場合は肝臓専門医に相談することが推奨される。

2. 核酸アナログ薬中止後に肝炎が再燃し再投与した場合、中止しなかった場合と比較し核酸アナログ薬耐性株の出現頻度が増加するか否かについては不明である。
3. HBV キャリアでは非活動性キャリア期（HBV DNA が 4.0 log copy/ml 未満かつ ALT が 30 IU/L 未満）となってもまれに肝炎の再燃がみられるので、中止に成功してもキャリアとしての経過観察は継続する必要がある。また、肝発癌に関しても同様に経過観察が必要である。
4. 今後の検討課題としては、核酸アナログ薬中止基準の精度をさらに高めること、本指針で用いた基準を前向き検討で検証すること、インターフェロン併用によるシークエンシャル療法で核酸アナログ薬を積極的に中止しようとする方法の検討などが挙げられる。

Ⅱ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻・号	ページ	出版年
Matsumoto A, Tanaka E, Morita S, Yoshizawa K, Umemura T, Joshita S.	Changes in the serum level of hepatitis B virus (HBV) surface antigen over the natural course of HBV infection.	J Gastroenterol			in press
田中榮司、松本晶博、鈴木義之、小林万利子、田中靖人、新海登、髭修平、八橋弘、長岡進矢、茶山一彰、柘植雅貴、横須賀收、今関文夫、西口修平、齋藤正紀、藤原圭、鳥居信之、平松直樹、狩野吉康	核酸アナログ薬中止に伴うリスク回避のための指針 2012 －厚生労働省「B型肝炎の核酸アナログ薬治療における治療中止基準の作成と治療中止を目指したインターフェロン治療の有用性に関する研究」の報告－.	肝臓			印刷中
Matsumoto A, Tanaka E, Suzuki Y, Kobayashi M, Tanaka Y, Shinkai N, Hige S, Yatsuhashi H, Nagaoka S, Chayama K, Tsuge M, Yokosuka O, Imazeki F, Nishiguchi S, Saito M, Fujiwara K, Torii N, Hiramatsu N, Karino Y, Kumada H.	Combination of hepatitis B viral antigens and DNA for prediction of relapse after discontinuation of nucleos(t)ide analogs in patients with chronic hepatitis B.	Hepatol Res	42(2)	139-149	2012
Tanaka E, Urata Y.	Risk of hepatitis B reactivation in patients treated with tumor necrosis factor-alpha inhibitors.	Hepatol Res	42(4)	333-339	2012
Yokosuka O, Kurosaki M, Imazeki F, Arase Y, Tanaka Y, Chayama K, Tanaka E, Kumada H, Izumi N, Mizokami M, Kudo M.	Management of hepatitis B: Consensus of the Japan Society of Hepatology 2009.	Hepatol Res	41(1)	1-21	2011
田中榮司	透析患者におけるB型肝炎ウイルスマーカー測定の意義	日本透析医学会雑誌	26(1)	55-61	2011
Kumada H, Okanoue T, Onji M, Moriwaki H, Izumi N, Tanaka E, Chayama K, Sakisaka S, Takehara T, Oketani M, Suzuki F, Toyota J, Nomura H, Yoshioka K, Seike M, Yotsuyanagi H, Ueno Y.	Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis B virus infection for the fiscal year 2008 in Japan.	Hepatol Res	40(1)	1-7	2010

Kumada H, Okanoue T, Onji M, Moriwaki H, Izumi N, Tanaka E, Chayama K, Sakisaka S, Takehara T, Oketani M, Suzuki F, Toyota J, Nomura H, Yoshioka K, Seike M, Yotsuyanagi H, Ueno Y.	Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan.	Hepatol Res	40(1)	8-13	2010
Matsumoto A, Maki N, Yoshizawa K, Umemura T, Joshita S, Tanaka E.	Comparison of hepatitis B virus DNA, RNA, and core related antigen as predictors of lamivudine resistance in patients with chronic hepatitis B.	Shinshu Med J	58(4)	153-162	2010
溝上雅史、田中榮司、茶山一彰、田中靖人、黒崎雅之、泉並木、荒瀬康司、熊田博光、今関文夫、横須賀收、工藤正俊	日本肝臓学会コンセンサス神戸 2009　B 型肝炎の診断と治療.	肝臓	51(5)	243-260	2010
田中榮司	B 型肝炎再活性化の病態と対策	日本消化器病学会雑誌	107(9)	1417-1425	2010
Matsumoto A, Tanaka E.	Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in patients with chronic hepatitis C, using viral and host factors: some concerns (reply).	Hepatology	49(6)	2125; author reply 2125-2126	2009
Matsuura K, Tanaka Y, Hige S, Yamada G, Murawaki Y, Komatsu M, Kuramitsu T, Kawata S, Tanaka E, Izumi N, Okuse C, Kakumu S, Okanoue T, Hino K, Hiasa Y, Sata M, Maeshiro T, Sugauchi F, Nojiri S, Joh T, Miyakawa Y, Mizokami M.	Distribution of hepatitis B virus genotypes among patients with chronic infection in Japan shifting toward an increase of genotype A.	J Clin Microbiol	47(5)	1476-1483	2009
Umemura T, Ichijo T, Yoshizawa K, Tanaka E, Kiyosawa K.	Epidemiology of hepatocellular carcinoma in Japan.	J Gastroenterol	44 Suppl 19	102-107	2009.
Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M.	Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C.	Nat Genet	41(10)	1105-1109	2009

保坂哲也、鈴木文孝、小林正宏、瀬古裕也、今井則博、平川美晴、川村祐介、瀬崎ひとみ、芥田憲夫、鈴木義之、斎藤聡、荒瀬康司、池田健次、小林万利子、熊田博光	B 型慢性肝疾患に対する核酸アナログ療法による HBs 抗原消失とその関連因子の検討	肝臓	52(4)	255-7	2011
小林万利子、鈴木文孝、鈴木義之、芥田憲夫、瀬崎ひとみ、川村祐介、瀬古裕也、保坂哲也、小林正宏、斎藤聡、荒瀬康司、池田健次、熊田博光	コバス TaqManHBV「オート」v2.0 における同一時の血清検体と血漿検体の HBVDNA 検出率の検討	肝臓	52(11)	756-7	2011
Kobayashi M, Suzuki F, Akuta N, Yatsuji H, Hosaka T, Sezaki H, Kobayashi M, Kobayashi M, Kawamura Y, Suzuki Y, Arase Y, Ikeda K, Mineta R, Iwasaki S, Watahiki S, Kumada H.	Correlation of YMDD mutation and breakthrough hepatitis with hepatitis B virus DNA and serum ALT during lamivudine treatment.	Hepatol Res	40(2)	125-134	2010
Hosaka T, Suzuki F, Kobayashi M, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Akuta N, Suzuki Y, Saitoh S, Arase Y, Ikeda K,Miyakawa Y, Kumada H.	Development of HCC in patients receiving adefovir dipivoxil for lamivudine-resistant hepatitis B virus mutants.	Hepatol Res	40(2)	145-152	2010
Hosaka T, Suzuki F, Kobayashi M, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Akuta N, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Kumada H.	HBcrAg is predictor of post-treatment recurrence of hepatocellular carcinoma during antiviral therapy.	Liv Int	30(10)	1461-1470	2010
八辻寛美、鈴木文孝、平川美晴、川村祐介、瀬崎ひとみ、保坂哲也、芥田憲夫、小林正宏、鈴木義之、斎藤聡、荒瀬康司、池田健次、岩崎里美、峰田理恵、綿引祥予、小林万利子、熊田博光	核酸ｱﾅﾛｸﾞ未使用の B 型慢性肝炎症例へのｴﾝﾃｶﾋﾞﾙ治療中にｒｔ A181T ｳｲﾙｽが増殖した一症例	肝臓	51(4)	196-198	2010
Ikeda K, Kobayashi M, Someya T, Saitoh S, Hosaka T, Akuta N, Suzuki F, Suzuki Y, Arase Y, Kumada H.	Occult hepatitis B virus infection inceases hepatocellular carcinogenesis by eight times in patients with non-B, non-C liver cirrhosis: a cohort study.	J Viral Hepatitis	16	437-443	2009
Suzuki Y, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Hosaka T, Akuta N, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Miyakawa Y, Kumada H.	Efficacy of entecavir treatment for lamivudine-resistant hepatitis B over 3 years: Histological improvement or entecavir resistance?	J Gastroenterol Hepatol	24	429-435	2009

