Research Article

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Treatment optimization and prediction of HCV clearance in patients with acute HCV infection

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Background & Aims: The lack of consensus on the optimal timing, regimen, and duration of treatment, in patients with acute HCV infection, stimulates the research on both favourable outcome predictors and individualized treatment regimens. This study aimed at investigating the impact of *IL28B* SNP rs12979860 alone or in combination with HLA class II alleles in both predicting spontaneous viral clearance and individualizing treatment strategies for patients with HCV persistence, after acute HCV exposure.

Methods: 178 patients with AHC, consecutively treated with interferon alone or in combination with ribavirin, starting within or after 48 weeks from the diagnosis of AHC, were tested for *IL28B* SNPs and HLA class II alleles.

Results: Spontaneous viral clearance was achieved in 28% of 169 patients available for genetic testing. Factors associated with HCV elimination were jaundice (OR 2.75, 95% CI 1.31–5.77) and *IL28B* CC (OR 3.87, CI 1.71–8.51), but not HLA alleles. In CT/TT patients without jaundice, NPV for virus persistence was 98%. In patients with *IL28B* CT/TT, starting treatment 48 weeks after the onset was significantly associated with lower rates of response (28% *vs.* 100%, $p = 0.027$). By contrast, no significant differences in the rate of SVR were observed for CC carriers who started treatment later (65% *vs.* 85%, $p = 1.0$).

Conclusions: In patients with acute HCV hepatitis, lack of viral clearance may be predicted by absence of jaundice and *IL28B* CT/TT genotype; in patients with these characteristics, treatment needs to be started immediately.

Keywords: HCV hepatitis; Acute hepatitis; Jaundice; IL28B.
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Abbreviations: HCV, hepatitis C virus; AHC, acute HCV infection; SVR, sustained virologic response; PPV, positive predictive value; ALT, alanine aminotransferase; NPV, negative predictive value; LR, likelihood ratio; RECPAM, recursive partitioning and amalgamation; SC, spontaneous clearance; PI, persistent infection; AUC, area under a curve.

Introduction

In patients with acute HCV infection (AHC), early antiviral therapy seems to be associated with higher rates of sustained virological response (SVR) [1,2]. However, there is no consensus on the optimal regimen and timing for starting interferon-based therapy in AHC [3,4]. Given that spontaneous recover is observed in up to 50% of AHC cases, mostly associated with symptoms, an early treatment might expose patients who may achieve spontaneous clearance, to expensive and heavy therapies [5]. Therefore, the choice to wait and see may be rational, in view of guidelines recommendation advice to wait no longer than 12 weeks after diagnosis or symptoms onset [6], in order to avoid response failure by deferring treatment. It should also be considered that, as 75–90% of AHC are asymptomatic and therefore less likely to be identified without a delay in diagnosis, in the real life, it is not infrequent to start treatment after 24 weeks from the onset. The question is therefore whether we can individualize the decision of treating early only patients with unfavourable predictors.

Seeking markers of lack of spontaneous viral clearance, male gender, absence of symptoms, ethnicity other than Caucasian and possibly HCV genotype have been identified [7,8]. Recently, *IL28B* polymorphisms on chromosome 19 were shown to be associated with outcome of AHC, regardless of race and routes of transmission [9,10], since carriers of *IL28B* CC genotype were more likely to spontaneously clear HCV after AHC. However, 30–40% of patients with the favourable *IL28B* genotype do not achieve spontaneous clearance, whereas a few with unfavourable *IL28B* genotype do [9,10]. Thus, additional parameters may interact with *IL28B* in determining HCV outcome.

Among genetic factors potentially associated with HCV clearance, HLA class II alleles have been explored [11,12]. In a cohort

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of 169 patients with thalassemia major, who acquired the infection by blood transfusion before 1990, we recently showed that the evaluation of HLA DQB1∗0301 increases the PPV of *IL28B* in predicting spontaneous HCV clearance [13].

In this study, we sought to investigate, in patients with AHC, the role of *IL28B* SNP rs12979860 alone or with *HLA DQB1* and *DRB1* alleles in both predicting HCV outcome after AHC and identifying the best timing to start treatment in patients with persistent infection.

Materials and methods

Patients and controls

A cohort of 178 Italian patients with AHC, formerly treated with either Peg-Interferon (PegIFN) alone or in combination with ribavirin, at 8 different Italian institutions representative of the Northern, Central, and Southern part of the country, was evaluated. 169 out of 178 patients enrolled in the original study gave their consent to do the genetic evaluation. Patients were matched by age ±5 years with 178 healthy control subjects from the same geographical area. Healthy volunteers had normal liver enzymes and no serological markers of HCV, HBV or HIV infection. The frequency of *IL28B* rs12979860 SNP and HLA class II alleles was investigated in the study cohort and in the control population. No statistical differences were observed between *IL28B* C allele frequency (73%) in patients and in 178 healthy control subjects (67%), although the frequency in AHC patients was numerically higher. Frequencies of *HLA DRB1*∗1101 and *DQB1*∗0301 did not differ from those observed in our control population group (Supplementary Table 1). All distributions were in Hardy Weinberg equilibrium.

In 5 out of 8 centers, all the consecutive patients with diagnosis of acute HCV hepatitis presenting at the center were enrolled in this study. In the remaining 3 centers, only 1, 3, and 2 AHC patients, respectively, were excluded because they were active IVDU.

Acute hepatitis C was defined by the presence of ALT elevation of more than 10 times the UNL, with or without jaundice in a patient with "*de novo*" appearance of HCV-antibodies (Ab) and positive HCV RNA.

