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Changes in serological biomarkers of liver function and connective tissue turnover in chronic hepatitis B during lamivudine therapy

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Abstract
Assessment of hepatic damage associated with chronic hepatitis B (CHB) currently relies on measurement of serum transaminases and assessment of hepatic histology. It was determined serum hepatic function tests and the liver fibrosis biomarkers type IV collagen (CIV), amino-terminal propeptide of type I procollagen (PINP), amino-terminal propeptide of type III procollagen (PIIINP) and carboxy-terminal telopeptide of type I collagen (ICTP) were of value in monitoring the effect of lamivudine therapy for CHB. Thirty-nine patients received orally 100 mg lamivudine daily for 48 weeks. Blood samples were obtained at baseline, 24 and 48 weeks. At the end of the treatment period, the patients were then divided into four groups according to the pattern of HBs and HBe antigens. At baseline, alanine aminotransferase, aspartate aminotransferase, PIIINP and the PINP/ICTP ratio and at 24 weeks alanine aminotransferase, aspartate aminotransferase and the PINP/ICTP ratio had lower values in the complete response compared with complete failure groups. Using receiver-operated curve analysis, only the PINP/ICTP ratio at baseline (area under the curve 0.806) and ALT and the PINP/ICTP ratio at 24 weeks (areas under the curve 0.803 and 0.776, respectively) had significant diagnostic ability in detecting responders. In conclusion, the PINP/ITCP ratio is sensitive and specific in detecting responders to treatment.

Keywords: *Chronic hepatitis, fibrosis, lamivudine, receiver operating characteristic (ROC) analysis*

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Introduction

Lamivudine has been shown in patients with chronic hepatitis B (CHB) to produce a satisfactory response with regard to reduction in viral replication rates, normalization of transaminases, improvement of hepatic histology and increased clearance of HBeAg (Lai et al. 1998, Suzuki et al. 1999, Tassopoulos et al. 1999, Hu 2005). Alanine (ALT) and aspartate (AST) transaminases are the most widely used serum markers of hepatocellular damage. However, they have a periportal distribution and in cases of centrilobular necrosis a delay in peak serum concentrations has been observed

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(Nelson et al. 1995). This finding has led to the search of other non-invasive indices of hepatic damage or fibrogenesis.

Liver biopsy is employed in HBV infection to confirm hepatic inflammation and fibrosis, to exclude other causes of hepatic disease and to assess the effect of treatment on the liver architecture (Shindo et al. 2004). However, it does not provide information on balance between fibrolysis and fibrogenesis. This problem has been addressed by the quantitation in serum of products of connective tissue turnover of which the most extensively investigated to date are the carboxy-terminal propeptide of type I procollagen (PICP), amino-terminal propeptide of type III procollagen (PIIINP) and type IV (basement membrane) procollagen (CIV) (Schuppan et al. 1986, Melkko et al. 1990, Ristelli et al. 1990) in relation to collagen synthesis and carboxy-terminal telopeptide of type I collagen (ICTP) in relation to degradation (Elomaa et al. 1992). In particular, intact collagen IV has shown great promise as a biomarker for predicting and monitoring therapy response in hepatitis C, alcoholic liver injury and other chronic liver diseases (Ueno et al. 1992, Tsutsumi et al. 1993, 1996, Yabu et al. 1994).

Little information is available regarding the effect of CHB infection and subsequent treatment with lamivudine on serum products of extracellular matrix (ECM) turnover. This study therefore sought to determine serum levels of a range of biomarkers of liver damage and fibrosis before, during and at the end of lamivudine therapy to determine if any had promise as predictors of response.

