した2試験の安全性については試験毎に記載したが，これらの試験において，TDFの安全性はADVと同様であり，忍容性が高いことが示された。これまでのHIV/HBV合併例におけるTDFの使用経験においても，特記すべき重篤な副作用は報告されていない。また，TDF投与中，ALTの上昇を示した症例が認められているが，肝不全に至った症例はない。

TDFの最も注意すべき副作用として，腎障害がHIV感染症患者で報告されているが，今回の2試験を