

Serum beta2-microglobulin levels in hepatitis B e antigen-negative chronic hepatitis B patients under long term lamivudine monotherapy: Relationship with virological breakthrough

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IS Elefsiniotis, N Scarmeas, I Glynou, KD Pantazis, H Kada, C Mavrogiannis. Serum beta2-microglobulin levels in hepatitis B e antigen-negative chronic hepatitis B patients under long term lamivudine monotherapy: Relationship with virological breakthrough. *Can J Gastroenterol* 2004;18(5):307-313.

OBJECTIVES: To evaluate the predictive value of serum beta2-microglobulin (β 2m) levels for virological breakthrough in hepatitis B e antigen-negative chronic hepatitis B patients under long term lamivudine monotherapy.

METHODS: Serum β 2m levels were calculated at baseline and every three months during lamivudine monotherapy in 25 patients with chronic hepatitis B, using microparticle enzyme immunoassay technology to investigate their association with biochemical, virological and histological outcome data. Cox proportional hazard models were used to investigate the association between serum β 2m levels and virological breakthrough.

RESULTS: Seven of 25 (28%), nine of 25 (36%) and 14 of 25 (56%) chronic hepatitis B patients exhibited virological breakthrough at months 12, 24 and 36 of treatment, respectively. All chronic hepatitis B patients who did not show virological breakthrough in the follow-up period exhibited β 2m elevation in month 3 of treatment. The duration (in months) of serum β 2m elevation was significantly higher in the responders group than the nonresponders group (7.3 ± 2.6 versus 3.8 ± 3.4 , $P=0.02$). In contrast to patients whose serum β 2m levels were increased at three months, patients whose β 2m levels were decreased had a 4.6 times higher risk of experiencing virological breakthrough (hazards ratio 4.6, 95% CI 1.22 to 17.36). When age, pretreatment serum alanine aminotransferase and hepatitis B virus DNA levels, and grade of liver disease were simultaneously included in the same Cox model, decreased β 2m status was still associated with increased risk of virological breakthrough (hazards ratio 12.2, 95% CI 1.28 to 116.8).

CONCLUSIONS: In hepatitis B e antigen-negative chronic hepatitis B patients under long term lamivudine monotherapy, serum β 2m levels at three months of treatment, compared with baseline levels, are good predictors of risk for virological breakthrough.

Key Words: Beta2-microglobulin; Chronic hepatitis B; Lamivudine; Virological breakthrough

Taux sérique de β -2 microglobuline chez des patients atteints d'hépatite B chronique, négative à l'égard de l'antigène HBe, et traités à la lamivudine en monothérapie prolongée : lien avec une percée du virus

OBJECTIF : Évaluer la valeur prédictive du taux de β -2 microglobuline chez des patients atteints d'hépatite B chronique, négative à l'égard de l'antigène HBe, et traités à la lamivudine en monothérapie prolongée : lien avec une percée du virus.

MÉTHODE : Nous avons mesuré le taux sérique de β -2 microglobuline au départ, puis aux trois mois, chez 25 patients atteints d'hépatite B et traités à la lamivudine en monothérapie, au moyen du dosage immuno-enzymatique de microparticules, afin d'étudier les liens avec les résultats biochimiques, virologiques et histologiques. Le modèle de régression des hasards proportionnels de Cox a servi à examiner le lien entre ce taux sérique et la percée virale.

RÉSULTATS : Sept (28 %), neuf (36 %) et quatorze patients (56 %) sur les 25 à l'étude ont présenté une augmentation de la charge virale au bout de 12, 24 et 36 mois respectivement. Tous les patients atteints d'hépatite B chronique chez qui le virus n'a pas fait de percée au cours du suivi ont vu le taux de β -2 microglobuline augmenter après trois mois de traitement. La période (mois) durant laquelle le taux sérique de β -2 microglobuline est resté élevé était sensiblement plus longue chez les sujets répondants que chez les sujets non répondants ($7,3 \pm 2,6$ contre $3,8 \pm 3,4$; $P=0,02$). Contrairement aux patients chez qui le taux sérique de β -2 microglobuline avait augmenté, ceux chez qui ce taux avait diminué avaient 4,6 fois plus de risques de connaître une percée virale (rapport des taux d'incidence : 4,6; IC à 95 % : 1,22 à 17,36). Même lorsque l'âge, le taux sérique d'alanine-aminotransférase avant le traitement, le taux d'ADN du virus de l'hépatite B et le degré d'atteinte hépatique étaient intégrés dans le même modèle de Cox, la diminution du taux de β -2 microglobuline était toujours associée à une augmentation du risque de percée du virus (rapport des taux d'incidence : 12,2; IC à 95 % : 1,28 à 116,8).

