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Hepatitis C virus, an important risk factor for tuberculosis in immunocompromised: experience with kidney transplantation

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Summary

Little is known about the role of hepatitis C virus (HCV) infection in the development of tuberculosis (TB) in patients with immunosuppression. We performed a retrospective case–control study (1:4) to investigate by univariate and multivariate logistic regression analysis the importance of HCV infection in the development of TB in a cohort of kidney transplant recipients (KTR). TB was diagnosed in 16 out of 2012 (0.8%) KTR between 1976 and 2004. The percentage of HCV-positive patients was significantly higher in cases than in controls (56.3% vs. 18.8%; $P = 0.02$). By multivariate analysis, the only two independent risk factors associated with the development of TB were the presence of HCV infection ($P = 0.003$; OR = 6.5; 95% CI 1.9–23) and serum creatinine over 1.5 mg/dl ($P = 0.03$; OR = 4.8; 95% CI 1.1–21). HCV infection and chronic graft dysfunction are important risks factors for TB in KTR.

Introduction

The incidence of tuberculosis (TB) in kidney transplant recipients (KTR) in Spain is 219/100 000 patients/year (data from the Spanish Network for Transplantation, RESITRA http://www.resitra.retics.net/web/index.asp, not published), an incidence almost six times higher than that in general population (38/100 000 habitants/year) [1]. There is scarce information on possible risk factors for TB in KTR [2]. In our clinical experience, many KTR who developed TB have had previous infection by hepatitis C virus (HCV). HCV is one of the causes of chronic renal insufficiency in our country [3] and chronic renal insufficiency is a well known risk factor for TB [4]. Otherwise we have demonstrated that HCV infection has an immunosuppressive effect in KTR, which contributes to reduce the rate of acute renal-transplant rejection and to increase the risk of opportunistic infections in these patients [5]. This immunosuppressive effect could act favoring the development of TB in HCV-infected patients. To prove this hypothesis, we performed a case–control study to evaluate the role played by HCV infection and by other potential risk factors for the development of TB in KTR.

Materials and methods

This retrospective case–control study was carried out at the Unit of Renal Transplantation (Department of Nephrology) of the University Hospital 12 de Octubre, a 1200-bed university-affiliated tertiary hospital in Madrid, Spain. We retrospectively reviewed the hospital- and outpatient follow-up records of patients over 18 years of age who received a kidney transplant between January 1976 and May 2004 and developed active TB after transplantation, even though they had a functioning graft. We selected four control patients for every TB case. Control patients had been transplanted at the same time (±2 months) as the cases and had a graft survival time

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equal to or higher than TB cases, in order to ensure that controls were exposed to immunosuppression for a similar period of time as the cases. Specifically were excluded all patients in whom TB was diagnosed before transplantation. Cases were identified by reviewing the files of the kidney transplantation program of our institution. In addition, we reviewed the microbiologic files searching for all *Mycobacterium tuberculosis*-positive specimens obtained from KTR during the period studied.

Data collection

Patients' clinical charts were reviewed for demographic information (age, gender), presence of diabetes mellitus, first or subsequent kidney transplantation, etiology of renal insufficiency, time in hemodialysis, HCV (in included patients who underwent transplant before 1990, retrospective HCV determination on archived sera was performed when available or otherwise HCV assumption based on subsequent serologic determinations), hepatitis B virus (HBV) and cytomegalovirus (CMV) serology, type of immunosuppression, purified protein derivative of tuberculin (PPD), previous history of TB, pretransplant abnormal chest X-ray, chemoprophylaxis, time to development of TB (early: ≤1 year, late: >1 year), clinical symptoms, laboratory data, radiographic and pathologic features, results of *Mycobacterium* cultures, sites of involvement of TB infection, methods for diagnosis, number of rejection episodes, microbiologic and clinical outcome, and cause of death.

Infection by HIV was an exclusion criterion for receiving a renal transplant and therefore none of the included patients in this study was infected.

