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High hepatitis B virus surface antigen levels and favorable interleukin 28B genotype predict spontaneous hepatitis C virus clearance in uremic patients

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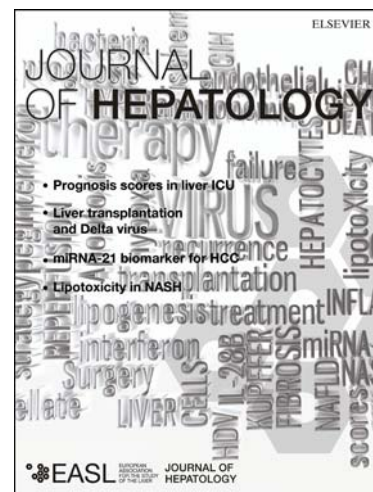
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Running title: IL-28B and HBV in HCV clearance

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Abbreviations

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; ESRD, end-stage renal disease; IL-28B, interleukin 28B; SNP, single nucleotide polymorphism

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Abstract

Background& Aims

Host and viral factors interplay in the spontaneous clearance of hepatitis C virus (HCV) infection. We aimed to explore the roles of interleukin-28B genotypes and hepatitis B virus (HBV) infections in spontaneous HCV seroclearance.

Methods

Interleukin-28B rs8099917 genotypes, HCV and HBV markers were determined in 290 patients who were seropositive for HCV antibodies from 1681 total uremic patients on maintenance hemodialysis.

Results

Persistent HCV viremia was observed in 74.6% (214/287) of patients. Logistic regression revealed that the strongest factors associated with spontaneous HCV seroclearance were carriage of rs8099917 TT-type (odds ratio/ 95% confidence intervals [OR/CI]: 6.22/1.41-27.35, $P=0.016$), followed by concurrent hepatitis B surface antigen (HBsAg) seropositivity (OR/CI: 2.37/1.06-5.26, $P=0.035$). The clearance rate was highest among patients with both positive-HBsAg/rs8099917 TT-type (44.8 %, OR/CI: 20.88/3.5-402.5), followed by positive-HBsAg/rs8099917 non-TT-type (28.6 %, OR/CI: 8.86/1.8-160.8), and negative-HBsAg/rs8099917

TT-type (26.7 %, OR/CI: 12.75/1.0-319.4), compared to 4% of negative-HBsAg/rs8099917 non-TT-type (trend P=0.0002). HBsAg levels, but not HBV DNA levels, were significantly associated with spontaneous HCV seroclearance. Spontaneous HCV seroclearance rate was 58.3% in patients with HBsAg >200 IU/mL/rs8099917 TT-type (OR/CI: 42.54/5.7-908.4), 28.0% in patients with HBsAg <200 IU/mL/rs8099917 TT-type or HBsAg >200 IU/mL/rs8099917 non-TT-type (OR/CI: 11.12/2.3-201.0), compared to only 3.3% in those with HBsAg <200 IU/mL/rs8099917 non-TT-type (trend P=0.0004). Five of 214 (2.3%) HCV viremic patients at enrollment had spontaneous HCV seroclearance during one-year follow-up, which was associated with baseline HCV RNA and HBsAg levels.

Conclusions

High HBsAg levels and favorable interleukin-28B genotype were additively associated with spontaneous HCV seroclearance in uremic patients.

Keywords: *IL-28B; HCV; HBsAg level; spontaneous clearance; uremia*

Introduction

Hepatitis C virus (HCV) infection is one of the most important causes of liver cirrhosis and hepatocarcinogenesis. Over 170 million people are chronically infected with HCV, one of the leading threats of public health worldwide[1]. Uremic patients on maintenance hemodialysis are at great risk for HCV infection. The prevalence and annual incidence of HCV infection in end-stage renal disease (ESRD) patients undergoing hemodialysis have been reported to be 10%-59% and 0.2%-6.2%, respectively [2, 3]. Taiwan has the highest prevalence and the second highest incidence of ESRD worldwide at 2,584 and 336 per million individuals, respectively[4]. Although the progression of HCV-associated liver fibrosis might be relatively slow in uremic patients, HCV-related morbidities and mortality remain the major disease burdens in the ESRD population [5, 6]. Even if interferon-based therapy is effective in some uremic patients, it remains unsatisfactory and leads to frequent and significant adverse events of HCV infection most cases[2]. As a consequence, controlling HCV infection and clarifying the driving force of HCV infection spontaneous HCV clearance in uremic patients would be particularly essential in the clinical setting.

