双方）に別紙の説明文書に沿って説明を行い、十分な理解を得た上で自由意思に基づいて文書による同意を得ることとする。同意は別紙の同意書に、説明を行った医師と説明を受けた患者（未成年者の場合は親権者の双方）の署名、捺印、同意を得た日付を記載し、カルテに保管するとともに患者へも一部渡す。尚、この研究については長崎大学病院倫理委員会にて了承を得ている。

C．研究結果

これら対象の年齢中央値は59歳、男性13例（64%）女性8例（36%）全例IL28bのSNP（rs8099917）がTTであった。これらの症例でrapid virologic response（RVR）達成例は、81%をしめた。これらでRVRに寄与する因子についてロジステック回帰分析による単変量解析で検討したが有意な因子を抽出することはできなかった。更に、導入4週以上経過観察可能な21例をテラプレビル2250mg投与群と1500mg投与分に分け検討したところ2250mg投与群と比較して、1500mg投与群では、ヘモグロビン値の低下、クレアチニン値の上昇が緩徐であり、投与4週後のRNA消失率は、2250mg投与群が75%であったのに対し、1500mg投与群は、85%と1500mg投与群においてRVRの達成率が高い傾向がみられた。

D．考察

① 三剤療法によるHCVRNA消失率は、2週で37%、4週で81%であった。
② 2250mg投与群と比較して、1500mg投与群では、ヘモグロビン値の低下、クレアチニンの上昇が緩徐であった。
③ 投与4週後のRNA消失率は、2250mg投与群が75%であったのに対し、1500mg投与群は、85%であった。

E．結論

PEG-IFN/RBV/テラプレビル三剤併用療法におけるRVR達成症率は、テラプレビル2250mg投与群と比較し1500mg投与群で高く、ヘモグロビンの低下、クレアチニンの上昇が緩徐であった。テラプレビル1500mg減量投与は、RVR達成率を維持したままクレアチニン上昇などの副作用が軽減できる可能性が示唆された。テラプレビル投与量とSVRとの関連について、今後症例の蓄積及び検討が必要である。

F．研究発表

1. 論文発表

1) Takeshita S, Ichikawa T, Taura N, Miyaaki H, Matsuzaki T, Otani M, Muraoka T, Akiyama M, Miuma S, Ozawa E, Ikeda M, Kato N, Isomoto H, Takeshima F, Nakao K. Geranylgeranylacetone has anti-hepatitis C virus activity via activation of mTOR in human hepatoma cells. Journal of gastroenterology 47(2):195-202, 2012

2) Muraoka T, Ichikawa T, Taura N, Miyaaki H, Takeshita S, Akiyama M, Miuma S, Ozawa E, Isomoto H, Takeshima F, Nakao K. Insulin-induced mTOR activity exhibits anti-hepatitis C virus activity. Molecular medicine reports 5(2):331-335, 2012

3) Kamihira S, Usui T, Ichikawa T, Uno N, Morinaga Y, Mori S, Nagai K, Sasaki D, Hasegawa H, Yanagihara K, Honda T, Yamada Y, Iwanaga M, Kanematu T, Nakao K. Paradoxical expression of IL-28B mRNA in peripheral blood in human T-cell leukemia virus Type-1 mono-infection and co-infection with hepatitis C Virus. Virology journal 9:40, 2012

4) Ichikawa T, Taura N, Miyaaki H, Matsuzaki T, Ohtani M, Eguchi S, Takatsuki M, Soyama A, Hidaka M, Okudaira S, Usui T, Mori S, Kamihira S, Kanematsu T, Nakao K. Human T-cell leukemia virus type 1 infection worsens prognosis of hepatitis C virus-related living donor liver transplantation. Transplant international 25(4):433-438, 2012

5) Eguchi S, Hidaka M, Soyama A, Takatsuki M, Miyaaki H, Ichikawa T, Nakao K, Kanematsu T. Is liver-targeted FOXp3 staining beneficial after living-donor liver transplantation? Transplant infectious disease 14(2):156-162, 2012

6) Miuma S, Ichikawa T, Arima K, Takeshita S, Muraoka T, Matsuzaki T, Ootani M, Shibata H, Akiyama M, Ozawa E, Miyaaki H, Taura N, Takeshima F, Nakao K. Branched-chain amino acid deficiency stabilizes insulin-induced vascular endothelial growth factor mRNA in hepatocellular carcinoma cells. Journal of cellular biochemistry 113(10):3113-3121, 2012

7) Yamaguchi T, Ichikawa T, Takeshita S, Taura N, Miyaaki H, Muraoka T, Shibata H, Honda T, Hamasaki K, Kato Y, Takeshima F, Nakao K. Anti-hepatitis C virus activity of geranylgeranylacetone treatment in hepatitis C-infected patients. Acta Medica Nagasakiensia 57(1):1-4, 2012

8) Matsuzaki T, Ichikawa T, Kondo H, Taura N, Miyaaki H, Isomoto H, Takeshima F, Nakao K. Prevalence of restless legs syndrome in Japanese patients with chronic liver disease. Hepatology research 42(12):1221-1226, 2012

9) Migita K, Abiru S, Ohtani M, Jiuchi Y, Maeda Y, Bae SK, Bekki S, Hashimoto S, Yesmembetov K, Nagaoka S, Nakamura M, Komori A, Ichikawa T, Nakao K, Yatsuhashi H, Ishibashi H, Yasunami M. HLA-DP gene polymorphisms and hepatitis B infection in the Japanese population. Translational research 443-444, 2012

10) Otani M, Honda N, Xia PC, Eguchi K, Ichikawa T, Watanabe T, Yamaguchi K, Nakao K, Yamamoto T. Distribution of Two Subgroups of Human T-Lymphotropic Virus Type 1 (HTLV-1) in Endemic Japan. Tropical medicine and health 40(2):55-58, 2012

