

Serum cytokine levels in chronic hepatitis B patients receiving peginterferon alpha-2a therapy

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BACKGROUND: The relationship between cytokines and responses to peginterferon α -2a treatment in chronic hepatitis B patients has not yet been fully elucidated. We analyzed the serum levels of interleukin (IL)-1 α , IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, vascular endothelial growth factor, interferon- γ , tumor necrosis factor- α , monocyte chemotactic protein-1 (MCP1) and epidermal growth factor during the treatment with peginterferon α -2a.

METHODS: Ninety-three serum samples from 20 chronic hepatitis B patients were collected before, during and after 48 weeks of peginterferon therapy and were assayed for 12 cytokines. The patients were categorized as either virologic responders (VRs) or non-responders (NRs) according to their HBV DNA levels taken at 6th month during treatment. The Evidence Investigator (Randox, Antrim, UK), a protein chip analyzer, was used to quantify cytokines.

RESULTS: Among the 12 cytokines, the levels of MCP1 were increased and the levels of IL-4 were decreased during the treatment in VRs. However these cytokines were not significantly changed in NRs in the treatment phases. Area under the receiver operating characteristic curve (AUROC) value of HBV DNA measured before the treatment was 0.81 in predicting VRs, and that of the baseline MCP1 was 0.76. IL-6 levels at 3rd and 6th months during the treatment also showed AUROC values 0.85 and 0.78 respectively in predicting sustained VRs.

CONCLUSION: Serum cytokine levels reflect the pathological differences of individual treatment phases and could also be useful in monitoring responses to peginterferon treatment in chronic hepatitis B patients.

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KEY WORDS: chronic hepatitis B; cytokine; hepatitis B virus; peginterferon; protein chip array

Introduction

Hepatitis B virus (HBV) is a common etiologic agent of chronic hepatitis. HBV is thought not to be cytolytic, but rather HBV infection induces a host immunoreaction against its viral products. In this process, cytokines are produced by various types of immune cells including lymphocytes, macrophages, natural killer cells and dendritic cells, and these cells play crucial roles in the course of chronic hepatitis B (CHB).^[1,2]

Inflammation in HBV-infected hepatocytes is caused by cytokines, such as interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α),^[3] and these molecules also trigger elimination of the virus. Cytokines such as transforming growth factor- β (TGF- β) promote fibrosis of the liver,^[4,5] and hepatocarcinogenesis is also associated with abnormal immune responses initiated by various types of cytokines including TGF- β and interleukin (IL)-10.^[6-8] In a recent study,^[9] IL-6 limited adaptive immune responses and protected HBV-infected hepatocytes from death. Another study^[10] reported that increased IL-6 expression was associated with liver dysfunction after HBV infection.

Studies^[11-13] also reported the relationship between the expression pattern of cytokines and the progression of CHB. TNF- α and IL-10 levels were found to be higher in the progress of the CHB phases.^[11] TNF- α , IL-4, and TGF- β levels were also higher in CHB patients than in controls, whereas IFN- γ level was lower in patients than in controls in another study.^[12] Hepatic flares in CHB patients were also associated with high serum levels of IFN- γ inducible chemokines such as chemokine (C-X-C

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motif) ligand (CXCL)-9 and CXCL-10.^[13] However, roles of various cytokines in the progression of CHB are not completely understood, and the association of cytokines with treatment responses to drugs in CHB has rarely been reported.^[14]

In the present study we analyzed serum levels of cytokines in CHB patients treated with peginterferon α -2a in their treatment phases and responses to the therapy. Epidermal growth factor (EGF), IL-1 α , IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, INF- γ , monocyte chemotactic protein-1 (MCP1), TNF- α , and vascular endothelial growth factor (VEGF) were simultaneously assayed in specimens obtained from the CHB patients. Predictive values of the cytokines for the responses to peginterferon α -2a were also assessed.

Methods

Study subjects

Altogether 93 serum samples were prospectively collected with written informed consents from 20 CHB patients treated with peginterferon α -2a between January 2009 and December 2011. CHB diagnosis was based on positive serum HBsAg for more than 6 months and positive HBeAg with HBV DNA over 20 000 IU/mL or negative HBeAg with HBV DNA over 2000 IU/mL along with persistent elevation of alanine aminotransferase (ALT).^[15] The patients showed serum HBV DNA levels greater than 20 000 IU/mL before initiation of the therapy, and were treated with 180 μ g of peginterferon α -2a per week for 48 weeks. From each patient, one or more samples were drawn at the following time points: before peginterferon therapy, during peginterferon therapy (at 3rd and 6th months after initiation of the therapy), and at 6th and 12th months after the therapy.