Materials and methods

Patients

Ethical approval for the study was obtained from the Bioethical Committee of the Medical University of Bialystok. Informed consent was obtained from 39 patients (12 females and 27 males; mean age 37.3 ± 5.9 years). All patients had proven CHB as determined by the presence of HBs and HBe antigens and stable elevations in ALT for at least 6 months. Patients with a history of alcohol abuse and who were positive for anti-HCV or anti-HIV antibodies were excluded. Disease activity was confirmed by viral replication demonstrated through the presence of HBV DNA isolated and detected qualitatively using PCR techniques with reagents from Sigma (St. Louis, MO, USA). Liver biopsy was performed for diagnostic management by means of the Hepafix System (Braun, Melsungen, Germany). Paraffin-embedded biopsy specimens were stained and evaluated using scoring system according to Scheuer (1991).

Lamivudine therapy and response definition

Patients received 100 mg lamivudine (Zeffix™, Glaxo-Smith-Kline) orally daily for 48 weeks. At the end of the treatment period, the patients treated with lamivudine were divided into four groups according to the pattern of HBs and HBe antigens and HBV DNA as follows: group 1 (complete response, $n = 5$) HBsAg (−), HBeAg (−), HBV DNA (−); group 2 ($n = 5$) HBsAg (+), HBeAg (−), HBV DNA (−); group 3 ($n = 6$) HBsAg (+), HBeAg (−), HBV DNA (+); and group 4 (no response, $n = 23$) HBsAg (+), HBeAg (+), HBV DNA (+).

Biomarkers of hepatocellular injury

The liver function tests alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), gamma-glutamyltranspeptidase (GGT), bilirubin and albumin serum levels were measured using Cobas Mira (Roche) and prothrombin index (PI) determined using Kselmed K-3002 (Poland).

Fibrosis biomarkers

Serum concentrations of PINP, PIIINP and ICTP were measured using radio-immunoassay techniques (Orion Diagnostica, Espoo, Finland). Serum concentrations of CIV were measured using a commercially available EIA kit (manufactured by Daiichi Fine Chemical Co, Japan; supplied by Biotrin International, Dublin, Ireland). The intra- and inter-assay coefficients of variation were for all assays <5.6 and $<7.1\%$.

Statistics

The Statview statistics package (SAS Institute, Inc., Cary, NC, USA) was used. Values are expressed as the mean $\pm$ SEM. The significance of the differences for each parameter was evaluated by analysis of variance (ANOVA) and by non-parametric Wilcoxon Rank test. Spearman Rank correlation coefficients were used to estimate significance of correlations. Receiver operating characteristic (ROC) analysis was used to determine the sensitivity and specificity of the assays in relation to response to therapy. ROC curves were plotted using the SPSS statistical program (SPSS, Inc., Chicago, IL, USA). Comparison of the area under the curve (AUROC) was performed using a two-tailed p test, which compares the AUROC to the diagonal line of no information (AUROC $= 0.5$). Values of $p < 0.05$ were considered statistically significant.

Results

Lamivudine therapy produced a complete response, i.e. inhibition of viral replication and clearance of HbsAg and HBeAg in 5/39 (13%) (group 1); partial response, i.e. inhibition of viral replication and clearance of HBeAg in 5/39 (13%) (group 2); clearance of HBeAg with sustained viral replication in 6/39 (15%) (group 3); and no response in 23/39 (59%) (group 4). Serum bilirubin, AST, ALT, ALP, GGT and PI values obtained at the various time points subdivided according to the degree of response to treatment are shown in Table I.

No significant difference ($p > 0.05$) was found between the serum concentrations of bilirubin, ALP and GGT activities and PI values at the three time points (0, 24 and 48 weeks) in relation to response group. Serum ALT activities at baseline showed no significant difference between groups. However, at 24 weeks, significantly higher serum ALT activities were found in group 4 subjects compared with groups 1 and 2 (Table I). As shown in Table I, at the end of the treatment period ALT activities were significantly higher in groups 3 and 4 than in complete or partial responders (groups 1 and 2). Serum AST demonstrated significant differences between the non-responders (group 4) and both response groups 1 and 2 only at week 48.

