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CLINICAL MICROBIOLOGY - RESEARCH PAPER

Hierarchical assessment of host factors influencing the spontaneous resolution of hepatitis C infection

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Abstract

Background Hepatitis C virus (HCV) infection is associated with chronic liver disease, resulting in cirrhosis and hepatocellular carcinoma. Approximately 20% of HCV infections are spontaneously resolved. Here, we assessed the hierarchical relevance of host factors contributing to viral clearance.

Methods DNA samples from 40 resolved infections and 40 chronic HCV patients paired by age were analyzed. Bivariate analysis was performed to rank the importance of each contributing factor in spontaneous HCV clearance.

Results Interestingly, 63.6% of patients with resolved infections exhibited the protective genotype CC for SNP rs12979860. Additionally, 59.3% of patients with resolved infections displayed the protective genotype TT/TT for SNP ss469415590. Moreover, a ranking of clearance factors was estimated. In order of importance, the IL28B CC genotype (OR 0.197, 95% CI 0.072–0.541) followed by the INFL4 TT/TT genotype (OR 0.237, 95% CI 0.083–0.679), and female gender (OR 0.394, 95% CI 0.159–0.977) were the main predictors for clearance of HCV infection.

Conclusions HCV clearance is multifactorial and the contributing factors display a hierarchical order. Identifying all elements playing role in HCV clearance is of the most importance for HCV-related disease management. Dissecting the relevance of each contributing factor will certainly improve our understanding of the pathogenesis of HCV infection.

Keywords Hepatitis C · HCV · MAVS · SNP rs12979860 · IL28B

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Background

Hepatitis C virus (HCV) is an enveloped RNA hepatotropic virus belonging to the *Flaviviridae* family [1]. The positive single–stranded RNA genome contains 9.6 kb and encodes a single polyprotein precursor, which is cleaved into several structural and nonstructural proteins [2, 3]. Polyprotein cleavage is performed by host and viral proteases, including the HCV NS3/4A protease complex [4]. HCV causes chronic liver diseases in approximately 75–85% of patients [1, 5], eventually progressing to severe liver damage 25% of those cases [6, 7]. Worldwide, more than 180 million individuals are chronically infected with HCV [6–8] and 3–4 million new cases occur each year [9]. However, approximately 20% of infected persons spontaneously clear the virus. Both, viral and host factors, such as nucleotide polymorphism (SNPs) in immune system genes, age, infecting viral genotype, virus nucleotide variation, and risk behavior, among others, are thought to play a role in HCV clearance [10–12]. After entry into host cell, the HCV RNA is recognized by the host, triggering an intrinsic innate immune response that initiates an antiviral state [13]. A series of interactions with the viral RNA and host proteins culminates in the activation of the mitochondrial antiviral signaling protein (MAVS), which regulates the expression of type-I interferons, such as IFN-β [13–17]. Nevertheless, HCV can prevent the host innate immune response via its NS3/4A protease. A catalytic interaction between MAVS amino acids (aa) residue cysteine 508 (Cys-508) and serine 139 (Ser-139) from the NS3/4A protease has been reported to result in MAVS cleavage from the mitochondria and peroxisomes organelles, impairing the interferon cascade [14–16, 18, 19]. However, the mutation C508A has been described as sufficient to maintain the mitochondrial localization of MAVS in the presence of NS3/4A [20].To date, polymorphisms at MAVS Cys-508 have not been described in vivo, although a single point mutation at this position would render resistance to MAVS cleavage by the HCV NS3/4A. The innate anti-viral immune response also relies on other types of interferons. The IL-28B gene, located on chromosome 19, codes for the type III interferon lambda 3 (IFN-3), which is part of the first line of host antiviral response [21]. Previous studies identified single-nucleotide polymorphisms (SNPs) in the region of the IFNL-3 gene (IL-28B, IFN-3) on chromosome 19q13.13 that correlate with spontaneous [12, 22] and IFN-mediated HCV clearance [22–25]. One of these SNPs in the IFNL3 region at position 12979860 (rs12979860) has been considered as a marker for HCV clearance [26]. Upstream of IFNL-3 (IL-28B) on chromosome 19q13.13, the dinucleotide variant ss469415590 (TT/ΔG) was also discovered [27]. This polymorphism is in linkage disequilibrium with rs12979860 [27]. The deletion of one nucleotide in the ΔG variant results in a frameshift change that creates the interferon lambda 4 (IFNL-4) new gene, while TT variant does not produce IFNL4 [27]. It was reported that IFNL-4 impairs spontaneous resolution and IFNα treatment–induced clearance of HCV infection [27]. Associated with SNPs, additional host factors, such as gender, age, and sexual behavior also seem to influence HCV clearance or persistence.