The patients were classified as virologic responders (VRs) when serum HBV DNA levels at 6th month after initiation of the therapy were decreased to less than 2000 IU/mL,^[15-18] and the other patients were classified as virologic non-responders (NRs). The VRs were subdivided into the sustained virologic responders (SVRs) when virologic breakthrough (increase in serum HBV DNA by $>1 \log_{10}$ [10-fold] above nadir after achieving virologic response) was not observed until 12 months off-treatment. This study was approved by the Institutional Review Board of Severance Hospital and conducted according to the ethical principles of the *Declaration of Helsinki*.

Measurement of cytokine levels

All serum samples were separated within 2 hours

after taking venous blood samples and stored at -70°C until use. A protein chip analyzer, Evidence Investigator and Cytokine Array I reagents (Randox, Antrim, UK) were used to measure 12 cytokines including IL-1 α , IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, INF- γ , TNF- α , MCP1 and EGF. The assay was carried out according to the manufacturer's instructions. Frozen serum specimens and the reagent carrier comprising 9 protein chips were left at room temperature for about 15 minutes when unpacked. Monoclonal antibodies for the 12 cytokines were attached on each protein chip surface. Assay diluent of 200 μ L was placed into each well of the chips, and then 100 μ L of serum was added. The chips were placed in a thermo shaker at 37°C , 370 rpm for 1 hour. After washing the chips with washing fluid for six times, 300 μ L of conjugate fluid containing enzyme-labeled antibody was added to each well. After another one-hour reaction in the same condition, the chips were washed again for six times. Substrate reagent containing luminol and peroxidase was added to each well of the chips and then after 2 minutes the carrier was inserted into the Evidence Investigator analyzer. A charge-coupled device camera detected the chip signals and saved images in the computer. The computer program then calculated cytokine levels from the chip signals using calibration curves for the respective cytokines.

Other assays

Serum HBV DNA concentrations were determined by Cobas AmpliPrep/Cobas TaqMan HBV test v2.0 (Roche Molecular Systems, Inc., Pleasanton, CA., USA). Aspartate aminotransferase (AST) and ALT levels were measured by a Hitachi 7600 DDP modular chemistry analyzer (Hitachi High-Technologies Co., Tokyo, Japan). Each assay was performed according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were made using Analyse-it Method Evaluation Edition software version 2.26 (Analyse-it Software Ltd., City West Business Park, Leeds, UK). Continuous variables between the two groups were compared using the Mann-Whitney *U* test. Multiple comparisons of continuous variables including levels of cytokines among the patient subgroups and among the treatment phases were performed using the Kruskal-Wallis test with Bonferroni correction to compensate for alpha-statistical errors. Friedman's test was also carried out to compare cytokine levels among the paired group. Categorical variables between the two groups were analyzed for the difference in proportion by Fisher's exact test. Area under the receiver operating

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characteristic curves (AUROCs) of the cytokines and HBV DNA were calculated to assess their predictive values for virologic responses. *P* values less than 0.05 were considered as statistically significant.

Results

Characteristics of the CHB patients

Characteristics of the patients are summarized in Table 1. Age and male proportions were not different between the VRs (*n*=11) and NRs (*n*=9). Serum AST and ALT levels were not different between the VRs and NRs before peginterferon therapy, but they were higher in the NRs during and after the therapy. Serum HBV DNA levels before, during and after the therapy were lower in the VRs than in the NRs. Among the VRs, four (36.4%) demonstrated HBeAg seroconversion during or after the therapy, and five (45.5%) showed recurrence of active chronic hepatitis at 6 to 12 months after the therapy, thus, six (54.5%) patients were classified into SVRs.

Serum levels of cytokines according to treatment phases and responses to the therapy

Serum levels of 12 cytokines in the phases of peginterferon therapy in the VR and NR groups are summarized in Table 2. The levels of all cytokines except for EGF, IFN- γ , IL-1 α , IL-4, IL-6, IL-8, and MCP1 were not different among the groups according to the responses to the therapy. The levels of MCP1 were higher

during the therapy than before the therapy at 3rd and 6th months in the VR group (*P*=0.0371 at 3rd month and *P*=0.0011 at 6th month) (Fig. 1), whereas IL-4 levels were lower during the therapy than before the therapy at 3rd and 6th months in the same group (*P*=0.0037 at 3rd month and *P*=0.0133 at 6th month) (Fig. 1). IL-6 levels were also higher during the therapy than before the therapy (*P*=0.0159 at 3rd month and *P*=0.0174 at 6th month). On the other hand, levels of those three cytokines were not significantly changed according to the phases of the therapy in the NR group. In addition, MCP1 levels were also higher in the VRs than in the NRs during and after the therapy (*P*<0.05), and EGF levels were decreased at 3rd month in the VR group during the therapy compared with those before the therapy (*P*=0.0080).

