Table 4. Applications of MVA and C4 as biomarkers for cholesterol and bile acid biosynthesis.

	MVA	C4	References
HMGCR inhibitors	decrease	–	(Nozaki, 1996; Naoumova, 1996; Yoshida, 1997; Naoumova, 1997; Pfohl, 1998; O'Neill, 2001; Pappu, 2002)
	–	no effect	(Yoshida, 1997; Naoumova, 1999; O'Neill, 2001)
HMGCR inhibitors with partial ileal resection	decrease	decrease	(Naoumova, 1999)
Insulin	decrease	–	(Lala, 1994; Scoppola, 1995; Naoumova, 1996)
Growth hormone	no effect	–	(Boyle, 1992)
	–	no effect	(Leonsson, 1999; Lind, 2004)
Thyroid hormone	–	no effect	(Sauter, 1997)
CDCA	–	decrease	(Einarsson, 2001)
DCA	–	decrease	(Einarsson, 2001)
UDCA	–	increase	(Sauter, 2004)
Rifampin	–	increase	(Lutjohann, 2004)
Gallstone	–	increase	(Muhrbeck, 1997; Gälman, 2004)
Liver cirrhosis	no change	decrease	(Yoshida, 1999)
Diarrhea	–	increase	(Eusufzai, 1993; Sauter, 1999)

rhythm of cholesterol biosynthesis. These results were different from previous studies. Further investigations will be required to elucidate the reason for the discrepancy.

HMGCR inhibitors

The measurement of plasma MVA concentration is very useful to evaluate the *in vivo* effects of HMGCR inhibitors. Pfohl et al. reported that the HMGCR inhibitor, simvastatin, rapidly down-regulated cholesterol biosynthesis, which was then up-regulated when the drug was withdrawn (Pfohl et al. 1998). Nozaki et al. investigated the difference in the effect of another HMGCR inhibitor, pravastatin, on cholesterol biosynthesis between the morning and the evening. They administered pravastatin to the same patients in the morning or evening, and found that morning and evening administrations of pravastatin elicited equivalent reductions in the plasma and urinary MVA concentrations (Nozaki et al. 1996). Pappu and Illingworth demonstrated that patients with familial hypercholesterolemia exhibited a diurnal pattern in plasma MVA levels similar to that reported previously in controls (Pappu and Illingworth, 2002). In addition, they reported that the administration of lovastatin in the evening reduced the nocturnal increases in MVA levels, and the administration of simvastatin completely abolished the nighttime rise. Naoumova et al. treated familial hypercholesterolemia patients with 3 different HMGCR inhibitors, pravastatin, simvastatin, and atorvastatin, and showed that the patients who responded well to statins exhibited higher basal plasma levels of MVA (Naoumova et al. 1996).

We investigated the short-term effects of pravastatin on cholesterol and bile acid biosynthesis by measuring MVA and C4 as biomarkers (Yoshida et al. 1997). Six male volunteers were administered 40 mg of pravastatin, and the plasma MVA and C4 levels were measured every 2 hours. The plasma MVA levels 2 hours after the administration of pravastatin were decreased compared with those in controls. While the decrease in MVA concentrations continued for 8 hours, the plasma C4 concentrations did not change during the initial 6 hours and then decreased 8 hours after the administration. Three-way analysis of this study indicated that the MVA level was influenced significantly by

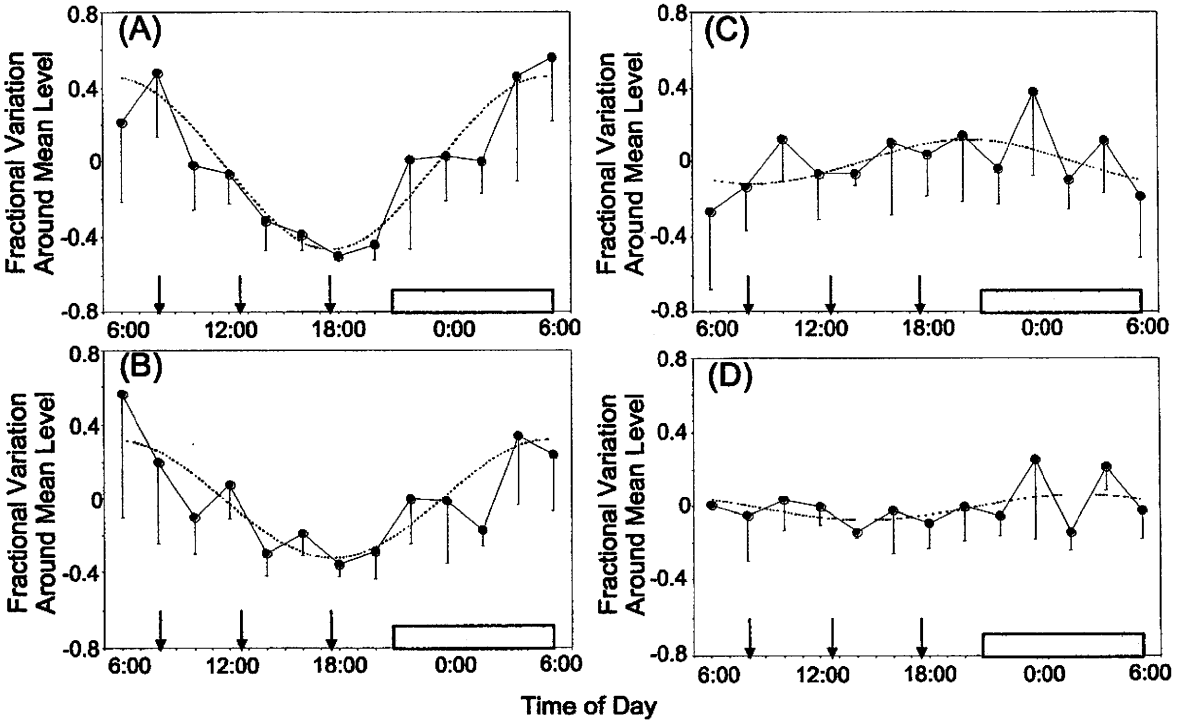

Figure 3. The circadian rhythm of the plasma levels of C4 and 7A in three normal volunteers. The volunteers consumed a normal hospital diet three times a day (shown by arrows), and slept from 21:00 on the first day to 6:00 on the second day (shown by the shaded box). The values are expressed as fractional variations around the mean levels (mean ± SD). Dashed lines represent the curves of best fit. **(A)** C4; **(B)** free 7A; **(C)** esterified 7A; **(D)** total 7A. Reprinted with minor modification from our previous paper (Yoshida et al. 1994), Copyright (1994), with permission from Elsevier.

both pravastatin treatment and the time-course. In contrast, C4 level was affected significantly by both inter-individual differences and time-course, but not by pravastatin treatment. These results indicated that cholesterol biosynthesis was inhibited by pravastatin treatment, but bile acid biosynthesis was not influenced in normal subjects (Yoshida et al. 1997). Naoumova et al. treated familial hypercholesterolemia patients with atorvastatin and partial ileal bypass (Naoumova et al. 1999). Atorvastatin decreased the rate of bile acid synthesis only when bile acid synthesis was up-regulated by partial ileal bypass or bile acid sequestrants, presumably by limiting the supply of newly synthesized free cholesterol.

Hormones

There are several reports that show the effects of hormones e.g. insulin, growth hormone and thyroid hormone, on *in vivo* cholesterol metabolism. Euglycemic hyperinsulinemia acutely decreased the circulating levels of MVA (Lala et al. 1994), which indicated that insulin could decrease cholesterol biosynthesis. Naoumova et al. also investigated the effects of hyperinsulinemia on the plasma MVA concentrations and reported that acute hyperinsulinemia decreased cholesterol biosynthesis less in the subjects with non-insulin-dependent diabetes mellitus compared with non-diabetic subjects, which suggests that the patients with non-insulin-dependent diabetes mellitus exhibit insulin resistance (Naoumova et al. 1996).

Because plasma growth hormone levels and cholesterol biosynthesis are both increased during sleep, Boyle et al. speculated that growth hormone might stimulate *de novo* cholesterol biosynthesis (Boyle et al. 1992). However, the peak nocturnal and fasting MVA concentrations did not correlate with the growth hormone levels, and they concluded that nocturnal growth hormone secretion was not related to the stimulation of cholesterol production during sleep.

Patients with hypothyroidism exhibit hypercholesterolemia, while those with hyperthyroidism exhibit hypocholesterolemia. Sauter et al. measured serum C4 concentrations before and after treatment for hypo- and hyperthyroidism and

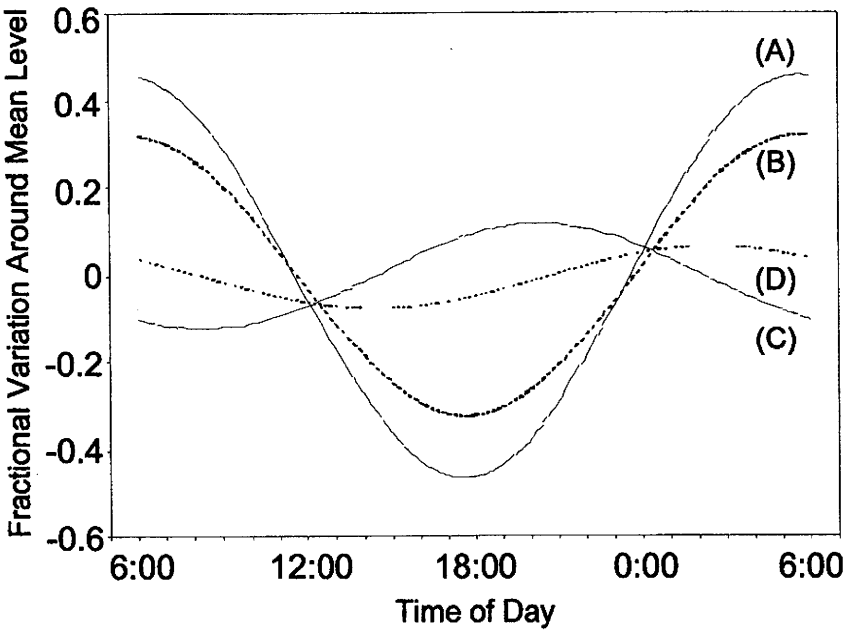

Figure 4. The curves of best fit for C4 and 7A. **(A)** C4, $y = 0.46 \cos((2\pi/24)t - 1.46)$ ($p < 0.005$); **(B)** Free 7A, $y = 0.32 \cos((2\pi/24)t - 1.48)$ ($p < 0.005$); **(C)** Esterified 7A, $0.12 \cos((2\pi/24)t + 0.97)$ (NS); **(D)** Total 7A, $0.07 \cos((2\pi/24)t - 0.65)$ (NS). Statistical significance was evaluated by a Zero-amplitude test by Nelson et al. (Nelson et al. 1979). Reprinted with minor modification from our previous paper (Yoshida et al. 1994), Copyright (1994), with permission from Elsevier.

showed that in humans, thyroid hormones influenced the serum cholesterol concentrations by mechanisms other than through modification of the CYP7A1 activity (Sauter et al. 1997).

Bile acids

Bile acids, particularly chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA), are physiological ligands for the farnesoid X receptor (FXR, NR1H4). They inhibit bile acid biosynthesis through activation of this nuclear receptor. In fact, Einarsson et al. reported that the treatment of healthy subjects with CDCA or DCA reduced the serum concentrations of C4 (Einarsson et al. 2001). They also found that CDCA reduced cholesterol biosynthesis while DCA did not when they evaluated *in vivo* cholesterol biosynthesis by measuring the serum 7-dehydrocholesterol concentrations. In contrast, UDCA treatment for 40 days did not affect cholesterol synthesis, as evaluated by urinary excretion of MVA, but the same treatment significantly increased bile acid biosynthesis determined by serum C4 concentrations (Sauter et al. 2004).

