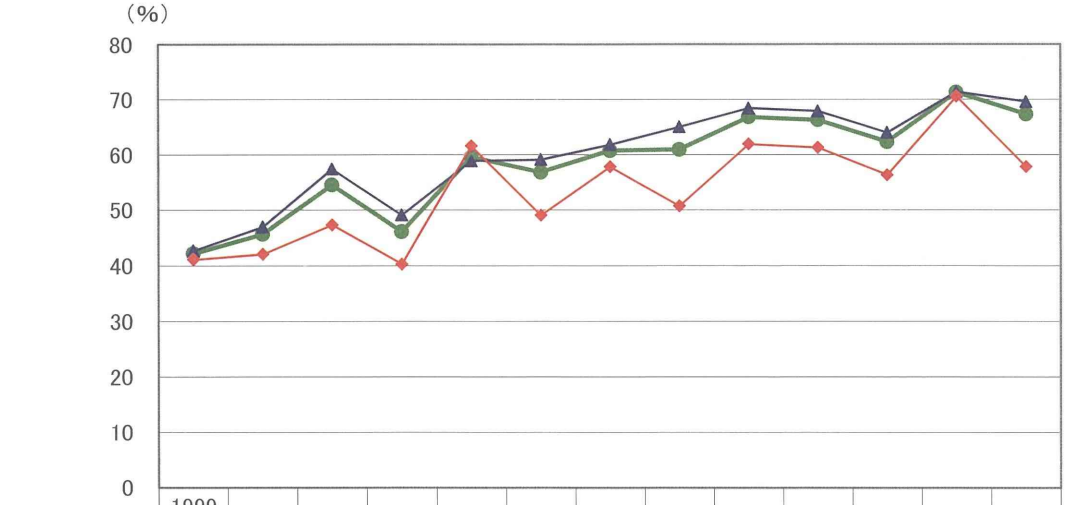
図5. B 型肝炎の性別・年別・性的接触*を感染経路とするものの割合　1999(4 月)-2011 年



	1999 (4月-)	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
男女合計	42.2	45.6	54.5	46.1	59.6	56.8	60.8	61.0	66.8	66.3	62.4	71.3	67.3
男性	42.7	46.9	57.4	49.1	58.9	59.1	61.8	65.0	68.5	67.9	64.0	71.4	69.6
女性	41.1	42.1	47.4	40.4	61.7	49.1	57.9	50.8	62.0	61.4	56.4	70.6	57.9

感染症発生動向調査 2012 年 1 月 20 日現在

*:性的接触には性的接触+αのものを含む

表1. B 型肝炎の 1 医療機関当たり届出数別にみた医療機関数　2009-2011 年

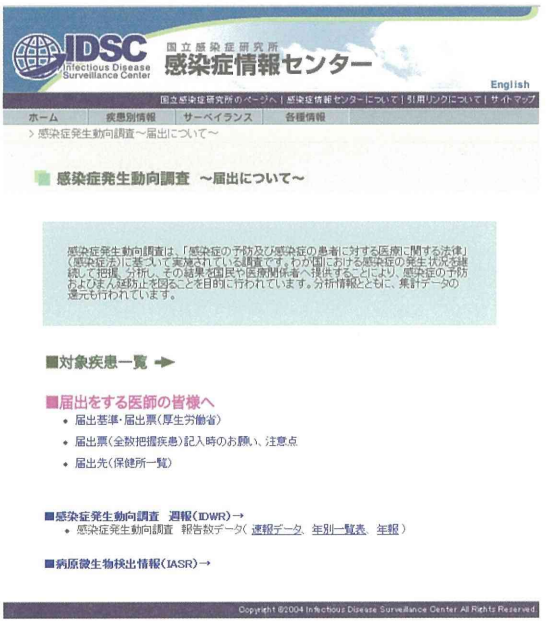
報告数	2009年 (178例)	2010年 (174例)	2011年 (196例)
1例	95	98	109
2例	29	18	24
3例	3	6	6
4例	1	1	2
5例	1	2	0
6例	0	0	1
7例	1	0	1
8例	0	1	0

