＜特別寄稿＞

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 ―

Guidelines for avoiding risks resulting from discontinuation of nucleos(t)ide analogues in patients with chronic hepatitis B (2012)

Running title：核酸アナログ薬中止の指針

著者：

田中榮司[1)]、松本晶博[1)]、鈴木義之[2)]、小林万利子[2)]、田中靖人[3)]、新海登[3)]、髭修平[4)]、八橋弘[5)]、長岡進矢[5)]、茶山一彰[6)]、柘植雅貴[6)]、横須賀收[7)]、今関文夫[7)]、西口　修平[8)]、齋藤正紀[8)]、藤原圭[9)]、鳥居信之[10)]、平松直樹[11)]、狩野吉康[12)]

Authors:

Eiji Tanaka[1], Akihiro Matsumoto[1], Yoshiyuki Suzuki[2], Mariko Kobayashi[2], Yasuhito Tanaka[3], Noboru Shinkai[3], Shuhei Hige[4], Hiroshi Yatsuhashi[5], Shinya Nagaoka[5], Kazuaki Chayama[6], Masataka Tsuge[6], Osamu Yokosuka[7], Fumio Imazeki[7], Shuhei Nishiguchi[8], Masaki Saito[8], Kei Fujiwara[9], Nobuyuki Torii[10], Naoki Hiramatsu[11], Yoshiyasu Karino[12]

所属：

1) 信州大学医学消化器内科
2) 虎の門病院肝臓内科
3) 名古屋市立大学大学院医学研究科病態医科学
4) 北海道大学大学院医学研究科消化器内科
5) 独立行政法人国立病院機構長崎医療センター臨床研究センター
6) 広島大学大学院分子病態制御内科学
7) 千葉大学大学院医学研究院腫瘍内科学
8) 兵庫医科大学肝胆膵内科
9) 名古屋第二赤十字病院消化器内科
10) 東京女子医科大学消化器内科
11) 大阪大学大学院医学系研究科消化器内科学
12) 札幌厚生病院消化器科

Affiliations:

1) Department of Medicine, Shinshu University School of Medicine
2) Department of Hepatology, Toranomon Hospital
3) Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences

4) Department of Gastroenterology and Hepatology, Graduate School of Medicine, Hokkaido University
5) The Clinical Research Center, NHO Nagasaki Medical Center
6) Programs for Biomedical Research, Division of Frontier Medical Science, Department of Medicine and Molecular Science, Graduate School of Biomedical Sciences, Hiroshima University
7) Department of Medicine and Clinical Oncology, Graduate School of Medicine, Chiba University
8) Division of Hepatobiliary and Pancreatic Diseases, Department of Internal Medicine, Hyogo College of Medicine
9) Gastroenterology Section, Nagoya Daini Red Cross Hospital
10) Department of Internal Medicine and Gastroenterology, Tokyo Women's Medical University
11) Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine
12) Department of Gastroenterology, Sapporo Kosei General Hospital

Corresponding author :
田中榮司
〒390-621　松本市旭 3-1-1　信州大学医学部内科学第二
電話 0263-37-2634、ファックス 0263-32-9412
e-mail address : etanaka@shinshu-u.ac.jp

索引用語：　核酸アナログ薬、治療中止、B 型肝炎、肝炎再燃、HBV cccDNA

Key Words: nucleos(t)ide analogue, discontinuation of treatment, hepatitis B, hepatitis relapse, HBV cccDNA

はじめに

近年B型肝炎の治療に導入された核酸アナログ薬はHBVの増殖を強力に抑制するため、多くの症例で血中HBV DNA量は速やかに低下しALT値の正常化がもたらされる[1]。さらに、組織学的な改善が得られ肝発癌率が低下することや[2,3]、経口薬で副作用も少ないことから臨床的に広く使用されている。しかし、核酸アナログ薬を使用してもウイルスを完全に排除することは困難であり、本治療薬には耐性株の出現や治療中止に伴う肝炎の再燃が問題点として残されている[4]。この原因の一つとして、血中のHBV DNA量が低下しても、HBV複製の起源となる肝細胞核内のHBV cccDNA量はほとんど減らず、これが長期に残存することが挙げられている[5]。

B型肝炎の核酸アナログ薬治療において、同薬の中止はしばしば肝炎の再燃を伴うため、安易な中止はすべきでないとされている。しかし、中止後、いつ頃どの様な形で肝炎が再燃するかは必ずしも明らかにされてはいない。また、中止後に肝炎が再燃しない症例や再燃しても軽度で最終的に安定化する症例も少なからず存在するが、この様な症例を効率よく見分ける方法も確立されていない。

我々は、厚生労働省の科学研究費により「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」（平成21年度～23年度）を行い、治療中止後の経過の特徴や肝炎再燃の定義、さらには再燃率の予測を検討した。本稿では、この研究成果を元に「核酸アナログ薬中止に伴うリスク回避のための指針 2012」をまとめたので報告する（表 1)。本指針は必ずしも核酸アナログ薬の中止を推奨するものではなく、様々な理由により中止を検討する必要がある場合の参考になるよう定めた。

Ⅰ．本指針の目指すもの

本指針は、核酸アナログ薬の中止を検討する際に、中止成功の可能性が高い症例や逆に治療を継続すべき症例を明らかにすること、さらに、中止後の経過観察の指標を設定することにより、核酸アナログ薬中止に伴うリスクを極力回避することを目指して作成した（表1-Ⅰ)。ここでの中止成功は、最終的に非活動性キャリアの状態、すなわちALTが30 IU/L未満かつ血中HBV DNAが4.0 log copies/ml未満に落ち着くこととした。この基準は日本のB型慢性肝炎治療ガイドラインに準拠して設定したが、このような非活動性キャリア状態になると肝病変の進行はなく発癌率も低下することが知られており[6,7]、適切なものと考えられる。

Ⅱ．肝炎再燃に伴う重症化のリスクを回避するための必要条件

現状では、核酸アナログ薬中止後の肝炎再燃を十分高い確率で予測することはできない。このため、重症化の危険性[8]が存在することを想定し、重症化防止のための必要条件を設定した（表1-Ⅱ)。肝炎再燃や重症化の危険性を主治医と患者が共に理解していること、さらに、中止後の経過観察体制があり、再燃しても適切な対処が可能であることは当然の条件と考えられる。また、肝硬変やこれに近い線維化の進行した慢性肝炎症例では重症化しやすいこと、さらには将来的に発癌の危険性が高いことを考慮すると、現状では安易に中止すべきでないと判断した。

Ⅲ．HBV増殖能の評価と再燃のリスクを低下させるための条件

これまでも、核酸アナログ薬中止時にHBV DNAが十分低下しない症例またはHBe抗原陽性の症例では中止後に肝炎が高率再燃することが経験されていたが、本研究班の検討でもこれが科学的に確認された[9]。肝

炎再燃を予測するHBV DNA量のcut-off値はROC解析で3.0 log copies/mlであり、これ以上の症例ではほとんど全例が1年以内に再燃したのに対し、3.0 log copies/ml未満の症例では長期に安定化する症例が30%近く存在した。さらに、HBV DNA量が3.0 log copies/ml未満の症例に限った場合、HBe抗原陽性例は1年以内に90%以上が再燃したのに対し、HBe抗原陰性例では長期に安定化する症例が少なからず存在した。この結果から、HBV DNA量の十分な低下とHBe抗原の陰性化は中止の必要条件として設定した。ここで、HBV DNA量の十分な低下の基準値については、実際の指針では3.0 log copies/ml未満ではなく、安全を考慮してリアルタイムPCR法で陰性であることとした。

明らかに中止後の肝炎再燃が予測される症例、すなわち、核酸アナログ薬中止時にHBV DNA量が3.0 log copies/ml以上またはHBe抗原陽性の症例を除いて中止後の肝炎再燃と関連する因子をさらに解析すると、核酸アナログ薬治療期間、中止時HBs抗原量、中止時HBコア関連抗原量が有意な因子として算出された[9]。治療期間のcut-off値は16ヶ月と算出されたため、本指針では余裕をもって2年以上経過していることが望ましいとの条件を設定した。