Among subjects with HCV infections, uremic patients might exert different immunological and clinical presentations from that of the general population.

1 Impaired phagocytic and cell-mediated immunity activities might lead to the less
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4 biochemical and inflammatory activities [7]. HCV RNA levels appeared to be lower
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7 in hemodialysis patients despite their relatively immune-compromised uremic status
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10 [8].
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13 It has been reported that 10-52 % of patients with acute HCV infection were able to
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16 clear HCV spontaneously, which might correlate to age at infection, symptoms at
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19 presentation, mode of infection, rapid HCV viral decline, and the pattern and
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22 magnitude of HCV-specific CD4/CD8 cell responses[9, 10]. Host interleukin-28B
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25 (IL-28B) genetic variants have been recently recognized as the most important host
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28 factor not only for predicting anti-HCV treatment efficacy [11-13], but also for
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31 spontaneous HCV clearance [14-17]. Furthermore, hepatitis B virus (HBV) carriers
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34 might have a higher likelihood of spontaneous HCV clearance[18]. However, the
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37 determinants of spontaneous clearance of acute HCV infection in
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40 immunocompromised patients, such as patients with uremia, have not been fully
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43 investigated. Taiwan possesses the unique background to elucidate this problem
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46 because it has the highest prevalence of uremia worldwide and both HBV and HCV
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49 infections are endemic[19]. We therefore prospectively recruited a multi-center, large
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52 cohort of uremic patients with comprehensive virological and genetic information to
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55 explore the determinants of self-limited HCV infection. We also aimed to explore the
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interplay between the host genetics and viral factors in this special population.

Methods

Patient enrollment

Of 1902 uremic patients from 15 hemodialysis units (one medical center, 3 regional core hospitals and 11 regional clinics) in Taiwan, 1681 uremic patients with maintenance hemodialysis who provided informed consents were prospectively enrolled for a hepatitis surveillance project (Uremic cohort, Clinical Trial NCT01766895, appendix figure 1). All participants were tested for HCV antibodies (anti-HCV), hepatitis B surface antigen (HBsAg), biochemistry, complete blood counts, and host IL-28B genotype. Of the 1681 patients, 290 (17.3%) who had anti-HCV seropositivity at enrollment were included in the current study and were prospectively followed for more than one year (HCV-uremic cohort). None of the patients had concurrent human immunodeficiency virus infection. The study was carried out according to the guidelines of the International Conference on Harmonization for Good Clinical Practice. The ethics committee of each participating site approved the protocol for sample collection in terms of virological, biochemical, and genetic testing.

All of the blood samples were collected before beginning the process of hemodialysis.

Anti-HCV was determined by a third-generation enzyme immunoassay (Abbott

Laboratories, North Chicago, IL). HCV RNA was measured by a real-time

polymerase chain reaction assay (RealTime HCV; Abbott Molecular, Des Plaines IL, USA; detection limit: 12 IU/ml)[20] at enrollment and 6-12 months after enrollment. Two consecutive serum samples, at least six months apart, were collected to confirm spontaneous HCV seroclearance by HCV RNA testing. HCV genotypes were determined using Okamoto's method [21]. Patients seronegative for HCV RNA at enrollment were considered to be patients without spontaneous HCV seroclearance if they had received a full course of interferon-based therapy for chronic HCV infection prior to enrollment. Pretreatment biochemical and virological features were retrieved as baseline characteristics for these patients. HBsAg was measured using a standard quantitative chemiluminescent microparticle immunoassay (ARCHITECT HBsAg, Abbott Diagnostics). The concentration of HBsAg (qHBsAg) was determined using a previously generated Architect HBsAg calibration curve (range, 0.05–250 IU/mL). Samples with serum HBsAg titer >250 IU/mL were diluted at 1:20 and 1:500 with the Architect HBsAg diluent and retested to expand the upper limit of the dynamic range from 250 to 125,000 IU/mL. Serum HBV DNA for patients seropositive for HBsAg was tested with a standardized automated quantitative PCR assay (COBAS TaqMan HBV test, Roche Diagnostics, Branchburg, NJ; detection limit 12 IU/ml) [22].

IL-28B Genotyping

The IL-28B rs8099917 genotype was selected as a candidate single nucleotide

polymorphism (SNP) in the current study based on our previous studies [11, 23, 24].