感染症発生動向調査 2012 年 1 月 20 日現在

参考図

1. 感染症情報センタートップページに
『感染症発生動向調査～届出について～』
のアイコンを新設

2.『感染症発生動向調査～届出について～』
のページ

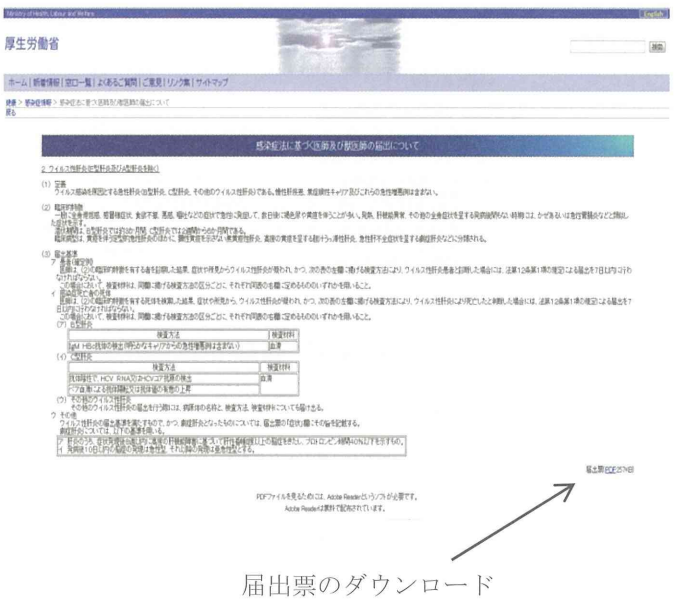


3. 対象疾患一覧

4. 届出をする医師の皆様へ

・届出基準・届出票(厚生労働省)⇒リンク

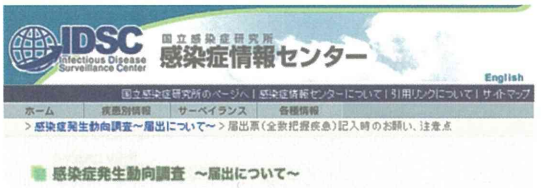
リンク先のページで、「全数報告対象」の「5類感染症の一部」から、「ウイルス性肝炎（E型肝炎及びA型肝炎を除く）」を選択する



届出票のダウンロード

・届出票（全数把握疾患）記入時のお願い、注意点

届出票記入時の注意点などを記載



届出票（全数把握疾患）記入時のお願い、注意点

印刷は**こちら**から
（PDF.180KB）

診断（検案）した者（死体）の類型：
初診時に死亡されている方は「感染症死亡者の死体」、「感染症死亡疑い者の死体」のいずれかとなります。初診時に生存されていた方が死亡された場合は「患者（確定例）」（あるいは「届出基準」「無症状病原体保有者」）です。

当該者職業：
診断のみならず公衆衛生対策上も重要な情報となります。公務員、会社員などにとどめず、できる だけ職種（調理師、保育士、医師、ツアーコンダクターなど）を記載してください。

当該者住所と当該者所在地：
住所は住民登録してある所、所在地は届る場所で、届出を受けた保健所等が連絡連絡のとれる場所（入院中なら病院、帰省中であれば帰省先）です。

症状：
自覚症状に限らず、他覚症状・所見が含まれます（例えば、肝腫大、肝機能異常値、X-P・内視鏡・超音波検査での異常所見、検便の潜血陽性など）。「その他」には、選択項目以外で重要と思われる症状や、基礎疾患、重症感染症の有無、内服中の薬剤などを記載してください（例えば、コレラでの制腸剤内服中、劇症型溶血性レンサ球菌感染症での紅斑など）。「症状なし」は無症状病原体保有者の扱いとなるものです（一一四類感染症はすべて無症状者も届出対象ですが、五類感染症では後天性免疫不全症候群、梅毒を除き、症状なしの場合は届出対象外です）。

