HCV Antibody Quantitative Levels in Liver Transplant Patients: Do They Have Any Relevance in Clinical Practice?

Ashok Jain,[1] Marilyn Menegus,[2] Ravi Mohanka,[1] Mark Orloff,[1] Peter Abt,[1] Parvez Mantry,[3] Adel Bozorgzadeh[1]

Objectives: **Hepatitis C virus (HCV) is not directly cytopathic to the hepatocytes; however, host immune response against the virus does cause hepatic injury. Production of the HCV antibody is a host immune response to a viral antigen. The currently used HCV antibody assay is a qualitative, not quantitative, assessment. In this study, we sought to quantitatively estimate HCV antibody levels in patients who had undergone liver transplantations at the University of Rochester Medical Center, Rochester, New York, and correlate these levels with HCV RNA viral load, genotype, severity of recurrence, and anti-HCV treatment.**

Materials and Methods: **From 39 liver transplantation patients, we obtained 141 blood samples for quantitative HCV RNA to measure HCV antibody levels quantitatively.**

Results**: Most antibody levels were within a narrow range with a mean of 32.9 ± 5.1. Samples with undetectable RNA had a mean antibody level of 31.4 ± 8.0, and samples with a positive RNA had mean level of 33.0 ± 4.6. The mean antibody levels were significantly higher for patients with genotype 1 (n = 33) compared with those with genotype 2 (n = 5) (33.2 vs 29.1; *P* = .007). No correlation was found between antibody levels and severity of hepatic injury with regard to hepatitis activity index or fibrosis score. Six patients with no response to anti-HCV treatment had no change in their mean antibody levels (33.7 vs 34.5). Ten patients who responded to anti-HCV therapy had lower mean levels after therapy, but the changes were not significant (34.2 vs 30.4).**

Conclusions**: Antibody levels in this study did not correlate with viral load or hepatic injury. However, genotype-2 patients had significantly lower levels compared with genotype-1 patients, and patients who responded to anti-HCV therapy demonstrated decreased antibody levels.**

Key words: *Hepatitis C recurrence, Liver transplant, Antibody levels*

Hepatitis C virus (HCV) infection is the most common indication for liver transplantation (LTx) in the United States. Recurrence of HCV infection is almost universal after LTx. With currently available anti-HCV therapy, less than 35% of patients achieve a sustained viral response [1-3]. Some of these patients develop slow and mild recurrent hepatitis, while others have disease with a more aggressive course [4]. Viral load is not predictive of severity of recurrence; however, genotype has been found to be predictive of the severity of recurrence, progression of disease, and response to anti-HCV therapy [5-11]. Hepatitis C virus is not a cytopathic virus and does not cause hepatocyte cell injury itself. Hepatic injury associated with hepatitis C viral infection is said to be due to the host immune response [12-15]. One of the host immune responses to HCV is the production of a specific antibody directed against the virus. In a vast majority of infections, similar antibodies provide immunity for the host from subsequent damage from the same pathogen. Unfortunately, in the case of HCV, although the antibody is diagnostic of infection, it is not protective to hepatocytes [16, 17]. Currently, HCV antibody measurements are reported qualitatively as

University of Rochester Medical Center, Departments of [1]Surgery (Division of Transplantation), [2]Microbiology, and [3]Gastroenterology and Hepatology, Rochester, NY, USA

Acknowledgments: *The study was supported in part by Roche Laboratories and in part by internal departmental funding. Data presented here were partly presented at the American Association for the Study of Liver Diseases, March 4-5, 2005, Chicago, Ill, USA, and at the 11th Meeting of the International Liver Transplantation Society, July 2005, Los Angeles, Calif, USA. Additionally, we acknowledge the help of Dr. Manoj Purohit in finalizing the manuscript.*

Address reprint requests to: *Ashok Jain, MD, University of Rochester Medical Center Division of Transplantation, Department of Surgery, Box SURG, 601 Elmwood Avenue, Rochester, NY 14642 USA*

