



RAPID COMMUNICATION

Factors influencing a low rate of hepatitis C viral RNA clearance in heroin users from Southern China

Rebecca J Garten, Sheng-Han Lai, Jin-Bing Zhang, Wei Liu, Jie Chen, Xiao-Fang Yu

Rebecca J Garten, Xiao-Fang Yu, Department of Molecular Microbiology and Immunology, The Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD 21205, United States

Sheng-Han Lai, Jin-Bing Zhang, Department of Epidemiology, The Johns Hopkins University Bloomberg School of Public Health, Baltimore, United States

Wei Liu, Jie Chen, Guangxi Centers for Disease Prevention and Control, Nanning 530000, Guangxi Zhuang Autonomous Region, China

Author contributions: Garten RJ, Lai SH, Liu W, Chen J, and Yu XF designed research; Garten RJ, Zhang JB, Lai SH, Liu W, Chen J, and Yu XF performed research; Garten RJ, Lai SH, Liu W, Chen J, and Yu XF analyzed data; and Garten RJ, Lai SH, and Yu XF wrote the paper.

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Correspondence to: Dr. Xiao-Fang Yu, Department of Molecular Microbiology and Immunology, The Johns Hopkins University Bloomberg School of Public Health, Room E5148, 615 N. Wolfe St, Baltimore, MD 21205, United States. xfyu@jhsph.edu
Telephone: +1-410-9553768 Fax: +1-410-9550105

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Peer reviewer: Yoshiaki Iwasaki, Dr, Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Okayama 700-8558, Japan

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Abstract

AIM: To study the virological and host factors influencing hepatitis C infection outcomes in heroin users in southern China.

METHODS: HCV RNA and associated factors were analyzed among 347 heroin users from Guangxi Zhuang Autonomous Region, southern China who were hepatitis C virus (HCV) EIA positive for two or more consecutive visits.

RESULTS: Using the COBAS AMPLICOR HCV TEST, a remarkably low HCV RNA negative rate of 8.6% was detected. After multivariate logistic regression analysis, HCV RNA clearance was significantly associated with the presence of HBsAg (OR = 8.436, $P < 0.0001$), the lack of HIV-1 infection (OR = 0.256, $P = 0.038$) and age younger than 25 (OR = 0.400, $P = 0.029$).

CONCLUSION: Our study suggests HCV infection among Chinese heroin users results in high levels of viral persistence even amidst factors previously found to enhance viral clearance. Prospective studies of a possible genetic component within the Chinese population and the pathogenicity of non-genotype 1 HCV infections are needed.

INTRODUCTION

The hepatitis C virus (HCV) epidemic now affects over 200 million people worldwide. HCV is a single-stranded RNA flavivirus that is responsible for the majority of non-A-non-B hepatitis infections^[1,2]. Natural history studies have found that 15%-59% of people who are infected with HCV will undergo spontaneous viral clearance with no further liver disease due to HCV^[3-11]. The remaining will develop chronic HCV infection that can lead to cirrhosis, hepatocellular carcinoma and the need for a liver transplant.

The exact mechanism of HCV RNA clearance is not well understood although recent studies have shown that clearance is associated with strong, broad cellular immune responses^[12-19]. Other factors such as younger age^[3,20], female gender^[21,22], presence of hepatitis B surface antigen (HBsAg)^[3,23], certain HLA alleles^[6,24-29], and low viral quasispecies diversity^[30] have been linked to increased HCV RNA clearance. While African American ethnicity^[3,25], HCV genotype 1^[31-33] and co-infections with human immunodeficiency virus-1 (HIV-1)^[3], Human T-Lymphotropic Virus-1^[34] and *Schistosoma mansoni*^[35] have been associated with lower HCV RNA clearance and higher HCV RNA levels. Understanding why and how individuals clear HCV is the key to developing new drugs and an effective vaccine^[21].

