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Hepatitis C virus Genotypes and reinfection of the graft during long-term follow-up in 35 liver transplant recipients

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Abstract To understand the clinical outcome of hepatitis C virus (HCV) recurrence, data from 35 liver transplant recipients who survived more than 6 months were reviewed. The presence of HCV-RNA was evaluated and genotyping was performed. On the basis of alanine aminotransferase (ALT) levels, patients were sorted into four groups. In 20 patients, a chronic elevation in ALT was found; HCV-RNA detection was positive in 17/17 and the following genotypes were found in 15 of them: 1b in ten patients, 2a in four patients, and 3a in one patient. In 11 patients, ALT levels remained normal throughout follow-up; in nine of them HCV-RNA was positive; HCV genotyping was available in eight patients and identified type 1b in two, type 2a in five, and type 3a in another patient. In two patients, ALT fluctuated above and below the upper limits of normality; type 1b HCV-RNA was found in one of them. In two patients, after an initial period of normality, ALT levels showed an abrupt rise; HCV-RNA was positive and type 1b was identified in both patients. Eight patients developed HCV-related deep jaundice and three of them spontaneously recovered. Progressive hepatic injury occurred in eight patients, six with chronic ALT elevation and two showing a late ALT elevation; genotype 1b was present in seven patients while in one, genotype 3a was found; sub-acute graft failure developed in five of them, leading to death in two and retransplantation in the others; the other three patients are alive with recurrent overt cirrhosis. The 1, 3, and 5 year actuarial survivals were 89 %, 79 %, and 63 % respectively. The 1, 3, and 5 year actuarial risks of progressive graft damage were 6 %, 7 %, and 15 %, respectively. In conclusion, HCV reinfection causes a slow decrease in the long-term patients' survival. Persistent elevation of ALT is more frequently observed in patients with genotype 1b infection.

Key words Liver transplantation · Hepatitis C virus · Polymerase chain reaction

Introduction

Liver cirrhosis in patients with hepatitis C virus (HCV) infection is a major indication for orthotopic liver transplantation (OLT) [15, 26]. HCV infection is responsible for a large number of postoperative graft impairments [7, 20]. In pretransplant HCV-positive recipients, a higher risk of disease is dependent from the primary infection because, differently from hepatitis B virus (HBV) disease, no prophylaxis is available.

The prevalence and the clinical course of HCV infection after liver transplantation is still not well established [1]. This is partly due to the lack of simple and valid tools for the diagnosis of HCV disease: HCV anti-

body (anti-HCV) testing seems to be of little use in the follow-up of pretransplant infected patients [7]. Direct detection in serum of the HCV genome (HCV-RNA) by the polymerase chain reaction (PCR) faces an array of technical problems that still prevent it from becoming a routine procedure [19]. HCV-RNA detection with viral genome typing [2, 7] and viremia quantification [4, 8, 17] are currently the only methods that can be used to study the course of HCV infection.

The aim of this retrospective study is to evaluate the role of HCV genotypes in the natural posttransplant history of pretransplant infected recipients and their different impact in longterm patients' and grafts' survival.

