Effect of season and sunlight on viral kinetics during hepatitis C virus therapy

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ABSTRACT

Background and aims: Rapid viral response (RVR) during antiviral treatment for hepatitis C virus (HCV) predicts sustained viral response (SVR). Recently, vitamin D levels have been associated with SVR. As sunlight is the most important source of vitamin D and shows seasonal variation, we evaluated the effect of season on viral kinetics during peginterferon/ribavirin-based therapy for HCV.

Methods: Consecutive HCV patients treated with peginterferon/ribavirin and boceprevir/ telaprevir (June 2011–July 2014) were included. Patients were grouped according to season when therapy was initiated (Season A: May–October and Season B: November–April) depending on hours of daily sunlight. Multiple logistic regression analysis included factors known to influence SVR to treatment. The dependent variables were undetectable viral load (VL) or VL ≤15 UI/mL (VL ≤15) at weeks 4, 8 and 12, end of treatment and SVR.

Results: The study included 930 patients (66.8% men; median 54 years) treated with telaprevir (n=537) or boceprevir, without (n=481) or with lead-in therapy of peginterferon/ribavirin. Baseline characteristics of patients in Season A (45.3%, n=421) and Season B groups were similar. Overall, a higher rate of RVR (23.5% vs 16.1%, p=0.005) and VL ≤15 (51.0% vs 38.6%, p≤0.001) was observed in patients starting treatment during Season A versus Season B. By logistic regression analysis, initiating treatment in Season A proved to be an independent predictor of RVR and VL ≤15.

Conclusions: In our setting, seasonality affects viral kinetics in HCV genotype 1 patients treated with peginterferon/ribavirin-based therapy. Our findings support the hypothesis that vitamin D influences viral response to peginterferon/ribavirin-based therapy.

INTRODUCTION

Vitamin D is known to play a key role in calcium and bone homeostasis, but there is emerging evidence that it also has antifibrotic and anti-inflammatory properties.1 2 In fact, latest studies have found an association between low vitamin D levels and risk of severe chronic hepatitis C (CHC) and cirrhosis,3 5 suggesting that vitamin D deficiency may be involved in the progression of chronic liver disease.6 7 In addition, vitamin D has immunomodulatory properties and in vitro experiments have shown that cholecalciferol has antihepatitis C virus (HCV) effects, which may theoretically improve response to antiviral therapy in patients with CHC.8 More recently, vitamin D has been shown to reduce anaemia induced by ribavirin avoiding dose reduction.9 Finally, recent studies have shown that vitamin D levels correlate with viral response to treatment.8 10–12 However, some investigators have not corroborated these findings; the discrepancy of results has been mainly attributed to methodological limitations and bias, and thus more evidence is needed on this issue.

During recent decades, CHC has been treated with regimens based on interferon, with variable effectiveness. This variability depends on viral and host-related factors which affect the likelihood of achieving a rapid viral response (RVR), defined as...
undetectable viral load (VL) after 4 weeks of treatment. RVR, in turn, predicts sustained viral response (SVR), defined as undetectable VL 24 weeks after the end of treatment.\textsuperscript{11,15} The search for new factors able to predict treatment response has been a constant during the therapeutic peginterferon/ribavirin era. Current evidence shows that IL-28B polymorphism, genotype, degree of hepatic fibrosis and baseline VL, among others, are solid independent factors associated with RVR and SVR.\textsuperscript{11,12,14,15}

Given that the major source of vitamin D is exposure to natural sunlight, and that sun-induced vitamin D synthesis is greatly influenced by season,\textsuperscript{1,16} our aim was to evaluate the effect of season on viral kinetics with peginterferon/ribavirin-based therapy for CHC.

PATIENTS AND METHODS

Patients

Patient data were obtained from a prospective multicentre registry promoted by the Spanish Association for the Study of the Liver (HepatiC Registry database). The present study included consecutive HCV genotype 1 patients attending the participating centres and treated between June 2011 and July 2014 with peginterferon/ribavirin and telaprevir or boceprevir. Patients were recruited from different geographical areas and classified accordingly: Northern, Central and Southern Spain.

The month of initiating therapy was obtained from the database and patients were grouped into Season A (from May to October) and Season B (from November to April), that is, the months with higher and lower hours of sunlight during 2012 according to the Spanish Meteorological Agency records\textsuperscript{17} (figure 1). In addition, to evaluate the effect of initiating treatment during the months with the highest versus the lowest daily hours of sunlight, intermediate months were excluded and patients were grouped into Season $A^+$(from July to September) and Season $B^+$ (from January to March).