Studies of heroin users from Guangxi Zhuang Autonomous Region in southern China have attributed the appearance and spread of HIV, HCV and other infectious agents to the change in heroin using patterns from smoking to injection^[36]. In Guangxi, two separate emerging HIV epidemics have sprung from different

heroin trafficking routes into the province^[37,38]. It is not clear how widespread the HCV epidemic is in China. Little seroprevalence data exists, mostly from the major cities of Beijing, Shanghai and Hong Kong and to our knowledge; no studies on HCV RNA clearance in China have been done. Studies in Yunnan Province, which borders Guangxi Zhuang Autonomous Region in the west, have found high HCV co-infection rates in HIV-1 positive drug users^[39]. Previous epidemiological studies in Guangxi not only found similar high rates of HCV co-infection in HIV-1 positive drug users, but a high HCV prevalence (72%), incidence (37.8 per 100 person years) in all heroin users enrolled in the study^[40]. To determine the clearance rate of HCV RNA and study factors that influence clearance, serum from a large cohort of heroin users from Guangxi Zhuang Autonomous Region, southern China was qualitatively tested for HCV RNA.

MATERIALS AND METHODS

Study participants

Heroin users over the age of 18 in Guangxi Zhuang Autonomous Region, China are currently being followed in a study of behavioral and virological features of HIV-1 in injection drug users conducted at the Guangxi Provincial Health and Anti-Epidemic Center. Over 600 participants are being followed at study sites in Pingxiang and Binyang City in the Guangxi Zhuang Autonomous Region. Study participants from Pingxiang have been followed since September 1999 while participants from Binyang have been followed since January 2000. The informed consent procedure was described previously^[40]. Briefly, at each visit, participants underwent blood draw and personal interviews and were counseled on the results of their serological tests. Baseline and follow-up questionnaires entailed a brief medical history including sexually transmitted diseases, history of drug use, sexual history, ethnic and economical backgrounds. Blood was collected and centrifuged and the serum either underwent serological assays or was stored at -70°C. Samples were then shipped from Guangxi to our laboratory in Baltimore, MD for HCV RNA testing and further analysis.

Serologic assays

At the Guangxi Health and Anti-Epidemic Center in Nanning, the presence of HIV-1 antibody was determined by enzyme-linked immunosorbent assay (ELISA) using the Vironostika HIV-1 Microelisa System (Organon Teknika). All ELISA-positive samples were not considered HIV-positive until confirmation by HIV-1/2 Western blot immune assay manufactured by Gene Lab (Singapore). Positivity for Hepatitis B surface antigen (HBsAg) and antibody to Hepatitis B surface antigen (HBsAb) were determined by HBV ELISA (Xiamen Xinchung Scientific, Xiamen, China). Hepatitis C antibody was analyzed by the Ortho HCV Version 3.0 ELISA Test System (Ortho Diagnostic Systems, Raritan, NJ).

HCV RNA detection and determination of HCV serotypes and genotypes

Available sera from 347 participants who were HCV

antibody positive for two consecutive visits (> 6 mo apart) were qualitatively tested for Hepatitis C RNA by the COBAS AMPLICOR HCV TEST KIT, sensitivity > 50 IU/mL (Version 2.0, Roche Diagnostics). Hepatitis C serotypes were determined for samples found HCV RNA negative using the Murex HC03 ELISA (Abbott Diagnostics, England). RNA for HCV genotyping was extracted from 100 µL of serum using the QIAamp Viral RNA kit (QIAGEN Inc, Valencia, CA). Reverse transcription and nested PCR was performed using primers to conserved regions of Core and E1 as previously described^[41]. After purification with the QIAquick PCR Purification kit (QIAGEN Inc, Valencia, CA), samples were sequenced using the inner forward primer on an automated sequencer (PRISM, version 2.1.1; ABI, Foster City, CA). Sequences were compiled using the BioEdit program, version 4.7 (T. Hall, North Carolina State University, Raleigh) and genotypes were assigned after alignment with known HCV genotypes as previously described^[41].