Patients and methods

Up to 30 September 1995, 284 liver transplants were performed at our center. HBsAg negative, IgM anti-HBcAg negative patients with a post-transplant follow-up of at least 6 months were selected from our series. Immunosuppressive treatment was based on administration of cyclosporine A or FK506, and low doses of prednisone and azathioprine, when possible on the basis of the white cell count. Administration of steroids was gradually tapered and was finally withdrawn during follow-up. Liver function tests were regularly determined over posttransplant time. Serum alanine aminotransferase (ALT) levels were assumed as expression of functional hepatic injury and patients were sorted according to the course of ALT mean monthly values. In order to enhance the specificity of ALT variations secondary to HCV infection, ALT levels were analyzed from the begining of the second postoperative month to the subsequent follow-up. Therefore, ALT variations due to ischemic or rejection injuries, which more frequently occur in the first postoperative month, were ignored. Functional graft impairment was defined as persistent when abnormal ALT levels lasted longer than 3 months. In the presence of biochemical dysfunction, both biliary and vascular technical complications were ruled out by appropriate diagnostic imaging procedures. Liver biopsy samples, when available, were evaluated for hepatitis. The histological diagnosis of hepatitis required the presence of portal inflammation, lobular mononuclear cell infiltration, and hepatocellular necrosis; the appearance of intralobular bridging fibrosis defined an active evolution. In order to establish the etiology of the allograft damage, causes of hepatitis other than HCV were excluded by serial analysis of patients' serum samples for HBV markers, P65 cytomegalovirus (CMV) antigen and antibodies against CMV, hepatitis A, Epstein-Barr, herpes simplex and herpes zoster viruses. Each liver sample was fixed in buffered formalin and routinely processed. Staining for HBV and in situ hybridization for CMV were performed to definitively rule out these infections. The presence of either histological hepatitis or persistent liver dysfunction together with HCV-RNA positivity and no evidence of other known causes of hepatitis was defined as HCV hepatitis. Usually the diagnosis of viral hepatitis led to a rapid reduction or withdrawal of steroids, while no antiviral therapy was undertaken.

Recipients' sera were tested using a second- or third-generation anti-HCV enzyme-linked immunosorbent assay (ELISA); supplementary testing with a second- or third-generation recombinant immunoblotting assay (RIBA) was performed in all ELISA-positive cases. Both anti-HCV assays were carried out according to the manufacturers' instructions, as described elsewhere [3]. All posttransplant sera were stored at −20 °C before being tested for periods of 1–12 months. RNA extraction was performed with the acid guanidinium thiocyanate-phenol-chloroform method [5] with the addition of 1 μg of glycogen as a carrier prior to isopropanol precipitation. Complementary DNA (cDNA) synthesis (Promega, Madison, USA) was primed from the core region of HCV-RNA. A 267-bp fragment from the 5′ untranslated region of the viral genome was amplified by heminested polymerase chain reaction (HNPCR) after cDNA synthesis by reverse transcription (RT). We employed a modification of methods previously described, achieving single-tube RT and amplification of viral sequences [21]. Strict measures to prevent contamination were adopted; reagent-negative, sample-negative, and sample-positive controls were included in each batch of RNA extraction and carried on through PCR. HCV genotyping was performed using a fraction of the PCR products, which was hybridized to oligonucleotides directed against the variable region of 5′ untranslated region and immobilized as parallel lines on membrane strips (line probe assay, LIPA, Innogenetics, Gent, Belgium). The reactivity of the amplified fragments with one or more lines in the strip allowed identification of the five major genotypes and their subtypes. Viral genotypes were denominated according to Simmonds [24].

Values were expressed as mean ± standard deviation (SD) and range between minimum and maximum values. Chi-square test and Fischer's exact test were used to analyze differences between groups. A P value less than 0.05 was considered to indicate a significant difference. Actuarial patients' survival and risk of HCV-related graft failure were calculated according to the method of Kaplan-Meir.

Table 1 Data of 35 pretransplant hepatitis C virus (HCV)-positive liver transplant recipients

Sex (male/female)	25/10
Age (years)	
Mean ± SD	39 ± 19
Range	2–63
Primary disease	
Posthepatitis cirrhosis	19
Hepatoma-cirrhosis	9
Primary biliary cirrhosis	3
Metabolic deficiency	2
Biliary atresia	2
Follow-up (months)	
Mean ± SD	41 ± 32
Range	6–118

Results

Data from 37 pretransplant HCV-positive recipients were reviewed. At the time of the last out-patient control (mean 41 months, SD 32, range 6–119) a biliary complication was found in two recipients, who were excluded from this study. Follow-up data from 35 patients were analyzed and are summarized in Tables 1 and 2. The majority of the patients could be divided into two patterns of follow-up ALT levels (Fig. 1): chronic persistent elevation (20 patients) or sustained normal levels (11 patients). In 16 of the 20 patients showing chronic ALT elevation, histologically acute hepatitis was pre-

