

Occult Hepatitis C Virus Infection in Spanish Patients with Lymphoproliferative Disorders

Roque Cuellar MC^{1*}, García Lozano JR², Sánchez B², Carrillo Cruz E³, De la Cruz Vicente F³, Núñez Roldán A² and Aguilar Reina J¹

¹Biomedicine Institute of Seville (IBIS), University of Seville, CSIC, University Hospital Virgen del Rocío, Seville, Spain

²Department of Immunology, IBIS, University of Seville, CSIC, University Hospital Virgen del Rocío, Seville, Spain

³Department of Hematology, IBIS, University of Seville, CSIC, University Hospital Virgen del Rocío, Seville, Spain

*Corresponding author: María del Carmen Roque Cuéllar, Biomedicine Institute of Seville, University of Seville, CSIC, University Hospital Virgen del Rocío, Seville, Spain, Tel: +34 646 17 99 22; E-mail: mroquecuellar@gmail.com

Received date: 03 May 2017; Accepted date: 24 Jul 2017; Published date: 31 Jul 2017.

Citation: Roque Cuéllar MC, García Lozano JR, Sánchez B, Carrillo Cruz E, De la Cruz Vicente F et al. (2017) Occult Hepatitis C Virus Infection in Spanish Patients with Lymphoproliferative Disorders. J Emerg Dis Virol 3(2): doi <http://dx.doi.org/10.16966/2473-1846.134>

Copyright: © 2017 Roque Cuéllar MC et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Occult hepatitis C virus (HCV) infection (OI) can be found in peripheral blood mononuclear cells (PBMCs) and serum of anti-HCV, HCV-RNA-negative individuals. As HCV is related with several lymphoproliferative disorders (LPD), the aim of this study was to determine the prevalence of OI in Spanish patients with LPD.

Sixty patients with LPD were included: 58 with B-cell non-Hodgkin lymphoma (B-NHL), 1 with Hodgkin lymphoma (HL) and 1 with T-cell non-Hodgkin lymphoma (T-NHL). RNA was extracted from CD19+ B cells positively isolated from PBMCs as well as CD19- fraction, mainly lymphocytes T+ monocytes and NK cells, negatively selected. The detection and quantitation of both HCV-RNA strands was performed by strand-specific RT-PCR. Furthermore, IgG antibody to an HCV core-derived peptide (anti-HCV core), was also analyzed. OI was detected in 8/60 (13.33%) patients with LPD: 6 with B-NHL and those with HL and T-NHL. In the B-NHL group, HCV-RNA positive strand was found in the CD19- fraction of 3 patients (one of them bearing the negative strand too), and in B-cells from the other 3. Both HCV-RNA strands were detected in the CD19- fraction of the HL patient, who showed certain anti-HCV core reactivity. In the T-NHL patient, the positive strand was detected in B-cells.

In conclusion, patients with LPD without signs of classic HCV infection may have OI in PBMCs. The study of the virus presence in these cellular sub populations is of interest, as HCV is known by its oncogenic properties.

Keywords: HCV-RNA positive and negative-strands; Lymphoproliferative disorders; Occult Hepatitis C virus infection; Peripheral blood mononuclear cells

Abbreviations: Anti-HCV: Antibodies against HCV; Anti-HCV core: IgG antibody to an HCV core-derived peptide; B-NHL: B-cell non-Hodgkin lymphoma; CD19- fraction/cells : T lymphocytes, NK cells and monocytes; CD19+ fraction/cells: B cells; HCV: Hepatitis C virus; HL: Hodgkin lymphoma; IgG : Immunoglobulin G ; LPD: Lymphoproliferative disorders; OI: Occult HCV infection; PBMCs : Peripheral blood mononuclear cells; T-NHL: T-cell non-Hodgkin lymphoma

Introduction

Hepatitis C virus (HCV) infection is a global pandemic. Following primary infection, a minority of individuals clear the infection predominantly *via* cellular immune mechanisms, whereas the majority become chronically infected [1].