Thus, spontaneous HCV clearance is a complex process involving multiple players resulting in rather intricate mechanism. Here, we assess the hierarchical relevance of virus and host factors contributing to viral clearance.

Material and methods

Population and samples

The study cohort consists of 80 ELISA HCV antibody positive patients, divided into two groups: (I) 40 RNA negative patients (resolved infections) and (II) 40 patients chronically infected. Co-infection with human immunodeficiency virus (HIV) and/or the hepatitis B virus (HBV) were considered exclusion criteria. The number of 80 anti-HCV antibody-positive individuals recruited was defined by the number of HCV-RNA–negative individuals obtained for the study. The number of HCV-RNA–positive individuals was selected to pair, by age, with the 40 HCV-RNA–negative individuals.

Group I (HCV antibodies positive, HCV RNA negative) were blood donor candidates at the Blood Center of the São José do Rio Preto School of Medicine (FAMERP) in São José do Rio Preto, São Paulo, Brazil and at the Hemocenter of the Botucatu Medical School, São Paulo State University in Botucatu, São Paulo, Brazil. These individuals were originally screened and found positive for anti-HCV antibodies. The presence of anti-HCV antibodies was evaluated and repeated using the ELISA III procedure for hepatitis C (*WIENER LAB)* at the *Hemocenter* of the *Botucatu* Medical School, São Paulo State University in Botucatu, São Paulo, Brazil. Serum samples were then screened for HCV RNA. Briefly, HCV was detected by polymerase chain reaction (PCR) targeting the 5′ UTR region of the HCV genome [28, 29] (lower detection limit is 50 UI/mL). To confirm the absence of HCV RNA, an additional PCR targeting the NS3 region was also performed [30]. Lack of viral RNA and presence of HCV antibodies characterized those individuals as resolved HCV cases.

Blood samples from group II (HCV antibodies positive, HCV RNA positive) were collected at the Hepatology Clinic of the São José do Rio Preto School of Medicine (FAMERP) in São José do Rio Preto, São Paulo, Brazil. HCV-positive patients underwent anti-viral combinatory treatment with pegylated interferon (PegIFN)-α and ribavirin for 24 or 48 weeks (according to genotype). All patients included in this group presented chronic hepatitis C confirmed by anti-HCV serological test and HCV RNA detected in plasma.

DNA and RNA extraction

Human genomic DNA (gDNA) was obtained from leukocytes using Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturer's instructions. HCV RNA from serum samples was isolated using QIAamp Viral RNA Mini Kit (QIAGEN Inc., Valencia, CA) and cDNA was synthesized using a High-Capacity cDNA Archive Kit (Applied Biosystems) according to the manufacturer's instructions.