Case definition

The diagnosis of TB was considered certain if *M. tuberculosis* was cultured from any clinical sample. TB was considered probable in patients who had a clinical picture highly suggestive of TB that resolved with specific antituberculous treatment, and in whom acid-fast bacilli were seen on smear, with or without caseating granulomas on histopathology. Disseminated TB was considered to be present when *M. tuberculosis* was isolated from two or more noncontiguous organs or from blood, or when there was isolation of the organism from one organ along with demonstration of acid-fast bacilli or granulomas at a different site.

Microbiologic identification

Samples were processed and organisms identified according to standard methods [6]. Ziehl-Neelsen stain and the auramine O-rhodamine fluorescent method were used for staining. Samples were cultured in different media according to the year of diagnosis.

Immunosuppression and immunologic tests

Immunosuppressive regimens varied according to the year of transplantation. Triple therapy with cyclosporine (CsA), azathioprine, and steroids was used preferentially until 1997. Since 1997, the majority of patients received mycophenolate mofetil (usual dose of 1 g twice daily) instead of azathioprine, and tacrolimus instead of CsA. Doses of CsA were adjusted to obtain trough plasma levels of 200–400 ng/ml (as determined by radioimmunoassay) during the first month and 100–200 ng/ml thereafter. The targeted trough level of tacrolimus was between 8 and 10 ng/ml. Rejection episodes were usually treated with boluses of steroids or antilymphocyte antibodies (OKT3) in the case of steroid-resistant rejection. Steroids were reduced slowly and in a stepwise fashion in the great majority of the patients, leading to steroid-free maintenance after 12 months.

Statistical analysis

We compared the demographic characteristics and clinical and laboratory variables between control group and KTR with TB. For the determination of risk factors for TB, the effects of each variable were examined in a univariate approach with comparison of averages and application of the Student's *t*-test, as well as with the analysis of contingency tables with χ^2-test or Fisher's exact test when necessary. Multivariate analysis was performed through the model of logistic regression with the odds ratio (OR) for each risk factor being identified with a 95% confidence interval (CI). The program used was SSPS for Windows, version 11.0 (SPSS Inc Headquarters, Chicago, IL, USA). A *P*-value of lower than 0.05 was established as the level of significance for all the tests.

Results

Between January 1976 and May 2004, a total of 2012 renal transplants were performed in our hospital. During this time 16 patients (0.8%) were diagnosed of TB and compared with 64 controls (ratio 1:4). The principal characteristics of patients with TB are shown in Table 1. There were 10 men and six women. The mean age of the patients was 39.8 ± 13.2 years. One patient had a past history of TB, but none of them had radiologic evidence of TB before transplantation. PPD skin test was done only in three out of 16 cases (18.7%) and in 10/64 control (15.6%). PPD was negative in all cases, and none of the patients received prophylaxis against TB. The time that

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Table 1. Clinical and diagnostic data in kidney transplant recipients with tuberculosis (TB).

Case	Age (years)	Previous TB	Year of transplant	Time from transplant (months)	Localization	Symptoms	Diagnostic method Sample/diagnostic method
1	37	No	1981	204	Pulmonary	Fever	Sputum/AFS, culture
2	29	Yes	1987	2	Pulmonary	Fever	Sputum/AFS
3	40	No	1988	6	Pulmonary	Fever,	Sputum/AFS
4	24	No	1990	102	Extrapulmonary*	Fever	Lymph node/AFS, granulomas
5	29	No	1990	139	Pleural	Fever, pleuritic pain	Pleural biopsy/AFS, granulomas
6	44	No	1991	52	Pulmonary	Fever, cough	Sputum, lung biopsy/AFS, culture
7	57	No	1991	24	Pulmonary	Fever	Lung biopsy/AFS, culture
8	38	No	1991	5	Disseminated†	Fever	Urine/AFS
9	52	No	1992	4	Pulmonary	Fever	Lung biopsy/culture
10	25	No	2002	34	Pulmonary	Fever, cough	Sputum/AFS
11	46	No	1995	23	Pleural	Cough	Pleural fluid/culture
12	60	No	1995	32	Disseminated†	Fever	Bone marrow, lung biopsy/AFS, culture
13	32	No	1999	36	Pulmonary	Fever, cough	Sputum/AFS
14	52	No	1992	4	Pulmonary	Fever, dyspnea	Sputum/AFS
15	31‡	No	2002	3	Disseminated†	Fever, asthenia	BAL/AFS
16	68	No	2003	1	Disseminated†	Fever, asthenia	BAL/AFS