Patients were also grouped according to the type of treatment, that is, with or without peginterferon/ribavirin lead-in therapy.

Clinical and laboratory data

Demographic, anthropometric and clinical data were collected for all patients. Body mass index was calculated based on weight in kilograms and height in metres. A cut-off body mass index of 30 kg/m$^2$ was used to group patients. Degree of fibrosis was assessed by liver biopsy (Metavir score) or ultrasound elastography when available. IL28B polymorphism (CC vs other result) and HCV genotype 1 subtype (1a, 1b or indeterminate) were determined. Previous treatments were also recorded and patients were categorised as naïve or experienced (no response, early discontinuation, unknown response, relapse, partial response and breakthrough). A cut-off baseline VL of 600 000 UI/mL was used to distinguish high from low VL.\textsuperscript{14,18} HCV viraemia (quantitative, qualitative and log RNA) was recorded at baseline and during treatment at weeks 4, 8, 12; at the end of treatment and 24 weeks after treatment completion. Viral responses were evaluated at different time points, and RVR defined as undetectable HCV RNA at 4 weeks and SVR defined as HCV RNA indetectability more than 24 weeks after treatment completion. We also recorded cases with VL $\leq$ 15 UI/mL (VL $\leq$ 15) measured at week 4.

Vitamin D levels

Blood samples drawn at the start of treatment were only available from a single centre in the Northern area and were used to evaluate vitamin D levels, determined by electrochemiluminescence. Values are expressed in nmol/L.

Ethics statement

Approval for the study protocol was obtained from the promoter’s research ethics committee and written informed consent was obtained from all participants. The study was conducted in accordance with the principles of the 1975 Declaration of Helsinki.

Statistics

Categorical variables are expressed as frequencies and percentages. Continuous variables are expressed as means and SDs.

The assumption of normality was tested with the Kolgomorov-Smirnov test. Comparisons of continuous variables between groups were carried out with Wilcoxon-Mann-Whitney test. We used $\chi^2$ or Fisher exact tests to compare proportions, as appropriate.

Two multiple logistic regression models were constructed. The dependent variable was RVR in the first model and VL $\leq$ 15 in the second. In both models, season of treatment initiation was the main independent variable, controlling for VL, genotype 1 subtype, IL-28B status, degree of fibrosis, age, type of treatment, presence of diabetes and previous treatment response. The logistic regression results are expressed as ORs and 95\% CIs.

Statistical analysis was performed using SPSS program V.15.0 for Windows (SPSS, Chicago, Illinois, USA). All $p$ values lower than 0.05 were considered statistically significant.

RESULTS

Characteristics of the study groups

The study included 930 consecutive patients (66.8\% men; median age 54 years, range 15–81 years) treated for HCV with at least 4 weeks of follow-up. Regarding treatment initiation, 45.3\% of patients (n=421) started peginterferon/ribavirin-based therapeutic regimen during Season A and 54.7\% (n=509) during Season B. Table 1 shows their baseline demographic, clinical and analytical characteristics. Regarding the main
variables previously described as influencing viral response to treatment, there were no significant differences between the two groups.

In our cohort, 51.7% of patients were treated without lead-in therapy (n=8, boceprevir; n=473, telaprevir) and 48.3% of patients with lead-in therapy (n=310, boceprevir; n=64, telaprevir; n=75, discontinued treatment after not achieving a decrease 1 log RNA of baseline VL after 4 weeks of lead-in phase).

Among the patients that discontinued treatment after 4 weeks, 26 of 157 patients (14.2%) initiated treatment during Season A and 49 of 217 (18.4%) during Season B (p=0.240).

Patients were recruited from different geographical areas of Spain (figure 2), and the season of treatment initiation was equally distributed within regions, except for the Southern area (Northern area, 48.9% vs 51.1%; Central area, 50.2% vs 49.8%; Southern area, 61.8% vs 38.2%, during Season A and Season B, respectively; p=0.003).

**Viral response to treatment and seasonality**

As expected, patients without lead-in therapy (98% received peginterferon/ribavirin and telaprevir) achieved higher rates of RVR (35.1% vs 2.7%, p≤0.001) and VL ≤15 (79.2% vs 6.8%, p≤0.001) compared with patients with lead-in therapy (69% received peginterferon/ribavirin and boceprevir). Patients with RVR achieved a higher rate of SVR (131 of 155, 84.5% vs 337 of 545, 61.8%; p≤0.001). Similarly, patients with VL ≤15 also achieved a higher rate of SVR (280 of 338, 82.8% vs 185 of 355, 52.1%; p≤0.001).

