LIVER

Host and Viral Determinants of the Outcome of Exposure to HCV Infection Genotype 4: A Large Longitudinal Study

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OBJECTIVES: **The objective of this study was to characterize the factors that influence the outcome of exposure to hepatitis C virus (HCV) genotype 4 (HCV-G4) and the course of recent infection.**

METHODS: **In this longitudinal study, we prospectively assessed the clinical, genetic, virological, and immunological parameters and retrospectively determined single-nucleotide polymorphisms at interleukin-28B (*IL-28B*) rs12979860 in a well-characterized large cohort recently exposed to HCV-G4.**

RESULTS: **A total of 136 subjects with acute HCV (new viremia, seroconversion, and HCV-specific T-cell responses) were identified. Forty-eight subjects (35%) had spontaneous viral clearance and 88 subjects developed chronic HCV of which 42 subjects were treated with pegylated interferon monotherapy, with a sustained virologic response (SVR) rate of 88%. Twenty-six subjects developed HCV-specific T-cell immune responses without detectable viremia or seroconversion. *IL-28B*-CC (odds ratio (OR) 14.22; $P<0.0001$), multispecific T-cell responses (OR = 11.66; $P<0.0001$), >300 IU/l alanine aminotransferase (ALT) decline within 4 weeks (OR = 6.83; $P<0.0001$), jaundice (OR = 3.54; $P=0.001$), female gender (OR = 2.39; $P=0.007$), and >2.5 $\log_{10}$ HCV-RNA drop within 8 weeks (OR = 2.48; $P=0.016$) were independently associated with spontaneous clearance. ALT normalization and undetectable HCV-RNA predicted SVR. Exposed apparently uninfected participants had a higher frequency of *IL-28B*-CC than patients with unresolved acute HCV ($P<0.001$). *IL-28B*-CC was associated with multispecific T-cell response ($r^2=0.0.835$; $P<0.001$).**

CONCLUSIONS: ***IL-28B-CC* genotype, multispecific HCV T-cell responses, rapid decline in ALT, and viral load predict spontaneous clearance and response to acute HCV-G 4 therapy. *IL-28B-CC* genotype correlates with developing early multispecific T-cell responses. These findings have important implications for predicting the outcome of HCV exposure and acute infection and identifying patients likely to benefit from therapy.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

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INTRODUCTION

Egypt has the highest incidence and prevalence of hepatitis C virus (HCV) infection worldwide (1–3), and hepatitis C represents a major public health and economic burden (3). Acute HCV represents 30% of acute hepatitis cases in Egypt (4–6). Genotype 4 is the most prevalent genotype in Egypt (7) and has recently begun spreading beyond its strongholds in the Middle East and Africa to Europe (1).

Exposure to hepatitis C usually goes unnoticed by individuals, and acute HCV infection is mostly undetectable (8,9). Although some acutely infected patients will experience a spontaneous resolution, the majority will develop silent chronic HCV infection,

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which may progress to cirrhosis, hepatic failure, and liver cancer (8,9). A number of host factors such as presence of symptoms and female gender have been related to spontaneous viral clearance in some studies (10–12). Growing evidence supports a crucial role of HCV-specific T-cell responses in controlling recent viremia and preventing establishment of chronic infection (13–17). However, it is not well understood why some individuals mount effective cellular immune responses to HCV and successfully clear the infection, whereas the majority fail to develop or maintain efficient responses and develop chronic HCV. Also, it is not clear why some individuals at high risk for HCV infection such as injection drug users, health-care workers, and sexual partners of HCV patients have detectable HCV-specific T-cell responses without detectable HCV-RNA or antibodies against HCV (18–23).

The susceptibility to primary HCV infection and subsequent clinical course is likely to be influenced by genetic factors that govern both the innate and adaptive immune responses against HCV (24,25). Genome-wide association studies have recently identified single-nucleotide polymorphisms in proximity to the interleukin-28B (*IL-28B*) gene that can predict spontaneous clearance of acute HCV infection (26–29).

To date, the correlates of exposure to HCV genotype 4 (HCV-G4) infections and the impact of *IL-28B* polymorphism and immune system interactions on the outcome of exposure have not been adequately identified. Therefore, the current cohort study of high-risk Egyptians recently exposed to HCV-G4 was set up to investigate the outcome of exposure to HCV-G4 infection, the natural course of acute HCV-G4 infection, and to identify the clinical, genetic, virologic, and immunologic parameters predictive of outcome of exposure, recent HCV-G4 infections, and acute HCV therapy.

METHODS

Study design and clinical cohorts

This prospective cohort study (**Figure 1**), which spanned 7 years, 2005 through 2012, is part of a larger surveillance program for screening, early detection, and management of HCV among high-risk subjects A cohort of high-risk individuals (health-care workers to sharp injuries or needle sticks, injection drug use (IDU), invasive medical procedure, sexual and non-sexual contact with HCV index cases, receiving blood components or organ transplants) was assembled and their HCV exposure status was determined at the start of the study. HCV antibody-positive individuals were excluded, and only subjects with undetectable HCV antibodies and confirmed HCV-PCR-negative status at the time of study entry were followed up to measure and compare the outcome of exposure and frequency of recent HCV infection. If exposed to a recent exposure, the individual was tested again for HCV antibodies (third-generation enzyme-linked immunosorbent assay: Abbott IMx; Abbott Diagnostics, Maidenhead, Berkshire, UK) and a serum sample from the potential "source" individual (if available) was tested to HCV. If the exposed subject had undetectable HCV antibody at baseline and the source individual was HCV-positive or his/her HCV status was unknown, HCV-RNA (transcription-mediated amplification VERSANT HCV RNA Qualitative assay, Bayer Diagnostics, Emeryville, CA; lower limit of detection: 5 IU/ml) and a screening interferon gamma (IFN-γ) ELISpot assay were performed.

Subjects were enrolled in the study only if infected with genotype 4, in order to avoid the confounding effect of various HCV genotypes on interpretation of results. They were also included if at baseline they tested negative for anti-HCV antibody, HCV-RNA, and HCV-specific T-cell responses, and then had detectable HCV viremia, antibody seroconversion, or HCV-specific T-cell responses during follow-up (**Figure 1**). Patients were excluded if they had pre-existing HCV or hepatitis B virus liver disease (history and appropriate serologic and virologic studies), HIV, or active *Schistosoma mansoni* (urine or stool analysis or rectal snip), autoimmune hepatitis, organ transplantation, neoplastic disease, or immunomodulatory therapy. Subjects with no detectable HCV-RNA or no HCV-specific T-cell responses within 12 weeks of exposure were not tested further.

Control subjects. Twenty healthy volunteers (10 men and 10 women; mean age 37±6 years) not infected with HCV were enrolled as a control cohort for the HCV-specific T-cell response assays. We also assessed the distribution of *IL-28B* single-nucleotide polymorphism (*rs12979860*) among 200 healthy Egyptians (100 men and 100 women, mean age 39±5.9 years), as the distribution of *IL-28B* rs12979860 allele among Egyptians was not known.

Methods

At enrollment, eligible subjects completed a structured confidential questionnaire and the HCV genotype (Roche LINEAR ARRAY HCV Genotyping; Roche Diagnostics, Pleasanton, CA) was performed. Alanine aminotransferase (ALT), HCV antibody (Abbott IMx; Abbott Diagnostics), HCV-PCR (COBASR AmpliPrep/COBASR TaqManR HCV Quantitative Test, v2.0; lower limit of detection: 15 IU/ml) and IFN-γ ELISpot assay were performed at baseline, at weeks 4, 8, 12, 24, and 48 weeks. Transcription-mediated amplification (VERSANT HCV RNA Qualitative Assay) retesting was performed at the end of the study. Genotyping for the *IL-28B* rs12979860 C/T polymorphism was retrospectively performed, using patients' stored archived samples.

