

# 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  $\leq 15$  UI/mL (VL  $\leq 15$ ) 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  $\leq 15$  (51.0% vs 38.6%, p $\leq 0.001$ ) 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  $\leq 15$ .

**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.<sup>1–2</sup> In fact, latest studies have found an association

## Summary box

- This is the first study to establish a link between the season of initiating interferon/ribavirin-based therapy for chronic hepatitis C and viral response to treatment.
- Patients starting treatment during the season with the highest daily hours of sunlight showed the highest rate of rapid viral response.
- Our results suggest that viral response to treatment is influenced by the hours of sunlight, and by inference, sunlight exposure, a surrogate marker of vitamin D levels.
- Our findings support the hypothesis that vitamin D influences the viral response to peginterferon/ribavirin-based therapy.

between low vitamin D levels and risk of severe chronic hepatitis C (CHC) and cirrhosis,<sup>3–5</sup> suggesting that vitamin D deficiency may be involved in the progression of chronic liver disease.<sup>6–7</sup> 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.<sup>8</sup> More recently, vitamin D has been shown to reduce anaemia induced by ribavirin avoiding dose reduction.<sup>9</sup> Finally, recent studies have shown that vitamin D levels correlate with viral response to treatment.<sup>3–10–12</sup> However, some investigators have not corroborated these findings;<sup>7</sup> 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.<sup>11–13</sup> 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.<sup>11–12–14–15</sup>

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,<sup>1–16</sup> 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<sup>17</sup> (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<sup>2</sup> 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.<sup>14–18</sup>

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 undetectability more than 24 weeks after treatment completion. We also recorded cases with VL ≤15 UI/mL (VL ≤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 Kolmogorov-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 ≤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

SUNLIGHT HOURS (h)		MONTHS													
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
GEOGRAPHICAL AREA	Northern	Asturias	79	101	204	83	182	126	158	230	155	184	91	85	
		Cantabria	90	106	215	97	243	174	243	219	214	188	119	89	
		Navarra	97	167	271	99	249	212	275	307	266	231	102	82	
		Cataluña	185	226	278	178	202	220	227	308	274	236	129	144	
						221.8 ± 46.1									
	137.8 ± 62														
	Central	Toledo	230	263	291	225	336	371	417	376	329	279	151	178	
		Segovia	155	165	274	107	313	324	401	351	279	263	128	142	
		Madrid	221	243	273	179	301	348	410	359	305	263	107	181	
		Ávila	145	177	277	153	295	311	353	327	253	213	118	159	
						324 ± 51.6									
	189.2 ± 57.8														
Southern	Murcia	225	255	284	258	344	359	370	330	296	251	138	217		
	Andalucía	252	290	280	237	342	376	411	375	302	300	198	218		
	Alicante	211	193	273	200	258	244	315	293	257	212	180	223		
	Canarias	216	247	284	237	341	329	342	310	294	245	154	203		
					312.3 ± 50.1										
228 ± 40.5															

**Figure 1** Number of hours of sunlight per month in 2012 according to geographical area (Northern, Central and Southern Spain). Data modified from original source available at: [http://www.aemet.es/documentos\\_d/conocermas/biblioteca/calendarios/cm-2013.pdf](http://www.aemet.es/documentos_d/conocermas/biblioteca/calendarios/cm-2013.pdf).