Table I. Liver function test results (mean ± SEM) obtained at baseline, 24 and 48 weeks of lamivudine treatment in patients divided according to the degree of response.

Group	Time (weeks)	Bilirubin (mg dl^{-1})	AST (U l^{-1})	ALT (U l^{-1})	ALP (U l^{-1})	GGT (U l^{-1})	PI (%)
1 ($n=5$)	0	0.90 ± 0.25	80 ± 14	87 ± 22	97 ± 14	108 ± 53	90.3 ± 5.3
	24	0.90 ± 0.22	30 ± 6	25 ± 4	82 ± 8	68 ± 33	90.6 ± 4.9
	48	0.85 ± 0.20	27 ± 5	23 ± 4	81 ± 8	59 ± 28	89.5 ± 3.4
2 ($n=5$)	0	1.10 ± 0.25	74 ± 26	119 ± 58	115 ± 25	48 ± 8	83.4 ± 4.3
	24	1.11 ± 0.14	46 ± 14	33 ± 11	88 ± 20	33 ± 7	88.5 ± 1.9
	48	1.00 ± 0.16	31 ± 4	33 ± 6	99 ± 16	30 ± 6	85.8 ± 2.7
3 ($n=6$)	0	1.39 ± 0.56	117 ± 43	127 ± 40	96 ± 11	77 ± 24	85.6 ± 7.7
	24	0.91 ± 0.21	39 ± 8	39 ± 7	88 ± 8	45 ± 8	95.4 ± 2.7
	48	1.13 ± 0.13	64 ± 20	92 ± 30*	82 ± 3	50 ± 11	93.3 ± 4.6
4 ($n=23$)	0	0.97 ± 0.09	66 ± 6	98 ± 12	101 ± 9	41 ± 10	88.9 ± 2.9
	24	1.06 ± 0.12	65 ± 21	79 ± 18*	97 ± 10	32 ± 6	89.1 ± 2.8
	48	1.00 ± 0.19	59 ± 4*	69 ± 4*	96 ± 11	44 ± 15	88.2 ± 3.1

*Significant difference against group 1 complete and group 2 partial responders ($p < 0.05$).

Liver biopsy data were available from 20 patients before the start of treatment. Correlations between inflammation grading, fibrosis staging and serum markers of liver function or damage and markers of ECM turnover are shown in Tables II and III. No correlation was found between AST, ALT and PI against hepatic histology scored according to Scheuer (1991). Significant correlations were found, however, between bilirubin, ALP, and GGT and lobular inflammation and fibrosis staging scores. With regard to hepatic histology, only serum PINP correlated with the degree of portal/periportal inflammation. No significant differences in portal/periportal inflammation baseline scores were found between subjects with different responses (data not shown). Significant differences with regard to lobular inflammation score and fibrosis staging score at baseline were, however, found between groups 2 and 4 (data not shown).

The serum values of ECM turnover PIIINP, PINP, ICTP and CIV are shown in Table IV. No significant differences were found in the serum concentrations of PIIINP, PINP, ICTP and CIV at the three time points within each response group or between response groups. Ratios of these analytes were determined for the various time points subdivided according to treatment response. Significant differences at baseline were found for the PINP/ICTP ratios (mean ± SEM) between groups 1 (6.7 ± 1.5) and 4 (16.9 ± 1.8) (Table IV).

The serum parameters ALT, AST, PIIINP and the PINP/ITCP ratio were found in some patients to be higher in the poor response groups at the various time points. Serum ALT levels above 175 U l^{-1}, AST levels above 136 U l^{-1}, PIIINP levels above

Table II. Correlation between serum markers of liver damage and histological score demonstrated through r.

Histological score	Bilirubin	AST	ALT	ALP	GGT	PI
Portal/periportal inflammation	0.255	0.164	0.080	0.151	0.443	0.042
Lobular inflammation	0.443*	0.150	0.103	0.444*	0.693*	0.091
Fibrosis	0.458*	0.308	0.172	0.495*	0.555*	0.243

*Statistical significance ($p < 0.05$).