Polymerase chain reaction

A portion of the β-globin gene was amplified by polymerase chain reaction (PCR) and used as an internal control in all samples [31–33]. A PCR method was designed to amplify and sequence a segment of the human MAVS gene containing residue 508. Oligonucleotides 5′-ATCTTGCCATCAGT GCCAG-3′ and 5′-TACAGCACCACCAGGAGT-3′ were used to amplify a product of 291 base pairs (bp). The IL28B SNPs rs12979860 and ss469415590 were genotyped as described previously [34]. One round PCR amplification was performed with the nucleotides IFNL-3F: 5′-CGC TTATCGCATACGGCTAGGCC-3′, IFNL-3R: 5′-CGCT ACGTAAGTCACCGCCCAGC-3′, IFNL-4F: 5′ ACTTACGTAGCGGTCCCTCAGGG-3′, IFNL-4:5′-TCTC TTTGGCTTCCCTGACGTCTC-3′. Briefly, with some variation, PCR reactions were carried out in a final volume of 25 μl, containing 100 ng of gDNA, 100 mM dNTP mix (Roche Applied Sciences, Indianapolis, IN), 25 mM MgCl2, 1× buffer, 5 U high-fidelity Taq DNA polymerase (Fermentas/Thermo Scientific, Waltham, MA) and appropriate forward and reverse primers (10 mM each). The cycling condition were 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 56 °C, and 30 s at 72 °C, followed by 10 min at 72 °C. The amplification products were purified with the QIAquick PCR Purification kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's recommendations.

HCV genotyping was performed by polymerase chain reaction (PCR) targeting the 5′UTR region and/or the NS3 region of the HCV genome, as reported previously [28–30]. Briefly, 5′UTR region was amplified in two round PCR, using the primers PTC1-5′CGTTAGTATGAGTGTCGTG3′, NCR2–5′ATACTCGAGGTGCACGGTCTACGAGACCT3′, PTC3–5′AGTGTCGTGCAGCCTCCAGG3′, and NCR4–5′ CACTCTCGAGCACCCTATCAGGCAGT3′, and the reactions were carried out as described [29]. The generated consensus sequence was compared with the sequences of all HCV genotypes deposited in GenBank database. The NS3 helicase domain (181–631 aa) was amplified using the specific primers 5′GGAATTCCATATGTCCCCATCTTTCTCTGACA ATTCAACT3′ and 5′CGCGGATCCTCAGGTGGTTA CTTCCAGATC3′. Internal primers for the complete helicase sequencing were also used (Seq787F 5′GCCAAA CTGACCTATTCCAC3′; Seq980F 5′AGCATCAC TGTGCCACATTC3′; Seq1216F 5′GTCGTAGTTTGCGC TACTG3′; Seq1654R 5′GCTTAGTCTGTGACAGAAAG TG3′; Seq1454R 5′ATTCCAGACGGTCTTTCACC3′; and Seq1005R 5′GTTAGAATGTGGCACAGTGATG3′. The helicase amplification reactions were performed in one round PCR as first described [30], and the consensus sequence was then compared with the sequences of all HCV genotypes deposited in GenBank database.

Sequencing

The fragments generated were sequenced using an ABI Sequencer Model 3130XL (Applied Biosystems) with the Big Dye terminator kit (Applied Biosystems) according to the manufacturer's instructions. Both, forward and reverse strands were sequenced for each amplicon to confirm all SNP. The sequences were aligned against the reference sequences gi193083235, rs12979860, and ss469415590 (rs368234815) (GenBank) to search for all corresponding mutations (Cys-508 residue in MAVS, IFNL-3, and IFNL-4 gene, respectively).

Sequence analysis

All sequences were assessed for quality using the PHRED/Phrap/CONSED software (http://www.bioinformatica.ucb.br/electro.html) and were checked for similarity with sequences deposited in GenBank using BLAST—Basic Local Alignment System (www.ncbi.nlm.nih.gov/blast/Blast.cgi) and Blat—Human Genome Search (http://genome.ucsc.edu/FAQ/FAQblat.html). The sequences were aligned using the Clustal W software program available from BioEdit—Biological Sequence Alignment Editor (www.mbio.ncsu.edu/BioEdit/page2.html). For IL28B SNPs rs12979860 and ss469415590 genotyping, the sequence analysis was performed as first described [34] using the SeqMan Pro (DNASTAR Lasergene software package 7.1.0). Polymorphic sites from contig sequences were checked manually and heterozygote results were confirmed by a new amplification and sequencing reaction.