CONCLUSION : Le taux sérique de β -2 microglobuline après trois mois de traitement, comparativement au départ, constitue un bon prédicteur de risque de percée virale chez les patients atteints d'hépatite B chronique, négative à l'égard de l'antigène HBe, et traités à la lamivudine en monothérapie prolongée.

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Received for publication October 9, 2003. Accepted March 19, 2004

Chronic hepatitis B (CHB) is a common disease with an estimated global prevalence of 350 million chronically infected patients, according to the World Health Organization (1). Hepatitis B e antigen (HBeAg)-negative CHB accounts for 7% to 30% of patients with CHB worldwide, with the highest rates reported in Mediterranean Europe and Asia (2). It results from infection with hepatitis B virus (HBV) mutants that are unable to produce HBeAg (3). In Greece, it is estimated that more than 95% of patients with HBeAg-negative CHB are infected with the precore mutant HBV variant (4). These patients often appear to have more severe liver disease than that observed in patients infected with the wild type form of the virus, and treatment with interferon (IFN)-alpha is associated with suboptimal responses, high relapse rates and poor compliance (5,6).

Lamivudine is an oral nucleoside analogue that has potent antiviral properties against HBV and human immunodeficiency virus (HIV). Studies in HBeAg-positive (7) and HBeAg-negative (8) CHB patients demonstrate that lamivudine treatment results in rapid and consistent suppression of serum HBV DNA levels with normalization of aminotransferases and significant improvement of liver histology in the majority of patients. Extended lamivudine treatment increases the emergence of HBV variants, which have changes in the tyrosine-methionine-aspartate-aspartate amino acid (YMDD) locus of the HBV DNA polymerase sequence and exhibit reduced susceptibility to the drug (9-12). However, the clinical impact of these mutations is controversial (9-11) and the prediction of virological breakthrough (VB) is an issue needing further investigation.

Beta2-microglobulin (β 2m) plays a key role in influencing the immune response to viral infections because it is an integrating part of the major histocompatibility complex or human leukocyte antigen (HLA) (13). In comparison with controls, patients with chronic viral hepatitis manifest an enhanced hepatocellular display of class I HLA antigens together with rising serum β 2m levels (13,14). Both hepatic class I HLA antigens and serum β 2m levels correlated positively with duration and severity of liver disease (13,14). Moreover, IFN treatment significantly increased serum β 2m levels, offering a Th1-dominant environment to patients with chronic viral hepatitis, irrespective of therapy outcome (14-16). However, in at least two studies, hepatocyte β 2m expression significantly decreased after IFN treatment (17,18) following the reduction of histological activity of liver disease. The alterations of serum β 2m levels in CHB patients under long term lamivudine treatment have not been investigated yet.

In the present study, we sought to record the alterations of serum β 2m levels in HBeAg-negative CHB patients under long term lamivudine monotherapy, and to investigate their association with biochemical, virological and histological outcome data. We hypothesized that serum β 2m levels may be a predictor of VB or biochemical breakthrough (BB).

METHODS

Between March 1998 and August 1999, a total of 25 consecutive hepatitis B surface antigen (HbsAg)-positive patients (23 males) were enrolled prospectively in the study (GRLM01, Glaxo-SmithKline, Greece). All patients were treated with oral lamivudine (100 mg daily) for 36 months. Patients were evaluated clinically, biochemically and serologically at entry and every three months during treatment. To allow for reasonable time for

breakthrough to occur, only patients with at least 18 months of follow-up were included in the current analyses.