Table 2 Sorting of recipients according to the four individualized patterns of posttransplant alanine aminotransferase (ALT) levels. Serum HCV-RNA positivity, HCV genotypes, and occurrence of graft failure due to HCV infection are shown. In the first two groups of patients, the pattern of ALT levels relative to the HCV genotypes (1b versus non-1b) at the Fischer's exact test showed $P = 0.0894$ and relative risk = 1.83, with a 95 % confidence interval between 0.915 and 3.675

Patterns of ALT	Patients	Positive HCV-RNA	Type 1b	Type 2a	Type 3a	Graft failure
Elevated	20	17/17[a]	10	4	1	6
Normal	11	9/11[b]	2	5	1	0
Late elevated	2	2/2	2	0	0	2
Fluctuating	2	1/1	1	0	0	0

[a] Genotyping of two HCV-RNA-positive patients was not available

[b] Genotyping of one HCV-RNA-positive patient was not available; two patients were HCV-RNA-negative

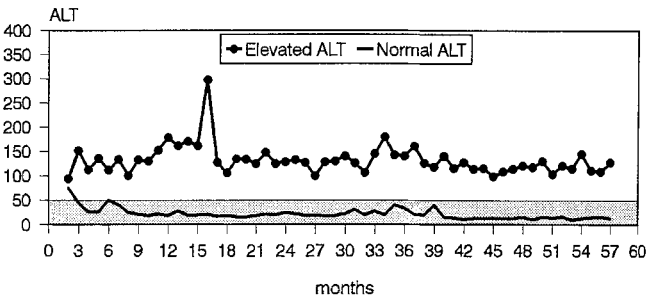

Fig. 1 Mean values of serum alanine aminotransferase *(ALT)* after liver transplantation: 20 patients showed chronic ALT elevation and 11 patients showed normal ALT levels

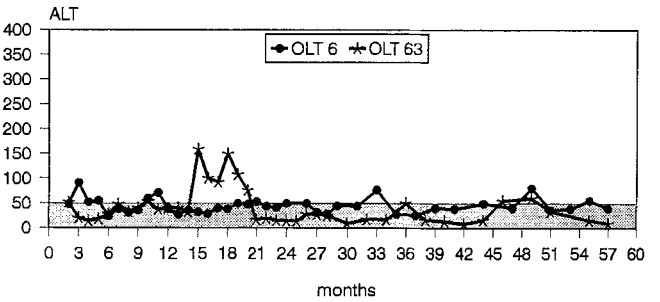

Fig. 2 Mean values of serum ALT after liver transplantation in two patients *(OLT6* and *OLT63)* with levels fluctuating above and below the normal limits

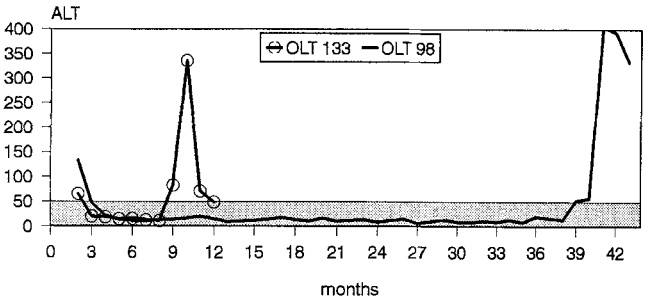

Fig. 3 Mean values of serum ALT after liver transplantation in two patients *(OLT98* and *OLT133)* with late elevation and death due to hepatitis C virus (HCV)-related sub-acute graft failure