保坂哲也、鈴木文孝、小林正宏、平川美晴、川村祐介、八辻寛美、瀬崎ひとみ、芥田憲夫、鈴木義之、斉藤聡、荒瀬康司、池田健次、小林万利子、熊田博光.	核酸ｱﾅﾛｸﾞ療法中の B 型関連肝癌に対する肝癌再発予測ﾏｰｶｰとして HB ｺｱ関連抗原の有用性	肝臓	50(10)	588-589	2009
Wai-Kay Seto, Yasuhito Tanaka, Danny Ka-Ho Wong, Ching-Lung Lai, Noboru Shinkai, John Chi-Hang Yuen, Teresa Tong, James Fung, Ivan Fan-Ngai Hung, Man-Fung Yuen	Evidence of serologic activity in chronic hepatitis B after surface antigen (HBsAg) seroclearance documented by conventional HBsAg assay	Hepatology International			In press
新海登，田中靖人，	HBV 遺伝子型と臨床像	日本臨床	69	422～427	2011
中村恵利那，角田浩一，松浦健太郎，菅内文中，新海登 ，田中靖人	HBs 抗原定量値の臨床的有用性の検討	臨床病理	59(9)	838 -843	2011
新海登，田中靖人，松浦健太郎，可児里美，長沼初江，溝上雅史	新しい超高感度 HBs 抗原定量試薬の基礎的・臨床的有用性	臨床病理	58(11)	1078-1084	2010
Kurokawa M, Hiramatsu N, Oze T, Yakushijin T, Miyazaki M, Hosui A, Miyagi T, Yoshida Y, Ishida H, Kiso S, Tatsumi T, Kanto T, Kasahara A, Iio S, Doi Y, Yamada A, Oshita M, Kaneko A, Mochizuki K, Hagiwara H, Mita E, Ito T, Inui Y, Katayama K, Yoshihara H, Imai Y, Hayashi E, Hayashi N, Takehara T.	Long-term effect of lamivudine treatment on the incidence of hepatocellular carcinoma in patients with hepatitis B virus.	J Gastroenterol.			in press
Hikita H, Takehara T, Kodama T, Shimizu S, Shigekawa M, Hosui A, Miyagi T, Tatsumi T, Ishida H, Li W, Kanto T, Hiramatsu N, Shimizu S, Tsujimoto Y, Hayashi N.	Delayed-onset caspase-dependent massive hepatocyte apoptosis upon fas activation in bak/bax-deficient mice.	Hepatology.	54(1)	240-51	2011
Kodama T, Takehara T, Hikita H, Shimizu S, Shigekawa M, Li W, Miyagi T, Hosui A, Tatsumi T, Ishida H, Kanto T, Hiramatsu N, Yin XM, Hayashi N.	BH3-only Activator Proteins Bid and Bim Are Dispensable for Bak/Bax-dependent Thrombocyte Apoptosis Induced by Bcl-xL Deficiency: molecular requisites for the mitochondrial pathway to apoptosis in platelets.	J Biol Chem.	22;286(16)	13905-13	2011

Uemura A, Takehara T, Miyagi T, Suzuki T, Tatsumi T, Ohkawa K, Kanto T, Hiramatsu N, Hayashi N.	Natural killer cell is a major producer of interferon gamma that is critical for the IL-12-induced anti-tumor effect in mice.	Cancer Immunol Immunother.	59(3)	453-463.	2010
Miyagi T, Takehara T, Uemura A, Nishio K, Shimizu S, Kodama T, Hikita H, Li W, Sasakawa A, Tatsumi T, Ohkawa K, Kanto T, Hiramatsu N, Hayashi N.	Absence of invariant natural killer T cells deteriorates liver inflammation and fibrosis in mice fed high-fat diet.	J Gastroenterol.	45(12)	1247-54.	2010
Kurashige N, Hiramatsu N, Ohkawa K, Yakushijin T, Kiso S, Kanto T, Takehara T, Kasahara A, Doi Y, Yamada A, Oshita M, Mita E, Hagiwara H, Nagase T, Yoshihara H, Hayashi E, Imai Y, Kato M, Kashihara T, Hayashi N.	Factors contributing to antiviral effect of adefovir dipivoxil therapy added to ongoing lamivudine treatment in patients with lamivudine-resistant chronic hepatitis B.	J Gastroenterol.	44(6)	601-7.	2009
Kurashige N, Ohkawa K, Hiramatsu N, Yakushijin T, Mochizuki K, Oze T, Kiso S, Kanto T, Takehara T, Kasahara A, Doi Y, Yamada A, Fukuda K, Oshita M, Mita E, Fukui H, Nagase T, Yoshihara H, Imai Y, Kato M, Kashihara T, Hayashi N.	Lamivudine-to-entecavir switching treatment in type B chronic hepatitis patients without evidence of lamivudine resistance.	J Gastroenterol.	44(8)	864-70.	2009
狩野吉康，小関至，豊田成司	ウイルス肝炎 どのように実際の治療をすすめるか B型肝炎 B型肝炎の抗ウイルス療法の実際	Med Pract	28(28)	1425-1429	2011
小関至，狩野吉康，豊田成司	B型肝炎に対する新治療戦略 Entecavirによる抗ウイルス療法	月刊消化器内科	53(3)	283-287	2011
Karino Y, Toyota J, Kumada H, Katano Y, Izumi N, Kobashi H, Sata M, Moriyama M, Imazeki F, Kage M, Ishikawa H, Masaki N, Seriu T, Omata M.	Efficacy and resistance of entecavir following 3 years of treatment of Japanese patients with lamivudine-refractory chronic hepatitis B.	Hepatol Int	4(1)	414-422	2010
Tsuge M, Takahashi S, Hiraga N, Fujimoto Y, Zhang Y, Mitsui F, Abe H, Kawaoka T, Imamura M, Ochi H, Hayes CN, Chayama K.	Effects of hepatitis B virus infection on the interferon response in immunodeficient human hepatocyte chimeric mice.	J Infect Dis	204	224-8	2011

Ohishi W, Fujiwara S, Cologne JB, Suzuki G, Akahoshi M, Nishi N, Tsuge M, Chayama K.	Impact of radiation and hepatitis virus infection on risk of hepatocellular carcinoma.	Hepatology	53	1237-45	2011
Chayama K, Hayes CN, Hiraga N, Abe H, Tsuge M, Imamura M.	Animal model for study of human hepatitis viruses.	J Gastroenterol Hepatol	26	13-8	2011
柘植雅貴 茶山一彰	B 型慢性肝炎における核酸アナログ中止例の検討	消化器内科	53(3)	300-308	2011
Tsuge M, Hiraga N, Akiyama R, Tanaka S, Matsushita M, Mitsui F, Abe H, Kitamura S, Hatakeyama T, Kimura T, Miki D, Mori N, Imamura M, Takahashi S, Hayes CN, Chayama K.	HBx protein is indispensable for development of viraemia in human hepatocyte chimeric mice.	J Gen Virol	91	1854-64	2010
Mitsui F, Tsuge M, Kimura T, Kitamura S, Abe H, Saneto H, Kawaoka T, Miki D, Hatakeyama T, Hiraga N, Imamura M, Kawakami Y, Aikata H, Takahashi S, Hayes CN, Igarashi H, Morimoto K, Shimizu M, Chayama K.	Importance of serum concentration of adefovir for Lamivudine-adefovir combination therapy in patients with lamivudine-resistant chronic hepatitis B.	Antimicrob Agents Chemother	54	3205-11	2010
Huang YW, Chayama K, Tsuge M, Takahashi S, Hatakeyama T, Abe H, Hu JT, Liu CJ, Lai MY, Chen DS, Yang SS, Kao JH.	Differential effects of interferon and lamivudine on serum HBV RNA inhibition in patients with chronic hepatitis B.	Antivir Ther	15	177-84	2010
Noguchi C, Imamura M, Tsuge M, Hiraga N, Mori N, Miki D, Kimura T, Takahashi S, Fujimoto Y, Ochi H, Abe H, Maekawa T, Tateno C, Yoshizato K, Chayama K.	G-to-A hypermutation in hepatitis B virus (HBV) and clinical course of patients with chronic HBV infection.	J Infect Dis	199(11)	1599-607	2009
Abe H, Ochi H, Maekawa T, Hatakeyama T, Tsuge M, Kitamura S, Kimura T, Miki D, Mitsui F, Hiraga N, Imamura M, Fujimoto Y, Takahashi S, Nakamura Y, Kumada H, Chayama K.	Effects of structural variations of APOBEC3A and APOBEC3B genes inchronic hepatitis B virus infection.	Hepatol Res	39(12)	1159-68	2009