To be eligible, patients had to fulfill the following criteria: age greater than 18 years; detectable HbsAg in serum for at least six months; HBeAg negativity; antibody to HBeAg positivity with signs of active viral replication (serum HBV DNA-positive); serum alanine aminotransferase (ALT) levels above the normal range on at least two separate occasions in the previous six months; and absence of previous immunomodulatory and/or antiviral treatment for hepatitis. HBV sequencing to confirm genotypic precore mutations was not performed. Patients were excluded if they were infected with hepatitis C and/or hepatitis D virus, were infected with HIV, had decompensated liver disease (Child-Pugh B or C), had evidence of autoimmune hepatitis (antinuclear antibody titre of at least 1:160) or had a positive clinical history for other chronic liver disease. Moreover, patients were excluded from the study if they had a positive clinical history for other chronic diseases or if they were under any other medication. Pregnant and breastfeeding women were also excluded.

Liver biopsy was done for all patients at baseline and at month 12 of the study. A single pathologist evaluated all biopsy specimens that were scored according to the Ishak scoring system (grade 0 to 18, stage 0 to 6) (19). Histological response was defined as at least a two-point reduction in the necroinflammatory score (grade) between the pretreatment and the month 12 biopsies, and at least the same fibrosis score (stage).

Routine biochemical and hematological tests were performed using automated techniques. Virological evaluation (HBV DNA) was done at baseline and at months six, 12, 24 and 36 of treatment. HbsAg, HBeAg, antibody to HBeAg and antibody to HbsAg were measured using routine commercially available enzyme immunoassays (Abbott Laboratories, USA). HBV DNA was quantified with the use of a commercially available polymerase chain reaction assay (Amplicor, Roche, Switzerland) with a lower limit of quantification of 400 copies/mL. Detection of HBV polymerase YMDD variants was performed as described by Lai et al (7), using a restriction fragment-linked polymorphism assay.

BB was defined as an ALT flare (ALT greater than 40 IU/L) during lamivudine treatment, after at least one previous test had been under normal range (ALT 40 IU/L or less).

VB was defined as an HBV DNA reappearance (HBV DNA greater than 400 copies/mL) during lamivudine treatment, after at least one previous test had been under normal range (HBV DNA 400 copies/mL or less).

β 2m

Serum was collected from patients at baseline and every three months during lamivudine treatment and was stored at -85°C . Serum β 2m levels were calculated using microparticle enzyme immunoassay technology (20), which used submicron microparticles coated with a capture molecule specific for the analyte being measured (Abbott Laboratories, USA). Serum sample and microparticles (captured molecules) were transferred to the incubation well of the reaction cell. During the incubation period, analytes bound to the microparticles, creating an immune complex. The reaction mixture was aspirated from the incubation well to another cell where it was washed to remove unbound materials. Alkaline phosphate-labelled conjugate was then transferred in the cell, where it bound to the immune complex to complete the antibody-analyte-conjugate 'sandwich'. The new complex was washed again and a dispenser added the substrate 4-methylumbelliferyl

phosphate. The alkaline phosphate conjugate catalyzed the hydrolysis of 4-methylumbelliferyl phosphate to 4-methylumbelliferone. The rate at which 4-methylumbelliferone was generated in the cell was proportional to the concentration of analyte in the serum sample.

Written informed consent was obtained from each patient for his or her participation in the study. The Hippokration Institutional Review Board reviewed and approved this project. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki.

Statistical analysis

β 2m levels were treated as a continuous variable in initial analyses. Two variables relating to β 2m were subsequently calculated. First, a continuous variable was created, reflecting the number of three-month intervals for which β 2m was rising, in relation to baseline levels. Second, a dichotomous variable reflecting the status of either β 2m elevation or decline at three-month intervals was created: this variable took the value of zero if β 2m increased at three months (compared with baseline) and the value of one if β 2m either remained the same or decreased at three months (compared with baseline).

Age, serum ALT levels and histological activity index (HAI, grade) of liver disease were all treated as continuous variables in the analyses. Initial HBV DNA levels were dichotomized into two groups: high viral load (greater than 10^6 copies/mL); and low viral load (10^6 copies/mL or less).

Student's *t* test and χ^2 analyses were used to examine the association between the presence of VB and other potentially interactive variables including age, ALT levels, BB, HAI, serum HBV DNA levels and serum β 2m levels. A $P < 0.05$ was considered significant.