Early after its discovery, it was shown that HCV is also a lymphotropic virus [2] being able to infect and replicate in established B cell lines and primary B lymphocytes [3], as well as in T cell lines and primary naïve T lymphocytes [4]. This lymphotropism had various effects, especially on cell development and proliferation [4-6]. Furthermore, B cells can harbor occult variants that have a poor translational efficiency in hepatocytes, suggesting their extra-hepatic origin [7]. Several lymphoproliferative disorders (LPD) have been associated with HCV, including B-cell non-Hodgkin lymphoma (B-NHL), between others [8]. However, the epidemiological estimates do not take into account the extra hepatic aspects of HCV infection, neither the third outcome: the occult HCV infection (OI) [1,8].

The genomic and antigenomic (replicative) HCV-RNA strands has been found in the liver, serum and peripheral blood mononuclear cells (PBMCs) of patients with antibodies against HCV (anti-HCV), years after spontaneous or treatment-induced resolution of HCV infection [9-12]. These situations are interpreted as OI, which has also been detected in subjects without anti-HCV or HCV-RNA in serum tested by routine methods: patients in whom the etiology of persistently abnormal results of liver-function tests is unknown [13] or population unselected for hepatic disease [14], between others cohorts as heterosexual partners of chronically infected patients or family of patients with OI [15,16]. The ultracentrifugation of serum samples, the analysis of immunoglobulin G (IgG) antibody to a dominant HCV core epitope (anti-HCV core), together with another non-invasive assay: test HCV-RNA in PBMCs, have allowed the detection of this form of HCV infection without the need of a liver biopsy [17-20]. However, the possible consequences of this type of infection remain unknown. The purpose of this study was to know the existence of OI in PBMCs and serum from Spanish patients with LPD, without anti-HCV or HCV-RNA in serum tested by routine methods.

Patients and Methods

Patients

This is a prospective study to determine the presence of OI in sixty patients diagnosed with LPD, who were recruited consecutively since March 2012 to June 2013, from Hematology Department belonging to University Hospital Virgen del Rocío of Seville. Selection criteria for patients was being negative for anti-HCV antibodies, HCV-RNA, hepatitis B virus (HBV) surface antigen, HBV core antibody, HBV-DNA and anti-human immunodeficiency virus (HIV) antibodies in serum. They were asked about HCV transmission risks factors: intravenous drug users; tattoos and piercing experience; professional risk factors: health care workers in contact with infected blood; familial risk factors: coexistence with intravenous drug users or HCV-infected patients and shared personal care items, like toothbrushes or razors; sexual risk factors: promiscuity or having sex with an HCV-infected patient that causes contact with the infected blood.

Epidemiological and laboratory data of the patients with LPD are depicted in Table 1. The majority of the patients selected were diagnosed of B-NHL (n=58), one patient of HL and another one of T-NHL. B-NHL patients were classified as: extra nodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) (n=4), splenic marginal zone B-cell lymphoma (n=6), follicular lymphoma (n=22), diffuse large B-cell lymphoma (n=17), small lymphocytic lymphoma (n=2), chronic lymphocytic leukemia (n=1), mantle cell lymphoma (n=3), Burkitt lymphoma (n=1), lymphoplasmacytic lymphoma (n=2).

Routine detection of HCV, HBV and HIV

HCV-RNA in serum was investigated by Cobas Amplicor HCV test version 5.0, with a lower limit of detection of 15 IU/ml (Roche Diagnostics, Mannheim, Germany). The analysis of anti-HCV in serum was done by the third-generation enzyme immunoassay ADVIA Centaur HCV assay (Siemens Healthcare Diagnostics, Deerfield, USA). HBV surface antigen and HBV core were analyzed by E170 and HIV by Cobas 680 (Roche both). The study was approved by the Ethical Committee of the aforementioned Hospital and conducted according to the Declaration

of Helsinki on human experimentation. Informed consent was obtained from all the patients.

