Relationship of **vitamin D status** with **advanced liver fibrosis** and **response** to **hepatitis C virus therapy**: a **meta-analysis**.
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Title: Relationship of vitamin D status with advanced liver fibrosis and response to hepatitis C virus therapy: A meta-analysis

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ABSTRACT

Background and Aims: There is growing evidence that vitamin D is related to chronic hepatitis C (CHC) pathogenicity. We analysed the relationship of vitamin D status with advanced liver fibrosis (ALF) in CHC treatment-naïve patients and sustained virologic response (SVR) in CHC patients on pegylated interferon alpha plus ribavirin (pegIFNα/ribavirin) therapy.

Methods: We performed a meta-analysis of all eligible studies published to date (April, 2014) in PubMed , SCOPUS, LILACS and the Cochrane Library , assessing plasma/serum vitamin D levels related to ALF and/or SVR. Pooled odds ratios were estimated by either fixed or random effects models.

Results: Fourteen studies were selected from the literature search, 7 for ALF (1083 patients) and 11 for SVR (2672 patients). For liver fibrosis, low vitamin D status was related to a diagnosis of ALF, with the cut-offs of 10 ng/mL (OR=2.37 (95%CI=1.20, 4.72)) and 30 ng/mL (OR=2.22 (95%CI=1.24, 3.97)) being significant, and a near-significance for 20 ng/mL (OR=1.44 (95%CI=0.99, 2.12)). Regarding SVR, a significant heterogeneity among studies was found ($p<0.001$), and we only found a significant association with SVR for a vitamin D cut-off of 20 ng/mL (OR=0.53 (95%CI=0.31, 0.91)). When meta-analysis was performed excluding the outliers, significant pooled ORs were found for all patients [10 ng/mL (OR=0.48 (95%CI=0.34, 0.67)) and 20 ng/mL (OR=0.58 (95%CI=0.45, 0.76))] and GT1/4 patients [10 ng/mL (OR=0.53 (95%CI=0.34, 0.81)) and 20 ng/mL (OR=0.54 (95%CI=0.39, 0.74))].

Conclusions: Low vitamin D status in CHC patients is associated with a higher likelihood of having ALF and lower odds of achieving SVR following pegIFNα/ribavirin therapy.

INTRODUCTION

Nearly 200 million people worldwide are chronically infected with hepatitis C virus (HCV), which leads to the development of cirrhosis, end-stage liver disease, hepatocellular carcinoma, and liver transplantation (1). Advanced liver fibrosis (ALF) in chronic hepatitis C (CHC) is believed to be progressive and largely irreversible (2). Such fibrosis progression is probably due to multifactorial interactions between viral and host characteristics such as age at HCV infection, gender, daily alcohol intake, intravenous drug use, obesity, metabolic syndrome, HCV genotype, and coinfection by other viral pathogens such as human immunodeficiency virus (HIV) (3).

Throughout the past decade, CHC therapy has consisted of pegylated interferon alpha plus ribavirin (pegIFNα/ribavirin). This treatment produces rates of sustained virologic response (SVR) of around 40-50% in patients infected with HCV genotype (HCV-GT) 1, 60% in HCV-GT 4, and 80% in HCV-GT 2/3. Additionally, recent analyses have revealed that the use of pegIFNα/ribavirin in HIV/HCV-coinfected patients may provide SVR rates which approximate to those of HCV-monoinfected patients (4). Current CHC therapy involves new direct-acting antivirals plus pegIFNα/ribavirin, which has further improved the SVR rate (5). Several host factors that influence the efficacy of IFN therapies have been identified, including age, sex, liver fibrosis, HCV genotype, HCV viral load, interleukin-28B (IL28B) polymorphisms, and obesity (6). However, an unexplained variability in HCV treatment outcomes still remains.

There is growing evidence that vitamin D status is related to chronic liver disease (7). Vitamin D receptor (VDR) is widely expressed in the liver and its expression is negatively associated with the severity of liver histology in CHC patients (8). Moreover, vitamin D has antiproliferative and antifibrotic effects on the liver, and may have potential therapeutic value (9).

Vitamin D is produced naturally during exposure to ultraviolet B radiation. It is then metabolized in the liver forming 25-hydroxyvitamin D (25(OH)D), which is later metabolized to the active form, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), in the kidneys. In the blood, 25(OH)D is the main circulating form of vitamin D, and its concentration in plasma is the most reliable indicator of vitamin D status (10). There is consensus that levels of 25(OH)D below 25 nmol/L (10 ng/mL) are qualified as deficient, and that over 75 nmol/L (30 ng/mL)

may be required for optimal health (11). In HCV infection, vitamin D status has been associated with CHC-related outcomes such as liver fibrosis progression in treatment-naïve patients and SVR in patients on pegIFNα/ribavirin therapy (10). This evidence suggests the potential for vitamin D supplementation as a preventive and/or early treatment strategy for CHC.

Recently, a high number of publications have been reported about vitamin D status and CHC, but some conflicting conclusions have been reached. To our knowledge, only one meta-analysis has been published to date about the association between vitamin D and SVR (12), but it only involved a literature search through March 2012. This study did not analyse liver fibrosis or any other factors that might affect the relationship between the study factor and outcome. For that reason, our aim was to carefully analyse the relationship between vitamin D status and ALF in HCV treatment-naïve patients and SVR in HCV-infected patients on pegIFNα/ribavirin therapy, by conducting a meta-analysis of all eligible studies published to date (April, 2014).

MATERIALS AND METHODS

Search strategy

Relevant studies were identified by a PubMed, SCOPUS, LILACS and the Cochrane Library literature search with the following terms: ("hepatitis C" or "HCV" or "chronic hepatitis C") and ("vitamin D" or "ergocalciferol" or "cholecalciferol"). The information contained in this report is based on articles published before April 2014.

The meta-analysis was conducted following guidelines from Sutton et al. (13), and the data were reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (14).

Study selection

We developed strict criteria for categorizing the studies by two independent reviewers:

a) Inclusion criteria: i) patients infected with HCV or coinfected with HCV/HIV, ii) ALF data were exclusively derived from hepatic biopsy (not from non-invasive markers) in treatment-naïve HCV patients and/or SVR data in patients undergoing pegIFNα/ribavirin therapy, and iii) data of serum or plasma 25(OH)D levels related to ALF and/or SVR.

b) Exclusion criteria: i) coinfection with hepatitis B virus (HBV); ii) absent or inadequate information about 25(OH)D levels, study population, HCV status, or not enough information to calculate the odds ratio (OR) and 95% confidence intervals (CI); iii) studies with sample size less than 40 subjects; iv) patients who received vitamin D supplementation; v) Reviews, letters, editorials or clinical cases.

In order to select the candidate studies, we carefully screened the title and abstract of each publication. When articles fulfilled the inclusion criteria, we examined the full text and extracted data from the study. When studies included several subgroups and some of them did not fulfil the inclusion criteria, we only incorporated into the meta-analysis those subgroups that did meet the inclusion criteria. When more than one paper studying the same cohort was found, only the study with the most extensive cohort was reviewed, excluding the remaining overlapping studies or data.

Data extraction

Data were extracted independently by two investigators (MGA and DPT) and then cross-checked. When data were unclear or required assumptions to be made, other investigators (MAJS, MGF, AFR, and SR) were consulted so that a consensus could be reached before recording an entry in the database. When the data were not explicitly reported, we contacted some authors of individual studies to request the data.