Phone*: 00 (585) 275 2924* ***Fax:*** *00 (585) 506 0054* ***E-mail:*** *ashok_jain@urmc.rochester.edu*

Experimental and Clinical Transplantation (2006) 1: 475-480

either positive or negative. Because hepatic injury is due to the host immune response, we felt that quantitative evaluation of the HCV antibody might predict the severity of hepatic injury and the response to anti-HCV therapy, and that it may be clinically relevant. Additionally, it may be particularly important in liver transplant patients who are immunosuppressed and may provide a permissive environment for the virus to multiply in the face of an altered immune response. We hypothesized that quantitative evaluation of the HCV antibody may have more clinical significance in these patients. We therefore sought to quantitatively estimate the amount of HCV antibody and correlate this with HCV viral load, genotype, hepatic injury, response to anti-HCV therapy, and presence of cryoglobulin in liver transplant patients infected with HCV.

Materials and Methods

Leftover blood samples originally collected to quantify hepatitis C viral load were used for quantitative estimation of HCV antibody levels by using a VITROS automated hepatitis assay (Ortho-Clinical Diagnostics, Raritan, New Jersey, USA) [18]. Antibody levels were estimated using the signal-to-cutoff (S/C) ratio. All LTx patients with HCV infection who attended the LTx clinic at the University of Rochester Medical Center, Rochester, New York, were given an opportunity to participate in this study (which had been approved by our institutional review board); the study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration. Leftover blood samples from consenting patients that had been collected for HCV RNA viral load estimations, as part of their routine follow-up, were used to quantitatively estimate HCV antibody levels.

In all, 141 samples from 39 patients with chronic HCV infection who underwent LTx between December 1996 and February 2004 (10 living-donor liver transplants, 29 deceased-donor liver transplants) were available for analysis. Five samples from 4 patients were prior to LTx, and the remaining samples were after LTx. Blood samples that were used for the study had been collected between October 2001 and December 2004. Mean age at the time of LTx was 51.76 ± 7.0 years. There were 9 women (23.1%) and 30 men (76.9%). Six patients in this study had genotype 1 with an undetermined subtype, 14 had subtype 1a, 13 had subtype 1b, 4 had subtype 2b, and 1 had genotype 2 with an undetermined subtype. In 1 patient, the genotype was not performed before LTx, and viral loads were undetectable after posttransplant treatment.

Thirty-two of 39 patients were given combination therapy with pegylated interferon 2b or 2a and ribavirin for the treatment of HCV infection. Those who did not clear the virus from their plasma (< 50 IU/mL), as detected by polymerase chain reaction (PCR), after 1 year of treatment were labeled *nonresponders.* Those who did clear the virus after starting treatment were labeled *responders.* Patients who could not complete the treatment, but achieved a 2 log reduction in viral load after starting treatment, also were considered responders for the purpose of analysis. HCV antibody levels were correlated with quantitative HCV RNA, HCV genotype and subtypes, hepatitis activity index (HAI), fibrosis score, and response to anti-HCV therapy.

Statistical analyses

The results are expressed as means ± standard deviation. Differences in mean antibody levels were analyzed using the Student *t* test and one-way analysis of variance. Differences in mean antibody levels before and after treatment were compared using the paired *t* test. Statistical analyses were performed with SPSS software (Statistical Package for the Social Sciences, version 13.0, SSPS Inc, Chicago, Ill, USA). Values for *P* less than .05 were considered significant.

Results

The overall antibody levels had a relatively narrow range from 24.8 to 41.2 except for 4 values, which were 7.61, 13.3, 13.7, and 17.8. The overall mean was 32.9 ± 5.1 (Figure 1).

Of 141 samples that were for analyzed for quantitative HCV RNA, the upper endpoint of detection was 0.5 million IU/mL for 21 samples and were reported as greater than 0.5 million IU/mL. The actual viral load in these samples could not be accurately determined. In the remaining 120 samples, there was no apparent correlation between HCV antibody level and HCV viral load (Figure 1).