HCV RNA detection and quantitation from PBMCs

PBMCs from patients with LPD were isolated from EDTA-treated blood by Ficoll-Hypaque density gradient centrifugation. CD19+ B cells were isolated from PBMCs by positive selection using CD19 Micro Beads and MS Columns (Miltenyi Biotec Inc. Auburn, CA, USA), whereas the others components of the PBMCs, mainly T lymphocytes and monocytes together with NK cells (CD19- fraction), were collected by negative selection in the same sample. Both populations were resuspended in Tripure (Roche) and stored at -80°C. RNA from the CD19+ and CD19- fractions were purified using the Direct-zol™ RNA MiniPrep kit (Zymo Research, Irvine, CA, USA), following manufacturer's instructions. RNA was stored at -80°C after measuring the concentration by spectrophotometry (NanoDrop ND-1000, Wilmington, USA).

Detection of the 5' non-coding region of the genomic and antigenomic HCV-RNA strands, was performed by a strand-specific real-time RT-PCR, using the Transcriptor First Strand cDNA Synthesis kit (Roche) according to the kit instructions, with 0.5 µg of the total RNA isolated, and 0.5 µmol L-1 of the antisense or sense primers belonging to the HCV untranslated region (UTR) (UTRLC2:5'-CAAGCACCTATCAGGCAGT-3';UTRL C1:5'CTTCACGCAGAAAGCGTCTA-3', respectively). Real-time PCR was done in LightCycler 2.0 (Roche Molecular Biochemicals, Mannheim, Germany), using fluorescence resonance energy transfer (FRET) probes, with the Light Cycler FastStart DNA Master HybProbe Kit (Roche Molecular Biochemicals), as described [18]. A standard curve constructed with 10-fold dilutions of synthetic genomic HCV-RNA was used for the quantification of HCV-RNA in PBMCs [21]. The sensitivity of this real-time PCR was ten genome copies per reaction.

Enzyme immunoassay to detect anti-HCV core in serum

Analysis of anti-HCV core was done by Kit DIATER ANTI VHC CORE ALTA SENSIBILIDAD (Diater Laboratorio de Diagnóstico y Aplicaciones Terapéuticas (Leganés, Madrid, Spain), following manufacturer's instructions [19]. In brief, serum samples (together with

Table 1: Epidemiological and laboratory data of the patients.

Lymphoproliferative disorders N=60	N	Age (years, mean ± SD ¹)	Gender Male/Female	AS ² (U/L)	ALT ³ (U/L)	Bilirubin (mg/dl)	Cholesterol (mg/dl)	Triglicerides (mg/dl)	Platelets (x103/µl)
Hodgkin lymphoma	1								
Follicular lymphoma		48	0/1	124	153	3.64	371	345	237
B-cell non-Hodgkin lymphoma	58								
Mucosa associated lymphoid tissue (MALT)	4	58.75 ± 11.32	4/0	22.75 ± 8.10	32.50 ± 17.41	0.54 ± 0.26	181.25 ± 47.12	140.25 ± 53.38	225.75 ± 58.03
Splenic marginal zone B-cell lymphoma	6	67.33 ± 11.62	6/0	21.17 ± 8.16	19.83 ± 9.85	0.51 ± 0.24	183.17 ± 28.14	144.67 ± 89.67	204.50 ± 59.62
Follicular lymphoma	22	60.95 ± 12.95	13/9	19.43 ± 5.68	22.14 ± 10.45	0.41 ± 0.18	225.86 ± 39.01	162.33 ± 202.97	266.05 ± 93.54
Diffuse large B-cell lymphoma	17	53.88 ± 10.43	9/8	20.71 ± 4.33	23 ± 11.74	0.48 ± 0.23	205.82 ± 55.85	116.76 ± 67.27	272 ± 58.97
Small lymphocytic lymphoma	2	60 ± 28.28	1/1	22.5 ± 6.36	28.5 ± 6.36	0.615 ± 0.09	194 ± 24.04	149 ± 97.58	218 ± 11.31
Chronic lymphocytic leukemia	1	49	1/0	27	33	0.45	282	164	386
Mantle cell lymphoma	3	55.67 ± 8.96	2/1	27 ± 9.54	40 ± 19.16	0.46 ± 0.16	244.33 ± 83.20	90 ± 41.68	267 ± 84.04
Burkitt lymphoma	1	28	1/0	24	64	0.55	125	230	297
Lymphoplasmacytic lymphoma	2	67.5 ± 13.44	1/1	22 ± 5.58	27 ± 7.32	0.71 ± 0.02	205 ± 54.96	70 ± 60.48	289 ± 64.01
T-cell non-Hodgkin lymphoma	1	30	1/0	18	18	0.71	181	107	185