11) Nakamura M, Nishida N, Kawashima M, Aiba Y, Tanaka A, Yasunami M, Nakamura H, Komori A, Nakamuta M, Zeniya M, Hashimoto E, Ohira H, Yamamoto K, Onji M, Kaneko S, Honda M, Yamagiwa S, Nakao K, Ichida T, Takikawa H, Seike M, Umemura T, Ueno Y, Sakisaka S, Kikuchi K, Ebinuma H, Yamashiki N, Tamura S, Sugawara Y, Mori A, Yagi S, Shirabe K, Taketomi A, Arai K, Monoe K, Ichikawa T, Taniai M, Miyake Y, Kumagi T, Abe M, Yoshizawa K, Joshita S, Shimoda S, Honda K, Takahashi H, Hirano K, Takeyama Y, Harada K, Migita K, Ito M, Yatsuhashi H, Fukushima N, Ota H, Komatsu T, Saoshiro T, Ishida J, Kouno H, Kouno H, Yagura M, Kobayashi M, Muro T, Masaki N, Hirata K, Watanabe Y, Nakamura Y, Shimada M, Hirashima N, Komeda T, Sugi K, Koga M, Ario K, Takesaki E, Maehara Y, Uemoto S, Kokudo N, Tsubouchi H, Mizokami M, Nakanuma Y,

Tokunaga K, Ishibashi H. Genome-wide Association Study Identifies TNFSF15 and POU2AF1 as Susceptibility Loci for Primary Biliary Cirrhosis in the Japanese Population. American journal of human genetics 91(4):721-728, 2012
12) Taura N, Fukuda S, Ichikawa T, Miyaaki H, Shibata H, Honda T, Yamaguchi T, Kubota Y, Uchida S, Kamo Y, Yoshimura E, Isomoto H, Matsumoto T, Takeshima F, Tsutsumi T, Tsuruta S, Nakao K. Relationship of α-fetoprotein levels and development of hepatocellular carcinoma in hepatitis C patients with liver cirrhosis. Experimental and therapeutic medicine 4(6):972-976, 2012
13) Taura N, Ichikawa T, Miyaaki H, Kadokawa Y, Tsutsumi T, Tsuruta S, Kato Y, Inoue O, Kinoshita N, Ohba K, Kato H, Ohata K, Masuda J, Hamasaki K, Yatsuhashi H, Nakao K. Baseline serum cholesterol is associated with a response to pegylated interferon alfa-2b and ribavirin therapy for chronic hepatitis C genotype 2. Gastroenterology research and practice (http://www.hindawi.com/journals/grp/2012/317580/), Epub 2012

2. 学会発表

なし。

G. 知的財産権の出願・登録状況

なし。

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分担研究報告書

# 種々の環境下の肝細胞癌におけるPIVKA-IIの分泌に関する検討

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研究要旨　PIVKA-IIは、肝細胞癌（以下肝癌）の腫瘍マーカーであるが、近年、肝癌の分子標的薬であるソラフェニブの有効症例で、治療開始後早期にPIVKA-IIの上昇が見られるという報告がある。今回、その機序解明を目的に、ソラフェニブとPIVKA-II分泌との関連性についてPIVKA-II産生性の中分化型肝癌細胞株KYN-2を用いて検討した。各種濃度（0.3125, 0.625, 1.25 μM）のソラフェニブ添加、非添加培地で24-72時間培養し、培養上清中のPIVKA-II、prothrombin、VEGFを測定した。また、低$O_2$（1%$O_2$）環境下でも検討を行った。その結果、1.25 μMのソラフェニブを加えた場合の細胞数は24時間目より有意に低下し72時間後にはコントロールの25%まで低下した。ソラフェニブ投与によりPIVKA-IIの分泌は48時間後より著明に低下し、prothrombinの分泌とVEGFの分泌は、24時間後より有意に増加した。各種濃度のソラフェニブの投与により、0.3125 μM以上の濃度で濃度依存性にPIVKA-IIの分泌が有意に低下し、1.25 μMでは、コントロールの16%まで低下した。一方、prothrombinは、逆に0.625 μMをピークに1.4倍増加し、VEGFも最高1.8倍まで増加した。1%$O_2$環境下では、通常状態に比べPIVKA-IIは約2-6倍に分泌量が増加した。また、低酸素下でもソラフェニブ投与によりPIVKA-IIの分泌は低下したが、prothrombin、VEGFの分泌は増加した。この結果に基づくとソラフェニブの治療後のPIVKA-IIの上昇の機序としては虚血による低酸素との関連性が最も考えられた。

## A．研究目的

PIVKA-IIは、1984年にLiebmanらにより肝細胞癌（以下肝癌）において発現することが初めて報告された腫瘍マーカーであるが、その後、肝癌の腫瘍径、脈管侵襲、肝内転移などと密接に関係することが報告されている。近年、肝癌の分子標的薬であるソラフェニブの有効症例で、治療開始後早期にPIVKA-IIの上昇が見られるという報告がある。このPIVKA-IIの上昇機序として、肝癌細胞の虚血（低酸素）との関連性が推察されているが、詳しい機序は不明である。今回、肝癌細胞株を使用して低酸素やソラフェニブのPIVKA-II産生などに及ぼす作用を検討した。

## B．研究方法

実験にはPIVKA-II産生性の中分化型肝癌細胞株KYN-2を使用した。(1)　ソラフェニブ（0.3125-20 μM）添加培地で培養し増殖抑制効果とアポトーシス誘導に関して形態的およびAnnexin V陽性細胞の有無により検討した。(2)　1.25 μMのソラフェニブ添加（S群）、非添加（C群）培地を加え経時的（24、48, 72時間）に培養し、培養上清中のPIVKA-II、prothrombin、VEGFを測定

した。(3) 各種濃度 (0.3125, 0.625, 1.25 μM) のソラフェニブ添加 (S群)、非添加 (C群) 培地で72時間培養し、培養上清中のPIVKA-II、prothrombin、VEGFを測定した。(4) (2) と (3) の実験を1%$O_2$の低酸素環境で行った。

C．研究結果

(1) KYN-2細胞に対するソラフェニブの50%増殖抑制濃度は2.1 μMであった。ソラフェニブ投与により、形態的にアポトーシスを生じた細胞が見られ、また、Annexin V染色で有意な陽性細胞の増加を認めた。以上より、ソラフェニブによるアポトーシス誘導が確認された。

(2) 1.25 μMのソラフェニブを加えたS群の細胞数は24時間目より有意に低下し72時間後にはC群の25%まで低下した。C群のPIVKA-IIの分泌は、24, 48, 72時間後でそれぞれ、0.2, 8.4, 48.2 mAU/mL/$10^4$ cellであったが、S群では、0.2, 0.3, 0.7 mAU/mL/$10^4$ cellと著明に低下していた。prothrombinの分泌は、C群が0.32, 0.48, 0.51 ng/mL/$10^4$ cellで、S群では0.47, 1.12, 0.75 ng//mL/$10^4$ cellと有意に増加していた。VEGFの分泌は、C群では3.9, 8.9, 9.4 pg/mL/$10^4$ cellだが、S群では10.7, 31.3, 26.2 pg/mL/$10^4$ cellと有意に増加していた。