Statistical analysis

Univariate logistic regression analyses were first performed to explore the crude associations between the clearance of HCV and related factors, including age, length of drug use, frequency of drug use, injection drug use, HIV-1 Ab, HBsAb, HBsAg, study site, ethnicity and gender. Variables with a $P < 0.1$ in the univariate model were then put into a multiple logistic regression model^[42]. Those that ceased to be significant in the multivariate model, $P > 0.05$, were eliminated in a stage-wise manner, yielding a final model in which all variables were independently associated with the clearance of HCV. P -values reported are two-sided. The age, frequency of drug use and length of drug use closest to the overall sample mean within a factor of five were used in the model.

RESULTS

A total of 347 study participants who were HCV EIA positive for two or more consecutive study visits were included in this study, 127 from Pingxiang City and 220 from Binyang City. Pingxiang City is in southern Guangxi and borders Vietnam while Binyang City is centrally located within the province. Survey and serology results from the study visit of HCV RNA analysis are listed in Table 1. This subset of the Guangxi cohort is predominantly male (96.25%) with a mean age of 27 (range 19-50). Two main ethnic groups are present in Guangxi, Han and the Zhuang minority. Approximately 67% of the study group is Han and 29% Zhuang. Less than half of the participants are married (32%). Over 90% of the participants have a middle school or lower level of education.

Approximately 93% of the heroin users admit to injection drug use (Table 1). Over half admit to sharing needles (data not shown). The participants have been injection drug users for an average of about 5 years (Table 1). The study group uses heroin at an average frequency of 74 times per month. Along with being HCV EIA positive, 25.94% of the study participants are also HIV Ab positive,

Table 1 Characteristics of consecutively HCV ELISA positive heroin users from Guangxi Zhuang Autonomous Region, China

	Number (%) HCV RNA		Total
	(+)	(-)	
Factor	317 (91.35)	30 (8.65)	347
Location			
Binyang	200	20	220
Pingxiang	117	10	127
Mean age (yr)			
Mean \pm SD	27.5 \pm 5.7	25.4 \pm 4.52	27.4 \pm 5.6
Range	(19-50)	(19-37)	(19-50)
Gender			
Male	307 (96.85)	27 (90.00)	334 (96.25)
Female	10 (3.15)	3 (10.00)	13 (3.75)
Ethnicity			
Han	212 (66.88)	20 (66.67)	232 (66.86)
Zhuang	94 (29.65)	7 (23.3)	101 (29.11)
Other	11 (3.47)	3 (10.0)	14 (4.03)
Marital status			
Single	215 (67.82)	21 (70.00)	236 (68.01)
Married	102 (32.18)	9 (30.00)	111 (31.99)
HIV Ab status			
Positive	87 (27.44)	3 (10.00)	90 (25.94)
HBsAg status			
Positive	32 (10.09)	14 (46.67)	46 (13.26)
HBsAb status			
Positive	142 (44.79)	6 (20.00)	148 (42.65)
Injection drug use			
Yes	294 (92.74)	29 (96.67)	323 (93.08)
Mean length of drug use			
Months \pm SD	63.7 \pm 26.4	54.72 \pm 28.2	62.9 \pm 26.6
Range	(9.0-165.2)	(13.1-124.2)	(9.0-165.2)
Mean frequency of drug use			
Per Month \pm SD	73.9 \pm 44.7	79.6 \pm 34.6	74.4 \pm 44.0
Range	(1.0-390.0)	(20.0-150.0)	(1.0-390.0)
Education level			
College or above	1 (0.32)	0	1 (0.29)
High school	26 (8.25)	2 (6.9)	28 (8.14)
Middle school	151 (47.94)	12 (41.38)	163 (47.38)
Primary school	133 (42.22)	15 (51.72)	148 (43.02)
Illiterate	4 (1.27)	0	4 (1.16)
Unknown	2	1	3 (0.86)

SD: Standard deviation; HCV: Hepatitis C virus; HIVAb: HIV-1 antibody; HBsAg: Hepatitis B surface antigen; HBsAb: Antibody to hepatitis B surface antigen.