In 12 patients (23 samples) with undetectable viral load (< 50 IU/mL), the mean antibody level was 31.4 ± 8.0. The mean antibody level for patients with a detectable viral load (> 50 IU/mL) was 33.2 ± 4.3; this difference was not significant ($P = .292$). Also, the mean antibody levels for groups based on HCV RNA levels of ≤ 1 million, > 1 million but ≤ 5 million, > 5

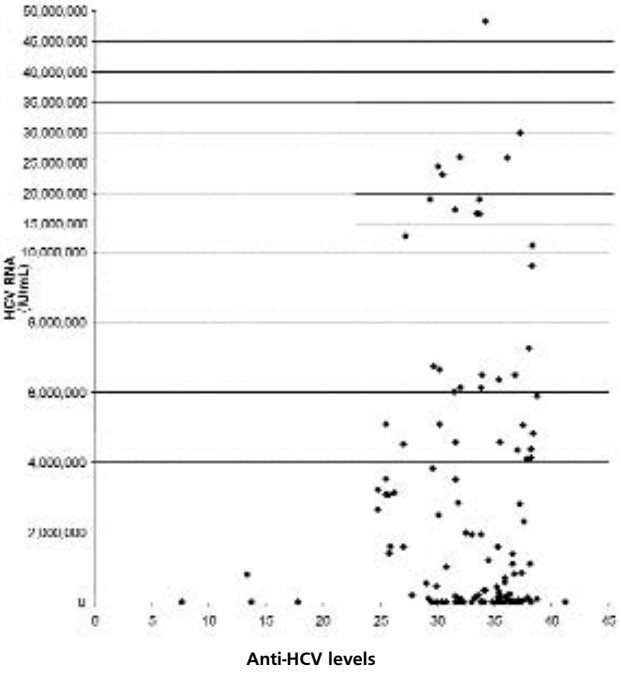

Figure 1. Scattergram; HCV RNA (IU/mL) against anti-HCV levels. Please note that the scale has been compressed from 10,000,000 upwards.

million, or > 0.5 million (upper endpoint) were 34.2 ± 4.4, 32.0 ± 5.0, 33.2 ± 3.6, and 33.1 ± 3.5, respectively (Table 1).

HCV genotype and HCV antibody levels

Genotype information was available for 38 of 39 patients. One patient cleared the virus before entering the study, and genotype was not determined prior to anti-HCV treatment. Of 38 patients, 33 (86.8%) had genotype 1, of whom 6 (15.8%) had an undetermined subtype, 14 (36.8%) had subtype 1a, and 13 (34.2%) had subtype 1b. Their antibody levels were 34.8 ± 3.37 (genotype 1: undetermined subtype), 34.6 ± 3.0 (genotype 1a), and 32.4 ± 4.24 (genotype 1b), respectively. The overall mean HCV antibody level for genotype-1 patients (including all subtypes) was 33.2 ± 5.08 (Table 2). The remaining 5 patients had genotype 2, of which 1 patient had an undetermined subtype, and 4 patients (10.2%) had subtype 2b. The mean antibody levels for these patients were 31.6 ± 1.6 (genotype 2), 28.2 ± 4.53 (genotype 2b), and 29.1 ± 4.2 (overall genotype 2) (Table 2). Overall mean HCV antibody levels were significantly lower for HCV genotype 2 than they were for genotype 1 (P = .007).

Hepatic injury

Thirty-nine patients underwent 47 biopsies during the study. The mean antibody titres were compared

