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Hepatitis C virus genotypes and the influence of the induction of immunosuppression with anti-thymocyte globulin (ATG) on chronic hepatitis in renal graft recipients

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Abstract Hepatitis C virus (HCV) exhibits a dramatic genetic variability and several mechanisms of immunological response are unable to control hepatic and extrahepatic replication. Genotype 1 b is associated with more severe clinical manifestations and is less responsive to interferon. In addition, we have reported an increase of HCV RNA viral load after renal transplantation. Anti-thymocyte globulin (ATG) is supposed to increase viral replication and liver dysfunction in chronically infected renal graft recipients. We evaluated the genotype profile in HCV + patients of our Renal Transplant Unit and studied the effects of ATG, as part of the induction of immunosuppression, on viral load and liver enzymes abnormalities. From 726 renal graft recipients, 104 patients, with a mean follow up of 3.9 ± 2.9 years, were anti-HCV + by ELISA II. HCV RNA was measured by quantitative PCR. We correlated the viral load and biochemical liver parameters with genotype, exposure to ATG as induction therapy, early acute rejection episode and the duration of infection. Of the 81 patients tested, 72 % were viraemic and genotype 1 b was the predominant viral strain (66 %). The majority of these patients (65 %) were coinfected by two or more strains.There was no correlation between HCV RNA blood levels and liver enzymes. We did not find higher viral load with genotype 1 b infection (68 ± 88 mEq/ml vs 75.8 ± 123 mEq/ml in the others) nor with ATG induction therapy (43.5 ± 71.3 mEq/ml vs 64.1 ± 110.5 mEq/ml). Early acute rejection and longer follow up were not associated with higher levels of HCV RNA. The biochemical liver profile showed no relationship with the variables studied. We concluded that genotype 1 b is the predominant strain in our HCV + population and there is a great prevalence of coinfection with several genotypes. Our results did not confirm a deleterious effect of the use of ATG as induction therapy in these HCV-infected patients. Prospective randomised studies with liver biopsy evaluation are needed to answer more fully the remaining questions about the best immunosuppressive therapy in renal graft recipients with chronic HCV infection.

Key words Hepatitis C virus · Kidney transplantation · Anti-thymocyte globulin

Introduction

Several issues related to hepatitis C virus (HCV) infection are still a matter of debate among nephrologists since there are no definitive conclusions about the risks of renal transplantation in chronically infected patients [1]. Viral replication increases [2–4] and liver disease may progress under immunosuppression [5]. We have reported a lower actuarial survival of HCV + renal graft recipients than that of the seronegative population [6]. Bouthot et al. [7] also showed higher infectious mortality of HCV + transplanted patients, but the majority of the studies could not detect any significant difference in either patient or graft survivals between HCV-infected and -noninfected patients [1, 8]. A recent study has even demonstrated that HCV + renal transplant recipients had a better survival than similar HCV + haemodialysed patients awaiting transplantation [9]. Probably, the duration of infection [7, 9], liver biopsy score [1] and induction of immunosuppression with anti-lymphocyte preparations [10, 11] may be major factors involved in the outcome of these patients. Genotype variability certainly contributes to the immunological escape and persistence of replication [12]. Genotype 1 b is associated with more severe histological lesions and failure of interferon therapy [13].

In the near future, it will be necessary to optimise the immunosuppressive schemes of HCV + patients in order to avoid the risk of overimmunosuppression, fatal sepsis and progressive liver failure in long-term renal graft recipients. The aim of this study was to evaluate the genotype profile in HCV + patients of our Renal Transplant Unit and study the effects of anti-thymocyte globulin (ATG), as part of the induction of immunosuppression, on viral load and liver enzymes abnormalities.

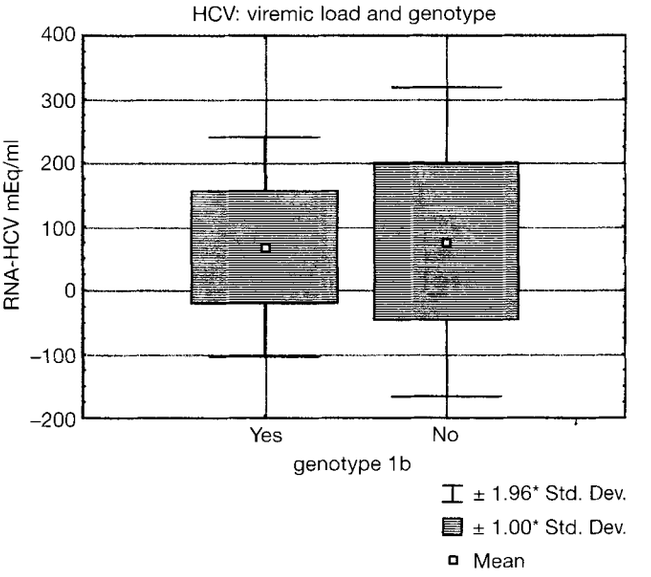

Fig. 1 Blood levels of hepatitis C virus (HCV) RNA were not higher in genotype 1b-infected patients