(3) 各種濃度のソラフェニブを添加したS群では、0.3125 μM以上の濃度で濃度依存性にPIVKA-IIの分泌が有意に低下し1.25 μMでは、C群の16%まで低下した。逆にprothrombinは、0.625 μMをピークに1.4倍増加した。VEGFも最高1.8倍まで増加した。

(4) 1%$O_2$環境下では、通常状態に比べPIVKA-IIは約2-6倍に分泌量が増加した。また、低酸素下でもS群では、PIVKA-IIの分泌低下とprothrombin、VEGFの分泌増加を認めた。

D．考察

PIVKA-IIは、プロトロンビンのグルタミン酸残基 (Glu基) のカルボキシル化 (Gla基化) 不全により生じる異常プロトロンビンで肝細胞癌の腫瘍マーカーとして汎用されている。PIVKA-IIの産生機序としては、γグルタミルカルボキシラーゼの異常、プロトロンビンの過剰産生、ビタミンKの濃度低下や取り込み低下、低酸素、そして上皮間葉転換などが報告されている。今回、ソラフェニブによる肝細胞癌の治療後、2, 3週目にPIVKA-IIが上昇する機序を解明するためPIVKA-II産生性の肝癌細胞株KYN-2を使用して、ソラフェニブの接触がPIVKA-II産生に及ぼす作用を検討した。その結果、ソラフェニブは、肝癌細胞のPIVKA-II産生を抑制し、プロトロンビンやVEGFの産生を亢進する事が明らかとなった。この結果に基づくと、ソラフェニブによる治療後にPIVKA-IIが上昇する減少は、ソラフェニブの肝癌細胞に対する直接的作用とは考えにくい。ソラフェニブは、VEGFやPDGFの受容体のリン酸化を阻害して血管新生の阻害作用を有している。低酸素によりPIVKA-IIの発現が亢進すると言う事実から推察すると、ソラフェニブ治療後のPIVKA-IIの上昇には、血管新生阻害による低酸素環境が腫瘍細胞によるPIVKA-IIの産生亢進を誘導したと推察される。今後、ソラフェニブによるPIVKA-II産生抑制機序については更に検討が必要である。

E．研究発表

1. 論文発表

1) Abe M, Koga H, Yoshida T, Masuda H, Iwamoto H, Sakata M, Hanada S, Nakamura T, Taniguchi E, Kawaguchi T, Yano H, Torimura T, Ueno T, Sata M: Hepatitis C virus core protein upregulates the expression of vascular endothelial growth factor via the nuclear factor-

kappaB/hypoxia-inducible factor-1alpha axis under hypoxic conditions, Hepatol Res 2012, 42:591-600.
2) Kondo R, Yano H, Nakashima O, Tanikawa K, Nomura Y, Kage M: Accumulation of platelets in the liver may be an important contributory factor to thrombocytopenia and liver fibrosis in chronic hepatitis C, J Gastroenterol 2012, in press.
3) Komuta M, Govaere O, Vandecaveye V, Akiba J, Van Steenbergen W, Verslype C, Laleman W, Pirenne J, Aerts R, Yano H, Nevens F, Topal B, Roskams T: Histological diversity in cholangiocellular carcinoma reflects the different cholangiocyte phenotypes, Hepatology 2012, 55:1876-1888.

2. 学会発表

1) 矢野博久. 肝移植のリスクファクターを考える－PIVKA-II発現の意味するところ－ JDDW（ランチョンセミナー）神戸 2012年10月10日

F．知的財産権の出願・登録状況

なし。

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難病・がん等の疾患分野の医療の実用化研究事業（肝炎関係研究分野）

# 病態別の患者の実態把握のための調査および肝炎患者の病態に即した相談に対応できる相談員育成のための研修プログラム策定に関する研究

平成24年度

総括研究報告書
分担研究報告書
（2／2）

論　　文　　集

研究代表者
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平成25（2013）年3月

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（2／2）

論　　文　　集

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# 研究成果の刊行に関する一覧表

雑誌

| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻号 | ページ | 出版年 |
|---|---|---|---|---|---|
| Bae SK, Yatsuhashi H, Hashimoto S, Motoyoshi Y, Ozawa E, Nagaoka S, Abiru S, Komori A, Migita K, Nakamura M, Ito M, Miyakawa Y, Ishibashi H. | Prediction of early HBeAg seroconversion by decreased titers of HBeAg in the serum combined with increased grades of lobular inflammation in the liver. | Med Sci Monit. | 18 (12) | CR698-705 | 2012 |
| Watanabe T, Sugauchi F, Tanaka Y, Matsuura K, Yatsuhashi H, Murakami S, Iijima S, Iio E, Sugiyama M, Shimada T, Kakuni M, Kohara M, Mizokami M. | Hepatitis C virus kinetics by administration of pegylated interferon-α in human and chimeric mice carrying human hepatocytes with variants of the IL28B gene. | Gut | | | 2012 |
| Izumi N, Asahina Y, Kurosaki M, Yamada G, Kawai T, Kajiwara E, Okamura Y, Takeuchi T, Yokosuka O, Kariyama K, Toyoda J, Inao M, Tanaka E, Moriwaki H, Adachi H, Katsushima S, Kudo M, Takaguchi K, Hiasa Y, Chayama K, Yatsuhashi H, Oketani M, Kumada H. | Inhibition of hepatocellular carcinoma by PegIFNα-2a in patients with chronic hepatitis C: a nationwide multicenter cooperative study. | J Gastroenterol. | | | 2012 |
| Nakamura M, Nishida N, Kawashima M, Aiba Y, Tanaka A, Yasunami M, Nakamura H, Komori A, Nakamuta M, Zeniya M, Hashimoto E, Ohira H, Yamamoto K, Onji M, Kaneko S, Honda M, Yamagiwa S, Nakao K, Ichida T, Takikawa H, Seike M, Umemura T, Ueno Y, Sakisaka S, Kikuchi K, Ebinuma H, Yamashiki N, Tamura S, Sugawara Y, Mori A, Yagi S, Shirabe K, Taketomi A, Arai K, Monoe K, Ichikawa T, Taniai M, Miyake Y, Kumagi T, Abe M, Yoshizawa K, Joshita S, Shimoda S, Honda K, Takahashi H, Hirano K, Takeyama Y, Harada K, Migita K, Ito M, Yatsuhashi H, Fukushima N, Ota H, Komatsu T, Saoshiro T, | Genome-wide Association Study Identifies TNFSF15 and POU2AF1 as Susceptibility Loci for Primary Biliary Cirrhosis in the Japanese Population. | Am J Hum Genet. | 91 (4) | 721-8 | 2012 |