Outcome variables

i) ALF according to Metavir (F≥3). Data containing a different score were transformed to the Metavir score (15).

ii) SVR was defined as an undetectable serum HCV-RNA level up through 24 weeks after the end of HCV treatment. HCV-GT 1/4 patients were considered as difficult-to-treat and HCV-GT 2/3 patients were considered as easy-to-treat (16).

Quality assessment

In order to evaluate the quality of the included studies, two investigators (MGA and DPT) appraised them independently using an evaluation system modified from the Newcastle–Ottawa Scale (17). The included studies were judged on several aspects, including the selection of study populations (for both deficiency and sufficiency of vitamin D), comparability between groups, ascertainment of exposure, and demonstration of the outcomes of interest. The full score was 9 stars, and a high-quality study was defined as a study with 7 or more stars.

Statistical analysis

All analyses were performed using Stata software (version 11.0; Stata Corporation, College Station, TX, USA). All p-values <0.05 were considered significant.

Overall, meta-analysis was performed only when two or more papers studying the same outcome were available. In all analyses, pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. The significance of the pooled OR was calculated by the Z-test. A fixed effect model (Mantel-Haenszel method) was used for homogeneous studies (18). When significant heterogeneity existed, a random effect model was applied (DerSimonian and Laird method) (19). The study heterogeneity was assessed using the Cochran's Q statistic and I^2 statistic, considering a Q statistic $p<0.1$ or $I^2>50\%$ as significant

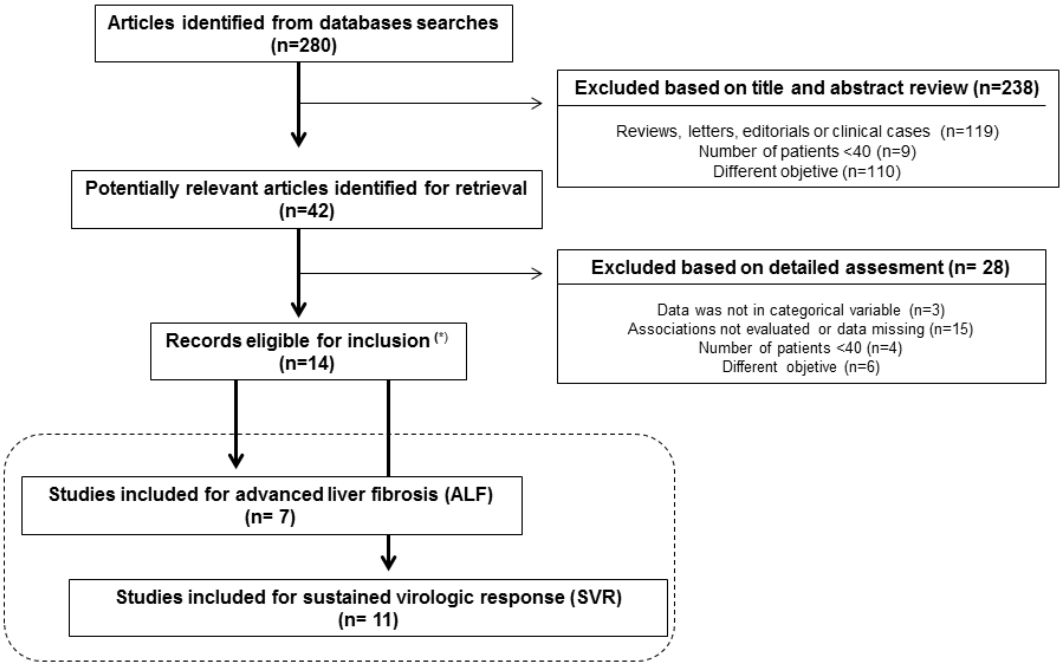
heterogeneity. In addition, when heterogeneity was detected, meta-regression analysis was also performed with the aim of defining the potential effect of the covariates on the outcome variables. The regression coefficients obtained describe how ALF or SVR changes with each unit increase in the covariate. Significance of the linear relationship was identified by the p-value (20). The covariables analysed were: the cut-off for insufficiency/deficiency of vitamin D, study design, HIV coinfection, country of the study, ethnicity, and methodology for vitamin D level estimation.

Publication bias was assessed by Begg´s funnel plot and the Egger linear regression test (21), which detects funnel plot asymmetry. Publication bias was assumed to exist when the Egger test reported a $p<0.05$. The Galbraith plot was used to detect possible outliers of the heterogeneity (22), which could have biased the combined estimate. This graphical method allowed us to check those studies that had a strong influence on the pooled results. The sensitivity analyses were also conducted to assess the consistency of results and to investigate the influence of one study on the overall meta-analysis (21). It was carried out by sequential omission of individual studies.

RESULTS

Search results

The search strategy yielded 280 entries (**Figure 1**). 42 of those 280 were considered to have potential value, and the full texts were retrieved for detailed evaluation. After exclusion based on detailed assessment, 14 studies were eligible for inclusion. These consisted of 7 that were selected for ALF meta-analysis (23-29) and 11 for SVR meta-analysis (23-25, 28, 30-36). Four studies were included in both analyses (23-25, 28).



Article characteristics

The main characteristics of the studies analysed are summarized in **Table 1** for ALF meta-analysis and **Table 2** for SVR meta-analysis. The publication year of the studies ranged from 2011 to 2014, and meta-analyses involved 1083 individuals for ALF outcome and 2672 for SVR outcome. Data were collected from several countries with different latitudes among long periods of time. The seasonal differences on vitamin D levels could not be considered because the majority of studies did not provide this information, and when there was, the information was very heterogeneous.

Table 1. Characteristics of studies included in meta-analysis for advanced liver fibrosis (ALF).

Year	First author	Design	N	Fibrosis score	Period (start/end)	Age (years)	Gender (% male)	Country	Ethnicity (%C-%AA)	HCV genotype	HIV	Cut-off (ng/mL)	Assay
2014	Guzmán-Fulgencio (25)	Cross-sectional	174	Metavir (51)	2000/2008	40.8	74.7	Spain	100% C	1-4	Yes	10	EIA
2013	Petta (26)	Cross-sectional	260	Scheuer (52)	NR	52.8	49.2	Italy	100% C	1	No	30	LC
2013	Ladero (27)	Cross-sectional	89	Metavir (51)	January/May 2012	54.3	62.03	Spain	100% C	1, non 1	No	20	CI
2013	Mandorfer (28)	Cross-sectional	65	Metavir (51)	NR	38.6	71	Austria	100% C	1-4	Yes	10/30	EIA
2012	Lange (29)	Cohort	303	Metavir (51)	2000/2005	45	63.62	Switzerland	100% C	1-4	No	20	RIA
2011	Milazzo (23)	Case-control	48	Ishak (53)	2007/2010	45	74.19	Italy	100% C	1, non 1	Yes	30	RIA
2011	Bitetto (24)	Cross-sectional	114	Ishak (53)	2005/2009	47	51.7	Italy	100% C	1-5	No	20	ICMA

Abbreviations: AA, african americans; C, caucasians; CI, chemiluminescent immunoassay; EIA, enzyme immunoassay; F, fibrosis stage; HCV, hepatitis C virus; HIV, human inmunodefficiency virus; ICMA, immunochemiluminometric; LC, liquid chromatography; NR, not reported; RIA, radio immuno assay; SVR, sustained virologic response.