診断方法：
診断の根拠となったものすべてに○をつけ、必要な内容を記入してください。検査中のものや、陰性結果のものは含まれません（但し、著しくは、例外的に、陰性結果も含めて記載していただくようになっています）。届出票には届出基準に示された診断方法があらかじめ書かれており、それ以外の方法で診断されたものは、原則、届出対象外となります。新しい検査法など場合によっては対象と判断できる方法もありますので、ご不明な場合には保健所または国立感染症研究所感染症情報センター感染症情報室（03-5285-1111）にご確認ください。

初診年月日：
当該疾患の初診日です。それ以前から他疾患で通院中・入院中である場合には、他疾患の初診日としないようご注意ください。

感染したと推定される年月日：
他の感染者の存在を把握するうえで公衆衛生対策上重要です。問診内容や潜伏期間などから感染機会をでできる限り判断して、記入してください。

発病年月日：
感染性の有る期間の把握や、集団発生時などでの発症曲線の描写などに必要となり、公衆衛生対策上重要です。忘れずに記入してください。なお、何をもって「発病」とするかの規定は定められていませんが、当該疾患の主となる症状が最初に出現した日について記入してください（例えば、発熱性疾患なら発熱出現日、消化器症状が主たる疾患はそれらの症状（腹痛、下痢など）の出現日）。

診断年月日：
届出基準を満たす結果が得られ、診断が確定した日です。

死亡年月日：
届出後に死亡された場合にも、保健所で追加入力ができるよう、できるだけ保健所にご連絡をお願い致します。

感染原因・感染経路・感染地域：
集団発生の探知や、拡大・再発防止策など、公衆衛生対策に直結する非常に重要な項目です。問診を含めた診断結果からできるだけ記載をお願いします。不明としか判断できない場合には、その他（　　）に（不明）と記載してください。

なお（確定・推定）の判断基準は示されていないので、状況により判断してください。

1. 感染原因・感染経路：それぞれ選択された項目の詳細内容（例えば、経口感染では飲食物の種類・状況、利用した飲食店など）をできるだけ具体的に記入してください。
2. 感染地域：詳細地域・場所（わかる場合には施設名なども）をできるだけ具体的に記入してください。複数の地域が考えられる場合などには、潜伏期間や現地の流行状況なども考慮して判断してください。渡航先や国内旅行先などでは、感染地域への滞在期間も問診し、把握できれば記入してください。

その他感染症のまん延の防止及び当該者の医療のために医師が必要と認める事項：
例えば、集団発生の可能性に関する情報、家族や接触者調査の必要性などの保健所へのアドバイス、入院の必要性や重篤度など、他の項目にかけなかった事項などを積極的に記入してください。