Hepatobiliary diseases

Cholesterol gallstone disease is caused by abnormal cholesterol and bile acid metabolism. The formation of cholesterol supersaturated bile is one of the important factors in the pathogenesis of this disease. Shoda et al. proposed an estimated biliary cholesterol saturation index $(CSI)_E = 1[MVL] + 0.7[C4]$ that was calculated by multivariate linear regression analysis using the plasma MVA and C4 concentrations of patients with hyperlipoproteinemia and demonstrated that this convenient calculation of $(CSI)_E$ corresponded well to actual biliary CSI (Shoda et al. 1997). However, the hypersecretion of biliary cholesterol in patients with gallstones does not seem to be due to increased hepatic synthesis of cholesterol or decreased catabolism of cholesterol to bile acids. This could be because the plasma levels of lathosterol were not significantly different between gallstone subjects and controls and the C4 levels were about 40% higher in the gallstone subjects compared with the controls (Muhrbeck et al. 1997). The increased bile acid biosynthesis determined by the plasma C4 levels, corrected for plasma cholesterol, was also reported in gallstone subjects and gallstone high-risk Mapuche Indians (Gälman et al. 2004).

Conversely, some hepatobiliary diseases affect cholesterol and bile acid metabolism. In patients with liver cirrhosis (LC), the blood cholesterol levels are relatively preserved, despite other

markers, including the serum albumin levels, show liver dysfunction. We studied the association between hepatic cholesterogenesis and bile acid synthesis in hepatocellular impairment using the plasma levels of MVA and C4 (Yoshida et al. 1999). There were no significant differences in the plasma MVA levels between chronic hepatitis (CH), LC and control groups. In contrast, plasma C4 levels were significantly lower in LC compared with the CH and control groups. Although the MVA levels did not correlate with the Child-Pugh's score, which reflects the severity of liver damage (Albers et al. 1989), there was a significant correlation between the C4 level and Child-Pugh's score. In addition, plasma C4 levels in the control subjects correlated positively with the MVA levels, but there was no significant correlation between these biomarkers in CH and LC patients. Therefore, it was concluded that in the patients with chronic liver disease, there was a tendency for hepatic cholesterogenesis to be sustained in the face of hepatocellular dysfunction, while bile acid synthesis declined in parallel with the severity of impairment.

Perspectives

Biological specimens contain many types of organic acids and sterols. While fatty acids and cholesterol are relatively abundant compounds, MVA and oxysterols (7A and C4) are minor components. To quantify the concentrations of such minor components, stable isotope dilution mass spectrometry (GC-MS or LC-MS/MS) is an ideal method because of its high sensitivity, specificity and accuracy.

Recently, LC-MS/MS has come to be used more readily than GC-MS. Because MS/MS is more specific than MS, sample preparation process for the elimination of interfering materials can be simplified. In addition, LC-MS/MS does not require a derivatization step, which is also advantageous for high-throughput analyses. However, simple and rapid procedures do not always produce good results for the microanalysis of biological samples. A careful sample purification can increase the sensitivity of an analyte by reducing matrix effect (Jemal et al. 2003). Derivatization is useful not only to increase the sensitivity by enhancing the ionization efficiency but also to give a prominent ion in the high mass region, which makes it possible to avoid interfering peaks and to increase the specificity. A thorough chromatographic separation is also important to distinguish between similar biological compounds, e.g. hydroxycholesterols that have the same molecular weight and a virtually identical MS/MS spectrum. Thus, the importance of basic analytical techniques, i.e. sample purification, derivatization and chromatographic separation will not be denied even if the performance of mass spectrometer is improved further.

In conclusion, the methods for the quantification of key intermediates in cholesterol and bile acid biosynthetic pathways using stable isotope dilution mass spectrometry exhibit superior accuracy and sensitivity. By using this technique, the MVA and oxysterols in blood were established as biomarkers for cholesterol and bile acid biosynthesis. The use of these biomarkers has made it possible to monitor *in vivo* cholesterol and bile acid synthesis without the need for invasive liver biopsy, which is very useful for basic or clinical studies of cholesterol metabolism in humans.

References

Abrar, M. and Martin, P.D. 2002. Validation and application of an assay for the determination of mevalonic acid in human plasma by liquid chromatography tandem mass spectrometry. *J. Chromatogr. B.*, 773:103–11.

Albers, I., Hartmann, H., Bircher, J. et al. 1989. Superiority of the Child-Pugh classification to quantitative liver function tests for assessing prognosis of liver cirrhosis. *Scand. J. Gastroenterol.*, 24:269–76.

Axelson, M., Aly, A. and Sjövall, J. 1988. Levels of 7α-hydroxy-4-cholesten-3-one in plasma reflect rates of bile acid synthesis in man. *FEBS Lett*, 239:324–8.

Axelson, M., Björkhem, I., Reihnér, E. et al. 1991. The plasma level of 7α-hydroxy-4-cholesten-3-one reflects the activity of hepatic cholesterol 7α-hydroxylase in man. *FEBS Lett.*, 284:216–8.

Björkhem, I. and Danielsson, H. 1974. Assay of liver microsomal cholesterol 7α-hydroxylase using deuterated carrier and gas chromatography-mass spectrometry. *Anal. Biochem.*, 59:508–16.

Björkhem, I., Miettinen, T., Reihnér, E. et al. 1987a. Correlation between serum levels of some cholesterol precursors and activity of HMG-CoA reductase in human liver. *J. Lipid Res.*, 28:1137–43.

Björkhem, I., Reihnér, E., Angelin, B. et al. 1987b. On the possible use of the serum level of 7α-hydroxycholesterol as a marker for increased activity of the cholesterol 7α-hydroxylase in humans. *J. Lipid Res.*, 28:889–94.

Boyle, P.J., Avogaro, A., Smith, L. et al. 1992. Role of GH in regulating nocturnal rates of lipolysis and plasma mevalonate levels in normal and diabetic humans. *Am. J. Physiol.*, 263:E168–72.

Buffalini, M., Pierleoni, R., Guidi, C. et al. 2005. Novel and simple high-performance liquid chromatographic method for determination of 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity. *J. Chromatogr. B.*, 819:307–13.

Cighetti, G., Santaniello, E. and Galli, G. 1981. Evaluation of 3-hydroxy-3-methylglutaryl-CoA reductase activity by multiple-selected ion monitoring. *Anal. Biochem.*, 110:153–8.

Del Puppo, M., Cighetti, G., Galli Kienle, M. et al. 1989. Measurement of mevalonate in human plasma and urine by multiple selected ion monitoring. *Biomed. Environ. Mass Spectrom.*, 18:174–6.

Dietschy, J.M. and Brown, M.S. 1974. Effect of alterations of the specific activity of the intracellular acetyl CoA pool on apparent rates of hepatic cholesterogenesis. *J. Lipid Res.*, 15:508–16.

Duane, W.C., Levitt, D.G., Mueller, S.M. et al. 1983. Regulation of bile acid synthesis in man. Presence of a diurnal rhythm. *J. Clin. Invest.*, 72:1930–6.

Duane, W.C. and Javitt, N.B. 1999. 27-hydroxycholesterol: production rates in normal human subjects. *J. Lipid Res.*, 40:1194–9.

Einarsson, C., Hillebrant, C.G. and Axelson, M. 2001. Effects of treatment with deoxycholic acid and chenodeoxycholic acid on the hepatic synthesis of cholesterol and bile acids in healthy subjects. *Hepatology.*, 33:1189–93.

Eusufzai, S., Axelson, M., Angelin, B. et al. 1993. Serum 7α-hydroxy-4-cholesten-3-one concentrations in the evaluation of bile acid malabsorption in patients with diarrhoea: correlation to SeHCAT test. *Gut.*, 34:698–701.

Everson, G.T. 1992. Bile acid metabolism and its role in human cholesterol balance. *Semin. Liver Dis.*, 12:420–8.

Galli Kienle, M., Galli, G., Bosisio, E. et al. 1984. Evaluation of enzyme activities by gas chromatography-mass spectrometry: HMGCoA reductase and cholesterol 7α-hydroxylase. *J. Chromatogr.*, 289:267–76.

Gälman, C., Arvidsson, I., Angelin, B. et al. 2003. Monitoring hepatic cholesterol 7α-hydroxylase activity by assay of the stable bile acid intermediate 7α-hydroxy-4-cholesten-3-one in peripheral blood. *J. Lipid Res.*, 44:859–66.

Gälman, C., Miquel, J.F., Perez, R.M. et al. 2004. Bile acid synthesis is increased in Chilean Hispanics with gallstones and in gallstone high-risk Mapuche Indians. *Gastroenterology*, 126:741–8.

Gälman, C., Angelin, B. and Rudling, M. 2005. Bile acid synthesis in humans has a rapid diurnal variation that is asynchronous with cholesterol synthesis. *Gastroenterology*, 129:1445–53.

Goldfarb, S. and Pitot, H.C. 1971. Improved assay of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Lipid Res.*, 12:512–5.

Hagenfeldt, L. and Hellström, K. 1972. Blood concentration and turnover of circulating mevalonate in the rat. *Life Sci.*, 11:669–76.

Hahn, C., Reichel, C. and von Bergmann, K. 1995. Serum concentration of 7α-hydroxycholesterol as an indicator of bile acid synthesis in humans. *J. Lipid Res.*, 36:2059–66.

Hiramatsu, M., Hayashi, A., Hidaka, H. et al. 1998. Enzyme immunoassay of urinary mevalonic acid and its clinical application. *Clin. Chem.*, 44:2152–7.

Hiraoka, K. and Kudaka, I. 1992. Nagative-mode electrospray-mass spectrometry using nonaqueous solvents. *Rapid Commun. Mass Spectrom.*, 6:265–8.

Honda, A., Shoda, J., Tanaka, N. et al. 1991. Simultaneous assay of the activities of two key enzymes in cholesterol metabolism by gas chromatography-mass spectrometry. *J. Chromatogr.*, 565:53–66.

Honda, A., Yoshida, T., Xu, G. et al. 2004. Significance of plasma 7α-hydroxy-4-cholesten-3-one and 27-hydroxycholesterol concentrations as markers for hepatic bile acid synthesis in cholesterol-fed rabbits. *Metabolism*, 53:42–8.

Honda, A., Mizokami, Y., Matsuzaki, Y. et al. 2007a. Highly-sensitive assay of HMG-CoA reductase activity by LC-ESI-MS/MS. *J. Lipid Res.*, 48:1212–20.

Honda, A., Yamashita, K., Numazawa, M. et al. 2007b. Highly-sensitive quantification of 7α-hydroxy-4-cholesten-3-one in human serum by LC-ESI-MS/MS. *J. Lipid Res.*, 48:458–64.

Hylemon, P.B., Studer, E.J., Pandak, W.M. et al. 1989. Simultaneous measurement of cholesterol 7α-hydroxylase activity by reverse-phase high-performance liquid chromatography using both endogenous and exogenous [4–14C]cholesterol as substrate. *Anal. Biochem.*, 182:212–6.

Ishihama, Y., Mano, N., Oda, Y. et al. 1994. Simple and sensitive quantitation method for mevalonic acid in plasma using gas chromatography/mass spectrometry. *Rapid Commun. Mass Spectrom.*, 8:377–80.

Jemal, M., Schuster, A. and Whigan, D.B. 2003. Liquid chromatography/tandem mass spectrometry methods for quantitation of mevalonic acid in human plasma and urine: method validation, demonstration of using a surrogate analyte, and demonstration of unacceptable matrix effect in spite of use of a stable isotope analog internal standard. *Rapid Commun. Mass Spectrom.*, 17:1723–34.

Kempen, H.J.M., Glatz, J.F.C., Leuven, J.A.G. et al. 1988. Serum lathosterol concentration is an indicator of whole-body cholesterol synthesis in humans. *J. Lipid Res.*, 29:1149–55.