Table III. Correlation between serum markers of ECM turnover and histological score demonstrated through r.

Histological score	PINP	PIIINP	ICTP	CIV
Portal/periportal inflammation	−0.566*	−0.306	−0.313	−0.311
Lobular inflammation	−0.362	−0.204	−0.108	0.281
Fibrosis	−0.404	0.074	−0.020	−0.047

*Statistical significance ($p < 0.05$).

20.0 μg l^{-1} and a PINP/ITCP ratio above 11.5 at baseline were associated with therapy failure. Using these baseline 'cut-off' values, the response rates in groups 2–4 were 18, 12 and 66%, respectively. At week 24, ALT levels above 33 U l^{-1}, AST levels above 50 U l^{-1} and a PINP/ITCP ratio above 14.8 were associated with therapy failure. Using these values, the response rates in groups 2–4 were 68, 33 and 55% of patients, respectively. Using ROC analysis, further examination of these results (Tables V and VI) demonstrated that only the PINP/ITCP ratio and ALT and the PINP/ITCP ratio were statistically significant at baseline and at week 24 in detecting treatment failure (Figures 1–3).

Discussion

Hepatitis B virus infection in adulthood leads to 10% of individuals failing to clear the virus and subsequently becoming chronic carriers (McMahon et al. 1985). The development of CHB infection may lead to extensive liver damage culminating in cirrhosis and hepatocellular carcinoma in 20–30% of such cases (Beasley et al. 1981, Liaw et al. 1988). Recently developed novel therapeutic regimens based on inhibiting viral replication via the use of nucleoside analogues have been shown to induce improvements in hepatic histology, decreases in viral replication rates, normalization

Table IV. Extracellular marker results (mean±SEM) obtained at baseline, 24 and 48 weeks subdivided according to the degree of response.

		Analyte				
Group	Time (weeks)	PIIINP (μg l^{-1})	PINP (μg l^{-1})	ICTP (μg l^{-1})	CIV (μg l^{-1})	PINP/ICTP ratio (μg l^{-1})
1 ($n=5$)	0	11.9±2.8	71±28	15.8±9.6	167±40	6.7±1.5*
	24	12.1±3.0	82±41	12.4±7.8	140±36	8.7±2.0
	48	10.0±1.5	43±20	13.4±8.9	136±33	5.7±1.6
2 ($n=5$)	0	11.3±2.7	63±20	5.7±0.4	151±40	12.3±4.7
	24	11.3±2.5	64±16	4.9±0.5	160±29	13.9±3.8
	48	7.9±1.7	48±12	4.6±0.5	124±21	12.5±2.9
3 ($n=6$)	0	11.3±2.4	63±11	8.3±2.9	176±29	12.7±4.8
	24	9.8±1.0	51±8	5.9±1.8	155±28	11.4±2.3
	48	9.7±1.2	56±12	6.2±2.2	143±25	12.9±4.0
4 ($n=23$)	0	13.2±0.9	88±11	9.4±3.7	234±105	16.9±1.8*
	24	12.3±1.1	89±14	10.0±3.7	220±103	16.7±2.0
	48	11.6±0.9	76±12	9.5±3.1	306±135	15.8±2.4

*Significance between groups ($p < 0.05$).

Table V. Area under the curve (AUROC) statistics for ALT, AST, PIIINP and PINP/ITCP ratio at baseline.