| | | | | | |
|---|---|---|---|---|---|
| Ishida J, Kouno H, Kouno H, Yagura M, Kobayashi M, Muro T, Masaki N, Hirata K, Watanabe Y, Nakamura Y, Shimada M, Hirashima N, Komeda T, Sugi K, Koga M, Ario K, Takesaki E, Maehara Y, Uemoto S, Kokudo N, Tsubouchi H, Mizokami M, Nakanuma Y, Tokunaga K, Ishibashi H. | | | | | |
| Ueda T, Tsuchiya K, Hashimoto S, Inoue T, Inao M, Tanaka A, Kaito M, Imazaki F, Nishiguchi S, Mochida S, Yokosuka O, Yatsuhashi H, Izumi N, Kudo M, for the RETRY Study Group. | Retreatment with Peginterferon α -2a + Ribavirin in Patients Who Failed Previous Peginterferon α-2b+Ribavirin Combination Therapy. | Dig Dis. | 30 (6) | 554-60 | 2012 |
| Migita K, Abiru S, Ohtani M, Jiuchi Y, Maeda Y, Bae SK, Bekki S, Hashimoto S, Yesmembetov K, Nagaoka S, Nakamura M, Komori A, Ichikawa T, Nakao K, Yatsuhashi H, Ishibashi H, Yasunami M. | HLA-DP gene polymorphisms and hepatitis B infection in the Japanese population. | Transl Res. | 160 (6) | 443-4 | 2012 |
| Kani S, Tanaka Y, Matsuura K, Watanabe T, Yatsuhashi H, Orito E, Inose K, Motojuku N, Wakimoto Y, Mizokami M. | Development of new IL28B genotyping method using Invader Plus assay. | Microbiol Immunol. | 56 (5) | 318-23 | 2012 |
| Migita K, Watanabe Y, Jiuchi Y, Nakamura Y, Saito A, Yagura M, Ohta H, Shimada M, Mita E, Hijioka T, Yamashita H, Takezaki E, Muro T, Sakai H, Nakamuta M, Abiru S, Komori A, Ito M, Yatsuhashi H, Nakamura M, Ishibashi H; the Japanese NHO-Liver-network study group. | Hepatocellular carcinoma and survival in patients with autoimmune hepatitis (Japanese National Hospital Organization-autoimmune hepatitis prospective study). | Liver Int. | 32 (5) | 837-44 | 2012 |
| Kurosaki M, Hiramatsu N, Sakamoto M, Suzuki Y, Iwasaki M, Tamori A, Matsuura K, Kakinuma S, Sugauchi F, Sakamoto N, Nakagawa M, Yatsuhashi H, Izumi N. | Age and total ribavirin dose are independent predictors of relapse after interferon therapy in chronic hepatitis C revealed by data mining analysis. | Antivir Ther. | 17 (1) | 35-43 | 2012 |

| | | | | | |
|---|---|---|---|---|---|
| Matsumoto A, Tanaka E, Suzuki Y, Kobayashi M, Tanaka Y, Shinkai N, Hige S, Yatsuhashi H, Nagaoka S, Chayama K, Tsuge M, Yokosuka O, Imazeki F, Nishiguchi S, Saito M, Fujiwara K, Torii N, Hiramatsu N, Karino Y, Kumada H. | Combination of hepatitis B viral antigens and DNA for prediction of relapse after discontinuation of nucleos(t)ide analogs in patients with chronic hepatitis B. | Hepatol Res. | 42 (2) | 139-49 | 2012 |
| Kohjima M, Enjoji M, Yoshimoto T, Yada R, Fujino T, Aoyagi Y, Fukushima N, Fukuizumi K, Harada N, Yada M, Kato M, Kotoh K, Nakashima M, Sakamoto N, Tanaka Y, Nakamuta M. | Add-on therapy of pitavastatin and eicosapentaenoic acid improves outcome of peginterferon plus ribavirin treatment for chronic hepatitis C. | J Med Virol. | 85 (2) | 250-60 | 2013 |
| Kotoh K, Fukushima M, Horikawa Y, Yamashita S, Kohjima M, Nakamuta M, Enjoji M. | Serum albumin is present at higher levels in alcoholic liver cirrhosis as compared to HCV-related cirrhosis. | Exp Ther Med. | 3 (1) | 72-5 | 2012 |
| Enjoji M, Kohjima M, Kotoh K, Nakamuta M. | Metabolic disorders and steatosis in patients with chronic hepatitis C: metabolic strategies for antiviral treatments. | Int J Hepatol. | | | 2012 |
| Oze T, Hiramatsu N, Mita E, Akuta N, Sakamoto N, Nagano H, Itoh Y, Kaneko S, Izumi N, Nomura H, Hayashi N, Takehara T. | A multicenter survey of re-treatment with pegylated interferon plus ribavirin combination therapy for patients with chronic hepatitis C in Japan. | Hepatol Res. | 43 (1) | 35-43 | 2013 |
| Higashitani K, Kanto T, Kuroda S, Yoshio S, Matsubara T, Kakita N, Oze T, Miyazaki M, Sakakibara M, Hiramatsu N, Mita E, Imai Y, Kasahara A, Okuno A, Takikawa O, Hayashi N, Takehara T. | Association of enhanced activity of indoleamine 2,3-dioxygenase in dendritic cells with the induction of regulatory T cells in chronic hepatitis C infection. | J Gastroenterol. | | | 2012 |
| Harada N, Hiramatsu N, Oze T, Yamada R, Kurokawa M, Miyazaki M, Yakushijin T, Miyagi T, Tatsumi T, Kiso S, Kanto T, Kasahara A, Oshita M, Mita E, Hagiwara H, Inui Y, Katayama K, Tamura S, Yoshihara H, Imai Y, Inoue A, Hayashi N, Takehara T. | Incidence of hepatocellular carcinoma in HCV-infected patients with normal alanine aminotransferase levels categorized by Japanese treatment guidelines. | J Gastroenterol. | | | 2012 |