Lala, A., Scoppola, A., Ricci, A. et al. 1994. The effects of insulin on plasma mevalonate concentrations in man. *Ann. Nutr. Metab.*, 38:257–62.

Leonsson, M., Oscarsson, J., Bosaeus, I. et al. 1999. Growth hormone (GH) therapy in GH-deficient adults influences the response to a dietary load of cholesterol and saturated fat in terms of cholesterol synthesis, but not serum low density lipoprotein cholesterol levels. *J. Clin. Endocrinol. Metab.*, 84:1296–303.

Lind, S., Rudling, M., Ericsson, S. et al. 2004. Growth hormone induces low-density lipoprotein clearance but not bile acid synthesis in humans. *Arterioscler. Thromb. Vasc. Biol.*, 24:349–56.

Lutjohann, D., Hahn, C., Prange, W. et al. 2004. Influence of rifampin on serum markers of cholesterol and bile acid synthesis in men. *Int. J. Clin. Pharmacol. Ther.*, 42:307–13.

Mitropoulos, K.A. and Balasubramaniam, S. 1972. Cholesterol 7α-hydroxylase in rat liver microsomal preparations. *Biochem. J.*, 128:1–9.

Miyazaki, H., Koyama, M., Hashimoto, M. et al. 1978. Assay for 3-hydroxy-3-methylglutaryl coenzyme A reductase by selected ion monitoring. In Stable Isotopes: Proceedings of the Third International Conference, Oak Brook, IL, May 23–26, 1978. Klein, ER. and Klein PD, editors. 267–73.

Muhrbeck, O., Wang, F.H., Björkhem, I. et al. 1997. Circulating markers for biosynthesis of cholesterol and bile acids are not depressed in asymptomatic gallstone subjects. *J. Hepatol.*, 27:150–5.

Naoumova, R.P., Cummings, M.H., Watts, G.F. et al. 1996. Acute hyperinsulinaemia decreases cholesterol synthesis less in subjects with non-insulin-dependent diabetes mellitus than in non-diabetic subjects. *Eur. J. Clin. Invest.*, 26:332–40.

Naoumova, R.P., Marais, A.D., Mountney, J. et al. 1996. Plasma mevalonic acid, an index of cholesterol synthesis in vivo, and responsiveness to HMG-CoA reductase inhibitors in familial hypercholesterolaemia. *Atherosclerosis*, 119:203–13.

Naoumova, R.P., Dunn, S., Rallidis, L. et al. 1997. Prolonged inhibition of cholesterol synthesis explains the efficacy of atorvastatin. *J. Lipid Res.*, 38:1496–500.

Naoumova, R.P., O'Neill, F.H., Dunn, S. et al. 1999. Effect of inhibiting HMG-CoA reductase on 7α-hydroxy-4-cholesten-3-one, a marker of bile acid synthesis: contrasting findings in patients with and without prior up-regulation of the latter pathway. *Eur. J. Clin. Invest.*, 29:404–12.

Ndong-Akoume, M.Y., Mignault, D., Perwaiz, S. et al. 2002. Simultaneous evaluation of HMG-CoA reductase and cholesterol 7α-hydroxylase activities by electrospray tandem MS. *Lipids*, 37:1101–7.

Nelson, W., Tong, Y.L., Lee, J.K. et al. 1979. Methods for cosinor-rhythmometry. *Chronobiologia*, 6:305–23.

Noshiro, M., Ishida, H., Hayashi, S. et al. 1985. Assays for cholesterol 7α-hydroxylase and 12α-hydroxylase using high performance liquid chromatography. *Steroids*, 45:539–49.

Nozaki, S., Nakagawa, T., Nakata, A. et al. 1996. Effects of pravastatin on plasma and urinary mevalonate concentrations in subjects with familial hypercholesterolaemia: a comparison of morning and evening administration. *Eur. J. Clin. Pharmacol.*, 49:361–4.

Oda, H., Yamashita, H., Kosahara, K. et al. 1990. Esterified and total 7α-hydroxycholesterol in human serum as an indicator for hepatic bile acid synthesis. *J. Lipid Res.*, 31:2209–18.

Ogishima, T. and Okuda, K. 1986. An improved method for assay of cholesterol 7α-hydroxylase activity. *Anal. Biochem.*, 158:228–32.

O'Neill, F.H., Patel, D.D., Knight, B.L. et al. 2001. Determinants of variable response to statin treatment in patients with refractory familial hypercholesterolemia. *Arterioscler. Thromb. Vasc. Biol.*, 21:832–7.

Pappu, A.S. and Illingworth, D.R. 1994. Diurnal variations in the plasma concentrations of mevalonic acid in patients with abetalipoproteinaemia. *Eur. J. Clin. Invest.*, 24:698–702.

Pappu, A.S. and Illingworth, D.R. 2002. The effects of lovastatin and simvastatin on the diurnal periodicity of plasma mevalonate concentrations in patients with heterozygous familial hypercholesterolemia. *Atherosclerosis*, 165:137–44.

Park, E.J., Lee, D., Shin, Y.G. et al. 2001. Analysis of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors using liquid chromatography-electrospray mass spectrometry. *J. Chromatogr. B. Biomed. Sci. Appl.*, 754:327–32.

Parker, T.S., McNamara, D.J., Brown, C. et al. 1982. Mevalonic acid in human plasma: relationship of concentration and circadian rhythm to cholesterol synthesis rates in man. *Proc. Natl. Acad. Sci.* U.S.A., 79:3037–41.

Parker, T.S., McNamara, D.J., Brown, C.D. et al. 1984. Plasma mevalonate as a measure of cholesterol synthesis in man. *J. Clin. Invest.*, 74:795–804.

Pettersson, L. and Eriksson, C.G. 1994. Reversed-phase high-performance liquid chromatographic determination of 7α-hydroxy-4-cholesten-3-one in human serum. *J. Chromatogr. B. Biomed. Appl.*, 657:31–6.

Pfohl, M., Naoumova, R.P., Kim, K.D. et al. 1998. Use of cholesterol precursors to assess changes in cholesterol synthesis under non-steady-state conditions. *Eur. J. Clin. Invest.*, 28:491–6.

Pooler, P.A. and Duane, W.C. 1988. Effects of bile acid administration on bile acid synthesis and its circadian rhythm in man. *Hepatology*, 8:1140–6.

Popjak, G., Boehm, G., Parker, T.S. et al. 1979. Determination of mevalonate in blood plasma in man and rat. Mevalonate "tolerance" tests in man. *J. Lipid Res.*, 20:716–28.

Saini, G.S., Wani, T.A., Gautam, A. et al. 2006. Validation of LC-MS/MS method for quantification of mevalonic acid in human plasma and an approach to differentiate recovery and matrix effect. *J. Lipid Res.*, 47:2340–5.

Sanghvi, A., Grassi, E., Bartman, C. et al. 1981. Measurement of cholesterol 7α-hydroxylase activity with selected ion monitoring. *J. Lipid Res.*, 22:720–4.

Saisho, Y., Kuroda, T. and Umeda, T. 1997. A sensitive and selective method for the determination of mevalonic acid in dog plasma by gas chromatography/negative ion chemical ionization-mass spectrometry. *J. Pharm. Biomed. Anal.*, 15:1223–30.

Sauter, G., Berr, F., Beuers, U. et al. 1996. Serum concentrations of 7α-hydroxy-4-cholesten-3-one reflect bile acid synthesis in humans. *Hepatology*, 24:123–6.

Sauter, G., Weiss, M. and Hoermann, R. 1997. Cholesterol 7α-hydroxylase activity in hypothyroidism and hyperthyroidism in humans. *Horm. Metab. Res.*, 29:176–9.

Sauter, G.H., Munzing, W., von Ritter, C. et al. 1999. Bile acid malabsorption as a cause of chronic diarrhea: diagnostic value of 7α-hydroxy-4-cholesten-3-one in serum. *Dig. Dis. Sci.*, 44:14–9.

Sauter, G.H., Thiessen, K., Parhofer, K.G. et al. 2004. Effects of ursodeoxycholic acid on synthesis of cholesterol and bile acids in healthy subjects. *Digestion*, 70:79–83.

Schwarz, M., Lund, E.G., Setchell, K.D. et al. 1996. Disruption of cholesterol 7α-hydroxylase gene in mice. II. Bile acid deficiency is overcome by induction of oxysterol 7α-hydroxylase. *J. Biol. Chem.*, 271:18024–31.

Scoppola, A., Maher, V.M., Thompson, G.R. et al. 1991. Quantitation of plasma mevalonic acid using gas chromatography-electron capture mass spectrometry. *J. Lipid Res.*, 32:1057–60.

Scoppola, A., Testa, G., Frontoni, S. et al. 1995. Effects of insulin on cholesterol synthesis in type II diabetes patients. *Diabetes Care*, 18:1362–9.

Shapiro, D.J., Imblum, R.L. and Rodwell, V.W. 1969. Thin-layer chromatographic assay for HMG-CoA reductase and mevalomic acid. *Anal. Biochem.*, 31:383–90.

Shefer, S., Hauser, S. and Mosbach, E.H. 1968. 7α-Hydroxylation of cholesterol by rat liver microsomes. *J. Lipid Res.*, 9:328–33.

Shefer, S., Hauser, S., Lapar, V. et al. 1972. HMG CoA reductase of intestinal mucosa and liver of the rat. *J. Lipid Res.*, 13:402–12.

Shefer, S., Nicolau, G. and Mosbach, E.H. 1975. Isotope derivative assay of microsomal cholesterol 7α-hydroxylase. *J. Lipid Res.*, 16:92–6.

Shoda, J., Tanaka, N., He, B.F. et al. 1993. Alterations of bile acid composition in gallstones, bile, and liver of patients with hepatolithiasis, and their etiological significance. *Dig. Dis. Sci.*, 38:2130–41.

Shoda, J., Miyamoto, J., Kano, M. et al. 1997. Simultaneous determination of plasma mevalonate and 7α-hydroxy-4-cholesten-3-one levels in hyperlipoproteinemia: convenient indices for estimating hepatic defects of cholesterol and bile acid syntheses and biliary cholesterol supersaturation. *Hepatology*, 25:18–26.

Siavoshian, S., Simoneau, C., Maugeais, P. et al. 1995. Measurement of mevalonic acid in human urine by bench top gas chromatography-mass spectrometry. *Clin. Chim. Acta.*, 243:129–36.

Vlahcevic, Z.R., Pandak, W.M., Heuman, D.M. et al. 1992. Function and regulation of hydroxylases involved in the bile acid biosynthesis pathways. *Semin. Liver Dis.*, 12:403–19.

Vlahcevic, Z.R., Stravitz, R.T., Heuman, D.M. et al. 1997. Quantitative estimations of the contribution of different bile acid pathways to total bile acid synthesis in the rat. *Gastroenterology*, 113:1949–57.

Woollen, B.H., Holme, P.C., Northway, W.J. et al. 2001. Determination of mevalonic acid in human urine as mevalonic acid lactone by gas chromatography-mass spectrometry. *J. Chromatogr.*, 760:179–84.

Yamashita, H., Kuroki, S. and Nakayama, F. 1989. Assay of cholesterol 7α-hydroxylase utilizing a silica cartridge column and 5α-cholestane-3β,7β-diol as an internal standard. *J. Chromatogr.*, 496:255–68.

Yoshida, T., Honda, A., Tanaka, N. et al. 1993. Simultaneous determination of mevalonate and 7α-hydroxycholesterol in human plasma by gas chromatography-mass spectrometry as indices of cholesterol and bile acid biosynthesis. *J. Chromatogr.*, 613:185–93.

Yoshida, T., Honda, A., Tanaka, N. et al. 1994. Determination of 7α-hydroxy-4-cholesten-3-one level in plasma using isotope-dilution mass spectrometry and monitoring its circadian rhythm in human as an index of bile acid biosynthesis. *J. Chromatogr. B. Biomed. Appl.*, 655:179–87.