Hiraga N, Imamura M, Hatakeyama T, Kitamura S, Mitsui F, Tanaka S, Tsuge M, Takahashi S, Abe H, Maekawa T, Ochi H, Tateno C, Yoshizato K,Wakita T, Chayama K.	Absence of viral interference and different susceptibility to interferon between hepatitis B virus and hepatitis C virus in human hepatocyte chimeric mice.	J Hepatol.	51(6)	1046-54	2009
Kanda T, Shinozaki M, Kamezaki H, Wu S, Nakamoto S, Arai M, Fujiwara K, Goto N, Imazeki F, Yokosuka O.	Efficacy of lamivudine or entecavir on acute exacerbation of chronic hepatitis B.	Int J Med Sci.	9(1)	27-32	2012
Togo S, Arai M, Tawada A, Chiba T, Kanda T, Fujiwara K, Imazeki F, Yokosuka O.	Clinical importance of serum hepatitis B surface antigen levels in chronic hepatitis B.	J Viral Hepat	18(10)	e508-515	2011
Kamezaki H, Kanda T, Wu S, Nakamoto S, Arai M, Maruyama H, Fujiwara K, Imazeki F, Yokosuka O.	Emergence of entecavir-resistant mutations in nucleos(t)ide-naive Japanese patients infected with hepatitis B virus: virological breakthrough is also dependent on adherence to medication.	Scand J Gastroenterol	46(9)	1111-1117	2011
Bekku D, Arai M, Imazeki F, Yonemitsu Y, Kanda T, Fujiwara K, Fukai K, Sato K, Itoga S, Nomura F, Yokosuka O.	Long-term follow-up of patients with hepatitis B e antigen negative chronic hepatitis B.	J Gastroenterol Hepatol.	26(1)	122-128	2011
Wu S, Imazeki F, Kurbanov F, Fukai K, Arai M, Kanda T, Yonemitsu Y, Tanaka Y, Mizokami M, Yokosuka O.	Evolution of hepatitis B genotype C viral quasi-species during hepatitis B e antigen seroconversion.	J Hepatol	54(1)	19-25	2011
Wu S, Fukai K, Imazeki F, Arai M, Kanda T, Yonemitsu Y, Yokosuka O.	Initial Virological Response and Viral Mutation with Adefovir Dipivoxil Added to Ongoing Lamivudine Therapy in Lamivudine-Resistant Chronic Hepatitis B.	Dig Dis Sci	56(4)	1207-14	2011
Wu S, Kanda T, Imazeki F, Arai M, Yonemitsu Y, Nakamoto S, Fujiwara K, Fukai K, Nomura F, Yokosuka O.	Hepatitis B virus e antigen downregulates cytokine production in human hepatoma cell lines.	Viral Immunol	23(5)	467-476.	2010

Ito K, Arai M, Imazeki F, Yonemitsu Y, Bekku D, Kanda T, Fujiwara K, Fukai K, Sato K, Itoga S, Nomura F, Yokosuka O.	Risk of hepatocellular carcinoma in patients with chronic hepatitis B virus infection.	Scand J Gastroenterol	45(2)	243-249	2010
Kobashi H, Takaguchi K, Ikeda H, Yokosuka O, Moriyama M, Imazeki F, Kage M, Seriu T, Omata M, Sakaguchi K, Shiratori Y.	Efficacy and safety of entecavir in nucleoside-naive, chronic hepatitis B patients: phase II clinical study in Japan.	J Gastroenterol Hepatol.	24(2)	255-261.	2009
髭修平、寺下勝巳、小林智絵、堀本啓大、中西満、中馬誠.	HBs抗原測定 - 新たな臨床応用の話題.	臨床消化器内科	27 (1)	125-129	2012
Matsuura K, Tanaka Y, Kusakabe A, Hige S, Inoue J, Komatsu M, Kuramitsu T, Hirano K, Ohno T, Hasegawa I, Kobashi H, Hino K, Hiasa Y, Nomura H, Sugauchi F, Nojiri S, Joh T, Mizokami M.	Recommendation of lamivudine-to-entecavir switching treatment in chronic hepatitis B responders: Randomized controlled trial.	Hepatol Res	41 (6)	505-511	2011
中西満、中馬誠、髭修平、小林智絵、中井正人、荘拓也、浅香正博	HBV関連肝細胞癌サーベイランスにおけるHBコア関連抗原の有用性	肝臓	52 (6)	387-389	2011
髭修平、荘拓也、中西満、中馬誠.	B 型慢性肝炎に対するインターフェロン療法.	日本臨床	69 (4)	481-485	2011
髭修平、中馬誠、中井正人、小林智絵、荘拓也、中西満、浅香正博.	B 型肝炎に対する IFN/核酸アナログ製剤の有効性.	消化器内科	52 (1)	108-114	2011
Hige S, Yamamoto Y, Yoshida S, Kobayashi T, Horimoto Hi, Yamamoto K, Sho T, Natsuizaka M, Nakanishi M, Chuma M, Asaka M.	Sensitive assay for the quantification of hepatitis B virus mutants by a minor groove binder probe and peptide nucleic acids.	J Clin Microbiol	48 (12)	4487-4494	2010
Matsuura K, Tanaka Y, Hige S, Yamada G, Murawaki Y, Komatsu M, Kuramitsu T, Kawata S, Tanaka E, Izumi N, Okuse C, Kakumu S, Okanoue T, Hino K, Hiasa Y, Sata M, Maeshiro T, Sugauchi F, Nojiri S, Joh T, Miyakawa Y, Mizokami M.	Distribution of hepatitis B virus genotypes among patients with chronic infection in Japan shifting toward an increase of genotype A.	J Clin Microbiol	47 (5)	1476-1483	2009