A risky exposure was defined as a health-care worker exposed to needle stick or sharp injury, being an injection drug user, exposure to an invasive medical procedure, sexual and non-sexual contacts of HCV index cases, or a recipient of blood components or organ transplants. Acute hepatitis C was defined on the basis of documented HCV conversion from HCV-RNA-negative to HCV-RNA-positive status within 2–6 months after exposure, elevated serum ALT >5 times upper limit of normal; with or without symptoms such as jaundice or fever. Although the convention is that two consecutive negative HCV-RNA tests define viral clearance, along with others, we observed in previous longitudinal studies that few patients have transient aviremia with detection of HCV-RNA after two consecutive HCV-RNA results (3,11). Thus, spontaneous viral clearance was defined in our study as three consecutive negative HCV-RNA (transcription-mediated amplification) results (3 months apart) during

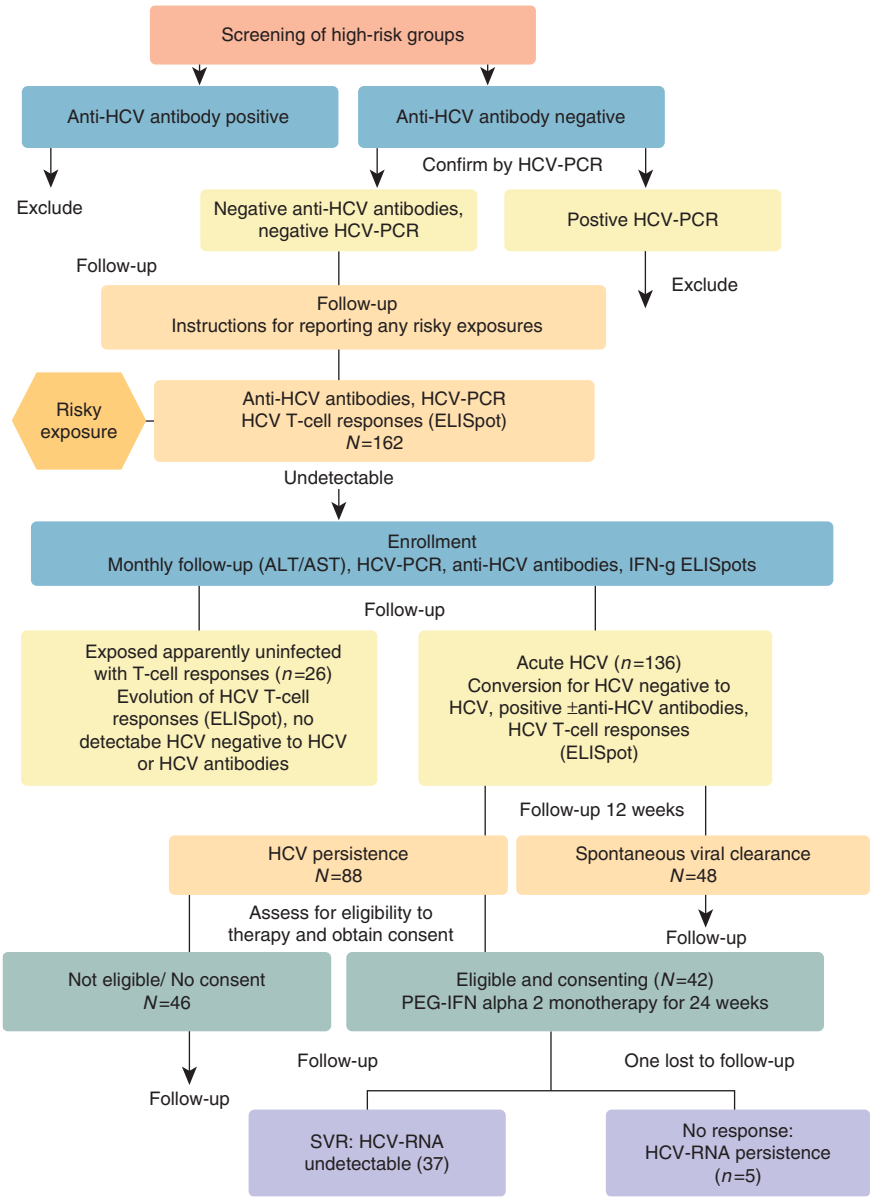

Figure 1. Longitudinal screening was performed for high-risk individuals. Individuals who reported a risky exposure such as needle sticks, sharp injuries, recent injection drug use (IDU), or contact with hepatitis C virus (HCV)-positive subjects were enrolled if at baseline they had undetectable anti-HCV antibody, HCV-RNA, and HCV immune responses. Enrolled subjects were prospectively followed for conversion from HCV-negative status to HCV-positive status and development of HCV-specific immune responses. ALT, alanine aminotransferase; PEG-IFN, pegylated interferon; SVR, sustained virologic response.

follow-up. The estimated date of infection was defined as midpoint from last negative HCV antibody/HCV-PCR to first positive HCV-RNA. The estimated date of clearance was the mid-point from last positive HCV to first of three consecutive negative HCV-RNA results.

Participants with persistent viremia beyond 12 weeks of enrollment were offered pegylated interferon (PEG-IFN) α-2a (Pegasys, Hoffmann-LaRoche, Basel, Switzerland) at a dose of 180 μg per week, or PEG-IFN-α-2b (1.5 mg/kg; PegIntron, Schering-Plough, Kenilworth, NJ) monotherapy for 24 weeks. Clinic visits for evaluation of clinical and biochemical laboratory values and adverse event monitoring were scheduled before treatment allocation, at allocation, at weeks 2 and 4, monthly thereafter during treatment, and at 6 months after treatment completion. Serum HCV-RNA assessments were performed before therapy, after 4 and 12 weeks, at the end of treatment, and end of follow-up. Safety was assessed through monitoring of patient-reported adverse events and, as indicated, by clinical and laboratory test results. The primary efficacy end point was sustained virologic response (SVR), defined as undetectable serum HCV-RNA, 24 weeks after completing treatment (HCV transcription-mediated amplification with a lower limit of detection of 5 IU/ml; Bayer VERSANT HCV RNA 3.0).

The study protocol was reviewed and approved by the Institutional Review Board on 2 April 2004 and amended on 10 January 2011 to test *IL-28B* polymorphisms on patients' stored samples.

The study was monitored by the Data Safety and Monitoring Board Committees of Ain Shams University School of Medicine, Al Azhar University School of Medicine and at each participating institution. All participants provided informed consent in writing at enrollment, before blood collection, immunological studies, treatment, and retrospective *IL-28B* genotyping. The protocol and all study procedures were conducted in conformity with the ethical guidelines of the Declaration of Helsinki of 1975.

Detection of HCV-specific T-cell responses

In this study, we longitudinally analyzed HCV-specific responses in un-fractionated peripheral blood mononuclear cell (PBMC) samples, using an IFN-γ ELISpot assay. T-cell subset analysis and fine mapping of antigenic targets were not part of the scope of this analysis.

Recombinant HCV proteins

Purified recombinant proteins (Core (aa1–115), NS3 (aa1007–1534), NS4 (aa1617–1864), and NS5a (aa2006–2264) (Mikrogen, Neuried, Germany)) were used in the ELISpot assays at a final concentration of 2 µg/ml. HCV proteins were expressed as COOH-terminal fusion proteins with human superoxide dismutase in yeast. Yeast and superoxide dismutase were used as controls for nonspecific stimulation. As positive controls, phytohemagglutinin (1:200 dilution; Murex Diagnostics, Chatillon, France) and tetanus toxoid (TT)/ml (Wyeth Laboratories, St Davids, PA) were used as positive controls at concentrations of 4 and 1 ìg/ml, respectively. Un-stimulated cells with media only were used as negative controls.

ELISpot assays

IFN-γ ELISpot assays were performed for study subjects and 20 HCV-negative volunteers at baseline, monthly for 3 months, and every 3 months thereafter. PBMCs were separated from whole blood by Ficoll–Hypaque density gradient centrifugation and immediately stored in liquid nitrogen until used in ELISpot assays. The ELISpot assay was carried out essentially as previously described (19,30). A response in a test well was considered positive if the spot forming units (SFUs) in the presence of antigen were at least double the Spot forming units in the medium control (medium alone) and if the number of spots per well, minus the background, was at least 25 SFUs/10^6 PBMCs. ELISpot assay is described in "**Supplementary Materials and Methods** online."

IL-28B genotyping

All enrolled participants were retrospectively genotyped for the rs12979860 allele. Genotyping was performed on stored baseline samples, using ABI TaqMan, Applied Biosystems, Foster City, CA, Roche Molecular Systems, Branchburg, NJ and the ABI7900HT sequence detection system (Applied Biosystems), according to the manufacturer's instructions.

Statistical analysis

Continuous variables were compared using Student's *t*-test or the Mann–Whitney *U*-test when appropriate. The frequencies were compared using the chi-square test or the Fisher's test if the expected frequency for any cell was five or lower. To assess independent associations between baseline factors, HCV outcomes, and viral clearance status, a multiple logistic regression model was built using stepwise selection technique (31). After adding each X variable, the effects of removing any of the other X variables was tested. Crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unadjusted/univariate and adjusted/multivariate model, respectively. The baseline and follow-up variables (age, sex, symptoms (jaundice), ALT, serum bilirubin, HCV-RNA, *IL-28B* genotype, number of ELISpot spot-forming units, and numbers of HCV antigens recognized) were introduced in the logistic model because of their association (<0.11) in the univariate analysis. The Kaplan–Meier plot was utilized to estimate the time-to-spontaneous resolution.

