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HIV co-infection accelerates decay of humoral responses in spontaneous resolvers of HCV infection

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SUMMARY. Acute hepatitis C virus (HCV) infection is primarily followed by chronic infection, while spontaneous recovery of HCV infection (SR-HCV) occurs in a minority of those infected. Identification of SR-HCV clinically depends on two combined indicators, persistently undetectable peripheral HCV RNA and positivity for anti-HCV. However, the characteristics of dynamic variation in anti-HCV antibodies in SR-HCV, especially in those patients co-infected with HIV, are still undefined. In this study, a cohort of patients infected with HCV through commercial blood collection practices was studied. We found that the annual decreasing rate of anti-HCV presented a gradually accelerated process in HCV resolvers. However, the variation in the decline of anti-HCV presented a slowly accelerated process within the early decrease stage and a gradually decelerated process within the latter decrease stage. In addition, we deduced that it expended approximately 16 years from natural HCV recovery to undetectable peripheral anti-HCV in HCV resolvers co-infected with HIV, while this time was estimated to be 20 years in SR-HCV without HIV co-infection. Our data indicated that the decay of anti-HCV was accelerated by HIV-related impairment of immune function. The prevalence of HCV infection may be severely underestimated in this large-scale retrospective epidemiologic investigation in an HIV-infected population.

Keywords: anti-HCV antibodies, CD4+T counts, HCV, HIV, spontaneous recovery.

INTRODUCTION

The typical chronically hepatitis C virus (HCV)-infected patient shows strong reactivity for HCV antibodies and high titres of circulating HCV RNA [1]. Diagnosis of spontaneous resolution of a prior HCV infection depends on continued negativity when monitoring for HCV RNA and positivity for anti-HCV responses[2,3]. Although the idea is widely accepted that SR-HCV patients may show a gradual attenuation, after years or decades, of their anti-HCV responses, a detailed chronology of the loss of the anti-HCV responses from the starting point of HCV recovery has been rarely documented. A study that followed a small size cohort of patients accidentally exposed to HCV concluded that 5 of 10 SR-HCV individuals cleared circulating HCV-specific humoral responses 18–20 years after infection [4]. However, whether differences in such factors as living environment, ethnicity and HIV status will alter the time taken for HCV-specific antibody responses to become undetectable in SR-HCV individuals is largely undefined.

In this study, a cohort that had become infected with HCV mainly as a result of unsanitary blood donation practices was recruited. Dynamic changes in anti-HCV were monitored in SR-HCV individuals, grouped as to whether they were co-infected with HIV or not. Our data provide valuable information in evaluating the incidence of anti-HCV seropositivity, especially in the HIV-positive population.

Abbreviations: CMIA, chemiluminescent microparticle immunoassay; HCV, hepatitis C virus; HPV, human papillomavirus; SR-HCV, spontaneous recovery of hepatitis C virus infection.

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MATERIALS AND METHODS

Initial investigation of chronic HCV infection, HCV recovery and follow-up

From 14 August 2009 to 27 August 2009, 335 patients with negative HBsAg and positive anti-HCV responses from a village in Shangcai county, Henan province of China,

were initially investigated. Subsequently, a follow-up study was performed between 15 August 2012 and 23 August 2012, when 212 of 335 patients were seen for follow-up investigation. The remaining 123 persons were either dead or lost contact. All of the enrolled patients had never received any form of HCV-specific antiviral therapy. Based on their anti-HCV, HCV RNA and anti-HIV status, measured in samples collected in both 2009 and 2012, the 212 individuals were divided into four groups: HIV-1^{neg} Chronic HCV carriers (HIV^{neg} chronic HCV) containing 73 subjects; HIV-1^{pos} Chronic HCV carriers (HIV^{pos} chronic HCV) containing 66 subjects; HIV-1^{neg} spontaneous HCV resolvers (HIV^{neg} SR-HCV) containing 40 subjects; and HIV-1^{pos} spontaneous HCV resolvers (HIV^{pos} SR-HCV) containing 33 subjects. The demographic characteristics of the 212 patients investigated in 2009 are presented in Table S1. There was gender imbalance in the frequency of HCV spontaneous recovery in women being significantly more likely to resolve their infection than men, independently of HIV infection [5–7], which is indicated in Figure S1. Additionally, a total of 18 cryopreserved HIV-positive sera collected in March 2005 from the same village were kindly provided by Dr. Zhang[8,9]. All of these patients belonged to the HIV^{pos} SR-HCV patient group and are included in the cohort investigated in 2009 and 2012. A flow diagram for recruited persons is indicated in Figure S2.