Genotypes of the patients were determined using pre-designed ABI TaqMan[®] SNP genotyping assays (ABI Assay ID: C__11710096_10, Applied Biosystems, Foster City, CA, USA). Briefly, PCR primers and two allele-specific probes were designed to detect the specific SNP target. The PCR reactions were performed in 96-well microplates with ABI 7500 real-time PCR (Applied Biosystems, Foster City, USA). Allele discrimination was achieved by detecting fluorescence using the System SDS software version 1.2.3.

Statistical analyses

Frequency was compared between groups using the χ^2 test with the Yates correction or Fisher exact test. Group means, presented as mean values \pm standard deviation, were compared using analysis of variance and Student's *t* test. The Mann-Whitney U test was performed for comparing the quantifications of HBsAg levels. Serum HCV RNA levels were expressed after logarithmic transformation of the original values. The frequencies of the rare allele of the rs8099917 genotype were too low and we therefore combined the rare homozygote (GG) and heterozygote (GT) alleles together when analyzing the SNPs. The area under the curve (AUC) was compared using ROC analysis. An attempt was made to derive a suitable clinical cutoff that would best predict the spontaneous HCV seroclearance. The cutoff point was determined by

1 choosing the point on the ROC curve with the closest distance to the point of (0,1).
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4 Stepwise logistic regression analysis was applied to assess the factors associated with
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7 spontaneous HCV clearance by using age, sex, hemogram, diabetes, IL-28B genotype,
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10 and HBV status as covariants. The interactive or additive effect of the variables that
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13 contributed to HCV clearance was analyzed using the Cochran Armitage trend test.
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16 The statistical analyses were performed using the SPSS 12.0 statistical package (SPSS,
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19 Chicago, IL, USA). All statistical analyses were based on two-sided hypothesis tests
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22 with a significance level of $p < 0.05$.
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Results

The prevalence rates of HBsAg, anti-HCV, and both HBsAg and anti-HCV in the Uremic cohort of 1681 patients were 13.7 % (230/1681), 17.3 % (290/1681) and 2.4 % (41/1681), respectively. Among the 290 patients in the HCV-uremic cohort, the prevalence rate of HBsAg (14.1 %, 41/290) was comparable to the entire Uremic cohort.

Spontaneous HCV seroclearance at enrollment

After excluding 3 patients who have received interferon-based antiviral therapy, 214 (74.6 %) of the 287 anti-HCV-positive patients were HCV viremic at enrollment; the remaining 73 (25.4 %) patients seronegative for HCV RNA were regarded as patients with HCV spontaneous clearance (Appendix figure 1). None of the 41 patients with HBV dual infection had history of prior anti-HBV therapy.

Factors associated with spontaneous HCV seroclearance

Table 1 depicts the patient characteristics, liver chemistry, virological features, and frequency of the IL28B rs8099917 genotype in HCV-uremic cohorts with and without spontaneous HCV seroclearance. Compared with patients without spontaneous HCV seroclearance, those with spontaneous HCV seroclearance had significantly lower levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), a higher proportion of concurrent HBV infection (21.9 % vs. 11.2 %, $P=0.02$) and a

higher rate of carriage of the rs8099917 TT genotype (95.6 % vs. 84.6 %, $P=0.02$).

Logistic regression analysis revealed that the strongest factors associated with spontaneous HCV clearance in uremic patients were the carriage of the rs8099917 TT genotype (odds ratio [OR]/ 95% CI: 6.22/1.41-27.35, $P=0.016$), followed by concurrent HBsAg-seropositivity (OR/CI: 2.37/1.06-5.26, $P=0.035$).

Effect of HBV DNA and qHBsAg levels on spontaneous HCV seroclearance

We further evaluated the effect of HBV activity in terms of HBV DNA levels and qHBsAg titers on spontaneous HCV seroclearance among HBV/HCV dually-infected uremia patients. The HBsAg levels (AUROC 0.70, $P=0.04$) but not HBV DNA levels (AUROC 0.55, $P=0.6$) provided value for differentiating patients with spontaneous HCV clearance from HCV viremia. We further analyzed the predictive values of qHBsAg titers with different cutoffs in predicting spontaneous HCV seroclearance. HBV carriers with HBsAg more than 100 IU/mL, 200 IU/mL and 500 IU/mL had a significantly higher likelihood of spontaneous HCV seroclearance compared with their counterparts (all $P < 0.05$, table 2). HBsAg titers > 200 IU/mL provided the best accuracy and positive predictive value in predicting spontaneous HCV seroclearance, with clearance rates of 64.3% (9/14) in patients with qHBsAg > 200 IU/mL and 23.4% (64/273) in those with qHBsAg < 200 IU/mL or seronegativity ($P = 0.002$).