Among those assigned to therapy, treatment efficacy analyses were performed using the intention-to treat population, defined as all enrolled patients who received at least one dose of any study medication. Receiver operating characteristic curves were calculated to determine the best cutoff of HCV-RNA decay to predict SVR and treatment failure. The Hardy–Weinberg disequilibrium test was performed for single-nucleotide polymorphism analysis. Linkage disequilibrium index (Lewontin's D′ and r^2) was calculated by Haploview version 4.2 software (MIT MediaLab, Boston, MA). Results are expressed as mean values ± s.d., *P* values and 95% CIs or median and interquartile range as appropriate. Statistical analysis was done using Statistical Analysis Software version 16 (SAS Institute, Cary, NC), MedCalc Software version 12 (MedCalc Software, Ostend, Belgium), and GraphPad Prism Software (La Jolla, CA).

RESULTS

Outcome of exposure to HCV

A total of 3,516 high-risk Egyptians (from Cairo, Giza, Minya, Beni Seuf, and Quena governorates) screened for HCV revealed that 283 participants (8.05%) were HCV antibody-positive, 2 (0.06%) were HCV-PCR-positive and HCV antibody-negative, and 3,231 participants (92.5%) were HCV-negative by antibody and HCV-PCR (**Figure 1**). Of the 3,231 exposed individuals, 136 (4%) had newly acquired acute HCV infection (patients were initially HCV-RNA-negative at baseline and became HCV-RNA positive ± seroconversion) and 26 (0.7%) developed HCV-specific T-cell responses without detectable viremia or anti-HCV antibodies (**Figure 1**).

Spontaneous viral clearance was achieved by 48 out of the 136 participants with proven acute HCV (35.3%), with a median time from the last negative HCV antibody/RNA to the first positive HCV-RNA of 90 days (range 30–180 days; interquartile range = 79) (*group A: resolved acute HCV*). In 26,136 participants (0.7%), HCV immune responses evolved gradually after exposure to HCV infection, although neither HCV-RNA nor anti-HCV antibody was detected during the scheduled follow-up points (*group B: exposed seronegative (ESN) individuals*). Eighty-eight participants with

proven acute HCV did not clear HCV spontaneously (*group C: unresolved acute HCV*). This group was further subdivided into patients treated with PEG-IFN α-2 ($n=42$) and patients who did not resolve acute infection and were not treated ($n=46$).

The median follow-up of study subjects was 15 months (range 11–19 months, interquartile range = 5). The demographic and clinical parameters of the study population are shown in **Table 1**. The highest proportion of participants with newly acquired infections was IDUs and individuals exposed to invasive medical procedure (mostly cardiac catheterization and hemodialysis). Spontaneous clearance was considerably higher among patients acquiring acute HCV infection through occupational exposure (36/48; 75%), compared with IDUs (8/48; 16.7%), who were also the least compliant during follow-up, compared with other study subjects (29 IDUs completed all follow-up time points while 19 IDUs fulfilled 5–7 follow-up points (data not shown)). Intrafamilial transmission was reported between parents and children (**Table 1**).

Sequential ALT and HCV-RNA measurements are shown in **Figure 2**. More women attained spontaneous viral clearance than men ($P=0.0007$; **Table 1**). More women mounted T-cell responses without detectable HCV viremia or seroconversion (**Table 1**). Also, the interval between the first positive HCV-PCR and the viral clearance (negative HCV-PCR) was significantly shorter in women compared with men ($P=0.036$; **Figure 3**). Patients with jaundice ($n=38$) had higher chance of achieving spontaneous viral clearance compared with patients with anicteric acute HCV ($P<0.0001$; **Table 1**).

Table 1. Baseline demographic and clinical characteristics of patients

Parameter	Exposed uninfected Group A (*N*=26)	Spontaneous clearance Group B (*N*=48)	No spontaneous clearance Group C (*n*=88)	*P* value (mean of difference between groups; 95% CI of difference)
Age (years) Mean±s.d. (95% CI of the mean)	37.08±8.97 (33.46–40.7)	36.48±7.64 (34.26 –38.69)	37.17±6.2 (35.86–38.48)	Group A vs. B: *P*=0.076 (–0.6; –4.54 to 3.344) Group A vs. C: 0.95 (–0.09; –3.14 to 2.96) Group B vs. C: *P*=0.56 (–0.69; –3.082 to 1.702)
Females (*n*; %)	17 (65.4%)	30 (62.5%)	37 (42.05)	Group A vs. B: *P*=0.98 (3.30% ; –21.74% to 26.3%) Group A vs. C: *P*=0.003 (34.70%; 11.07% to 54.47%) Group B vs. C: *P*=0.0007 (31.80%; 13.23% to 48.29%)
Risk factors for HCV transmission: n *(%)*				
Occupational exposure	16 (61.5)	35 (72.9)	26 (29.5)	
Intravenous drug use	5 (19.2)	8 (16.7)	35 (39.8)	
Sexual activity	4 (15.4)	2 (4.16)	9 (10.2)	
Medical procedures	1 (3.8)	2 (4.16)	10 (11.4)	
Cardiac catheterization	0	0	4/10 (40)	
Hemodialysis	0	0	3/10 (30)	
Home delivery	1/1 (100)	2/2 (100)	0	
Dental procedures	0	0	3/10 (30)	
Intrafamilial	0	1 (2.1)	8 (9.1)	
Mean time between exposure and first positive HCV-PCR (days) (95% CI of the mean)	0	35.21±10.58 (10.1–59.31)	38.36±12.7 (11.42–60.3)	Group B vs. C: 0.09 (3.02; –0.49 to 6.52)
Symptoms (jaundice±fever; *n*, %)	0	30 (62.5)	8 (9.1)	Group B vs. C: *P*<0.0001
Mean total bilirubin±s.d. (mg/dl) (95% CI of the mean)	1.2±1.003 (0.8 to 1.6)	4.445±2.1 (3.8–1.9)	3.04±1.2 (2.6–3.5)	Group A vs. B: <0.001 (3.18; 2.32 to 4.06) Group A vs. C: <0.001 (–1.83; –2.71 to –0.96) Group B vs. C: <0.001 (1.35; 0.585 to 2.11)
Mean ALT±s.d. (U/l) (95% CI of the mean)	36.53±20.81 (26.01 to 77.05)	423.58±132.4 (385.14–462.02)	441.5±93.96 (42.6–461.37)	Group A vs. B: <0.001 Group A vs. C: <0.001 Group B vs. C: 0.361
Mean AST±s.d. (U/l) (95% CI of the mean)	39.7±25.3 (23.3 to 106.08)	480.5±118.3 (446.14–514.85)	437.15±128.2 (409.94–464.26)	Group A vs. B: <0.001 Group A vs. C: <0.001 Group B vs. C: 0.0550
HCV-RNA ($\times 10^6$ IU/ml) (95% CI of the mean)	0	1.219±0. 57 (1.05–1.38)	1.126±0. 636 (0.9–1.3)	Group B vs. C: 0.3998 (0.093; –0.125 to 0.311)
HCV genotype Genotype 4 (*n*, %)	0	48 (100)	88 (100)	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; HCV, hepatitis C virus.