Statistical analysis

The statistical analysis was performed using the software R version 2.13.0—the R Foundation for Statistical Computing, Auckland, New Zealand. Depending on the nature of the variables, statistical tests were used; qualitative variables were analyzed by Pearson's χ^2, and means of quantitative variables were compared by *t* test, or, when recommended, by Kruskal-Wallis test, considering the median as centrality parameter and interquartile range (IQR) as dispersion

measure. To identify the predictors of clearance, odds ratios (OR) were derived by performing bivariate logistic regression analysis, with criterion to remain in the model when p value ≤ 0.10. The adopted significance level was $\alpha = 0.05$. Each factor was considered as an independent variable, and bivariate analysis was performed. Thus, the association among the various factors and chronicity was estimated. The OR calculated for each variable were listed according to their association, and grouped according to their hierarchy.

Ethics approval

The project was approved by the in-house Ethics Committee of Sao Paulo State University (IBILCE-UNESP, São José do Rio Preto) (No. 049/09), and all participants signed an informed consent form.

Results

The study included 80 anti-HCV antibody positive individuals, including 40 individuals who cleared virus (group I) and 40 chronically infected HCV patients (group II). Logistic regression analysis ranking the factors contributing to viral clearance accounted for viral genotypes, demographic risk factors associated with HCV infection, mutation in the MAVS coding sequence, and genotypes in SNPs rs12979860 and ss469415590. In the chronic group, the predominant infecting HCV genotype (GT) was GT1, accounting for 26 patients (65.0%), followed by the GT3, detected in 10 cases (25.0%), and GT2 present in 4 individuals (10.0%). Association with the infecting viral genotype and HCV clearance could not be established since infecting genotype in all 40 patients who cleared the virus was not available.

The patients' demographic information and risk factors were collected from both groups and are listed on Table 1. A positive association ($p = 0.011$) between HCV chronicity and the reporting of venereal disease was observed. The number of male patients was also significantly higher among chronic patients than clearance patients ($p = 0.043$). Otherwise, patients from group I showed a higher surgery incidence than chronic patients ($p = 0.019$). The frequency of men who have sex with men (MSM) was higher among chronic patients ($p = 0.056$, if considering the likelihood ratio χ^2 of 4.038, then $p = 0.044$). No significant association with number of sexual partners was seen ($p = 0.364$). Furthermore, no other statistically significant association between risk factors and chronicity or clearance could be found in this cohort.

All patients were genotyped for substitutions at MAVS Cys-508 codon. Sequences derived from all patients were aligned with the GenBank reference sequence gi193083235 in the search for any mutation at Cys-508. All patients displayed the TGC codon in the MAVS gene encoding for cysteine at position 508.

Table 1 Patients' demographics and existing risk factors

Categorical variables	Group I (Cleared)	Group II (Chronic)	p value
Gender[1]	18 (M)/22 (F)	27 (M)/13 (F)	0.043*
Mean age[2]	49.0 (12.9)[£]	47.8 (12.2)[£]	0.651
Age 1st intercourse[2]	17 (6)[§]	17 (4)[§]	0.202
Occupational exposure	11/40	7/40	0.284
Blood transfusion (BT)	8/37	9/36	0.733
Surgery	37/40	29/40	0.019*
Number sexual partners[3]	2.0 (3)[†]	2.0 (2) [†]	0.364
MSM**	1/38	6/40	0.044*
STD[¥]	8/39	19/40	0.011*
Drug use	13/37	14/40	0.990
Injection drug use	6/13	10/14	0.182
Personal contact—drug user	5/40	7/40	0.531
Sexual contact—drug user	11/34	6/36	0.126
Personal contact—HCV carrier	3/40	4/40	0.692
Sexual contact—HCV carrier	4/36	5/29	0.477
Personal contact—(BT)	6/40	7/40	0.762
Sexual contact—(BT)	6/27	2/19	0.303