Table 2. Characteristics of studies included in meta-analysis for the analysis of response to HCV treatment.

Year	First author	Design	N	HCV Treatment	Period (start/end)	Age (years)	Gender (% male)	Country	Ethnicity (%C-%AA)	HCV genotype	HIV	Cut-off (ng/mL)	Assay
2014	Guzmán-Fulgencio (25)	Cross-sectional	125	pegIFNα/RBV	2000/2008	40.8	74.7	Spain	100% C	1-4	Yes	10/30	EIA
2013	Mohamed (31)	Case-control	50	pegIFNα/RBV	2010/ 2011	40	68	Egypt	100% NA	4	No	30	ELISA
2013	Mandorfer (28)	Cross-sectional	65	pegIFNα/RBV	NR	38.6	71	Austria	100% C	1-4	Yes	10/30	CI
2013	Bitetto (32)	Case-control	190	pegIFNα/RBV	2005/2009	47	51.7	Italy	100% C	1-5	No	10/20	ICMA
2012	Weintraub (33)	Cross-sectional	171	pegIFNα/RBV	2001/2009	47.25	41.52	USA	62% AA 38% C	1	No	20/30	RIA
2012	Terrier (34)	Cross-sectional	106	pegIFNα/RBV	2001/2010	58,2	48.93	France	100% C	1,4, other	No	12/30	RIA
2012	Falleti (35)	Cross-sectional	206	pegIFNα/RBV	2005/2009	48	51.5	Italy	100% C	1-5	No	20	ICMA
2011	Milazzo (23)	Case-control	51	pegIFNα/RBV	2007/2010	45	74.19	Italy	100% C	1, non 1	Yes	30	RIA
2011	Bitetto (24)	Cross-sectional	211	pegIFNα/RBV	2005/2009	47	51.7	Italy	100% C	1-5	No	10/20	ICMA
2011	Terrier (30)	Cross-sectional	189	pegIFNα/RBV	4^{th} trimesters	39,5	77.24	France	100% C	1-4, other	Yes	10/20/30	RIA
2011	Bitetto (36)	Cohort	42	IFNα/RBV; pegIFNα/RBV	1996/2006	54	71.42	Italy	100% C	1, other	No	10/20	ICMA

Abbreviations: AA, african americans; C, caucasians; CI, chemiluminescent immunoassay; EIA, enzyme immunoassay; F, fibrosis stage; HCV, hepatitis C virus; HIV, human inmunodefficiency virus; ICMA, immunochemiluminometric; LC, liquid chromatography; NA, north africans; NR, not reported; RIA, radio immuno assay; SVR, sustained virologic response.

The quality scores of the included studies are summarized in **Supplemental Table 1** (case-control and cross-sectional analysis) and **Supplemental Table 2** (cohort studies). Overall, the quality score was high (7.83±0.71 and 7.5±0.71; respectively).

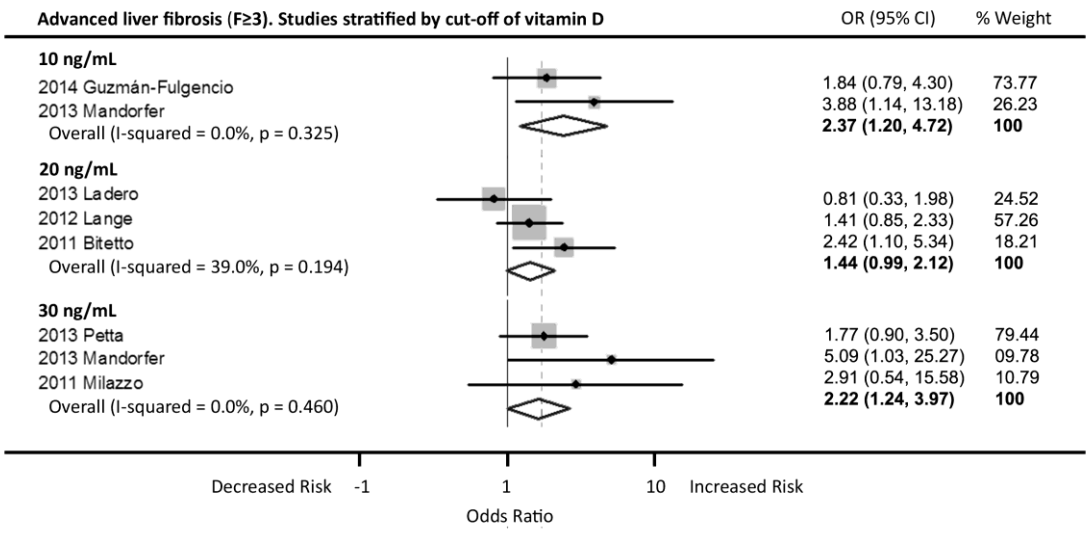
Plasma/serum vitamin D levels

Nearly 70% of all patients had suboptimal 25(OH)D levels (below 20 or 30ng/mL, depending on the cut-off of the study), and almost 50% of the HCV-infected patients had deficient 25(OH)D levels (less than 10 or 20 ng/mL). Vitamin D status was lower in HIV/HCV-coinfected patients, as 82.7% of them had suboptimal levels, compared to 66.2% of HCV-monoinfected patients.

Meta-analysis of advanced liver fibrosis

A total of 7 studies examined the association between vitamin D status and ALF. No publication bias was observed by Begg's Funnel plot and Egger's test (**Supplemental Figure 1**).

Figure 2 shows the pooled ORs for each different threshold used to define the deficiency or insufficiency of vitamin D. From 7 studies analysed, 6 of them showed a direct relationship between deficiency or insufficiency of vitamin D and ALF, but only 3 of them were statistically significant. Overall, we found a significant association between vitamin D status and ALF, including cut-offs of 10 ng/mL (OR=2.37 (95%CI=1.20, 4.72)) and 30 ng/mL (OR=2.22 (95%CI=1.24, 3.97)). The cut-off of 20 ng/mL was very close to significance (OR=1.44 (95%CI=0.99, 2.12)) possibly due to the low number of articles included.



The Cochran's Q statistic and I^2 statistic did not show heterogeneity among the studies (p>0.1; **Figure 2**). This finding was supported by Galbraith´s plots (**Supplemental Figure 2**) and by meta-regression analysis (**Supplemental Table 3**).

Table 3. Meta-regression analysis for SVR according to vitamin D deficiency.