Routine blood tests, anti-HIV and CD4+/CD8+ T-cell counts were performed by the local CDC. The study was approved by the Institutional Review authorities of Peking University Health Science Center, and informed consent forms were signed by all participants.

Recruitment of acute HCV-infected patients

A total of 45 outpatients with acute HCV infection in the Sixth subsidiary Sun Yat-sen University Hospital from April 2011 to December 2012 were included in our study. HIV- and HBV-infected patients were excluded from our cohort. The time range from possible time of HCV infection to positive anti-HCV was estimated to be <6 months. The basic characteristics of these 45 patients are shown in Table S2.

Detection of HCV RNA and HCV genotyping

Hepatitis C virus RNA level from EDTA anticoagulated plasma was measured with the Abbott Realtime™ HCV Amplification Kit (Abbott Molecular Inc., Des Plaines, IL, USA) according to the manufacturer's instructions. Hepatitis C virus genotyping was performed by RT-PCR as described previously[10].

Detection and analysis of anti-HCV antibody response

All sera were tested for the presence of anti-HCV using the Abbott Architect anti-HCV assay (product code: 6C37; Abbott GmbH & Co. KG, Wiesbaden, Germany), which is a semiquantitative method based on chemiluminescent microparticle immunoassay (CMIA) technology. Sample/cut-off (S/CO) ratios of greater than or equal to 1.0 were considered reactive for anti-HCV. Anti-HCV status of all SR-HCV persons was confirmed by HCV-RIBA assay (Wantai Biological Pharmacy, Beijing, China), which utilizes recombinant proteins (Core, NS3, NS4 and NS5) immobilized as individual bands onto test strips. The intensity of the HCV bands was scored in relation to the intensities of the internal IgG controls as five different levels: 0(none), +, ++, +++, ++++, according to the manufacturer's instructions.

Predicting time of anti-HCV decay at different ranges of anti-HCV in SR-HCV individuals

The time (n, years) from baseline S/CO value to lower level S/CO values was assessed using the following formula A:

$$n(\text{years}) = log_{10}(\text{lower level}S/CO \div baselineS/CO) \div log_{10}(1 - \text{annual decreasing rate}\%)$$

Statistical analysis

Spearman's rank-correlation, Wilcoxon matched-pairs, Mann–Whitney U-tests and Pearson Chi-Square tests (Yates' correction for continuity is used in certain situations) were performed using SPSS 18.0 software (Chicago, IL, USA) when necessary.

RESULTS

Co-infection with HIV enhances the decrease in anti-HCV in SR-HCV individuals

To provide evidence in support of the widely held belief that anti-HCV decreases gradually with time after an individual acquires SR-HCV status, anti-HCV titres of patients in the four different groups obtained in 2009 were compared with those measured in 2012. This revealed that anti-HCV gradually decreased over a 3-year period in both HIV^{neg} SR-HCV ($P < 0.001$) and HIV^{pos} SR-HCV ($P < 0.001$) (Fig. 1a). By contrast, no such differences were found in the HIV^{neg} chronic HCV and HIV^{pos} chronic HCV groups (Fig. 1a). The profiles of the anti-HCV in HCV resolvers were retested by RIBA assay subsequently. We found that anti-HCV responses induced by the core and NS3 proteins were the primary components of anti-HCV as measured by the CMIA assay (Figure S3).