・届出先（保健所一覧）

都道府県を選択する

保健所の住所、FAX 番号等

	名称	〒	所在地	電話	Fax	区分
1	西多摩	198-0042	青梅市東青梅5-19-6	0428(22)6141	0428(23)3987	
2	南多摩	206-0025	多摩市永山2-1-5	042(371)7661	042(375)6697	
3	多摩立川	190-0023	立川市柴崎町2-21-19	042(524)5171	042(524)7813	
4	多摩府中	183-0034	府中市美好町2-51-1	042(362)2334	042(360)2144	
5	多摩小平	187-0002	小平市花小金井1-31-24	042(450)3111	042(450)3261	
6	島しょ	163-8001	新宿区西新宿2-8-1	03(5320)4342	03(5388)1428	
7	町田市	194-0021	町田市中町2-13-3	042(722)0621	042(722)3249	政令市
8	八王子市	192-0083	八王子市旭町13-18	042(645)5111	042(644)9100	政令市
9	千代田	101-0054	千代田区神田錦町3-10	03(3291)3641	03(3291)3650	特別区
10	中央区	104-0044	中央区明石町12-1	03(3541)5930	03(3546)9554	特別区
11	みなと	108-0073	港区三田1-4-10	03(3455)4701	03(3798)4619	特別区
12	新宿区	160-8484	新宿区歌舞伎町1-4-1	03(5273)3024	03(5273)3930	特別区
13	文京	112-8555	文京区春日1-16-21	03(3812)7111	03(5803)1386	特別区
14	台東	110-0015	台東区東上野4-22-8	03(3847)9401	03(3841)4325	特別区
15	墨田区	130-8640	墨田区吾妻橋1-23-20	03(5608)1111	03(5608)6404	特別区
16	江東区	135-0016	江東区東陽2-1-1	03(3647)9855	03(3615)7171	特別区
17	品川区	142-0063	品川区荏原2-9-6	03(3788)2000	03(3788)7900	特別区
18	目黒区	153-8573	目黒区上目黒2-19-15	03(5722)9501	03(5722)9508	特別区
19	大田区	144-8621	大田区蒲田5-13-14	03(5744)1262	03(5744)1523	特別区
20	世田谷	154-8504	世田谷区世田谷4-22-35	03(5432)1111	03(5432)3022	特別区

平成 23 年度　分担研究報告書

HBV 関連体外診断用医薬品の性能比較調査

分担研究者：水落利明　国立感染症研究所　血液・安全性研究部　室長

研究要旨：HBV 感染動態の重要な指標となる３種のマーカー：HBs 抗原、抗 HBs 抗体、抗 HBc 抗体について、それらを検出／測定する体外診断用医薬品の性能比較調査を国内で承認を受け販売されている高感度キットについて実施している。なお、本調査には日本赤十字社中央血液研究所より譲渡を受けた献血由来の実検体を用いた。本年度は抗 HBs 抗体と抗 HBc 抗体測定キットについての調査結果を報告する。

A. 研究目的

B 型肝炎ウイルス（HBV）のキャリアおよび既感染者においては、血液悪性疾患等に対する化学療法や免疫抑制剤投与により HBV の再活性化（再燃）による B 型肝炎が発症することがあり、時にはそれが劇症化することから細心の注意が必要である。そこで厚生労働省による２つの研究班「肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究」および「難治の肝・胆道疾患に関する調査研究」が合同でワーキンググループを立ち上げ、ガイドラインを作成した（2009 年作成、2011 年 9 月改訂）。ここではまず HBV キャリアであるかを確認するために全例で HBs 抗原を測定する。そして HBs 抗原が陰性の場合には抗 HBc 抗体と抗 HBs 抗体を測定し、既感染者かどうかを確認する。ここで各マーカーを測定する体外診断用医薬品（キット）の性能が重要となる。現在国内では様々なキットが承認され販売されているが、検査に使用するキットにより判定が乖離することがあれば、HBV キャリアあるいは HBV 既感染者であるかどうかを判定する上での大きな障害となる。そこで本研究の目的は、国内献血由来の検体を用いて、現在国内で使用されている各マーカー（HBs 抗原、抗 HBs 抗体、抗 HBc 抗体）測定キットの性能比較調査を実施することにある。

B. 研究方法

日本赤十字社中央血液研究所より供与された以下の検体を用いた。

1. 抗 HBs 抗体陽性 172 検体（抗 HBc 抗体陽性検体を含む）
2. 低力価（<100mIU/mL）抗 HBs 抗体陽性 50 検体
3. 低力価（COI=1.0-5.0）抗 HBc 抗体陽性 44 検体

日本臨床検査薬協会を通じて参加を募ったキットの製造／販売各社へ上記検体を配布し測定を依頼した。

本調査に用いた各キット（抗 HBs 抗体測定、抗 HBc 抗体測定）の一覧を表１に示す。

C. 研究結果

抗 HBs 抗体測定キット：抗 HBs 抗体陽性 172 検体中 4 検体において、キット間での判定乖離が見られた（表２）。また、判定は陽性でもキットによって測定値が大きく異なる検体があった。