¹SD: Standard deviation; ²AST: Aspartate aminotransferase; ³ALT: Alanine aminotransferase; Clinical determinations were performed at the time of this study.

positive and negative controls supplied by the kit) were diluted 1/10 in blocking buffer and pre-incubated for 1 h at 37°C with shaking. After wash twice the 96-well micro titre plate, coated with anti-HCV core, duplicate samples reacted with it for 1h at 37°C. Wells were washed five times with the appropriate buffer and, after removing liquid excess, the conjugated serum was added. After another incubation and wash cycle in the same conditions, the revelation solution was added. Finally, after 30 min incubation at room temperature, dark and shaking, the absorbance values were measured at 405 nm with a reference at 620 nm in the Microplate autoreader EL 309 (Bio-tek instruments, Cultek, Madrid, Spain). The samples were considered non reactivities, potentially reactivities or reactivities following the kit instructions: after validating the assay with absorbance means for blank wells, positive and negative controls, the cut-off point was calculated and finally the absorbance index (AI), dividing the absorbance mean of the sample in duplicate between the cut-off point. Samples with $AI \geq 1.2$ are considered reactivities, $1.1 \leq AI < 1.2$ are considered potentially reactivities and $AI < 1.1$ non reactivities.

Results

HCV-RNA detection and quantitation in the CD19+ cells or CD19-fractions from patients with LPD have been summarized in Table 2. Table 3 shows epidemiological and laboratory data of patients with OI. Genomic (positive) HCV-RNA strand was detected in PBMCs of 8 patients (13.33%): one patient diagnosed of HL, 6 of B-NHL and another one of T-NHL. Six of these 8 patients had sample enough to analyze the HCV-RNA anti genomic (negative) strand too, being detectable in two of them (2/6=33.33%). Seven of the 60 patients with LPD had HCV

transmission risks (familial and professional, Table 2), and 4 of them had OI. With regard to anti-HCV core detection in serum, the HL patient, who had the highest values of HCV viral load of both HCV-RNA strands in the CD19- fraction, was potentially reactive (AI=1.13). In the other patients, an AI lower than 1.1 was obtained, corresponding to non-reactive sample for anti-HCV core detection in serum.

Discussion

The relationship between the classic HCV infection and LPD was documented as consequence of the viral lymphotropism [2,22]. However, HCV replicates in the same cells in the classic and occult infection [23,24]. As the lymphoproliferation studies have been performed on cohorts of patients chronically infected [8,25,26], the etio pathogenic link between HCV-associated lymphoproliferative disorders may be still undiscovered. In the present work, OI was detected in the 13.33% of patients with LPD, negative for anti-HCV antibodies or HCV-RNA in serum analyzed routinely.

Three general theories have emerged to understand the HCV-induced lymphomagenesis: (a) continuous external stimulation of lymphocyte receptors by viral antigens and consecutive proliferation: (b) HCV replication in B cells with oncogenic effect mediated by intracellular viral proteins and (c) "hit and run" theory or permanent B-cell damage caused by a transiently intracellular virus [27]. And all these theories could also meet on the OI stage. It has been found a higher expression of low-density lipoprotein receptors involved in PBMCs HCV entry, as well as viral replication, in lymphocytes and monocytes from sustained

Table 2: OI in patients with LPD.