sent 1 month after surgery; in three others it was recognized during the second and third postoperative months and in the latter after 9 months of follow-up. HCV-RNA detection was positive in 17/17 of them and the following genotypes were identified in 15: 1b in ten patients, 2a in four patients, and 3a in one patient. In the 11 patients who showed normal ALT levels throughout follow-up, a graft biopsy during the long-term follow-up was not available, but in three of them the acute hepatitis had been histologically proven during the first postoperative month. In nine of these 11 patients, HCV-RNA was positive; HCV genotyping identified type 1b in two patients, type 2a in five patients, and type 3a in another patient; genotyping was not available in a HCV-RNA-positive patient. Of the two patients in this group who were HCV-RNA-negative, one was found anti-HCV negative at 69 months after surgery. The four remaining recipients showed two different patterns of presentation: ALT fluctuating above and below the normal limits (two patients) and an abrupt rise in ALT levels after a sustained period of normal values (two patients). In two patients, ALT levels were found fluctuating above and below the upper limits of normality (Fig. 2) during a follow-up of 82 and 119 months, respectively (histology not available). HCV-RNA was positive in 1/1 of them and type 1b was identified. In the last two patients, after an initial period of normal liver biochemistry, ALT levels showed an abrupt rise (Fig. 3) with rapidly progressive graft failure and death at 11 and 52 months after transplantation; HCV-RNA was found positive and type 1b was identified in both patients. Comparing the first two groups of patients, there was a consistent trend for patients in the group with persistent elevation in ALT to be infected by genotype 1b versus other types in patients in the group with normal ALT levels. However, the difference was not significant ($P = 0.0894$), maybe owing to the small sample size, even if the relative risk for ALT elevation was almost doubled in the presence of genotype 1b infection.

Eight out of all patients examined developed a deep jaundice episode (serum total bilirubin above 10 mg% for a mean of 4 months, range 2–10); six of them had persistent elevated ALT and the others were the two patients with the late ALT elevation; all these patients were infected by genotype 1b. Five of them developed marked cholestasis as a sign of sub-acute HCV-related

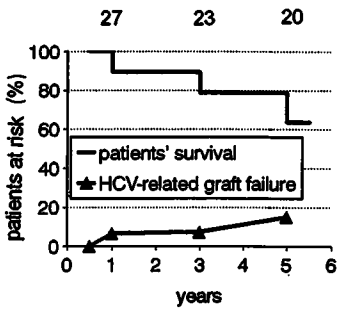

Fig. 4 Actuarial patients' survival curve and risk of HCV-related graft failure after liver transplantation. Data from 32 patients surviving at least 6 months (three deaths for recurrent hepatocarcinoma were excluded)

graft failure, while in three patients jaundice lasted 2, 3, and 10 months, respectively and then spontaneously resolved. After a mean time of 28 months from transplantation (range 1–88), progressive hepatic injury occurred in eight patients, six with chronic ALT elevation and two with the late ALT elevation; the genotype 1b was identified in seven of them and in the remaining patient genotype 3a was present. Sub-acute hepatic failure developed in five recipients after a mean of 12 months (range 3–50) leading to death in two and retransplantation (re-OLT) in the others; re-OLT was performed elsewhere abroad in a patient who died shortly afterwards, and at our center in the remaining two patients (one received her first transplant at another Italian center), who are currently alive 14 and 34 months after re-OLT, respectively, despite a HCV infection in the new liver in both of them. The other three patients have recurrent cirrhosis: one patient was not considered for re-OLT due to her poor respiratory condition, a Le Veen shunt was placed 84 months after surgery and she is now showing a progressively deteriorating condition (follow-up 108 months); another patient improved after medical treatment for ascites, which she had developed 24 months after the transplant (follow-up 35 months); the latter patient spontaneously improved after a hepatic decompensation episode occurred 48 months after surgery and he is stable 79 months after transplantation. Moreover, another patient who has persistent elevated ALT and a genotype 1b infection had been known to have recurrent overt histological cirrhosis but he is still in good general condition with a well-functioning graft 24 months after transplantation.

Fifty-seven graft biopsies from 25 patients were reviewed. Acute hepatitis was found in histological specimens during the early posttransplant period in 21 patients. In five patients the acute phase rapidly progressed to a graft failure with morphological signs of wide disruption of the hepatic structure by marked infiltrates. Subsequent late histology samples were available from eight recipients after a mean time of 10 months after the acute hepatitis and histological progression to cirrhosis was found in four of them, one of whom remained in good clinical status but expressed repeat marked alteration in ALT levels during 18 months of histological follow-up.