Yoshida, T., Honda, A., Shoda, J. et al. 1997. Short-term effects of 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor on cholesterol and bile acid synthesis in humans. *Lipids*, 32:873–8.

Yoshida, T., Honda, A., Matsuzaki, Y. et al. 1999. Plasma levels of mevalonate and 7α-hydroxy-4-cholesten-3-one in chronic liver disease. *J. Gastroenterol. Hepatol.*, 14:150–5.

ウルソデオキシコール酸

松﨑　靖司*
まつざき　やすし

- 抗ウイルス療法が無効であったり，副作用などで抗ウイルス療法が行えない場合，肝庇護療法を行うことで肝線維化抑制や肝発癌を抑制する必要がある．肝庇護療法は，肝炎を沈静化し肝細胞の再生を促すことにより，肝線維化進展を抑える治療法である．
- ウルソデオキシコール酸（UDCA）は胆石溶解剤として使用されていたが，後に原発性胆汁性肝硬変やC型慢性肝炎に対する有効性が確認された．
- 現在，C型慢性肝炎に対する効果的なUDCA投与量は600～900 mg/日であり，従来の150 mg/日は効果がきわめて少ない．
- ALT値30～70 IU/lの患者さんがUDCA投与でALT値正常化が得られやすい．ALT値が30 IU/lを超えたらUDCA投与を開始したほうがよい．

Key Words　肝庇護療法，ウルソデオキシコール酸（UDCA），強力ミノファーゲンC®（SNMC），ALT値

□ 肝庇護療法の位置づけ

C型慢性肝炎の治療目標は肝癌の発現阻止にほかならない．この目標を達成するためにいくつかの治療法があるが，そのなかで一番はじめに考慮されるのは，抗ウイルス療法である．これはC型肝炎ウイルスを排除する治療法で，現在のところ国内外ともにペグインターフェロンとリバビリンの併用療法が標準療法とされている．しかしその有効率は完全なものとはいえないのが現状である．また副作用の問題などで十分な抗ウイルス療法が行えない場合も少なくない．このように抗ウイルス療法を行えない場合には，肝庇護療法にて肝炎を鎮静化し，肝発癌を抑制する必要がある．

肝庇護療法はHCVを排除しないものの，肝炎を鎮静化し肝細胞の再生を促すことにより，肝線維化進展を抑える治療法である．C型慢性肝炎で肝庇護療法の適応になるのは，肝臓の炎症マーカーであるALTが異常値を示す患者さんで，抗ウイルス療法にてウイルス排除ができなかった患者さん，IFN療法の副作用により抗ウイルス療法を実施できない患者さん，実施できても規定の投与期間を完遂できない患者さん，また抗ウイルス療法を望まない患者さんがおもな対象者となる．

□ 肝庇護療法：ウルソデオキシコール酸

肝庇護療法の歴史は古く，これまで多くの治療法が試みられている．そのなかでもウルソデオキシコール酸（UDCA）とグリチルリチン製剤の注射薬の先発品であるウルソ®と強力ミノファーゲンC®（SNMC）は，有用性において科学的な根拠を有して使用されだした治療法とされている．

経口肝庇護療法の第一選択薬としては，UDCA（商品名：ウルソ®）があげられる．UDCAは胆汁酸製剤であり，古来より動物性生薬として珍重された「熊胆」の成分である．本邦において1970年代後半より胆石溶解剤として使用されるようになった．ウルソ®はすでに，胆石溶解療法剤として1978年に600 mg/日投与が保険適応認可となり，慢性肝疾患に対しては，原発性胆汁性肝硬変（PBC）に対して1999年に600～900 mg/日が保険適応となっている．これらは本邦において，二重盲検試験により有効性が確認され認可された科学的な根拠に基づく治療法である[1,2]．UDCAのPBCに対する有効性は，1987年フランスのPouponらにより初めて示された[3]．その後，著者らも同様にUDCAのPBCに対する有効性を報告した[4,5]．われわれの成績

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でもPBCにUDCA 600 mg/日投与開始1ヵ月後より，γ-GTP，Al-p，AST，ALTそれぞれ開始前に比し有意に低下しだし，2年後においても同様に有意に低下をする[5]．またさらに，慢性肝炎に対してわれわれはUDCA 300 mg/日を投与し，投与前に比べAST，ALTが有意に改善することも見いだした[4]．

これらPBCや慢性肝炎に対するUDCAの有効性の成績は，二重盲検法により本邦を含め世界から報告された[2,6,7]．作用機序について，われわれは臨床例からの検討した．原発性胆汁性肝硬変患者にUDCAを投与した時の血清胆汁酸分画の検討より，体内胆汁酸プールの変換の重要性を考えている[2,5]．UDCAの肝細胞保護作用に関しては，さまざまな角度より検討されている．しかし，いまだUDCA作用発現機序にはナゾの部分が多く存在していることも実状である．以下に現在考えられている作用機序をまとめてみる．UDCAの投与により，上記のごとく細胞障害性の胆汁酸がUDCAに置き換わり肝細胞膜が保護されることが第一に考えられている．またUDCAには抗酸化ストレス作用，免疫調整作用，抗アポトーシス作用もあり，肝細胞の保護に働いているとも報告されている．これら複合的な機序により，PBCばかりでなく，C型慢性肝炎に対してもUDCAは肝機能の改善効果を発揮するものとされる．

2007年3月にウルソ®はC型慢性肝疾患に対す

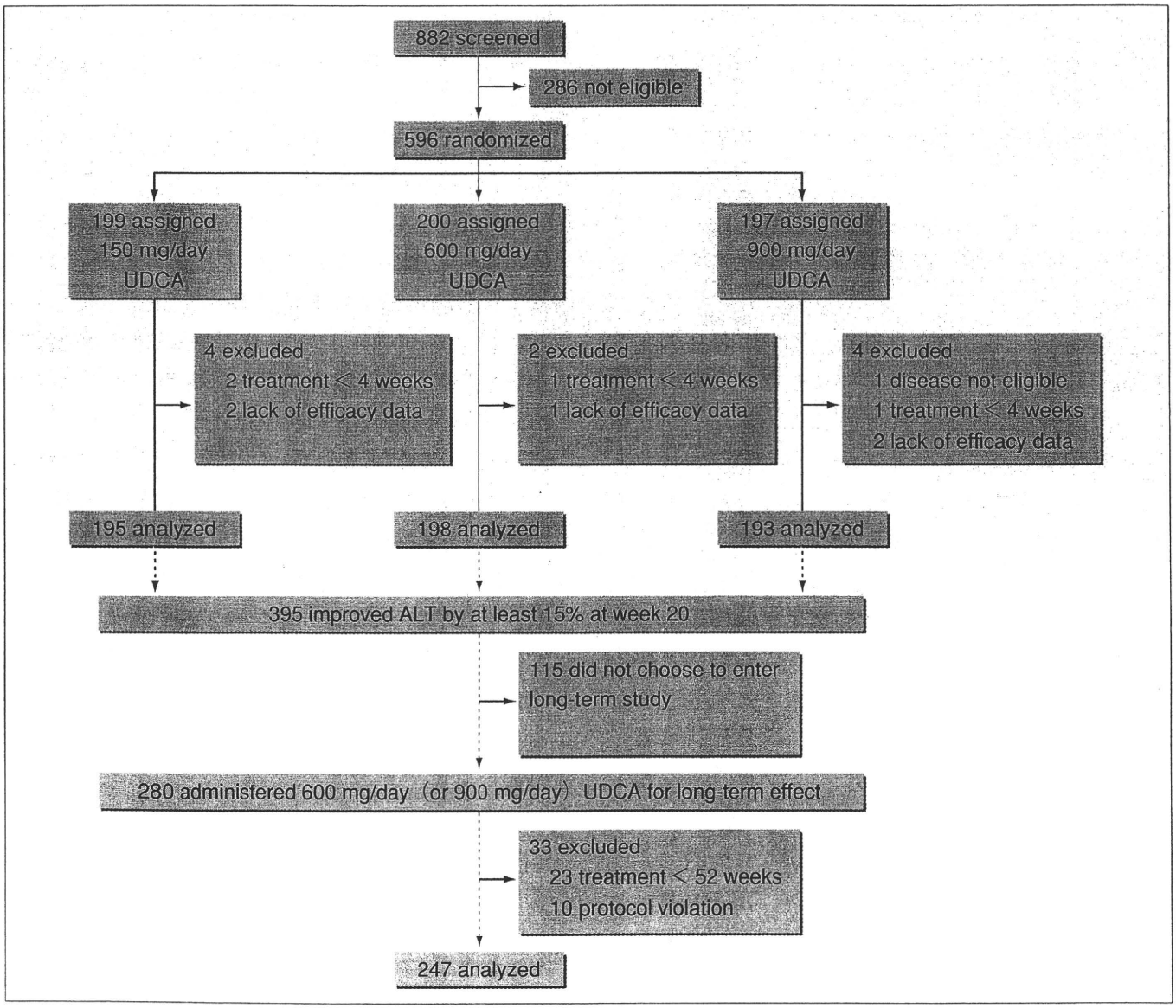

図1　慢性C型肝炎におけるUDCA二重盲験試験解析

（Omata M, et al：Gut 2007 Aug 30；[Epub ahead of print][8]より）

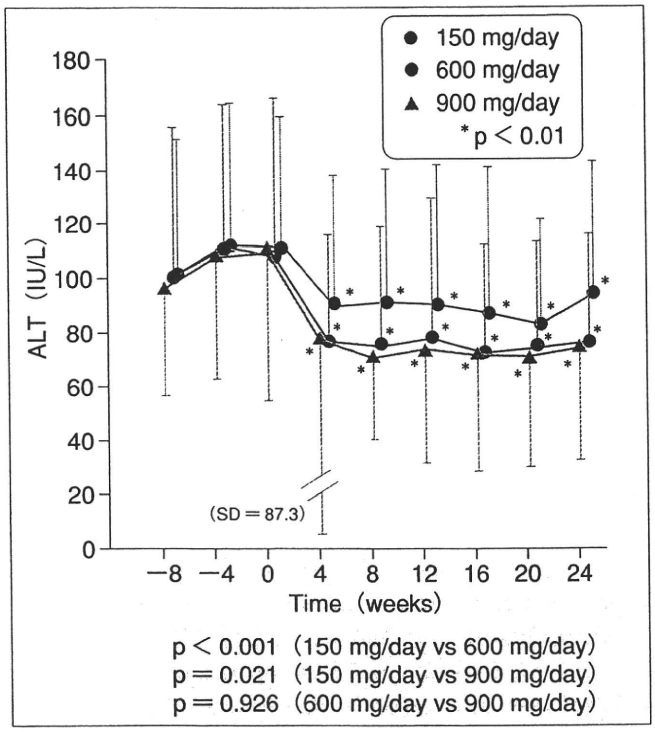

図 2　慢性 C 型肝炎における UDCA 二重盲験試験における，ALT の変動

(Omata M, et al : Gut 2007 Aug 30 ; [Epub ahead of print][8] より)