抗 HBc 抗体測定キット：日本赤十字社がスクリーニングで使用している CL-4800 による測定値が COI=3.0 以上の検体についてはほとんどのキットが陽性と判定したが、低力価（COI<3.0）の検体では明らかにキット間での判定乖離が見られた（表３）。

D. 考察

低力価の抗 HBs 抗体陽性検体においては、キット間での判定乖離が見られたが、陽性と判定された検体が実際には擬陽性の可能性がある。そ

こでリコンビナント HBs 抗原を用いた吸収確認試験を実施したところ、adr, adw の両抗原による吸収が確認され、陽性判定が非特異ではなく正しいことが明らかになった。つまり、それらの低力価検体を陰性と判定したキットの測定感度が低いと考えられた。今回の調査結果から、キットで用いている抗体捕捉用 HBs 抗原の subtype によって検出感度に差が生じることが示唆された。つまり adr 抗原を使用しているキットのほうが ad/ay 抗原を使用しているキットに比較して感度が高い傾向が見られた。これは国内での HBV 感染者の大多数が adr 型の HBs 抗原陽性であることに起因する可能性を示している。

低力価の抗 HBc 抗体陽性検体においてもキット間での判定乖離が見られた(表3)。これについても今後 HBc 抗原による吸収確認試験を実施する予定である。

HBs 抗原検出／測定キットについては今後性能比較調査を実施する予定である。2001 年に当時国内で販売されていた HBs 抗原キットについての再点検を、厚生労働省審査管理課の要請で感染研が実施している(医薬品・医療用具等安全性情報 170 号:平成 13 年)。その調査結果から明らかに感度の低いキットは市場から撤退した経緯がある。今回の性能調査では、検出感度のみではなく、HBV genotype の違いおよび mutant(変異) HBs 抗原についても考慮した解析を行うことを予定している。

E. 結論

国内献血由来の実検体を用いて抗 HBs 抗体および抗 HBc 抗体測定キットの性能比較調査を行った。その結果、低力価検体においては、キット間での判定乖離が見られた。この結果から HBV キャリアあるいは HBV 既感染者であるかどうかを判定するために用いる体外診断用キットの感度については細心の注意が求められる。

F. 研究発表(本研究に関わるもの)

1. 論文発表
   なし
2. 学会発表
   なし

G. 知的財産権の出願・登録状況

1. 特許取得
   なし
2. 実用新案登録
   なし
3. その他
   なし

表1：HBs抗体測定キットとHBc抗体測定キット一覧

No.	試薬名	メーカー名	単位	陽性判定基準
1	アーキテクト®・オーサブ	アボットジャパン	mIU/mL	10mIU/mL
2	オーサブ・ダイナパック（アキシム）	アボットジャパン	mIU/mL	5mIU/mL
3	スフィアライトHBs抗体	和光純薬	mIU/mL	6mIU/mL
4	HISCL HBcAb試薬	日本凍結乾燥（株）	mIU/mL	5mIU/mL
5	ルミパルスHBsAb	富士レビオ	mIU/mL	5mIU/mL
6	ルミパルスプレストHBsAb	富士レビオ	mIU/mL	5mIU/mL
7	ルミパルスプレストHBsAb-N	富士レビオ	mIU/mL	10mIU/mL
8	ルミスポット'栄研' HBs抗体	栄研化学	mIU/mL	10mIU/mL
9	ビトロス HBs抗体	オーソ	mIU/mL	12mIU/mL
10	エクルーシス試薬 Anti-HBs	ロシュ	mIU/mL	10mIU/mL
11	ケミルミ Centaur-HBs抗体	シーメンス	mIU/mL	7.5mIU/mL
12	エンザイグノスト　Anti-HBs II	シーメンス	mIU/mL	8mIU/mL
13	Eテスト「TOSOH」II（HBsAb）	東ソー	mIU/mL	6.4mIU/mL