Chuma M, Hige S, Kamiyama T, Meguro T, Nagasaka A, Nakanishi K, Yamamoto Y, Nakanishi M, Kohara T, Sho T, Yamamoto K, Horimoto H, Kobayashi T, Yokoo H, Matsushita M, Todo S, Asaka M.	The influence of hepatitis B DNA level and antiviral therapy on recurrence after initial curative treatment in patients with hepatocellular carcinoma.	J Gastroenterol	44 (9)	991-999	2009
髭修平、堀本啓大、小原俊央、中西満、中馬誠、浅香正博.	新しいB型肝炎ウイルスマーカー：HBコア関連抗原.	臨床消化器内科	24 (5)	132-136	2009
Tamada Y, Yatsuhashi H, Masaki N, Nakamuta M, Mita E, Komatsu T, Watanabe Y, Muro T, Shimada M, Hijioka T, Satoh T, Mano Y, Komeda T, Takahashi M, Kohno H, Ota H, Hayashi S, Miyakawa Y, Abiru S, Ishibashi H.	Hepatitis B virus strains of subgenotype A2 with an identical sequence spreading rapidly from the capital region to all over Japan in patients with acute hepatitis B.	Gut.			2011 (in press)
Enomoto H, Inoue SI, Matsuhisa A, Aizawa N, Imanishi H, Saito M, Iwata Y, Tanaka H, Ikeda N, Sakai Y, Takashima T, Shimomura S, Iijima H, Nakamura H, Nishiguchi S	Development of a new in situ hybridization method for the detection of global bacterial DNA to provide early evidence of a bacterial infection in spontaneous bacterial peritonitis	J Hepatol.	Aug	9	2011
Shimomura S, Ikeda N, Saito M, Ishii A, Takashima T, Sakai Y, Yoshikawa S, Aizawa N, Tanaka H, Iwata Y, Enomoto H, Imanishi H, Yamamoto T, Jomura H, Nakamura H, Iijima H, Nishiguchi S.	Long-term interferon therapy after radiofrequency ablation is effective in treating patients with HCV-associated hepatocellular carcinoma.	Hepatol Int	5	559-66	2011
Liu W, Nakamura H, Deng H, Enomoto H, Yamamoto T, Iwata Y, Koh N, Saito M, Imanisi H, Shimomura S, Nishigushi S.	A higher expression of hepatoma-derived growth factor in hepatocellular carcinoma cells and more tumor growth in vivo.	Trends in Cancer Research	5	29-36	2010
Yoshikawa S, Iijima H, Saito M, Tanaka H, Imanishi H, Yoshimoto N, Yoshimoto T, Futatsugi-Yumikura S, Nakanishi K, Tsujimura T, Nishigami T, Kudo A, Arii S, Nishiguchi S.	Crucial role of impaired Kupffer cell phagocytosis on the decreased Sonazoid-enhanced echogenicity in a liver of a nonalchoholic steatohepatitis rat model.	Hepatol Res.	40	823-31	2010

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Changes in the serum level of hepatitis B virus (HBV) surface antigen over the natural course of HBV infection

Akihiro Matsumoto · Eiji Tanaka · Susumu Morita · Kaname Yoshizawa · Takeji Umemura · Satoru Joshita

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Abstract
Background Despite its status as a potential biomarker of hepatitis B virus (HBV) response to interferon treatment, the changes in hepatitis B surface antigen (HBsAg) levels over the natural course of HBV carriers have not been analyzed sufficiently.
Methods A total of 101 HBV carriers were followed prospectively from 1999 to 2009. HBsAg level was measured yearly during the followed period.
Results HBsAg levels at baseline ranged from −1.4 to 5.32 log IU/ml, with a median value of 3.2 log IU/ml. Lower HBsAg levels were significantly associated with higher age and lower HBV replication status. The rate of change of HBsAg levels showed two peaks, with a cut-off value of −0.4 log IU/year. Based on this, patients were tentatively classified into rapid decrease (rate of change <−0.4 log IU/year) and non-rapid decrease groups. All baseline levels of HBsAg, HB core-related Ag, and HBV DNA were lower in the rapid decrease group than in the non-rapid decrease group. Patients with persistently positive HBeAg were all classified into the non-rapid decrease group. In patients with persistently negative HBeAg, HBV DNA levels were significantly ($P = 0.028$) lower in the rapid decrease group than in the non-rapid decrease group.
Conclusions Lower baseline HBsAg levels were significantly associated with older age and lower viral activity. Both a loss of HBeAg detection as well as inactive replication of HBV are suggested to be fundamental factors contributing to a rapid decrease in HBsAg over the natural course of HBV infection.

Keywords Hepatitis B virus · Hepatitis B surface antigen · Hepatitis B core-related antigen · Serum level · Natural course

Abbreviations

HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
IU	International unit
HBcrAg	Hepatitis B virus core-related antigen
HCC	Hepatocellular carcinoma
NA	Nucleos(t)ide analogue
HBeAg	Hepatitis B e antigen
CLEIA	Chemiluminescent enzyme immunoassay
Da	Dalton
HR	Hazard ratio
cccDNA	Covalently closed circular DNA

Introduction

With an estimated 350–400 million cases of chronic infection, hepatitis B virus (HBV) infection is a major worldwide health problem [1]. Chronic infection of HBV often leads to chronic hepatitis and eventually to liver cirrhosis and hepatocellular carcinoma [2, 3]. During infection, hepatitis B surface antigen (HBsAg), which is a component of the virion envelope, is secreted into the

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bloodstream in large amounts as subviral particles. Thus, serum HBsAg is routinely used as a marker for detection of HBV infection.

Recently, several groups have reported that HBsAg levels can be used as an indicator of the response to peg-interferon in chronic hepatitis B similarly to the conventional markers of HBV DNA level and hepatitis B (HB) e antigen/antibody status [4, 5]. Since HBV carriers who clear HBsAg usually have a better prognosis than those who do not [6–8], it may be worthwhile to monitor HBsAg levels in the natural disease course of HBV infection. However, such changes need to be clarified more thoroughly to validate their clinical significance. In the present study, we analyzed the changes in HBsAg levels in a cohort of HBV carriers who were followed prospectively and compared them with those of HBV DNA and HB core-related antigens (HBcrAg) levels.

Patients and methods

Patients

A total of 101 HBV carriers were followed prospectively from 1999 to 2009. Patients were selected consecutively between 1997 and 1999 and met the following conditions: (1) HBsAg was positive in at least two examinations performed over 1 year apart; (2) no complications of hepatocellular carcinoma (HCC) or signs of hepatic dysfunction, such as jaundice or ascites, were observed; (3) nucleos(t)ide analogues (NAs) were not administered at the start of follow-up; and (4) patients were negative for hepatitis C and human immunodeficiency virus antibodies. The clinical and virological characteristics of our cohort are shown in Table 1. The 101 patients consisted of 57 men and 44 women with a median age of 50 years (range 15–83 years). Hepatitis B e antigen (HBeAg) was positive in 38 (38%) patients and negative in 63 (63%). Of the 38 patients with HBeAg, 15 remained positive and 23 became negative during the follow-up period. Alanine aminotransferase (ALT) level flares of over 1,000 IU/L were observed in four (17%) of the 23 patients with HBeAg loss, but in none of the 15 patients with persistent HBeAg ($P = 0.138$). HBV genotype distribution was A in three (3%) patients, B in nine (9%), C in 87 (86%), and undetermined in two (2%). All patients were seen at Shinshu University Hospital or one of its affiliated hospitals. Our cohort tended to have a higher prevalence of cirrhosis (19%) and HCC (14%). These tendencies may be attributed to the higher age distribution in our cohort than that in other cohorts of HBsAg studies [6, 9, 10].

Patients were seen at least once a year during the 10 years of follow-up. The presence of cirrhosis was judged by histological findings and/or typical findings seen in cirrhosis, such as esophageal varices and splenomegaly. Screening for HCC was done using ultrasonography (US), computed tomography (CT), and/or magnetic resonance (MR) imagining at least once a year. The presence of complicating HCC was judged by evidence of characteristic hepatic masses on liver CT, MRI, and/or hepatic angiography. Serum samples were collected on a yearly basis and immediately stored at −20°C or below until assayed. This study was approved by the Ethics Committee of Shinshu University.