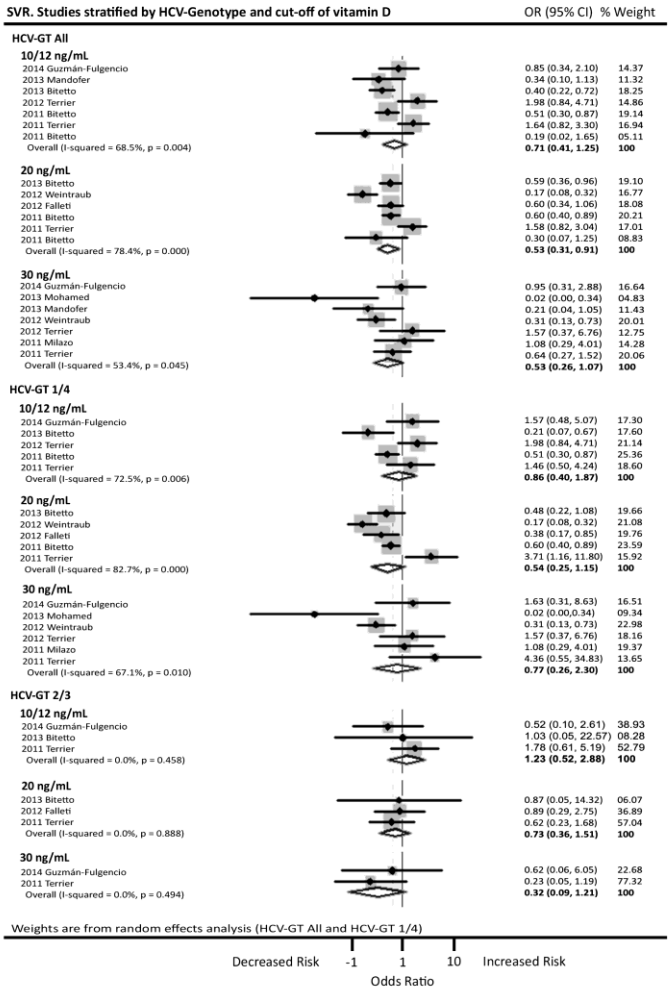
	Coefficient	Standard error	P-value	95% CI
A) SVR (HCV-GT All)				
Cut-off	0.093	0.327	0.781	-0.834, 0.646
Study design	-0.124	0.279	0.642	-0.766, 0.497
HIV Coinfection	-0.219	0.387	0.584	-1.096, 0.656
Region	0.047	0.153	0.766	-0.301, 0.395
Racial descent	-0.981	0.425	**0.047**	**-1.944, -0.018**
Method Vitamin D	0.088	0.303	0.779	-0.597, 0.773
B) SVR (HCV-GT 1/4)				
Cut-off	0.076	0.332	0.821	-0.640, 0.793
Study design	-0.620	0.777	0.451	-2.459, 1.219
HIV Coinfection	-1.218	0.636	0.097	-2.723, 0.287
Region	0.259	0.332	0.460	-0.526, 1.045
Racial descent	-1.196	0.635	0.102	-2.698, 0.305
Method Vitamin D	0.510	0.500	0.342	-0.672, 1.693
C) SVR (HCV-GT 2/3)				
Cut-off	-0.628	0.391	0.169	-1.634, 0.377
Study design	0.417	1.525	0.810	-6.144, 6.979
HIV Coinfection	0.998	0.861	0.366	-2.707, 4.703
Region	-0.661	0.689	0.438	-3.625, 2.302
Method Vitamin D	-0.661	0.689	0.438	-3.625, 2.302

Abbreviations: CI, confidence interval; GT, genotype; HCV, hepatitis C virus; HIV, human inmunodefficiency virus; SVR, sustained virologic response.

Meta-analysis of SVR

A total of 11 studies examined the association between vitamin D status and SVR. No publication bias was observed by Begg´s Funnel plot and Egger´s test (**Supplemental Figure 3**).

Figure 3 shows the pooled ORs for each different threshold used to define vitamin D status. From 11 studies analysed, 9 of them showed a direct relationship between deficiency or insufficiency of vitamin D and SVR. Overall, the pooled OR only indicated a significant association between the cut-offs of 20 ng/mL and SVR for patients not stratified by HCV genotype (OR=0.53 (95%CI=0.31, 0.91)). The Cochran's Q statistic and I^2 statistic suggested a strong heterogeneity among the studies included in this analysis (**Figure 3**).



A meta-regression analysis was conducted to examine the source of heterogeneity (**Table 3**), and only ethnicity had a potential effect on SVR (p=0.047). Galbraith´s plots identified 4 outliers as sources of heterogeneity (**Supplemental Figure 4**): Mohamed et al. (31), Weintraub et al. (33), Terrier et al. (2011) (30), and Terrier et al. (2012) (34). When the meta-analysis was performed excluding these outliers, the heterogeneity disappeared (**Supplemental Figure 5**). Thus, significant pooled ORs were found for all patients [10 ng/mL (OR=0.48 (95%CI=0.34, 0.67)) and 20 ng/mL (OR=0.58 (95%CI=0.45, 0.76))] and in patients infected with HCV-GT 1/4 [10 ng/mL (OR=0.53 (95%CI=0.34, 0.81)) and 20 ng/mL (OR=0.54 (95%CI=0.39, 0.74))] (**Supplemental Figure 5**).

Additionally, we replicated the analysis only considering the studies of HCV-monoinfected patients (**Supplemental Figure 6**). The Cochran's Q statistic and I^2 statistic suggested a strong heterogeneity, which was similar to the observed among the studies of HIV/HCV-coinfected patients. Nevertheless, the pooled OR indicated a significant relationship between the cut-

off of 20 ng/mL and SVR for both, patients not stratified by HCV genotype (OR=0.44 (95%CI=0.27, 0.71)) and patients infected with HCV-GT 1/4 (**Supplemental Figure 6**).

DISCUSSION

The two major results of our meta-analysis results were: (1) a significant direct association between plasma vitamin D deficiency/insufficiency and ALF and (2) a significant inverse association between low vitamin D status and achieving successful virologic response to pegIFNα/RBV therapy.

HCV-infected subjects usually have vitamin D deficiencies, which are related to liver disease severity and a lower chance of responding to pegIFNα/RBV therapy (7, 9). However, there is inconsistency in the published data possibly due to the heterogeneity in the study designs, such as characteristics of patients (HCV infection or HIV/HCV coinfection, ethnicity), characteristics of HCV infection (genotype), and characteristics of vitamin D assessment (seasonality, cut-off values, and methodology). For this reason, our meta-analysis focused on displaying general conclusions about the trend of these associations (overall OR), while serving as a robust tool to investigate heterogeneous results.

Based on the global analysis, there was a significant association between suboptimal vitamin D levels and ALF. However, not all studies detected significant differences between groups maybe due to the different criteria considered in each individual study. However, the heterogeneity between studies was low and the covariates included in the meta-analysis did not have significant values (ethnic origin, study design, HCV genotype, HIV coinfection, vitamin D cut-off value and methodology to measure vitamin D).

The role of vitamin D in CHC patients is controversial and the mechanisms by which these associations occur are not well established (10). On the one hand, since the liver is responsible for 25(OH)D production, patients with liver injury may have low levels of 25(OH)D due to low vitamin D absorption and impaired 25(OH)D synthesis. However, liver function needs to be severely compromised for this impairment to occur (37). On the other hand, low vitamin D levels have been associated with the more severe histologic changes in liver disease (38). This effect could be explained by the antiproliferative and antifibrotic effects of vitamin D on the liver (39) and the role of vitamin D as an immune modulator that reduces inflammation and enhances protective immune responses (40). Thus, it probably is a feedback mechanism. Severe liver failure impairs hydroxylation of vitamin D and causes vitamin D deficiency, which in turn increases hepatic fibrosis, leading to liver failure.

However, since almost all of the included studies have a cross-sectional design, it is impossible to dissect the temporal relationship between 25(OH)D levels and liver disease severity.