Correlation between the serum levels of HBV DNA and cytokines

Serum HBV DNA levels were weakly correlated with the serum levels of EGF (*r*=0.33, 95% CI, 0.13 to 0.50, *P*=0.0016), IL-4 (*r*=0.31; 95% CI, 0.11 to 0.49; *P*=0.0025), IL-10 (*r*=0.31; 95% CI, 0.11 to 0.49; *P*=0.0025), and IL-1 β (*r*=0.28; 95% CI, 0.08 to 0.46; *P*=0.0068) regardless of treatment phases and groups according to the treatment responses. Whereas, the serum levels of MCP1 (*r*=-0.49; 95% CI, -0.64 to -0.32; *P*<0.0001), IL-6 (*r*=-0.39; 95% CI, -0.55 to -0.20; *P*=0.0002), and IFN- γ (*r*=-0.26; 95% CI, -0.44 to -0.06; *P*=0.0127) were inversely correlated with HBV DNA levels.

Table 1. Characteristics of patients with chronic hepatitis B

Parameters	Virologic responders (<i>n</i> =11)	Virologic non-responders (<i>n</i> =9)	<i>P</i> value
Age (yr)	32.0 (26.3-36.0)	32.0 (26.0-35.0)	0.6556
Gender (<i>n</i> of male, %)	5 (45.5)	4 (44.4)	1.0000
Recurrence after Tx (<i>n</i> , %)	5 (45.5)	-	-
HBeAg seroconversion during or after Tx (<i>n</i> , %)	4 (36.4)	0	0.1362
AST (IU/L)			
Before Tx	120.0 (75.5-205.7)	83.5 (69.5-125.2)	0.3950
During Tx*	33.0 (29.3-40.3)	59.0 (34.3-85.0)	0.0105
After Tx [#]	25.0 (20.7-37.7)	45.0 (29.0-98.3)	0.0237
ALT (IU/L)			
Before Tx	143.0 (73.0-212.8)	170.5 (117.8-233.8)	0.5448
During Tx*	30.0 (24.7-50.3)	67.0 (43.5-122.5)	0.0013
After Tx [#]	27.0 (19.7-48.0)	69.0 (29.0-148.0)	0.0294
Serum HBV DNA (IU/mL)			
Before Tx	2.15 $\times 10^7$ (7.72 $\times 10^5$ -6.94 $\times 10^7$)	>1.70 $\times 10^8$ (7.33 $\times 10^7$ ->1.70 $\times 10^8$)	0.0259
During Tx*	1.74 $\times 10^2$ (1.00 $\times 10^1$ -1.22 $\times 10^3$)	9.01 $\times 10^6$ (1.13 $\times 10^6$ -4.59 $\times 10^7$)	<0.0001
After Tx [#]	1.94 $\times 10^3$ (2.81 $\times 10^2$ -3.06 $\times 10^4$)	2.75 $\times 10^7$ (4.64 $\times 10^4$ -7.60 $\times 10^7$)	0.0091

Data are shown as median (1st to 3rd quartiles). *: Samples were obtained at 3rd and 6th months from peginterferon α -2a therapy initiation; #: Specimens were collected at 6 to 12 months after 48 weeks of peginterferon α -2a therapy had finished. AST: aspartate aminotransferase; ALT: alanine aminotransferase; Tx: peginterferon α -2a therapy for 48 weeks.