Overall, patients initiating treatment during Season A showed higher rates of RVR (23.5% vs 16.1%, p=0.005) and VL ≤15 (51.0% vs 38.6%, p=0.001) than those initiating treatment during Season B. There were also differences between patients initiating treatment during Season A’ (n=147) and patients treated during Season B’ (n=290) (RVR, 25.2% vs 18.3%, p=0.104; VL ≤15, 53.3% vs 38.2%, p=0.004).

Analysis of viral response according to seasonality and type of treatment showed that patients treated during Season A with lead-in therapy exhibited a higher rate of viral response (RVR, 4.3 vs 1.5 in Season B, p=0.07; VL ≤15, 10.4 vs 4.2 in Season B, p=0.013; table 2). Seasonality in patients without lead-in therapy had less impact in viral response, although a trend was observed.

**Table 3** shows the results of logistic regression analysis. Season of initiating treatment (Season A vs Season B) together with type of therapy (lead-in therapy, no vs yes), HCV RNA (low vs high VL) and IL-28 polymorphism (CC vs no CC) proved to be independent predictors...
of RVR and VL \( \leq 15 \). No interaction between seasonality and INF lead-in therapy was found regarding RVR \( (p=0.145) \) and VL \( \leq 15 \) \( (p=0.300) \).

Furthermore, because the two treatments (lead-in vs no lead-in) differ in terms of efficacy, we performed a multivariate analysis of these groups separately, observing that the season of initiating treatment remained a predictor of RVR (table 4).

Viral response was not statistically different between patients initiating treatment in Season A compared with Season B assessed when VL was available at week 8 \( (n=262 \text{ in Season A, } n=341 \text{ in Season B; VL } \leq 15, 43.8\% \text{ vs } 56.2\%, p=0.703) \), week 12 \( (n=395 \text{ in Season A, } n=465 \text{ in Season B; VL } \leq 15, 45.9\% \text{ vs } 54.1\%, p=0.923) \), end of treatment \( (n=163 \text{ in Season A, } n=200 \text{ in Season B; HCV RNA undetectable, } 46\% \text{ vs } 54\%, p=0.437) \) and SVR \( (n=328 \text{ in Season A, } n=372 \text{ in Season B; HCV RNA undetectable, 48.1\% vs 51.9\%, } p=0.358) \).

Baseline VL was significantly different between the two periods considered. Nonetheless, this difference was clinically irrelevant and was adjusted for in the multivariate analysis. Analysis was performed considering baseline VL as categorical and continuous variable (quantitative RNA, online supplementary table S1), finding that seasonality remained as an independent predictor variable.

Since relapsers do have a different response to retreatment more like naïve patients, a subanalysis was performed considering both group of patients together compared with the others. Seasonality remained an independent predictor (see online supplementary table S2).

### Vitamin D levels and seasonality

As shown in figure 3, vitamin D levels in our small sample oscillated according to season, but were not significantly different between patients initiating treatment in Season A compared with Season B \( (49.2 \pm 23.4 \text{ vs } 47.8 \pm 20.7 \text{ nmol/L, } p=828, n=115) \).

### DISCUSSION

This is the first study to establish a link between the season of initiating interferon/ribavirin-based therapy...
for CHC and viral response to treatment. Our results suggest that viral response to treatment was influenced by the hours of sunlight, and by inference, sunlight exposure, a surrogate marker of vitamin D levels.

The most striking finding in our cohort of patients from different latitudes was that a different viral response was observed according to the season of initiating peginterferon/ribavirin-based treatment. In fact, patients starting treatment during the season with the highest daily hours of sunlight, according to the State Meterological Agency, showed the highest rate of RVR. We believe our findings are coherent for different reasons. First, the rate of viral response correlated well with the effectiveness of treatment since the largest difference in the response pattern was observed in patients receiving lead-in therapy instead of direct-acting antiviral agents from the outset. Second, multivariate analysis adjusted for variables influencing viral response clearly revealed that seasonality was an independent predictor for viral response. This was observed no matter what VL cut-off value was considered, either undetectable, ≤15 UI or both. In addition, other variables previously shown to influence RVR such as baseline VL >600 000 IU/mL and IL-28B gene polymorphism also appeared to be independent factors in our cohort of patients. Finally, our subanalysis of peak months regarding sunlight exposure confirmed the results, albeit with a smaller sample size, and reinforces the overall results.