13.26% are HBV surface antigen positive (HBsAg) and 42.65% are antibody positive for the HBV surface antigen (HBsAb). Of the 347 consecutively HCV EIA positive samples tested, only 30 had undetectable levels of HCV RNA (less than 50 IU/mL) resulting in an HCV RNA clearance rate of 8.6% (Table 1).

Results of univariate logistic regression analyses and final multivariate logistic regression analyses for Hepatitis C Viral RNA clearance are shown in Table 2. Univariate analysis revealed HCV RNA clearance to be associated with age younger than 25 (OR = 0.472), lack of HIV-1 infection (OR = 0.294), presence of HBsAg (OR = 7.793), lack of HBsAb (OR = 0.308), female gender (OR = 3.411) and acknowledgement of injection drug use (OR = 0.441). In the final model, only three factors were independently associated with HCV RNA clearance; being HBsAg

positive (OR = 8.436, $P < 0.0001$), lacking HIV-1 infection (OR = 0.256, $P = 0.038$) and age younger than 25 (OR = 0.400, $P = 0.029$).

A comparison of previously published HCV RNA clearance rate can be found in Table 3. Our HCV RNA clearance rate is most similar to African American injection drug users in the Baltimore ALIVE cohort^[3], but remarkably less than the remaining studies listed.

DISCUSSION

Injection drug practices in Guangxi Zhuang Autonomous Region, China continue to efficiently spread HCV, HIV and HBV resulting in large numbers of co-infections and multi-infections, the full impact of which has yet to be determined. Analysis of this cohort found a very low spontaneous HCV clearance rate (8.6%) among heroin users who have been HCV EIA positive for two or more consecutive study visits. The natural history of HCV in Chinese individuals has not been studied to this extent before.

Hepatitis C viral clearance was strongly associated with co-infection by the hepatitis B virus, specifically participants currently HBsAg positive. Other cohorts have seen trends between HBsAg positivity and HCV clearance^[3,23], but none with an association as convincing as is shown in our cohort. Previous studies of HBV and HCV co-infections have revealed viral interference between these hepatotropic viruses resulting either in one dominant virus^[43] or in some cases resolution of both infections^[44]. The exact mechanism of this interference is not known, although data suggest it is the result of inhibition of replication by viral proteins^[45,46]. It is also plausible that the existing activation of non-specific immune responses within the liver during the current HBV infection enhances the clearance of the HCV infection.

HCV is now a major opportunistic infection for those with co-infected HIV-1^[47]. Previous studies have shown that co-infections with HIV and HCV do not increase the progression to AIDS^[48], but do increase the HCV viral load and progression to end-stage liver disease (ESLD)^[49,50]. Two separate subtypes of HIV, A/E and a B/C recombinant, were likely to enter Guangxi Zhuang Autonomous Region in 1996^[38]. With high HCV prevalence and incidence rates, almost all injection drug users in Guangxi who become HIV-1 positive will be co-infected with HCV. This study found a significant association between HIV co-infection, defined by the presence of HIV-1 antibody, and the inability of the individual to clear HCV RNA. Only 3/90 HIV-1 antibody positive individuals were able to clear their HCV RNA. Two of these participants were new HIV-1 seroconverters and the third was HBsAg positive (data not shown). The mechanism of how HIV-1 infection inhibits HCV RNA clearance is likely due to immune suppression caused by HIV-1. It is less likely that the two HIV-1 seroconverters who cleared HCV RNA were immunocompromised (data not shown). Thomas *et al* did not see a significant association between HIV-1 co-infection and HCV clearance in the ALIVE cohort until HIV-1 infected people were broken down by CD4 levels^[3].