Survival analyses (Cox proportional hazard [21]) were used to investigate the association between serum β 2m levels (predictor) and VB (outcome). Participants were considered to have failed at the time of the first follow-up visit at which VB was documented. For participants who did not manifest VB, the last follow-up evaluation was used for censoring. Therefore, the Cox model's time axis was the duration of follow-up until either VB or the last evaluation without VB. The initial Cox proportional hazard models used either the duration of β 2m elevation (continuous variable) or the elevation versus decline status at three-month intervals of β 2m (dichotomous variable) as a predictor. In subsequent Cox models, potential confounders (age, ALT, HBV DNA, HAI) were simultaneously included along with β 2m status at three months of follow-up in the same Cox model.

In addition, the changes of β 2m levels over time were explored, as well as their association with VB using generalized estimating equations (GEE) (22). GEE take into account the multiple assessments per subject and the fact that the characteristics of the same individual over time (β 2m scores for this analysis) are likely to be correlated. The repeated measures for each subject are treated as a cluster. β 2m was the dependent variable in this model. The model initially considered the effect of time (in months) for β 2m measurements. In line with the primary hypothesis (that changes of β 2m in the beginning of the lamivudine treatment period would predict VB), only the first six-month β 2m measurements were included. The model also considered the effects of the group (VB versus no VB) and their interaction. A significant time effect indicated a marked change in β 2m measurements over time (across both groups combined). A significant group effect indicated a significant difference in β 2m values at baseline. A significant

TABLE 1
Demographic, biochemical, virological and histological features at baseline for all chronic hepatitis B (CHB) patients and for the subgroups of patients who did (YMDD) and did not (responders) exhibit virological breakthrough

	All CHB patients (n=25)	Responders (n=11)	YMDD (n=14)	P
Age (years)	42.8±13.6	43.9±5	41.9±12.9	0.75 (NS)
ALT (IU/L)	218±106.1	263.9±115.8	183.6±87.7	0.09 (NS)
HBV DNA×10 ⁶ (copies/mL)	6.83±14.22	1.27±2.57	11.00±17.85	0.09 (NS)
Grade (0 to 18)	7.0±3.3	6.8±3.5	7.1±3.3	0.84 (NS)
Stage (0 to 6)	3.2±1.5	3.1±1.3	3.4±1.9	0.65 (NS)
β 2m (mg/dL) (baseline levels)	1695.5±348.1	1588.8±391.6	1775.5±304.1	0.23 (NS)
Duration of β 2m elevation (months)	5.3±3.5	7.3±2.6	3.8±3.4	0.02 (S)
β 2m elevation in 3 months (%)	19/25 (76%)	11/11 (100%)	8/14 (57.1%)	0.03 (S)

With the exception of the last row, mean values \pm SD are presented. ALT Alanine aminotransferase; β 2m beta2-microglobulin; HBV Hepatitis B virus; NS Not significant, $P > 0.05$; S Significant, $P < 0.05$; YMDD Tyrosine-methionine-aspartate-amino acid locus of the HBV DNA polymerase sequence

time \times group effect indicated significantly different rates of change of β 2m over time for one group versus the other.

RESULTS

All CHB patients tolerated lamivudine therapy well and completed the treatment period. None of the patients was lost from the follow-up. Mean overall follow-up (since start of lamivudine treatment) for the whole group was 31.4±6.0 months (range 24 to 36 months). Mean follow-up for the patients who exhibited VB (31±6.2 months) did not differ from follow-up for patients without VB (32±6.0 months) ($P = 0.71$). None of the treated CHB patients lost HbsAg and none showed HbsAg seroconversion during the lamivudine treatment period. Mild elevation of serum amylase levels, with no clinical significance, was observed in three of 25 (12%) treated patients. One patient developed a macular skin rash during the first two weeks of treatment, which disappeared without intervention in the following two weeks while he was on lamivudine treatment.

The demographic, laboratory and histological features of CHB patients at entry are shown in Table 1. There was no association between histological findings (grade, stage) and either serum ALT levels ($P = 0.719$ and $P = 0.486$, respectively) or serum HBV DNA levels ($P = 0.784$ and $P = 0.542$, respectively). Patients with a higher level of fibrosis (stage) at entry were older ($P = 0.021$). Three of 25 CHB patients (12%) had evidence of cirrhosis with ongoing inflammatory activity at baseline liver biopsy (Child-Pugh A).