No.	試薬名	メーカー名	単位	陽性判定基準
1	アーキテクト®・HBcII	アボットジャパン	S/CO	1.00以上
2	ランリーム HBcAb	シスメックス	mU/mL	10mU/ml
3	エルジア　F-HBc抗体	シスメックス	INH%	70以上陽性（50-70%未満保留）
4	HISCL HBcAb試薬	日本凍結乾燥（株）	C.O.I	1.0以上
5	ルミパルスHBcAb-N検討品	富士レビオ	C.O.I	1.0以上
6	ルミパルスプレストHBcAb-III検討品	富士レビオ	C.O.I	1.0以上
7	ビトロス HBc抗体	オーソ	C.O.I.	1.0より（1.0以上、1.2未満　保留、4.8以上再検査）
8	エクルーシス試薬 Anti-HBc	ロシュ	C.O.I	1.0以上
9	ケミルミ Centaur-HBc抗体	シーメンス	Index	0.5以上
10	エンザイグノスト　Anti-HBc monoclonal	シーメンス	INH%orS/CO	Asample =<cut off値
11	Eテスト「TOSOH」II（HBcAb）	東ソー	InH%	60以上（40INH%以上60INH%未満再検査）

表2：HBs抗体測定キットにおける判定乖離

	日赤	A	B	C	D	E	F	G	H	I	J	K	L	M
No.4	＋	＋	＋	－	＋	＋	＋	＋	－	－	＋	＋	＋	＋
No.7	＋	－	＋	－	＋	－	＋	－	－	－	－	＋	＋	＋
No.15	＋	＋	＋	＋	＋	＋	＋	＋	＋	＋	－	＋	＋	＋
No.17	＋	－	＋	＋	＋	＋	＋	－	－	＋	－	＋	＋	＋

表3：HBc抗体測定キットにおける判定乖離

No.	日赤(CL4800)	A	B	C	D	E	F	G	H	I	J	K
1	1	+	+	+	+	+	+	+	+	+	+	+
2	1	+	−	−	−	+	+	−	+	−	−	−
3	1	+	+	+	+	+	+	+	+	+	+	+
4	1.1	+	+	+	+	+	+	+	+	+	+	+
5	1.1	+	+	+	+	+	+	+	+	+	+	+
6	1.2	+	+	−	−	+	+	+	+	+	−	indeterminant
7	1.2	+	−	+	−	+	+	+	+	+	+	+
8	1.2	+	+	−	−	+	+	−	+	+	−	indeterminant
9	1.3	+	+	+	−	+	+	+	+	+	+	+
10	1.5	+	−	−	−	+	+	+	+	+	indeterminant	+
11	1.6	+	+	−	+	+	+	+	+	+	+	+
12	1.6	+	+	+	+	+	+	+	+	+	+	+
13	1.7	+	−	+	−	+	+	+	+	+	+	+
14	1.8	+	+	−	−	+	+	+	+	+	+	indeterminant
15	1.8	+	+	+	+	+	+	+	+	+	+	+
16	2	−	−	−	−	+	+	−	−	−	−	−
17	2.1	+	−	−	−	+	+	−	−	−	−	−
18	2.2	+	+	−	−	+	+	+	+	+	+	indeterminant
19	2.2	+	+	+	+	+	+	+	+	+	+	+
20	2.3	+	+	+	+	+	+	+	+	+	+	+
21	2.3	+	+	−	+	+	+	+	+	+	+	+
22	2.4	+	+	−	+	+	+	+	+	+	−	indeterminant
23	2.6	+	+	+	+	+	+	+	+	+	+	+
24	2.6	+	+	+	−	+	+	+	+	+	+	+
25	2.7	+	+	+	+	+	+	+	+	+	+	+
26	2.7	+	+	+	+	+	+	+	+	+	+	+
27	2.7	+	+	+	−	+	+	+	+	+	+	+
28	3	+	+	+	+	+	+	+	+	+	+	+
29	3.1	+	+	+	+	+	+	+	+	+	+	+
30	3.2	+	+	+	+	+	+	+	+	+	+	+
31	3.3	+	+	+	+	+	+	+	+	+	+	+
32	3.3	+	+	+	+	+	+	+	+	+	+	+
33	3.4	+	+	+	+	+	+	+	+	+	+	+
34	3.5	+	+	+	+	+	+	+	+	+	+	+
35	3.5	+	+	+	−	+	+	+	+	+	+	+
36	3.5	+	+	+	+	+	+	+	+	+	+	+
37	3.5	+	+	−	+	+	+	+	+	+	+	+
38	3.5	+	+	+	+	+	+	+	+	+	+	+
39	3.6	+	+	+	+	+	+	+	+	+	+	+
40	3.6	+	+	+	+	+	+	+	+	+	+	+
41	3.7	+	+	+	+	+	+	+	+	+	+	+
42	4.1	+	+	+	−	+	+	+	+	+	+	+
43	4.4	+	+	+	+	+	+	+	+	+	+	+
44	4.5	+	+	+	+	+	+	+	+	+	+	+