Table 2. Serum levels of cytokines according to responses and treatment phases in patients with CHB treated with peginterferon α -2a

Cytokines (pg/mL)	Virologic responders (n=11)										Virologic non-responders (n=9)										Overall				
	Before Tx (n=11)		3 mon during Tx (n=11)		6 mon during Tx (n=11)		6 mon after Tx (n=11)		12 mon after Tx (n=9)		P value*		Before Tx (n=8)		3 mon during Tx (n=9)		6 mon during Tx (n=9)		6 mon after Tx (n=9)		12 mon after Tx (n=5)		P value*		
	Tx	P value	Tx	P value	Tx	P value	Tx	P value	Tx	P value	P value	Tx	P value	Tx	P value	Tx	P value	Tx	P value	Tx	P value	Tx	P value		
EGF	114.9 (28.4-123.6)		22.7 (11.3-44.8)		48.0 (36.3-55.8)		58.0 (32.6-83.4)		118.2 (79.9-183.5)		0.0628	0.0157	66.6 (43.6-99.0)		56.9 (31.9-73.9)		37.2 (30.2-73.9)		96.0 (72.0-116.5)		44.3 (33.8-88.4)		0.0533	0.0075	0.0007
IFN-γ	0.0 (0.0-0.7)		1.3 (0.0-1.5)		1.0 (0.0-1.4)		1.0 (0.0-1.1)		2.2 (1.0-3.7)		0.3139	0.0064	0.0 (0.0-0.5)		0.9 (0.0-1.1)		1.0 (0.0-1.5)		0.0 (0.0-0.3)		0.0 (0.0-1.0)		0.5394	0.4605	0.0161
IL-1α	0.0 (0.0-0.5)		0.3 (0.0-0.4)		0.0 (0.0-0.2)		0.0 (0.0-0.2)		0.0 (0.0-0.0)		0.0155	0.0939	0.1 (0.0-0.4)		0.3 (0.0-0.3)		0.0 (0.0-0.3)		0.0 (0.0-0.3)		0.3 (0.0-0.8)		0.2495	0.8285	0.3508
IL-1β	1.0 (0.7-1.2)		0.7 (0.0-1.3)		0.9 (0.1-1.0)		0.8 (0.0-2.1)		0.9 (0.5-1.9)		0.9787	0.7477	1.2 (1.0-6.4)		1.2 (0.0-3.1)		1.5 (0.5-4.4)		1.0 (0.5-4.0)		4.9 (1.6-5.6)		0.1442	0.3950	0.1238
IL-2	3.4 (0.6-7.5)		3.5 (0.5-4.2)		0.0 (0.0-3.5)		3.8 (3.3-4.8)		3.5 (0.0-5.7)		0.2894	0.4454	0.0 (0.0-18.5)		3.3 (0.0-5.8)		3.8 (3.3-4.8)		0.0 (0.0-5.3)		5.7 (2.7-18.0)		0.2914	0.6580	0.6470
IL-4	2.8 (0.0-7.4)		0.0 (0.0-0.0)		0.0 (0.0-0.0)		0.0 (0.0-2.2)		0.0 (0.0-0.0)		0.0088	0.0033	2.1 (0.9-2.6)		2.1 (0.0-2.5)		0.0 (0.0-0.7)		2.2 (0.0-2.6)		2.2 (0.0-2.8)		0.0663	0.3080	0.0025
IL-6	0.7 (0.6-1.0)		1.5 (1.1-2.3)		1.4 (1.2-2.8)		1.0 (0.7-1.5)		1.2 (0.8-1.6)		0.1834	0.0112	0.6 (0.5-1.4)		1.0 (0.8-1.4)		0.8 (0.5-1.4)		0.8 (0.6-1.2)		2.0 (0.7-2.6)		0.6151	0.4352	0.0242
IL-8	72.2 (45.7-112.8)		20.4 (12.9-36.8)		30.4 (27.3-54.1)		43.1 (20.8-142.9)		302.5 (25.3-939.7)		0.3084	0.0640	42.0 (31.7-115.5)		29.1 (13.2-101.0)		14.1 (10.9-31.2)		26.5 (16.2-508.0)		273.2 (72.1-808.4)		0.1257	0.0527	0.0224
IL-10	1.0 (0.6-1.5)		0.8 (0.6-1.1)		0.8 (0.5-0.9)		0.7 (0.4-0.9)		0.6 (0.4-0.7)		0.2942	0.1964	1.3 (1.0-1.6)		0.9 (0.7-1.2)		1.0 (0.4-1.2)		0.6 (0.4-0.7)		1.0 (0.6-1.6)		0.4830	0.0623	0.0600
MCPI	260.4 (220.3-325.9)		362.8 (333.9-400.0)		395.0 (333.1-463.3)		347.1 (294.8-375.1)		396.7 (366.7-588.1)		0.0206	0.0010	190.5 (174.4-245.3)		263.6 (206.2-328.0)		269.3 (205.1-312.9)		181.7 (160.1-245.7)		255.4 (218.3-278.5)		0.1132	0.3369	<0.0001
TNF-α	2.3 (1.8-2.9)		3.3 (2.2-3.6)		2.5 (2.0-3.4)		1.9 (1.4-2.9)		2.3 (1.7-3.3)		0.0553	0.3243	3.4 (1.8-4.9)		3.8 (1.9-4.6)		2.2 (1.5-4.4)		2.5 (1.4-3.0)		3.4 (1.8-4.5)		0.0995	0.3957	0.4699
VEGF	72.3 (46.6-162.1)		51.6 (33.5-140.5)		88.2 (30.4-117.7)		51.6 (34.0-157.0)		50.5 (42.2-148.6)		0.1324	0.9149	98.7 (67.1-174.4)		63.8 (60.1-118.0)		66.7 (34.5-92.0)		63.1 (39.7-138.7)		77.2 (68.4-205.4)		0.1132	0.5229	0.8759