The 1, 3 and 5 year actuarial patients' survival, excluding from the analysis three patients who died of cancer recurrence, were 89 %, 79 % and 63 %, respectively (Fig. 4). The 1, 3, and 5 year actuarial risks of progressive graft decompensation were 6 %, 7 %, and 15 %, respectively (Fig. 4).

Discussion

Hepatitis C virus infection is a well-recognized cause of chronic graft damage in liver transplant recipients [9]. In common with pretransplant HBV-positive liver allograft recipients, pretransplant HCV-positive patients are at high risk of recurrence because of the extra-hepatic sites of viral replication [14]. Differently from HBV disease [22], pretransplant HCV viremia does not seem to be a relevant independent factor for the risk of posttransplant infection [26]. Virtually all patients with pretransplant HCV-related disease develop recurrent viral infection during follow-up, as expressed by the positivity of serum HCV-RNA detection [7, 11]. Moreover, the reappearance of graft dysfunction is common [7, 13, 25] and it occurs soon after transplantation [9, 12, 26], as diagnosed by either histological evidence of hepatitis or persistent elevations in liver enzymes in the absence of other known causes.

In the present study we have described the biochemical appearance and the long-term clinical course of HCV recurrence in 35 pretransplant infected patients. In the majority of patients, two distinct patterns of ALT levels were observed, namely persistent elevation or normal values, whereas in a few patients fluctuating ALT levels above and below the norm and sustained normal ALT levels followed by an abrupt rise associated with graft failure were found. Out of 30 patients in whom HCV-RNA detection was performed, only two were found negative and both had normal ALT levels over time; in one of them, anti-HCV was found negative after the transplant, and we suggest that this patient cleared the viral infection, a rare event that has been reported in the literature [25]. In three patients with long-term normal ALT values, acute hepatitis was found by the end of the first postoperative month. The possibility that normal ALT values do not exclude an underlying hepatitis had been previously reported [11]. However, in our series, no patient having ALT levels within the normal limits showed progressive hepatic damage.

Three different HCV genotypes have been found in 26 patients analyzed and the various types were present both in the elevated and normal ALT level groups of patients. Patients infected by genotype 1b more commonly

showed elevated ALT levels, but this finding was not statistically significant. In our patients, regardless of the genotype of the infection, an early recurrent acute hepatitis was the rule. In most patients, the evolution of the acute phase of viral reinfection was favorable, but in rare cases it rapidly worsened, leading to early graft loss. Elevated ALT levels were present in six patients who showed hepatic decompensation; genotype 1b infection was present in all these patients but one, who was infected by type 3a. In the two other patients who experienced HCV-related graft failure, ALT presented a sharp elevation after a long period of normality: a change in the quasispecies mixture of the individual viral population can be hypothesized in these recipients, as recently demonstrated by Gretch et al. [10]. Finally, our data, even without recognizing the genotype 1b infection as a statistically significant negative independent prognostic factor after transplantation [8], showed that the occurrence of HCV-related hepatic decompensation was strikingly linked to this genotype when associated with abnormal ALT levels.

It has been reported that, in some patients, HCV recurrence can cause a marked cholestatic syndrome and this was identified as a negative prognostic factor [25]. Yet, in some series, the occurrence of HCV-associated severe jaundice has been denied [11] or other etiologic factors have been found than the viral infection [23]. In this series a deep jaundice episode appeared in eight patients infected by genotype 1b, and cholestasis spontaneously resolved during a variable length of time in three of them who did not show a rapid deteriorating evolution with loss of hepatic synthesis.

One potential shortcomming of the present study is the lack of sequential histological follow-up in our patients. It is not our policy to perform "protocol" liver biopsies and, therefore, the risk of an underestimation of the HCV recurrent hepatitis may be present in this series. Ferrel et al. [9] showed how difficult it is to achieve a histological diagnosis of HCV hepatitis in grafted patients because of the high frequency of atypical microscopic features, regardless of aspartate aminotransferase levels; in their study the authors emphasized the central role of HCV-RNA positivity in the diagnosis of the cause of liver damage.