The cutoff point of qHBsAg, 200 IU/ml, was comparable to the best cutoff point by

choosing the point on the ROC curve with the closest distance to the point of (0,1),
138 IU/ml.

Additive effect of the IL-28B rs8099917 TT genotype and HBV dual infection in spontaneous HCV clearance

Because both host IL-28B rs8099917 TT genotype and HBV dual infection were strongly associated with spontaneous HCV clearance, we sought to evaluate the interplay between the two determinants. The clearance rate was highest among patients with positive-HBsAg and the IL-28B TT genotype (44.8 %, OR/CI: 20.88/3.5-402.5), followed by negative-HBsAg and the IL-28B TT genotype (26.7 %, OR/CI: 12.75/1.0-319.4), positive-HBsAg and the IL-28B non-TT genotype (28.6 %, OR/CI: 8.86/1.8-160.8), and lastly, negative-HBsAg and the IL-28B non-TT genotype (4.0%, reference, trend $P=0.0002$, figure 1 and table 3, model 1).

Interaction of the IL-28B rs8099917 TT genotype and HBsAg levels in spontaneous HCV seroclearance

Because HBsAg levels were significantly and independently predictive of HCV seroclearance, we further analyzed the complementary role of HBsAg levels and IL-28B genotype to predict spontaneous HCV seroclearance. The clearance rate was highest in patients with HBsAg levels >200 IU/mL and the IL28B-TT genotype (58.3 %, OR/CI: 42.54/5.7-908.4), followed by HBsAg levels <200 IU/mL and the

IL28B-TT genotype or HBsAg levels > 200 IU/mL and the IL28B-non-TT genotype (28.0%, OR/CI: 11.12/2.3-201.0) and HBsAg levels < 200 IU/mL and the IL28B-non-TT genotype (3.3%, reference, trend $P=0.0004$, figure 2 and table 3, model 2).

We further used qHBsAg levels instead as a co-variant in the logistic regression analysis to predict spontaneous HCV seroclearance (table 4). The carriage of the rs8099917 TT genotype remained the strongest factor predictive of spontaneous HCV seroclearance (OR/CI: 6.42/1.42-29.01), closely followed by qHBsAg > 200 IU/mL (OR/CI: 6.06/1.77-20.82). The status of HBsAg seropositivity with qHBsAg < 200 IU/mL was not predictive of spontaneous HCV seroclearance. The seroclearance rate was similar between those seropositive for HBsAg with qHBsAg < 200 IU/mL and those seronegative for HBsAg (26.9 % vs. 23.1%, $P = 0.66$).

Virological features at the end of follow-up

At the end of follow-up, all of the 73 HCV non-viremic patients at enrollment remained seronegative for HCV RNA. None of the 41 HBV carriers in the HCV-uremic cohort experienced HBsAg loss or HBsAg seroconversion during the follow-up period. Five of the 214 (2.3%) HCV viremic patients, including three HCV genotype 1b and two genotype 2a, became HCV RNA seronegative for > 6 months (Appendix Table 1). All of the five patients were female and carried IL-28B

rs8099917 TT genotype. The only one patient with HBV dual infection had a high HBsAg level of 1129 IU/mL. Logistic regression analysis revealed that qHBsAg > 200 IU/mL (OR/CI: 33.87/1.99-577.08, P = 0.015) and low HCV RNA levels at enrollment (OR/CI: 0.41/0.20-0.83, P = 0.013) were independently associated with HCV RNA seronegativity during follow-up (Table 5).

Discussion

In the current study, we demonstrated that the HCV spontaneous seroclearance rate in uremic patients was 25.4 %, which was comparable to that of the general population in a community-based setting (25.5%)[25]. One-third of uremic patients carrying the favorable host-IL28B genotype had spontaneous HCV seroclearance, in contrast to only 9.4% of those with unfavorable IL28B genotypes. Our results demonstrated that the concurrence of HBV infection not only had an effect on spontaneous HCV seroclearance, but also acted additively with the favorable IL28B genotype on HCV seroclearance. Notably, the spontaneous HCV seroclearance rate reached 64.3% in patients with HBsAg levels >200 IU/mL. Our study therefore provides novel evidence regarding viral interference in uremic patients.

Interestingly, spontaneous HCV seroclearance, although only 2.3% in HCV viremic patients with subsequent one-year follow-up, was associated with high HBsAg levels and low HCV viral loads.