Hepatitis B viral markers

Serological markers for HBV, including HBsAg, HBeAg, and HBe antibody, were tested using commercially

Table 1 Clinical and virological characteristics of patients with respect to HBeAg status

Characteristic	Overall ($n = 101$)	HBeAg-positive ($n = 38$)	HBeAg-negative ($n = 63$)	P
At baseline				
Age (years)[a]	50 (15 to 83)	42 (15 to 72)	53 (25 to 83)	<0.001
Male[b]	57 (56%)	22 (58%)	35 (56%)	>0.2
With cirrhosis[b]	19 (19%)	10 (26%)	9 (14%)	0.188
ALT (IU/L)[a]	31 (10 to 447)	47 (13 to 447)	29 (10 to 81)	0.002
HBV genotype (A:B:C:UD)	3:9:87:2	1:0:36:1	2:9:51:1	0.144
HBsAg (log IU/ml)[a]	3.2 (−1.4 to 5.3)	3.7 (1.6 to 5.3)	2.9 (−1.4 to 4.3)	<0.001
HBcrAg (log U/ml)[a]	3.8 (<3.0 to >6.8)	6.8 (<3.0 to >6.8)	3.1 (<3.0 to >6.8)	<0.001
HBV DNA (log copies/ml)[a]	4.7 (neg. to >9.5)	7.4 (2.4 to >9.5)	3.6 (neg. to 8.3)	<0.001
During follow-up				
Followed period (years)[a]	5 (1 to 10)	6 (1 to 10)	5 (1 to 10)	>0.2
Clearance of HBsAg[b]	20 (20%)	3 (8%)	17 (27%)	0.022
Complication of HCC[b]	14 (14%)	8 (21%)	6 (10%)	0.139
Introduction of NAs[b]	23 (23%)	11 (29%)	12 (19%)	>0.2

UD undetermined

[a] Data are expressed as median (range)

[b] Data are expressed as positive number (%)

available enzyme immunoassay kits (Abbott Japan Co., Ltd., Tokyo, Japan). Quantitative measurement of HBsAg was done using an HISCL® HBsAg assay based on the chemiluminescence enzyme immunoassay (CLEIA) (Sysmex Co. Ltd., Kobe, Japan), which had a quantitative range from −1.5 to 3.3 log IU/ml. End titer was determined by diluting samples with normal human serum when initial results exceeded the upper limit of the assay range. Changes in HBsAg levels during the natural course of HBV infection were calculated as: difference in HBsAg level at baseline and at last visit (not undergoing NA treatment) divided by the corresponding follow-up time. Results were expressed as log change per year. Points when patients were negative for HBsAg were omitted in calculations; thus, three patients who had cleared HBsAg by the first follow-up were excluded from the study. Changes in HBsAg levels during NA treatment were calculated similarly using the differences in HBsAg levels between the start and either the end of NA treatment or the last visit.

Serum HBcrAg levels were measured using a CLEIA-based HBcrAg assay kit with a fully automated Lumipulse System analyzer (Fujirebio Inc., Tokyo, Japan) as described previously [11]. Briefly, 150 μl of serum was incubated with 150 μl of pretreatment solution containing 15% sodium dodecyl sulphate at 60°C for 30 min. After heat treatment, 120 μl of pretreated specimen was added to a ferrite microparticle suspension in an assay cartridge. Ferrite particles were coated with a monoclonal antibody mixture (HB44, HB61, and HB114) against denatured HBcAg, HBeAg, and the 22 kDa precore protein. [12] After 10 min of incubation at 37°C and washing, further incubation was carried out for 10 min at 37°C with alkaline phosphatase conjugated with two kinds of monoclonal antibodies (HB91 and HB110) against denatured HBcAg, HBeAg, and the 22 kDa precore protein. After washing, 200 μl of substrate solution was added to the test cartridge, which was then incubated for 5 min at 37°C. The relative chemiluminescence intensity was measured, and HBcrAg concentration was calculated by a standard curve generated using recombinant pro-HBeAg (amino acids −10 to 183 of the precore/core gene product). The immunoreactivity of pro-HBeAg at 10 fg/ml was defined as 1 U/ml. HBcrAg was expressed in terms of log U/ml, and the quantitative range was set at 3.0–6.8 log U/ml.

Serum concentration of HBV DNA was determined using an AccuGene m-HBV kit (Abbott Japan Co., Ltd.) with a quantitative range of 1.7–9.5 log copies/ml when tested in a sample volume of 0.2 ml. Six HBV genotypes (A–F) were evaluated according to the restriction patterns of DNA fragments from the method reported by Mizokami et al. [13].

Statistical analyses

Correlations between variables were calculated using the Spearman correlation coefficient test. The Fisher's exact and Pearson's Chi-square tests were adopted to test for differences between subgroups of patients. To compare continuous data, the Mann–Whitney *U* test was employed. To compare paired continuous data, the Wilcoxon signed-rank test for matched pairs was used. The Kaplan–Meier method was used to estimate positive rates of HBsAg and the occurrence rate of HCC. Multivariate analyses were performed using the Cox regression model. Variables associated with a P value of <0.2 in univariate analyses were included in a stepwise Cox regression analysis to identify independent factors associated with clearance of HBsAg. All tests were performed using the IBM SPSS Statistics Desktop for Japan ver. 19.0 (IBM Japan Inc., Tokyo, Japan). *P* values of less than 0.05 were considered to be statistically significant.

Results

Follow-up of patients

Twenty three (23%) of the 101 patients enrolled dropped out of the study for reasons of changing addresses (11 patients) or halting hospital visits (12 patients). Among the remaining 78 patients, six died (four from HCC, one from hepatic failure, and one from old age) and one underwent liver transplantation due to hepatic failure. Thus, 71 patients completed the full follow-up period of 10 years.

Long term treatment with NAs, such lamivudine, was introduced in 23 patients (23%) during the study period.

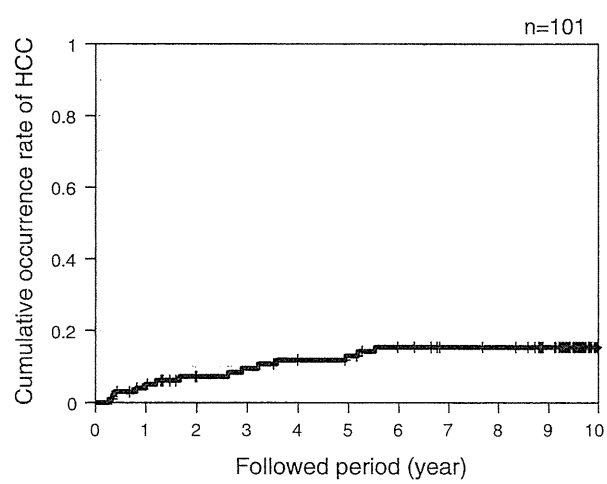

Fig. 1 Changes in the cumulative occurrence rate of HCC during the follow-up period

These patients commenced treatment after showing clinical and/or histological features of chronic active hepatitis B. The treatment period with NAs was excluded from our analysis of HBsAg level changes during the natural disease course.

Complicating HCC was seen in 14 patients within 6 years of their first visit (Fig. 1), leading to an annual rate of HCC occurrence of 2.3% per year for the first 6 years of follow-up. HCC was seen after the disappearance of HBsAg in a 90-year-old woman with negative HBeAg and HBV DNA at the time of diagnosis.

HBsAg levels at baseline and during clinical course

Baseline HBsAg levels ranged from −1.4 to 5.32 log IU/ml, with a median value of 3.2 log IU/ml (Fig. 2). Table 2 shows a comparison of clinical and virological characteristics between patients with lower and higher HBsAg levels divided at the median level. Older patients were significantly more prevalent in the lower level group than in the higher level group. Genotype B was only seen in the lower level group. Detection of HBeAg and median levels of both HBV DNA and HBcrAg were significantly lower in the lower level group. Clearance of HBsAg during the follow-up period was more frequent in the lower level group, but the occurrence of HCC was comparable between the two groups. Of the 52 patients with higher HBsAg levels at baseline, five lost HBsAg positivity and the remaining 47 did not. Median levels of HBV DNA (3.2 vs. 6.0 log copies/ml, $P = 0.023$), HBsAg (3.5 vs. 3.8 log IU/ml, $P = 0.091$), and HBcrAg (3.2 vs. 5.5 log U/ml, $P = 0.095$) tended to be lower in the former than in the latter group of patients at baseline, but the difference was statistically significant for HBV DNA level only. Median age (49 vs. 46 years, $P > 0.2$), male gender (80 vs. 45%, $P = 0.183$), and median ALT level (32 vs. 40 IU/L, $P > 0.2$) did not differ between the two groups at baseline.