The average annual decreasing rate of anti-HCV in HIV^{pos} SR-HCV and HIV^{neg} SR-HCV patients from 2009 to 2012 was calculated as 14.26% and 11.31%, respectively. The difference in these two rates of decline was statistically significant ($P = 0.037$) (Fig. 1b). Importantly, the annual decrease in anti-HCV of HIV-infected patients was found to be negatively correlated with their CD4+ T-cell counts

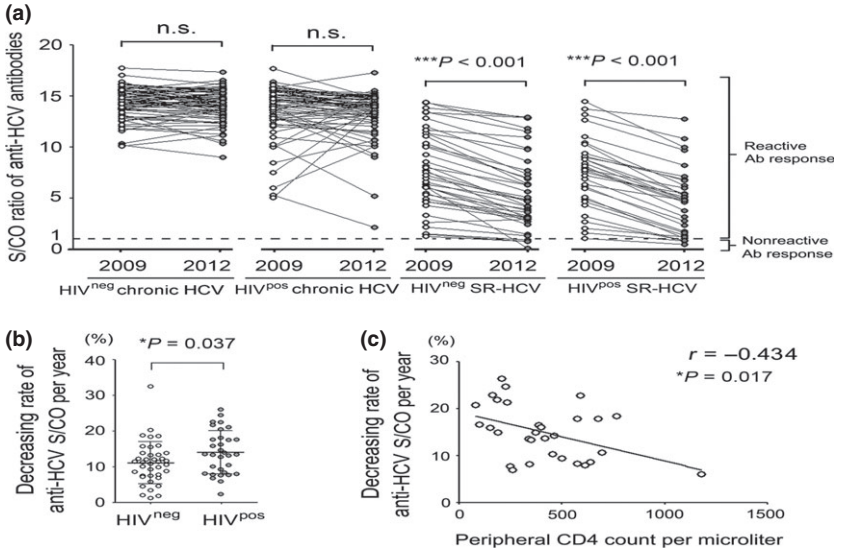

Fig. 1 HIV-related immune dysfunction enhanced the decreasing rate of hepatitis C virus (HCV)-specific antibody responses in HCV resolvers. **(a)** HCV-specific antibody responses decreased as time progressed in HCV spontaneous resolvers, while no decreasing trends of HCV antibody titres were found in chronic HCV carriers. The anti-HCV titres in four different groups (HIV^{neg} chronic HCV, HIV^{pos} chronic HCV, HIV^{neg} SR-HCV and HIV^{pos} SR-HCV) between 2009 and 2012 were analysed by the paired comparison method. **(b)** Comparison of the annual decreasing rate of HCV-specific antibody S/CO values between HIV-uninfected and HIV-infected subjects. **(c)** The annual decreasing rates of HCV-specific antibodies of HIV-infected patients correlate negatively with peripheral CD4+ T-cell counts in HIV-infected patients. $*P < 0.05$; $***P < 0.001$.

($r = -0.434$, $P = 0.017$)(Fig. 1c). These data imply that the immune dysfunction induced by HIV co-infection can enhance the rate of decrease in anti-HCV responses in SR-HCV individuals.

The annual decreasing rate of anti-HCV is gradually accelerating in SR-HCV individuals

In our study, a significant negative correlation was found between the annual rate of decrease in anti-HCV S/CO values and the initial anti-HCV S/CO values in both the HIV^{neg} SR-HCV ($R^2 = 0.316$, $P < 0.001$, Fig. 2a) and HIV^{pos} SR-HCV ($R^2 = 0.470$, $P < 0.001$, Fig. 2b) groups. Linear regression equations are shown as '$y = -0.894x + 18.35$' and '$y = -1.192x + 23.35$' respectively.

Further, to support the idea that the annual decreasing rate of anti-HCV is gradually accelerating in SR-HCV individuals, from the 33 HIV^{pos} SR-HCV individuals in the study cohort, 18 sera collected in March 2005 were retrospectively traced (Fig. 2c). The annual rate of decrease in S/CO values from 2005 to 2009 was significantly lower than the corresponding rate between 2009 and 2012 ($P < 0.001$, Fig. 2d).

Predicting annual decay rate and time of anti-HCV S/CO values in SR-HCV individuals

Depending on the linear regression equations '$y = -0.894$x $+18.35$' and 'y $= -1.192$x$+23.35$' shown in Fig. 2a, b, the annual decreasing rate of anti-HCV at different ranges of anti-HCV S/CO values (e.g. 15–14, 14–13, 13–12……) is predicted in Fig. 3a, which clearly shows that the annual decreasing rates of anti-HCV increased gradually over time and HIV co-infection significantly enhanced this decrease in SR-HCV individuals ($P < 0.001$).