中止時のHBs抗原量とHBコア関連抗原量については、ROC解析の結果からそれぞれ2つのcut-off値の存在が示唆され、HBs抗原量は1.9と2.9 log IU/ml、HBコア関連抗原量は3.0と4.0 log U/mlであった[9]。この事から、表1-Ⅲに示す如くHBs抗原量とHBコア関連抗原量をスコア化し、総スコアから低リスク群、中リスク群、高リスク群の3群を設定した。それぞれの予測成功率は低リスク群が80～90%、中リスク群が約50%、高リスク群が10～20%であった。各群の中で肝炎再燃と関連する因子をさらに検討すると、低リスク群と中リスク群では新しい因子はなかったが、高リスク群では年齢が有意な因子であった。すなわち、予測成功率が10～20%と低い高リスク群であっても、年齢が35歳未満ではこの成功率がやや高く30～40%であった。

以上の如く、治療期間やウイルスマーカーの結果から核酸アナログ薬中止後の経過を予測することが可能であり、治療中止を計画する際の指標となると考えられた。近年、HBs抗原量の測定は新しいマーカーとして注目されており、インターフェロン治療効果の予測などに有用なことが報告されている[10,11]。一方、HBコア関連抗原量は核酸アナログ薬使用下においても肝細胞核内のHBV cccDNA量を反映することが報告され[12-14]、この量が中止後の肝炎再燃と関連することはこれまでも報告されていた[15,16]。今回、これらの抗原量の組み合わせが中止の指針作成に有用であった点は興味深い[9]。

Ⅳ．中止後の経過観察方法と再治療開始の条件

核酸アナログ薬中止後の経過観察は、定期的にHBV DNA量（リアルタイムPCR法）とALT値を測定することにより行う。中止後の再燃は、中止直後から1年以内が多く、その後徐々に減少し、3年目以降はまれになることが今回の研究で明らかになった[9]。このため、特に中止直後は再燃に対する注意が必要であると判断した。具体的には、中止後16週までは2週毎、その後は4週毎の血液検査による経過観察が望ましいとした。

肝炎再燃をどのように定義し、中止後の経過観察をどのように行うかは本指針の要点の一つである。最終的に非活動性キャリア状態に落ち着く症例においても、約2/3では一過性のALTまたはHBV DNAの異常値が出現する。このため、中止後の経過観察でALTまたはHBV DNAの異常値が出現しても、軽度の上昇であれば再治療を行わずに経過をみることが可能である。しかし、どこまでなら経過をみて良いのかの基準はこれまで明らかにされていない。この点を明らかにするため我々は、核酸アナログ薬中止後のALT値とHBV DNA量の推移を平均値と最高値で評価した。この結果、両者とも平均値と最高値の間にきわめて強い

相関があることが明らかになった[9]。ROC解析の結果より、平均ALT値の30 IU/Lは最高ALT値の79 IU/Lに、一方、平均HBV DNA量の4.0 log copies/mlは最高HBV DNA量の5.7 log copies/mlに相当することが明らかになった。すなわち、中止後にALT値が80 IU/L以上になる場合は平均値が30 IU/Lを超える可能性が高く、最終的に中止成功の基準を満足しないことが予測される。同様に、中止後のHBV DNA量が5.8 log copies/ml以上となる場合は平均値が4.0 log copies/mlを超える可能性が高く、中止成功の基準を満足しないことが予測される。これらの結果より、中止後にALT値が80 IU/L以上、またはHBV DNA量が5.8 log copies/ml以上となる場合は最終的に非活動性キャリア状態に落ち着く可能性は低く、核酸アナログ薬による再治療を考慮するとする条件を設けた。この条件設定により、より効率的で具体的な中止が可能になると考えられる。安全を考慮し、主治医の判断でこの基準をより厳しく設定することは可能である。逆に、この基準を緩く設定することも可能であるが、その場合は漫然と経過観察は行わず、何らかの方針を立てて対処することが望ましい。

V．注意点と今後の課題

核酸アナログ薬中止の指針についてはこれまで本格的なものはなく、その意味で本指針は初めてのものとも言える。しかし、多くは後向きの検討データを基に作成したものであり、まだ不明な点も多く残されている。そのため、注意点や今後の課題を一つの項目としてまとめた（表1-V）。本指針では核酸アナログ薬の中止に関する判断材料を提供したが、実際に中止するかは否かの判断は主治医が行うべきと考える。それは、核酸アナログ薬を継続した場合と中止した場合の長期の予後が必ずしも明らかになっていないことが大きな要因であり、患者の希望と主治医の判断が優先されると考えられる。

中止が成功しなかった場合、核酸アナログ薬を再投与することが選択肢の一つとなる。しかし、この場合、継続投与した場合と比較して耐性株出現率が高くなるか否かについては明らかになっておらず、この点は患者に同意を得ておく必要があると考えられる。

今後の検討課題としては、中止後の肝炎再燃の予測精度を向上させることが一つである。このためには新しい因子の検討が必要であり、候補としては、より高感度なHBV DNA量の測定、HBV RNA量の測定[17,18]、HBV遺伝子型、HBV遺伝子変異の検出などが挙げられている。また、今回の指針は後ろ向き研究で作成された基準であり、今後、前向き検討でこれを検証する必要がある。さらに、インターフェロン併用によるシークエンシャル治療で核酸アナログ薬を積極的に中止しようとする方法も今後の重要な検討課題と考えられる。

おわりに

核酸アナログ薬が日本で使用可能になって12年目になるが、本格的な中止の指針作成は今回が最初である。難しい課題であるが、第一歩を踏み出さないことにはその次はない。すなわち、本指針はまだ十分とは言えないが、少なくとも、今後より良いものを作るための出発点になることが期待される。

謝辞

本指針の作成は、厚生労働科学研究費補助金、肝炎等克服緊急対策研究事業（平成21年度～23年度）の助成金により行った。

文献

1. Ghany M, Liang TJ. Drug targets and molecular mechanisms of drug resistance in chronic hepatitis B. Gastroenterology 2007; 132: 1574-1585
2. Liaw YF, Sung JJ, Chow WC, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004; 351: 1521-1531
3. Matsumoto A, Tanaka E, Rokuhara A, et al. Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: A multicenter retrospective study of 2795 patients. Hepatol Res 2005; 32: 173-184
4. Lok AS, Zoulim F, Locarnini S, et al. Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. Hepatology 2007; 46: 254-265
5. Werle-Lapostolle B, Bowden S, Locarnini S, et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology 2004; 126: 1750-1758
6. Iloeje UH, Yang HI, Su J, et al. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006; 130: 678-686
7. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006; 295: 65-73
8. Lim SG, Wai CT, Rajnakova A, et al. Fatal hepatitis B reactivation following discontinuation of nucleoside analogues for chronic hepatitis B. Gut 2002; 51: 597-599
9. Matsumoto A, Tanaka E, Suzuki Y, et al. 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 2012; 42: 139-149
10. Chan HL, Wong VW, Tse AM, 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: 1462-1468
11. Moucari R, Lada O, Marcellin P. Chronic hepatitis B: back to the future with HBsAg. Expert Rev Anti Infect Ther 2009; 7: 633-636
12. Suzuki F, Miyakoshi H, Kobayashi M, et al. 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
13. Tanaka E, Matsumoto A, Yoshizawa K, et al. Hepatitis B core-related antigen assay is useful for monitoring the antiviral effects of nucleoside analogue therapy. Intervirology 2008; 51 Suppl 1: 3-6
14. Wong DK, Tanaka Y, Lai CL, et al. Hepatitis B virus core-related antigens as markers for monitoring chronic hepatitis B infection. J Clin Microbiol 2007; 45: 3942-3947
15. Matsumoto A, Tanaka E, Minami M, 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: 661-666
16. Shinkai N, Tanaka Y, Orito E, 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: 272-276

17. Rokuhara A, Matsumoto A, Tanaka E, et al. Hepatitis B virus RNA is measurable in serum and can be a new marker for monitoring lamivudine therapy. J Gastroenterol 2006; 41: 785-790
18. Hatakeyama T, Noguchi C, Hiraga N, et al. Serum HBV RNA is a predictor of early emergence of the YMDD mutant in patients treated with lamivudine. Hepatology 2007; 45: 1179-1186

表 1：核酸アナログ薬中止に伴うリスク回避のための指針　2012

Ⅰ．本指針の目指すもの

B 型慢性肝炎の核酸アナログ薬治療において、同薬の中止により drug free を目指すことは重要な治療目標の一つである。しかし、同薬の中止によりしばしば肝炎が再燃し、時に重症化することがある。このため、中止に際してはその危険性に十分配慮する必要がある。