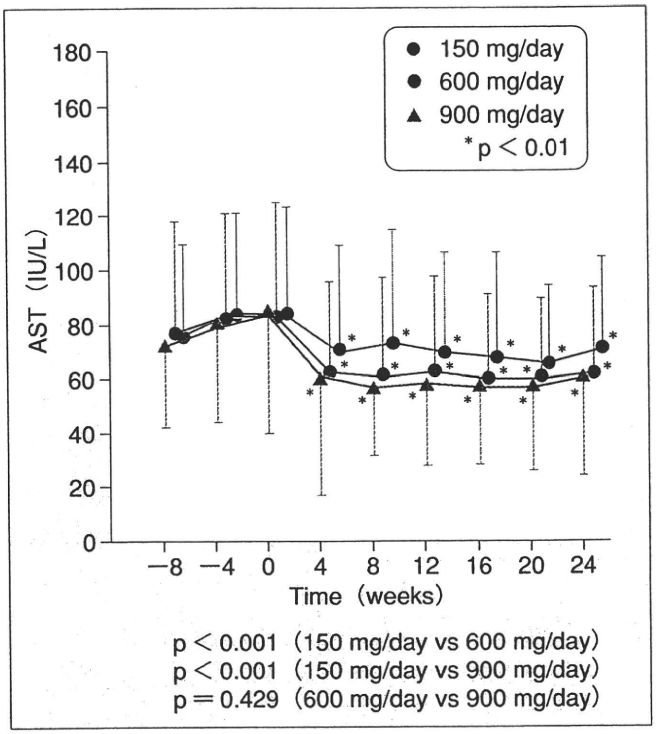

図 3　慢性 C 型肝炎における UDCA 二重盲験試験における，AST の変動

(Omata M, et al : Gut 2007 Aug 30 ; [Epub ahead of print][8] より)

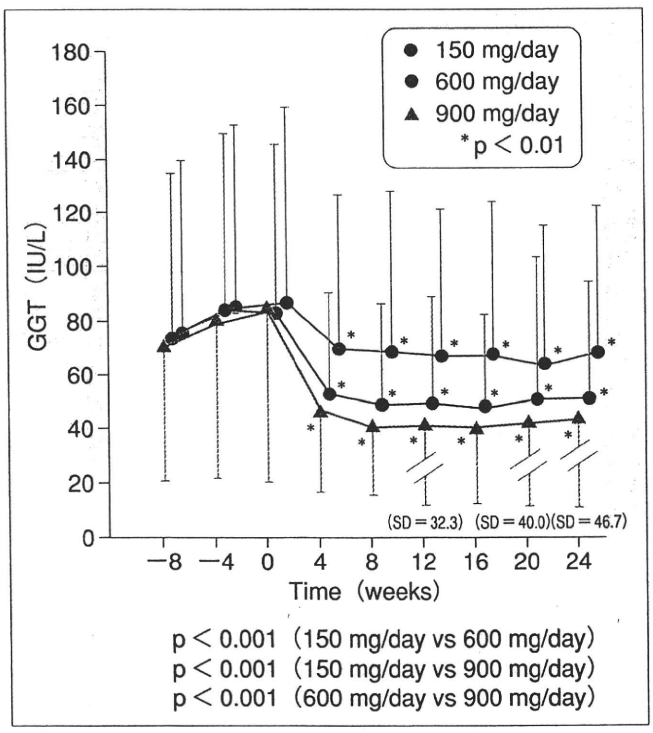

図 4　慢性 C 型肝炎における UDCA 二重盲験試験における，GGTP の変動

(Omata M, et al : Gut 2007 Aug 30 ; [Epub ahead of print][8] より)

る効能追加が承認された．以前からウルソ®は 150 mg/日の使用が可能であったが，今回二重盲検法であるコントロール試験を国内 63 施設において実施した（**図 1**）．その結果，ウルソ® 150 mg/日投与群に比べ 600 mg/日および 900 mg/日投与群での投与開始 4〜24 週後における AST，ALT および γ-GTP 値の改善が有意の差をもって認められた（**図 2〜4**）．このような有効性が確認され，併せて安全性に問題ないことが確認され[8]，承認に至った．現在，C 型慢性肝疾患に対する効果的なウルソ投与量は 600〜900 mg/日である．副作用については，胃不快感，下痢，便秘などの消化器症状が時にみられるが，その程度は軽微なものである．

現在では，UDCA は C 型慢性肝炎における効果と，大腸癌の発癌予防という新しい利用法が模索されている．今後の臨床成績の蓄積によりさらに興味ある知見が得られるものと考えられる．

□ UDCA 投与のタイミング：ALT 値から

C 型慢性肝炎における UDCA の使用現況は，

肝炎等克服緊急対策研究事業の平成13年度～15年度報告書によると[9]，IFN無効・非適応例に対するUDCA単独治療成績は，ALT値正常化が37%，正常値の1.5倍以下まで改善が30%で，目標値までの改善は合計67%とされている．現在では，ガイドライン上でも，C型慢性肝炎の患者さんのALT値を基準値（30IU/*l*）以下にすることが推奨されている．よって，基準値30IU/*l*を超え，さらにALT値が70IU/*l*以下くらいの比較的低値の患者さんが，ウルソ®投与でALT値正常化が得られやすいとされている．つまりALT値が30IU/*l*を超えたらウルソ®は開始したほうがよいということになるであろう．

虎の門病院の報告によると，C型慢性肝炎に対しての肝炎沈静効果を無作為コントロール試験にて，SNMC 100m*l*/日×3/週とSNMC 100m*l*/日×3/週にUDCA 600mg/日連日服用群の2群で比較している．その結果，併用群がALT改善率は有意にSNMC 100m*l*/日に比し良好であることが報告されている[10]．SNMCとUDCAの併用が炎症の沈静化に有効であると考えられる．このような肝庇護薬の使用方法もあり，UDCAの投与タイミングを工夫することで，より炎症の沈静化をはかる努力をする必要があろう．

まとめ

C型慢性肝炎に対する肝庇護療法について，その治療の位置づけと具体的な治療法を述べた．C型慢性肝炎に対する「抗ウイルス療法」と「肝庇護療法」は治療の両輪である．2つの治療法のターゲットは，それぞれウイルスの複製阻害と肝の炎症抑制であり，その役割は異なる．ただし，繰り返しになるが，C型慢性肝炎に対する真の治療目標は肝発癌進展抑止であるので，患者さんに応じた治療法の選択が必要であると考える．

文　献

1) Tokyo Cooperative Gallstone Study Group：Efficacy and indication of ursodeoxycholic acid treatment for dissolving gallstones. A multicenter double blind trial. Gastroenterology 78：542-548, 1980
2) Oka H, Toda G, Ikeda Y, et al：A multi-center double-blind controlled trial of ursodeoxycholic acid for primary biliary cirrhosis. Gastroenterol Jpn 25：774-780, 1990
3) Poupon R, Chretien Y, Poupon RE, et al：Is ursodeoxycholic acid an effective treatment for primary biliary cirrhosis? Lancet i：834-836, 1987
4) Osuga T, Tanaka N, Matsuzaki Y, et al：Effect of ursodeoxycholic acid in chronic hepatitis and primary biliary cirrhosis. Dig Dis Sci 34（Suppl 12）：49-51, 1989
5) Matsuzaki Y, Tanaka N, Osuga T, et al：Improvement of biliary enzyme levels and itching as a result of long-term administration of ursodeoxycholic acid in primary biliary cirrhosis. Am J Gastroenterol 85：15-23, 1990
6) Takikawa H, Yamanaka M, Miyake K, et al：Ursodeoxycholic acid therapy for chronic type C hepatitis：A multicenter, dose-finding trial. Curr Ther Res 55：16-21, 1994
7) Poupon RE, Balkou B, Eschwege E, et al：A multicenter, controlled trial of ursodiol for the treatment of primary biliary cirrhosis. N Engl J Med 324：1548-1554, 1991
8) Omata M, Yoshida H, Toyota J, et al：A large-scale, multicentre, double-blind trial of ursodeoxycholic acid in patients with chronic hepatitis C. Gut Aug 30；[Epub ahead of print], 2007
9) 熊田博光：「C型肝炎ウィルスの感染者に対する治療の標準化に関する臨床的研究」肝炎等克服緊急対策研究事業　平成13年度～15年度　総合研究報告書，2004
10) Tsubota A, Kumada H, Arase Y, et al：Combined ursodeoxycholic acid and glycyrrhizin therapy for chronic hepatitis C virus infection：a randomized controlled trial in 170 patients. Eur J Gastroenterol Hepatol 11：1077-1083, 1999

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ウイルス性肝炎のプライマリケア

慢性ウイルス性肝炎の診断と経過観察

肝細胞癌のスクリーニングと早期発見

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Key Words

腹部超音波検査
TVDT
腫瘍マーカー
Occult HBV
線維化マーカー

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近年，わが国においては肝細胞癌（HCC）患者の発生が増加している．年間発生率は近年2万例を越している．薬害肝炎訴訟の和解により，政府公報として血液製剤の全使用医療機関名が先日公表された．これを機会に少しでも多くの潜在的B, C型肝炎の患者をみつけ治療することが重要である．

近年，HCV抗体陽性HCC患者の発生が70～80%と増加している．しかし，住民健診の受診率が低いこと，職域健診の状況が把握できないことなどから，潜在的C型肝炎の患者の掘り起こしが十分でない現況である．また健診で陽性とわかっても放置されている方々も少なくない．

C型肝炎ウイルス陽性者の肝癌発生率はB型肝炎ウイルス陽性者の約5倍近くとなる換算である．肝細胞癌による死亡者数は世界中で年間100万人に及ぶと推測され，未治療の場合，平均生存期間は数か月といわれている．日本における肝細胞癌患者数は，わが国における肝細胞癌患者は2003年の国民衛生の動向によれば，癌による死亡のなかで男性では3位，女性では4位の位置を占めており，年々その患者数は増加しており，肝癌予防，早期診断，そして治療法の改善は大変重要なこととなっている．

C型肝炎ウイルス（HCV）陽性キャリアーの患者は，全国で200万人ともいわれている．またHCV抗体陽性者の自然経過は，C型肝炎ウイルス暴露から高率に慢性化し，20～30年後に肝硬変，そして肝癌へと移行することが明らかとなっている．慢性肝炎は，肝臓の炎症が長期間持続している状態で，その炎症によって肝臓の細胞が破壊され線維化を起こす．自覚症状に乏しく，患者は自分が知らないうちに，病気が徐々に進行してしまう場合が少なくない．

C型慢性肝炎から不幸にも肝硬変になった患者の場合は肝癌の早期発見を目指し，早期治療することが重要課題である．いかに慢性C型肝炎，肝硬変の患者を綿密に経過観察するかが問われている．

筆者らの臨床データの解析より，慢性C型肝疾患患者のフォローアップの方法として肝癌のハイリスク群の絞り込み方法，肝細胞癌をスクリーニングするかの戦略法について述べる．

0370-999X/08/¥100/頁/JCLS

HCV 抗体陽性慢性肝疾患患者におけるHCC 発生危険因子の予測

● HBV の感染の既往と大量喫煙について

不幸にして肝硬変になってしまった患者の場合，HCC が発生する確立が高くなることはすでに述べた．HCC のフォローアップに関しては，まずは肝硬変からの癌発生の危険因子を疫学的に検討し危険因子を探ることから，早期発見，早期治療に努めることが重要である．そこで，肝癌発生の危険因子を疫学的に検討し，危険因子を探り，早期発見に努める一助とする必要がある．

そこで，prospective study として HCV 抗体陽性慢性肝疾患（慢性肝炎，肝硬変）412 例の患者において，Cox 比例ハザードモデルによる解析を行った．その結果，表 1 に示すように肝硬変は慢性肝炎に比し 5.14 倍の危険率で，anti-HBs 陽性かつあるいは anti-HBc 陽性例において陰性例に比し 2.14 倍の危険率，大量喫煙者（SI ＞ 400）は非喫煙者に比し 2.46 倍の危険率で有意に HCC の発現が高率に認められることが明らかとなった．つまり，C 型慢性肝疾患患者において肝硬変であると，HBV 関連抗体陽性者，大量喫煙者が HCC の発生の危険性が高いことが示唆された[1]．同様の成績はほかのグループなどからも慢性肝疾患患者においての HCC 発癌危険因子として同様な結果を報告している[2]．近年，anti-HBs 陽性かつあるいは anti-HBc 陽性例において有意に HCC 発生例が多いということは，以下の理由が示唆される．いわゆる occult HBV といわれるものである[3]．一般的には HBV 持続感染の可能性が低いと考えられる HBsAg（－）HCV（＋）HCC においても，少なからず HBV の持続感染が成立している可能性が明らかとなってきた．筆者らの検討では，HBsAg 陽性 HCC の多くの例では HBVDNA の組み込み，非癌部への組み込みをも認めた．組み込み部位はランダムであるという特徴がある[4,5]．以上より，現在 HBV の関与する肝発癌機序としては，HBVDNA の組み込みにより，遺伝子産物によるトランス活性化，癌抑制遺伝子の不活化，組み込みによる染色体不安定性などが多段階に関与していると考えられる．

HBsAg（－）HCV（＋）HCC に，肝癌発癌に HBV が関与している可能性が示唆され

表 1　C 型慢性肝疾患における肝癌発生危険因子の検討（Cox 比例ハザードモデル解析）

Variable	Adjusted Rate Ratio	95%CI	χ^2
Stage of disease			
Liver cirrhosis	5.14	2.52 ～ 10.46	0.0001
Chronic hepatitis	1.00		
Anti-HBs and/or Anti-HBc			
Positive	2.14	1.13 ～ 4.07	0.02
Negative	1.00		
Smoking			
Smoking index*≧ 400	2.46	1.11 ～ 5.49	0.03
Smoking index ＜ 400	1.67	0.75 ～ 3.73	0.21
Nonsmoking	1.00		

* Smoking index; average of cigarettes per day multiplied by total years of smoking.