Our data are of considerable importance as RVR is highly correlated with SVR in peginterferon/ribavirin-based regimens. In addition, our results can be considered as proof of concept that vitamin D levels may influence response to treatment for HCV.

Although the regimens used in this study are not those recommended in current clinical practice, it is important to note that, in some countries of Eastern Europe, Asia, the Middle East and South America, with a high prevalence of HCV, DAA regimens without...
Peginterferon have not been implemented as the only therapeutic regimen, and treatments that include peginterferon and ribavirin are still present.22–24 Since the first observation published in 2010 about low vitamin D serum level being related to poor response to peginterferon/ribavirin-based therapy in genotype 1 CHC,3 several studies have supported the association of vitamin D and viral response to treatment,25 26 attributing the rate of early virological response to the role of vitamin D in regulating the immune response. In recent years, there has been increasing interest in the role of vitamin D, in terms of bone homeostasis as well as in different tissues and its relationship with non-skeletal effects.12 Vitamin D plays an important role in the immune system and some immune cells possess vitamin D receptors, suggesting a regulatory role for vitamin D in innate and adaptive immune responses.27 28

Several studies have shown that vitamin D supplementation significantly improves the rate of RVR, early viral response and SVR.29–33 In contrast, other studies have not confirmed better outcomes of antiviral therapy after vitamin D supplementation 33 34 or any correlation between vitamin D levels and viral response.35 However, methodological bias regarding serum vitamin D measurement has been noted, such as differences in circulating vitamin D binding protein,36 the absence of an optimal and clinically relevant vitamin D level cut-off37 or the accuracy of vitamin D assays.38 These and other difficulties may theoretically preclude the possibility of finding any correlation trend even if it were present.

In addition, the benefits of supplementation may go beyond hepatitis C treatment response. In this regard, higher vitamin D levels have been associated with less inflammation and liver fibrosis in HCV patients.37

| Table 3 Multivariate logistic analysis | Rapid viral response | Undetectable or VL ≤15 UI |
|--------------------------------------|----------------------|--------------------------|
|                                      | OR 95% CI p Value     | OR 95% CI p Value        |
| Treatment initiation (Season A vs Season B) | 2.03 1.27 to 3.25 0.003 | 1.93 1.14 to 3.29 0.014 |
| Type of therapy (lead-in no vs yes)   | 23.70 10.66 to 52.69 <0.0001 | 90.1 46.54 to 174.6 <0.0001 |
| HCV RNA (≤600 000 vs >600 000 IU/mL) | 2.14 1.29 to 3.55 0.003 | 4.66 2.36 to 9.17 <0.0001 |
| IL-28 polymorphism (CC vs no CC)     | 1.94 1.14 to 3.30 0.014 | 2.24 1.17 to 4.29 0.015 |
| Naïve vs experienced                 | 1.55 0.91 to 2.63 0.10  | 0.74 0.41 to 1.31 0.30  |
| Genotype (1b vs 1a)                  | 1.32 0.73 to 2.40 0.35 | 0.71 0.37 to 1.36 0.30 |
| Diabetes mellitus (no vs yes)        | 1.28 0.63 to 2.61 0.49 | 1.42 0.66 to 3.05 0.36 |
| Age (≥50 vs <50 years)               | 0.92 0.55 to 1.52 0.75  | 1.01 0.58 to 1.78 0.95  |
| Fibrosis (≥F3 vs <F3)                | 0.92 0.51 to 1.66 0.79 | 1.63 0.80 to 3.30 0.17 |

HCV, hepatitis C virus; VL, viral load.

| Table 4 Multivariate logistic analysis grouped by type of therapy | Rapid viral response | Lead-in | Undetectable or VL ≤15 UI |
|---------------------------------------------------------------|----------------------|--------|--------------------------|
|                                                              | OR 95% CI p Value    | OR 95% CI p Value |
| Treatment initiation (Season A vs Season B)                   | 43.31 1.88 to 996.65 0.019 | 3.36 1.10 to 10.16 0.031 |
| HCV RNA (≤600 000 vs >600 000 IU/mL)                          | 23.95 2.75 to 208.41 0.004 | 8.86 3.02 to 25.94 <0.001 |
| IL-28 polymorphism (CC vs no CC)                              | 7.29 0.94 to 56.17 0.056 | 4.00 1.34 to 11.94 0.013 |
| Naïve vs experienced                                          | 5.68 0.591 to 54.56 0.132 | 1.02 0.37 to 2.86 0.959 |
| Genotype (1b vs 1a)                                           | 0.18 0.02 to 1.62 0.128 | 0.55 0.17 to 1.799 0.328 |
| Diabetes mellitus (no vs yes)                                 | 0.27 0.02 to 3.62 0.326 | 0.98 0.19 to 0.87 0.985 |
| Age (≥50 vs <50 years)                                        | 2.78 0.44 to 17.65 0.276 | 1.38 0.48 to 3.97 0.542 |
| Fibrosis (≥F3 vs <F3)                                          | 3.69 0.22 to 62.58 0.365 | 0.98 0.17 to 5.63 0.987 |