Both serum ALT levels and serum HBV DNA titres showed a sharp decline during the first six months of therapy. Serum ALT levels were under the upper normal limits (less than 40 IU/L) and serum HBV DNA were negative (less than 400 copies/mL) in all CHB patients who participated in the study, at month 6 of lamivudine treatment. In the first year of treatment, seven of 25 (28%) CHB patients exhibited VB and

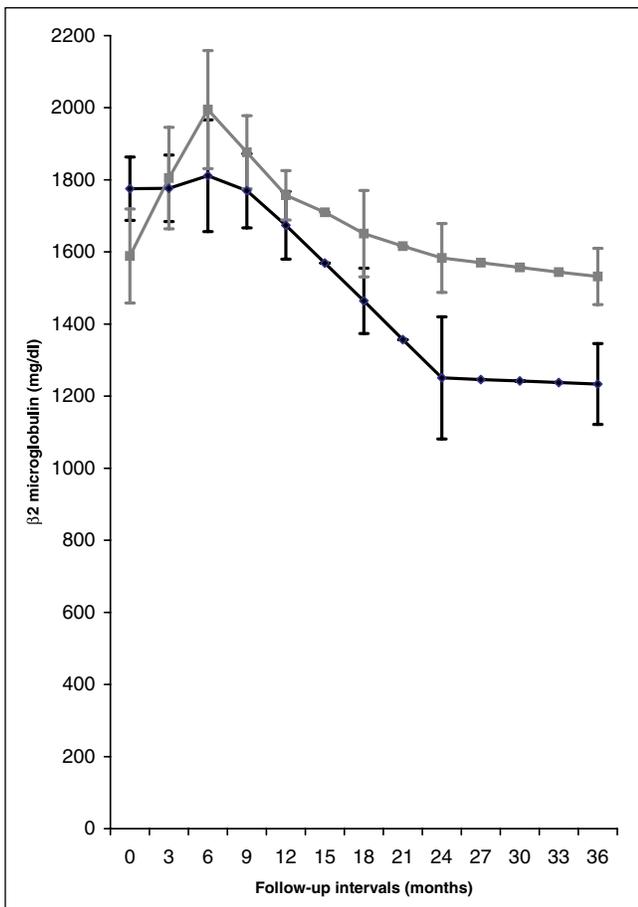


Figure 1 Mean serum beta 2 (β 2)-microglobulin (β 2m) levels for each follow-up interval, plotted separately for the group of patients who exhibited virological breakthrough (rhombuses-black line) and for the group of patients who did not (squares-grey line). As evidenced from the chart, mean serum β 2m levels remained stable (did not increase) for approximately nine months in patients who exhibited virological breakthrough, and steeply increased for approximately six months in patients who did not. Subsequently, serum β 2m levels declined in both groups

four of the seven patients (16% of the total CHB patients) showed both VB and BB. At month 12, the proportion of lamivudine-treated patients who were complete responders (neither VB nor BB) was as high as 72%. Both serum ALT levels and serum HBV DNA levels were significantly reduced after the 12-month treatment period. Moreover, there was a significant reduction in the histological score both for grade and stage of liver disease. In particular, 21 of 25 CHB patients (84%) showed at least a two-point reduction in the histological score for grade, three of 25 (12%) showed no improvement of liver histology and only one (4%) showed worsening of the necroinflammatory activity of liver disease. All four patients with no improvement or worsening of liver histology appeared with both VB and BB, whereas the other three patients who appeared with VB but no BB showed a significant improvement of liver histology.

Nine of 25 (36%) and 14 of 25 (56%) CHB patients exhibited VB at months 24 and 36 of lamivudine monotherapy, respectively.