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

**Table 1** Baseline characteristics of patients according to the season of initiating hepatitis C virus treatment

	Season A n=421	Season B n=509	p Value
Age (years) mean±SD	58.8±9.4	54.4±8.3	0.45
Age >50 years, n (%)	261 (62)	333 (65.4)	0.30
Gender (male), n (%)	277 (65.8)	344 (67.5)	0.57
Body mass index*			
≤30 kg/m <sup>2</sup>	119 (78.8)	143 (79)	0.10
>30 kg/m <sup>2</sup>	32 (21.2)	38 (21)	
Diabetes mellitus, n (%)	65 (12.8)	65 (15.4)	0.25
HCV genotype			
1a, n (%)	89 (21.1)	118 (23.2)	0.68
1b, n (%)	283 (67.2)	347 (68.2)	
Others, n (%)	49 (11.6)	44 (8.6)	
Low viral load (<600 000 UI), n (%)	109 (26.2)	111 (21.9)	0.14
Basal viral load IU, mean±SD	2.459.627±4.553.883	3.383.240±5.406.573	0.006
Log RNA, mean±SD	6.06±0.60	6.18±0.65	0.007
Fibrosis†			
≤F2, n (%)	67 (12)	66 (13)	0.22
≥F3–F4, n (%)	351 (84)	438 (87)	
IL28B rs polymorphism CC, n (%)‡	69 (49.3)	71 (50.7)	0.29
Previous treatment			
Naïve, n (%)	139 (33)	160 (31)	0.62
Experienced, n (%)	282 (67)	349 (69)	
No response	45 (16)	70 (20.1)	
Early discontinuation	13 (4.6)	17 (4.9)	
Unknown response	47 (16.7)	83 (23.8)	
Relapse	122 (43.3)	120 (34.4)	
Partial response	50 (17.7)	48 (13.8)	
Breakthrough	5 (1.8)	11 (3.2)	
AST, UI, mean±SD	78±51	82±54	0.32
ALT, UI, mean±SD	99±67	101±70	0.76
GGT, UI, mean±SD	109±96	101±88	0.19

\*In 64% of the patients, body mass index was not calculated.

†In 8 (0.86%) patients, fibrosis degree was not available.

‡In 27% of the patients, IL28B polymorphism was not available.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT,  $\gamma$ -glutamyltransferase; HCV, hepatitis C virus.

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 ( $n=262$  in Season A,  $n=341$  in Season B; VL  $\leq 15$ , 43.8% vs 56.2%,  $p=0.703$ ), week 12 ( $n=395$  in Season A,  $n=465$  in Season B; VL  $\leq 15$ , 45.9% vs 54.1%,  $p=0.923$ ), end of treatment ( $n=163$  in Season A,  $n=200$  in Season B; HCV RNA undetectable, 46% vs 54%,  $p=0.437$ ) and SVR ( $n=328$  in Season A,  $n=372$  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 naive 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).

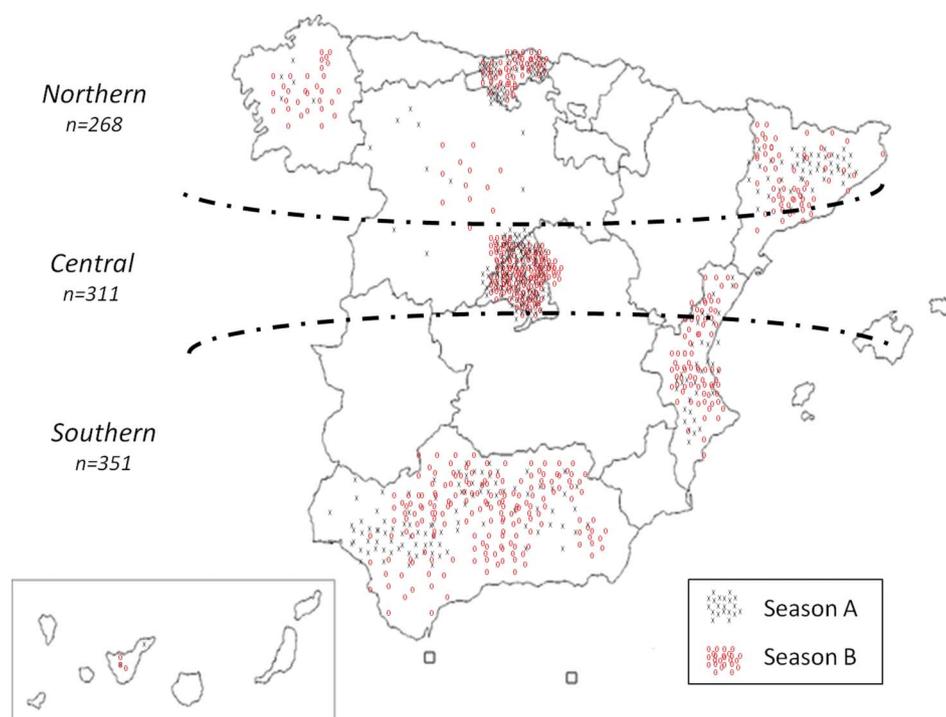
### Vitamin D levels and seasonality

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 ( $49.2\pm 23.4$  vs  $47.8\pm 20.7$  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