（Chiba T, et al.: Am J Gastroenterol 1996 より一部改変）

（文献 1 より引用）

る．一概にこの事象で発癌を説明できないが，今後の重要な検討課題の一つであろう．よってHBsAg陰性であっても，anti-HBs陽性，かつあるいはanti-HBc陽性例においては，HCC発癌高危険群であり，注意して経過観察を行わねばならないと考えている．

さらにC型慢性肝疾患患者において大量喫煙者が非喫煙者に比べHCCの発生の危険性が高いことが明らかとなった．しかし，大量喫煙と肝細胞癌発癌に関してはいくつかの同じ報告がなされている．たばこの成分の解毒，代謝に肝臓内でのcytochrome P450が関与していることは明らかであり，なんらかの関連があることが示唆される．しかし，いまだ過剰喫煙と肝細胞癌発癌機序に関する明確な実証はない．この問題も今後の検討課題である．大量喫煙者においては，発癌を念頭におきフォローする必要があろう．そして，発癌予防としては禁煙も必要であろう．

アルコール飲酒と発癌の関係

アルコール飲酒と発癌については，大酒家においてウイルス感染がHCC発癌に重要な役割をしていることが明らかとなっている．肝細胞癌とアルコール飲酒との発癌の関係は多く報告されている[6]．HCV感染において，大量飲酒は肝組織像や，臨床症状の進行に重要な危険因子であることもいわれている．肝硬変の患者が，長期に大量飲酒をすると，cytochrome P450 2E1（CYP2E1）が誘導されることが明らかとなっている．CYP2E1がN-nitrosodimethylamine（NMDA）を代謝し，発癌物質となる．大量飲酒者のHCC発癌において，HBV, HCVが関与するという報告もあり[7]，いまだ一定した見解は得られていない．生涯飲酒量が常習量よりも多いLCの患者の場合危険因子となると推測され，HCCの出現に注意し経過観察を行う必要があろう．今後のさらなる検討が必要であるものの，禁酒はHCCの出現予防となるものと考えたほうがよい．

腹部超音波検査の徹底

一般に，腫瘍体積は指数関数的に増大し，その増加速度は一定であるとされている．この理論を用いて，図1，2に示すごとくHCC

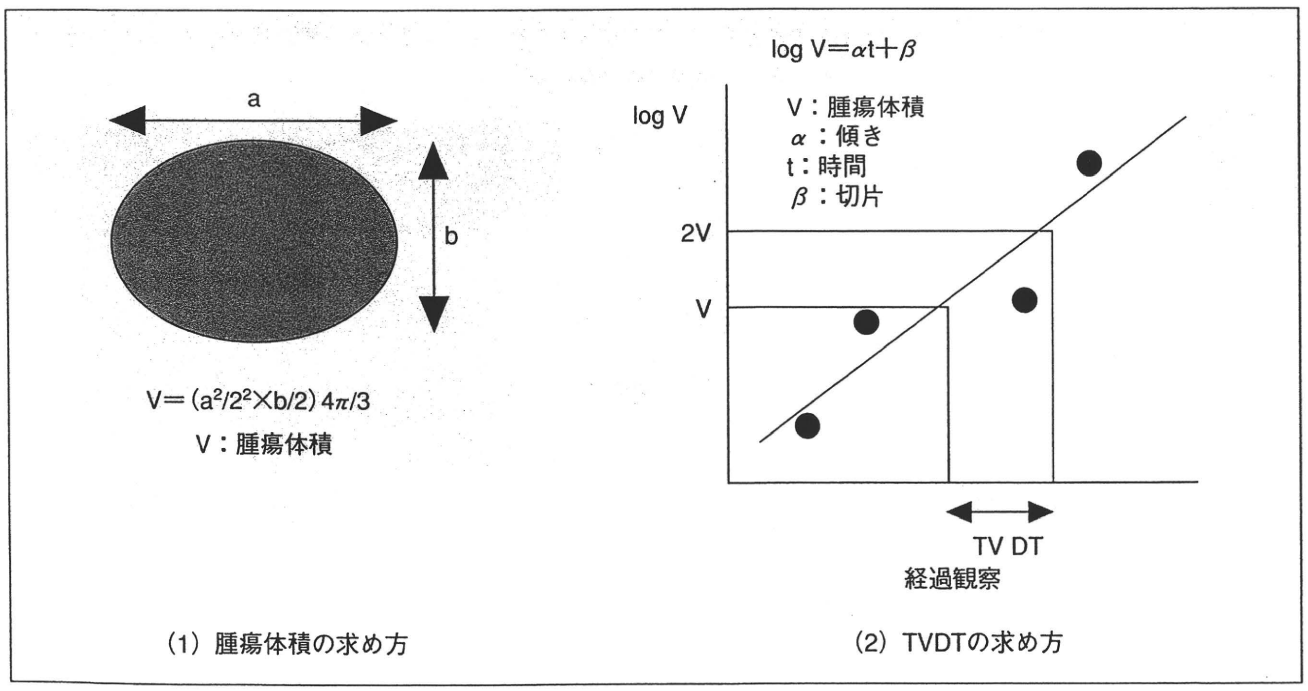

図1　腫瘍体積およびTVDTの求め方

において画像より求めた仮想腫瘍体積より腫瘍倍加時間（TVDT）を求めることが検討されてきた．さらにTVDTと予後との関連性を検討した報告が散見されている[8]．

また，USは患者に対し非侵襲的であるため反復して検査しやすく，かつ肝内の小腫瘤の描出に優れている．近年US診断装置の発達，普及および慢性肝疾患患者のfollow upの徹底によって，径5～20 mm程度のいわゆる小HCCが発見されるようになった．現在，わが国においてHCCはUSで発見されることが最も多くHCC診断・治療における重要な地位を占めるようになった．しかし，US像の違い，あるいはその経時的変化からみたHCCの増大速度等の特性については一定の見解がいまだない．このため，経時的なUS所見の変化をはじめとする，USからみたHCCの特性を知ることは重要であると考えられる．USを用いて腫瘍径を測定する場合，検者や操作角度の違いによりかなり誤差を含んでしまう可能性を考慮しなくてはならず，数mmの腫瘍径変化を有意とするか否かについて苦慮するところである．また，背景に進行した肝硬変を有していると肝内エコーが不整となり腫瘍を明瞭に検出しづらくなる．これらの点も誤差を生じる要因であると考える．

筆者らはTVDTの予後に及ぼす影響についてのCox比例ハザードモデルを用いた検討を表2に示した．様々な理由によりUS経過観察を行わざるを得なかった18名の患者の予後の検討である．70歳未満であること（P＝0.015），Child-Pugh分類がgrade Aであること（P＝0.035），TVDTが3か月未満であること（P＝0.003）が有意な因子であった．無治療の直径3 cm未満の小HCC患者において，TVDT，年齢，腫瘍径，肝硬変の重症度が有意な予後因子であったと報告されている．またdysplastic noduleの厳重なfollow up中，TVDTを慎重に計測することは，癌化の時期を知る方法として有用である可能性もあるとされている．

図3に示すように筆者らは直径3 cm未満

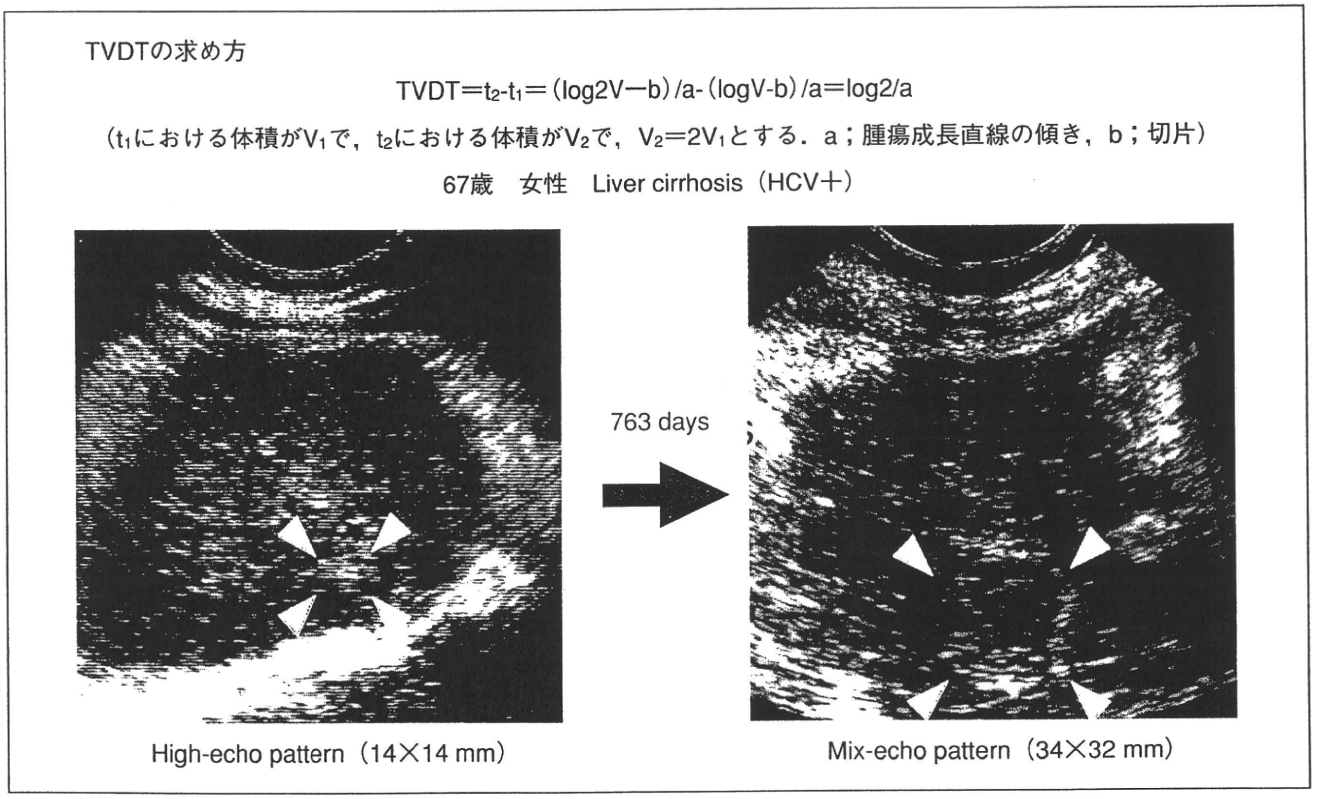

図2　TVDTの算出方法

の小 HCC 患者において，TVDT が細胞増殖の指標となる Ki-67 抗原の表出と相関することを確認しており，腫瘍の増大の経過を観察する重要性を示唆するものである[9]．以上より TVDT の短い腫瘤に関しては十分悪性化や治療後の再発の可能性を考慮し厳重に経過観察する必要があると考えられる．