| | | | | | |
|---|---|---|---|---|---|
| Nishida N, Sawai H, Matsuura K, Sugiyama M, Ahn SH, Park JY, Hige S, Kang JH, Suzuki K, Kurosaki M, Asahina Y, Mochida S, Watanabe M, Tanaka E, Honda M, Kaneko S, Orito E, Itoh Y, Mita E, Tamori A, Murawaki Y, Hiasa Y, Sakaida I, Korenaga M, Hino K, Ide T, Kawashima M, Mawatari Y, Sageshima M, Ogasawara Y, Koike A, Izumi N, Han KH, Tanaka Y, Tokunaga K, Mizokami M. | Genome-wide association study confirming association of HLA-DP with protection against chronic hepatitis B and viral clearance in Japanese and Korean. | PLoS One. | 7 (6) | | 2012 |
| Sawai H, Nishida N, Mbarek H, Matsuda K, Mawatari Y, Yamaoka M, Hige S, Kang JH, Abe K, Mochida S, Watanabe M, Kurosaki M, Asahina Y, Izumi N, Honda M, Kaneko S, Tanaka E, Matsuura K, Itoh Y, Mita E, Korenaga M, Hino K, Murawaki Y, Hiasa Y, Ide T, Ito K, Sugiyama M, Ahn SH, Han KH, Park JY, Yuen MF, Nakamura Y, Tanaka Y, Mizokami M, Tokunaga K. | No association for Chinese HBV-related hepatocellular carcinoma susceptibility SNP in other East Asian populations. | BMC Med Genet. | 13 | | 2012 |
| Toyama T, Ishida H, Ishibashi H, Yatsuhashi H, Nakamuta M, Shimada M, Ohta H, Satoh T, Kato M, Hijioka T, Takano H, Komeda T, Yagura M, Mano H, Watanabe Y, Kobayashi M, Mita E. | Long-term outcomes of add-on adefovir dipivoxil therapy to ongoing lamivudine in patients with lamivudine-resistant chronic hepatitis B. | Hepatol Res. | 42 (12) | 1168-74 | 2012 |
| Kurokawa M, Hiramatsu N, Oze T, Yakushijin T, Miyazaki M, Hosui A, Miyagi T, Yoshida Y, Ishida H, Tatsumi T, Kiso S, Kanto T, Kasahara A, Iio S, Doi Y, Yamada A, Oshita M, Kaneko A, Mochizuki K, Hagiwara H, Mita E, Ito T, Inui Y, Katayama K, Yoshihara H, Imai Y, Hayashi E, Hayashi N, Takehara T. | Long-term effect of lamivudine treatment on the incidence of hepatocellular carcinoma in patients with hepatitis B virus infection. | J Gastroenterol. | 47 (5) | 577-85 | 2012 |

| | | | | | |
|---|---|---|---|---|---|
| 坂根貞嗣、榊原祐子、由雄敏之、中水流正一、外山隆、石田　永、三田英治 | Telaprevir/Peg-IFN-α 2b/Ribavirin併用療法導入直後の腎機能低下機序に関する検討. | 肝臓 | 53 (7) | 434-5 | 2012 |
| Takaguchi K, Moriwaki H, Doyama H, Iida M, Yagura M, Shimada N, Kang M, Yamada H, Kumada H. | Effects of branched-chain amino acid granules on serum albumin level and prognosis are dependent on treatment adherence in patients with liver cirrhosis. | Hepatol Res. | | | 2012 |
| 正木尚彦. | 肝炎をめぐる医療政策. | 医学のあゆみ | 240 (12) | 997-9 | 2012 |
| Miyagi Y, Nomura H, Yamashita N, Tanimoto H, Ito K, Masaki N, Mizokami M, Shibuya T. | Estimation of two real-time RT-PCR assays for quantitation of hepatitis C virus RNA during PEG-IFN plus ribavirin therapy by HCV genotypes and IL28B genotype. | J Infect Chemother. | 19 (1) | 63-9 | 2013 |
| Saito H, Ito K, Sugiyama M, Matsui T, Aoki Y, Imamura M, Murata K, Masaki N, Nomura H, Adachi H, Hige S, Enomoto N, Sakamoto N, Kurosaki M, Mizokami M, Watanabe S. | Factors responsible for the discrepancy between IL28B polymorphism prediction and the viral response to peginterferon plus ribavirin therapy in Japanese chronic hepatitis C patients. | Hepatol Res. | 42 (10) | 958-65 | 2012 |
| Ito K, Kuno A, Ikehara Y, Sugiyama M, Saito H, Aoki Y, Matsui T, Imamura M, Korenaga M, Murata K, Masaki N, Tanaka Y, Hige S, Izumi N, Kurosaki M, Nishiguchi S, Sakamoto M, Kage M, Narimatsu H, Mizokami M. | LecT-Hepa, a glyco-marker derived from multiple lectins, as a predictor of liver fibrosis in chronic hepatitis C patients. | Hepatology | 56 (4) | 1448-56 | 2012 |
| Nomura H, Miyagi Y, Tanimoto H, Yamashita N, Ito K, Masaki N, Mizokami M. | Increase in platelet count based on inosine triphosphatase genotype during interferon beta plus ribavirin combination therapy. | J Gastroenterol Hepatol. | 27 (9) | 1461-6 | 2012 |
| 都築智之, 岩瀬弘明, 島田昌明, 平嶋　昇, 日比野祐介, 龍華庸光, 齋藤雅之, 玉置　大, 神谷麻子, 横井美咲, 横幕能行, 藤崎誠一郎, 杉浦　亙, 後藤秀実. | 当院におけるHIV, HCV重複感染症例に対するペグインターフェロン, リバビリン併用療法の治療成績. | 日本消化器病学会雑誌 | 109 (7) | 1186-96 | 2012 |
| 杉　和洋. | クリティカルパスを活用した肝臓病チーム医療の実践. | Medical QOL | | 26-9 | 2013 |

