Published in final edited form as:

Gastroenterology. 2017 February ; 152(3): 550–553.e8. doi:10.1053/j.gastro.2016.11.002.

Detection of Occult Hepatitis C Virus Infection in Patients Who Achieved a Sustained Virologic Response to Direct-acting Antiviral Agents for Recurrent Infection After Liver Transplantation

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Abstract

Occult infection with the hepatitis C virus (HCV) is defined as the presence of the HCV genome in either liver tissue or peripheral blood monocytes (PBMCs), despite constant negative results

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Conflicts of Interest: The authors disclose no conflicts.

Authors contribution:

Study Design; JK, LC, KRJ, and TS.

Data Acquisition and Interpretation; SE, BB, LC, BK, TH, SC, GK, KRJ, JK, and TS.

Drafting of the Manuscript; SE, SW, JK, and TS.

Statistical Analysis; SE, BB, and TS.

Technical Support; ZF, BK, TH.

Study Supervision; JK and TS.

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from tests for HCV RNA in serum. We investigated whether patients who maintained a sustained virologic response 12 weeks after therapy (SVR12) with direct-acting antiviral (DAA) agents for recurrent HCV infection following liver transplantation had occult HCV infections. We performed a prospective study of 134 patients with recurrent HCV infection following liver transplantation who were treated with DAAs, with or without ribavirin, from 2014 through 2016 (129 patients achieved an SVR12). In more than 10% of the patients who achieved an SVR12 (n=14), serum levels of aminotransferases did not normalize during or after DAA therapy, or normalized transiently but then increased sharply after DAA therapy. Of these 14 patients, 9 were assessed for occult HCV infection by reverse transcription quantitative PCR. This analysis revealed that 55% of these patients (n=5) had an occult infection, with the detection of negative strand viral genome, indicating viral replication. These findings indicate the presence of occult HCV infection in some patients with abnormal levels of serum aminotransferases in spite of an SVR 12 to DAAs for HCV infection following liver transplantation.

Keywords

viral hibernation; treatment outcome; persistence; limit of detection

Recurrent HCV infection after OLT occurs in nearly all patients with viremia at the time of transplantation¹. DAA treatment for recurrent HCV infection is highly indicated as it may prevent graft failure^{1, 2}. At our institution, between the years of 2014 to 2016, a total of 134 cases of recurrent HCV infections were treated with interferon-free, DAA therapy with or without Ribavirin. During treatment, serum AST/ALT decreased rapidly, as did serum HCV-RNA in the majority of cases. Of those 134 cases, 129 patients achieved SVR12. The SVR12 patients were further subdivided into two groups (Table1): 1) biochemical remission (BR): serum aminotransferase values rapidly normalized during and after antiviral therapy as did serum HCV-RNA, and 2) biochemical non-responders (BNR): serum aminotransferases either flared after the end of therapy or did not improve in parallel with HCV-RNA reduction. Based on these classifications, 14 patients were included in the BNR.

Given the deregulated immunity due to concomitant immunosuppressant, we hypothesized that the post-OLT liver is susceptible to develop OCI, which may contribute to the abnormal aminotransferases seen after achieving SVR. Consequently, this study was designed to investigate the occurrence of OCI in these 14 cases of BNR. Of those 14 cases, 9 patients were enrolled upon obtaining informed consent under approved IRB. The majority of these 9 cases of BNR demonstrated a transient improvement of the serum aminotransferases during antiviral therapy in parallel with the reduction of serum HCV-RNA. However, the transaminase values flared shortly after the end of antiviral treatment (subjects 1, 3-5, 7-9) (Supplemental Figure1). The remaining subjects (subjects 2 and 6) exhibited either negligible improvement or worsening of the serum transaminases (Supplemental Figure1). These enrolled 9 subjects underwent liver biopsies in order to seek an explanation for the ongoing hepatocellular injury (Supplemental Figure1). A portion of liver tissue, serum, and PBMC were simultaneously collected and were subjected to HCV-genome detection by a high-sensitive Taqman RT-qPCR assay developed in our laboratory (Supplemental Figure2). The results revealed that HCV-RNA was detected in a total of 5 subjects (55%): in the liver

tissue of 4 subjects (44%), in the PBMC of two subjects (22%), and in both in 1 subject (Figure1). A grossly identical result was obtained with Abbott RealTime HCV viral load assay (Supplemental Figure3).