つまり，US は 3 か月ごとに肝硬変患者には行ったほうがよいということになる．以上のことから，筆者らの施設ではこれらの危険因子を考慮し，多中心性発癌を考え経過観察に充分注意を払うこととしている．慢性肝炎の場合の観察期間は最低 4 ～ 6 か月ごとが妥当であろう．

血液マーカーのチェック

HCC 発癌率を高める独立要因の多変量解析により，HCV 陽性肝硬変であり，血小板数が 10 万/mm² 未満，男性で，AFP 値が 20 ng/mL 以上，年齢が 55 歳以上，ICG15 分値が 30% 以上の場合，有意に HCC 発癌危険率が高くなることが報告されている[10]．AFP とレクチン分画である L3 分画，PIVKA-II は有用な腫瘍マーカーである．HCC は，血小板の減少，線維化の進行，トランスアミナーゼの高値（正常値 30 以下にならない場合）のときは，HCC 累積発癌率が増加することが明らかとなっている．このなかで肝線維化を想定するマーカーとして，血中 IV 型コラーゲン値（ラテックス法），ヒアルロン酸が線維化の程度と相関する．線維化の進行をみるうえで，有用なマーカーの一つとなる．以上より，肝

表 2　Cox 比例ハザードモデルによる予後関連因子の検討

検討因子		ハザード比	P 値
年齢	70 歳未満	0.094	0.015
Child 分類	A	0.218	0.035
TVDT	3 か月未満	52.670	0.003
治療の有無	無治療	0.465	0.452

N ＝ 18

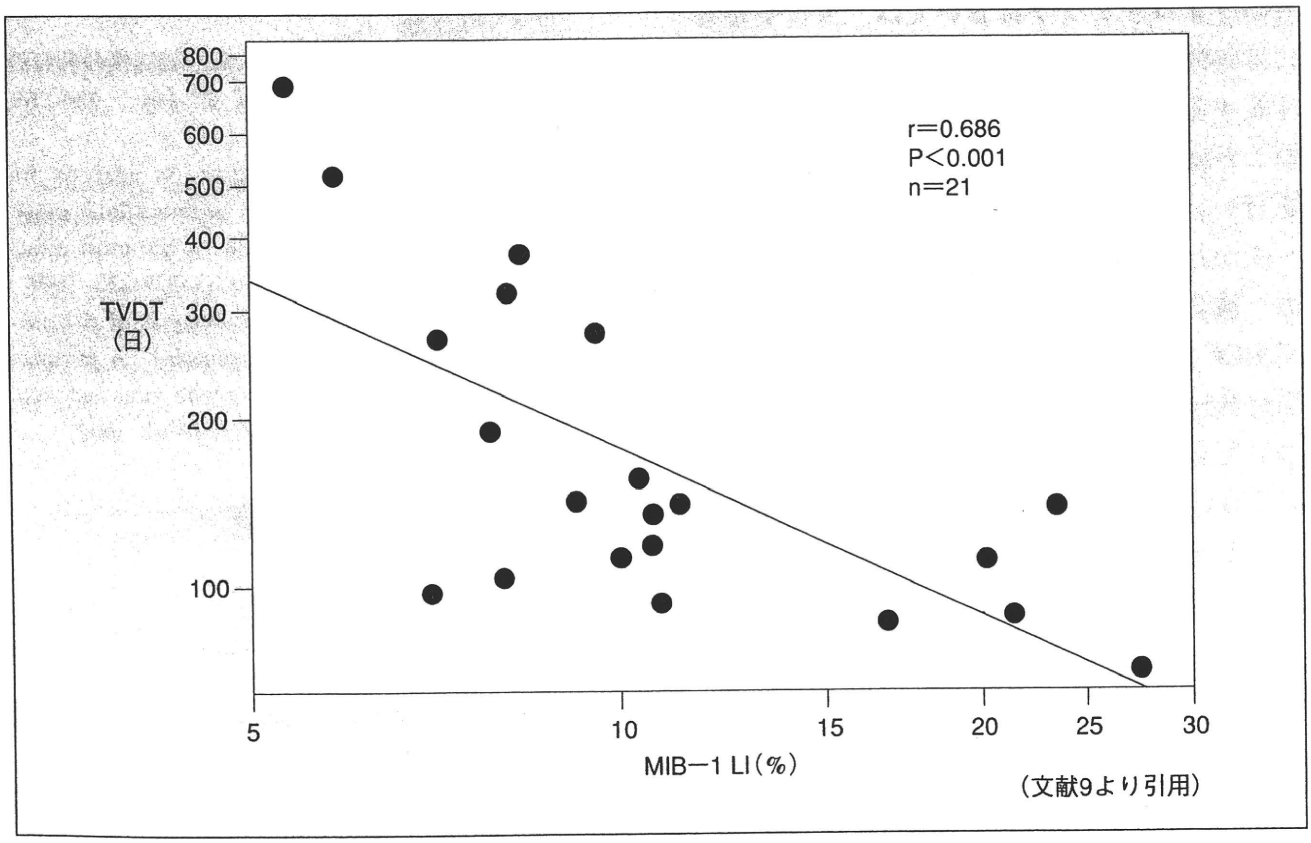

図 3　TVDT と MIB-1 の相関
－小 HCC における重回帰分析より－

炎から肝硬変，肝細胞癌への進行を観察するには，血小板数，血中IV型コラーゲン値，年齢の高齢化，AFP, PIVKA-IIなどの腫瘍マーカーなどの推移などが重要な観察マーカーとなると考える．これらの推移を観察することが，肝癌早期発見のための重要な経過観察指標となると考えられる．慢性肝炎の線維化の進行例や，肝硬変の場合，2～3か月ごとに腫瘍マーカーを組み合わせ，交互に検査を行うことがよい．

総括

慢性C型肝疾患患者においては，HCCの発生に十分注意をすることがまず肝要である．肝機能の変動のある慢性肝炎の患者においては，肝硬変への移行を注意深く観察する．飲酒者には禁酒をさせること．血小板数，血中IV型コラーゲン値，年齢の高齢化，などが重要な観察項目である．特にanti-HBV（anti-HBs, anti-HBc）陽性者で，C型肝硬変患者のフォローアップにおいては，大量喫煙者には禁煙を，飲酒者には禁酒をさせ，発癌に注意することである．そしてなによりも重要なことは毎月肝機能，血小板などのチェックを行い，3～6か月毎の腹部超音波検査，2～3か月毎にAFP, PIVKA-IIなどの腫瘍マーカー検査を施行し，より綿密に観察することでHCC早期発見に努めることが重要である．無症候性キャリアーの場合や，IFN著効例においても発癌例があるので，年1～2回は検査をうける指導をすることが重要なことであることを最後に付け加える．

文献

1) Chiba T, et al.: The role of previous hepatitis B virus infection and heavy smoking in hepatitis C virus-related hepatocellular carcinoma. Am J Gastroenterol 91: 1195-1203, 1996
2) Paterlini P, et al.: Persistence of hepatitis B and hepatitis C viral genomes in primary liver cancers from HBsAg-negative patients: a study of a low-endemic area. Hepatology 17: 2-29, 1993
3) Brechot C, et al.: Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: Clinically significant or purely "Occlut". Hepatology 34: 194-203, 2001
4) Koike K, et al.: Hepatitis B virus DNA integration frequency observed in the hepatocellular carcinoma DNA of hepatitis C virus-infected patients. Int J Oncol 8: 781-784, 1996
5) Matsuzaki Y, et al.: HBV genome integration and genetic instability in HBsAg-negative and anti-HCV-positive hepatocellular carcinoma in Japan. Cancer Letters 119（1）: 53-61, 1997
6) Kubo S, et al.: Alcohol abuse as an etiologic factor for hepatocellular carcinoma in Japan. Hepatolgy Res 31: 73-78, 2005
7) Matsuzaki Y: Alcohol abuse and occult HBV-a risk factor for hepatocellular carcinoma. Hepatolgy Res 32: 67-70, 2005
8) 真島康雄：超音波断層法による肝細胞癌の発育速度とその臨床的意義．肝臓 25（6）: 754-765, 1984
9) Saito Y, et al.: Multiple regression analysis for assessing the growth of small hepatocellular carcinoma: the MIB-1 labeling index is the most effective parameter. J. Gastroenterol 33: 229-235, 1998
10) Ikeda K, et al.: A multivariate analysis of risk factors for hepatocellular carcinogenesis] A prospective observation of 795 patients with viral and alcoholic cirrhosis. Hepatology 18: 457-481, 1993

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Original Article

The associated markers and their limitations for the primary screening of HCV carriers in public health examination

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Aim: Although the anti-hepatitis C virus (HCV) antibody test has been recommended to the whole Japanese population, most countries have not implemented it. The present study aims to re-evaluate the usefulness of markers examined in the general health examination for the initial screening of HCV carriers.

Methods: Of the overall population, 25 142 individuals (8876 males, 16 266 females) participated in health examinations with HCV tests in 2005, and the most commonly associated markers for HCV-positive subjects were explored by multivariate analysis, based on blood biochemical, physical, sphygmomanometric and hematological parameters. Thereafter, the efficiencies of the markers were estimated from a total population of 85 013 individuals (29 502 males, 55 511 females) in 2003–2005.

Results: The most significantly associated markers for HCV positivity were aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Optimal limits of ALT and AST by receiver–operator characteristic (ROC) analysis were 24 and 27 IU (male, 33 and 28 IU; female, 22 and 26 IU), respectively. However, one-quarter of HCV carriers were not found to be positive using the optimal limits of aminotransferases.

Conclusion: The present study confirmed the limitation of serum aminotransferase levels as markers of HCV for primary screening. Therefore, at present, an anti-HCV antibody test is required for the efficient screening of HCV carriers in all health examinations.