Table 1. HCV RNA and antibody levels*

	Patients	Observations	Mean antibody levels
HCV RNA			
Undetectable (< 50 IU/mL)	12	23	31.4 ± 8.0
Detectable (> 50 IU/mL)	38	118	33.2 ± 4.3
≤ 1 million	20	38	34.2 ± 4.4
> 1 million to ≤ 5 million	18	31	32.0 ± 5.0
> 5 million	15	28	33.2 ± 3.6
> 0.5 million†	19	21	33.1 ± 3.5
Hepatic injury			
HAI			
≤ 3	14	18	34.3 ± 4.0
> 3 to ≤ 6	17	25	34.7 ± 4.2
> 6	4	4	34.7 ± 2.5
Fibrosis			
0	20	30	34.1 ± 3.9
≥ 1	12	16	35.4 ± 4.1
Total injury			
≤ 3	13	14	33.3 ± 3.8
> 3 to ≤ 6	15	20	35.0 ± 4.1
> 6 to ≤ 10	9	11	35.1 ± 4.4
> 10	2	2	34.1 ± 3.2

*Some patients had more than 1 sample with values in the same category
†These samples not analyzed up to the endpoint

Table 2. Mean antibody levels grouped by genotype

Genotype	1			2		*P*
No. of samples (patients)	125 (33)			12 (5)		
Antibody levels	33.2 ± 5.08			29.1 ± 4.22		.007
Subtype	**U**	**1a**	**1b**	**U**	**2b**	
No. of samples (patients)	25 (6)	48 (14)	52 (13)	3 (1)	9 (4)	
Antibody levels	34.9 ± 3.3	34.2 ± 4.3	31.6 ± 6.0	31.6 ± 1.6	28.2 ± 4.5	

U, Undetermined subtype within major genotype group

in relation to hepatitis activity index (HAI: ≤ 3, 3-6, > 6), fibrosis score (0, ≥ 1), and total hepatic injury (Table 1). Mean antibody levels for HAI of ≤ 3 (n = 18), > 3 and ≤ 6 (n = 25), and > 6 (n = 4) were almost identical [34.3 ± 4.0, 34.7 ± 4.2, and 34.7 ± 2.5, respectively (P = .967)]. Similarly, patients with fibrosis scores of 0 (n = 30), or ≥ 1 (n = 17) had almost identical mean antibody levels [34.1 ± 4 and 34.7 ± 2, respectively (P = .513)]. Mean HCV antibody levels for total hepatic injury (combined HAI and fibrosis scores) ≤ 3 (n = 14), > 3 and ≤ 6 (n = 20), > 6 and ≤ 10 (n = 11), or > 10 (n = 2), were also very similar [33.3 ± 3.8, 35.0 ± 4.1, 35.1 ± 4.4, and 34.1 ± 3.2, respectively (P = .790)]. Hepatic activity index, fibrosis score and total hepatic injury against anti-HCV level are shown in Figure 2.

Antibody levels before and after LTx

In 4 patients, antibody levels were available both before and after LTx (Figure 3A). The mean antibody levels before and after transplant in these patients

Table 3. Antibody levels before and after interferon (IFN) treatment

	Before IFN	After IFN	*P*
All treated (n = 16)	34.0 ± 3.7	32.0 ± 9.0	.279
Responders (n = 10)	34.2 ± 4.4	30.4 ± 11.0	.207
Nonresponders (n = 6)	33.7 ± 2.6	34.5 ± 3.8	.319