Analyte	AUROC	Sensitivity (%)	Specificity (%)	Asymptotic significance	Criterion (cut-off for detecting treatment failure)
ALT ($U\,l^{-1}$)	0.542	53	80	0.788	70
AST ($U\,l^{-1}$)	0.280	56	100	0.156	59
PIIINP ($\mu g\,ml^{-1}$)	0.538	94	40	0.790	6.9
PINP/ITCP (ratio)	0.806	66	100	0.029*	11.52

*Significance ($p < 0.05$).

of liver function tests and increased sero-conversion of HBeAg (Malaguarnera et al. 2001, Regev & Schiff 2001). There is, however, a lack of information regarding the effect of such therapy on serum markers of ECM turnover. This study, therefore, sought to provide information on the effect of lamivudine treatment for CHB infection on such biomarkers of hepatic dysfunction and damage.

In this study, response rates following 48 weeks of treatment for CHB with lamivudine are comparable with previously published studies citing inhibition of viral replication and HBeAg sero-conversion rates of 16–22% (Zoulim 2002). Pretreatment liver function test serum values, particularly ALT, have previously been shown to indicate the likely outcome of 1 year's therapy with lamivudine. Studies have shown that the more elevated the pretreatment serum ALT, the better the HBeAg sero-conversion rate (Liaw 2002, Perrillo et al. 2002). This study, however, did not demonstrate significant differences between baseline ALT activities that could be predictive for treatment success.

Baseline serum ALT activities found in this study were not significantly different between the response groups. The results suggest that the baseline serum ALT level per se may not have sufficient discriminative power to enable prediction of response in all patients. One possible explanation is interindividual variation in immune response. Changes in serum ALT during treatment was, however, evident at 24 and 48 weeks with the non-responder group serum ALT remaining significantly elevated compared with other groups. The evaluation of serum ALT activity at 24 weeks was, however, significant with 68% of patients failing to respond to treatment having a serum ALT $>33\ U\,l^{-1}$. These results suggest that although pretreatment assessment of ALT may not be sufficient to identify a significant proportion of non-responders, assessment mid-treatment may allow for the discontinuation of treatment in patients who are not going to respond to further treatment.

Table VI. Area under the curve (AUROC) statistics for ALT, AST, PIIINP and PINP/ITCP ratio at week 24.

Analyte	AUROC	Sensitivity (%)	Specificity (%)	Asymptotic significance	Criterion (cut-off for detecting treatment failure)
ALT ($U\,l^{-1}$)	0.803	68	100	0.031*	33
AST ($U\,l^{-1}$)	0.709	59	80	0.136	34
PIIINP ($\mu g\,ml^{-1}$)	0.485	79	60	0.914	13.9
PINP/ITCP (ratio)	0.776	64	80	0.049*	12

*Significance ($p < 0.05$).

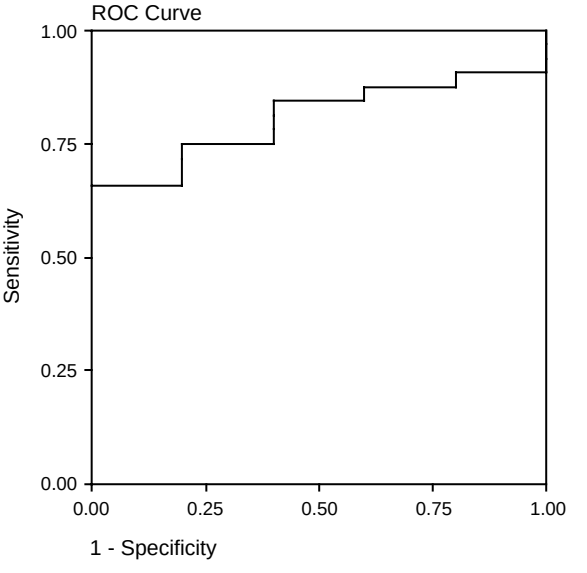

Figure 1. ROC analysis for the baseline PINP/ITCP ratio.

Measurement of other indices of hepatic integrity such as bilirubin, ALP, GGT and PI offered no additional information compared with ALT as to the predicted outcome. Serum AST in this study exhibited a similar result profile to serum ALT, being indicative of 12% of subject treatment failures at baseline.