Lymphoproliferative disorders N=60	N	Risk factors for HCV transmission Familial/professional risks	HCV-RNA positive in PBMCs	HCV-RNA copies/ μ g of total RNA in PBMCs			
				CD19+ cells		CD19- fraction	
				+ strand	-strand	+ strand	-strand
Hodgkin lymphoma	1						
Follicular lymphoma		1/1	1			986	387
B-cell non-Hodgkin lymphoma	58						
Mucosa associated lymphoid tissue (MALT)	4		1			2.91	
Splenic marginal zone B-cell lymphoma	6	1/-	2	75.9			
				12.3			
Follicular lymphoma	22		2	35.3		799	50.4
Diffuse large B-cell lymphoma	17	2/1	0				
Small lymphocytic lymphoma	2		0				
Chronic lymphocytic leukemia	1		0				
Mantle cell lymphoma	3	1/-	1			48.3	
Burkitt lymphoma	1		0				
Lymphoplasmacytic lymphoma	2		0				
T-cell non-Hodgkin lymphoma	1	1/-	1	23.4			

Table 3: Epidemiological and laboratory data of the patients with OI.

OI patients N=8	N	Age (years, mean \pm SD ¹)	Gender Male/Female	AST ² (U/L)	ALT ³ (U/L)	Bilirubin (mg/dl)	Cholesterol (mg/dl)	Triglicerides (mg/dl)	Platelets (x103/ μ l)
Hodgkin lymphoma	1								
Follicular lymphoma		48		124	153	4	371	345	237
B-cell non-Hodgkin lymphoma	6								
Mucosa associated lymphoid tissue (MALT)	1	67		15	16	1	171	211	148
Splenic marginal zone B-cell lymphoma	1	70		29	22	0	226	116	250
	1	68		16	12	0	158	118	181
Follicular lymphoma	1	80		17	15	0	187	82	265
	1	55		17	17	0	202	66	241
Mantle cell lymphoma	1	66		33	49	0	179	111	216
T-cell non-Hodgkin lymphoma	1	30		18	18	1	181	107	185

virological responders (SVR) with HCV persistence in PBMCs [28]. On the other hand detection of high viral loads had been described in B-cells from patients with HCV persistence in PBMCs, which resolved the HCV infection spontaneously or after achieving a sustained virological response [23,24]. Furthermore, an enhanced expression of lymphoma genesis-related genes in B-cells from SVR with OI, as recently observed by our group [29].

Assessment of the existence of OI in LPD patients had been documented in Egypt and worldwide in 2012 [30]. The authors studied anti-HCV as well as HCV-RNA positive and negative strands from serum and PBMCs in 50 LPD patients, and in 50 apparently healthy volunteers. Anti-HCV and serum HCV-RNA antibodies were detected in 13 out of 50 LPD patients (26%). The HCV-RNA positive strand was detected in the PBMCs from 18 out of 50 LPD patients (36%), among them 10 (20%) patients were negative for serum HCV infection markers, representing the cohort with OI. The HCV-RNA negative strand was undetectable in patients with the positive one. Finally, in two healthy controls (HC), the HCV-RNA positive strand was detected (2/50=4%). So, the occurrence of OI in Egyptian with LPD in comparison with HCV chronically infected patients with LPD and HC were 20, 26 and 4%, respectively; therefore, less incidence than in the HCV chronically infected patients but more that the probability found in the healthy population.