Interestingly, severe liver disease is usually associated with vitamin D deficiency (10), while ALF usually results in a lower SVR rate in CHC patients on HCV therapy (41). Therefore, it would be expected that vitamin D levels would be associated with SVR. In our meta-analysis, we found a general trend towards a direct relationship between low vitamin D levels and decreased SVR rate. However, this is not always so, for example not for all HCV genotypes nor for all vitamin D cut-offs. This may be due to the degree of heterogeneity detected during the analysis of SVR ($p<0.05$; $I^2>50\%$). Additionally, the ethnicity was detected as a covariate that affects the results found in the SVR meta-analysis. In fact, when the meta-analysis was performed excluding the four outlier articles (Mohamed et al. (31), Weintraub et al. (33), Terrier et al. (2011) (30), and Terrier et al. (2012) (34)), only patients of Caucasian ethnicity remained. As a consequence, the heterogeneity disappeared and the statistical associations were stronger. Thus, low vitamin D levels were linked to lower odds of achieving SVR after pegIFNα/RBV therapy for all patients as well as for patients infected with HCV-GT 1/4 (difficult-to-treat). On the other hand, this meta-analysis did not detect significant associations for HCV-GT2/3 (easy-to-treat), possibly due to the smaller number of patients with these HCV genotypes and the high SVR rate. Another hypothesized cause of heterogeneity was the presence of HIV coinfection. Terrier et al. (2011) (30) detected a lack of significant association between vitamin D levels and response to pegIFNα/RBV therapy in HIV/HCV coinfected patients. This discordancy could be due to the more complex situation of HIV/HCV coinfected patients. However, although vitamin D metabolism is affected by HIV infection and combined antiretroviral therapy (cART) (37), we found similar association values for HCV monoinfected and HIV coinfected patients. In fact, HIV coinfection was not detected as a significant covariate in meta-regression analysis.

The major source of vitamin D is the exposure to natural sunlight, and therefore, the major cause of vitamin D deficiency is the inadequate exposure to sunlight (42). Thus, the influence of race, country latitude or study region, and seasonality in vitamin D status have been previously described (42). People with a naturally dark skin tone have natural sun protection and require at least three to five times longer exposure to make the same amount of vitamin

D as a person with a white skin tone. Besides, vitamin D deficiency has been proposed as a cause of the racial differences in response rates to antiviral therapy for CHC (33, 43). Moreover, the season variability had a substantial impact on 25(OH) levels (44), but, unfortunately, most studies did not establish the relationship between the seasonal levels of vitamin D and the outcome variables. Only 4 items studied this effect, finding that both HCV infected individuals (29, 34)and HIV/HCV coinfected patients (23, 25)suffered vitamin D insufficiency during all seasons. The other factor that might influence is the study region, but we did not find values in the meta-regression.

In this meta-analysis, a high prevalence of vitamin D insufficiency (25(OH)D <30 ng/mL) and deficiency (25(OH)D <10 ng/mL) was found in HCV-infected patients. Vitamin D deficiency is almost universal among patients with chronic liver disease (45), and specifically in HCV-infected patients (45-47), as well as in HIV/HCV-coinfected patients (23, 28, 30). Besides, prevalence of vitamin D deficiency in HCV-infected (46) and HIV/HCV-coinfected patients (23) is higher than in the general population. However, the prevalence of insufficiency or deficiency of vitamin D has been found in the general population is not so far from the values found in patients infected with HCV. In fact, the National Diet and Nutrition Survey (NDNS) provides evidence of low vitamin D status, as defined by a plasma 25(OH)D concentration less than 10 ng/mL, in most age groups in the United Kingdom population, and either highlights a greater risk of vitamin D deficiency in population subgroups (11). Moreover, a major point of controversy is the establishment of optimal 25(OH)D levels. The latest recommendations suggest that a 25(OH)D level over 20 ng/mL is sufficient to meet the vitamin D requirement (44). However, the Endocrine Society Clinical Practice Guideline (ESCPG) suggested that vitamin D requirements may be greater for sick patients than for healthy individuals and blood vitamin D level above 30 ng/mL may have additional health benefits in reducing the risk of various disease conditions (42).

Moreover, the ESCPG also recommended screening for vitamin D deficiency in individuals at risk for deficiency, including ones with hepatic failure, and supplementation with vitamin D for these deficient patients. In our meta-analysis, the cut-off 10 ng/mL showed higher pooled OR for liver fibrosis and SVR, although the cut-off 30 ng/mL also showed significant values for ALF and were nearly significant for SVR. Thus, our study suggests that the cut-off 30 ng/mL may be an appropriate threshold to prevent fibrosis and HCV therapy failure. In

CHC patients, vitamin D supplementation has been suggested as a preventive and/or early treatment strategy (9). Other major point is the vitamin D supplementation to improve the SVR rate in patients treated with pegIFNα/ribavirin (9). However, there are conflicting data in the scientific literature. Thus, a small number of studies have reported the positive impact of vitamin D supplementation on SVR rate in patients treated with pegIFNα/ribavirin (36, 48-50). However, further clinical investigations on the effect of vitamin D supplementation in treating CHC are needed to confirm this item.

Finally, in order to properly interpret our results, some considerations have to be taken into account. Firstly, our meta-analysis was performed by using the unadjusted raw data provided from each study, whereas most of the results given by the authors were adjusted by age, gender, HCV viral load, and/or other factors. For this reason, the pooled ORs may differ slightly from those cited by the original articles. Secondly, the seasonal differences in vitamin D levels, dietary intake, polymorphisms of vitamin D hydroxylating enzymes, and other variables involved in vitamin D metabolism, such as parathyroid hormone, have not been considered. *IL28B* polymorphisms have not been taken into account for evaluating SVR. Thirdly, only data of blood 25(OH)D levels were used to evaluate vitamin D status rather than the $1,25(OH)_2D$ assay. The serum $1,25(OH)_2D$ assay is only recommended for monitoring certain conditions, such as acquired and inherited disorders of vitamin D and phosphate metabolism (42, 44). Fourthly, multiple methodologies for measuring 25(OH)D were used, including enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), high performance liquid chromatography (HPLC), and liquid chromatography tandem mass spectroscopy. However, the vitamin D detection methodology was not a source of heterogeneity in this meta-analysis. Fifthly, the majority of the studies had a cross-sectional design and the number of studies in some subgroup analyses was small, which might have led to weak results. Consequently, these results should be interpreted with caution.

In conclusion, this meta-analysis shows that a low vitamin D status in CHC patients is associated with a higher likelihood of having ALF and lower odds of achieving SVR, suggesting the utility of vitamin D screening in HCV-infected patients.

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AUTHORS CONTRIBUTIONS

SR designed and supervised the study.

MGA and DPT collected all data, performed the statistical analysis and drafted the report.

MAJS, MGF, and AFR participated in the interpretation of the data and critically reviewed the report.

All authors have approved the final version of the manuscript.

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Supplemental Table 1. Methodological quality of case-control and cross-sectional studies included in the meta-analysis, which was assessed using a method based in the 9-star Newcastle-Ottawa Scale (17).