Data are shown as median (1st to 3rd quartiles). *: P value was calculated by the Friedman test for the comparison among the paired groups according to the treatment phases. #: P value was calculated by the Kruskal Wallis test for the unpaired comparison among the groups. Tx: peginterferon α -2a therapy for 48 weeks; EGF: epidermal growth factor; IL: interleukin; IFN: interferon; MCPI: monocyte chemoattractant protein-1; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor.

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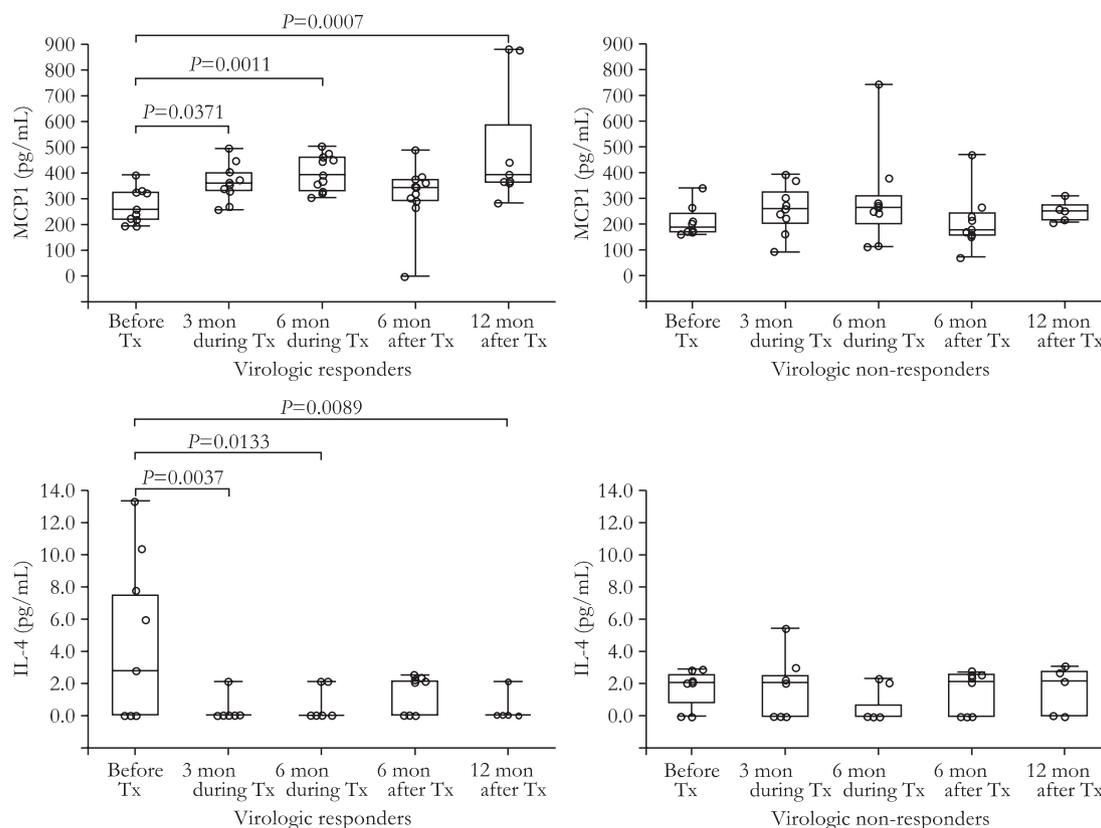


Fig. 1. Serum levels of cytokines in CHB patients according to treatment phases and responses to peginterferon α -2a. Levels of MCP-1 were increased at 3rd and 6th months after treatment had been started, and at 12th month after treatment had been finished, while IL-4 levels were decreased during and after treatment in the same group. Levels of these two cytokines were not different according to the treatment phases in virologic non-responders. *P* values in the figures were calculated by Mann-Whitney *U* test. The upper, lower ends of the boxes and box inner lines correspond to the upper and lower quartiles and median values, respectively. Whiskers denote extreme distribution values, and circles represent individual concentrations.