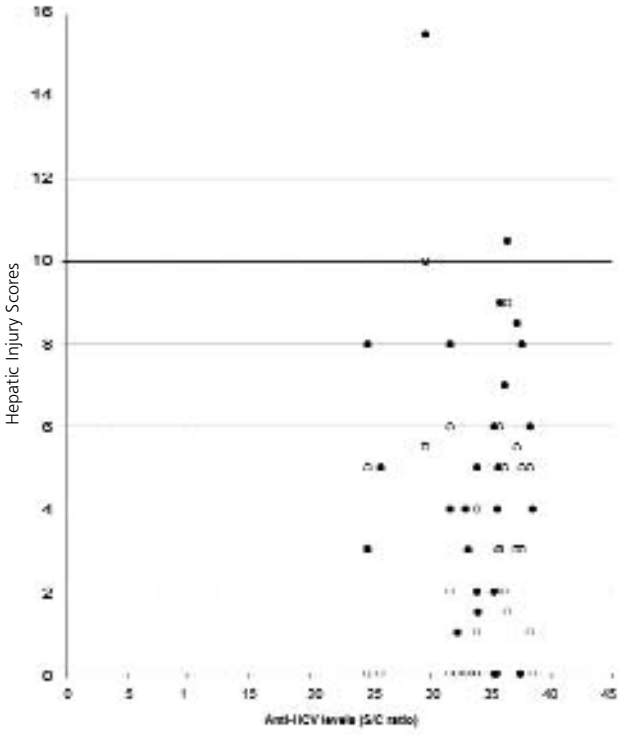

Figure 2. Hepatic activity index (HAI) score (○), Hepatic fibrosis score (□) and total hepatic injury (HAI + fibrosis) score (●) against HCV antibody levels (S/C ratio).

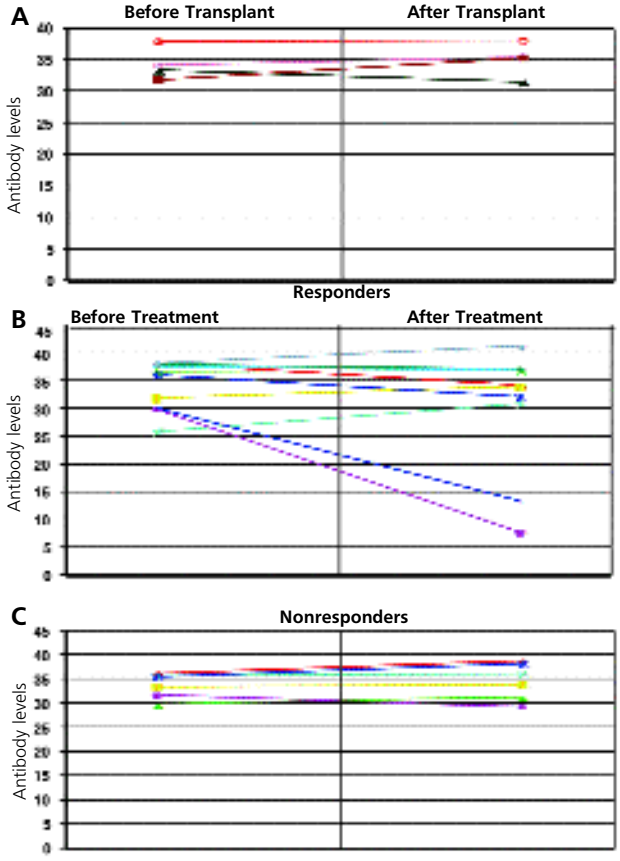

Figure 3. **A**: HCV antibody level before and after liver transplant (n = 4), 1 patient received anti-HCV treatment before LTx. **B**: LTx HCV antibody level before and after acute HCV treatment. Responders (n = 10) (refer to text). **C**: LTx antibody level before and after anti-HCV treatment nonresponders (n = 6) (refer to text).

were 33.9 ± 1.1 and 34.8 ± 1.1 respectively. The difference was not statistically significant (P = .323).

Antibody levels before and after interferon and ribavirin treatment

Out of 32 patients who received pegylated interferon and ribavirin combination therapy, 18 completed the therapy for 1 year; the other 14 did not complete treatment because of adverse effects. Thirteen of the 32 patients had a response of either clearing the virus or a 2 log reduction in viral load. Of 32 treated patients, antibody levels were available both before and after interferon and ribavirin therapy in 16. Ten of these patients responded to treatment and 6 did not. Of the 10 responders, 5 had mild to moderate decreases in antibody levels, and the remaining 5 had either the same, or a mild rise in, level. Overall mean antibody level in these 10 responders was 34.2 ± 4.4 before anti-HCV therapy, which decreased to 30.4 ± 11.0 (Figure 3B, Table 3, P = .207). In 6 patients who did not respond to anti-HCV treatment, the mean antibody level was 33.7 ± 2.6 and did not change much after completion of therapy 34.5 ± 3.8 (P = .319) (Figure 3C).