HBsAg levels at baseline were further analyzed according to age and HBeAg status, and the trend of HBsAg distribution was compared to those of HBcrAg and HBV DNA (Fig. 3). HBsAg levels in HBeAg-positive patients were distributed in a higher range, and the association of HBsAg with age was faint ($r = -0.291$, $P = 0.076$). On the other hand, HBsAg levels were distributed in a higher range in patients younger than 50 years of age, but were distributed more widely in patients 50 years or older. HBsAg levels in HBeAg-negative patients decreased significantly ($r = -0.453$, $P < 0.001$) with age. Furthermore, whereas HBcrAg levels in HBeAg-positive patients ($r = -0.260$, $P = 0.115$) were distributed in a higher range among all

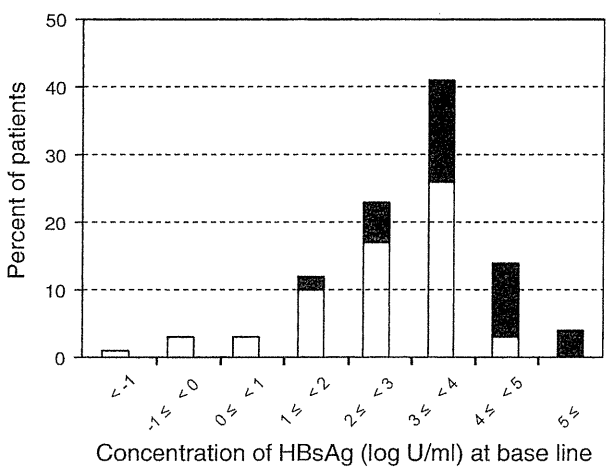

Fig. 2 Distribution of HBsAg concentration at baseline. *Closed bars* indicate patients with detectable HBeAg and *open bars* indicate those without

Table 2 Comparison of clinical and virological characteristics between patients with serum HBsAg levels less than 3.2 log IU/ml and those with levels equal to or higher than 3.2 log IU/ml

Characteristic	HBsAg level at baseline		*P*
	<3.2 log IU/ml ($n = 49$)	≥3.2 log IU/ml ($n = 52$)	
At baseline			
Age (years)[a]	55 (32 to 83)	45 (15 to 72)	<0.001
Male[b]	32 (65%)	25 (48%)	0.108
With cirrhosis[b]	13 (27%)	6 (12%)	0.075
ALT (IU/L)[a]	28 (10 to 119)	39 (12 to 447)	0.089
HBV genotype (A:B:C:UD)	1:9:38:1	2:0:49:1	0.018
HBeAg[b]	11 (22%)	27 (52%)	0.004
HBcrAg (log U/ml)[a]	3.3 (<3.0 to >6.8)	5.5 (<3.0 to >6.8)	<0.001
HBV DNA (log copies/ml)[a]	3.7 (<1.7 to 8.3)	6.0 (neg. to >9.5)	0.001
During follow-up			
Followed period (years)[a]	4 (1 to 10)	8 (1 to 10)	0.001
Clearance of HBsAg[b]	15 (31%)	5 (10%)	0.012
Occurrence of HCC[b]	9 (18%)	5 (10%)	>0.2
Introduction of NAs[b]	9 (18%)	14 (27%)	>0.2

UD undetermined

[a] Data are expressed as median (range)

[b] Data are expressed as positive number (%)

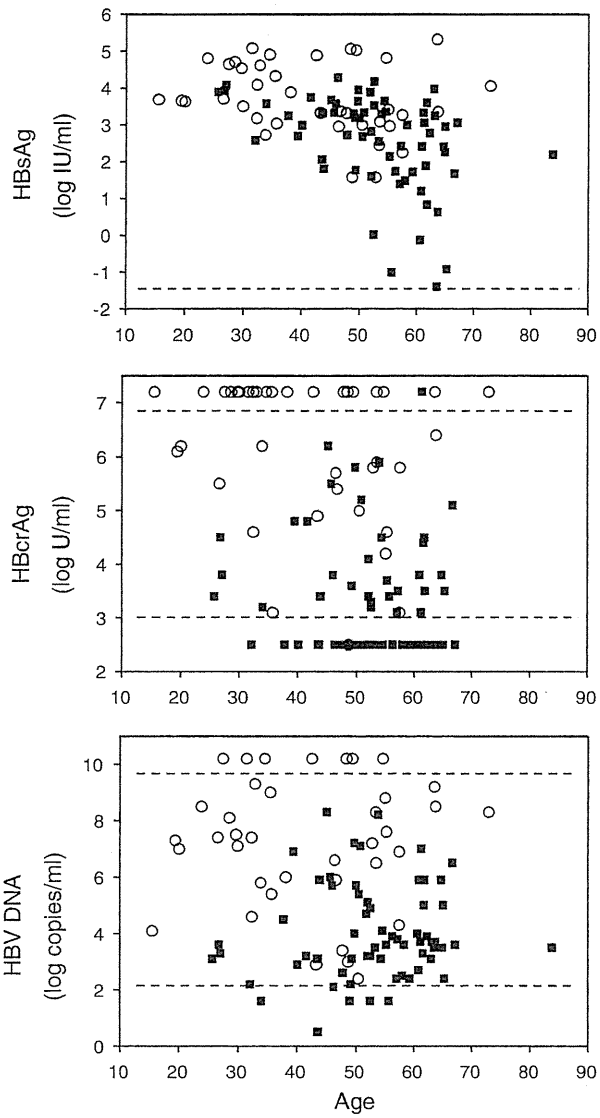

Fig. 3 HBsAg, HBcrAg, and HBV DNA levels analyzed according to HBeAg status and patient age. *Open circles* indicate patients with detectable HBeAg and *closed squares* indicate those without

ages, those in HBeAg-negative patients ($r = -0.103$, $P > 0.2$) were found in a lower range. A similar trend was seen for HBV DNA level distribution ($r = 0.015$, $P > 0.2$ and $r = 0.146$, $P > 0.2$, respectively).

Changes in HBsAg levels during the follow-up period

Positivity for HBsAg decreased gradually over the follow-up period (Fig. 4). A total of 20 patients cleared HBsAg during the follow-up period, for a disappearance rate of 2.1% per year. Clinical and virological backgrounds were compared between patients with and without clearance of HBsAg in Table 3. Patients losing HBsAg positivity were

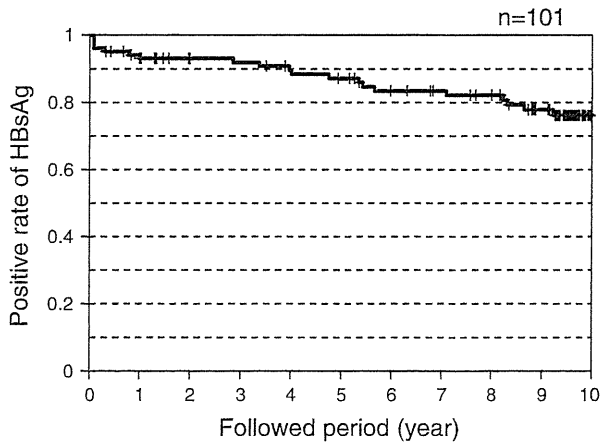

Fig. 4 Changes in HBsAg positivity during the follow-up period

significantly older than those who did not. Baseline levels of HBsAg, HBcrAg, and HBV DNA were significantly lower in these patients as well. Clearance of HBsAg was significantly associated with HBV DNA (HR 3.6, 95% CI 1.1–11.4, $P = 0.033$) and HBcrAg (HR 4.0, 95% CI 1.1–14.9, $P = 0.036$) levels at baseline by multivariate analysis. Of the 20 patients who cleared HBsAg, seven were positive for HBV DNA (range, positive 3.0 log copies/ml) and three were positive for HBcrAg (range 3.0–3.2 U/ml).