Year	First author	Deficiency of vitamin D		Sufficiency of Vitamin D		Comparability between groups on the Basis of the Design or Analysis [a]	Ascertainment of Exposure [b]	Non-Response Rate [c]	Total scores
		Adequate definition	Representativeness	Adequate selection	Adequate definition				
2014	Guzmán-Fulgencio (25)	*	*	*	*	**	**	*	9
2013	Petta (26)	*	*	*	*	**	*	-	7
2013	Mohamed (31)	*	*	*	*	**	*	-	7
2013	Ladero (27)	*	*	*	*	**	*	*	8
2013	Mandorfer (28)	*	*	*	*	**	*	*	8
2013	Bitetto (32)	*	*	*	*	**	*	*	8
2012	Weintraub (33)	*	*	*	*	**	*	-	7
2012	Terrier (34)	*	*	*	*	**	**	*	9
2012	Falleti (35)	*	*	*	*	**	*	*	8
2011	Milazzo (23)	*	*	*	*	**	**	-	8
2011	Bitetto (24)	*	*	*	*	**	*	-	7
2011	Terrier (30)	*	*	*	*	**	**	-	8
						Quality average ± standard deviation:			**7.83 ± 0.71**

(a), A maximum of 2 stars could be awarded for this item. Studies that controlled for age received 1 star. Presence of any other controlled factor received an additional star.
(b), A maximum of 2 stars could be awarded for this item. Studies that consider the issues related to vitamin D levels, as dietary supplements or HCV treatment received 1 star. Studies that consider the geolocation of the population and seasonal effect received an additional star.
(c), One star was assigned if there was no significant difference in the response rate between case and control subjects by using the chi-square test ($P > 0.05$).

Supplemental Table 2. Methodological quality of cohort studies included in the meta-analysis, which was assessed using a method based in the 9-star Newcastle-Ottawa Scale (17).

Year	Study	Deficiency of vitamin D		Ascertainment of exposure	Outcome of interest not present at start of study	Control for important factor [(a)]	Assessment of outcome	Follow-up period long enough for outcomes to occur [(b)]	(c) Adequacy of follow-up evaluation of cohorts	Total scores
		Representativeness of the exposed cohort	Selection of the nonexposed cohort							
2011	Bitetto (36)	*	*	*	-	**	*	*	*	8
2012	Lange (29)	*	*	*	-	**	*	-	*	7
									Quality average ± standard deviation:	**7.5±0.71**

(a), A maximum of 2 stars could be awarded for this item. Studies that controlled for age received 1 star. Presence of any other controlled factor received an additional star.
(b), A cohort study with a follow-up time longer than 5 years was assigned 1 star.
(c), A cohort study with a follow-up rate greater than 80% was assigned 1 star.

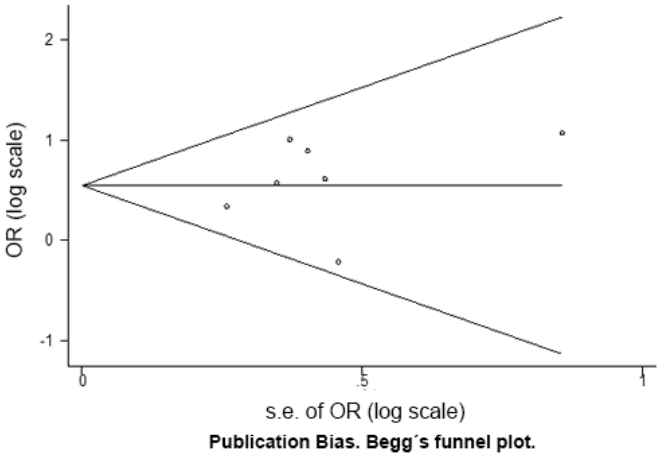
Supplemental Table 3. Meta-regression analysis for advanced liver fibrosis according to vitamin D deficiency.

	Coefficient	Standard error	P value	95% CI
Advanced liver fibrosis				
Cut-off	0.001	0.027	0.979	-0.065, 0.066
Study design	0.214	0.324	0.538	-0.619, 1.047
HCV genotype	-0.285	0.316	0.408	-1.098, 0.527
HIV Coinfection	-0.443	0.317	0.222	-1.259, 0.373
Region	0.034	0.155	0.834	-0.364, 1.539
Method Vitamin D	-0.144	0.120	0.285	-0.454, 0.165

Note: There are not enough studies to perform the F2 meta-regression.
Abbreviations: CI, confidence interval; HCV, hepatitis C virus; HIV, human inmunodefficiency virus.

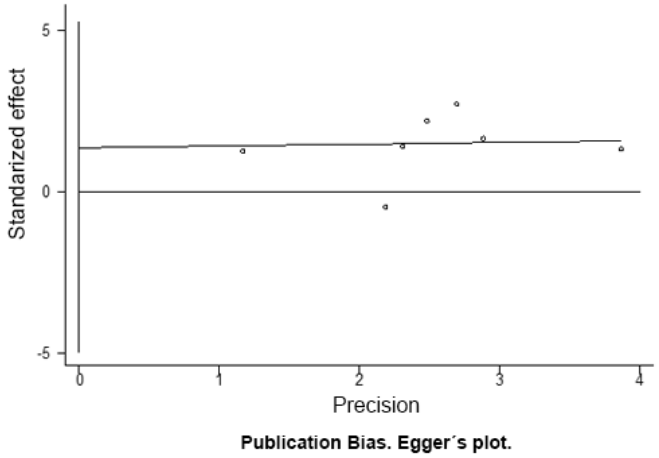
Supplemental Figure 1. Publication bias for liver fibrosis as Begg´s funnel plots (**A**) and Egger´s plot (**B**). The analyses were performed considering an advanced stage of fibrosis (F≥3) and the higher cut-off employed in each study. Table (**C**) shows the Egger´s test results bias considering stratification by stage of fibrosis and cutoff for insufficiency/deficiency of vitamin D. **Abbreviations:** Coef., asymmetry regression coefficient; F3, advanced fibrosis; Std.Err., standard error; t, statistic; P>|t|, significance; and CI, confidence interval.

A



Publication Bias. Begg´s funnel plot.

B



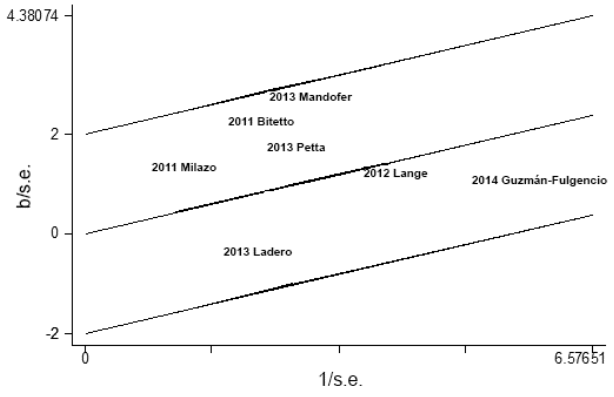
Publication Bias. Egger´s plot.

C

Fibrosis Stage	Cut-off (ng/mL)	Studies (n)	Coef.	Std. Err.	t	P>|t|	95% CI
F≥3	Higher	7	1.365	1.585	0.86	0.429	-2.712, 5.442
	10	2	Insufficient studies for the analysis				
	20	3	0.132	3.669	0.04	0.977	-46.48, 46.74
	30	3	1.615	0.654	2.47	0.245	-6.693, 9.923

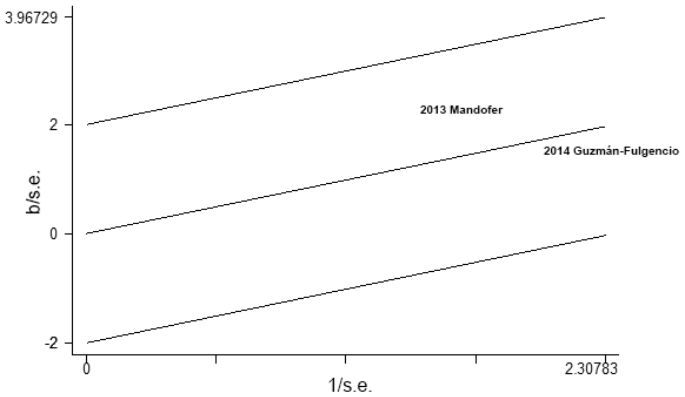
Supplemental Figure 2. Galbraith´s plots for publication heterogeneity for all advanced liver fibrosis (**A**) and cut-offs for vitamin D insufficiency/deficiency of 10 ng/mL (**B**) 20 ng/mL (**C**) and 30 ng/mL (**D**). The analyses were performed considering the higher cutoff employed in each study. **Abbreviations** 1/s.e, precision; b/s.e, standardized effect.