Based on the predicted decreasing rate of anti-HCV indicated in Fig. 3a and using Formula A, the deduced decaying time of anti-HCV in SR-HCV subjects at different ranges of anti-HCV S/CO values is shown in Fig. 3b. Clearly, a significant difference in the deduced decaying time of anti-HCV between the HIV^{neg} SR-HCV and HIV^{pos} SR-HCV groups was observed ($P < 0.001$) (Fig. 3b).

Interestingly, the decaying time in anti-HCV followed a slowly accelerating process within the early decrease stage (S/CO value ≥9). However, the decaying time of anti-HCV was shown to gradually decelerate if S/CO values of anti-HCV dropped to the latter decrease stage (S/CO value <9) (Fig. 3b).

Predicting the overall time for loss of detectable anti-HCV reactivity in SR-HCV individuals

The deduced decaying time of anti-HCV in SR-HCV individuals to different ranges of anti-HCV S/CO values was calculated (Fig. 3b), and then, the overall time for the anti-HCV in these individuals to drop from a detectable level at the point of resolution of the infection to undetectable levels was predicted. As shown in Fig. 3c (for HIV^{neg} population)

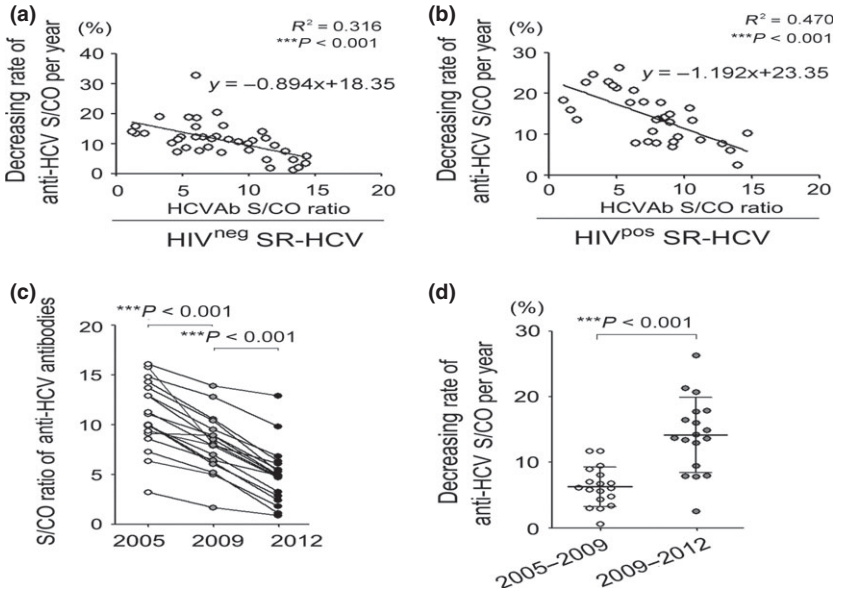

Fig. 2 The decrease in anti-HCV presented a gradually accelerated process in HCV spontaneous resolvers. (a, b). Significant correlations ($P < 0.001$) between the annual decreasing rates and the initial anti-HCV S/CO values were observed in HIV-uninfected **(a)** and HIV-infected **(b)** HCV resolvers, respectively. Linear regression equations were analysed by SPSS 18.0 software. **(c)** The HCV antibody titres of 18 HIV-infected HCV resolvers presented a significant decreasing trend from 2005 to 2009 and 2012. (D). The annual decreasing rate of anti-HCV from 2005 to 2009 is lower than the annual rate from 2009 to 2012. $^{**}P < 0.01$; $^{***}P < 0.001$.

and Fig. 3d (for HIV^{pos} population), the overall time of positive anti-HCV from different detectable levels to 1 was calculated by adding up the corresponding deduced decaying time at different ranges of anti-HCV S/CO values (Fig. 3c).