Figure 5 shows the distribution of patients according to the rate of change of HBsAg levels. Of the 98 patients analyzed, 79 (81%) showed a decrease in HBsAg. Although this level increased in 19% of patients, such changes were less than 0.2 log IU/year. The rate of change of HBsAg levels peaked at a cut-off value of −0.4 log IU/year. Accordingly, patients were tentatively classified into the rapid decrease group (rate of change <-0.4 log IU/year) and the non-rapid decrease group (rate of change ≥ -0.4 log IU/year). Median age, gender distribution, prevalence of cirrhosis, ALT level, and genotype distribution did not differ between the two groups (Table 4). Levels of HBsAg, HBcrAg, and HBV DNA were significantly lower in the rapid decrease group than in the non-rapid one. Whereas all patients with persistently positive HBeAg were classified into the non-rapid group, patients with persistently negative HBeAg fell more frequently into the rapid decrease group (77%) than into the non-rapid decrease group (54%). In those patients, HBV DNA levels were significantly ($P = 0.028$) lower in the rapid decrease group (median 3.4, range <2.1–5.9 log copies/ml) than in the non-rapid decrease group (median 3.8, range <2.1–8.1 log copies/ml). Complicating HCC was lower in the rapid decrease group, but this difference was not statistically significant.

The median change in HBsAg level before NA treatment (−0.117 log IU/ml/year; range −2.4 to 1.41 log

Table 3 Comparison of clinical and virological characteristics between patients with and without clearance of HBsAg

Characteristic	Clearance of HBsAg		*P*
	Positive ($n = 20$)	Negative ($n = 81$)	
At baseline			
Age (years)[a]	56 (30 to 65)	50 (16 to 84)	0.038
Male[b]	8 (40%)	36 (44%)	>0.2
With cirrhosis[b]	4 (20%)	15 (18%)	1.000
ALT (IU/L)[a]	26 (10 to 108)	35 (13 to 447)	0.057
HBV genotype (A:B:C:UD)	0:2:18:0	3:7:69:2	>0.2
HBeAg[b]	3 (15%)	35 (43%)	0.022
HBsAg (log IU/ml)[a]	1.7 (−1.7 to 4.2)	3.3 (0.83 to 5.3)	<0.001
HBcrAg (log U/ml)[a]	3.0 (3.0 to >6.8)	4.7 (3.0 to >6.8)	<0.001
HBV DNA (log copies/ml)[a]	3.0 (<1.7 to 7.6)	5.7 (neg. to >9.5)	<0.001
During follow-up			
Followed period (years)[a]	4.4 (0.31 to 10.0)	5.2 (0.1 to 10.0)	>0.2
Occurrence of HCC[b]	1 (5.0%)	13 (16.0%)	>0.2
Introduction of NAs[b]	0 (0%)	23 (28%)	0.006

UD undetermined

[a] Data are expressed as median (range)

[b] Data are expressed as positive number (%)

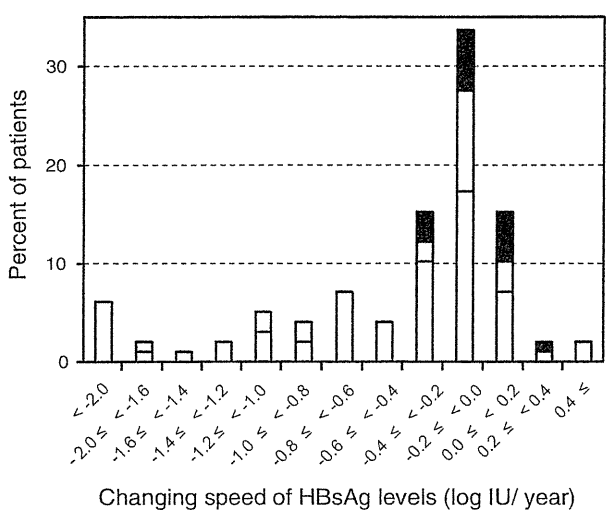

Fig. 5 Distribution of patients classified according to rate of change of HBsAg levels (log IU/year) during follow-up period. *Closed bars* indicate patients with persistent HBeAg-positive status. *Shaded bars* indicate patients who became negative for HBeAg during follow-up period. *Open bars* indicate patients with persistent HBeAg-negative status

IU/ml/year) was similar ($P > 0.2$) to that after starting NA treatment (−0.017 log IU/ml/year; range −5.18 to 0.17 log IU/ml/year) in the 20 patients who commenced therapy with NAs during the study period.

Discussion

During the natural course of HBV infection, HBsAg levels showed almost normal distribution, making a sharp peak at a median value of 3.2 log IU/ml. Lower HBsAg levels were significantly associated with older age and lower viral activity, but not with gender or genotype. A similar trend was observed in patients who cleared HBsAg in our cohort. Chan et al. [10] reported that HBsAg levels were significantly lower in HBeAg-negative patients than in HBeAg-positive ones and tended to fall in accordance with decreases in HBV DNA levels. Simonetti et al. [6] reported that clearance of HBsAg was associated with older age, but not with gender or genotype, in a prospective population-based cohort study. Chu et al. [9] also reported that HBsAg clearance was associated with older age, in which the cumulative probability of clearance increased disproportionally with a longer follow-up period. In light of these results as well as of our own, it appears that lower HBsAg levels are closely associated with older age and lower activity of HBV replication. The HBsAg clearance rate of 2.1% per year in the current study was three times higher than that of the 0.7% per year reported by Simonetti et al. [6]. However, the median age at the start of their follow-up (20 years) was considerably lower than that in our report (50 years). Chu et al. [9] followed 1965 asymptomatic HBV carriers that were positive for HBe antibodies in whom the mean age at baseline was 35.6 years, revealing a HBsAg clearance rate of 0.8% per year after 10 years of follow-up that increased to 1.8% per year over a 25-year observation period. HBsAg clearance appeared to increase as patients aged in that cohort, which may at least partly explain the higher clearance rate found in the present study.

Because HBsAg level is closely associated with age, we analyzed this relationship and compared it with those of HBcrAg and HBV DNA. HBsAg levels decreased in association with age in HBeAg-negative patients. A similar but faint association was also seen in HBeAg-positive patients. On the other hand, HBcrAg and HBV DNA levels

Table 4 Comparison of clinical and virological characteristics between patients with rapid and non-rapid decrease of HBsAg

Characteristic	Rapid decrease ($n = 31$)	Non-rapid decrease ($n = 67$)	P
At baseline			
Age (years)[a]	52 (15 to 65)	49 (19 to 83)	0.338
Male[b]	17 (55%)	38 (57%)	1.000
With cirrhosis[b]	6 (19%)	13 (19%)	1.000
ALT (IU/L)[a]	27 (10 to 108)	36 (13 to 447)	0.230
HBV genotype (A:B:C:UD)	1:4:26:0	2:4:59:2	0.617
HBeAg-positive[b]	7 (23%)	31 (46%)	0.028
HBsAg (log IU/ml)[a]	2.8 (−1.0 to 5.0)	3.3 (0.8 to 5.3)	0.001
HBcrAg (log U/ml)[a]	<3.0 (<3.0 to >6.8)	5.1 (<3.0 to >6.8)	<0.001
HBV DNA (log copies/ml)[a]	3.7 (<1.7 to >9.5)	5.9 (neg. to >9.5)	0.002
During follow-up			
Followed period (years)[a]	3 (1 to 9)	6 (1 to 10)	<0.001
Change in HBeAg status			0.012
Persistent positive[b]	0 (0%)	15 (22%)	
Became negative[b]	7 (23%)	16 (24%)	
Persistent negative[b]	24 (77%)	36 (54%)	
Clearance of HBsAg[b]	18 (58%)	0 (0%)	<0.001
Complication of HCC[b]	2 (7%)	12 (18%)	0.214
Introduction of NAs[b]	4 (13%)	19 (28%)	0.125

UD undetermined

[a] Data are expressed as median (range)

[b] Data are expressed as positive number (%)

were more uniformly distributed with age in both HBeAg-positive and -negative patients. Therefore, it can be inferred that HBsAg level is affected by age in the natural course of HBV, even when the factor of viral activity is excluded. The precise mechanism of this trend is at present unclear, but may be attributed to the character of HBsAg itself, and not to that of HBV antigens, because HBcrAg levels showed a similar trend as HBV DNA levels. Chan et al. [10] reported that a stronger correlation between HBV DNA and HBsAg was found in the HBeAg-positive phase than in the HBeAg-negative phase. This observation was clearly confirmed by our results in that the distribution pattern analyzed by age was similar between HBsAg and HBV DNA levels in HBeAg-positive patients but differed in HBeAg-negative ones.