Table 1 Genotype variability in renal graft recipients with hepatitis C virus infection

Genotype	*n*	Genotype	*n*
1 a	3	2 a + 3 a	1
1 a + 5	1	2 a	1
1 b	6	3 a	1
1 b + 4 + 5	2	3 a + 4	2
1 b + 5	16	4	2
1 b + 1 a + 3 a	1	4 + 5	2
1 b + 2 a + 3 a	1	5	1
1 b + 3 a + 5	1		

Patients and methods

Of 726 renal graft recipients, 104 patients were anti-HCV + by ELISA II. HCV RNA was measured by quantitative PCR in 81 patients, with a mean posttransplantation follow up of 3.9 ± 2.9 years. The genotype identification was performed by nested RT–PCR of the core region and characterisation with type-specific oligonucleotides (Sorin Biomédica). Fifty-two (50 %) HCV + patients were given ATG, 3–4 mg/kg per day for 7–10 days after transplantation, either as triple [ATG + cyclosporin A (CsA) + prednisolone (Pred)] or quadruple (ATG + CsA + Pred + azathioprine) induction therapy. Twenty-eight patients had at least one episode of acute rejection in the first 3 months after transplantation and received a course of methylprednisolone boluses.

We correlated the viral load and the biochemical liver parameters (alanine aminotransferase) with genotype, exposure to ATG as induction therapy, early acute rejection episodes and the duration of infection.

Statistical analysis was performed using Student's *t*-test and multiple regression methods with the STAT (Statistics for Windows) software program. Results are expressed as mean ± SD $P < 0.05$ was considered significant.

Results

Of the 81 patients tested, 72 % were viraemic and genotype 1 b was the predominant viral strain, 28/42 (66 %) patients. The majority of these patients (65 %) were co-infected by two or more strains (Table 1). HCV RNA blood levels averaged 105.5 mEq/ml (median 67.7, range 455–4.1 mEq/ml). Serum alanine aminotransferase averaged 63 mg/dl (median 41, range 6–564 mg/dl), being elevated in 50 % of the patients.

We found no correlation between HCV RNA blood levels and liver enzymes ($r = 0.16$, $P = 0.13$). The patients exposed to ATG did not differ from the others in age (38 ± 11 versus 40 ± 15 years, P not significant) but had a shorter follow up (36 ± 29 versus 60 ± 39 months, $P = 0.004$). We did not find higher viral loads with genotype 1 b infection (Fig. 1). Neither an early acute rejection episode (Fig. 2) nor exposure to ATG (Fig. 3) was associated with higher viraemia. We could not find an