核酸アナログ薬治療は HBs 抗原の陰性化を目標とするが、必ずしも容易ではない。このため、HBs 抗原が陰性化しなくても治療の中止を考慮する場合がある。本指針は、この様な状況下で核酸アナログ薬を中止し、最終的に非活動性キャリアの状態（ALT＜30 IU/L かつ血中 HBV DNA＜4.0 log copies/ml）に落ち着くことを目標として作成した。

核酸アナログ薬の中止と継続のどちらが生命予後や肝発癌に対して有利かは現在のところ明らかではない。このため、本指針は様々な理由により中止を検討する必要がある場合の参考になるよう定めた。この際、中止成功の可能性が高い症例を見いだすことや逆に治療を継続すべき症例を明らかにすること、さらに、中止後の経過観察の指標を設定することにより、核酸アナログ薬中止に伴うリスクを極力回避することを目指した。

Ⅱ．肝炎再燃に伴う重症化のリスクを回避するための必要条件

重症化のリスクをあらかじめ想定し、これを回避するため、以下を中止の必要条件とした。

1. 核酸アナログ薬中止後には肝炎再燃が高頻度にみられ、時に重症化する危険性があることを主治医、患者共に十分理解している。
2. 中止後の経過観察が可能であり、再燃しても適切な対処が可能である。（専門医が関与することが推奨される。）
3. 肝線維化が軽度で肝予備能が良好であり、肝炎が再燃した場合でも重症化しにくい症例である。（肝硬変やこれに近い線維化の進行した慢性肝炎の症例では中止すべきでない。）

Ⅲ．HBV 増殖能の評価と再燃のリスクを低下させるための条件

1. 核酸アナログ薬中止の必要条件

HBV 増殖能が高い症例では中止後の再燃はほぼ必発である。この様な症例で中止を行わないことが肝要であり、このための必要条件を以下に示す。

中止の必要条件
✧　中止時、血中 HBV DNA（リアルタイム PCR 法）が陰性。
✧　中止時、血中 HBe 抗原が陰性。

2. 核酸アナログ薬治療期間の条件

核酸アナログ薬治療期間が短いと再燃しやすいため、以下の条件を満たすことが望ましい。

治療期間の条件
✧　核酸アナログ薬投与開始後 2 年以上経過している。

3. ウイルス抗原量のスコア化による再燃の危険性の評価

中止の必要条件（中止時 HBV DNA 陰性かつ HBe 抗原陰性）を満たす症例について、中止時の HBs 抗原量と HB コア関連抗原量をスコア化し、合計スコアから再燃のリスクを以下の 3 群に分けて予測することが可能である。この予測リスクを参考に中止の可否を決定することにより再燃のリスクを低下させることを目指す。

中止時 HBs 抗原量	スコア	中止時 HB コア関連抗原量	スコア
1.9 log IU/ml 未満　(80 IU/ml 未満)	0	3.0 log U/ml 未満	0
1.9～2.9 log IU/ml　(80～800 IU/ml)	1	3.0～4.0 log U/ml	1
2.9 log IU/ml 以上　(800 IU/ml 以上)	2	4.0 log U/ml 以上	2

再燃リスク	総スコア	予測成功率	評価
低リスク群	0	80-90%	中止を考慮しても良い群。ただし、低リスク群でも肝炎再燃症例が存在するため、再燃に対する注意は必須である。
中リスク群	1-2	約 50%	状況によって中止を考慮しても良い群。この群では、中止の条件や方法を今後さらに検討する必要がある。
高リスク群	3-4	10-20%	治療の継続が推奨される群。ただし、35 歳未満では中止成功例が比較的高く 30-40%である。

Ⅳ. 中止後の経過観察方法と再治療開始の条件

1. 核酸アナログ薬中止後は定期的に HBV DNA（リアルタイム PCR 法）と ALT を測定し、HBV の再増殖とこれに伴う肝炎再燃に注意を払う。
2. 中止後の再燃は、中止直後から 1 年以内が多く、その後徐々に減少し、3 年目以降はまれになる。このため、特に中止直後は再燃に対する注意が必要である。具体的には、中止後 16 週までは 2 週毎、その後は 4 週毎の血液検査による経過観察が望ましい。
3. 中止が成功し、最終的に非活動性キャリア状態に落ち着く症例においても、約 2/3 では一過性の ALT または HBV DNA の異常値が出現する。このため、中止後の経過観察で ALT または HBV DNA の異常値が出現しても、軽度の上昇であれば再治療を行わずに経過をみることが可能である。ただし、以下の条件では、最終的に非活動性キャリア状態に落ち着く可能性は低く、核酸アナログ薬による再治療を考慮する。

核酸アナログ薬の再投与を考慮する条件
✧　中止後 ALT ≧ 80 IU/L または HBV DNA ≧ 5.8 log copies/ml となる場合

Ⅴ. 注意点と今後の課題

1. 患者の状況は個々に異なる。また、中止の目的や意義も個々に異なるため、実際に中止するか否かの判断は、これらの条件を考慮し主治医が行う。また、中止を考慮する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。

Hepatology Research

Hepatology Research 2012; 42: 139–149 doi: 10.1111/j.1872-034X.2011.00910.x

Original Article

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

Akihiro Matsumoto,[1] Eiji Tanaka,[1] Yoshiyuki Suzuki,[2] Mariko Kobayashi,[2] Yasuhito Tanaka,[4] Noboru Shinkai,[4] Shuhei Hige,[6] Hiroshi Yatsuhashi,[8] Shinya Nagaoka,[8] Kazuaki Chayama,[9] Masataka Tsuge,[9] Osamu Yokosuka,[10] Fumio Imazeki,[10] Shuhei Nishiguchi,[11] Masaki Saito,[11] Kei Fujiwara,[5] Nobuyuki Torii,[3] Naoki Hiramatsu,[12] Yoshiyasu Karino[7] and Hiromitsu Kumada[2]

[1]Department of Medicine, Shinshu University School of Medicine, Matsumoto, [2]Department of Hepatology, Toranomon Hospital, [3]Department of Internal Medicine and Gastroenterology, Tokyo Women's Medical University, Tokyo, [4]Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, [5]Gastroenterology Section, Nagoya Daini Red Cross Hospital, Nagoya, [6]Department of Gastroenterology and Hepatology, Graduate School of Medicine, Hokkaido University, [7]Department of Gastroenterology, Sapporo Kosei General Hospital, Sapporo, [8]The Clinical Research Center, NHO Nagasaki Medical Center, Omura, [9]Program for Biomedical Research, Division of Frontier Medical Science, Department of Medicine and Molecular Science, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, [10]Department of Medicine and Clinical Oncology, Graduate School of Medicine, Chiba University, Chiba, [11]Division of Hepatobiliary and Pancreatic Diseases, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, and [12]Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Osaka, Japan

Aim: The factors associated with hepatitis recurrence after discontinuation of nucleos(t)ide analogs (NAs) in patients with chronic hepatitis B were analyzed to predict the risk of relapse more accurately.

Methods: A total of 126 patients who discontinued NA therapy were recruited retrospectively. The clinical conditions of a successful discontinuation were set as alanine aminotransferase (ALT) below 30 IU/L and serum hepatitis B virus (HBV) DNA below 4.0 log copies/mL.

Results: Relapse of hepatitis B were judged to occur when maximal serum ALT became higher than 79 IU/L or when maximal serum HBV DNA surpassed 5.7 log copies/mL following NA discontinuation since these values corresponded with mean values of ALT (30 IU/L) and HBV DNA (4.0 log copies/mL), respectively. At least 90% of patients with either detectable hepatitis B e antigen or serum HBV DNA higher than 3.0 log copies/mL at the time of NA discontinuation relapsed within one year. In the remaining patients, higher levels of both hepatitis B surface and core-related antigens at the time of discontinuation, as well as a shorter course of NA treatment, were significantly associated with relapse by multivariate analysis.

Conclusions: It appears that negative results for hepatitis B e antigen and serum HBV DNA lower than 3.0 log copies/mL are essential for successful NA discontinuation, which may be attained by a longer treatment period. Levels of hepatitis B surface and core-related antigens are also significant factors independently associated with relapse of hepatitis.