TABLE 2
Cox proportional hazard model predicting virological breakthrough by serum beta2-microglobulin (β 2m) levels

	Variable	HR	95% CI
β 2m	Continuous	1.25	1.03 to 1.52*
	Decline versus elevation	4.6	1.22 to 17.36*

Results are presented with β 2m used as a continuous variable (number of months of β 2m elevation) or as a dichotomous variable (decline versus elevation of serum β 2m levels at three months, compared with baseline levels). The hazard ratio (HR) for β 2m as a continuous variable reflects risk for virological breakthrough for each month less of β 2m elevation. *Indicates that the HR does not include 1

Demographics, and biochemical, virological and histological information variables of patients who did and did not exhibit VB, as well as for the whole group of patients, are presented in Table 1. The two groups were comparable for all the baseline parameters (age, ALT, HBV DNA, grade, stage, β 2m) but they differed significantly in the pattern of β 2m curve during treatment. In particular, all patients who did not show VB in the follow-up period exhibited β 2m elevation in month three of treatment, whereas only 57.1% of those who exhibited VB showed β 2m elevation ($P=0.03$). Moreover, the duration (in months) of serum β 2m elevation was significantly higher in the responders than the nonresponders (7.3 ± 2.6 versus 3.8 ± 3.4 , $P=0.02$). The different patterns of β 2m in relation to recurrence are presented in Figure 1. It is evident that mean serum β 2m levels remained stable for about nine months in patients who exhibited VB, while there were steep increases for about six months in the responders group. Subsequently, β 2m values declined in both groups.

Mean baseline β 2m levels were slightly higher in patients with high initial HBV DNA compared with patients with low initial HBV DNA (1878 mg/dL versus 1622 mg/dL, $t=-1.58$, $P=0.13$). Patients with higher baseline serum β 2m levels had slightly higher baseline HAI ($r=0.39$, $P=0.08$) and slightly higher baseline ALT levels ($r=0.25$, $P=0.28$). Patients with high initial HBV DNA levels had a shorter duration of β 2m elevation (6.2 months for the low versus three months for the high initial HBV DNA group, $t=1.49$, $P=0.186$). Moreover, 99.3% of the initial low HBV DNA group of patients exhibited β 2m elevation at three months, while only 33% of the initial high HBV DNA group did the same ($\chi^2=8.5$, $P=0.004$).

Cox models

Survival analyses have the advantage of combining information about frequency of events and time of their occurrence. The reported hazard ratios (HR) indicate risk of having VB for each time unit of follow-up. Therefore, they reflect not only how likely it is for a patient to recur but also how quickly he or she may be expected to do so.

When the duration of β 2m elevation was used as a predictor, a lower score (decreased duration of β 2m elevation) was associated with an increased risk of VB (HR=1.25, 95% CI 1.03 to 1.52) (Table 2). It is of importance to note that, in this model, the HR reflects the increase in risk for VB for each additional unit of the predictor (eg, each month less for which serum β 2m failed to elevate). According to this result, in comparison with a patient whose serum β 2m levels continued to increase for 12 months, a patient whose β 2m continued to increase for only nine months had 1.95 times higher risk of

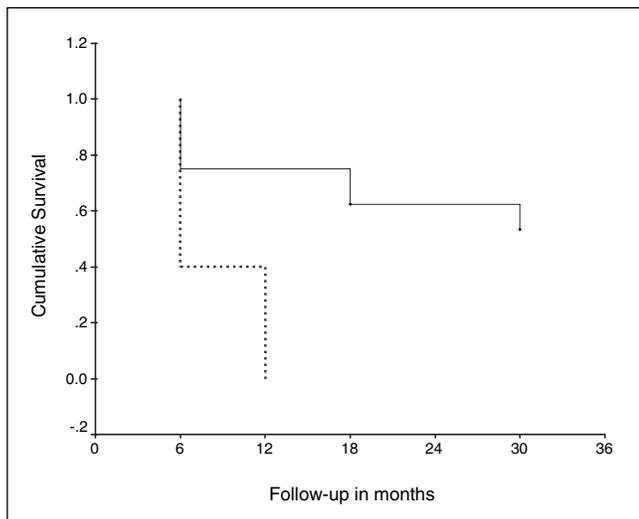


Figure 2 Kaplan-Meier survival curves comparing cumulative survival (percentage of patients remaining free of virological breakthrough) in chronic hepatitis B patients with elevated serum beta2-microglobulin (β 2m) levels at three months (solid line) versus decreased β 2m levels at three months (dashed line). All patients with decreased serum β 2m levels at three months experienced virological breakthrough in month 12 of lamivudine treatment

developing VB, a patient whose β 2m continued to increase for only six months had a 3.8 times higher chance of developing VB, and a patient whose β 2m continued to increase for only three months had a 7.4 times higher risk for developing VB.