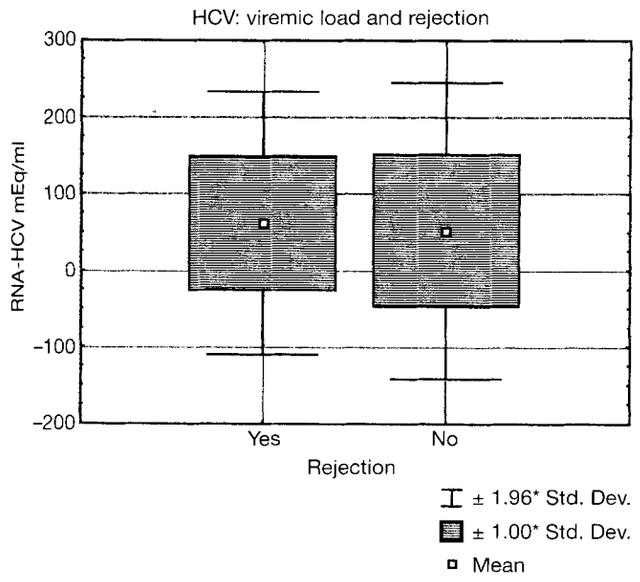

Fig. 2 Previous early acute rejection was not associated with higher viremia

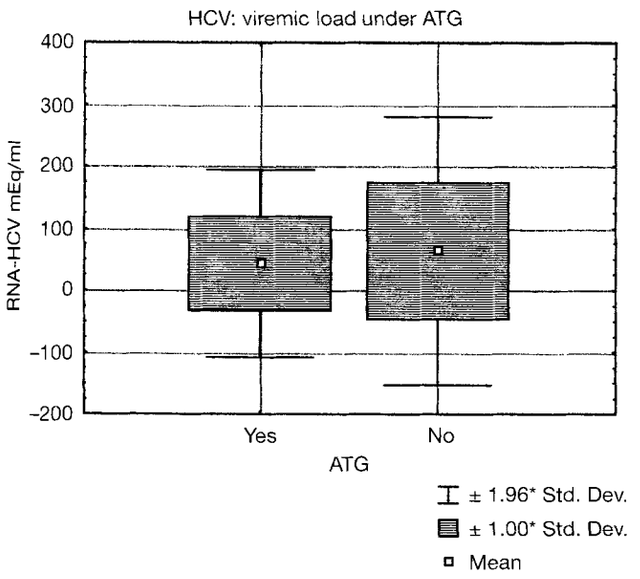

Fig. 3 HCV virus replication does not seem to have been enhanced by anti-thymocyte globulin (*ATG*) as part of the induction therapy

increase of blood HCV RNA with time after transplantation ($r = 0.13$, $P = 0.21$; Fig. 4).

Discussion

Several issues related to the interaction between HCV and immunosuppressive therapy remain to be clarified [14]. We still have no definite answers about the safest immunosuppressive treatment to use in infected renal graft recipients, and the assumption that progressive liver failure and infectious mortality are enhanced by transplantation has been questioned [1, 9, 15]. Unless serious lesions or cirrhosis are detected in liver biopsy, the majority of the studies agree that it is acceptable to transplant HCV + patients, since no major effects on patients and graft survivals have been reported. Those that have mentioned a deleterious effect on patient morbidity and mortality [1, 6, 7] have probably studied populations with a longer duration of infection before transplantation.

However, the management of these patients after transplantation deserves some considerations. Our study and others [16] emphasise that liver enzymes cannot be used as a surrogate marker for severity of chronic HCV infection and viral load. Like Ghany et al. [17], we have found no correlation between the liver parameters and HCV RNA blood levels. There is a great variability in HCV RNA blood levels among patients and it is our experience that even a single patient usually shows highly fluctuating levels of viraemia with time. Besides, we could also not find a correlation between the duration of infection under immunosuppression (follow up) and HCV RNA blood levels. Therefore, liver biopsy is required to assess liver damage in this population and guide the immunosuppressive therapy. Azathioprine, for instance, may have to be reduced or withdrawn [18], and it may be useful to reduce prednisolone and/or add micophenolate mophetyl instead of azathioprine in those patients on triple maintenance therapy. On the other hand, we must consider that viral infection may be immunosuppressive by itself and allow for the reduction of the drugs in protocols. In fact, it seems that graft survival is similar in HCV + patients to that in seronegative patients and some studies reported less rejection episodes. Therefore, we urge for better schemes of immunosuppression in this particular population to avoid overimmunosuppression and progressive liver disease.

Notably, we have documented increased circulating viral titres during postransplantation follow up [3] but, in this study, viral replication was not enhanced following exposure to ATG. We used the episode of early acute rejection as a surrogate of heavier immunosuppression, but this also was not shown to induce a higher viraemic load or liver dysfunction in the patients studied. However, we must be aware that these biological parameters may be insensitive for estimating liver lesions. The histological liver score would have given us a more definite answer about the safety of heavier induction and anti-rejection immunosuppressive drugs in this population. We think, therefore, that prospective randomised studies with liver biopsy evaluation are needed, not only to stage the hepatitis but also to guide immunosuppressive therapy in HCV + renal graft recipients.

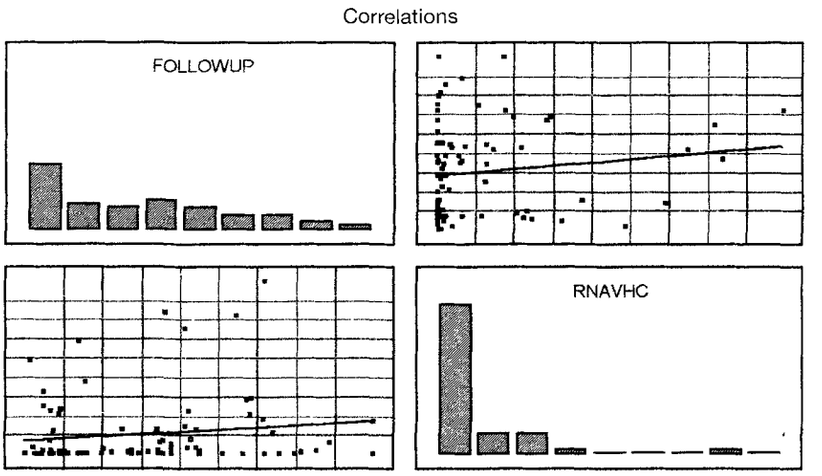

Fig. 4 HCV: no correlation found between viral load and duration of infection under immunosuppression (follow up)

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