A



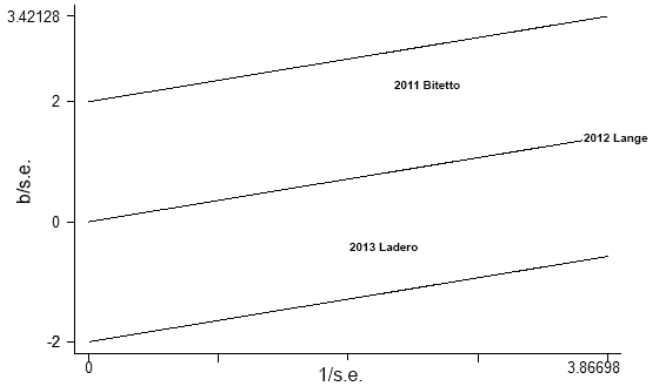
Publication Heterogeneity for Advanced Fibrosis (F3). Galbraith plot.

B



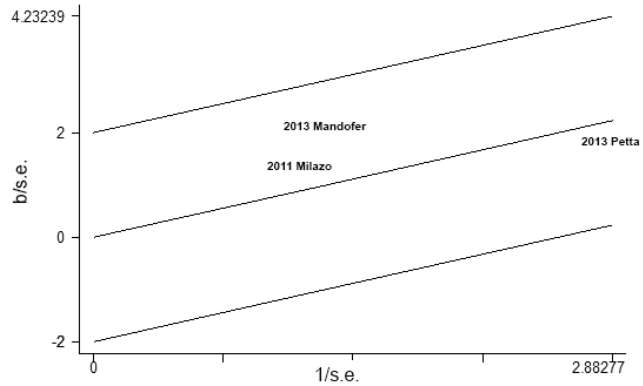
Publication Heterogeneity for Advanced Fibrosis (F3) and cut-off of 10 ng/mL. Galbraith plot.

C



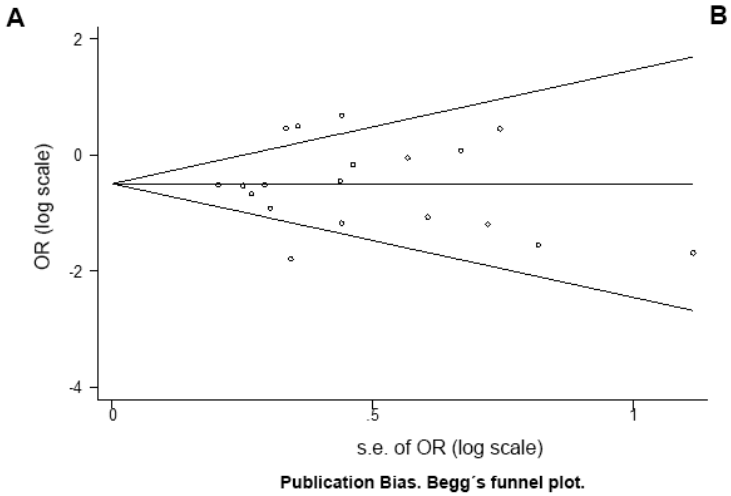
Publication Heterogeneity for Advanced Fibrosis (F3) and cut-off of 20 ng/mL. Galbraith plot.

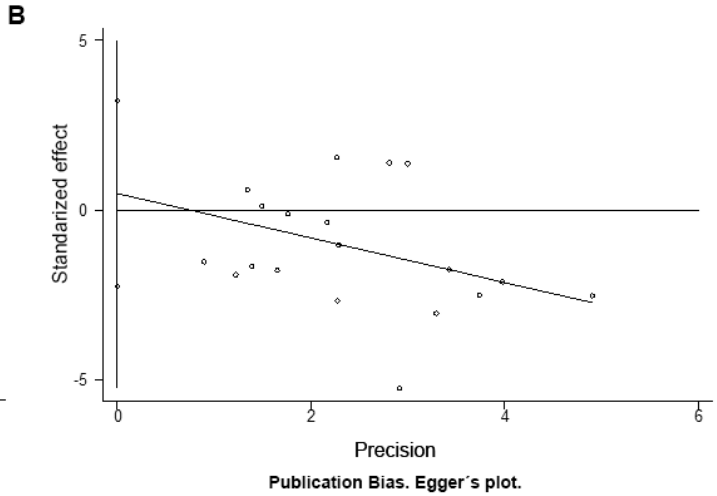
D



Publication Heterogeneity for Advanced Fibrosis (F3) and cut-off of 30 ng/mL. Galbraith plot.

Supplemental Figure 3. Publication bias for SVR as Begg´s funnel plots (**A**) and Egger´s plot (**B**). The analyses were performed considering the higher cut-off employed in each study. Table (**C**) shows the Egger´s test results bias considering stratification by cutoff for insufficiency/deficiency of vitamin D. **Abbreviations:** Coef., asymmetry regression coefficient; F2, significant fibrosis; F3, advanced fibrosis; Std.Err., standard error; t, statistic; P>|t|, significance; and CI, confidence interval.