HCV infection of the graft has been recognized as a crucial factor affecting the long-term result of liver transplantation [2, 7, 9, 20, 26]. It is known that the clinical evolution of posttransplant HCV disease over time may range from long-term asymptomatic carriers to progressive liver damage [4, 7, 16, 20], even with rapid developing graft failure [6, 17]. In our experience, pretransplant HCV infection commonly led to graft dysfunction and the recurrence of the disease caused a slow decrease in the long-term patients' survival, maybe because of the indolent rate of progression of the graft damage. We believe that although HCV-related graft loss has been rarely reported [1, 2, 6, 12, 26], it is likely to become more prevalent with longer follow-up studies [18]. In this series the risk of graft failure due to HCV disease showed a progressive increase throughout follow-up, leading to death or need for retransplantation. The latter was successful in term of patients' survival, but it was always followed by HCV infection in the new graft, as reported by others [1, 16, 17, 20].

In conclusion, from our results it appears that HCV genotype 1b could have a relevant prognostic role in the posttransplant HCV-recurrent disease. Monitoring long-term postoperative ALT levels is a simple and reliable test in the evaluation of the progression of HCV graft damage. Spontaneous resolution of HCV-related severe cholestatic episodes is possible when hepatic synthesis is maintained. HCV reinfection of the graft seems to affect the long-term survival because of increasing risk of HCV-related graft derangement and failure over time.

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References

1. Ascher NL, Lake JR, Emond J, Roberts J (1994) Liver transplantation for hepatitis C virus related cirrhosis. Hepatology 20: 24S–27S
2. Belli LS, Caccamo L, Mazzaferro V, Silini E, Alberti A, Melada E, Regalia E, Gridelli B, Rubino A, Gennari L, Galmarini D, Ideo G, Belli L (1994) Milan multicenter experience in liver transplantation for hepatitis C related cirrhosis: report of 105 cases. Transplant Proc 26: 3582–3584
3. Caccamo L, Colledan M, Gridelli B, Rossi G, Doglia M, Gatti S, Ghidoni P, Lucianetti A, Lunghi G, Maggi G, Paone G, Reggiani P, Galmarini D, Fassati LR (1993) Hepatitis C virus infection in liver allografts recipients. Arch Virol Suppl 8: 291–304
4. Chazouilleres O, Kim M, Combs C, Ferrel L, Bacchetti P, Roberts J, Ascher NL, Neuwald P, Wilber J, Urdea M, Quan S, Sanchez-Pescador R, Wright TL (1994) Quantitation of hepatitis C virus RNA in liver transplant recipients. Gastroenterology 106: 994–999
5. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium-thiocyanate-phenol-chloroform extaction. Anal Biochem 162: 156–169
6. Donataccio M, Lerut J, Ciccarelli O, Comu CH, Laterre PF, Geubel A, Otte JB (1994) Hepatitis C viral infection and adult liver transplantation: a difficult clinical problem. Transplant Proc 26: 3588–3590