Predictive values of cytokines and HBV DNA

Diagnostic performances of HBV DNA and some cytokines are summarized in Table 3. The AUROC value of serum HBV DNA levels measured before peginterferon therapy was 0.81 ($P=0.0017$) in predicting VR ($n=11$) (Fig. 2A), and the lower baseline HBV DNA level was related to the higher probability of VR. The sensitivity and specificity of baseline HBV DNA levels in predicting VR were 72.7% and 87.5%, respectively, when the cutoff DNA level was 7.33×10^7 IU/mL. Meanwhile, the AUROC value of baseline MCP1 levels was 0.76 ($P=0.0230$) in detecting VR ($n=11$), and the higher MCP1 level was related to VR. The sensitivity and specificity of the baseline MCP1 level in discriminating VRs from NRs were 81.8% and 75.0%, respectively, with the cutoff value of 213.9 pg/mL. The AUROC values for all other cytokines quantified in the samples before the therapy were not statistically significant in predicting VR.

In addition, the AUROC value of HBV DNA levels, which was measured at 3rd month during the treatment

was 0.85 ($P=0.0001$) in predicting SVR ($n=6$) (Fig. 2B), and the sensitivity and specificity of HBV DNA levels at that time point were 100.0% and 61.5%, respectively (cutoff, 3.28×10^5 IU/mL). The AUROC values of IL-6 and IL-8 determined at 3rd month during the treatment were 0.85 ($P=0.0001$) and 0.81 ($P=0.0020$) respectively in predicting SVR, and the higher IL-6 and IL-8 levels were associated with SVRs. The sensitivity and specificity of IL-6 were 100.0% and 69.2%, respectively, at the cutoff value of 1.05 pg/mL, and those of IL-8 were 76.9% and 83.3% (cutoff, 18.55 pg/mL).

Similarly, the AUROC value of HBV DNA taken at 6th month during the treatment was 0.92 ($P<0.0001$) in predicting SVR, and the sensitivity and specificity of HBV DNA were 100.0% and 76.9% respectively, when the cutoff value was 1.18×10^3 IU/mL. The AUROC value of IL-6 assessed at 6th month during the treatment was 0.78 ($P=0.0058$) in predicting SVR. The sensitivity and specificity of IL-6 were 100.0% and 69.2%, respectively when the cutoff value was 1.09 pg/mL.

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Table 3. Predictive values of HBV DNA and cytokines for virologic responses

Marker	Measured time	Target of prediction	AUROC	P value	Cutoff*	Sensitivity (%)	Specificity (%)
HBV DNA	Before Tx	VR	0.81	0.0017	$<7.33 \times 10^7$ IU/mL	72.7	87.5
MCP1	Before Tx	VR	0.76	0.0230	>213.9 pg/mL	81.8	75.0
HBV DNA	3 mon during Tx	SVR	0.85	0.0001	$<3.28 \times 10^5$ IU/mL	100.0	61.5
IL-6	3 mon during Tx	SVR	0.85	0.0001	>1.05 pg/mL	100.0	69.2
IL-8	3 mon during Tx	SVR	0.81	0.0020	>18.55 pg/mL	76.9	83.3
HBV DNA	6 mon during Tx	SVR	0.92	<0.0001	$<1.18 \times 10^3$ IU/mL	100.0	76.9
IL-6	6 mon during Tx	SVR	0.78	0.0058	>1.09 pg/mL	100.0	69.2
IL-6 increase#	6 mon-baseline	VR	0.76	0.0351	>0.34 pg/mL	77.8	83.3
IFN- γ increase#	6 mon-baseline	SVR	0.80	0.0098	<1.15 pg/mL	100.0	54.5
MCP1 increase#	6 mon-baseline	SVR	0.77	0.0161	>102.0 pg/mL	100.0	63.6
IL-8 decrease#	3 mon-6 mon	VR	0.76	0.0339	<17.55 pg/mL	100.0	57.1
EGF increase#	6 mon-3 mon	VR	0.83	0.0020	>5.23 pg/mL	90.0	71.4
EGF increase#	6 mon-3 mon	SVR	0.73	0.0451	>5.78 pg/mL	100.0	53.8
MCP1 increase#	6 mon-3 mon	SVR	0.79	0.0051	>2.2 pg/mL	100.0	69.2
IL-4 decrease#	Baseline-3 mon	SVR	0.78	0.0307	>2.09 pg/mL	80.0	90.9
IL-1 α decrease#	Baseline-3 mon	SVR	0.82	0.0011	>0.00 pg/mL	60.0	90.9