Table 2 Factors associated with hepatitis C viral clearance among 347 consecutively HCV antibody positive heroin users from Guangxi Zhuang Autonomous Region, China

Factor	Number (% clearance)	Univariate	Final multivariate model	
		Adjusted OR (95% CI)	Adjusted OR (95% CI)	P
Age (yr)				
≤ 25	125 (13.6)	1.0	1.0	0.029
> 25	222 (5.9)	0.472 (0.221-1.006)	0.400 (0.176-0.909)	
HIV Ab status				
Negative	257 (10.5)	1.0	1.0	0.038
Positive	90 (3.3)	0.294 (0.087-0.993)	0.256 (0.071-0.924)	
HBsAg status				
Negative	301 (5.3)	1.0	1.0	< 0.0001
Positive	46 (30.4)	7.793 (3.484-17.430)	8.436 (3.646-19.520)	
HBsAb status				
Negative	199 (12.1)	1.0		
Positive	148 (4.1)	0.308 (0.123-0.744)		
Gender				
Male	334 (8.1)	1.0		
Female	13 (23.1)	3.411 (0.885-13.143)		
Location				
Binyang	220 (9.1)	1.0		
Pingxiang	127 (7.9)	0.855 (0.387-1.888)		
Ethnicity				
Zhuang minority	115 (8.7)	1.0		
Han	232 (8.6)	0.991 (0.448-2.192)		
Injection drug use				
Yes	323 (9.0)	1.0		
No	24 (4.2)	0.441 (0.057-3.384)		
Length of drug use				
< 5 yr	176 (10.8)	1.0		
≥ 5 yr	171 (6.4)	0.568 (0.262-1.233)		
Frequency of drug use				
< 75 times per month	217 (7.4)	1.0		
≥ 75 times per month	130 (10.8)	1.516 (0.714-3.219)		

OR: Odds ratio; CI: Confidence Interval; HCV: Hepatitis C virus; HIVAb: HIV-1 antibody; HBsAg: Hepatitis B surface antigen; HBsAb: Antibody to hepatitis B surface antigen.

Age of the individual also affected the HCV clearance rate in our cohort. A meta-analysis by Mathei *et al* found a linear relationship between mean age and HCV RNA clearance rates^[20]. The higher prevalence of HCV RNA in older individuals was suggested as a result of continuous re-exposure to HCV for a prolonged period of time. The ages found in our cohort are much younger than previous HCV studies where HCV RNA clearance rates were less than 15%^[3,20]. We attempted to address whether our high levels of HCV RNA persistence were due to the length and frequency of drug use, but these factors proved non-significant.

HCV genotype 1 has been considered a more aggressive genotype, associated with lower clearance rates, decreased susceptibility to current treatments^[32-34] and in a few cases, associated with a faster progression of HIV disease^[51]. Many HCV cohort studies, including the ALIVE cohort in Baltimore are primarily genotype 1 infections^[5]. Examination of our cohort found three major HCV subtypes present in chronic infections, genotypes 6a (38%), 3b (37%) and 1a (19%)^[52]. It is unclear whether HCV genotypes in our cohort are responsible for the low HCV RNA clearance rate.

Age, HBsAg positivity and lack of HIV-1 co-infection were the most significant factors resulting in HCV RNA

clearance. In comparison with the ALIVE cohort^[3], our lower age, lower prevalence of HIV-1 and higher prevalence of HBsAg, would predict higher rates of HCV RNA clearance than was seen. After removing HIV-1 and HBsAg positive individuals, only 15 of the remaining 221 participants underwent HCV clearance, at a rate of 6.8% (data not shown). This suggests that other host and viral factors are present in the Guangxi cohort resulting in high rates of HCV persistence. Studies of the ALIVE cohort by Thomas *et al* found the lower HCV RNA clearance levels in African American injection drug users to be linked to differences in certain HLA-frequencies^[3,25]. There is also speculation of differences in TH1/TH2 cytokine balances between Caucasian and African Americans^[53]. Whether HLA or cytokine profiles of Chinese individuals account for the low HCV RNA clearance rates has yet to be seen.