Ⅲ．研究成果の刊行一覧

研究成果の刊行に関する一覧表

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Ⅳ. 研究成果の刊行物・別刷

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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

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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

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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

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

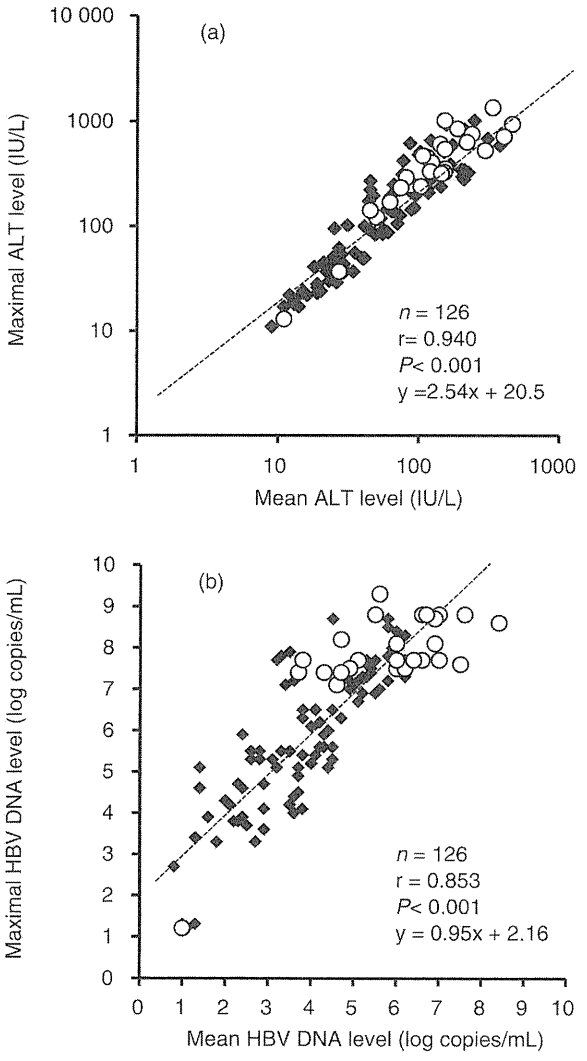

Figure 1 Correlation between maximal and mean levels of alanine aminotransferase (ALT) (a) and hepatitis B virus (HBV) DNA (b) after discontinuation of nucleos(t)ide analogs (NAs). Open circles indicate patients with detectable hepatitis B e antigen (HBeAg) and closed squares indicate patients without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