Key words: discontinuation, hepatitis B core-related antigen, hepatitis B surface antigen, nucleos(t)ide analogs, relapse of hepatitis

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is a major health concern that has an estimated 350 to 400 million carriers worldwide. Chronic infection with HBV can cause chronic hepatitis, and may eventually develop into liver cirrhosis and hepatocellular carcinoma.[1–3] Over the last decade, major advances in the treatment of chronic hepatitis B have been made with nucleos(t)ide

Correspondence: Professor Eiji Tanaka, Department of Medicine, Gastroenterology Division, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan. Email: etanaka@shinshu-u.ac.jp

Financial support
This research was supported in part by a research grant from the Ministry of Health, Labor and Welfare of Japan.
Received 7 August 2011; revision 31 August 2011; accepted 5 September 2011.

analogs (NAs) such as lamivudine (LVD), adefovir dipivoxil (ADV), and entecavir (ETV).[4] NAs are orally administered and are associated with low rates of adverse effects. Treatment with NAs shows strong suppression of HBV replication and consequently rapid improvement of elevated ALT levels. Furthermore, these drugs have been reported to lower the risk of complicating cirrhosis and hepatocellular carcinoma,[5–7] and so NAs are becoming widely used to treat patients with chronic hepatitis B. On the other hand, NAs carry the risk of developing drug-resistance;[8] drug-resistant viruses emerging during treatment may be associated with hepatitis flare-ups. Hepatitis B patients are also required to undergo prolonged treatment with NAs because early discontinuance often leads to relapse of hepatitis and ensuing hepatic failure following rises in alanine aminotransferase (ALT) level.[9,10]

Serum HBV DNA is normally used to monitor the antiviral effect of NAs. HBV DNA decreases rapidly and becomes undetectable in the majority of patients who are treated with NAs,[11–13] but relapse after discontinuation is not rare.[14–17] Since it is also true that favorable virological and biochemical responses to NAs may continue indefinitely in some patients,[9,15] reliable markers that can predict relapse of hepatitis after NA discontinuation are needed. Such markers would benefit not only patients who are considering discontinuation of NA treatment, but also clinicians, hospitals, and the medical economy.

In the present study, we assessed several factors associated with relapse of hepatitis after discontinuation of NAs in patients with chronic hepatitis B, including hepatitis B viral antigens, which have been reported as new and promising markers for monitoring the effect of antiviral agents, such as interferon and NAs.

METHODS

Patients

A TOTAL OF 126 patients with chronic hepatitis B who underwent and completed NA treatment between 2000 and 2010 were enrolled in this study. Patients were recruited retrospectively from 11 hospitals across Japan (Toranomon Hospital, Hokkaido University Hospital, Nagoya City University Hospital, Shinshu University Hospital, Hiroshima University Hospital, National Hospital Organization Nagasaki Medical Center, Chiba University Hospital, The Hospital of Hyogo College of Medicine, Japanese Red Cross Nagoya Daini Hospital, and Tokyo Women's Medical University Hospital, Sapporo Kosei General Hospital) and met the following conditions: (i) serum ALT higher than 30 IU/L and serum HBV DNA higher than 4.0 log copies/mL were observed at least twice within the 6 months prior to administration of NAs; (ii) stored serum samples at initiation and discontinuation of NAs were available for measurements of viral markers; (iii) clinical outcomes were followed for at least 6 months after the discontinuation of NAs; and (iv) tests for hepatitis C and human immunodeficiency virus antibodies were negative. Hepatitis B surface antigen (HBsAg) was confirmed to be positive on at least two occasions at least 6 months apart in all patients before treatment. Patients complicated with hepatocellular carcinoma or signs of hepatic failure at treatment discontinuation were excluded from the study. Our cohort consisted of 83 men and 43 women with a median age of 46 (range, 19 to 79) years when NA administration was discontinued. Hepatitis B e antigen (HBeAg) was positive in 64 patients (51%) at the initiation of treatment and in 24 patients (19%) at its discontinuation. HBV genotype was A in two (2%) patients, B in five (4%), C in 102 (81%), and undetermined in 17 (13%). Thirty-five of the 126 patients in this study were younger than 35 years old. Although not recommended as the first line treatment for this group by Japanese guidelines,[18] NA treatment was commenced since chronic active hepatitis had been persisting in all cases irrespective of their HBeAg status (26 positive and nine negative) at the initiation of treatment.

The decision to discontinue NAs was made by individual physicians using similar, but not uniform, conditions. Four patients who halted NAs for financial reasons were included. No patient underwent interferon treatment during or after NA treatment. The decision to recommence NA administration was also made by individual physicians, essentially when relapse of hepatitis became obvious. With few exceptions, patients were seen at least once a month during the first year after discontinuation of NAs, and at least once every several months afterwards. Stored serum samples were kept frozen at −20 °C or below until assayed. This study was approved by the Ethics Committees of all participating institutions.

Hepatitis B viral markers

Serological markers for HBV, including HBsAg, HBeAg, and antibody to HBe (anti-HBe) were tested using commercially available enzyme immunoassay kits (Abbott Japan Co., Ltd, Tokyo, Japan; Fujirebio Inc., Tokyo, Japan; and/or Sysmex Co., Kobe, Japan) at each hospital. Quantitative measurement of HBsAg[19] was done using a chemiluminescence enzyme immunoassay

(CLEIA)-based HISCL HBsAg assay manufactured by Sysmex Corporation (Kobe, Japan). The assay had a quantitative range of −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.

Serum concentration of HBV DNA was determined using an Amplicor HBV monitor kit (Roche, Tokyo, Japan),[20] which had a quantitative range of 2.6 to 7.6 log copies/mL. Serum HBV DNA was also determined using a COBAS TaqMan HBV kit (Roche, Tokyo, Japan)[21] with a quantitative range of 2.1 to 9.0 log copies/mL in 43 patients whose serum samples were available at the time of NA discontinuation. According to the manufacturer's instructions, detection of a positive signal below the quantitative range was described as a positive signal, and no signal detection was described as a negative signal. Six HBV genotypes (A–F) were evaluated according to the restriction patterns of DNA fragments from the method reported by Mizokami *et al.*[22]

Serum hepatitis B core-related antigen (HBcrAg) levels were measured using a CLEIA HBcrAg assay kit with a fully automated Lumipulse System analyzer (Fujirebio Inc., Tokyo, Japan) as described previously.[23,24] Briefly, 150 μL of serum was incubated with pretreatment solution and then added to a ferrite microparticle suspension in an assay cartridge. Ferrite particles were coated with a monoclonal antibody mixture against denatured HBcAg, HBeAg, and the 22 kDa precore protein. After incubation and washing, further incubation was carried out with alkaline phosphatase conjugated with two kinds of monoclonal antibodies against denatured HBcAg, HBeAg, and the 22 kDa precore protein. Following washing, a substrate solution was added to the test cartridge and then incubated. The relative chemiluminescence intensity was measured, and HBcrAg concentration was calculated by a standard curve generated using recombinant pro-HBeAg. The immunoreactivity of pro-HBeAg at 10 fg/mL was defined as 1 U/mL. We expressed HBcrAg in terms of log U/mL, with a quantitative range set at 3.0 to 6.8 log U/mL.

Statistical analyses

A linear regression model was used to examine for associations between mean and maximal values of both ALT and HBV DNA. Correlations between variables were calculated using the Spearman's rank correction correlation coefficient test. Each cut-off value was decided using receiver operating characteristic curve (ROC) analysis and results were evaluated by measuring the area under the curve (AUC). The Fisher's exact and Pearson's χ^2 tests were adopted to test for differences between subgroups of patients. To compare continuous data, the Mann–Whitney *U*-test was used. The Kaplan–Meier method was used to estimate rates of non-relapse observations, and the log-rank test was used to test hypotheses concerning differences in non-relapse observations between selected groups. Multivariate analyses were performed using the Cox regression model. Variables associated with a *P*-value < 0.2 in univariate analyses were included in a stepwise Cox regression analysis to identify independent factors associated with relapse of hepatitis after discontinuation of NAs. 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

Definition of hepatitis relapse after discontinuation of NAs

THE CLINICAL CONDITIONS of a successful discontinuation of NAs were set at serum HBV DNA below 4.0 log copies/mL and ALT below 30 IU/L according to the Japanese guidelines for the treatment of hepatitis B.[18] However, these criteria could not be directly applied to our cohort as post-therapy fluctuations in ALT and HBV DNA were difficult to evaluate consistently. In total, 26 (76%) of 34 patients with successful discontinuation of NAs showed transient abnormal levels of ALT and/or HBV DNA, especially during the early phase after cessation. We therefore used mean and maximal values of these markers to evaluate relapse of hepatitis B in this study; mean values were used to evaluate relapse of hepatitis as a whole, and maximal values were used to dynamically assess relapse during the follow-up period after NA discontinuation. Both ALT and HBV DNA were measured 11.0 times per year on average during the first year and 4.1 times per year on average thereafter.