| Type of therapy | Rapid viral response | Without lead-in | Undetectable or VL ≤15 UI |
|-----------------|----------------------|-----------------|--------------------------|
| Treatment initiation (Season A vs Season B)                   | 1.78 1.93 to 2.90 0.021 | 1.61 0.86 to 3.01 0.131 |
| HCV RNA (≤600 000 vs >600 000 IU/mL)                          | 1.76 1.04 to 3.00 0.035 | 3.11 1.32 to 7.30 0.009 |
| IL-28 polymorphism (CC vs no CC)                              | 1.81 1.03 to 3.16 0.037 | 1.84 0.83 to 4.08 0.132 |
| Naïve vs experienced                                          | 1.51 0.87 to 2.63 0.138 | 0.66 0.32 to 1.36 0.267 |
| Genotype (1b vs 1a)                                           | 1.52 0.81 to 2.84 0.191 | 0.74 0.33 to 1.64 0.488 |
| Diabetes mellitus (no vs yes)                                 | 1.36 0.64 to 2.85 0.416 | 1.57 0.67 to 3.67 0.296 |
| Age (≥50 vs <50 years)                                        | 0.83 0.49 to 1.40 0.488 | 0.87 0.44 to 1.69 0.684 |
| Fibrosis (≥F3 vs <F3)                                          | 0.88 0.48 to 1.60 0.681 | 1.89 0.82 to 4.38 0.133 |

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Indeed, adequate levels of vitamin D reduce oxidative stress, influence the migration, proliferation and gene expression of fibroblasts as well as reduce the fibrogenic activity of liver stellate cells. Additionally, a recent study supports the notion that supplementation with vitamin D in peginterferon/ribavirin-based regimen significantly improves viral response and helps to prevent other problems like the risk of emerging bone fragility in children. Vitamin D may also reduce anaemia induced by ribavirin. Finally, a recent meta-analysis of randomised controlled trials has suggested a reduction in all-cause mortality with vitamin D supplementation.

Several studies have demonstrated that seasonal changes and ultraviolet light exposure correlate with vitamin D levels, as sunlight is the main source of vitamin D, especially in sunny countries such as Spain. Not surprisingly, in our study, a consistent association was observed between vitamin D levels and seasonal variation, surprisingly, in our study, a consistent association was observed between vitamin D levels and seasonal variation, suggesting decreased risks and severity of diseases after moderate exposure to ultraviolet radiation. Thus, it is reasonable to assume a correlation between vitamin D levels and VL in hepatitis B has also been reported.

Although we did observe an association between seasonality and RVR, when later time points were assessed including SVR, no such relationship was found. This is not surprising since direct-acting NS3 protease inhibitors are very effective drugs and other well-known predictors of SVR also fail to have predictive value. Similarly, no association between vitamin D levels and SVR has been found in easy-to-treat patients such as those with HCV genotype 2 and 3.

To the best of our knowledge, this is the first epidemiological study to compare two groups of HCV patients treated in different periods of the year. Previously, attempts have been made to compare cure rates between different countries in different latitudes, but it is difficult to draw valid and reliable conclusions from them due to the influence of inherent differences such as race, genetic factors, etc., which are difficult to control.

Our study has certain limitations. First, there is a lack of data on drugs that interfere with vitamin D levels, supplements or dietary intake. Also, data about mutation ultraviolet meteo-geographic statistics.

In conclusion, seasonality in our setting had an impact on viral kinetics in HCV genotype 1 patients treated with peginterferon/ribavirin-based therapy. Our findings provide further support for the hypothesis that vitamin D influences viral response to treatment.

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Patient consent Obtained.

Ethics approval Approval for the study protocol was obtained from the promoter’s research ethics committee and written informed consent was obtained from all participants. The study was conducted in accordance with the principles of the 1975 Declaration of Helsinki.
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