Given that HCV is a single-stranded (+) RNA virus, the detection of the negative (–) strand genome serves as a signature of HCV replication³. Subsequently, the liver and PBMC samples that were positive for OCI were subjected to a strand-specific RT-qPCR for the quantification of (+) and (–) strand HCV-genome. In general, the number of copies of (+) strand is 1-3 logs greater than (–) strand in HCV replicating liver tissue due to a higher demand for (+) strand as it is responsible for both viral protein translation and virion assembly^{4, 5}. A similar trend was observed in our study using the liver tissue of subjects with active viremia or in the in vitro cultured HCV replicating cells (Figure1). Of great interest, the OCI had a greater proportion of (–) strand, thus the ratio between (+) and (–) was much lower (Figure1). Accordingly, we speculate that the altered (+)/(–) strand ratio may, at least in part, result in the interference of virion assembly/budding and, thus results in the accumulation of HCV-RNA within the infected cells while it sheds only trace amounts of virion into the serum.

The genotype of SNPs near interleukin 28B (IL28B) gene at rs12979860 (IL28B-genotype) is known to have a great influence on the outcomes of HCV infection. Thus, we tested whether the IL28B-genotype is associated with the presence of OCI. The results revealed that all cases of OCI had the unfavorable genotype (TT) while the non-OCI subjects had either favorable (CC) or intermediate (CT) genotypes (Table1). In addition, we did not find any correlation between OCI and the IL28B-genotype of the grafted liver, indicating that the TT genotype of the host and not the donor, might be a contributing factor to the development of OCI.

Next, we found that the histopathology of only two of the four cases of OCI in the liver were consistent with “recurrent viral hepatitis C”. In addition, one subject who had OCI in PBMC, but not in the liver, was reported to have “recurrent viral hepatitis C” (Supplemental Table1). Furthermore, the histology of one subject who had OCI in the liver demonstrated features of acute cellular rejection and de novo autoimmune hepatitis. Taken together, we conclude that the histopathological evaluation does not serve as a predictor for OCI.

We found the presence of OCI in 5 out of 9 BNR subjects. At the same time, the causality of abnormal aminotransferases in the remaining cases remains elusive. Given concomitant immunosuppression, we speculate that other viral pathogens could be responsible for the abnormal AST/ALT and thus we screened all samples for Parvoviridae, HBV, HEV, and Herpesviruses⁶⁻¹⁰. The results found that one case of OCI was superinfected with Parvo-B19 and AAV3 although the contribution of these pathogens to the abnormal AST/ALT remains unclear (Subject 1) (Supplemental Table1).

The occurrence of OCI in the immunocompetent population remains controversial as it depends on the detection methods employed, ranging as high as 75%¹¹⁻¹³. This is the first study to address the occurrence of OCI in the immunocompromised host and we found a relatively high incidence of OCI in post-OLT, DAA-treated subjects with abnormal

aminotransferases. Of note, we did not find any correlation between the type and dose of immunosuppressant and the onset of OCI. Moreover, it remains elusive whether OCI can be the causality of these abnormal aminotransferases. This question can also be extended to consider the implication of OCI in PBMC alone in hepatic inflammation, such as in subject 3 (Figure1). These are important questions because up to 30 percent of patients who are infected with HCV have normal serum aminotransferases¹⁴. Moreover, a recent exciting observation with explanted livers treated with DAA suggested that the AST/ALT may not be a predictor of OCI¹⁵. To precisely address these questions, further investigations with normal AST/ALT subjects are necessary; however, the acquisition of such samples is hampered by the lack of justification for a liver biopsy. Another intriguing question that needs to be explored is the longitudinal outcomes of OCI and the identification of potential resistance-associated variants that may be associated with the development of OCI. In conclusion, our study results urge the utilization of tissue sample-based detection of HCV-genome, at least for those with abnormal aminotransferases, especially with the unfavorable IL28B genotype. For the cases of OCI with sustained abnormal aminotransferases, an additional course of antiviral therapy can be considered to preserve the longevity of the graft due to possible inflammation caused by occult HCV infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial support: This work was supported by funds from SC-CTSI Pilot Funding (TS and JAK), NIH USC RCLD pilot grant (5P30DK048522), NIH NIAAA (R21AA022751:TS), and NIH NIDDK (RO1DK101773:TS).