The precise mechanisms of spontaneous HCV clearance remain unclear. Genetic variants of cytokines have been continuously reported.[26] Recently, a favorable IL28B genotype has been shown to be the most important host factor predictive of spontaneous HCV seroclearance across different ethnicities and HCV genotypes [14-17, 27, 28]. The exact mechanisms of HCV susceptibility or viral clearance in uremic patients also remain unclear.

The impairment or insufficiency of phagocytic, chemotactic and cell-mediated immunological

activities may be involved. Female gender has been reported to play either a synergistic or an independent role with IL-28B genotype in associating with HCV clearance in general population [27, 28]. In the current study, we did not observe the role of gender on spontaneous HCV seroclearance in uremic patients. However, the effect of IL-28B genotypes on the viral clearance of HCV held true in uremic patients as with the general population. Carriage of the favorable IL-28B genotype was the single best host factor predictive of self-limited HCV infection in the current study.

The interactive and suppressive effects among hepatotropic viruses on the same host might be dynamic and complicated [29, 30]. HBV co-infection has been associated with spontaneous HCV seroclearance. We have previously demonstrated that HBV/HCV dual infection was associated with a higher likelihood of spontaneous HCV clearance in the general population [18]. A six-fold chance of HCV seroclearance was reported in intravenous drug abusers with HBV co-infection compared with those who were never exposed to HBV, but the spontaneous clearance rate did not differ between patients negative for both HBsAg/antibody to HBV core antigen (anti-HBc) and patients with negative-HBsAg but positive-anti-HBc [28]. However, the REVEAL study pointed out that although the HCV RNA levels were substantially lower among HCV patients with positive-HBsAg compared with those with negative-HBsAg, the difference was not significant [31]. In the current study, concurrent HBV infection remained a predictive factor for self-limited HCV infection in a uremic

population. Importantly, we determined that the effect of HBV-related HCV seroclearance was associated with and determined by serum HBsAg levels but not by HBV DNA levels.

HBV carriers with HBsAg > 200 IU/mL had a significantly higher chance to have HCV clearance compared with those with HBsAg < 200 IU/mL (64.3 % vs. 26.9 %). By contrast, the rate of HCV viremia was similar between HBV carriers with HBsAg < 200 IU/mL and those without HBV dual infection. Our findings might explain, in part, the different observation among previous studies.

Currently, there are two quantitative serum markers representing the status of HBV infection, the HBsAg levels and HBV DNA levels. There is a positive correlation between both markers in the course of HBV infection or in patients undergoing antiviral therapy[32].

Serum HBV DNA levels represent the HBV replication. By contrast, serum HBsAg levels might represent transcriptionally active cccDNA and are considered a surrogate marker of viral activity in infected cells [32]. Unlike serum HBV DNA levels that reflect viral replication, a change in HBsAg levels represents variation in the translation of mRNAs produced from transcriptionally active cccDNA or integrated sequences, which in part represent the interaction between HBV and host immune control. Therefore, HBsAg levels represent the complex equilibrium between the host's immune system and the virus as well as the product of the transcription of specific mRNAs rather than only viral replication [33].

Furthermore, in vitro study showed that there is little direct interaction of HBV and HCV in

co-infected hepatocytes. However, extensive colocalization of HBsAg and HCV E2 in cells that harboured both viruses were observed, indicating that some aspects of the viral lifecycles cross paths [34]. The spontaneous clearance of HCV depends not only on viral-viral interaction, as reflected by HBV DNA levels, but also the eliciting of host immunity by the presence of HBV[10, 26]. Further fundamental studies are warranted to truly clarify the pathophysiology to explain the clinical observation.

Importantly, we also noted an additive effect between HBV infection and favorable IL-28B genotype in HCV clearance. Almost all patients with the unfavorable IL-28B genotype and without HBV infection developed chronicity once they were exposed to HCV. Conversely, half of patients with the favorable genotype and concurrent HBV infection could clear HCV spontaneously. Furthermore, two-thirds of HBV carriers with the favorable IL-28B genotype could clear HCV if their HBsAg levels were greater than 200 IU/mL. These results provide information for decision-making in the management of HCV infection in clinical settings.

