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ORIGINAL ARTICLE

Anti-E1E2 antibodies status prior therapy favors direct-acting antiviral treatment efficacy

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Sustained virological response;
E1E2 envelope

Summary
Introduction: Presence of anti-E1E2 antibodies was previously associated with spontaneous cure of hepatitis C virus (HCV) and predictive before treatment of a sustained virological response (SVR) to bi- or tri-therapy in naïve or experienced patients, regardless of HCV genotype. We investigated the impact of anti-E1E2 seroprevalence at baseline on treatment response in patients receiving direct-acting antiviral (DAA) therapy.
Material and methods: We screened anti-E1E2 antibodies by ELISA in serum samples collected at treatment initiation for two groups of patients: 59 with SVR at the end of DAA treatment and 44 relapsers after DAA treatment. Nineteen patients received a combination of ribavirin (RBV) or PEG-interferon/ribavirin with sofosbuvir or daclatasvir and others received interferon-free treatment with DAA ± RBV. HCV viral load was measured at different time points during treatment in a subgroup of patients.

Abbreviations: HCV, hepatitis C virus; DAA, direct-acting antiviral; SVR, sustained virological response; RBV, ribavirin; PEG-IFN, pegylated-interferon; SOF, sofosbuvir; DCV, daclatasvir; BOC, boceprevir; TVR, telaprevir; GT, genotype; R, relapsers; SMV, simeprevir; ASUNA, asunaprevir; ELISA, enzyme-linked immunosorbent assay.

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Results: A significant association was observed between presence of anti-E1E2 and HCV viral load < 6log10 prior treatment. Among patients with anti-E1E2 at baseline, 70% achieved SVR whereas among patients without anti-E1E2, only 45% achieved SVR. Conversely, 66% of patients experiencing DAA-failure were anti-E1E2 negative at baseline. In the multivariate analysis, presence of anti-E1E2 was significantly associated with SVR after adjustment on potential cofounders such as age, sex, fibrosis stage, prior HCV treatment and alanine aminotransferase (ALT) level.
Conclusions: The presence of anti-E1E2 at treatment initiation is a predictive factor of SVR among patients treated with DAA and more likely among patients with low initial HCV viral load (< 6log10). Absence of anti-E1E2 at baseline could predict DAA-treatment failure.

Introduction

Hepatitis C Virus (HCV) is a major cause of liver morbidity and mortality [1]. A majority of infected individuals progress to chronic hepatitis after acute infection, but spontaneous clearance may occur in around 25% of subjects without treatment [2]. We previously identified a protective/neutralizing anti-E1E2 response targeting a single epitope, which is only expressed on the surface of serum-derived HCV particles [3,4] and associated with spontaneous clearance/cure of HCV [5]. Anti-E1E2 response was also predictive before treatment of a sustained virological response (SVR) to bi- or tri-therapy (PEG-interferon/ribavirin [PEG-IFN/RBV] ± protease inhibitors: boceprevir or telaprevir) in naïve or experienced patients who failed prior treatments [6,7]. Current HCV treatment with direct-acting antivirals (DAAs) leads to SVR in over 90% of patients. Unfortunately, high costs are currently still limiting worldwide access to treatment [8]. A final challenge of scaling-up HCV treatment is the risk of subsequent recurrence of HCV, either from late relapse or reinfection following treatment [8].

In this context, (i) we studied anti-E1E2 seroprevalence prior DAA treatment and its potential predictive value on SVR and relapse, (ii) we correlated presence of anti-E1E2 at treatment baseline and HCV RNA viral load and kinetics.

Material and methods

Serum samples

A total of 103 cases with available serum or plasma at DAA treatment initiation, 59 (57%) who achieved SVR after DAA treatment (SVR group) and 44 (43%) who experienced a relapse (R group), were included in this multicentric retrospective study. All SVR patients and twenty-eight patients (64%) of the R group were from the Department of Hepatology, Croix-Rousse Hospital, Lyon, France and had available serum sample at the ''Biobanque INSERM CRCL Hépatologie (U1052)'', France (#DC2008-235) under the French IRB ''CPP Sud-Est IV'' approval #11/040 (2011). Sixteen patients (36%) of the R group had available serum sample from the Department of Hepatology, Virology collection (#DC2008-680), Grenoble-Alpes Hospital, Grenoble, France. Among these 103 patients, patients from the SVR group also had available serum samples during HCV treatment, i.e. at week 4, 8, 12, 24 of treatment, and at 12 weeks after the end of treatment.

Detection of anti-E1E2 antibodies by ELISA

Anti-E1E2 antibodies were measured with a validated enzyme-linked immunosorbent assay (ELISA), as previously described [5–7]. Three biotinylated peptides covering amino acids 292-306 (E1), 480-494 (E2A) and 608-622 (E2B) were used as capture phase through interaction with streptavidin coated in microtiter plates. Human serum samples were tested at 1/250 dilution. Mean optical densities (OD) from triplicate wells containing either E1, E2A or E2B, and the mean OD of at least four negative (N) controls (normal human serum [NHS]) were calculated for each experiment. For standardization, we set a cut-off of absorbance ≥ 2 times the NHS mean value as indicating a significantly positive result.

HCV RNA level monitoring by real-time PCR

HCV-RNA levels were assessed with a real-time polymerase chain reaction (PCR)-based assay, COBAS AmpliPrep/COBAS TaqMan (CAP/CTM, Roche Molecular Systems, Pleasanton, CA, USA). Measurements were performed at baseline (Day 0) in all serum samples available ($n = 103$) and at week 4, 8, 12, 24 of therapy, and at week 12 after the end of treatment (EOT) in samples of the SVR group for the longitudinal analysis. SVR was defined as undetectable HCV RNA 12 weeks after the end of treatment. The lower limit of quantification (LOQ) was 12 IU/mL.

Statistical methods

The nominal and categorical parameters were expressed as absolute numbers and percentages and were compared using the two-sided Chi^2 test. Medians and interquartile ranges (IQ) were calculated for the quantitative variable and *t*-test for normally distributed variables or Mann-Whitney test for non-normally distributed variables were used to compare the quantitative variables. A binary multivariate logistic regression was conducted to evaluate the impact of anti-E1E2 presence at baseline on the SVR after adjusting on several cofactors such as age, sex, fibrosis, initial HCV viral load, ALT and prior HCV treatment. The association between

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