The rate of change of HBsAg in the present study suggested the existence of two groups centered around a value of −0.4 log IU/year. A necessary decline in HBV replication was evident in the rapid decrease group, whose median HBV DNA level was lower than the 4.0 log copy/ml usually seen in inactive carriers of HBV. Since no patient with persistently positive HBeAg was classified into the rapid increase group, we presume that a loss of HBeAg is essential for a rapid decrease in HBsAg. In patients with persistently negative HBeAg, HBV DNA levels were significantly lower in the rapid decrease group than in the non-rapid decrease group. Therefore, not only a loss of HBeAg, but also a decline in HBV replication, appears to be fundamental factors necessary for a rapid decrease in HBsAg. Chan et al. [10] concluded that HBs antigen level remained stable in HBe antigen-positive patients and reduced slowly in HBe antigen-negative patients. Our results are similar, but further imply that a decline in HBV replication is also required. The rate of HBsAg level decrease was similar before and after starting NA treatment in the present study. However, additional studies in larger cohorts will be required to determine this particular relationship.

We analyzed HBcrAg in addition to HBsAg as an HBV-related antigen in the present study to further clarify the characteristics of HBsAg. The HBcrAg assay measures serum levels of HBcAg, HBeAg, and the 22 kDa precore protein [12] simultaneously using monoclonal antibodies that recognize the common epitopes of these denatured antigens. Since the assay measures all antigens transcribed from the pre-core/core gene, it is regarded as core-related [14]. It is possible that levels of HBsAg and HBcrAg have different properties because transcriptions of these two antigens are regulated by alternative enhancer-promoter systems in the HBV genome [15]. Recent studies have shown that HBsAg quantification may represent a surrogate marker of cccDNA concentration in the liver and a potential tool to monitor virologic response to interferon treatment [4, 5, 16]. On the other hand, serum HBcrAg has been reported to accurately reflect intracellular levels of HBV cccDNA even during nucleos(t)ide treatment [11, 17, 18], and was found to be useful for identifying patients who were likely to show relapse of hepatitis after the discontinuation of NAs [19, 20] or who had a higher possibility to develop hepatocellular carcinoma even under NA treatment [17]. Our results here suggest that there exists a

difference in natural course changes between HBsAg and HBcrAg levels. We recently reported that the combined use of these two antigens was useful for predicting the occurrence of hepatitis relapse after cessation of NAs [21]. Such results also indicated that levels of HBsAg and HBcrAg had different clinical significance despite the fact that both antigen levels are generally considered to reflect the amount of HBV cccDNA in hepatocytes.

Complicating HCC occurred during the first 6 years of follow-up in our study at an annual occurrence rate of 2.3% per year for that period. This complication was seen at similar frequencies in patients with high and low baseline HBsAg levels as well as in patients who showed rapid and non-rapid decreases in HBsAg. Patients with lower HBsAg levels and those with rapid decreases in HBsAg have been shown to have lower levels of HBV replication, which would indicate a lower risk of complicating HCC. However, such patients also tend to be older and presumably more predisposed to HCC. The similar occurrence of HCC irrespective of HBsAg status may be attributed to the existence of these two contrary factors. Yuen et al. [7] reported that the risk of HCC in patients with HBsAg seroclearance was higher in those older than 50 years of age; indeed, the single patient who developed HCC after HBsAg seroclearance in the present study was a 90 year-old woman.

In conclusion, lower HBsAg levels were significantly associated with older age and lower viral activity, but not with gender or genotype. Both a loss of HBeAg positivity and a decline in HBV replication are suggested to be fundamental factors necessary for a rapid decrease in HBsAg. Furthermore, the clinical significance of HBsAg may be different from that of HBcrAg with regard to age. Future studies are required to clarify the difference between the two antigens.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat. 2004;11:97–107.
2. Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: summary of a clinical research workshop. Hepatology. 2007;45:1056–75.
3. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology. 2007;45:507–39.
4. Brunetto MR, Moriconi F, Bonino F, Lau GK, Farci P, Yurdaydin C, et al. Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. Hepatology. 2009;49:1141–50.
5. Moucari R, Mackiewicz V, Lada O, Ripault MP, Castelnau C, Martinot-Peignoux M, et al. Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology. 2009;49:1151–7.
6. Simonetti J, Bulkow L, McMahon BJ, Homan C, Snowball M, Negus S, et al. Clearance of hepatitis B surface antigen and risk of hepatocellular carcinoma in a cohort chronically infected with hepatitis B virus. Hepatology. 2010;51:1531–7.
7. Yuen MF, Wong DK, Fung J, Ip P, But D, Hung I, et al. HBsAg Seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. Gastroenterology. 2008;135:1192–9.
8. Tseng TC, Kao JH. HBsAg seroclearance: the more and earlier, the better. Gastroenterology. 2009;136:1842–3. author reply 3–4.
9. Chu CM, Liaw YF. HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. Hepatology. 2007;45:1187–92.
10. Chan HL, Wong VW, Wong GL, Tse CH, Chan HY, Sung JJ. A longitudinal study on the natural history of serum hepatitis B surface antigen changes in chronic hepatitis B. Hepatology. 2010;52:1232–41.
11. Suzuki F, Miyakoshi H, Kobayashi M, Kumada H. Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. J Med Virol. 2009;81:27–33.
12. Kimura T, Ohno N, Terada N, Rokuhara A, Matsumoto A, Yagi S, et al. Hepatitis B virus DNA-negative dane particles lack core protein but contain a 22-kDa precore protein without C-terminal arginine-rich domain. J Biol Chem. 2005;280:21713–9.
13. Mizokami M, Nakano T, Orito E, Tanaka Y, Sakugawa H, Mukaide M, et al. Hepatitis B virus genotype assignment using restriction fragment length polymorphism patterns. FEBS Lett. 1999;450:66–71.
14. Tanaka E, Matsumoto A, Yoshizawa K, Maki N. Hepatitis B core-related antigen assay is useful for monitoring the antiviral effects of nucleoside analogue therapy. Intervirology. 2008;51(Suppl 1):3–6.
15. Lee WM. Hepatitis B virus infection. N Engl J Med. 1997;337:1733–45.
16. Chan HL, Wong VW, Tse AM, Tse CH, Chim AM, Chan HY, et al. Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response. Clin Gastroenterol Hepatol. 2007;5:1462–8.
17. Hosaka T, Suzuki F, Kobayashi M, Hirakawa M, Kawamura Y, Yatsuji H, et al. HBcrAg is a predictor of post-treatment recurrence of hepatocellular carcinoma during antiviral therapy. Liver Int. 2010;30:1461–70.
18. Wong DK, Tanaka Y, Lai CL, Mizokami M, Fung J, Yuen MF. Hepatitis B virus core-related antigens as markers for monitoring chronic hepatitis B infection. J Clin Microbiol. 2007;45:3942–7.
19. Matsumoto A, Tanaka E, Minami M, Okanoue T, Yatsuhashi H, Nagaoka S, et al. Low serum level of hepatitis B core-related antigen indicates unlikely reactivation of hepatitis after cessation of lamivudine therapy. Hepatol Res. 2007;37:661–6.
20. Shinkai N, Tanaka Y, Orito E, Ito K, Ohno T, Hirashima N, et al. Measurement of hepatitis B virus core-related antigen as predicting factor for relapse after cessation of lamivudine therapy for chronic hepatitis B virus infection. Hepatol Res. 2006;36:272–6.
21. Matsumoto A, Tanaka E, Suzuki Y, Kobayashi M, Tanaka Y, Shinkai N, et al. Combination of hepatitis B viral antigens and DNA for prediction of relapse after discontinuation of nucleos(t)ide analogues in patients with chronic hepatitis B. Hepatol Res. 2012;42:139–49.