AFS, acid-fast stain; BAL, bronchoalveolar lavage.
*Tuberculous lymphadenitis.
†Miliary TB.
‡Pancreas-kidney transplant.

elapsed from the date of transplantation to the onset of the symptoms ranged from 30 days to 17 years (mean 41.9 months ± 18.2). We observed a bimodal distribution in time: seven out of 16 patients (44%) developed TB early (during the first year after transplantation), all of them in the first 6 months, whereas 56% developed late TB, all cases occurring 2 or more years after transplantation. Pleuropulmonary involvement was seen in 11 patients (69%), disseminated TB in four cases (25%) and one patient had extrapulmonary (lymphadenitis) TB (6%). The most common initial symptom was fever, which was present in the majority of patients. The diagnosis of TB was considered certain in six cases and probable in the remaining 10 cases.

Both demographics (gender, mean age) and clinical characteristics (time in hemodialysis, etiology of chronic renal insufficiency, presence of diabetes mellitus, second RT, HBV serology, donor/recipient CMV serologic status, and data on previous TB) were similar in cases and controls (Table 2). Both the type of primary immunosuppressive drugs received as other data reflecting the grade of underlying inmunosuppression (≥episode of acute rejection, use of steroid boluses, use of OKT3 antibodies, and opportunistic infection) were also similar in both groups. Patients diagnosed of TB had higher serum creatinine levels at the time of diagnosis than that in controls at the same time (1.83 ± 0.53 vs. 1.43 ± 0.6 mg/dl; $P = 0.017$), and the percentage of HCV-positive patients was significantly higher in cases than in controls (56.3% vs. 18.8%; $P = 0.02$) as was the presence of any degree of liver dysfunction (44% vs. 0%; $P < 0.00001$).

On account of paucity of events in the study cohort, we could not perform a unique multivariate logistic regression model including all the major potential risk factors. We performed an exploratory analysis of major significant variables obtained in the univariate analysis adjusting each variable with all other variables by performing different logistic regression models that included a maximum of two variables. The potential risk factors that were constantly retained in the different models were the presence of HCV infection ($P = 0.003$; OR = 6.5; 95% CI 1.9–23) and a serum creatinine over 1.5 mg/dl ($P = 0.03$; OR = 4.8; 95% CI 1.1–21).

Discussion

Tuberculosis is an important infection in patients with solid organ transplantation [7], especially in developing countries, where there are high incidences of morbidity and mortality [8]. The estimated incidence of TB among KTR in Spain has been 219/100 000 patients/year (data from the Spanish Network for Transplantation, RESITRA http://www.resitra.retics.net/web/index.asp, not published), a figure almost six times higher than our national average for the general population, which has been estimated to be 38 cases per 10^5 habitants/year [1].

The risk factors for TB in transplant patients are as yet poorly defined. We performed a case–control study

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Table 2. Characteristics of kidney transplant recipients with tuberculosis (TB) compared with controls.