**Figure 2** Schematic representation of patients included in the study according to geographical area. Grey crosses represent patients starting treatment during Season A (May–October) and red circles represent patients starting treatment during Season B (November–April).



**Table 2** Viral response according to lead-in therapy with interferon and season of initiating hepatitis C virus treatment

	Without lead-in			Lead-in		
	Season A	Season B	p Value	Season A	Season B	p Value
Rapid viral response, n (%)	91 (38.2)	78 (32)	0.18	8 (4.3)	4 (1.5)	0.07
RNA undetectable or VL $\leq$ 15 UI at 4 weeks, n (%)	193 (82.5)	181 (76)	0.09	19 (10.4)	11 (4.2)	0.013
Viral response at 8 weeks, n (%)	79 (64)	85 (58.2)	0.38	60 (44.1)	87 (44.6)	0.99
VL $\leq$ 15 UI at 8 weeks, n (%)	119 (97)	137 (94)	0.39	95 (70)	136 (70)	0.99
Viral response at 12 weeks, n (%)	151 (66.6)	145 (62)	0.83	94 (56.3)	126 (54)	0.74
VL $\leq$ 15 UI at 12 weeks, n (%)	215 (94)	213 (91.4)	0.48	135 (81)	200 (85.5)	0.86
Sustained viral response, n (%)	147 (74.2)	147 (72)	0.57	78 (60)	96 (57.5)	0.72

HCV, hepatitis C virus; VL, viral load.

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 Meteorological Agency,<sup>17</sup> 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,  $\leq$ 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,<sup>11 18 19</sup> 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.<sup>15 20 21</sup> In fact, in our cohort of patients, RVR was associated with SVR. 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

**Table 3** Multivariate logistic analysis

	Rapid viral response			Undetectable or VL $\leq$ 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 ( $\leq$ 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 ( $\geq$ 50 vs $<$ 50 years)	0.92	0.55 to 1.52	0.75	1.01	0.58 to 1.78	0.95
Fibrosis ( $\geq$ 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

Type of therapy	Rapid viral response			Lead-in p Value	Undetectable or VL $\leq$ 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 ( $\leq$ 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 ( $\geq$ 50 vs $<$ 50 years)	2.78	0.44 to 17.65	0.276		1.38	0.48 to 3.97	0.542
Fibrosis ( $\geq$ F3 vs $<$ F3)	3.69	0.22 to 62.58	0.365		0.98	0.17 to 5.63	0.987

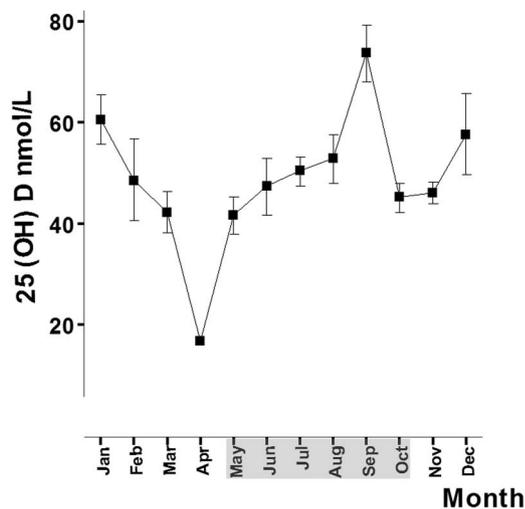
Type of therapy	Rapid viral response			Without lead-in p Value	Undetectable or VL $\leq$ 15 UI		
	OR	95% CI	p Value		OR	95% CI	p Value
Treatment initiation (Season A vs Season B)	1.78	1.93 to 2.90	0.021		1.61	0.86 to 3.01	0.131
HCV RNA ( $\leq$ 600 000 vs $>$ 600 000 IU/mL)	1.76	1.04 to 3.00	0.035		3.11	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	0.468
Diabetes mellitus (no vs yes)	1.36	0.64 to 2.85	0.416		1.57	0.67 to 3.67	0.296
Age ( $\geq$ 50 vs $<$ 50 years)	0.83	0.49 to 1.40	0.488		0.87	0.44 to 1.69	0.684
Fibrosis ( $\geq$ F3 vs $<$ F3)	0.88	0.48 to 1.60	0.681		1.89	0.82 to 4.38	0.133