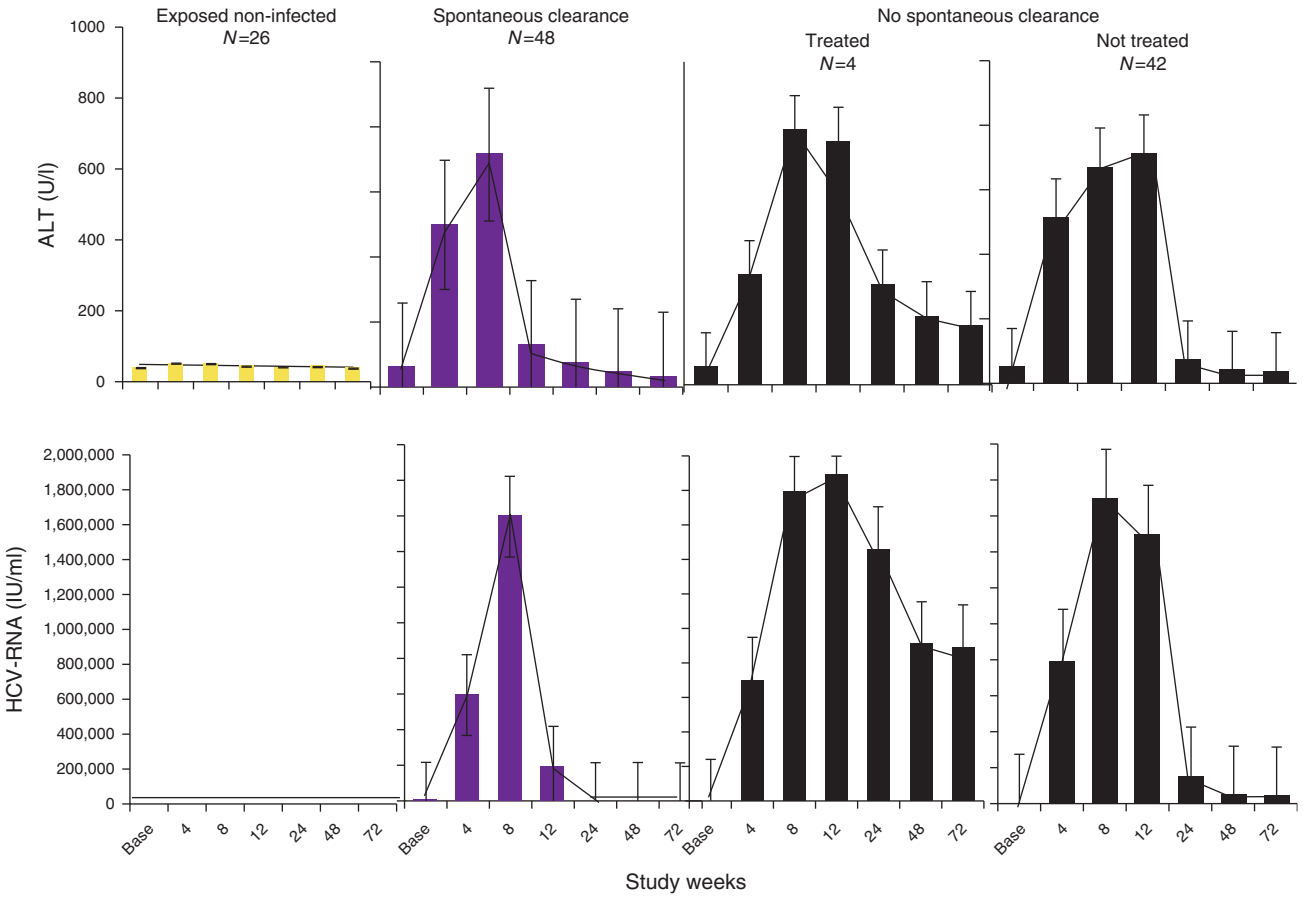

Figure 2. Kinetics of alanine aminotransferase (ALT) and hepatitis C virus (HCV)-RNA levels in 26 subjects who mounted HCV-specific T-cell responses without detection of anti-HCV antibodies or HCV-RNA at the scheduled testing time points, 48 subjects with spontaneous resolution of acute HCV, and 88 patients with unresolved acute HCV; 42 of whom were treated with pegylated interferon monotherapy during the acute phase.

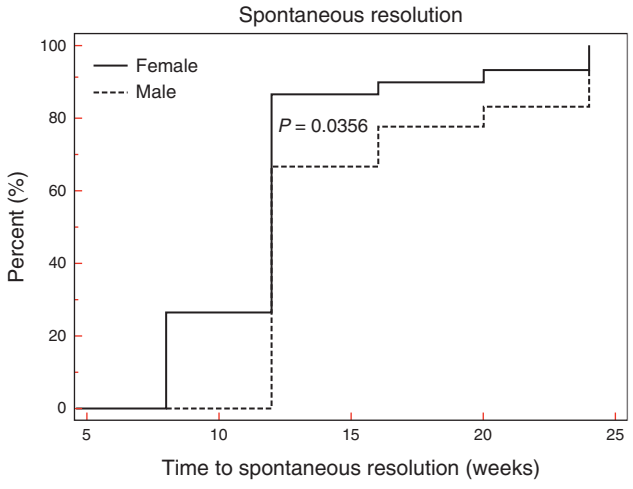

Figure 3. Time to spontaneous resolution of acute hepatitis C virus (HCV) in men and in women. It was observed that more women achieved spontaneous viral clearance within a shorter time frame compared with men.

HCV-specific T-cell responses

Consistent with previous work, the frequency, magnitude, and breadth of HCV-specific T-cell responses were greater in participants with spontaneous recovery, compared with those with chronic HCV evolution who initially developed significantly weaker IFN-γ responses that gradually waned with time (**Figure 4a**). The mean peak SFUs was significantly higher in spontaneous resolvers (776.7±204.2/106 PBMCs) and apparently uninfected participants with immune responses (743.27±188.7/106), compared those with chronic HCV evolution (197.56±60.41/106 PBMCs) ($P=0.0362$ between groups A and B; $P<0.0001$ between groups A and C and groups B and C, respectively). A cutoff of 270 SFUs/10^6 PBMCs distinguished subjects with spontaneous viral clearance from participants with viral persistence with a sensitivity of 83.3% and specificity of 97.8% (**Supplementary Table 2**).

The specificity and breadth of T-cell responses also differed among groups. During the acute phase, multispecific T-cell responses to three or more HCV antigens were observed in 46/48 (85.8%) subjects with spontaneous viral clearance, 25/26 (96.2%) exposed apparently uninfected subjects, and 23/88 (26.13%)

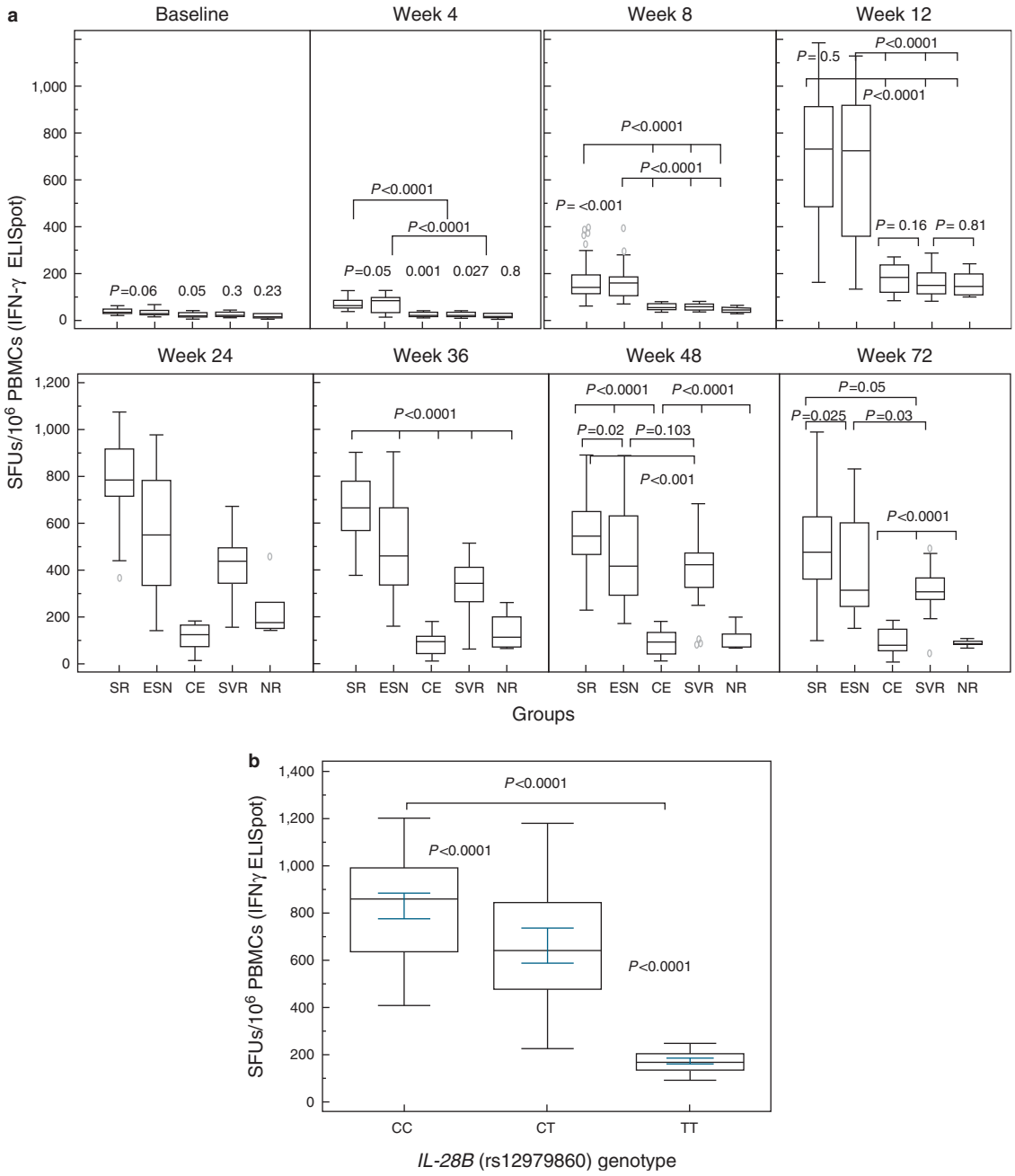

Figure 4. (**a**) Strength of hepatitis C virus (HCV)-specific T-cell response in an ELISpot assay in the different study groups. The spot-forming units in each group are represented at the indicated time points. The box extends from the first quartile to the third quartile. The horizontal line within the box represents the median of the data set. Overall, the magnitude and breadth of HCV-specific T-cell responses observed in acute HCV with spontaneous recovery and exposed apparently uninfected individuals were significantly higher than the responses detected in individuals with HCV persistence not receiving pegylated interferon monotherapy. Treatment was associated with enhancement of HCV-specific T-cell responses during therapy. (**b**) Strength of anti-HCV T-cell response tested by IFN-γ ELISpot assay, according to *IL-28B* genotype allele. X axis**:** *IL-28B* rs12979860 single-nucleotide polymorphism (CC, CT, TT). Y axis: number of spot-forming cells. The horizontal line within the box represents the median of the data set. The box extends from the first quartile (Q1) to the third quartile (Q3). The range is indicated by the distance between the smallest value and the largest value, including outliers. The interquartile range is Q3 minus Q1. CE, patients with chronic evolution; ESN, exposed seronegative uninfected subjects with immune responses; IFN-γ, interferon gamma; NR, non-responders; PBMC, peripheral blood mononuclear cell; SFUs, spot forming units; SR, spontaneous resolution (patients with spontaneous recovery; SVR, treated patients with sustained virologic response.