The mean values of HBV DNA were significantly ($P < 0.001$) correlated with maximal values with a correlation coefficient of 0.853. Similarly, the mean values of ALT were significantly ($P < 0.001$) correlated with maximal values with a correlation coefficient of 0.940 (Fig. 1). The mean HBV DNA value of 4.0 log copies/mL corresponded to a maximal HBV DNA value of 5.7 by ROC analysis (AUC = 0.930, $P < 0.001$), and the mean ALT value of 30 IU/L corresponded to a maximal ALT value of 79 IU/L (AUC = 0.988, $P < 0.001$). These results suggested that patients having serum HBV DNA higher

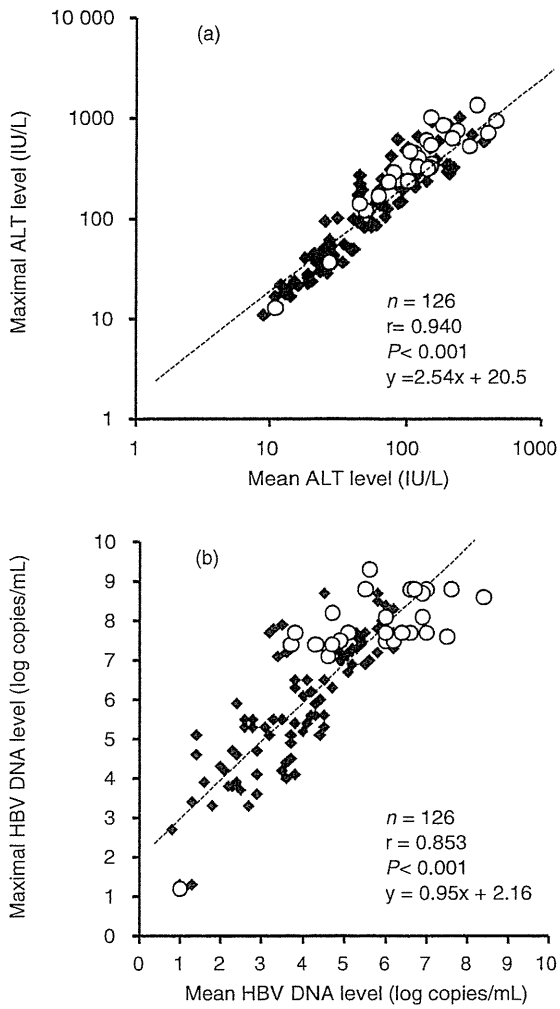

Figure 1 Correlation between maximal and mean levels of alanine aminotransferase (ALT) (a) and hepatitis B virus (HBV) DNA (b) after discontinuation of nucleos(t)ide analogs (NAs). Open circles indicate patients with detectable hepatitis B e antigen (HBeAg) and closed squares indicate patients without detectable HBeAg.

than 5.7 log copies/mL during the follow-up period after NA discontinuation were not likely to achieve the HBV DNA criterion of a successful discontinuation of below 4.0 log copies/mL. Similarly, it could be inferred that patients reaching ALT levels higher than 79 IU/L would also not likely achieve the ALT criterion of a successful discontinuation of below 30 IU/L.

Based on our findings, we judged that a relapse of hepatitis B occurred when serum ALT exceeded 79 IU/L or when serum HBV DNA exceeded 5.7 log copies/mL following NA discontinuation. Accordingly, 92 (73%) of the 126 patients enrolled in the present study showed a relapse. We set the follow-up period as discontinuation to relapse for relapse patients and as discontinuation to the last recorded examination for patients without relapse. Whereas re-administration of NAs due to relapse was commenced in 70% of relapse patients in the follow-up period, none was performed in non-relapse patients during that time.

Elimination of cases likely to show relapse of hepatitis

As it is generally believed that patients who are positive for HBeAg and/or have a higher level of HBV DNA at discontinuation of NAs are likely to relapse, these factors were assessed first. The progression of analyses in the present study and the population structure of each analysis are shown in Figure 2.

The non-relapse rate was compared using the Kaplan–Meier method between 31 patients with HBV DNA equal to or higher than 3.0 log copies/mL and 95 patients with levels lower than 3.0 log copies/mL when NAs were discontinued (Fig. 3). The revised cut-off value of 3.0 log copies/mL was determined by ROC analysis (AUC = 0.709, $P < 0.001$). Thirty (97%) of 31 patients with HBV DNA equal to or higher than 3.0 log copies/mL relapsed within one year of discontinuation. On the other hand, approximately 30% of patients with levels lower than 3.0 log copies/mL showed prolonged non-relapse. Thus, the 31 patients with high HBV DNA at the time of discontinuation were eliminated from the following analyses.

In the remaining 95 patients, the non-relapse rate was compared using the Kaplan–Meier method between 10 patients with detectable HBeAg and 85 patients without HBeAg when NAs were discontinued (Fig. 4). Ninety percent of patients with HBeAg experienced relapse within one year, which was significantly ($P = 0.005$) higher than in cases without HBeAg. In patients without HBeAg, the non-relapse rate decreased rapidly during the first year to approximately 45%, and then decreased relatively slowly over the following 3 years to nearly 30%. It is noteworthy that this subgroup did not relapse afterwards. Since the relapse rate was high among patients with detectable HBeAg, they were excluded from the following analyses as well.

Factors associated with relapse of hepatitis after discontinuation of NAs

Additional factors associated with relapse of hepatitis were analyzed in the remaining 85 patients who were

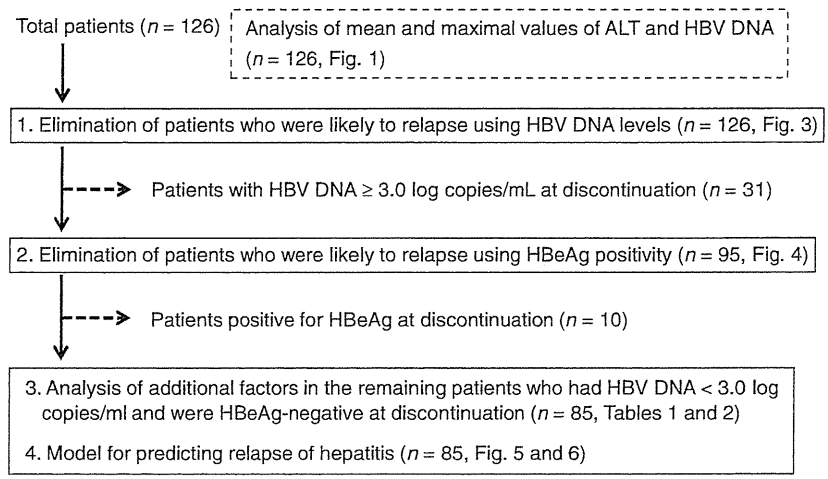

Figure 2 The progression of analyses in the present study and population structure of each analysis.

both negative for HBeAg and whose serum HBV DNA was lower than 3.0 log copies/mL at NA cessation. Table 1 shows the comparison of clinical and virological backgrounds between the 53 relapse and 32 non-relapse patients using univariate analysis. Age and gender distributions were similar between the groups. Approximately 75% of the 85 patients had HBV genotype C, but the distribution of genotypes did not differ between the groups. Approximately 90% of patients were being treated with LVD alone at the time of discontinuation, compared with 6% of patients being given ETV. The median duration of NA treatment was about two times longer in patients without relapse. Levels of both HBsAg and HBcrAg were significantly lower in non-relapse patients than in relapse patients at the time of NA discontinuation. The difference between serum HBsAg was also significant at the initiation of NAs, but not that of HBcrAg. As only patients with HBV DNA lower than 3.0 log copies/mL were analyzed, the majority of these cases showed levels below the 2.6 log copies/mL lower detection limit of the Amplicor assay at NA discontinuation. We therefore also tested HBV DNA with a TaqMan assay, which had a higher sensitivity than the Amplicor assay, in 43 patients whose serum samples were available. The prevalence of patients having a negative detection signal did not differ between the two groups. The number of

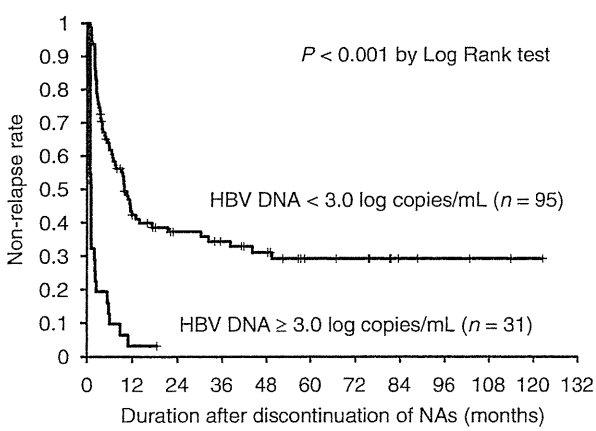

Figure 3 Comparison of non-relapse rates using the Kaplan–Meier method between 31 patients with serum hepatitis B virus (HBV) DNA equal to or higher than 3.0 log copies/mL and 95 patients with serum HBV DNA lower than 3.0 log copies/mL at the time of nucleos(t)ide analog (NA) discontinuation.