Nevertheless, the results should be further validated across ethnicities in the general population. There were some limitations in the current study. Firstly, we did not check HCV RNA for anti-HCV-negative individuals to determine the status of anti-HCV-negativity but HCV-viremia [35]. However, based on our previous study, there were no uremic patients seronegative for anti-HCV but seropositive for HCV RNA using current anti-HCV assays [36]. Therefore, we did not check HCV RNA for individuals seronegative for anti-HCV in the

current study. Secondly, our results did not appear to clarify the acquisition sequence of the two viruses. Nonetheless, most dual viral infections are in an HBV-then-HCV order in Taiwan. Third, the time course of acute HCV infection is often obscured due to the asymptomatic clinical presentation. The definition of chronic HCV infection remains challenging. It has been reported that up to 17% of patients who have self-limited HCV infection cleared the virus beyond 1 year of exposure [37]. We observed five HCV viremic patients at enrollment had HCV seroclearance during the subsequent one-year follow-up. All carried IL-28B favorable genotype; the only one patient with HBV dual infections had high HBsAg levels. Although baseline HBsAg and HCV RNA levels were shown to be associated with subsequent spontaneous HCV seroclearance in the current study, more patients with longer duration of follow-up is needed to explore the conclusive results.

In conclusion, we demonstrated that the spontaneous HCV seroclearance rate in uremic patients was comparable to that of the general population. Both the favorable host IL-28B genotype and high HBsAg levels were significant determinants of spontaneous HCV clearance in uremic patients. The concurrence of HBV infection could additively determine self-limited HCV infection with the favorable IL28B genotype in clinical settings.

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Figure legends

Fig 1. The complementary influence of host Interleukin-28B genetic variants and HBV dual infection status in the spontaneous seroclearance of HCV infection in uremic patients. HBV, hepatitis B virus; TT, interleukin-28B rs8099917 TT genotype.

Fig 2. The complementary influence of host Interleukin-28B genetic variants and HBsAg levels in the spontaneous seroclearance of HCV infection. qsAg>200, HBsAg levels >200 IU/ml; TT, interleukin-28B rs8099917 TT genotype.

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Table 1. Characteristics of the 287 HCV-uremic patients at enrollement and factors associated with spontaneous HCV seroclearance

	All patients (N=287)	Spontaneous HCV seroclearance (n=73, 25.4 %)	No spontaneous HCV seroclearance (n=214, 74.6 %)	P value	Logistic regression analysis		
					OR	95% CI	P value
Age (years, mean(SD))	62.0 (11.6)	60.9 (10.5)	62.4 (12.0)	0.35			
Male, n (%)	115 (40.1)	27 (37.0)	88 (41.1)	0.53			
Duration of hemodialysis (years, mean(SD))	9.9 (8.2)	8.9 (7.8)	10.3 (8.4)	0.25			
BMI (kg/m ² , mean (SD))	21.2 (3.5)	21.2 (3.0)	21.2 (3.7)	0.97			
AST (IU/L, mean (SD))	26.1 (13.2)	20.7 (8.3)	27.8 (14.1)	<0.001			
ALT (IU/L, mean (SD))	26.3 (18.5)	17.1 (6.8)	29.5 (20.0)	<0.001			
White blood cell count (x10 ³ u/L, mean (SD))	6.52 (2.40)	6.35 (2.12)	6.58 (2.50)	0.53			
Hemoglobin (g/dL, mean± SD)	10.7 (1.4)	10.7 (1.4)	10.7 (1.5)	0.79			
Platelet count (x10 ³ u/L, mean± SD)	174 (63)	173 (55)	175 (66)	0.79			
Diabetes, n/N (%)*	93/266 (35.0)	18/67 (26.9)	75/199 (37.7)	0.11			
HBsAg (+), n (%)	40 (13.9)	16 (21.9)	24 (11.2)	0.02	2.37	1.06-5.26	0.035
IL28B rs8099917 TT genotype, n/N (%)^	224/256 (87.5)	65/68 (95.6)	159/188 (84.6)	0.02	6.22	1.41-27.35	0.016
HCV RNA (log IU/mL, mean (SD))		-	5.37 (1.31)				
HCV genotype, n (%)		-					
1			116 (54.2)				
Non-1			98 (45.8)				

Note : SD: standard deviation; OR: odds ratio; CI: confidence intervals; BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase.* history and data available in 266 patients. ^Data available in 256 patients.