Key words: aminotransferases, HCV, health examination

INTRODUCTION

INFECTION WITH HEPATITIS C virus (HCV) has been the leading cause of liver cirrhosis, and the consequent development of hepatocellular carcinoma, for the past few decades. The number of HCV carriers has increased worldwide. Indeed, the World Health Organization (WHO) estimates that about 180 million people, that is 3% of the world's population, are infected with HCV, and 3–4 million people are newly infected every year, 70% of whom develop chronic hepatitis.[1]

Based on early detection and treatment, it is very important to detect HCV carriers as early as possible, for example, in public health examinations. HCV carriers are diagnosed by the detection of HCV-RNA and/or anti-HCV antibody using the judgment system of HCV infection established since 2002 in Japan (Fig. 1). Generally, subjects who have abnormally high levels of serum alanine aminotransferase (ALT) as well as aspartate aminotransferase (AST) are recommend to take thorough examinations for liver diseases, including the HCV tests. However, there is an issue that most HCV carriers are considered to be asymptomatic and paucisymptomatic, and that approximately 30% of chronic HCV carriers persistently exhibit normal ALT levels (PNAL), while another 40% exhibit minimally elevated ALT levels.[2–6] Consequently, these asymptomatic and paucisymptomatic HCV carriers fail to be detected by the primary screening using serum ALT levels in public health examinations. Importantly, these asymptomatic HCV carriers with PNAL have significant histological

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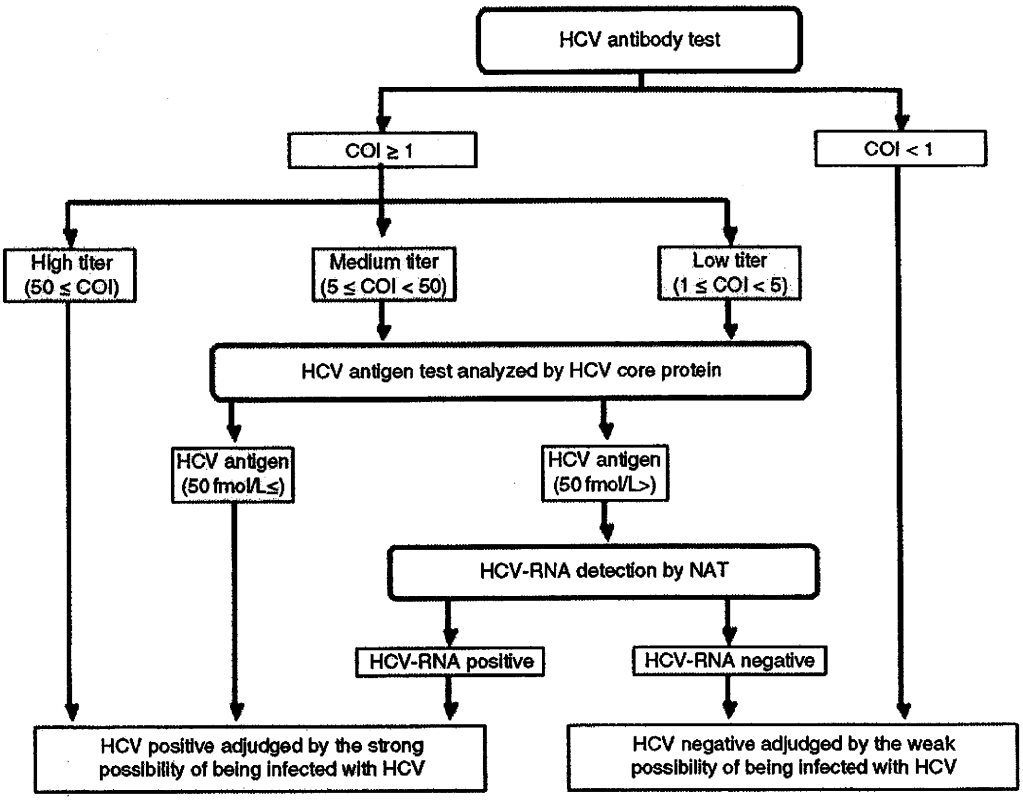

Figure 1 Flow chart showing the course of medical examination for hepatitis C virus (HCV). The diagnosis of HCV infection was conducted in accordance with the guidelines for the medical examination of HCV issued by the Japanese Ministry of Health, Labour and Welfare. COI, cut off index; HCV, hepatitis C virus; NAT, nucleic acid amplification test.

liver damage, similar to that in HCV carriers with raised ALT levels, and moderate to severe hepatitis has frequently been found in asymptomatic HCV carriers compared with HCV carriers with raised ALT levels.[7]

Accordingly, the optimal serum ALT limits for the screening of HCV carriers have been subject to debate.[8–10] However, what is considered a healthy range of ALT levels compared with liver disease differs between medical institutes, centers, hospitals, regions and countries. Almost all of the normal ALT ranges for liver disease are less than 40 IU,[7] however, Prati *et al.* reported that the upper limits of the healthy range differed between genders; 30 IU and 19 IU for males and females, respectively; calculated as the value of the 95th percentile in normal subjects from a population at the lowest risk for liver disease.[8] Furthermore, Okanoue *et al.* defined asymptomatic HCV carriers as those with PNAL less than 30 IU based on the histological fibrosis stage in a follow-up study.[11] In Japan, serum ALT levels under 35 IU had been considered to be within the healthy limit for diagnosis of liver diseases, but in 2008, the health limit was reduced to under 30 IU, for both ALT and AST, as suggesting liver disease in public health examinations, based on the guidance for antivirus therapy of HCV.[12] Based on these facts, it has not been actually clarified whether these markers are effective and whether the optimal limit points are useful or not for the detection of asymptomatic and paucisymptomatic HCV carriers. Therefore, in Japan, the anti-HCV antibody test has been recommended to the whole of the population during public health examinations.[13,14]

The purpose of the present study was to re-evaluate the effectiveness of serum aminotransferase levels as markers in the primary screening for HCV carrier detection in over 85 000 subjects in the annual public medical health examination for 3 years between 2003 and 2005.

METHODS

Population in the health examination

A TOTAL OF 85 013 individuals (29 502 males, 55 511 females), including non-employees, local residents, self-employed persons, farmers, housewives and retired persons participated in the biochemical examination of serum ALT and AST levels as part of the annual public health examination and HCV testing during the 3 years from 2003 to 2005 in Ibaraki Prefecture, Japan. HCV testing was carried out based in part on a project of urgent comprehensive countermeasures against hepatitis and HCC at the ages of 40, 45, 50, 55, 60, 65, or 70 for five years supported by the Japanese Ministry of Health, Labour and Welfare. In the health examination in 2005, in addition to the measurement of serum ALT and AST levels, 25 142 subjects (8876 males, 16 266 females) underwent examination of γ-GFP level, diastolic and systolic blood pressure, hemoglobin, hematocrit, red blood cell count (RBC), total cholesterol, triglyceride, glucose and glycohemoglobin (HbA_{1c}). Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters.[15] All of the health examinations with serum biochemical analyses, as well as the HCV tests, were carried out with the Ibaraki Health Service Association (Mito, Japan).

The determination of HCV carrier status

The determination of the presence or absence of HCV infection was performed in accordance with the guidelines for the medical examination of HCV issued by the Japanese Ministry of Health, Labour and Welfare, as summarized in Figure 1. Serum collected from the subjects during the medical examination was first measured for the HCV titer using a chemiluminescent enzyme immunoassay for HCV antibody (Lumipulse®, Fujirebio Inc, Tokyo, Japan). Subjects with serum HCV titer beneath a cut-off index (COI) of 1 were determined to be HCV negative. Those subjects with a COI > 1 were divided into three classes dependent on the levels of the HCV titer: low titer, COI under 5 and more than 1; medium titer, COI under 50 and more than 5; high titer, COI more than 50. The subjects in the high titer class were determined to be HCV positive. The subjects classified to the low and medium titers underwent the HCV antigen test analysis for the HCV core protein. Subjects with more than 50 fmol/L of HCV antigen were determined to be HCV positive. When the HCV antigen was under 50 fmol/L, a nucleic acid amplification test (NAT) was conducted for HCV-RNA detection. The subjects with positive and negative results by the NAT were finally determined to be HCV positive and negative, respectively.

Other investigated data in 2005

In the data from 2005, the most relevant factor for HCV positive status was determined statistically by multivariate analysis and the ROC curve. As a result of the ROC curve in 2005 (Table 1), the ROC curves for ALT and AST levels in serum were drawn from data for 3 years between 2003 and 2005 to evaluate the effective cut-off points to avoid false negative and positive findings for HCV.

Statistical analysis

Data are presented as the mean ± SE, the percentage and the percentiles. Significant differences were determined by unpaired Student's *t*-test or one-way ANOVA with Bonferonni's post-hoc test for comparisons between two groups or among multiple groups, respectively. The statistical analysis was performed using SPSS II software version 11.0 (SPSS Inc, Chicago, IL, USA). Multiple regression analyses were made using the stepwise method. The upper-left cut points for the HCV positive were chosen from likelihood value based on the ROC curve. ROC comparison was performed by calculation of the area under the curve and 95% confidence intervals using the technique described by Hanley and McNeil.[16]

RESULTS

Basic data, and the ROC and multivariate analyses of the health examinations in 2005

BASIC DATA OF all examined parameters in the health examinations in 2005 are shown in Table 2. The levels of serum ALT, AST and γ-GPT were significantly and markedly higher in the HCV positive than in the HCV negative subjects, for both genders. These serum levels were significantly higher in males than in females in all of the HCV negative and positive cases.

Table 1 presents the results of ROC and multivariate analyses in the respective parameters for HCV positive status from data in 2005. The most significant relevant parameter for HCV positive status was the serum AST level, followed by the serum ALT level. There were other significant parameters ($P < 0.05$), but the areas of the ROC curve for these parameters were less than 0.7. BMI and serum levels of triglyceride and total cholesterol

Table 1 Area under the receiver-operator characteristic (ROC) curve and multivariate analysis and the respective parameter for HCV positive subjects examined in 2005

Parameter	ROC curve area	SE	P-value	95% CI
AST	0.849	0.018	0.000	0.814–0.884
ALT	0.788	0.021	0.000	0.747–0.829
Hemoglobin	0.654	0.028	0.000	0.598–0.709
Age	0.642	0.026	0.000	0.591–0.692
Hematocrit	0.642	0.027	0.000	0.589–0.695
γ-GTP	0.622	0.028	0.000	0.508–0.677
Glucose	0.613	0.025	0.000	0.564–0.662
RBC	0.558	0.029	0.029	0.501–0.614
Systolic pressure	0.547	0.029	0.077	0.491–0.603
Diastolic pressure	0.528	0.028	0.289	0.473–0.583
Height	0.519	0.027	0.474	0.466–0.572
Weight	0.505	0.027	0.860	0.451–0.558
HbA1c	0.504	0.028	0.872	0.449–0.559
BMI	0.492	0.025	0.760	0.443–0.457
Triglyceride	0.409	0.025	0.001	0.361–0.330
Total cholesterol	0.278	0.027	0.000	0.226–0.330

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CI, confidence interval; HbA1c, glycohemoglobin; RBC, red blood cell count; γ-GTP, gamma-glutamyl transferase.

were related to HCV negative status but not HCV positive status. In particular, the total cholesterol level was the most relevant parameter for the HCV negative status. Based on the results of these analyses, the ROC curves for the serum AST and ALT levels of the HCV positive subjects among each gender were drawn from data for 3 years between 2003 and 2005.

As result of stepwise discrimination analysis, a combination of four parameters, AST, ALT, age and total cholesterol, gave the maximum likelihood. The established discrimination formula was as follows: $Z = 10.472 - 0.001 \times (\text{AST, IU}) + 0.027 \times (\text{ALT, IU}) + 0.057 \times (\text{age, year}) - 0.025 \times (\text{total cholesterol, mg/dL})$. However, the calculated predictive value for HCV positive ratio was only 6.61% using the formula.

HCV positive ratio and distribution of aminotransferases in HCV positive populations for 3 years

There were 787 HCV positive subjects (male, 406; female, 381) and the positive ratio was 0.93% (male, 1.38%; female, 0.69%) for 3 years between 2003 and 2005. The range of ages for the HCV positive subjects was 29–87 years old (male, 29–87 years; female, 40–84 years).

The distributions of serum ALT and AST levels were expressed as percentiles by age in the HCV positive populations for both genders (Fig. 2). In males, the levels of both aminotransferases, in particular ALT, were elevated in those aged less than 65 years, and there were large variations of these levels in all age ranges (Fig. 2a). However, there were no differences in the distribution of levels of both aminotransferases among the age ranges in females, and the variations of these levels were small compared to those in males (Fig. 2b).

The distribution of HCV positive subjects using the current limit points

Those who were detected as being HCV positive in 2003–2005 were divided into four cut-off ranges (A–D) by ALT and AST levels at 30 IU, that is the current limit point (Fig. 3). There was significant difference in the ratio balance of HCV positive between genders assessed by χ^2 analysis ($P < 0.0001$). In range A, which means the false-negative of HCV positive, the HCV positive rates of male and female were 25.9% and 47.0%, respectively. In range A, almost of half of female HCV positive subjects were classified as false-negative. In contrast, the ratios in range D were 56.3 and 39.8% in males and females, respectively, and over half of HCV positive subjects in males were included in range D. In males, the ratio in range B was 10.5% and higher than that (7.4%) in range C. In contrast, in females, the ratio in range B was only 4.3% and lower than that (9.0%) in range C.