Our study also included a total of 45 acute HCV-infected outpatients from April 2011 to December 2012. The median value and interquartile range of anti-HCV S/CO values for these 45 patients were calculated as 13.88 and 13.37–14.35, respectively (Table S2). Herein, we hypothesized that the hepatic and circulating HCV particles in these patients were spontaneously cleared from the acute stage of infection immediately. Alternatively, approximately 14.0 was assumed to be the average starting value of anti-HCV S/CO of viral clearance for acute HCV infections recruited in this study. Thus, anti-HCV reactivity can be expected to become undetectable in HIV^{neg} SR-HCV individuals in 19.8 years (approximately 20 years) indicated by a red arrow in Fig 3c, whereas in an HIV^{pos} SR-HCV person, the corresponding time is 15.8 years (approximately 16 years) indicated by a blue arrow in Fig. 3d.

DISCUSSION

The exact time point of infection for each subject in the study cohort has not been established as the acute phase of infection is usually asymptomatic and therefore rarely detected. As a consequence, calculating the time span required for clearance of anti-HCV antibodies in those SR-HCV individuals has previously proved difficult, as defining the potential starting point for virus clearance, that is: the time of initial HCV infection has not been usually possible. In this study to avoid the requirement for long-term monitoring of SR-HCV patients, reasonable assumptions were made following analysis of serum samples from a cohort of SR-HCV individuals containing both HIV^{neg} and HIV^{pos} members. This allowed a conclusion to be drawn that on average, it will take approximately 20 years for HCV antibodies to become undetectable in SR-HCV patients. Also, the presence of HIV^{pos} individuals in the SR-HCV group in the present larger patient cohort has also allowed the impact of HIV co-infection on the loss of HCV antibodies to be assessed. This has revealed a significant shortening of on average 4 years in the time taken for circulating HCV antibodies to be lost.

Although the annual decreasing rate of anti-HCV was gradually accelerating in HCV resolvers, the decaying time of anti-HCV presented a different mode of variation, which was characterized by a slowly accelerated process within the early decrease stage and a gradually decelerated process within the latter decrease stage. Similar modes in decay of antibodies were also found in persistence of