Abbreviations Footnote

HCV	hepatitis C virus
OLT	orthotopic liver transplant
DAAs	Direct-Acting Antivirals
HEV	hepatitis E virus
HSV	herpes simplex virus
EBV	Epstein-Barr virus
CMV	Cytomegalovirus
AAV	adeno-associated virus
AST	aspartate aminotransferase
ALT	alanine aminotransferase
SNPs	single nucleotide polymorphisms

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Figure 1. RT-qPCR analysis for the detection of HCV genome

The RNA extracted from the liver tissue or PBMC were subjected to one-step (left) or two-step strand- specific RT-qPCR (right). The number of copies of each strand is displayed as a percentage of the total abundance (right). *; The strand specific quantification was achieved only for (+) strand but not for (–) strand. Negative and positive control samples were obtained from alcoholic liver disease and active HCV infection patients or Huh7 SGR cells respectively.

Table 1

Characteristics of post-OLT recurrent HCV infection who achieved SVR with DAAs

Parameter		Biochemical Remission N = 115	Biochemical Non-Responder N = 9	
			Total	Presence of Occult HCV N = 5
				Absence of Occult HCV N = 4
Age (years), median		60	58	57.6
Male, n (%)		80 (70)	6 (66.7)	3 (60)
Ethnicity category, n (%)				3 (75)
Hispanic		69 (60)	5 (55.6)	3 (60)
White		35 (30.4)	2 (22.2)	1 (20)
Asian		11 (9.6)	0	0
African-American		0	2 (22.2)	1 (20)
Pre-treatment serum HCV RNA (copies/mL), median		2,820,000	1,044,309	766,038
Pre-treatment serum HCV RNA (copies/mL), mean		6,260,779	10,335,730	3,467,414
Genotype category, n (%)				18,577,709
1a		66 (57.4)	5 (55.6)	2 (40)
1b		25 (21.7)	1 (11.1)	1 (20)
2		8 (7.0)	0	0
3a		13 (11.3)	1 (11.1)	0
4		1 (0.9)	2 (22.2)	2 (40)
6		3 (2.6)	0	0
Laboratory values *				
Platelet count (K/cumm), mean (normal 141-401)		151.2 ± 60.3	125.6 ± 54.1	99.4 ± 27.4
ALT (IU/L), mean (normal 14-54)		25.6 ± 15.7	187.7 ± 85.1	201.4 ± 37.9
AST (IU/L), mean (normal 15-41)		25.5 ± 23.9	173.5 ± 78.8	198.6 ± 58.6
ALP (IU/L), mean (normal 38-126)		131.1 ± 147.6	119.3 ± 59.6	116.6 ± 65.5
TB (mg/dL), mean (normal 0-1)		0.8 ± 0.8	1.8 ± 3.1	2.52 ± 3.8
Treatment regimen category, n (%)				0.8 ± 0.3
LDV/SOF	12 weeks	12 (10.4)	3 (33.3)	1 (20)
LDV/SOF + RBV	12 weeks	26 (22.6)	3 (33.3)	2 (40)
SMV + SOF	12 weeks	39 (33.9)	1 (11.1)	1 (20)
DCV + SOF	12 weeks	4 (3.5)	0	0
DCV + SOF + RBV	12 weeks	2 (1.7)	0	0
SOF + RBV	12 weeks	3 (2.6)	0	0
SOF + RBV	16 weeks	1 (0.9)	0	0
SOF + RBV	20 weeks	0	1 (11.1)	1 (20)
DCV + SOF	20 weeks	2(1.7)	0	0
LDV/SOF	24 weeks	11 (9.6)	0	0
SIM + SOF	24 weeks	1 (0.9)	0	0
DCV + SOF	24 weeks	8 (7)	1 (11.1)	0
				1 (25)

Parameter			Biochemical Non-Responder N = 9		
			Total	Presence of Occult HCV N = 5	Absence of Occult HCV N = 4
SOF + RBV	24 weeks	5 (4.3)	0	0	0
SIM + SOF	32 weeks	1 (0.9)	0	0	0
IL28B Genotype (PBMC), n (%)					
CC		n/a	1	0	1 (25)
CT		n/a	3	0	3 (75)
TT		n/a	5	5 (100)	0
IL28B Genotype (grafted liver), n (%)					
CC		n/a	9	0	0
CT		n/a	0	5 (100)	4 (100)
TT		n/a	0	0	0

LDV = ledipasvir, SOF = sofosbuvir, RBV = ribavirin, SMV = simeprevir, DCV = daclatasvir.

* : Peak value after achieving SVR12.