patients with viral persistence. The mean numbers of recognized HCV antigens in groups A, B, and C were 3.61±1.2, 3. 3±1.1, and 1.6±1.02, respectively; ($P=0.2787$ between groups A and B; $P<0.0001$ between groups A and C and groups B and C, respectively). Among subjects with spontaneous viral clearance and ESNs, the strongest response in terms of SFUs/million cells was

Table 2. Frequency of the *IL-28B rs12979860-CC*, *CT*, *and TT* genotype in spontaneous resolvers, exposed apparently uninfected, treated patients, and patients with chronic evolution

			No spontaneous clearance Group C (*n*=88)			
rs12979860 Genotype (*n*; %)	Exposed uninfected Group A (*N*=26)	Spontaneous clearance Group B (*N*=48)	Untreated *N*=46	Treated SVR *N*=37	Treated NR *N*=5	*P* value
C/C	18 (70)	31 (65)	2 (4.3)	10 (27.1)	0	Group A vs. B: *P*=0.799 Group A vs. C: *P*<0.001* Group B vs. C: *P*<0.001*
C/T	7 (27)	13 (27)	11 (23.9)	14 (37.8)	1 (20)	Group A vs. B: *P*=1.000 Group A vs. C: *P*=1.000 Group B vs. C: *P*=0.844
T/T	1 (4)	4 (8.3)	33 (72)	13 (35)	4 (80)	Group A vs. B: *P*=0. 0.651 Group A vs. C: *P*<0.0001* Group B vs. C: *P*<0.0001*

HCV, hepatitis C virus; NR, Non-responders; SVR, sustained virologic response.

Group A: Twenty-six participants with detectable HCV-specific responses despite persistently negative HCV viremia or HCV antibodies.

Group B**:** Forty-eight participants with proven symptomatic or asymptomatic acute HCV (conversion from HCV-RNA-negative to HCV-RNA-positive status with or without seroconvesion) who mounted HCV responses and cleared infection spontaneously.

Group C: Eighty-eight participants with unresolved acute HCV with chronic evolution of whom 42 participants were treated with paginated interferon monotherapy.

*P<0.001 or P<0.0001: Highly significant.

elicited by the non-structural HCV-encoded proteins (NS_3, NS_4, and NS_5).

Impact of *IL-28B* polymorphism on the outcome of exposure to HCV Infection and T-cell responses

The C/C genotype of the rs12979860 polymorphism was detected in 79 (39.5%) healthy subjects. In the study population, the *C/C*, *C/T*, *and T/T* genotypes were detected in 61 (37.7%), 46 (28.4), and 55 (33.9%), respectively. The frequency of the *C/C* genotype was significantly higher in subjects with spontaneous viral clearance (group A) compared with those who failed to resolve the acute infection spontaneously (group B) (64.6% vs. 20.5%, respectively, $P<0.0001$). In contrast, T/T genotype was more prevalent among individuals with persistent HCV infection as compared with the spontaneous resolvers (50% vs. 8.3%, respectively, $P<0.0001$) or ESNs (50% vs. 3.8%, respectively, $P<0.0001$; **Table 2**). However, there was no significant difference in the CC homozygosity (64.6% vs. 69.2%, respectively; $P=0.7990$), *CT* heterozygosity (27.1% vs. 26.9%, respectively; $P=1.000$) or *TT* homozygosity (8.3% vs. 3.8%, respectively; $P=0.0.651$) between spontaneous resolvers and exposed apparently uninfected subjects with HCV-specific T cell-responses (**Table 2**). Subjects with the CC genotype mounted robust, multispecific T-cell responses as compared with the TT genotype (**Figure 4b**) with a significant correlation between carrying the C/C genotype and developing strong, broad HCV-specific T-cell responses ($r^2=0.0.835$; $P<0.001$), as well as between C/C and spontaneous HCV clearance ($r^2=0.97$; $P<0.001$) (data not shown).

Predictors of spontaneous resolution of acute HCV and HCV-specific T-cell responses

We identified certain predictors of spontaneous viral clearance (i.e., just focusing on group A participants). By univariate analysis, *IL-28B* rs12979860 CC, developing =/>270 SFUs/10^6 cells in response to 3 or more HCV proteins, female gender, presence of symptoms (jaundice), >300 IU/l ALT decline and >2.5 log10 HCV-RNA drop within 8 weeks were associated with spontaneous resolution. Multivariate analyses confirmed the independent roles for rs12979860 CC (OR=14.22 ; 95% CI: 5.42–38.35), $P<0.0001$), magnitude and breadth of HCV-specific T-cell responses (≥=/>270/106 SFUs to 3 or more HCV antigens; OR=11.66 ; 95% CI: 4.57–30.49, $P<0.0001$), ALT decline >300 IU/l within 4 weeks (OR=6.83; CI: 2.25–21.69; $P<0.0001$), female gender (OR=2.39; $P=0.007$), peak bilirubin level >3 mg/dl (OR=5.54; $P<0.001$) in spontaneous viral clearance. The sensitivity of *IL-28B*, >2.5 log10 HCV-RNA drop within 8 weeks, ALT decline >300 IU/l within 4 weeks, and peak bilirubin (>3 mg/dl) in predicting spontaneous resolution were 91.67%, 89.36%, 83.33%, and 81.25%, respectively. The specificities of *IL-28B*, >2.5 log10 HCV-RNA drop within 8 weeks, ALT decline >300 IU/l within 4 weeks, and peak bilirubin (>3 mg/dl) were 95.65%, 82.61%, 73.33%, and 67.39%, respectively (**Supplementary Table 2**). Neither age nor mode of infection was an independent factor for spontaneous viral clearance (**Table 3**). *IL-28B*-CC was also a strong predictor of developing strong broad T-cell responses (OR=11.58; 95% CI: 4.35–23.68; $P<0.0001$; **Table 4**).

Treatment response

Of the 88 patients who did not clear viremia within 12 weeks after enrollment (group C), 42 consented to treatment during the acute phase, whereas the remaining 46 preferred to defer therapy. The intention-to treat analysis that included all members of this cohort demonstrated a SVR rate of 88% (37 of 42 treated patients; **Figure 1**) and, per protocol, SVR was 90.4% (38/42). Treatment was well tolerated with few manageable adverse events **(Supplementary Table 3**). Females were more likely to attain SVR than males

Table 3. Association of between baseline/follow-up factors and spontaneous viral clearance

Variable	Unadjusted analysis		Adjusted analysis	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
IL-28B rs12979860 (CC vs. others)	17.78 (3.74–40.84)	<0.0001***	14.22 (5.42–38.35)	<0.0001***
=/>270 IFN-γ SFUs/10^6 cells to=/>3 HCV antigens	10.96 (3.46–0.65)	<0.0001***	11.66 (4.57–30.49)	< 0.0001***
ALT decline >300 IU/l within 4 weeks	3.078 (0.23–7.84)	<0.0001***	6.83 (2.25–21.69)	< 0.0001***
Peak bilirubin (>3 mg/dl)	3.99 (2.53–16.30)	<0.0001	5.54 (2.40–12.93)	< 0.0001***
Symptoms (jaundice±fever)	3.92 (1.37–9.87)	0.003	3.54 (1.57–8.02)	0.001**
Gender (females relative to males	2.80 (1.18–6.75)	0.006	2.39 (1.27–5.53)	0.007**
>2.5 log10 HCV-RNA drop within 8 weeks	2.96 (1.29–7.16)	0.0363	2.48 (1.09–8.64)	0.016*
Baseline age (per decade, (older vs. younger))	0.76 (0.58–0.99)	0.049	0.75 (0.53–1.06)	0.109

ALT, alanine aminotransferase; CI, confidence interval; IFN-γ, interferon gamma; IL-28B, interleukin-28B; HCV, hepatitis C virus; OR, odds ratio; SFUs, spot forming units.
*Significant (*P*<0.05).
**Highly significant (*P*<0.01).
***Highly significant (*P*<0.001).