Improvements in hepatic histology have previously been shown to occur with 1 year of lamivudine therapy (Lai et al. 1998). There are some data available regarding the

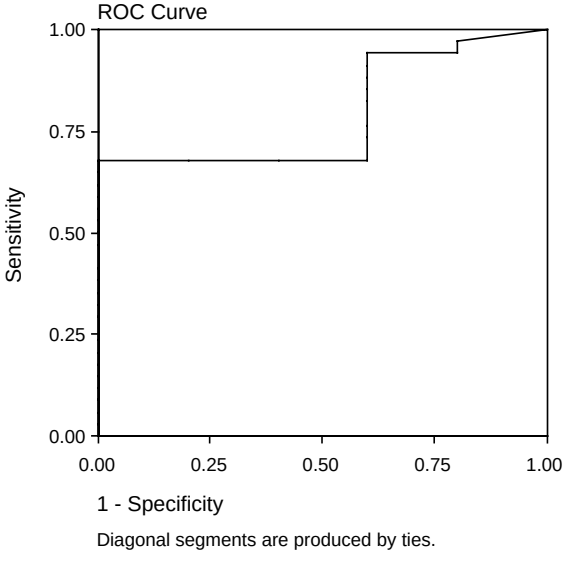

Figure 2. ROC analysis for week 24 serum ALT.

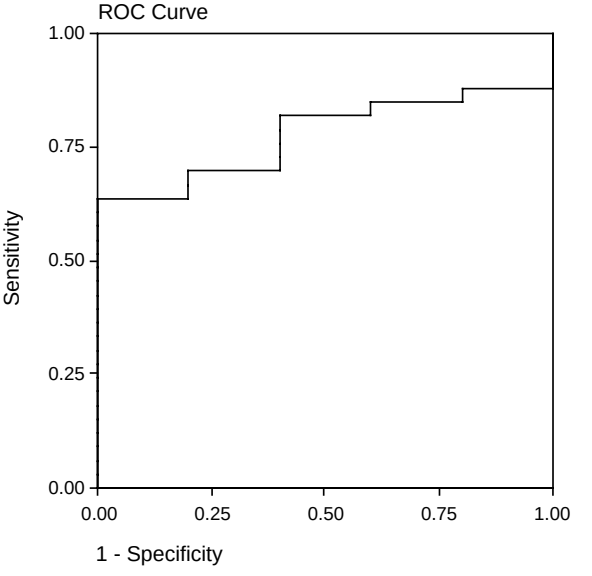

Figure 3. ROC analysis for week 24 PINP/ITCP ratio.

use of measurement of these serum collagen cleavage products such as PICP, PINP, ICTP, PIIINP and CIV in this subject group (Flisiak et al. 2002). Little information is available, however, regarding the estimation of such analytes as indices of response to treatment. This study shows that for all ECM parameters investigated, there were no significant changes within response groups during therapy. In relation to PIIINP, however, a serum baseline concentration of >20 μg l^{-1} may indicate treatment failure.

It is possible that such estimations may be used as a non-invasive index of non-response to treatment, elevations in PIIINP indicating failure to respond. Such estimations have already proved to be of value in the assessment of hepatic fibrogenesis in patients with psoriasis receiving methotrexate therapy (Zachariae et al. 2001).

The use of ECM collagen cleavage product ratios has previously been shown to be a better indicator of progression from hepatic inflammation to cirrhosis, with the PICP/PIIINP ratio performing better than either PICP or PIIINP alone (Lin et al. 1995). The present study found that the serum PINP/ICTP ratio at baseline and at week 24 was significantly different between the full response and partial–complete failure groups, identifying 66 and 55% of patients. As such, the estimation of this ratio before and during treatment may prove to be a valuable non-invasive test of response to therapy.

Conclusion

The results demonstrate that a low baseline PINP/ICTP ratio may help to indicate those CHB patients most likely to respond to therapy.

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