Antibody levels and cryoglobulinemia

Of 39 patients, cryoglobulin status was examined in 34. Of these, 31 had no detectable cryoglobulin, while 3 had trace to moderate amounts of cryoglobulin. Patients with positive cryoglobulin had a mean antibody level of 36.8 ± 3.1 while those with negative cryoglobulin had a mean antibody level of 32.4 ± 4.4.

Discussion

Until our ability to detect the HCV antibody, the disease was often described as non-A non-B hepatitis. In early 1990s, the HCV antibody was first detected using the ELISA-1 assay (Abbott Laboratories. Abbott Park, Illinois, USA). Later, the ELISA-2 assay was developed, which detected more epitopes of viral

antigen and subsequently, immunoblot assays RIBA-1 and then RIBA-2 techniques were introduced to improve the sensitivity and specificity [19-24]. It became an important test to screen potential blood donors and organ donors to reduce transmission of the HCV virus. It was a major landmark in prevention of disease transmission while providing life-saving treatments. At the same time, development of PCR techniques enabled detection of HCV with almost 100% accuracy. Further developments enabled us to quantify the number of virus copies in peripheral blood. This development rendered antibody testing useful only for screening purposes, and patients testing positive for the antibody underwent PCR for confirmation and quantification of the virus. This development halted further improvements in methods of antibody testing. This interest was recently renewed owing to the possibility of using the HCV antibody for passive immunity against HCV infection, based on the success of hyperimmune hepatitis B globulin (HBIg). Initial results with HCV immune globulin in chimpanzees were encouraging [25, 26]. However, a randomized trial with human hepatitis-C–immune globulin has failed to provide any benefits in humans [27].

Although the HCV antibody does not provide any protection, it is still a host immune response to infection. It is well known that hepatic damage from HCV varies between subjects. The degree of damage is believed to be due to host response rather than the any direct effect of the virus [15]. The present study was conceptualized to determine if HCV antibody levels correlate with the response to anti-HCV treatment, severity of disease, or hepatic injury. We already know that the immune response in chronic HCV infection is a T-cell–mediated response and therefore, an antibody-mediated response has only limited implications. The primary intent of this study was to examine HCV-infected LTx patients and determine the effect(s) of known predictors of severity and progression of disease (HCV RNA viral load, genotype, and HAI) on fibrosis score on liver biopsy, response to anti-HCV therapy, and effects of immunosuppressive drugs on HCV antibody levels.

Unfortunately, unlike quantitative HCV RNA, the HCV antibody levels exist in a very narrow range in most subjects, and the differences between various groups of known predictors of severity and progression of disease (HCV RNA viral load, genotype, and HAI) studied were not very prominent. Nonetheless, several interesting observations may be taken from this study that may have potential clinical implications and could be further explored. Genotype 2 HCV (mild type) and its subtypes have lower antibody levels than do genotype 1 HCV (aggressive type) and its subtypes. Patients with an undetectable HCV viral load have lower antibody levels compared with those with a measurable viral load, and some of the patients who responded to treatment had a decrease in their antibody levels after anti-HCV therapy. At the same time in this study, antibody levels did not correlate with a higher HCV viral load, hepatic injury, or immunosuppression.

It may be hypothesized from this study that patients with genotype 2 have a more robust T-cell response as compared with patients with genotype 1; therefore, these patients may have a less pronounced B-cell response, and therefore, lower antibody levels. Unfortunately, in this study, we did not have any subjects with genotype 3 for comparison. Further, sequential quantitative antibody level estimations in large populations with longer follow-ups may shed light on these subtle differences. Perhaps a study to access the T-cell response of genotype-2 patients after transplant would be warranted. Nonetheless, it was disappointing not to find a correlation between quantitative viral load and hepatic injury to HCV antibody levels.