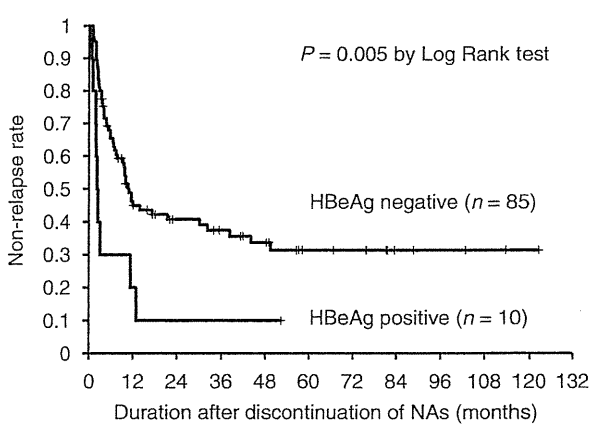

Figure 4 Comparison of non-relapse rates using the Kaplan–Meier method between 10 patients with detectable hepatitis B e antigen (HBeAg) and 85 patients without detectable HBeAg at the time of nucleos(t)ide analog (NA) discontinuation.

Table 1 Comparison of clinical and virological backgrounds between patients with and without relapse of hepatitis at initiation and discontinuation of nucleos(t)ide analogs (NAs)

Background	Non-relapse patients ($n = 32$)	Relapse patients ($n = 53$)	*P*-value
At initiation of NAs			
Age (years)†	47 (17–75)	48 (26–74)	>0.2
Gender (M : F)	23:9	32:21	>0.2
ALT (IU/L)†	183 (9–1182)	187 (20–2052)	>0.2
Genotype (A : B : C : UD)	1:2:21:8	0:3:44:6	0.193
HBeAg (positive)‡	11 (34%)	16 (30%)	>0.2
HBV DNA			
Amplicor assay (log copies/mL)†	6.2 (<2.6->7.6)	6.5 (<2.6->7.6)	0.099
HBsAg (log IU/mL)†	2.7 (0.1–4.3)	3.3 (1.6–3.9)	0.018
HBcrAg (log U/mL)†	5.2 (<3.0->6.8)	5.6 (<3.0->6.8)	>0.2
At discontinuation of NAs			
Age (years)†	50 (21–78)	49 (26–79)	>0.2
NAs (LVD : LVD+ADV : ETV : ADV)	28:1:3:0	50:0:2:1	>0.2
Duration of NA treatment (months)†	36 (4–129)	17 (4–84)	0.007
Follow-up period after discontinuation of NAs (months)†	45 (6–123)	12 (1–111)	0.002
ALT (IU/L)†	16 (7–38)	20 (9–65)	0.002
HBV DNA			
Amplicor assay (log copies/mL)†	<2.6 (<2.6–2.9)	<2.6 (<2.6–2.9)	>0.2
TaqMan assay (negative signal)‡	5 (23%) ($n = 22$)	3 (14%) ($n = 21$)	>0.2
TaqMan assay (negative or positive signal)‡	13 (59%) ($n = 22$)	13 (62%) ($n = 21$)	>0.2
HBsAg (log IU/ml)†	2.0 (<–1.5–4.3)	3.1 (0.6–4.0)	0.001
HBcrAg (log IU/mL)†	3.4 (<3.0–4.9)	4.3 (<3.0->6.8)	0.003

†Data are expressed as the median (range)
‡Data are expressed as a positive number (%)
ADV; adefovir dipivoxil; ALT, alanine aminotransferase; ETV, entecavir; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; LVD, lamivudine; UD, undetermined.

patients with a negative detection signal or a positive signal also did not vary significantly. The follow-up period after discontinuation of NAs was significantly shorter in patients with relapse than in those without because formal follow-up ended once patients relapsed. The median period of follow-up was 45 months in patients without relapse.

Multivariate analyses revealed that a shorter duration of NA treatment and higher levels of HBsAg and HBcrAg at discontinuation were significantly associated with the occurrence of hepatitis relapse (Table 2). The cut-off values that showed the highest significance by ROC analysis were 1.9 log IU/mL for HBsAg (AUC = 0.707, $P = 0.001$), 4.0 log U/mL for HBcrAg (AUC = 0.692, $P = 0.003$), and 16 months (AUC = 0.674, $P = 0.007$) for treatment duration.

Model for predicting relapse of hepatitis using levels of HBsAg and HBcrAg

The existence of a second cut-off value was suggested by ROC analysis for both of HBsAg (2.9 log IU/mL) and HBcrAg (3.0 log IU/mL) to discriminate between

Table 2 Multivariate analysis of factors associated with relapse of hepatitis after discontinuation of nucleos(t)ide analogs (NAs)

Factor	Hazard ratio	95%CI	*P*-value
HBsAg at discontinuation ≥ 1.9 log IU/mL	5.21	1.87–14.55	0.002
HBcrAg at discontinuation ≥ 4.0 log U/mL	2.20	1.25–3.87	0.006
Duration of NA treatment ≥ 16 months	0.54	0.31–0.93	0.027

CI, confidence interval; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen.

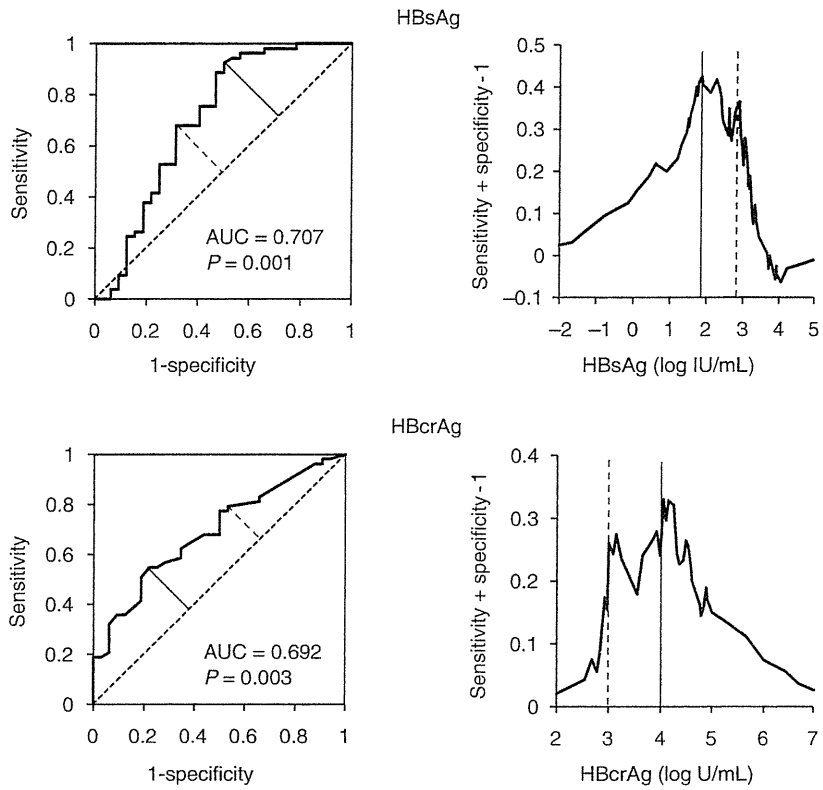

Figure 5 Receiver operating characteristic curve (ROC) analysis of hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) to discriminate between patients with and without hepatitis relapse. The existence of two inflection points is suggested for both HBsAg and HBcrAg. Short diagonal lines indicate main inflection points and short broken diagonal lines indicate second inflection points. Vertical lines indicate actual values of antigens that correspond to the main inflection points and vertical broken lines indicate actual values of antigens that correspond to the second inflection points.

patients with and without relapse (Fig. 5). Thus, we set cut-off values as 1.9 and 2.9 log IU/mL for HBsAg and 3.0 and 4.0 log U/mL for HBcrAg in our model for predicting hepatitis relapse.