| | | | | | |
|---|---|---|---|---|---|
| Matsuzaki T, Ichikawa T, Kondo H, Taura N, Miyaaki H, Isomoto H, Takeshima F, Nakao K. | Prevalence of restless legs syndrome in Japanese patients with chronic liver disease. | Hepatol Res. | 42 (12) | 1221-6 | 2012 |
| Miuma S, Ichikawa T, Arima K, Takeshita S, Muraoka T, Matsuzaki T, Ootani M, Shibata H, Akiyama M, Ozawa E, Miyaaki H, Taura N, Takeshima F, Nakao K. | Branched-chain amino acid deficiency stabilizes insulin-induced vascular endothelial growth factor mRNA in hepatocellular carcinoma cells. | J Cell Biochem. | 113 (10) | 3113-21 | 2012 |
| Ichikawa T, Taura N, Miyaaki H, Matsuzaki T, Ohtani M, Eguchi S, Takatsuki M, Soyama A, Hidaka M, Okudaira S, Usui T, Mori S, Kamihira S, Kanematsu T, Nakao K. | Human T-cell leukemia virus type 1 infection worsens prognosis of hepatitis C virus-related living donor liver transplantation. | Transpl Int. | 25 (4) | 433-8 | 2012 |
| Eguchi S, Hidaka M, Soyama A, Takatsuki M, Miyaaki H, Ichikawa T, Nakao K, Kanematsu T. | Is liver-targeted FOXp3 staining beneficial after living-donor liver transplantation? | Transpl Infect Dis. | 14 (2) | 156-62 | 2012 |
| Yamaguchi T, Ichikawa T, Takeshita S, Taura N, Miyaaki H,Muraoka T, Shibata H, Honda T, Hamasaki K, Kato Y, Takeshima F, Nakao K. | Anti-hepatitis C virus activity of geranylgeranylacetone treatment in hepatitis C-infected patients. | Acta Medica Nagasakiensia. | 57 (1) | 1-4 | 2012 |
| Takeshita S, Ichikawa T, Taura N, Miyaaki H, Matsuzaki T, Otani M, Muraoka T, Akiyama M, Miuma S, Ozawa E, Ikeda M, Kato N, Isomoto H, Takeshima F, Nakao K. | Geranylgeranylacetone has anti-hepatitis C virus activity via activation of mTOR in human hepatoma cells. | J Gastroenterol. | 47 (2) | 195-202 | 2012 |
| Muraoka T, Ichikawa T, Taura N, Miyaaki H, Takeshita S, Akiyama M, Miuma S, Ozawa E, Isomoto H, Takeshima F, Nakao K. | Insulin-induced mTOR activity exhibits anti-hepatitis C virus activity. | Mol Med Report. | 5 (2) | 331-5 | 2012 |
| Kamihira S, Usui T, Ichikawa T, Uno N, Morinaga Y, Mori S, Nagai K, Sasaki D, Hasegawa H, Yanagihara K, Honda T, Yamada Y, Iwanaga M, Kanematu T, Nakao K. | Paradoxical expression of IL-28B mRNA in peripheral blood in human T-cell leukemia virus type-1 mono-infection and co-infection with hepatitis C virus. | Virol J. | 9 | | 2012 |

| | | | | | |
|---|---|---|---|---|---|
| Otani M, Honda N, Xia PC, Eguchi K, Ichikawa T, Watanabe T, Yamaguchi K, Nakao K, Yamamoto T. | Distribution of Two Subgroups of Human T-Lymphotropic Virus Type 1 (HTLV-1) in Endemic Japan. | Trop Med Health. | 40 (2) | 55-8 | 2012 |
| Taura N, Fukuda S, Ichikawa T, Miyaaki H, Shibata H, Honda T, Yamaguchi T, Kubota Y, Uchida S, Kamo Y, Yoshimura E, Isomoto H, Matsumoto T, Takeshima F, Tsutsumi T, Tsuruta S, Nakao K. | Relationship of α-fetoprotein levels and development of hepatocellular carcinoma in hepatitis C patients with liver cirrhosis. | Exp Ther Med. | 4 (6) | 972-6 | 2012 |
| Taura N, Ichikawa T, Miyaaki H, Kadokawa Y, Tsutsumi T, Tsuruta S, Kato Y, Inoue O, Kinoshita N, Ohba K, Kato H, Ohata K, Masuda J, Hamasaki K, Yatsuhashi H, Nakao K. | Baseline serum cholesterol is associated with a response to pegylated interferon alfa-2b and ribavirin therapy for chronic hepatitis C genotype 2. | Gastroenterol Res Pract. | | | 2012 |
| Kondo R, Yano H, Nakashima O, Tanikawa K, Nomura Y, Kage M. | Accumulation of platelets in the liver may be an important contributory factor to thrombocytopenia and liver fibrosis in chronic hepatitis C. | J Gastroenterol. | | | 2012 |
| Torimura T, Ueno T, Taniguchi E, Masuda H, Iwamoto H, Nakamura T, Inoue K, Hashimoto O, Abe M, Koga H, Barresi V, Nakashima E, Yano H, Sata M. | Interaction of endothelial progenitor cells expressing cytosine deaminase in tumor tissues and 5-fluorocytosine administration suppresses growth of 5-fluorouracil- sensitive liver cancer in mice. | Cancer Sci. | 103 (3) | 542-8 | 2012 |
| Naito Y, Kusano H, Nakashima O, Sadashima E, Hattori S, Taira T, Kawahara A, Okabe Y, Shimamatsu K, Taguchi J, Momosaki S, Irie K, Yamaguchi R, Yokomizo H, Nagamine M, Fukuda S, Sugiyama S, Nishida N, Higaki K, Yoshitomi M, Yasunaga M, Okuda K, Kinoshita H, Nakayama M, Yasumoto M, Akiba J, Kage M, Yano H | Intraductal neoplasm of the intrahepatic bile duct: clinicopathological study of 24 cases. | World J Gastroenterol. | 18 (28) | 3673-80 | 2012 |
| Komuta M, Govaere O, Vandecaveye V, Akiba J, Van Steenbergen W, Verslype C, Laleman W, Pirenne J, Aerts R, Yano H, Nevens F, Topal B, Roskams T. | Histological diversity in cholangiocellular carcinoma reflects the different cholangiocyte phenotypes. | Hepatology | 55 (6) | 1876-88 | 2012 |

| | | | | | |
|---|---|---|---|---|---|
| Inoue K, Torimura T, Nakamura T, Iwamoto H, Masuda H, Abe M, Hashimoto O, Koga H, Ueno T, Yano H, Sata M. | Vandetanib, an inhibitor of VEGF receptor-2 and EGF receptor, suppresses tumor development and improves prognosis of liver cancer in mice. | Clin Cancer Res. | 18 (14) | 3924-33 | 2012 |
| Abe M, Koga H, Yoshida T, Masuda H, Iwamoto H, Sakata M, Hanada S, Nakamura T, Taniguchi E, Kawaguchi T, Yano H, Torimura T, Ueno T, Sata M. | Hepatitis C virus core protein upregulates the expression of vascular endothelial growth factor via the nuclear factor-kappaB/hypoxia-inducible factor-1alpha axis under hypoxic conditions. | Hepatol Res. | 42 | 591-600 | 2012 |