peginterferon have not been implemented as the only therapeutic regimen, and treatments that include peginterferon and ribavirin are still present.<sup>22–24</sup>

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,<sup>3</sup> several studies have supported the association of vitamin D and viral response to treatment,<sup>25–26</sup> 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.<sup>1–2</sup> 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.<sup>27–28</sup>

Several studies have shown that vitamin D supplementation significantly improves the rate of RVR, early viral response and SVR.<sup>29–33</sup> In contrast, other studies have not confirmed better outcomes of antiviral therapy after vitamin D supplementation<sup>33–34</sup> or any correlation between vitamin D levels and viral response.<sup>35</sup> However, methodological bias regarding serum vitamin D measurement has been noted, such as differences in circulating vitamin D binding protein,<sup>36</sup> the absence of an optimal and clinically relevant vitamin D level cut-off value<sup>37</sup> or the accuracy of vitamin D assays.<sup>38</sup> 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.<sup>3–7</sup>



**Figure 3** Seasonal variation of vitamin D. Vitamin D levels oscillated during the year depending on the season. The months of Season A (May–October) are shaded in grey.

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.<sup>3</sup> 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.<sup>32</sup> Vitamin D may also reduce anaemia induced by ribavirin.<sup>9</sup> Finally, a recent meta-analysis of randomised controlled trials has suggested a reduction in all-cause mortality with vitamin D supplementation.<sup>39</sup>

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.<sup>40–42</sup> Not surprisingly, in our study, a consistent association was observed between vitamin D levels and seasonal variation, although vitamin D levels were only available in a minority of our large cohort of patients. In addition, several studies have suggested decreased risks and severity of autoimmune, endocrine, cardiovascular and oncology diseases after moderate exposure to ultraviolet radiation. Thus, it is reasonable to assume a correlation between vitamin D levels and sunlight exposure.<sup>43–45</sup> Conversely, increased severity and prolonged hospitalisation rates have been associated with low sunlight exposure in patients with inflammatory bowel diseases<sup>46</sup> or sepsis.<sup>47</sup>

There were no differences in baseline characteristics between patients grouped according to season. However, baseline VL was significantly different between the two periods considered. Although the difference was clinically irrelevant and was adjusted for in the multivariate analysis, the finding deserves a comment. It is tempting to speculate that longer hours of sunlight may have anti-HCV effects, targeting the assembly of the infectious virus and reducing intracellular and extracellular

infectivity titres, since vitamin D has shown to play a role as a natural antiviral mediator.<sup>8 48–51</sup> It is important to note that an inverse relationship between vitamin D levels and VL in hepatitis B has also been reported.<sup>52 53</sup>

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.<sup>35 54–56</sup> 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.<sup>7</sup>

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.<sup>7</sup>

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 CYP27B1,<sup>29</sup> which has been found to affect vitamin D levels, were not available. Finally, individual sunlight exposure was not available and patients were therefore grouped according to seasons established according to ultraviolet meteo-geographic statistics.

Although peginterferon and ribavirin have been displaced by new DAAs and their use is increasingly limited in the treatment of hepatitis C, we should not forget that interferon is still used to treat hepatitis B and is the only existing therapy for hepatitis D. So the present study opens the possibility of exploring the effect of daylight hours on initiation of treatment and, by inference, vitamin D metabolism, which is poorly studied.<sup>57–59</sup>

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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