We tentatively defined three groups using the sum of the scores for HBsAg and HBcrAg levels at the time of NA discontinuation for our model. Conversions were made by assigning a score of 0 for an HBsAg level lower than 1.9 log IU/mL, 1 for a level from 1.9 to 2.8 log IU/mL, and 2 for a level equal to or higher than 2.9 log IU/mL. HBcrAg was scored as 0 for a level lower than 3.0 log U/mL, 1 for a level from 3.0 to 3.9 log U/mL, and 2 for a level equal to or higher than 4.0 log U/mL. Overall, group 1 consisted of patients with a total score of 0, group 2 of patients with a total score of 1 or 2, and group 3 of patients with a total score of 3 or 4.

Patients whose HBV DNA was lower than 3.0 log copies/mL and in whom HBeAg was negative at the time of NA discontinuation were assigned to one of the three groups. Figure 6 shows the comparison of non-relapse rates among the three groups using Kaplan–Meier analysis, which differed significantly. The non-relapse rate was approximately 90% in group 1, as low as 10% in group 3, and intermediate in group 2. When factors associated with relapse were analyzed in group 3 patients, an age of over 40 years at the time of discontinuation was calculated as a significant factor (hazard

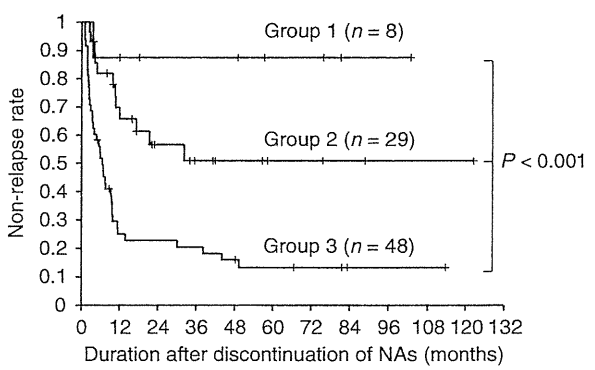

Figure 6 Comparison of non-relapse rates using the Kaplan–Meier method among three groups classified by the sum of the scores of hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) levels at the time of nucleos(t)ide analog (NA) discontinuation.

ratio = 5.25, range 2.37–11.65, $P < 0.001$). No significant factors were associated with relapse in group 2 patients.

DISCUSSION

THE EUROPEAN ASSOCIATION for the Study of the Liver recommends continuation of NA treatment until HBsAg is cleared.[25] Liu *et al.* came to a similar conclusion in their study of chronic hepatitis B patients treated with LVD.[14] Indeed, the clearance of HBsAg is a reliable marker for the safe discontinuation of NAs, but the rate of patients who can clear HBsAg is relatively low (1–3%/year).[26–28] Thus, additional factors associated with relapse of hepatitis B after discontinuation of NAs were analyzed in the present study to better identify candidates who could achieve drug-free status. Such studies are relatively few, possibly because patients who discontinue NAs prematurely often experience severe complicating relapse and hepatic failure.[9] Although prospective studies are desirable to obtain accurate results, retrospective studies, such as ours, are also necessary to minimize the risk of adverse complications.

Since HBV cannot be completely eradicated in hosts, the primary goal in treating chronic hepatitis B is to convert symptomatic patients into inactive carriers in whom HBeAg is negative (usually anti-HBe-positive), serum HBV DNA is low, and serum ALT is normal.[1,2,18,29] Thus, we set the clinical conditions of a successful discontinuation of NAs as serum HBV DNA level below 4.0 log copies/mL and ALT below 30 IU/L following NA cessation. Patients who satisfy these conditions are not recommended for treatment by the Japanese guidelines for hepatitis B,[18] and it is also widely accepted that the risk of developing cirrhosis or complicating hepatocellular carcinoma is very low in such patients.[30,31] We used our cohort's mean and maximal values of HBV DNA and ALT for relapse analyses. Mean values were useful for evaluating relapse of hepatitis as a whole since parameter levels often fluctuated after discontinuation, and maximal values were used to evaluate relapse in a real-time fashion during the follow-up period. It is noteworthy that the mean and maximal values correlated very closely for both HBV DNA and ALT. The mean HBV DNA value of 4.0 log copies/mL corresponded to the maximal HBV DNA value of 5.7 by ROC analysis, and similarly the mean ALT value of 30 IU/L corresponded to the maximal ALT value of 79 IU/L. Thus, relapse of hepatitis B was judged to occur when serum ALT became higher than 79 IU/L or when serum HBV DNA surpassed 5.7 log copies/mL after the time of NA discontinuation. Such criteria may also be useful for physicians to detect relapse at an early phase and avoid the occurrence of severe reactivation or unnecessary discontinuation of NAs.

It is generally understood that patients with a higher level of HBV DNA at the time of NA discontinuation are likely to relapse, but this cut-off value has not been analyzed sufficiently. Our findings using ROC analysis showed that patients with levels lower than 3.0 log copies/mL have a good possibility to achieve successful discontinuation. The presence of HBeAg is also generally accepted as a reliable factor to predict relapse of hepatitis. Our study showed that patients with detectable HBeAg at the time of NA discontinuation were likely to relapse, even if their HBV DNA levels were lower than 3.0 log copies/mL. Therefore, we next analyzed additional factors associated with a relapse of hepatitis after discontinuation of NAs by selecting patients who met both of these criteria.

Nucleos(t)ide analog treatment produces a rapid decrease in serum HBV DNA by suppressing reverse transcription of pregenomic HBV RNA. However, the key intrahepatic HBV replicative intermediate, covalently closed circular DNA (cccDNA), tends to remain and is capable of reinitiating replication once NAs are ceased.[32] Measurement of HBV cccDNA has been reported to be useful for monitoring and predicting responses to antiviral treatments.[33] However, its measurement is difficult in the clinical setting as it requires a liver biopsy. Due to the mechanism of action of NAs mentioned above, serum HBV DNA does not reflect intrahepatic HBV cccDNA in patients undergoing NA treatment.[34] To address this, quantitative measurement of HBV antigens has been reported to be useful for predicting the effect of antiviral treatment in patients with chronic hepatitis B. Although HBsAg is usually used as a serum marker for the diagnosis of HBV infection, several groups have shown that HBsAg levels can also be reflective of the response to peg-interferon in chronic hepatitis B.[28,35,36] The HBcrAg assay measures serum levels of HB core and e antigens simultaneously using monoclonal antibodies that recognize the common epitopes of these two denatured antigens. Since the assay measures all antigens transcribed from the pre-core/core gene, it is regarded as core-related.[37] Serum HBcrAg has been reported to accurately reflect intracellular levels of HBV cccDNA even during NA treatment,[24,34,38] and was found to be useful for identifying patients who were likely to show relapse of hepatitis after the discontinuation of NAs.[39,40] It is possible that levels of HBsAg and HBcrAg have different roles in

monitoring antiviral effects because the transcription of these two antigens are regulated by alternative enhancer-promoter systems in the HBV genome.[3] Therefore, we analyzed both of these antigens to elucidate their ability to predict relapse of hepatitis after discontinuation of NAs.

Multivariate analysis demonstrated that levels of HBsAg and HBcrAg at the time of NA discontinuation were independent factors significantly associated with relapse of hepatitis. Thus, we believe these factors can also be applied for predicting relapse in patients whose HBV DNA is lower than 3.0 log copies/mL and whose HBeAg is negative at NA discontinuation. HBV DNA levels were further analyzed using a highly sensitive assay based on real-time polymerase chain reaction (PCR). However, even the level of a negative signal did not ensure successful discontinuation of NAs. The results obtained here indicate that the combined use of HBV-related antigens are useful makers for monitoring the effect of anti-viral treatment in ways different from HBV DNA. Finally, since prolonged NA administration was also a significant factor associated with safe discontinuation, physicians are advised to continue patient treatment for at least 16 months for the best possible outcome.