Conclusions

HCV antibody levels were distributed within a narrow range in most of our observations. There were no significant differences in antibody levels with regard to viral load, severity of hepatic injury, immunosuppression, or anti-HCV therapy. However, genotype-2 patients had significantly lower antibody levels than did genotype-1 patients. Patients with undetectable viral loads had lower antibody levels compared with those patients with a detectable viral load. Also, some of the patients who responded to anti-HCV treatment had lower antibody levels after therapy. Prospective sequential and multiple measurements of antibody levels in large populations are necessary to establish the utility of the test and its relevance in clinical practice.

References

1. Abdelmalek MF, Firpi RJ, Soldevila-Pico C, Reed AI, Hemming AW, Liu C, et al. Sustained viral response to interferon and ribavirin in liver transplant recipients with recurrent hepatitis C. Liver Transpl 2004; 10 (2): 199-207

2. Mukherjee S, Lyden E, McCashland TM, Schafer DF. Interferon alpha 2b and ribavirin for the treatment of recurrent hepatitis C after liver transplantation: cohort study of 38 patients. J Gastroenterol Hepatol 2005; 20 (2): 198-203
3. Neff GW, O'Brien CB, Cirocco R, Montalbano M, de Medina M, Ruiz P, et al. Prediction of sustained virological response in liver transplant recipients with recurrent hepatitis C virus following combination pegylated interferon alfa-2b and ribavirin therapy using tissue hepatitis C virus reverse transcriptase polymerase chain reaction testing. Liver Transpl 2004; 10 (5): 595-598
4. Bonino F, Brunetto MR, Negro F, Baldi M, Saracco G, Abate ML, et al. Hepatitis C virus infection and disease. Diagnostic problems. J Hepatol 1993; 17 Suppl 3: S78-S82
5. Charlton M, Wiesner R. Natural history and management of hepatitis C infection after liver transplantation. Semin Liver Dis 2004; 24 Suppl 2: 79-88
6. Lopez-Labrador FX, Berenguer M, Sempere A, Prieto M, Sirera R, Gonzalez-Molina A, et al. Genetic variability of hepatitis C virus NS3 protein in human leukocyte antigen-A2 liver transplant recipients with recurrent hepatitis C. Liver Transpl 2004; 10 (2): 217-227.
7. Rodriguez-Luna H, Khatib A, Sharma P, De Petris G, Williams JW, Ortiz J, et al. Treatment of recurrent hepatitis C infection after liver transplantation with combination of pegylated interferon alpha2b and ribavirin: an open-label series. Transplantation 2004; 77 (2): 190-194
8. Yedibela S, Schuppan D, Muller V, Schellerer V, Tannapfel A, Hohenberger W, Meyer T. Successful treatment of hepatitis C reinfection with interferon-alpha2b and ribavirin after liver transplantation. Liver Int 2005; 25 (4): 717-722
9. Booth JC, Foster GR, Kumar U, Galassini R, Goldin RD, Brown JL, Thomas HC. Chronic hepatitis C virus infections: predictive value of genotype and level of viremia on disease progression and response to interferon alpha. Gut 1995; 36 (3): 427-432
10. Kobayashi M, Tanaka E, Sodeyama T, Urushihara A, Matsumoto A, Kiyosawa K. The natural course of chronic hepatitis C: a comparison between patients with genotypes 1 and 2 hepatitis C viruses. Hepatology 1996; 23 (4): 695-699
11. Tassopoulos NC, Papatheodoridis GV, Katsoulidou A, Delladetsima JK, Sypsa V, Touloumi G, et al. Factors associated with severity and disease progression in chronic hepatitis C. Hepatogastroenterology 1998; 45 (23): 1678-1683
12. Bach N, Thung SN, Schaffner F. The histological features of chronic hepatitis C and autoimmune chronic hepatitis: a comparative analysis. Hepatology 1992; 15 (4): 572-577