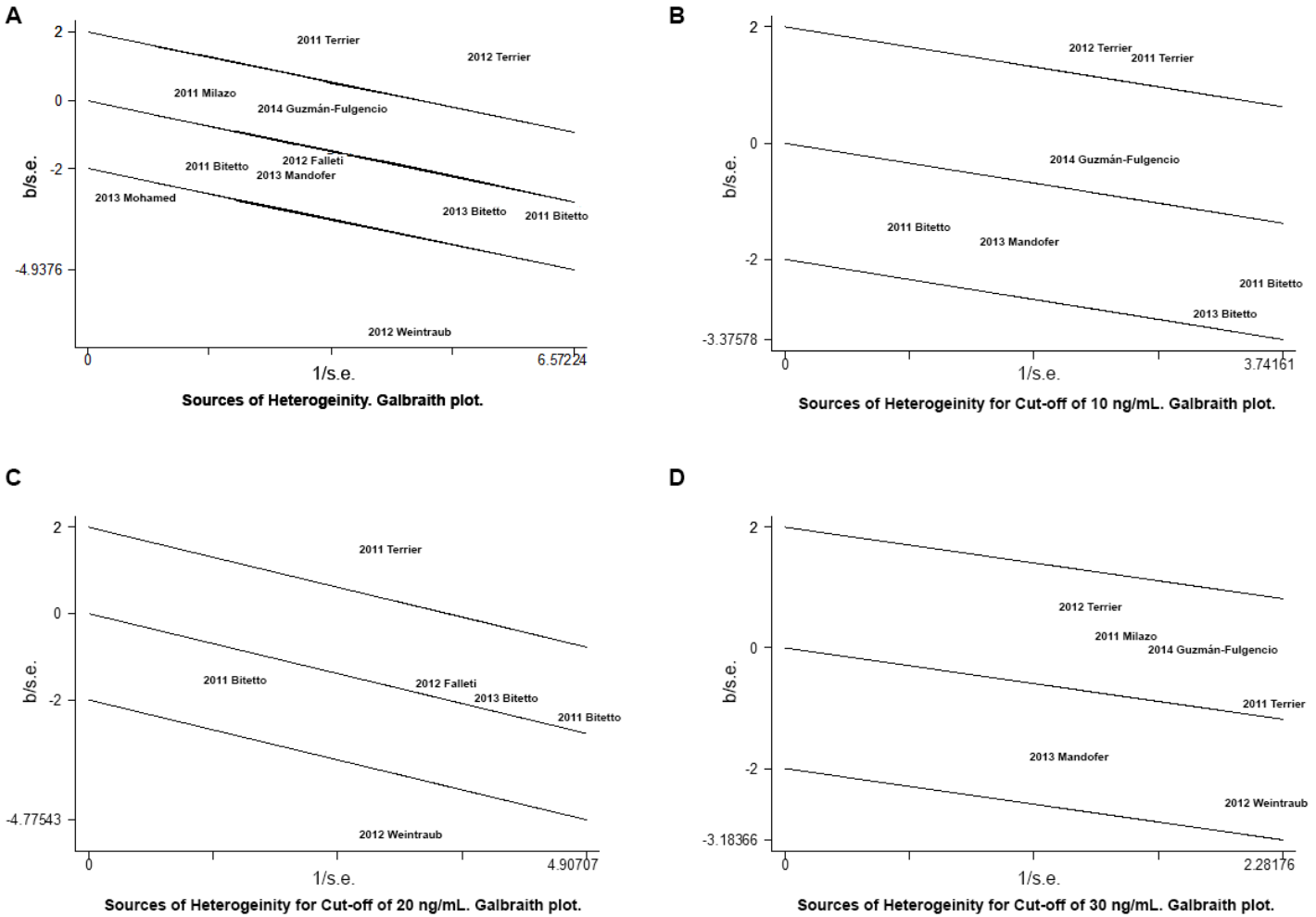




C

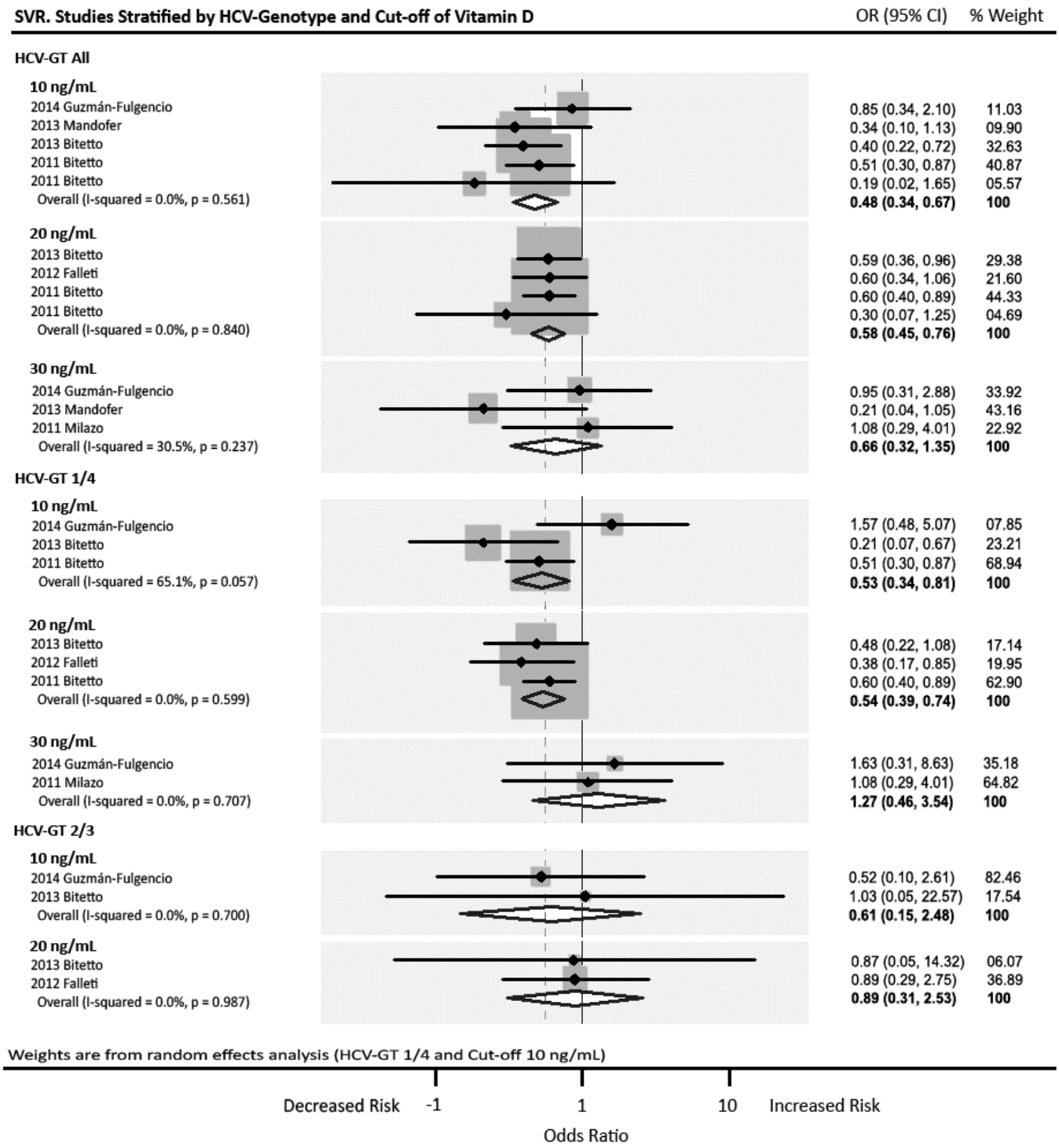
Cut-off (ng/mL)	Studies (n)	Coef.	Std. Err.	t	P>|t|	95% CI
Higher	11	0.494	1.292	0.38	0.707	-2.233, 3.222
10	7	3.131	2.901	1.08	0.330	-4.337, 10.601
20	6	-0.959	3.737	-0.26	0.810	-11.335, 9.416
30	7	2.162	2.263	0.96	0.393	-4.119, 8.444

Supplemental Figure 4. Galbraith´s plots for publication heterogeneity for all SVR (**A**) and cut-offs for vitamin D insufficiency/deficiency of 10 ng/mL (**B**) 20 ng/mL (**C**) and 30 ng/mL (**D**). The analyses were performed considering the higher cutoff employed in each study. **Abbreviations** 1/s.e, precision; b/s.e, standardized



effect.

Supplemental Figure 5. Forest plot of the meta-analysis performed to investigate the association between vitamin D levels and sustained virologic response (SVR) after removal of the outliers of the heterogeneity.
Abbreviations: CI, confidence intervals; OR, odds ratio.



Supplemental Figure 6. Forest plot of the meta-analysis performed to investigate the association between vitamin D levels and sustained virologic response (SVR) only considering the studies of HCV-monoinfected patients. **Abbreviations:** CI, confidence intervals; OR, odds ratio.