*: The cutoff at which the sum of sensitivity and specificity was maximized. #: Increase or decrease of a cytokine level was calculated from subtracting a value, which was measured at the time point displayed, and from the value quantified at the time shown at first. AUROC: area under the receiver operating characteristic curve; Tx: peginterferon α -2a therapy for 48 weeks; VR: virologic response at 6th month during treatment; MCP1: monocyte chemotactic protein-1; SVR: sustained virologic response until 12 months off-treatment; IL: interleukin; EGF: epidermal growth factor; IFN: interferon.

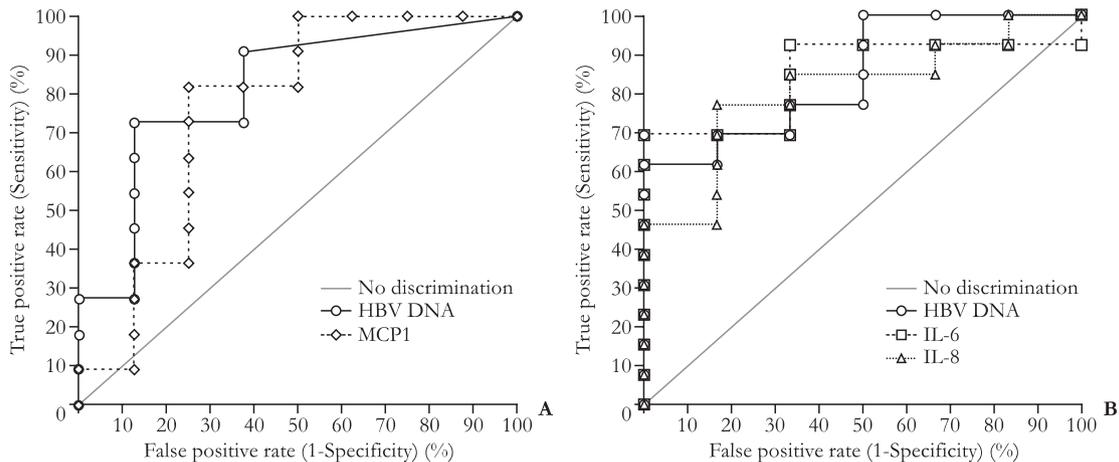


Fig. 2. Receiver operating characteristic (ROC) curves of HBV DNA and cytokines for predicting virologic responses. **A:** AUROC of HBV DNA levels measured before treatment was 0.81 ($P=0.0017$) for the discrimination between VRs ($n=11$) and NRs ($n=9$) at 6th month during therapy, and that of MCP1 was 0.76 ($P=0.0230$). **B:** AUROC value of HBV DNA quantified at 3rd month during treatment was 0.85 ($P=0.0001$) in predicting SVR ($n=6$) until 12 months off-treatment, and that of IL-6 were 0.85 ($P=0.0001$). IL-8 level at 3rd month also demonstrated AUROC of 0.81 ($P=0.0020$) in predicting SVR.

cytokines during the treatment were also significantly associated with VR and SVR (Table 3).

Discussion

Liver damage after HBV infection is mediated by host immune responses. Acquired and innate immunity is thought to be related to the pathogenesis of CHB. In

this study, we analyzed the serum levels of 12 cytokines in CHB patients to evaluate the patterns of changes in cytokines according to the treatment phases and responses to peginterferon α -2a, which is a widely used therapeutic agent for the treatment of CHB. In our study, the serum levels of MCP1, EGF, IFN- γ , IL-4, and IL-6 changed in the CHB patients according to the phases of peginterferon therapy. This would imply that

the levels of those cytokines during each treatment phase may reflect the immunological status of patients undergoing peginterferon therapy. It is plausible that the host's immune responses can be influenced by the administration of peginterferon since the drug has a dual mode of action which acts not only as an antiviral agent but also as a regulator of the immune system.