When serum β 2m levels were used in their dichotomous form (reflecting elevated versus declined status at three-month intervals), there was again an inverse association between β 2m levels and VB (HR=4.6, 95% CI 1.22 to 17.36) (Table 2). In other words, in comparison with patients whose serum β 2m levels were increased at three months, subjects whose β 2m levels were decreased at three months had a 4.6 times higher risk of experiencing VB. Cumulative survival curves for VB for patients with three-month β 2m elevation or decline are presented in Figure 2.

When age, baseline ALT levels, initial serum HBV DNA levels and grade of liver disease (HAI) were simultaneously included in the same Cox model (along with β 2m status at three months of follow-up), decreased β 2m status was still associated with an increased risk of VB (HR=12.23, 95% CI 1.28 to 116.80) (Table 3).

GEE models

There was a significant time effect (β =67.7, P <0.0001), indicating an overall significant increase of β 2m over time (for the first six months). The group effect was nonsignificant (β =182.5, P =0.21), indicating similar β 2m values at baseline across the two groups. The interaction (time \times group) effect (β =-61.7) indicated a higher slope of increase of β 2m over the first six months for patients who did not exhibit VB (compared with those who exhibited VB); the effect was borderline nonsignificant (P =0.08). In a subsequent GEE model, the following predictors were simultaneously included: time, group, time \times group, age, baseline β 2m values, baseline HBV DNA, baseline ALT levels and grade (HAI). The results were unchanged.

TABLE 3
Cox proportional hazard model predicting virological breakthrough by beta2-microglobulin (β 2m) at three months as a dichotomous variable

	Variable	HR	95% CI
Age (years)	Continuous	0.98	0.92–1.05
ALT (IU/L)	Continuous	0.99	0.98–1.00
Grade (0 to 18)	Continuous	1.26	0.98–1.63
HBV DNA (copies/mL)	High versus low	1.94	0.26–14.26
β 2m (mg/dL)	Decline versus elevation	12.23	1.28–116.80*

The model simultaneously includes age, baseline values for alanine aminotransferase (ALT), grade of liver disease and initial serum hepatitis B virus (HBV)-DNA as covariates. *Indicates that the hazard ratio (HR) does not include 1

Therefore, the associations between changes in β 2m levels and VB were similar to the Cox analyses. The borderline nonsignificant effect of the interaction term in the GEE analyses may be due to lower power compared with survival analyses, which take into account not only failure status (VB versus no VB), but also time to failure (duration until either VB or last evaluation without VB).

DISCUSSION

Lamivudine is an oral nucleoside analogue with potent antiviral activity against HBV and exhibits high response rates, usually at the end of the first year of treatment (7,8). However, response rates tend to decrease with the prolongation of lamivudine monotherapy (23) and breakthroughs due to YMDD mutant accumulation, culminating in the development of BBs in most HbeAg-negative CHB patients (11). Early prognosis of VB during lamivudine treatment is very useful in order to predict the clinical outcome and to modify the medication. Initial serum HBV DNA levels seem to be a good predictor of virological response (7-9). Similarly, quantitative HBV DNA testing during lamivudine treatment provides prognostic information: there is a low likelihood of response in patients who remain positive at month three of treatment (24). However, HBV DNA testing is expensive and requires highly qualified laboratories. In contrast, the calculation of serum β 2m levels using microparticle enzyme immunoassay technology is obviously a cheaper and easier method to use.

Chronic HBV infection is characterized by T cell hyporesponsiveness, and stimulation of HBV-specific T cell responses in patients with CHB is believed to represent a rational strategy to treat persistent infection (25). Activation of T cell immune responses leads to elimination of intracellular virus by cytolytic destruction of infected hepatocytes and suppression of viral gene expression, which is caused by cytokines such as IFN-gamma and tumour necrosis factor-alpha, which are secreted by activated T cells (Th1 subset) at the site of infection (26-28). This is involved in the T-cell response, providing help to cytotoxic T lymphocytes, and mediating HBV elimination by cytolytic and noncytolytic mechanisms (29). Serum β 2m levels are significantly elevated in patients with chronic viral hepatitis compared with healthy controls (13,15) and increase significantly on IFN-alpha treatment (14-16), representing a Th1-dominant immune response. To our knowledge, serum β 2m levels in CHB patients under long term lamivudine

monotherapy, which represents a classical antiviral treatment, has not been investigated.