Spontaneous clearance was defined as undetectable HCV RNA and anti-HCV positivity. Minimum time of observation after diagnosis to be classified as spontaneous resolver was 9 weeks. Patients identified as spontaneous resolvers were followed for at least one year to avoid misclassification in case of late HCV RNA reappearance. Only one patient of this group, after one year, was lost to follow-up. All the patients continue to be seen annually at our unit. In the remaining centers, spontaneous resolvers were re-evaluated after one year of observation. Only 5/89 untreated patients (47 with SC plus 42 who did not consent to treatment) were lost to follow-up (5.6%), one of them was a spontaneous resolver (2.1%).

All patients were HCV mono-infected. Persistence of HCV infection was defined as anti-HCV positivity and detectable HCV RNA during the follow-up.

In the absence of a spontaneous clearance, patients received, according to the physicians preference, PegIFN alone or in combination with ribavirin, starting not before week 10 after diagnosis. The parental study had been approved by the local ethics committee in 1999.

Samples collection

For genetic testing, patients were recalled and, if they agreed, EDTA blood was drawn. EDTA blood was centrifuged at 1500*g* for 20 min at room temperature and aliquots were immediately frozen after centrifugation at −80 °C until testing was performed.

Virologic testing

Antibodies to HCV were determined in serum by a commercial third-generation enzyme immunoassay test (Ortho Diagnostic Systems, Rochester, NY, USA). Serum HCV RNA was tested with the qualitative and quantitative COBAS Amplicor® HCV Test (Roche Molecular Systems, Branchburg, NJ, USA; lower limit of detection: 50 IU/ml; lower limit of quantification: 600 IU/ml), and since 2007 with COBAS® AmpliPrep/COBAS TaqMan® HCV Test (CAP/CTM, Roche Diagnostics, Pleasanton, CA, USA; lower limit of detection 10 IU/ml). HCV genotype was determined using a solid phase reverse hybridization Genotype 2.0 Assay (LiPA) (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA). All assays were performed according to the manufacturer's instructions.

Genetic testing for IL28B SNPs

Genomic DNA was isolated from peripheral blood according to the QIAamp DNA Blood Mini Kit from Qiagen (Hilden, Germany). We selected SNP rs12979860 in the region of the *IL28B* gene for genotyping, using the allele-specific discrimination kit (ABI TaqMan) and ABI 7700 Sequence Detection System (Applied Biosystems, Carlsbad, CA, USA). Genotyping was conducted in a blinded fashion relative to HCV RNA status and subjects characteristics. Hardy-Weinberg equilibrium was assessed in patients and the control population.

HLA genotyping

DRB1 and *DQB1* loci were molecularly defined by high-resolution reverse-line probe assay by means of hybridization technology. A set of 37 sequence-specific oligonucleotides was used for specific *DRB* and *DQB* hybridization in Line probe assay (Innogenetics). A total of 33 alleles for *DRB1* and 16 alleles for *DQB* were identified.

Statistical analysis

Patient baseline characteristics were reported as means ± standard deviations (SD) or frequencies and percentages for continuous and categorical variables, respectively. Baseline comparisons between groups were performed using the *t*-test for continuous variables and Pearson Chi-squared test or Fisher exact test for categorical variables, as appropriate. Normal distribution assumption was checked by means of Q-Q plot and Shapiro-Wilks and Kolmogorov-Smirnov tests. Deviations from HWE were investigated by exact χ^2 test [14].

Univariate and multivariate logistic regression analyses were performed both on spontaneous HCV and on SVR. Results were reported as odds ratios (OR) along with their 95% confidence interval (95% CI). Possible interactions between *DQB1*∗0301 and age at infection and route of transmission, in determining viral clearance, including into a logistic model also the specific product term (*DQB1*∗0301 x age at infection or *DQB1*∗0301 x route of transmission), were calculated.

Sensitivity, specificity, positive and negative predictive values (PPV and NPV), positive and negative likelihood ratios (LR+ and LR−), and accuracy of prognostic models were evaluated.

A Recursive Partitioning and Amalgamation (RECPAM) [15] classification tree analysis was used to evaluate interactions between clinical variables and to identify distinct and homogeneous subgroups of patients in terms of treatment responsiveness. This tree-based method integrates the advantages of main effects logistic regression and tree-growing techniques. At each partitioning step, the method chooses the covariate and its best binary split to maximize the difference in the outcome of interest. The algorithm stops when user defined conditions (stopping rules) are met. To obtain more robust and stable splits, a permutation approach was adopted to choose the best splitting variable.

A p value <0.05 was considered as significant. All analyses were performed using SAS Release version 9.3.

Results

Patient characteristics

One hundred and seventy-eight patients with AHC infection were enrolled. In 9 of them, samples for DNA evaluation were not available; therefore, final analysis was done on 169 patients. The workflow of the patients by different outcome is reported in Fig. 1. Of 169 patients originally enrolled in this observational study cohort, 47 (27.8%) achieved spontaneous clearance (SC). Treatment was offered to patients with persistent infection (PI), but only 80 accepted it. Baseline demographic data of the overall cohort or segregated by different outcomes, SC or PI, are given in Table 1. The majority of patients were male (65%). Mean age at diagnosis was 44.0 years, with SC patients being slightly older. Mean age at infection was 44.0 ± 15.5 years, without significant differences by different outcomes. As expected, at diagnosis the mean ALT value was 1329.1 ± 972 U/ml; ALT were higher in patients with viral clearance than in those with persistent infection ($p = 0.07$).