13. Fukumoto T, Berg T, Ku Y, Bechstein WO, Knoop M, Lemmens HP, et al. Viral dynamics of hepatitis C early after orthotopic liver transplantation: evidence for rapid turnover of serum virions. Hepatology 1996; 24 (6): 1351-1354
14. Lefkowitch JH, Schiff ER, Davis GL, Perrillo RP, Lindsay K, Bodenheimer HC Jr, et al. Pathological diagnosis of chronic hepatitis C: a multicenter comparative study with chronic hepatitis B. The Hepatitis Interventional Therapy Group. Gastroenterology 1993; 104 (2): 595-603
15. Molmenti EP, Klintmalm GB. Hepatitis C recurrence after liver transplantation. Liver Transpl 2000; 6 (4): 413-414
16. Diepolder HM, Gerlach JT, Zachoval R, Hoffmann RM, Jung MC, Wierenga EA, et al. Immunodominant CD4+ T-cell epitope within nonstructural protein 3 in acute hepatitis C virus infection. J Virol 1997; 71 (8): 6011-6019
17. Diepolder HM, Zachoval R, Hoffmann RM, Wierenga EA, Santantonio T, Jung MC, et al. Possible mechanism involving T-lymphocyte response to non-structural protein 3 in viral clearance in acute hepatitis C virus infection. Lancet 1995; 346 (8981): 1006-1007
18. Oethinger M, Mayo DR, Falcone J, Barua PK, Griffith BP. Efficiency of the ortho VITROS assay for detection of hepatitis C virus-specific antibodies increased by elimination of supplemental testing of samples with very low sample-to-cutoff ratios. J Clin Microbiol 2005; 43 (5): 2477-2480
19. Aoki SK, Kuramoto IK, Anderson S, Schoening V, Rodriguez R, Fernando L, et al. Evidence that use of a second-generation hepatitis C antibody assay prevents additional cases of transfusion-transmitted hepatitis. J Viral Hepat 1994; 1 (1): 73-77
20. Baath L, Widell A, Nordenfelt E. A comparison between one first generation and three second generation anti-HCV ELISAs: an investigation in high- and low-risk subjects in correlation with recombinant immunoblot assay and polymerase chain reaction. J Virol Methods 1992; 40 (3): 287-296
21. Leahy D, Kink J, Byrne R, Shah D, Preisel-Simmons B, Laska S, et al. Improved serologic detection of hepatitis C virus with a paramagnetic microparticle assay using multiple antigenic sequences. Transfusion 1992; 32 (6): 548-553
22. Lee S, McHutchinson J, Francis B, DiNello R, Polito A, Quan S, Nelles M. Improved detection of antibodies to hepatitis C virus using a second generation ELISA. Adv Exp Med Biol 1992; 312: 183-189
23. Mitchel LS, Jeffers LJ, Reddy KR, Cheinquer H, Coelho-Little E, Moreda R, et al. Detection of hepatitis C virus antibody by first and second generation assays and polymerase chain reaction in patients with autoimmune chronic active hepatitis types I, II, and III. Am J Gastroenterol 1993; 88 (7): 1027-1034
24. Rowan BP, Smith A, Gleeson D, Hunt LP, Warnes TW. Hepatitis C virus in autoimmune liver disease in the UK: aetiological agent or artefact? Gut 1994; 35 (4): 542-546
25. Farci P, Alter HJ, Wong DC, Miller RH, Govindarajan S, Engle R, et al. Prevention of hepatitis C virus infection in chimpanzees after antibody-mediated in vitro neutralization. Proc Natl Acad Sci USA 1994; 91 (16): 7792-7796
26. Farci P, Shimoda A, Wong D, Cabezon T, De Gioannis D, Strazzera A, et al. Prevention of hepatitis C virus infection in chimpanzees by hyperimmune serum against the hypervariable region 1 of the envelope 2 protein. Proc Natl Acad Sci USA 1996; 93 (26): 15394-15399
27. Davis GL, Nelson DR, Terrault N, Pruett TL, Schiano TD, Fletcher CV, et al. A randomized, open-label study to evaluate the safety and pharmacokinetics of human hepatitis C immune globulin (Civacir) in liver transplant recipients. Liver Transpl 2005; 11 (8): 941-949