In our study, we found a significant correlation between the decline of serum $\beta 2m$ levels (especially during the first three months of treatment) and the risk for VB in HBeAg-negative CHB patients under long term lamivudine monotherapy. Moreover, we found that the longer duration of $\beta 2m$ elevation is associated with a reduced risk of VB. In particular, we found that for each additional month where serum $\beta 2m$ continued to elevate, the risk for VB reduced by approximately 80% (or for each month less of $\beta 2m$ elevation, the risk increased by 1.25 times). The serum $\beta 2m$ elevation in the responders group possibly represents an elevated endogenous immune response in this group of patients. The majority (99.3%) had low initial serum HBV DNA levels, a finding that supports this hypothesis. These patients show an 'IFN-like' $\beta 2m$ curve following the first months of lamivudine treatment.

Serum $\beta 2m$ levels have been anticipated to represent the turnover of HLA antigens and are associated with lymphocyte proliferation and activation (30). Boni et al (31) suggest that lamivudine treatment can overcome cytotoxic T cell hyporesponsiveness in treated CHB patients by reducing the levels of viremia. High viral load reduces the number and the potential activity of circulating HBV-specific T lymphocytes compared with low viral load in CHB patients (32), suggesting the dominant role of the control of viral replication. High viral load and intrahepatic viral replication result in decreased hepatocellular presentation of class I HLA molecules and act as a negative modulator of natural killer cell activity (33). Lamivudine treatment reduced the intrahepatic viral load (34), possibly resulting in the modulation of T cellular immune response. On the other hand, Marinou et al (35) suggest that the profound inhibition of HBV replication by nucleoside analogues does not restore the impaired virus-specific T cell responses.

The association between the initial low HBV DNA levels and serum $\beta 2m$ elevation observed in our study implies an activation of host T cellular immune responses before the beginning of lamivudine treatment in the responders group, which leads to the control of viremia in the long term. The comparable baseline ALT levels and histological findings between the

two groups and the ALT normalization during the first months of treatment in both groups suggest an additive T cellular-mediated, noncytolytic inhibition of virus replication in the responders group, as has been shown by other studies (36), a possibility that needs further investigation. In a recent study, Santantonio et al (37) found that combination treatment (lamivudine plus IFN) in HBeAg-negative CHB patients was as beneficial as lamivudine monotherapy, but the combination regimen appeared to delay or prevent the emergence of YMDD variants. The significant elevation of serum $\beta 2m$ levels following IFN treatment and the results of our study suggest that close monitoring of serum $\beta 2m$ levels in HBeAg-negative CHB patients under lamivudine monotherapy could result in early recognition of the group of relapsers and the possible addition of immunomodulatory and/or other antiviral drugs.

Initial serum HBV DNA levels differed between the two groups (responders versus nonresponders) as expected from the literature (7-9), but not significantly (Table 1). Also, initial serum HBV DNA status was not a significant predictor in the adjusted Cox model (Table 3). This may be because of decreased power of the current study due to the small number of patients. However, serum $\beta 2m$ status remained significant in the prognosis of VB, in the adjusted Cox model (which was adjusted for baseline HBV DNA status). These results suggest that there is a predictive value of serum $\beta 2m$ levels compared with baseline ones for the emergence of HBV DNA polymerase mutants in CHB patients under lamivudine treatment.

In conclusion, serum $\beta 2m$ levels during the first months of treatment are a good predictor of VB in HBeAg-negative CHB patients under long term lamivudine monotherapy. Decreased serum $\beta 2m$ levels at month three of lamivudine treatment are associated with 4.6-times higher risk of virological relapse in comparison with patients whose serum $\beta 2m$ levels increased at the same time. The shorter duration of serum $\beta 2m$ elevation in these patients is associated with an increased risk of VB in the long term, suggesting frequent follow-up visits and possibly reinforcement of treatment strategies.

ACKNOWLEDGEMENTS: The study was partially supported by Glaxo-Smith Kline (GRLM01 protocol).

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