Injection heroin use in China is rapidly distributing HCV, HBV and HIV. The natural history of HCV in Chinese heroin users results in little spontaneous clearance of HCV RNA. Current HIV infection further debilitates the individual's ability to control HCV infection. And although HBV is endemic in China and viral interference between HBV and HCV may eliminate one of the hepatropic viruses, it may not decrease the possibility for further liver disease. Further studies within the Guangxi

Table 3 HCV RNA clearance rates among previously published cohort studies

Principal investigator	Cohort	HCV RNA			
		Number tested	clearance rate (%)	Percent HIV Ab +	Percent HBsAg +
This study Thomas ^[3]	Chinese IDU	347	8.7 ¹	25.9%	13.3%
	African				
	American IDU	729	9.3		
	Non-African				
	American IDU	44	36.0		
Alric ^[5]	Overall	773	10.9 ¹	45.7%	3.5%
	Caucasian				
	French	123	25 ¹	ND	ND
Minton ^[6]	Caucasian				
	English	172	20.3 ¹	ND	ND
Yee ^[7]	English				
Alter ^[8]	Hemophiliacs	200	14 ¹	40.3%	ND
	American blood				
	Donors	248	14 ²	ND	ND
Kenny-Walsh ^[9] Alter ^[10]	(42% IDU)				
	Irish women	704	44.6 ²	ND	ND
	Non-Hispanic				
	African				
	American	196	14.0		
Sagnelli ^[11]	Non-Hispanic				
	Caucasian	119	32.0		
	Hispanic				
	Americans	132	26.0		
	NHANES				
	Overall	447	26.1 ²	ND	ND
	Italian liver				
	Patients	336	21.7 ³	ND	28%

¹HCV RNA by Roche AMPLICOR HCV Qualitative Assay-Sensitivity > 50 IU/mL; ²HCV RNA by In-house RT-PCR- Sensitivity Unknown; ³HCV RNA by HEPA-Check C - Sensitivity Unknown; HCV: Hepatitis C virus; IDU: Injection drug user; HIV: Human immunodeficiency virus 1; HBsAg: HBV surface antigen; ND: Not determined.

cohort and other Chinese Provinces will better define the pathogenesis of HCV in Chinese ethnicities and non-genotype 1 infections. In order to decrease the spread of HIV-1 and HCV, education on safe-needle practices and the illnesses transmitted by injection drug use is urgent in China.

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COMMENTS

Background

Hepatitis C virus (HCV) is quickly spread through injection drug use. A proportion of individuals infected with HCV undergo viral clearance while the remaining individuals develop a chronic infection which can lead to cirrhosis, hepatocellular carcinoma and the need for a liver transplant. Injection drug use is a major risk factor for HCV infections. This research studies the rate of HCV clearance in injection drug users from Southern China and the potential associated factors for clearance.

Research frontiers

This research studies HCV clearance in Chinese ethnicities with non-genotype 1 infections.

Innovations and breakthroughs

A low rate of HCV clearance was found in injection drug users of Chinese ethnicities. The majority of HCV infections were non-genotype 1 and many of the participants had current or previous co-infections with Hepatitis B viruses. Together these factors have previously been found to increase the level of viral clearance, suggesting other factors in the cohort are driving the low rate of viral clearance.

Applications

This research highlights the need for further studies of HCV infections in Chinese ethnicities which concentrate on the immunogenetics of the host.

Peer review

This paper describes the rate of hepatitis C viral RNA clearance in heroin users from south China and analysis of factors associated with it. The authors found a remarkably low rate of hepatitis C viral RNA clearance in the cohort and some possibly relating factors. The data presented are epidemiologically important, and the authors discussed the results appropriately.

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