Table 4. Association between baseline/follow-up factors and development of strong (=/>270 IFN-γ SFUs/10^6 cells) and broad (=/>3 antigens with positive response) HCV-specific T-cell response regardless of group

	OR	95% CI	*P* value	OR	95% CI	*P* value
IL-28B rs12979860 (CC vs. others)	8.43	3.27–28.51	0.002	11.58	4.35–23.68	<0.0001**
Gender (females relative to males)	3.45	1.05–8.21	0.041	2.32	1.24–3.45	0.003*
>2.5 log10 HCV-RNA drop within 8 weeks	5.20	1.17–21.5	0.021	5.43	0.08–2.51	0.035*
Age (per decade, (older vs. younger))	0.86	0.63–3.87	0.25	0.75	0.53–1.07	0.11
Jaundice	3.8	0.4–11.7	0.42	Removed by stepwise selection		
ALT decline >300 IU/l within 4 weeks	1.06	0.95–1.89	0.29	Removed by stepwise selection		
Mode of transmission (IDU vs. others)	0.27	0.38–5.95	0.52	Removed by stepwise selection		

ALT, alanine aminotransferase; CI, confidence interval; IDU, injection drug use; IFN-γ, interferon gamma; IL-28B, interleukin-28B; HCV, hepatitis C virus; OR, odds ratio; SFUs, spot forming units.
*Significant (*P*<0.05).
**Highly significant (*P*<0.01).

(OR = 1.75; 95% CI: 0.18–5.49; *P* = 0.02) and patients with >2 log10 HCV-RNA drop within the first 4 weeks of therapy (OR = 4.92; 95% CI: 0.376–10.86; *P* = 0.002) (**Supplementary Table 4**).

SVR was associated with gradual improvement in the magnitude and breadth of T-cell responses during therapy, although, with less intensity and durability compared with responses detected in subjects with self-limited disease (**Figure 4a**). Patients with the C/C or the C/T genotypes had more than a threefold higher rate of SVR compared with patients with the T/T genotype (**Table 2**) and were more capable of maintaining HCV-specific T-cell responses during therapy.

DISCUSSION

The mostly unrecognized HCV exposure and subclinical nature of acute HCV infection limited the conduct of large studies investigating the outcome and course of recent infection. To bridge this knowledge gap, we conducted this prospective cohort study to analyze the correlates of exposure to HCV infection and the natural history of acute infection. The large cohort of subjects recently exposed to HCV, our stringent protocol of sequential assessment of transaminases, anti-HCV antibodies, HCV, PCR using a sensitive technique, in addition to the long follow-up, enabled us to monitor with reasonable accuracy the time of exposure, the timing of HCV-PCR, subsequent anti-HCV antibody conversion and viral clearance, in addition to assessing the various host and virologic factors that determine the outcome exposure to HCV.

Overall, the proportions of newly reported HCV cases after risky exposure in this study were similar to some reports (4–6,10,32–34) and a bit higher than others (35,36). Discrepancies in estimates of spontaneous viral clearance among studies may be attributed to differences in definitions of acute HCV and

viral clearance as well as variations in the sensitivities of the diagnostic methods used.

We observed a variation in the proportion of newly acquired infections, according to the mode of transmission. Our study identified IDU, invasive medical procedures, and occupational exposure as the main modes of HCV transmission in our cohort, in agreement with previous reports (4–6,10,33,34,36,37). Thus, our findings further underscore the crucial need for strict implementation of infection control measures in Egyptian health facilities. Similar to other studies from Egypt (19,38,39), this study showed that transmission within households also has a role in transmission of HCV. Given that the subjects enrolled in this study were mostly young or middle-aged; our findings suggest an ongoing active HCV transmission in Egypt via routes that differ from the historical antischistosomal therapy campaigns that officially ceased more than four decades ago.

In our study, spontaneous resolution occurred in 35.3% of patients with acute HCV infection, a figure not far from the 30 to 36% in previous studies (6,10,33,38,40). Some of the predictors of spontaneous viral clearance found in our study have been identified before by our group and others (10–12,35–37). Stratifying patients according to the presence or absence of jaundice demonstrated that two-thirds of symptomatic patients in the study achieved spontaneous viral clearance, in agreement with previous reports (3,11,35–38). In accordance with previous observations (27,38,39), we showed that spontaneous resolution was more frequent in women. Interestingly, we also found that women eradicated HCV more rapidly than men, although a larger sample size is required to validate this finding. We also observed that an early rapid significant decline in ALT and HCV-RNA titers could predict spontaneous viral clearance and may distinguish those who will develop viral persistence from those who will go on to spontaneous viral clearance. Thomson *et al.* (41) also reported that the best diagnostic test for the prediction of spontaneous resolution was a maximum log10 drop in viral load within 100 days.

As previously reported by our group and others (13–15,17,42,43), our study showed that subjects who achieved spontaneous viral clearance mounted polyclonal, strong, and broad virus-specific T-cell responses, whereas individuals who failed to mount or sustain such responses developed persistent viremia. To test if the quantitative assessment of the magnitude and breadth of T-cell responses could serve as a biomarker of self-limited disease, we attempted to define a reliable cutoff level of T-cell responses that may discriminate between those who may achieve spontaneous resolution from patients liable to develop chronic infection. In this study, we found that using a cutoff point of 270 SFUs/10^6 to 3 or more HCV antigens efficiently predicted spontaneous viral clearance, a finding that supports the results of a previous study (44), which suggested that assessing T-cell thresholds might be useful in predicting the clinical outcome of acute HCV infection.

Among individuals reporting definite HCV exposure, we identified 26 individuals with no baseline HCV-RNA, HCV antibodies, or HCV-specific T-cell responses, who gradually mounted immune responses against HCV a few weeks after exposure without any detectable HCV-RNA or seroconversion during follow-up. These data further support our previous report (19) on partners of acute HCV patients, and previous studies (20–23) on IDUs and household contacts of HCV patients who had cellular immune responses without detectable anti-HCV antibodies or RNA. The origin, patterns, and functions of such responses are intriguing. One may argue that the apparently uninfected subjects with immune responses may have had prior resolved HCV infection and lost anti-HCV antibodies, hence the detected responses represented memory responses. Takaki *et al.* (45) demonstrated that cellular immune responses existed without corresponding humoral immune responses, many years after resolution of acute HCV infection, suggesting that antibodies could be lost over time. In our study, the possibility that those subjects had a previous infection is unlikely, given the absence of HCV-RNA, anti-HCV antibodies, and T-cell responses at baseline and the gradual development of immune responses without detectable HCV-RNA or seroconversion at the scheduled assessment time points. It is unlikely that those subjects had very low baseline levels of chronic cryptic viremia, not detected by the HCV-PCR test, as we used a sensitive technique for detection of HCV-G4 viremia. In this study, one may speculate that exposure to HCV infection may provoke distinct, non-synergistic, innate immune responses such as natural killer cells, resulting in production of antiviral-cytokines that eliminated HCV virus at an early stage, leading to priming CD4+ and CD8+ adaptive immune responses as suggested in recent studies (46,47). Although we assessed HCV viremia at monthly intervals, we cannot completely exclude brief transient viremia that might have resolved in the time between our scheduled HCV-RNA, and anti-HCV antibodies measurement (42,48), or potential bias in reporting the date of exposure.