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# Prediction of early HBeAg seroconversion by decreased titers of HBeAg in the serum combined with increased grades of lobular inflammation in the liver

**Authors' Contribution:**
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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## Summary

**Background:** Hepatitis B e antigen (HBeAg) seroconversion is an important hallmark in the natural course of chronic hepatitis B. This study was designed to predict early HBeAg seroconversion within 1 year, by not only biochemical and virological markers, but also pathological parameters in patients with chronic hepatitis B.

**Material/Methods:** In a retrospective cohort study, 234 patients with HBeAg were reviewed for demographic, biochemical, virological and pathological data at the time of liver biopsy. Then, the patients who accomplished HBeAg seroconversion within 1 year thereafter were compared with those who did not, for sorting out factors predictive of early HBeAg seroconversion.

**Results:** Early HBeAg seroconversion occurred in 58 (24.8%) patients. In univariate analysis, factors predictive of early HBeAg seroconversion were: alanine aminotransferase (ALT) (p=0.002), IP-10 (p=0.029), HBsAg (p=0.003), HBeAg (p<0.001), HBV DNA (p=0.001), HBcrAg (p=0.001), core-promoter mutations (p=0.040), fibrosis (p=0.033) and lobular inflammation (p=0.002). In multivariate analysis, only serum HBeAg levels <100 Paul Ehrlich Institute (PEI) U/ml and grades of lobular inflammation >2 were independent factors for early HBeAg seroconversion (odds ratio 8.430 [95% confidence interval 4.173–17.032], p<0.001; and 4.330 [2.009–9.331], p<0.001; respectively).

**Conclusions:** HBeAg levels < 100 PEIU/ml combined with grades of lobular inflammation >2 are useful for predicting early HBeAg seroconversion. In patients without liver biopsies, high ALT levels (>200 IU/L) can substitute for lobular inflammation (grades >2).

**key words:** alanine aminotransferase • chronic hepatitis • hepatitis B virus • hepatitis B e antigen • lobular inflammation • seroconversion

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## Background

Worldwide, an estimated 350 million people are infected with hepatitis B virus (HBV) persistently [1,2]. HBV infection is a major global concern, because up to 40% of patients can develop grave complications, such as decompensated cirrhosis and hepatocellular carcinoma (HCC) [3]. In the natural course of chronic hepatitis B, HBeAg seroconversion, defined by the loss of HBeAg and development of the corresponding antibody (anti-HBe), is an important hallmark, because it is highly correlated with a favorable long-term outcome. Seroconversion is usually followed by sustained suppression of HBV DNA, normalization of alanine aminotransferase (ALT) levels, and clinical remission accompanied by ameliorated necro-inflammatory activities in the liver [4–6].

To date, a number of factors have been found to predispose patients to spontaneous HBeAg seroconversion [7–19]. However, few studies have evaluated pathological factors for predicting early HBeAg seroconversion. In a small series of patients from Spain, the Knodell's index of histological activity was one of the independent predictors of early HBeAg seroconversion [14]. Recently, novel markers of the replication of HBV were introduced, such as levels of HBsAg, HBeAg and HBcrAg (HBV core-related antigen), which can replace HBV DNA levels. These serological markers of HBV replication have been evaluated for sensitive and reliable prediction of early HBeAg seroconversion [20–23]. In the present study, an attempt was made to select factors predictive of early HBeAg seroconversion, from among many biochemical, virological and pathological parameters, based on the data of 234 HBeAg-positive patients with chronic hepatitis B.

## Material and Methods

### Patients and study design

This is a retrospective cohort study with use of stored sera and liver biopsy specimens from patients with chronic hepatitis B who were taken care of in the Hepatology Department, Nagasaki Medical Center, Japan, during 1991 through 2005. The clinical database was reviewed to identify consecutive patients who underwent liver biopsies and had been followed for longer than 1 year. The inclusion criteria were presence of hepatitis B surface antigen (HBsAg) for 6 months or longer, positivity for HBeAg at the time of liver biopsy, and lack of antiviral treatments before receiving liver biopsies. The exclusion criteria were co-infection with hepatitis C virus (HCV) or human immunodeficiency virus type-1, serological markers suggestive of autoimmune disease, daily intake of alcohol >50 g, recent exposure to hepatotoxic drugs, and no stored sera available. They were followed every 3 months or more frequently, if indicated clinically, and their serum samples were monitored for liver biochemistry and serologic markers of HBV infection, including HBsAg, HBeAg, anti-HBe, HBV DNA and HBcrAg. Serum samples had been stored at –20°C until use.

Antiviral therapy was commenced immediately in the patients with: (1) significant fibrosis/cirrhosis detected by liver biopsy; and (2) evidence of decompensation, such as ascites, varices and hepatic encephalopathy.

To identify predictors of early HBeAg seroconversion, clinical, biological, virological and pathological data at the time of liver biopsy were compared between patients who did and who did not achieve early HBeAg seroconversion, within 1 year after receiving liver biopsies, by univariate and multivariate analyses. Further, patients were stratified by independent factors for HBeAg seroconversion, and the cumulative incidence of HBeAg seroconversion was compared between groups using the Kaplan-Meier method. The study protocol complied with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the review board of the institution. Each patient gave a written informed consent before participating in this study.