[1] *M*, Male; *F*, Female. *Statistically significant. [2] Range within parenthesis. [£] For the mean age, criterion of the value between parentheses represents standard deviation (SD). [§] For the median age of first intercourse, criterion of the value between parentheses represents inter-quarter range (IQR). [3] Median number. [†] For the median of number of sexual partners, criterion of the value between parentheses represents inter-quarter range (IQR). **MSM, men who have sex with men. [¥] Self-report of sexually transmitted disease (STD)

IL28B SNP rs12979860 was successfully genotyped in 90.0% of individuals ($N = 72$). The frequencies of the CC genotype in clearance patients were 63.6% and 25.6% in chronic patients. In this case, we observed a statically significant difference between the frequencies of CC allele in the two groups ($p = 0.005$). As expected, there was a positive association between frequency of CC genotype and rates of spontaneous HCV clearance, confirmed by the odds ratio analysis (OR 0.197, 95% CI 0.072–0.541, $p = 0.001$) (Table 2). Interestingly, the CC genotype frequencies among male and females in both groups were not statistically significant (data not shown).

The IFNL4 SNP ss469415590 was successfully genotyped in 82.5% of individuals ($N = 66$). The frequencies of TT/TT in clearance patients were 59.3% and 25.6% in chronic patients. In this case, we observed a statically significant difference between the frequencies of TT/TT allele in the two groups ($p = 0.014$). Thus, a strong statistical association between the TT/TT genotype and the spontaneous resolution of HCV was observed (OR 0.237, 95% CI 0.083–0.679, $p = 0.006$) (Table 2).

Table 2 Frequency and comparison of the IL28B and IFNL4 genotypes in resolving and chronically infected HCV patients

All subjects		Frequency of clearance (%)	Frequency of persistence (%)	Comparison	OR (95% CI)	*p* value
IL28B GT	CC	21 (63.6%)	10 (25.6%)			0.005*
	TT	02 (6.1%)	03 (7.7%)	CC vs CT + TT	0.197 (0.072–0.541)	0.001*
	CT	10 (30.3%)	26 (66.7%)	CT vs CC + TT	4.6 (1.697–12.469)	0.002
	CT + TT	12 (36.4%)	29 (74.4%)	TT vs CC + CT	1.292 (0.203–8.236)	0.786
INFL4 GT	ΔG/ΔG	01 (3.7%)	07 (17.9%)	ΔG/ΔG vs ΔG/TT + TT/TT	5.688 (0.657–49.231)	0.081
	ΔG/TT	10 (37%)	22 (56.4%)	ΔG/TT vs ΔG/ΔG + TT/TT	2.200 (0.850–6.012)	0.122
	TT/TT	16 (59.3%)	10 (25.6%)†*	TT/TT vs ΔG/ΔG + ΔG/TT	0.237 (0.083–0.679)	0.006*
Protective GT	CC-TT/TT	13 (54.17%)	09 (23.68%)			0.015*
Deleterious GT	nCC-nTT/TT	11 (45.83%)	29 (76.32%)			

GT genotype. *Statistically significant. †**P* = 0.014

Remarkable differences were also observed in the analysis of genotype frequencies when the CC + TT/TT allele combination was tested versus non-CC + non-TT/TT genotype. The frequency of the favorable CC + TT/TT genotypes was significantly higher in resolving patients (54.17%) than in chronic patients (23.68%; $p = 0.015$) (Table 2).