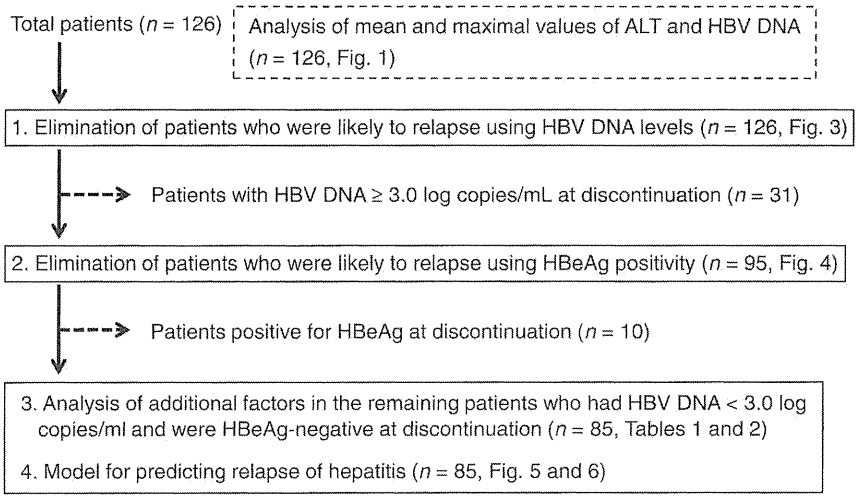

Figure 2 The progression of analyses in the present study and population structure of each analysis.

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

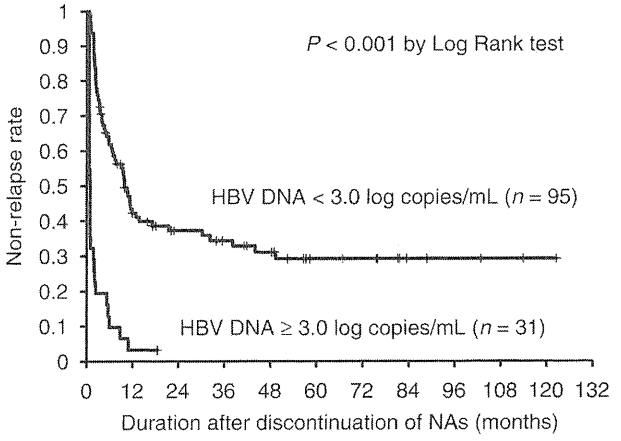

Figure 3 Comparison of non-relapse rates using the Kaplan–Meier method between 31 patients with serum hepatitis B virus (HBV) DNA equal to or higher than 3.0 log copies/mL and 95 patients with serum HBV DNA lower than 3.0 log copies/mL at the time of nucleos(t)ide analog (NA) discontinuation.

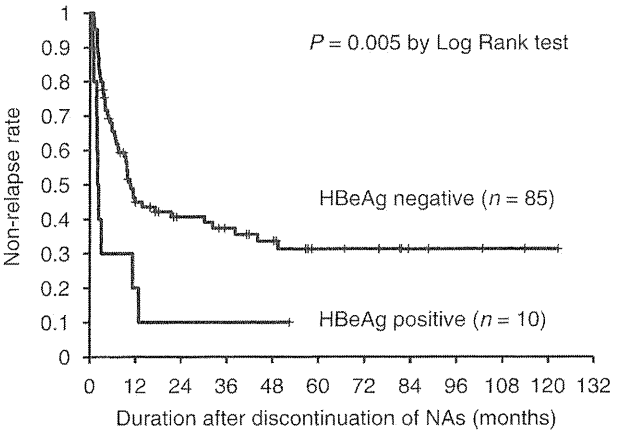

Figure 4 Comparison of non-relapse rates using the Kaplan–Meier method between 10 patients with detectable hepatitis B e antigen (HBeAg) and 85 patients without detectable HBeAg at the time of nucleos(t)ide analog (NA) discontinuation.