Table 2. Influence of HBV DNA and HBsAg levels in spontaneous HCV seroclearance among HBsAg-seropositive uremic patients

Variables	clearance n (%)	viremia n (%)	<i>P value</i>	SEN %	SPE %	PPV %	NPV %	ACC %
HBV DNA level*	n=15	n=24						
detectable	7 (46.7)	12 (50.0)	0.84	47	50	37	60	49
>2000 IU/mL	5 (33.3)	6 (25.0)	0.72	33	75	46	64	59
>10,000 IU/mL	5 (33.3)	6 (25.0)	0.72	33	75	46	64	59
HBsAg levels	n=16	n=24						
>50 IU/mL	11 (68.8)	10 (41.7)	0.09	69	58	52	74	63
>100 IU/mL	10 (62.5)	7 (29.2)	0.04	63	71	59	74	68
>200 IU/mL	9 (56.3)	5 (20.8)	0.02	56	79	64	73	70
>500 IU/mL	8 (50.0)	5 (20.8)	0.05	50	79	62	70	68
>1000 IU/mL	6 (37.5)	5 (20.8)	0.30	38	79	55	66	63
>2000 IU/mL	4 (25.0)	2 (8.3)	0.20	25	92	67	65	65

*data unavailable in 1 patient.

Table 3. Interaction of host IL-28B genotype and HBsAg on spontaneous HCV clearance in HCV-uremic patients

	Clearance rate, n/N (%)	OR	95% CI	Adjusted P value*	Trend P value
Model 1					
HBsAg (-)/IL28B-non-TT	1/25 (4.0)	1		Ref	0.002
HBsAg (+)/IL28B-non-TT	2/7 (28.6)	8.86	1.8-160.8	0.0039	
HBsAg (-)/IL28B-TT	52/195 (26.7)	12.75	1.0-319.4	0.0506	
HBsAg (+)/IL28B-TT	13/29 (44.8)	20.88	3.5-402.5	0.0002	
Model 2					
qHBsAg < 200 IU/ml/IL28B-non-TT	1/30 (3.3)	1		Ref	0.0004
qHBsAg < 200 IU/ml/IL28B-TT or qHBsAg > 200 IU/ml/IL28B-non-TT	60/214 (28.0)	11.12	2.3-201.0	0.0008	
qHBsAg > 200 IU/ml/IL28B-TT	7/12 (58.3)	42.54	5.7-908.4	<0.0001	

Note: *adjust age and sex. qHBsAg, HBsAg levels, qHBsAg < 200 IU/mL included HBsAg (-). CI, confidence intervals. IL28B-TT, IL-28B rs8099917 TT genotype.

Table 4. Logistic regression analysis of factors associated with HCV spontaneous clearance by using serum HBsAg levels as co-variant in uremic patient

Variables	OR	95% C.I.	<i>P</i> value
HBsAg status			
HBsAg (-)	1		
HBsAg (+)/qHBsAg < 200 IU/mL	1.20	0.40-3.55	0.75
HBsAg (+)/qHBsAg > 200 IU/mL	6.06	1.77-20.82	0.004
Rs8099917			
GT/GG-genotype	1		
TT genotype	6.42	1.42-29.01	0.02

Note: qHBsAg, HBsAg levels. OR, odds ratio. C.I., confidence interval

Table 5. Factors associated with HCV RNA seronegativity during one-year follow-up among the 214 HCV-viremic uremic patients at enrollment

	Persistent viremia (n=209)	HCV RNA seronegativity (n=5)	P value	Logistic regression analysis		
				OR	95% CI	P value
Age (years, mean(SD))	62.3 (12.0)	62.4 (12.0)	0.39			
Male, n (%)	88 (42.1)	0 (0)	0.08			
BMI (kg/m ² , mean (SD))	21.3 (3.1)	18.3 (3.2)	0.25			
AST (IU/L, mean (SD))	27.8 (14.2)	29.5 (5.2)	0.81			
ALT (IU/L, mean (SD))	29.6 (20.3)	23.2 (5.9)	0.48			
White blood cell count (x10 ³ u/L, mean (SD))	6.59 (2.51)	5.87 (2.32)	0.62			
Hemoglobin (g/dL, mean± SD)	10.7 (1.5)	10.9 (0.6)	0.72			
Platelet count (x10 ³ u/L, mean± SD)	175 (66)	176 (39)	0.98			
Diabetes, n/N (%)*	75/194 (38.7)	0 (0)	1			
HBsAg (+), n (%)	23 (11.0)	1 (20.0)	0.45			
HBsAg > 200 IU/mL in HBV carriers, n/N (%)	4/23 (17.4)	1/1 (100)	0.21			
HBsAg > 200 IU/mL, n (%)	4 (1.9)	1 (20.0)	0.11	33.87	1.99-577	0.015
HCV genotype 1b/non-1b	113/96	3/2	1			
IL-28B rs8099917 TT genotype, n/N (%)^	154/183 (84.2)	5/5 (100)	1			
HCV RNA at enrollemnt (log IU/mL, mean (SD))	5.41 (1.30)	3.97 (0.70)	0.015	0.41	0.20-0.83	0.013

Note : SD: standard deviation; OR: odds ratio; CI: confidence intervals; BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase.* history and data available in 194 patients. ^Data available in 188 patients.