The present work has a limitation due to the lack of internal controls performed in parallel on apparently healthy donors using the same methodology. However, in spite of the lack of studies in Spain, this results are between those found in other European (Italian) cohorts with regard to LPD in patients with chronic HCV infection (19.8%) and the incidence of OI in HC (3.3%) [14,31]. In this work a cohort of HC was not included since in previous studies, 72 were analyzed and OI in their PBMCs were not found. Demographic data from these cohorts in comparison with the present one, patients with LPD, are as follows: HC, Gender=39 men and 33 women; age (mean age \pm standard deviation)=44.9 \pm 8.56; patients with LPD, Gender=39 men and 21 women; age (mean age \pm standard deviation)=47.1 \pm 13.85 [15,28,29,32]. On the other hand, in 31 sexual partners of chronically HCV infected individuals with HCV transmission risk, (Gender=9 men and 22 women; age (mean age \pm standard deviation)=42.74 \pm 9.89), a similar percentage of HCV-RNA presence in PBMCs (13%) than in patients with LPD (13.33%) was observed [15].

In an Iranian cohort, HCV-RNA was found in PBMCs from 2 (1.9%) of the 104 patients with LPD studied, but taking into account the prevalence of HCV infection in general population (<0.5%), the prevalence of OI in LPD patients was approximately 4 times the rate in Iran [33]. Kisiel E et al. [34] also analyzed seronegative HCV infection in 77 anti-HCV-negative patients with LPD, studying HCV-RNA by RT-PCR in plasma, PBMCs and bone marrow as well as the presence of viral nonstructural protein 3 (NS3) by immunostaining in PBMCs and bone marrow. HCV-RNA was detected in at least one compartment in 27 (35.1%) patients: in bone marrow in 22 patients (28.6%), in PBMCs in 13 (16.9%) and in plasma in 10 (13%). Viral load in HCV-RNA-positive plasma ranged from 15 to 1.17 $\times 10^3$ IU/mL. NS3 was detected in all but two HCV-RNA-positive bone marrow and one HCV-RNA-positive PBMCs samples [34]. So this paper add evidences that HCV can be present among patients with LPD despite the lack of specific antibodies, and highlights the importance to check it in the bone marrow compartment, where the highest HCV-RNA prevalence was found.

Therefore, despite the HCV-RNA negative strand only was studied in the present work together with the Egyptians [30], the discovery of the HCV-RNA positive one is indicative that an OI may exist in patients with LPD.

In the present work, load of genomic and anti genomic HCV-RNA in PBMCs ranged from 2.91 to 986 and 50.4 to 387 copies/ μ g of total RNA

in PBMCs, respectively. Furthermore, the patient with HL (follicular lymphoma), who had the highest values of HCV-RNA positive and negative strands in PBMCs, was potentially reactive for the detection of anti-HCV core in serum by the enzyme immunoassay too. Further studies are required to analyze this phenomenon, to identify these patients as well as to evaluate the possibility that it might be related with the development of lymphoproliferative disorders.

Acknowledgements and Disclosures

We greatly appreciate the collaboration of the individuals under this study. We thank the nurses who generously enrolled in this study for their excellent technical assistance: Manuela Falcón Ortega and Ana María Pérez Mairén. We thank Miguel Ojeda Núñez and José Luis Gallardo Hidalgo for their collaboration in this work.

This study was supported by Consejería de Salud (Junta de Andalucía); Grant number: PI0371/2010; as well as by Instituto de Salud Carlos III; Grant number: PI10/ 02373. MCRC was sponsored by Plan Andaluz de Ayuda a la Investigación (PAI), CTS-102 and by the Instituto de Salud Carlos III, through the aforementioned grant.