### Routine laboratory tests for HBV markers

Quantitative measurements of HBsAg and HBeAg were carried out using commercial enzyme-linked immunosorbent assay (ELISA) kits in the ARCHITECT ANALYSER i2000 (Abbott Japan Co., Ltd., Tokyo, Japan) in accordance with the manufactures' instructions in Nagasaki Medical Center. The sensitivity of HBsAg assay ranged from 0.05 to 250 IU/ml. Sera with HBsAg >250 IU/ml were serially diluted 100-fold so as to include them within the dynamic range. HBeAg was quantified by a two-step immunoassay with use of chemiluminescence microparticles. Briefly, undiluted samples were mixed with paramagnetic beads coated with anti-HBe. After a washing step, conjugate and reactants were added for exciting emission of the light that is proportional to the concentration of HBeAg. The result was expressed by the ratio of relative light unit (RLU) of the sample to the cut-off RLU (S/CO). Samples with S/CO values >1.0 were regarded positive for HBeAg. Then, serial dilutions of the reference standard of PE HBeAg (Paul-Ehrlich Institute, Langen, Germany) were used to define the linear range of the assay and create a reference curve for linear regression. The linear range was 0.024–100 PEIU/ml. A standard curve was produced, and linear regression was used to convert assay results into appropriate units (PEIU/ml). For samples that fell outside the linear range of the assay, the assay was performed on serial dilutions to ensure the linearity.

### HBV DNA and HBcrAg

HBV DNA was determined by the COBAS Taqman HBV test (Roche Diagnostics K.K., Tokyo, Japan). Values under or over the detection range were recorded as 2.1 or 9.1 log copies/ml. HBcrAg was measured by the CLEIA HBcrAg assay kit (Fujirebio, Inc., Tokyo, Japan) in a fully automated analyzer (Lumipulse system, Fujirebio, Inc.). Values under or over the detection range were recorded as 3.0 or 7.0 log copies/ml. Assays for HBV DNA and HBcrAg were performed in a commercial clinical laboratory (SRL, Inc., Tokyo, Japan). Sera with values over the detection range were diluted to include them within the dynamic range.

### Interferon-inducible protein 10 (IP-10)

IP-10 was quantified by the Invitrogen Human IP-10 ELISA (Invitrogen Corporation, Carlsbad, CA, USA) according to the manufacturer's protocol in Nagasaki Medical Center.

### HBV genotyping

HBV DNA was extracted from serum (100 µl) with use of the SMITEST EX R&D extraction kit (MBL Co., Ltd., Nagoya, Japan). It was amplified for determination of genotypes by

**Table 1.** Histological evaluation of liver biopsy specimens.

| (A) Fibrosis staging | |
|---|---|
| **Stage** | **Fibrosis** |
| 0 | None |
| 1 | Enlarged, fibrotic portal tracts |
| 2 | Periportal or portal-portal septa but intact architecture |
| 3 | Fibrosis with architectural distortion without obvious cirrhosis |
| 4 | Probable or definite cirrhosis |

| (B) Inflammation grading | | | |
|---|---|---|---|
| **Grade** | **Portal/periportal activity** | | **Lobular inflammation** |
| | **Piecemeal necrosis** | **Lymphocyte aggregation** | |
| 0 | None or minimal | None | None |
| 1 | Inflammation only | < 1/3 in portal triad | Inflammation alone |
| 2 | Mild | 1/3–2/3 in portal areas | Focal necrosis or acidphil bodies |
| 3 | Moderate | > 2/3 in portal areas | Severe focal cell damages |
| 4 | Severe | Entire portal triad | Damage with bridging necrosis |

the SMITEST HBV Genotyping Kit (MBL Co., Ltd.) based on hybridization with type-specific probes immobilized on a solid-phase support [24].

### Precore stop codon (G1896A) and core promoter (A1762T/G1764A) mutations

A1896 mutation in the precore (PreC) region was detected by the enzyme-linked minisequence assay (SMITEST HBV PreC ELMA, Roche Diagnostics, Tokyo, Japan), and mutations in the core promoter (CP) region for T1762/A1764 by the enzyme-linked specific probe assay (SMITEST HBV Core Promoter Mutation Detection Kit, Roche Diagnostics K.K.). The results were recorded as "the wild-type" and "mutant types" dominantly expressed by HBV isolates [25].

### Histological examination

Liver biopsy was taken by fine-needle aspiration (16G sonopsy) guided by ultrasonography. Biopsy specimens were fixed in 10% neutral formalin, cut at 3- to 4-μm thickness, and stained with Hematoxyline-Eosin and Azan-Mallory, as well as for silver to visualize reticuline fibers. Tissue sections were examined independently by two senior liver pathologists. For each biopsy specimen, a protocol was filled out for grading necro-inflammation and staging fibrosis by the criteria of Desmet et al. [26] and Scheuer [27] (Table 1). As for the portal activity, not only piecemeal necrosis, but also lymphocytic aggregation was categorized into 5 (0–4) grades in the respective area involved.

### Statistical analysis

Continuous variables were compared between groups by the Mann-Whitney *U* test, and categorical variables by $\chi^2$ and Fisher's exact tests. The cumulative incidence of HBeAg seroconversion was calculated using the Kaplan-Meier method, and the difference was evaluated by the log-rank test. Multiple logistic regression analysis was performed to identify independent factors in significant association with early HBeAg seroconversion. A p value <0.05 was considered significant. Statistical analyses were performed using the SPSS version 17.0 software package (SPSS Inc., Chicago, IL, USA).

## Results

### Baseline characteristics of patients

Among the 673 patients with HBsAg who had received liver biopsies in our hospital during 1991 through 2005, 234 (34.8%) patients who met the inclusion criteria were enrolled in this study. Demographic and laboratory characteristics at the time of liver biopsy are listed in Table 2. They had a median age of 37 years (range: 12–74), and 161 (69%) were men. Of them, 231 (99%) were infected with HBV of genotype C. The median serum ALT level at the baseline was 141 IU/l (range: 13–2644 IU/l), and the median duration of follow-up was 86.5 months (range: 12.0–213.0 months). During the follow-up, 91 (39%) received antiviral treatment, with interferon (IFN) or lamividine, or the combination thereof.

### Comparison of clinical features between patients with and without early HBeAg seroconversion

Early HBeAg seroconversion, within 1 year after receiving liver biopsies, was achieved by 58 of the 234 (24.8%) patients. In univariate analysis, factors predictive of early HBeAg seroconversion were: ALT (p=0.002), IP-10 (p=0.029), HBsAg (p=0.003), HBeAg (p<0.001), HBV DNA (p=0.001), HBcrAg (p<0.001), CP mutations (p=0.040), fibrosis (p=0.033) and lobular inflammation (p=0.002). Other factors including age, albumin, platelets, AFP, PreC mutation, cell infiltration and