IL-28B (*IFN-*λ (α)-3), a member of the IFN-α family (49), is probably involved in the immune response to HCV infection and viral clearance following acute HCV (26–29,50) or anti-viral therapy (51,52). In this cohort of Egyptians with acute HCV-G4, this study demonstrates a significant association between C/C genotype and spontaneous HCV clearance, lending further credence to previous reports on patients with HCV-4 (50) and non-4 HCV acute infections (51–53). The study also provides novel data on the impact of *IL-28B* polymorphism on the evolution and functions of HCV-specific T-cell responses following risky exposures and during the early phase of recent HCV infection. In our study, *IL-28B*-CC was also a strong predictor of developing strong broad T-cell responses. Thus, robust broad immune responses and rs12979860 CC synergized to significantly increase the likelihood of achieving viral clearance over either factor alone. In this study, the frequency of the protective genotype (rs12979860 CC) among apparently uninfected subjects with immune responses (69.2%) was not different from that in subjects of spontaneously resolved acute viral hepatitis. However, Knapp *et al.* (54) (41.9%) reported that the *IL-28B* genotypes frequencies among ESNs were distinct from those among individuals with self-limited disease. The difference between the two studies may reflect variation in the characteristics of the cohorts. Knapp *et al.* (54) only enrolled IDUs repeatedly exposed to HCV who might have had remote infections that resolved. In this study, ESNs initially tested negative for HCV antibodies, viremia, and immune responses, shortly after

exposure, but mounted T-cell responses only during longitudinal monitoring. One may speculate that the C/C genotype may favor early stimulation of strong efficient innate immune responses that prime adaptive immune responses resulting in different scenarios. In some individuals, the innate immune response may blunt the acute HCV infection at a very early phase, leading to efficient viral eradication. In other exposed individuals, the CC genotype might not protect against acute HCV disease but favors spontaneous resolution through stimulation of strong, effective innate immunity that may in turn prime efficient adaptive immune responses capable of containing apparent acute HCV infection. Recent reports showed that rs12979860 CC carriers more likely have robust innate immune function (55,56). We may speculate that the ESNs in this study are very early resolvers whose genetic background enabled them to develop efficient, innate, and HCV-specific immune responses that induced viral eradication but was not sufficient to stimulate a humoral response. The similarity in *IL-28B* genotype distribution in spontaneous resolvers and ESNs in the study may support the previous premise.

Rates of SVR in the subset of study patients treated with PEG-IFN were comparable to those reported following treatment of patients with acute HCV-G4a (30,57). As we previously reported (30), PEG-IFN therapy during the acute phase was associated with enhancement of T-cell responses, although with less magnitude and breadth when compared with responses observed in spontaneous viral clearance (30). A significant association was observed between the CC and CT variants and achieving SVR, suggesting that *IL-28B* genotype determination can have an important role in treatment decision making in acute HCV infection and can predict SVR, following PEG-IFN alpha-2 therapies during acute HCV. Identifying the *IL-28B* genotype may have important clinical applications by prioritizing therapy to subgroups of acute HCV patients who are vulnerable to chronic evolution of HCV infection, and defining the adequate timing and duration of therapy. On the basis of our *IL-28B* genotype findings, we are currently conducting a clinical trial to optimize the duration and onset of PEG-IFN alpha therapy based on *IL-28B* genotype.

The longitudinal study design, the well-characterized large cohort of subjects recently exposed to HCV-G4, the long follow-up, the stringent definitions of acute HCV, SVR, the monitoring of natural history, and outcome of therapy are among the strengths of this study. However, the study has limitations. We enrolled only Egyptians infected with genotype 4 because this is the prevalent genotype in Egypt. Further studies are required to investigate the applicability of our findings in other HCV genotypes and other ethnicities. The estimate of spontaneous clearance maybe overestimated in this study since participants were offered treatment after 12 weeks following enrollment and some individuals may have gone on to spontaneously clear infection if they were not treated. Given the large cohort, the different parameters investigated, and the serial ELISpot assays performed, it was not possible to perform T-cell subset analysis and fine mapping of antigenic targets to all patients enrolled. We did not investigate the innate immune system in this cohort. It would be interesting to longitudinally monitor the impact of interplay between *IL-28B,* polymorphisms, and innate and adaptive immune responses on the outcome of acute HCV infection.

In conclusion, our study identified several host and viral factors that influence the outcome of exposure to HCV infection. *IL-28B* CC genotype, robust T-cell responses, rapid decline in ALT and HCV-RNA levels, and female gender could be predictors of spontaneous and treatment-induced resolution of acute HCV infection. These data have important clinical implications for identifying subjects eligible for therapy during the acute phase, to reduce HCV transmission, and chronic liver disease, with its serious complications.

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CONFLICT OF INTEREST

Guarantors of the article: Sanaa M. Kamal, MD, PhD and Samar K. Kassim, MD, PhD.

Specific author contributions: Sanaa M. Kamal planned and designed the study, conducted patients' recruitment, clinical assessment, follow-up and data collection, data interpretation, and drafting of the manuscript. Kamal has approved the final draft submitted. Samar Kassim performed the serial biochemical studies, HCV-PCR, and HCV genotyping, interpreted data, drafted the manuscript, and provided important intellectual content. Kassim has approved the final draft submitted. Amany Ahmed Ibrahim shared in patients' enrollment, clinical examination, follow-up, data collection and interpretation, and drafting of the manuscript. Ahmed has approved the final draft submitted. Sara Mahmoud shared in patients' enrollment, clinical examination, follow-up, data collection and interpretation, and drafting of the manuscript. Mahmoud has approved the final draft submitted. Ibrahiem A. Aziz shared in patients' enrollment, clinical examination, follow-up, and drafting of the manuscript. Abdel Aziz has approved the final draft submitted. Khaled Bahnasy contributed to data entry, processing, statistical analysis, and presentation. Bahnasy has approved the final draft submitted. Tamer Hafez performed retrospective *IL-28B* analysis, interpreted data, and has approved the final draft submitted. Iman Fathelbab has conducted the immunological studies, interpreted data, and has approved the final draft submitted. Hoda Mansour has conducted the immunological studies, interpreted data, shared in drafting the manuscript, and has approved the final draft submitted.

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LIVER

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Egypt has the highest prevalence of hepatitis C virus (HCV) worldwide: 15%.
- ✓ Acute HCV infection is mostly asymptomatic and not easily identified clinically.
- ✓ Given the scarcity of symptoms in many cases of acute HCV, it has largely escaped the focus of studies investigating the pathogenesis of viral clearance and persistence.
- ✓ Treatment of recent HCV infection is associated with high SVR rates.

WHAT IS NEW HERE

- ✓ This study is the largest, investigating the outcome and the natural history of exposure to HCV-G4 infection and the predictors of either spontaneous resolution or persistence.
- ✓ Quantitative determination of alanine aminotransferase (ALT), HCV-RNA, and T-cell responses can predict the outcome of acute HCV.
- ✓ *IL-28B* genotype is a powerful predictor of the outcome of exposure to HCV, the fate of acute natural HCV infection, and efficiency of HCV-specific T-cell responses.
- ✓ *IL-28B* polymorphisms assessment can be one useful tool for individualizing acute HCV-G4 therapy to identify acute HCV patients.
- ✓ Evolution of HCV-specific T-cell responses and undetectable HCV-RNA or antibody responses may imply an active role played by innate immune responses, leading to early viral clearance without developing anti-HCV antibodies.