References

1. Sugden PB, Cameron B, Bull R, White PA, Lloyd AR (2012) Occult infection with hepatitis C virus: friend or foe? *Immunol Cell Biol* 90: 763-773.
2. Zignego AL, Macchia D, Monti M, Thiers V, Mazzetti M, et al. (1992) Infection of peripheral mononuclear blood cells by hepatitis C virus. *J Hepatol* 15: 382-386.
3. Sung VM, Shimodaira S, Doughty AL, Picchio GR, Can H, et al. (2003) Establishment of B-lymphoma cell lines persistently infected with hepatitis C virus *in vivo* and *in vitro*: the apoptotic effects of virus infection. *J Virol* 77: 2134-2146.
4. Kondo Y, Ueno Y, Kakazu E, Kobayashi K, Shiina M, et al. (2011) Lymphotropic HCV strain can infect human primary naïve CD4+ cells and affect their proliferation and IFN- γ secretion activity. *J Gastroenterol* 46: 232-241.
5. Kondo Y, Machida K, Liu HM, Ueno Y, Kobayashi K, et al. (2009) Hepatitis C virus infection of T cells inhibits proliferation and enhances fas-mediated apoptosis by down-regulating the expression of CD44 splicing variant 6. *J Infect Dis* 199: 726-736.
6. Kondo Y, Sung VM, Machida K, Liu M, Lai MM (2007) Hepatitis C virus infects T cells and affects interferon-gamma signaling in T cell lines. *Virology* 361:161-173.
7. Durand T, Di Liberto G, Colman H, Cammas A, Boni S, et al. (2010) Occult infection of peripheral B cells by hepatitis C variants which have low translational efficiency in cultured hepatocytes. *Gut* 59: 934-942.
8. Zignego AL, Giannini C, Gagnani L (2012) HCV and lymphoproliferation. *Clin Dev Immunol* 2012: 980942.
9. Pham TN, MacParland SA, Mulrooney PM, Cooksley H, Naoumov NV, et al. (2004) Hepatitis C virus persistence after spontaneous or treatment-induced resolution of hepatitis C. *J Virol* 78: 5867-5874.
10. Radkowski M, Horban A, Gallegos-Orozco JF, Pawelczyk A, Jablonska J, et al. (2005) Evidence for viral persistence in patients who test positive for anti-hepatitis C virus antibodies and have normal alanine aminotransferase levels. *J Infect Dis* 191: 1730-1733.
11. Radkowski M, Gallegos-Orozco JF, Jablonska J, Colby TV, Walewska-Zielecka B, et al. (2005) Persistence of hepatitis C virus in patients successfully treated for chronic hepatitis C. *Hepatology* 41:106-114.
12. Castillo I, Rodríguez-Iñigo E, López-Alcorocho JM, Pardo M, Bartolomé J (2006) Hepatitis C virus replicates in the liver of patients who have a sustained response to antiviral treatment. *Clin Infect Dis* 43: 1277-1283.