Bivariate logistic regression analysis (LRA) was performed to estimate the predictive values of SNPs rs12979860 and ss469415590 genotypes and risk factors for HCV clearance. When the characteristics were inputted into the model, it was possible to establish a ranking based on odds ratio for predictive factors that contribute to HCV clearance, factors that do not contribute for HCV clearance and factors that do not present any association with HCV clearance or persistence (Table 3). The LRA ranked, in this order, the IL28B CC genotype (0.197, 95% CI 0.072–0.541), followed by INFL4 TT/TT genotype (0.237, 95% CI 0.083–0.679), and female sex (0.394, 95% CI 0.159–0.977) as the most relevant predictive factors for HCV clearance (Table 3). On the other side, the infestation by *Phthirus pubis* (14.407, 95% CI 1.892–125.499), IL28B genotype CT (4.6, 95% CI 1.697–12.469), infection by STDs (3.506, 95% CI 1.297–9.479), especially *Condyloma acuminatum* (2.1, 95% CI 1.621–2.721), and genital herpes (1.892, 95% CI 1.516–2.360) are predictive factors for HCV persistence (Table 3).

Discussion

Here, we have established a hierarchical order of factors influencing the clearance of HCV infection. Association between HCV clearance and IL28B genotype was observed, where 63.6% of patients who spontaneously cleared HCV infection had the protective CC genotype ($p = 0.005$). The advantageous C allele for HCV therapy has been well documented in previous studies [7, 8, 23, 25, 35–41]. Likewise, SNP in IFNL4 has been strongly associated with HCV clearance [27, 42, 43], and with poor response to IFN-α treatment [44–47]. Our results showed that the protective TT/TT genotype occurred in 59.3% of patients who cleared the infection ($p = 0.014$), corroborating the contribution of ss469415590 [ΔG] SNP for HCV persistence. Importantly, in our cohort, more than half of resolving patients (54.1%) bear the combinatory CC-TT/TT protective alleles.

Hierarchical ranking of HCV clearance factors was estimated using odds ratio by logistic regression analysis, where IL28B CC genotype, INFL4 TT/TT genotype, and being female were identified as predictor criteria for hepatitis C resolution. Overall, our analysis corroborate the contribution of rs12979860 and ss469415590 SNP for hepatitis C clearance [23, 42–44, 47–49] and shows that IL28B act as the most important factor when spontaneous resolution response for hepatitis C is been assessed. Nonetheless, the presence of the

Table 3 Risk analysis by logistic regression, ranking the contributing factors for HCV clearance and persistence

Risk factor ranking	OR (CI 95%*)
Protective factors for chronic	
IL28B CC genotype	0.197 (0.072–0.541)
INFL4 TT/TT genotype	0.237 (0.083–0.679)
Sex, being female is protective for chronic	0.394 (0.159–0.977)
Risk factors for chronic	
Phthirus pubis	15.407 (1.892–125.499)
IL28B CT genotype	4.6 (1.697–12.469)
All sexually transmitted disease (STD)	3.506 (1.297–9.479)
Condyloma acuminatum	2.1 (1.621–2.721)
Herpes genital	1.892 (1.516–2.360)
Other STDs	1.816 (1.467–2.247)
No association	
Syphilis	1.789 (0.155–20.626)
Haemophilus ducreyi infection	0.821 (0.049–13.642)
Gonorrhea	1.8 (0.541–5.984)
MSM relationship	6.529 (0.747–57.050)
Number of regular partners nowadays	1.0 (0.350–2.856)
Drug abuse	0.994 (0.390–2.537)
Injection drug use	2.917 (0.594–14.327)
Blood transfusion	1.208 (0.407–3.583)
Occupational exposure	0.559 (0.192–1.632)
Personal contact with drug users	1.485 (0.429–5.143)
Sexual contact with drug users	0.418 (0.135–1.299)
Personal contact with people with hepatitis C	1.370 (0.286–6.559)
Sexual contact with people with hepatitis C	1.667 (0.404–6.877)
Personal contact with people who have made blood transfusion	1.202 (0.365–3.955)
Sexual contact with people who have made blood transfusion	0.412 (0.073–2.307)
IL28B TT genotype	1.292 (0.203–8.236)
INFL4 ΔG/ΔG genotype	5.688 (0.657–49.231)
INFL4 ΔG/TT genotype	2.200 (0.805–6.012)