In our study, MCP1 was significantly increased during the therapy (362.8 pg/mL at 3rd month and 395.0 pg/mL at 6th month) compared with that before therapy (260.4 pg/mL) in VRs but not in NRs. This would imply that serum MCP1 levels reflect treatment phase and response to the agent in CHB patients. A previous study^[19] suggested that MCP1 plays an anti-inflammatory role in T cell-mediated hepatitis by inhibiting T cell-derived IL-4 production through direct stimulation of its specific receptor chemokine (C-C motif) receptor 2 (CCR2). In our study, the serum levels of MCP1 were increased, but those of IL-4 were decreased during the treatment in the VR group. In addition, the serum MCP1 level was also increased during the treatment of patients with chronic hepatitis C by using IFN α -2b plus ribavirin, but in contrast to our study, the increase of MCP1 levels in non-responders was significant.^[20] This discrepancy may be attributed to the difference of host immune responses to different viruses. MCP1, called as chemokine (C-C motif) ligand 2 (CCL2), small inducible cytokine A2 (SCYA2), and monocyte chemotactic and activating factor (MCAF), is known to act as a pro-inflammatory molecule, which plays a key role in the recruitment of monocytes to the site of infection. In a previous study,^[21] the 2518G>A polymorphism of the MCP1 gene was reported to be associated with whether HBV infected individuals recovered spontaneously or became a chronic HBV carrier. Plasma MCP1 levels were also higher in patients with chronic hepatitis caused by HBV or HCV infection (median 111.4 pg/mL) than in healthy controls (median 49.7 pg/mL).^[22] However, there are no reports on the role of MCP1 in monitoring CHB patients on peginterferon therapy. The results of our study provide a clue for the use of MCP1 as a biomarker in monitoring the efficacy of peginterferon therapy in CHB patients. Because MCP1 levels before treatment in our study showed an AUROC value of 0.76 in predicting virologic responses at 6th month during the treatment.

IL-6 is an endogenous pyrogen which causes fever as an inflammatory response in the body and acts as pro-inflammatory and anti-inflammatory cytokines. Its level was elevated during the therapy in the VR group in our study. IL-6 was reported to suppress HBV replication by preventing the formation of genome-

containing nucleocapsids, which is similar to the effect of interferons.^[23] In contrast, low serum IL-6 level was reported as an early predictor for acute exacerbation of CHB.^[24] Therefore, IL-6 also could be a marker for responses to peginterferon therapy. In our study IL-6 levels measured at 3rd and 6th months during the treatment were helpful in predicting SVR during the 12-month off-treatment. In addition, IFN- γ as one of the well-known cytokines has anti-viral effects, and the increase of IFN- γ level during the therapy in our study seems to be reasonable.

EGF was decreased during the early phase of peginterferon therapy compared to that before the therapy in our VR group. The finding indicates that the early decrease of serum EGF level during the therapy is a favorable prognostic factor for the peginterferon treatment. A study^[25] reported that the EGF-dependent upregulation of integrin beta-1 chains and increased adhesion to extracellular matrix were additional cytoprotective mechanisms of HBV infected hepatocytes. This study suggested that EGF can interfere with CD95-mediated apoptosis and the action of cytotoxic T-cells through multiple mechanisms in human hepatocytes. However, EGF levels were increased in the later phase of peginterferon therapy (at 6th month during treatment) compared with those in the early phase, and this increase was also related to VRs in our patients. Thus, the dynamics of cytokines in CHB seems to be complex and needs further investigation.

Cytokines are key molecules in the complex signaling network of cell-mediated immunity. Analyses of changes in cytokine expression patterns during the treatment help to understand the pathogenesis of CHB and predict treatment responses. However, a small number of subjects were enrolled in this study, and thus the statistical power was not adequate for assessment of the usefulness of cytokines in monitoring CHB patients, and the levels of some cytokines were changed or unchanged according to statistical analysis. Also, VRs to the treatment of CHB patients can be influenced by various factors including viral genotypes as well as host's genetic factors such as HLA types and polymorphisms of many genes. Our data show changes in the levels of particular cytokines according to treatment phases. Further large-scale studies to control various confounding factors are necessary to prove the usefulness of cytokine levels as predictive factors in CHB patients treated with peginterferon or other agents.

We simultaneously evaluated 12 cytokines in the sera from CHB patients in order to detect their expression patterns according to treatment phases and responses to therapy. The serum levels of cytokines,

particularly MCP1, EGF, IL-4, IL-6, and IL-8 reflected the pathological differences of the treatment phases. They could serve as indices for monitoring responses to peginterferon treatment in CHB patients.

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