13. Castillo I, Pardo M, Bartolome J, Ortiz-Movilla N, Rodríguez-Iñigo E, et al. (2004) Occult hepatitis C virus infection in patients in whom the etiology of persistently abnormal results of liver-function tests is unknown. *J Infect Dis* 189: 7-14.
14. De Marco L, Gillio-Tos A, Fiano V, Ronco G, Krogh V, et al. (2009) Occult HCV infection: An unexpected finding in a population unselected for hepatic disease. *PlosONE* 4: e8182.
15. Roque-Cuéllar MC, Sánchez B, García-Lozano JR, Praena-Fernández JM, Núñez-Roldán A, et al. (2011) Cellular immune responses and occult infection in seronegative heterosexual partners of chronic hepatitis C patients. *J Viral Hepat* 18: e541-e549.
16. Castillo I, Bartolomé J, Quiroga JA, Barril G, Carreño V (2009) Hepatitis C virus infection in the family setting of patients with occult hepatitis C. *J Med Virol* 81: 1198-1203.
17. Castillo I, Bartolomé J, Quiroga JA, Barril G, Carreño V (2010) Diagnosis of occult hepatitis C without the need for a liver biopsy. *J Med Virol* 82: 1554-1559.
18. Bartolomé J, López-Alcorocho JM, Castillo I, Iñigo ER, Quiroga JA (2007) Ultracentrifugation of serum samples allows detection of hepatitis C virus RNA in patients with occult hepatitis C. *J Virol* 81: 7710-7715.
19. Quiroga JA, Castillo I, Llorente S, Bartolomé J, Barril G, et al. (2009) Identification of serologically silent occult hepatitis C virus infection by detecting immunoglobulin G antibody to a dominant HCV core peptide epitope. *J Hepatol* 50: 256-263.
20. Carreño V (2006) Occult hepatitis C virus infection: a new form of hepatitis C. *World J Gastroenterol* 12: 6922-6925.
21. De Felipe B, Leal M, Soriano-Sarabia NA, Gutiérrez, López-Cortés L, et al. (2009) HCV RNA in peripheral blood cell subsets in HCV-HIV co infected patients at the end of PegIFN/RBV treatment is associated with virologic relapse. *Journal of Viral Hepatitis* 16: 21-27.
22. Zignego AL, Ferri C, Giannini C, La Civita L, Carecchia G, et al. (1997) Hepatitis C virus infection in mixed cryoglobulinemia and B-cell non-Hodgkin's lymphoma: evidence for a pathogenetic role. *Arch Virol* 142: 545-555.
23. Pham TN, King D, Macparland SA, McGrath JS, Reddy SB, et al. (2008) Hepatitis C virus replicates in the same immune subsets in chronic hepatitis C and occult infection. *Gastroenterology* 134: 812-822.
24. Pham TN, Michalak TI (2008) Occult persistence and lymphotropism of hepatitis C virus infection. *World J Gastroenterol* 14: 2789-2793.
25. Ito M, Murakami K, Suzuki T, Mochida K, Suzuki M, et al. (2010) Enhanced expression of lymphomagenesis-related genes in peripheral blood B cells of chronic hepatitis C patients. *Clin Immunol* 135: 459-465.
26. Zignego AL, Piluso A, Giannini C (2008) HBV and HCV chronic infection: autoimmune manifestations and lymphoproliferation. *Autoimmun Rev* 8: 107-111.
27. Peveling-Oberhag J, Arcaini L, Hansmann ML, Zeuzem S (2013) Hepatitis C-associated B-cell non-Hodgkin lymphomas. *Epidemiology, molecular signature and clinical management. J Hepatol* 59: 169-177.
28. Roque-Cuéllar MC, Sánchez B, García-Lozano JR, Garrido-Serrano A, Sayago M, et al. (2012) Expression of CD81, SR-BI and LDLR in lymphocytes and monocytes from patients with classic and occult hepatitis C virus infection. *J Med Virol* 84: 1727-1736.
29. Roque-Cuéllar MC, García-Lozano JR, Sánchez B, Martínez Sierra C, Praena-Fernández JM, et al. (2016) Lymphomagenesis-related gene expression in B cells from sustained virological responders with occult hepatitis C virus infection. *J Viral Hepat* 23: 606-613.
30. Youssef SS, Nasr AS, El Zanaty T, El Rawi RS, Mattar MM, et al. (2012) Prevalence of occult hepatitis C virus in Egyptian patients with chronic lymphoproliferative disorders. *Hepat Res Treat* 2012: 429784.
31. Romero-Gómez M, García-Romero D (2008) Hepatitis C: cryoglobulinemia and non-Hodgkin lymphoma. *Rev Esp Enferm Dig* 100: 164-170.
32. Roque-Cuéllar MC, Sánchez B, García-Lozano JR, Praena-Fernández JM, Márquez-Galán JL, et al. (2014) Hepatitis C virus-specific cellular immune response in sustained virological responders with viral persistence in peripheral blood mononuclear cells. *Liver International* 34: e80-e88.
33. Farahani M, Bokharaei-Salim F, Ghane M, Basi A, Meysami P, et al. (2013) Prevalence of occult hepatitis C virus infection in Iranian patients with lymphoproliferative disorders. *J Med Virol* 85: 235-240.
34. Kisiel E, Radkowski M, Pawelczyk A, Horban A, Stanczak J, et al. (2014) Seronegative hepatitis C virus infection in patients with lymphoproliferative disorders. *J Viral Hepat* 21: 424-429.