OR, odds ratio; *CI*, confidence interval. *CI considered a $p < 0.05$. Data is listed accordingly to the assessment of the association. Non-exposure for each variable was used as reference for comparisons

INFL4 TT/TT genotype and being female are also strong predictors for HCV clearance. When both protective SNP genotypes were analyzed in conjunction, a significant correlation was found in patients with resolved infection. In our cohort, female gender and clearance showed a strong association ($p = 0.043$). In a systematic review of longitudinal studies about predictors of spontaneous viral clearance, the female gender has also been considered predictive for spontaneous HCV resolution [50]. Interestingly, it has been proposed that the higher rate of HCV clearance observed among women may be owed to estrogen levels [51, 52].

Despite ranking factors that contribute for chronic hepatitis C, the regression logistic analysis evidenced the importance of sexual transmitted diseases (STDs) for viral persistence and reinforce the occurrence of HCV sexual transmission. However, HCV sexual transmission is somehow infrequent [36, 41]. The risk of HCV sexual transmission is different depending on the type of sexual intercourse [53]. Low rates of transmission have been identified in monogamous, heterosexual, and anti-HCV discordant couples [41]. Heterosexual transmission of HCV within this context is estimated to occur at a rate of 0–0.6% per year [41, 54, 55], but is higher for heterosexuals with multiple partners or within the context of coexisting sexually transmitted diseases (STDs) (0.4–1.8% per year) [53]. These disparities reflect differences in sexual risk behaviors [53].

MSM behavior was statically different between the two groups being more frequently reported among chronic patients than in clearance patients ($p = 0.044$, likelihood ratio $\chi^2 = 4.038$), and 83.0% of MSM chronic patients also had STDs ($p = 0.057$, if likelihood ratio $\chi^2 = 3.875$, than $p = 0.049$—data not shown). Despite of it, MSM behavior has no implications for clearance or chronicity (OR 6.529, 95% CI 0.747–57.050). Sexually acquired acute HCV infections have been observed

in MSM co-infected with HIV [37, 56, 57], which are mainly associated with drug use [58] and high-risk sexual practices [59, 60]. While our patients were not co-infected with HIV; the results, however, are in agreement with previous reports observing an increased in HCV incidence among MSM [8, 61]. In this setting, the risk behavior could lead to multiple re-infections or super infection events, thus contributing to persistence of HCV in this population [62, 63].

Several factors including host polymorphisms and viral factors affecting the IFN-induced cascade seem to contribute to HCV persistence. MAVS cleavage by HCV is prevented by a point mutation at residue Cys-508. However, no SNP or SNV have reported to occur at residue 508 in the human MAVS gene [39]. Our results are in agreement with those findings since no mutations at residue 508 were found in our patients. Therefore, the HCV clearance mechanism in our patients was not associated with MAVS cleavage in vivo. Nonetheless, the IFN cascade is of critical importance for viral clearance.

In summary, HCV clearance is multifactorial and the contributing factors display a hierarchical order. Identifying all elements playing role in HCV clearance is of the most importance for HCV-related disease management. Dissecting the relevance of each contributing factor is likely to guide the development of genetic predictor panels; besides helping us better understand the pathogenesis of HCV infection.

Author contributions PJSP, PR, LMGR, GV, and BMC wrote of the manuscript. PJSP, PR, and MLN conceived and designed the experiments; PJSP, BMC, VCM, PCRR, LRC, and STQA performed the experiments; RMF, RMTG, GFS, CRV, and PSN performed the management of samples collection and patients' data; PJSP, PR, LMGR, BMC, and JAC analyzed the data.

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Compliance with ethical standards

The project was approved by the in-house Ethics Committee of Sao Paulo State University (IBILCE-UNESP, São José do Rio Preto) (No. 049/09), and all participants signed an informed consent form.

Conflict of interest The authors declare that they have no conflict of interest.

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