REFERENCES

1. Lavanchy D. Evolving epidemiology of hepatitis C virus. Clin Microbiol Infect 2011;13:107–15.
2. El-Zanaty F, Way A. Egypt Demographic and Health Survey 2008: Egyptian: Ministry of Health. El-Zanaty and Associates, and Macro International: Cairo, 2009.
3. Guerra J, Garenne M, Mohamed MK *et al.* HCV burden of infection in Egypt: results from a nationwide survey. J Viral Hepatitis 2012;19:560–7.
4. Talaat M, El-Sayed N, Kandeel A *et al.* Sentinel surveillance for patients with acute hepatitis in Egypt, 2001-04. East Mediterr Health J 2010;16:134–40.
5. El Gaafary MM, Rekacewicz C, Abdel-Rahman AG *et al.* Surveillance of acute hepatitis C in Cairo, Egypt. J Med Virol 2005;76:520–5.
6. Meky FA, Stoszek SK, Abdel-Hamid M *et al.* Active surveillance for acute viral hepatitis in rural villages in the Nile Delta. Clin Infect Dis 2006;42:628–33.
7. Ray SC, Arthur RR, Carella A *et al.* Genetic epidemiology of hepatitis C virus throughout Egypt. J Infect Dis 2000;182:698–707.
8. Hoofnagle JH. Course and outcome of hepatitis C. Hepatology 2002;36:S21–9.
9. Micallef JM, Kaldor JM, Dore GJ. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. J Viral Hepat 2006;13:34–41.
10. Santantonio T, Medda E, Ferrari C *et al.* Risk factors and outcome among a large patient cohort with community-acquired acute hepatitis C in Italy. Clin Infect Dis 2006;43:1154–9.
11. Wang CC, Krantz E, Klarquist J *et al.* Acute hepatitis C in a contemporary US cohort: modes of acquisition and factors influencing viral clearance. J Infect Dis 2007;196:1474–82.
12. van den Berg CH, Grady BP, Schinkel J *et al.* Female sex and IL28B, a synergism for spontaneous viral clearance in hepatitis C virus (HCV) seroconverters from a community-based cohort. PLoS One 2011; 6:e27555.
13. Lechner F, Wong DK, Dunbar PR *et al.* Analysis of successful immune responses in personsm infected with hepatitis C virus. J Exp Med 2000;191:1499–512.
14. Thimme R, Oldach KM, Chang R *et al.* Determinants of viral clearance and persistence during acute hepatitis C virus infection. J Exp Med 2001;194:1395.
15. Thimme RJ, Bukh HC, Spangenberg S *et al.* Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. Proc Natl Acad Sci USA 2002;99:15661.
16. Cox AL, Mosbruger T, Mao Q *et al.* Cellular immune selection with hepatitis C virus ersistence in humans. J Exp Med 2005;201:1741–52.
17. Cox AL, Mosbruger T, Lauer GM *et al.* Comprehensive analyses of CD8+ T cell responses during longitudinal study of acute human hepatitis C. Hepatology 2005;42:104–12.
18. Freeman A, French J, Post C *et al.* Prevalence of production of virus-specific interferon-gamma among seronegative hepatitis C-resistant subjects reporting injection drug use. J Infect Dis 2004;190:1093.
19. Kamal SM, Amin AM, Madwar MA *et al.* Cellular immune responses in seronegative sexual contacts of acute hepatitis C patients. J Virol 2004;78:12252.
20. Scognamiglio PD, Accapezzato MA, Casciaro A *et al.* Presence of effector CD8+ T cells in hepatitis C virus-exposed healthy seronegative donors. J Immunol 1999;162:6681.
21. Post JJ, Pan Y, Freeman AJ *et al.* Clearance of hepatitis C viremia associated with cellular immunity in the absence of seroconversion in the hepatitis C incidence and transmission in prisons study cohort. J Infect Dis 2004;189:1846–55.
22. Mizukoshi E, Eisenbach C, Edlin BR. Hepatitis C virus (HCV)-specific immune responses of long-term injection drug users frequently exposed to HCV. J Infect Dis 2008;198:203–12.
23. Thurairajah PH, Hegazy D, Chokshi S. Hepatitis C virus (HCV)-specific T-cell responses in injection drug users with apparent resistance to HCV infection. J Infect Dis 2008;198:1749–174 1755.
24. Thursz M, Yallop R, Goldin R. Influence of MHC class II genotype on outcome of infection with hepatitis C virus: the HENCORE group. Hepatitis C European Network for Cooperative Research. Lancet 1999;354:2119–24.
25. Knapp S, Hennig BJ, Frodsham AJ. Interleukin-10 promoter polymorphisms and the outcome of hepatitis C virus infection. Immunogenetics 2003;55:362–9.
26. Houldsworth A, Metzner M, Rossol S. Polymorphisms in the *IL-12B* gene and outcome of HCV infection. J Interferon Cytokine Res 2005;25: 271–6.
27. Thomas DL, Thio CL, Martin MP *et al.* Genetic variation in *IL28B* and spontaneous clearance of hepatitis C virus. Nature 2009;461: 798–801.
28. Tillmann HL, Thompson AJ, Patel K *et al.* A polymorphism near *IL28B* is associated with spontaneous clearance of acute hepatitis C virus and jaundice. Gastroenterology 2010;139:1586–92.
29. di Iulio J, Ciuffi A, Fitzmaurice K *et al.* Estimating the net contribution of *Interleukin-28B* variation to spontaneous hepatitis C virus clearance. Hepatology 2011;53:1446–54.
30. Kamal SM, Ismail A, Graham CS *et al.* Pegylated interferon a therapy in acute hepatitis C: relation to hepatitis C virus specific T cell response kinetics. Hepatology 2004;39:1721–31.
31. Hosmer DW, Lemeshow S. Applied Logistic Regression. Wiley: New York, 2000.
32. Sharaf Eldin N, Ismail S, Mansour H *et al.* Symptomatic acute hepatitis C in Egypt: diagnosis, spontaneous viral clearance, and delayed treatment with 12 Weeks of pegylated interferon alfa-2a. PLoS One 2008;3:1–7.
33. Saleh DA, Shebl F, Abdel-Hamid M *et al.* Incidence and risk factors for hepatitis C infection in a cohort of women in rural Egypt. Trans R Soc Trop Med Hyg 2008;102:921–8.
34. Talaat M, Kandeel A, El-Shoubary W *et al.* Occupational exposure to needlestick injuries and hepatitis B vaccination coverage among health care workers in Egypt. Am J Infect Control 2003;31:469–74.
35. Mariano A, Scalia Tomba G, Tosti ME *et al.* Estimating the incidence, prevalence and clinical burden of hepatitis C over time in Italy. Scand J Infect Dis 2009;41:689–99.

36. Page K, Hahn J, Evans J *et al.* Acute hepatitis C virus infection in young adult injection drug users: a prospective study of incident infection, resolution, and reinfection. J Infect Dis 2009;200:1216–26.
37. Santantonio T, Sinisi E, Guastadisegni A *et al.* Natural course of acute hepatitis C: a long-term prospective study. Dig Liver Dis 2003;35:104–13.
38. Bakr I, Rekacewicz C, El Hosseiny M *et al.* Higher clearance of hepatitis C virus infection in females compared with males. Gut 2006;55:1183–7.
39. Saleh DA, Shebl FM, El-Kamary SS *et al.* Incidence and risk factors for community-acquired hepatitis C infection from birth to 5 years of age in rural Egyptian children. Trans R Soc Trop Med Hyg 2010;104:357–63.
40. Gerlach JT, Diepolder HM, Zachoval R *et al.* Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. Gastroenterology 2003;125:80–8.
41. Thomson EC, Fleming VM, Main J *et al.* Predicting spontaneous clearance of acute hepatitis C virus in a large cohort of HIV-1-infected men. Gut 2011;60:837–45.
42. Kamal SM, Rasenack JW, Bianchi L *et al.* Acute hepatitis C without and with schistosomiasis: correlation with hepatitis C-specific CD4(+) T-cell and cytokine response. Gastroenterology 2001;121:646–56.
43. Spada E, Mele A, Berton A *et al.* Multispecific T cell response and negative HCV-RNA tests during acute HCV infection are early prognostic factors of spontaneous clearance. Gut 2004;53:1673–81.
44. Smyk-Pearson S, Tester I, Klarquist J *et al.* Spontaneous recovery in acute human hepatitis C virus infection: functional T-cell thresholds and relative importance of CD4 Help. J Virol 2008;82:1827–37.
45. Takaki A, Wiese M, Maertens *et al.* Cellular immune responses persist and humoral responses decrease two decades after recovery from a single-source outbreak of hepatitis. Nat Med 2000;6:578.
46. Riviere Y, Montange T, Janvier G *et al.* Hepatitis C virus-specific cellular immune responses in individuals with no evidence of infection. Virol J 2012;9:76.
47. Golden-Mason L, Cox AL, Randall JA *et al.* Increased natural killer cell cytotoxicity and NKp30 expression protects against hepatitis C virus infection in high-risk individuals and inhibits replication in vitro. Hepatology 2010;52:1581–9.
48. Gerlach JT, Diepolder HM, Jung M C *et al.* Recurrence of hepatitis C virus after loss of virus specific CD4(+) T-cell response in acute hepatitis C. Gastroenterology 1999;117:93341.
49. Kelly C, Klenerman P, Barnes E. Interferon lambdas: the next cytokine storm. Gut 2011;60:1284–93.
50. Kurbanov F, Abdel-Hamid M, Latanich R *et al.* Genetic polymorphism in *IL28B* is associated with spontaneous clearance of hepatitis C virus genotype 4 infection in an Egyptian cohort. J Infect Dis 2011;204:1391–4.
51. Ge D, Fellay J, Thompson AJ *et al.* Genetic variation in IL28B predicts hepatitis C treatment induced viral clearance. Nature 2009;461:399–401.
52. Suppiah V, Moldovan M, Ahlenstiel G *et al. IL28B* is associated with response to chronic hepatitis C interferon-α and ribavirin therapy. Nat Genet 2009;41:1100–4.
53. Moghaddam A, Melum E, Reinton N *et al. IL 28B* genetic variation and treatment response in patients with hepatitis C virus genotype 3 infection. Hepatology 2011;53:746–54.
54. Knapp S, Warshow U, Ho KM *et al.* A polymorphism *in IL28B* distinguishes exposed, uninfected individuals from spontaneous resolvers of HCV infection. Gastroenterology 2011;141:320–5.
55. Naggie S, Osinusi A, Katsounas A *et al.* Dysregulation of host innate immunity in HCV genotype 1 infected *IL28B* CT/TT haplotype patients with depressed viral kinetics and therapeutic response. J Hepatol 2012;56:444–54.
56. Dring MM, Morrison MH, McSharry BP *et al.* Innate immune genes synergize to predict increased risk of chronic disease in hepatitis C virus infection. Proc Natl Acad Sci USA 2011;108:5736–41.
57. Kamal SM, Moustafa KN, Chen J *et al.* Duration of peginterferon therapy in acute hepatitis C: a randomized trial. Hepatology 2006;43:923–31.