7. Feray C, Gigou M, Samuel D, Paradis V, Wilber J, David MF, Urdea M, Reynes M, Brechot C, Bismuth H (1994) The course of hepatitis C virus infection after liver transplantation. Hepatology 20: 1137–1143
8. Feray C, Gigou M, Samuel D, Paradis V, Mishiro S, Maertens G, Reynes M, Okamoto H, Bismuth H, Brechot C (1995) Influence of genotypes of hepatitis C virus on the severity of recurrent liver disease after liver transplantation. Gastroenterology 108: 1088–1096
9. Ferrell LD, Wright TL, Roberts J, Ascher N, Lake J (1992) Hepatitis C viral infection in liver transplant recipients. Hepatology 16: 865–876
10. Gretch DR, Wilson JJ, Faulkner G, Corey L, Perkins J, Carithers RL (1994) Tracking hepatitis C virus quasispecies in liver transplant recipients (abstract). Hepatology 20: 132A
11. Gugenheim J, Baldini E, Mazza D, Fabiani P, St. Paul MC, Goubaux B, Ouzan D, Mouiel J (1994) Recurrence of hepatitis C virus after liver transplantation. Transplant Int 7 (suppl 1): S224–S226
2. Knoop M, Lusebrink R, Langrehr JM, Konig F, Berg T, Wedell A, Hopf U, Neuhaus P (1994) Incidence and clinical relevance of recurrent hepatitis C infection after orthotopic liver transplantation. Transplant Int 7 (suppl 1): S221–S223
3. Konig V, Bauditz J, Lobeck H, Lusebrink R, Neuhaus P, Blumhardt G, Bechstein WO, Neuhaus R, Steffen R, Hopf U (1992) Hepatitis C virus reinfection in allografts after orthotopic liver transplantation. Hepatology 16: 1137–1143
14. Li X, Jeffers L, Reddy R, De Medina M, Parker T, Schiff ER (1992) Detection of HCV-RNA by RT-PCR in lymphocyte subsets (abstract). Gastroenterology 102 (part 2): A842
15. Mateo R, Demetris A, Sico E, Frye C, Wang L-F, El-Sakhawi Y, Reilly M, Ehrlich GD, Cooper D, Fung J (1993) Early detection of de novo hepatitis C infection in patients after liver transplantation by reverse transcriptase polymerase reaction. Surgery 114: 442–448
16. Muller H, Otto G, Goeser T, Arnold J, Pfaff J, Thielmann L (1992) Recurrence of hepatitis C virus infection after orthotopic liver transplantation. Transplantation 54: 743–745
17. Nery J, Esquenazi V, Weppler D, Gomez C, Cirocco R, Gharagozloo H, Zucker K, Reddy R, Casella J, Parker I, Faria W, Jeffers L, Carreno M, Smith J, Markow M, Allouch M, Babischkin S, Bourke G, Hill M, Schiff E, LaRue S, Miller J (1994) Hepatitis C in liver transplantation: preliminary study of prognostic factors. Transplant Int 7 (suppl 1): S229–S231
18. Pappo O, Ramos H, Starzl TE, Fung JJ, Demetris AJ (1995) Structural integrity and identification of causes of liver allograft dysfunction occurring more than 5 years after transplantation. Am J Surg Pathol 19: 192–206
19. Persing DH, Rakela J (1992) Polymerase chain reaction for detection of hepatitis viruses: panacea or purgatory? Gastroenterology 103: 1098–1099
20. Pouterucha JJ, Rakela J, Lumeng L, Lee C-H, Taswell HF, Wiesner RH (1992) Diagnosis of chronic hepatitis C after liver transplantation by detection of viral sequences by polymerase chain reaction. Hepatology 15: 42–45
21. Sampietro M, Salvadori S, Badalamenti S, Graziani G, Fiorelli G (1995) Single tube reverse transcription and heminested polymerase chain reaction of hepatitis C virus RNA to detect viremia in serologically negative hemodialyzed patients. Int J Clin Lab Res 25: 52–54
22. Samuel D, Muller R, Alexander G, Fassati LR, Ducot B, Benhamou J-P, Bismuth H and the investigators of the European Concerted Action on Viral Hepatitis Study (1993) Liver transplantation in European patients with the hepatitis B surface antigen. N Engl J Med 329: 1842–1847
23. Shiffman ML, Contos MJ, Luketic VA, Santal AJ, Purdum PP, Mills AS, Fisher RA, Posner MP (1994) Biochemical and histologic evaluation of recurrent hepatitis C following orthotopic liver transplantation. Transplantation 57: 526–532
24. Simmonds P (1995) Variability of hepatitis C virus. Hepatology 21: 570–583
25. Vargas V, Comas P, Castella LI, Quer J, Esteban JI, Allende E, Esteban R, Guardia J, Margarit C (1994) Incidence and outcome of hepatitis C virus infection after liver transplantation. Transplant Int 7 (suppl 1): S216–S220
26. Wright TL, Donegan E, Hsu HH, Ferrel L, Lake JR, Kim M, Combs C, Fennessy S, Roberts JP, Ascher NL, Greenberg HB (1992) Recurrent and acquired hepatitis C viral infection in liver transplant recipients. Gastroenterology 103: 317–322