Fig. 1

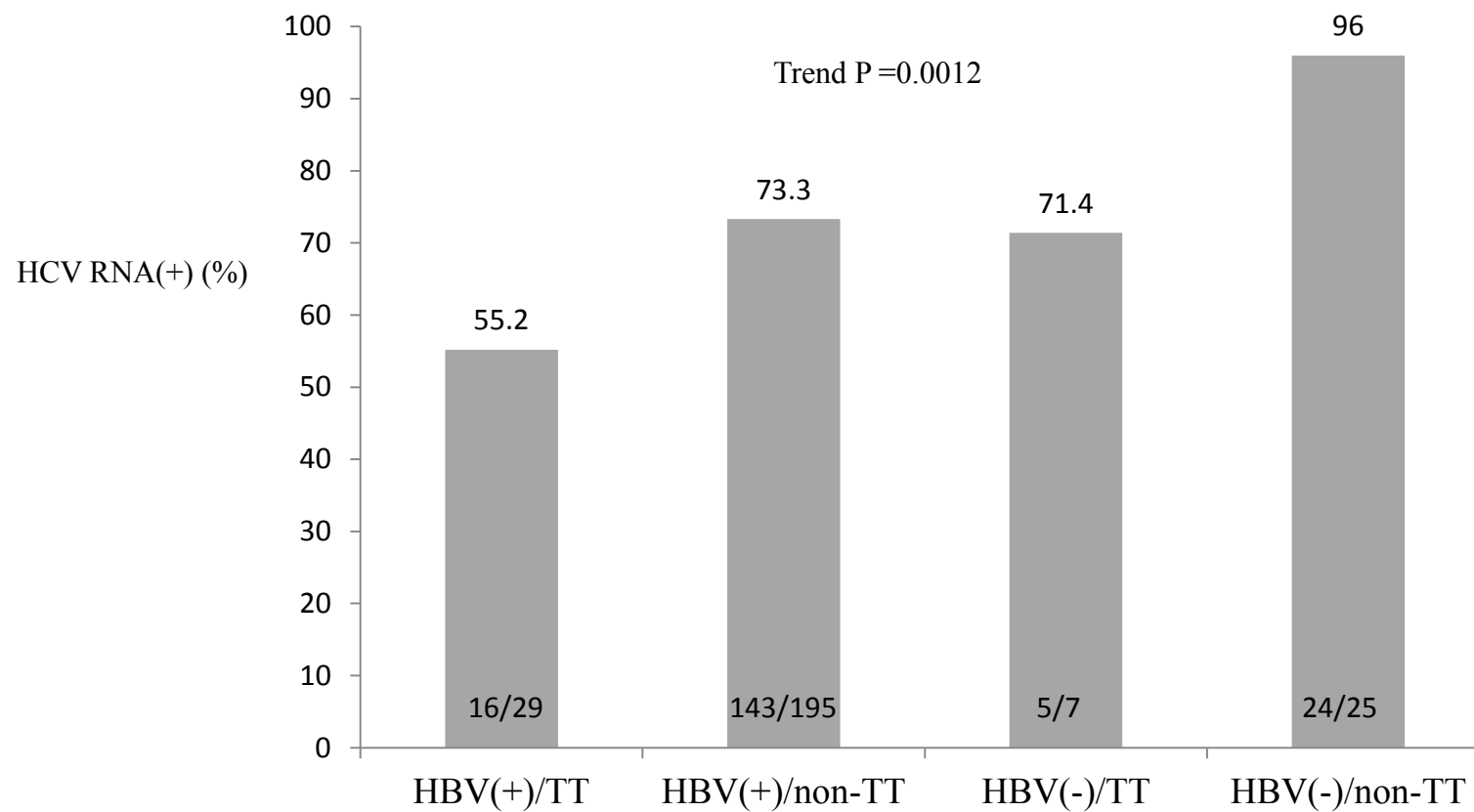


Fig. 2