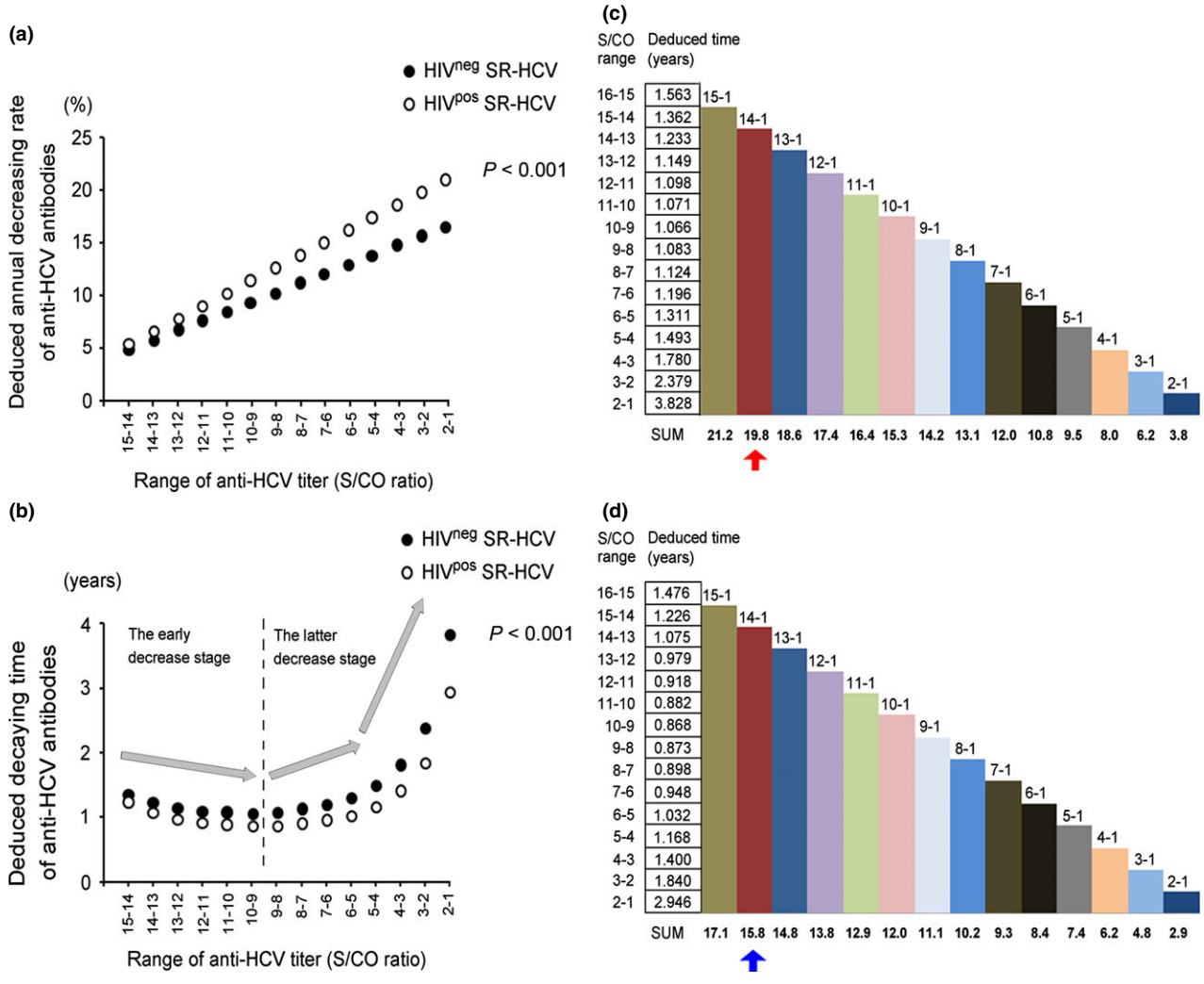

Fig. 3 Predicting overall time for loss of detectable anti-HCV in SR-HCV individuals. **(a)** The average annual decreasing rate of anti-HCV of HIV^{neg} SR-HCV (●) and HIV^{pos} SR-HCV (○) groups at different ranges of anti-HCV S/CO values was predicted based on the corresponding linear regression equations. **(b)** The deduced decaying time of anti-HCV of HIV^{neg} SR-HCV (●) and HIV^{pos} SR-HCV (○) groups at different ranges of anti-HCV S/CO values was predicted based on the corresponding predicted annual decreasing rate of anti-HCV antibodies. A significant difference of the deduced decaying rate ($P < 0.001$) and time ($P < 0.001$) of anti-HCV between HIV^{neg} SR-HCV and HIV^{pos} SR-HCV groups was verified by Wilcoxon matched-pairs *t*-test ($P < 0.001$). **(c) (d)** The overall time ranges for the level of anti-HCV in SR-HCV individuals to drop from a detectable level at the point of resolution of the infection to S/CO values equal to one were predicted both for HIV-uninfected population **(c)** and HIV-infected population **(d)**.

anti-HAV after hepatitis A vaccination [11–13] and long-term antibody responses followed by a human papillomavirus (HPV) vaccination [14]. Evaluating decaying dynamics of long-term persistent virus-specific antibody responses can be made by using a variety of mathematical models. However, the underlying reason for this decaying mode of anti-HCV responses in SR-HCV is still unknown.

To our knowledge, this is the first study to analyse the characteristics of the decrease in HCV-specific antibodies following spontaneous viral clearance, and how concomitant HIV infection influences this. At present, a positive HCV-specific antibody response is still regarded as a pivotal and nonsubstitutable indicator in diagnosing a prior HCV infection. This being the case, in large-scale retrospective epidemiologic investigations, the prevalence of HCV infection may be underestimated particularly in the HIV-infected population. Some adjustment should be made to correct the true rate of HCV infection and novel biomarkers, and easier-to-use manipulation procedures should also be explored.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1: Comparison of genders distribution in chronic hepatitis C patients and HCV spontaneous resolvers in the cohort.

Figure S2: Flow diagram for persons tested for HCV infection in our study.

Figure S3: HCV antibody responses induced by core and NS3 proteins are a pivotal component of S/CO ratios of HCV-Abs measured by the chemiluminescent microparticle immunoassay.

Table S1: Characteristics of patients investigated in August 2009 in the study, including persons with chronic HCV infection and persons who recovered from HCV, and persons with HIV-infected and uninfected status.

Table S2: Basic information of 45 outpatients with acute HCV infection in Sixth subsidiary Sun Yat-sen University Hospital (April 2011- December 2012).