From our data, a tentative model for predicting relapse of hepatitis after discontinuation of NAs was constructed using levels of HBsAg and HBcrAg at discontinuation. A negative result for HBeAg and HBV DNA lower than 3.0 log copies/mL at the time of NA discontinuation are the essential conditions in this system. Levels of HBsAg and HBcrAg were each converted into scores from 0 to 2 partly because two cut-off values were needed for each antigen and partly because a scoring system may be more convenient for clinical use. The sum of the two scores, which ranged from 0 to 4, was used to prospect relapse. We found that group 1 patients who had a low score (0) could be recommended to discontinue NAs because nearly 90% of this group achieved successful discontinuation. Further analysis of factors associated with relapse are needed for group 2 patients who had middle range scores (1 or 2), since the odds of achieving successful discontinuation were approximately 50%. Continuation of NA treatment is recommended for group 3 patients having high scores (3 or 4) because nearly 90% of this group relapsed. However, this recommendation may be reconsidered in patients younger than 40 years; such cases tended to have a lower relapse rate in group 3. It is also noteworthy that relapse occurred mainly during the first and second years following NA discontinuation in all groups, similarly to a report by Liu *et al.*[14] Thus, clinicians should be vigilant in the early phase after discontinuation.

This study has several limitations. The patients who discontinued NAs were recruited retrospectively, and thus the decision to halt NA treatment was made by individual physicians without uniformly established criteria. Based on this, prospective studies are required to confirm our results. Furthermore, as over 90% of the patients we enrolled had genotype C and over 90% of cases were treated with LVD until discontinuation, the results obtained here can not be applied directly to other HBV genotypes or other types of NAs.

In conclusion, the present study showed that maximal levels of serum ALT and HBV DNA were useful for defining relapse patients after discontinuation of NAs. Along with serum HBV DNA of less than 3.0 log copies/mL and negative serum HBeAg, serum levels of HBsAg and HBcrAg at the time of NA discontinuation were able to predict relapse of hepatitis B and should therefore be considered when establishing uniform guidelines regarding the safe withdrawal of NA treatment. To this end, NA administration of more than 16 months is advisable to achieve successful discontinuation.

ACKNOWLEDGMENTS

THIS RESEARCH WAS supported in part by a research grant from the Ministry of Health, Labor and Welfare of Japan.

We thank Ms. Hiroe Banno for her secretarial assistance and thank Ms. Nozomi Kamijo and Ms. Etsuko Iigahama for their technical assistance. We also thank Mr Trevor Ralph for his English editorial assistance.

REFERENCES

1 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.
2 Lok AS, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; 45: 507–39.
3 Lee WM. Hepatitis B virus infection. *N Engl J Med* 1997; 337: 1733–45.
4 Ghany M, Liang TJ. Drug targets and molecular mechanisms of drug resistance in chronic hepatitis B. *Gastroenterology* 2007; 132: 1574–85.
5 Liaw YF, Sung JJ, Chow WC *et al.* Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004; 351: 1521–31.
6 Matsumoto A, Tanaka E, Rokuhara A *et al.* Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic

hepatitis B: a multicenter retrospective study of 2795 patients. *Hepatol Res* 2005; **32**: 173–84.
7 Suzuki Y, Kumada H, Ikeda K *et al.* Histological changes in liver biopsies after one year of lamivudine treatment in patients with chronic hepatitis B infection. *J Hepatol* 1999; **30**: 743–8.
8 Lok AS, Zoulim F, Locarnini S *et al.* Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. *Hepatology* 2007; **46**: 254–65.
9 Honkoop P, de Man RA, Niesters HG, Zondervan PE, Schalm SW. Acute exacerbation of chronic hepatitis B virus infection after withdrawal of lamivudine therapy. *Hepatology* 2000; **32**: 635–9.
10 Honkoop P, de Man RA, Heijtink RA, Schalm SW. Hepatitis B reactivation after lamivudine. *Lancet* 1995; **346**: 1156–7.
11 Chang TT, Gish RG, de Man R *et al.* A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006; **354**: 1001–10.
12 Lai CL, Shouval D, Lok AS *et al.* Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2006; **354**: 1011–20.
13 Lai CL, Chien RN, Leung NW *et al.* A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 1998; **339**: 61–8.
14 Liu F, Wang L, Li XY *et al.* Poor durability of lamivudine effectiveness despite stringent cessation criteria: a prospective clinical study in hepatitis B e antigen-negative chronic hepatitis B patients. *J Gastroenterol Hepatol* 2011; **26**: 456–60.
15 Reijnders JG, Perquin MJ, Zhang N, Hansen BE, Janssen HL. Nucleos(t)ide analogues only induce temporary hepatitis B e antigen seroconversion in most patients with chronic hepatitis B. *Gastroenterology* 2010; **139**: 491–8.
16 Liaw YF, Leung N, Kao JH *et al.* Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int* 2008; **2**: 263–83.
17 Leung N. Recent data on treatment of chronic hepatitis B with nucleos(t)ide analogues. *Hepatol Int* 2008; **2**: 163–78.
18 Kumada H, Okanoue T, Onji M *et al.* Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis B virus infection for the fiscal year 2008 in Japan. *Hepatol Res* 2010; **40**: 1–7.
19 Schuttler CG, Wend UC, Faupel FM, Lelie PN, Gerlich WH. Antigenic and physicochemical characterization of the 2nd International Standard for hepatitis B virus surface antigen (HBsAg). *J Clin Virol* 2010; **47**: 238–42.
20 Dai CY, Yu ML, Chen SC *et al.* Clinical evaluation of the COBAS Amplicor HBV monitor test for measuring serum HBV DNA and comparison with the Quantiplex branched DNA signal amplification assay in Taiwan. *J Clin Pathol* 2004; **57**: 141–5.
21 Ronsin C, Pillet A, Bali C, Denoyel GA. Evaluation of the COBAS AmpliPrep-total nucleic acid isolation-COBAS TaqMan hepatitis B virus (HBV) quantitative test and comparison to the VERSANT HBV DNA 3.0 assay. *J Clin Microbiol* 2006; **44**: 1390–9.
22 Mizokami M, Nakano T, Orito E *et al.* Hepatitis B virus genotype assignment using restriction fragment length polymorphism patterns. *FEBS Lett* 1999; **450**: 66–71.
23 Kimura T, Rokuhara A, Sakamoto Y *et al.* Sensitive enzyme immunoassay for hepatitis B virus core-related antigens and their correlation to virus load. *J Clin Microbiol* 2002; **40**: 439–45.
24 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.
25 European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol* 2009; **50**: 227–42.
26 Gish RG, Lok AS, Chang TT *et al.* Entecavir therapy for up to 96 weeks in patients with HBeAg-positive chronic hepatitis B. *Gastroenterology* 2007; **133**: 1437–44.
27 Marcellin P. Hepatitis B and hepatitis C in 2009. *Liver Int* 2009; **29** (Suppl 1): 1–8.
28 Moucari R, Lada O, Marcellin P. Chronic hepatitis B: back to the future with HBsAg. *Expert Rev Anti Infect Ther* 2009; **7**: 633–6.
29 Yokosuka O, Kurosaki M, Imazeki F *et al.* Management of hepatitis B: consensus of the Japan Society of Hepatology 2009. *Hepatol Res* 2011; **41**: 1–21.
30 Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006; **130**: 678–86.
31 Chen CJ, Yang HI, Su J *et al.* Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65–73.
32 Werle-Lapostolle B, Bowden S, Locarnini S *et al.* Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. *Gastroenterology* 2004; **126**: 1750–8.
33 Sung JJ, Wong ML, Bowden S *et al.* Intrahepatic hepatitis B virus covalently closed circular DNA can be a predictor of sustained response to therapy. *Gastroenterology* 2005; **128**: 1890–7.
34 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.
35 Brunetto MR, Moriconi F, Bonino F *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.
36 Moucari R, Mackiewicz V, Lada O *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.
37 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.
38 Hosaka T, Suzuki F, Kobayashi M *et al.* HBcrAg is a predictor of post-treatment recurrence of hepatocellular carcinoma during antiviral therapy. *Liver Int* 2010; **30**: 1461–70.
39 Matsumoto A, Tanaka E, Minami M *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.
40 Shinkai N, Tanaka Y, Orito E *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.