	Cases (n = 16)	Controls (n = 64)	P-value
Male, *n* (%)	10 (62.5)	39 (70)	0.8
Age, mean (SD)	39.8 (13.2)	41.7 (13.6)	0.6
Diabetes mellitus, *n* (%)	1 (6.3)	2 (3.1)	0.8
Time in hemodialysis (months), mean (SD)	39.4 (36.4)	33.9 (28.8)	0.5
Etiology of CRI, *n* (%)			
Glomerulonephritis	6 (37.5)	21 (32.8)	0.9
Other cause	10 (62.5)	43 (67.2)	0.9
Second transplant, *n* (%)	2 (12.5)	6 (9.4)	0.9
HBV+, *n* (%)	0 (0)	5 (7.8)	0.2
HCV+, *n* (%)	9 (56.3)	12 (18.8)	0.02
Liver dysfunction	7 (44%)*	0	<0.00001
CMV+, *n* (%)	15 (94)	59 (92)	0.9
Previous TB, *n* (%)	1 (6.3)	1 (1.6)	0.8
Alteration in chest X-ray, *n* (%)	0 (0)	3 (4.6)	0.8
Previous prophylaxis against TB, *n* (%)	0 (0)	0 (0)	
Primary immunosuppressive drug, *n* (%)			
Cyclosporine	10 (62.5)	41 (64)	0.9
Tacrolimus	3 (18.8)	16 (25)	0.8
Azathioprine	3 (18.8)	7 (11)	0.9
Serum creatinine (mg/dl)†, mean (SD)	1.83 (0.53)	1.43 (0.6)	0.007
Serum creatinine > 1.5 mg/dl†, *n* (%)	12 (75)	25 (39)	0.02
≥1 episode of acute rejection†, *n* (%)	6 (37.5)	25 (39.1)	0.8
Use of steroids boluses†, *n* (%)	9 (56.2)	28 (43.7)	0.5
Use of OKT3 or ATG†, *n* (%)	2/6 (33)	9/25 (36)	0.9
Opportunistic infections†, *n* (%)	2 (12.5)	5 (7.8)	0.7

HBV, hepatitis B virus; HCV, hepatitis C virus; CMV, cytomegalovirus; CRI, chronic renal insufficiency.

*All of them with mild liver dysfunction (Child-Plug A).

†At the time TB was diagnosed (in patients) and at the same time after transplantation (in controls).

in order to clear the role of several possible factors influencing the onset of TB in KTR.

Although elder people are relatively immunocompromised, the mean age of persons with TB was similar to the control group, showing that age, *per se*, may not be an independent risk factor. Similarly, TB was seen with the same frequency in men and women in our study. As it has been suggested by other authors [8,9] it appears to be plausible that intensification of immunosuppression for a failing graft has an important role for developing TB, but this point was not entirely clear from our data. Although mean serum creatinine was significantly higher in case patients than in controls, which suggests that these patients could have needed a more intensive immunosuppression for controlling renal function, the type of immunosuppressive drugs used, the percentage of patients with more than one acute rejection, and the number of acute rejection episodes per patient requiring the use of steroid boluses were similar in both groups. Other authors [2] have reported an increased number of post-transplant rejection episodes in KTR who develops TB, but the degree of immunosuppression in these patients (evaluated as the total number and doses of immunosuppressive drugs received) was not higher than that recognized for the control group. Use of CsA have been related to early post-transplant TB [10,11], although in other reports, immunosuppression with tacrolimus and mycophenolate mofetil was also associated with an early development of TB after transplantation [12]. Some authors have suggested that the use of antilymphocyte antibodies (especially OKT3) increases the risk of TB dissemination [13,14], as occurs in mice [15]. Our data do not concur with this suggestion, because the number of steroid-resistant rejection episodes requiring OKT3 use was also similar in patients and controls. Other surrogate markers of underlying immunosuppression in our study as are the presence of opportunistic infections or a more prolonged duration of pretransplant hemodialysis were not associated with a higher risk of TB.

The two statistically significant risk factors favoring the development of TB in KRT in our series was the presence of HCV infection and chronic graft dysfunction (serum creatinine over 1.5 mg/dl). This is apparently a nonsurprising result because chronic renal insufficiency has been

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Table 3. Multivariate analysis of risk factors for tuberculosis (TB) in kidney transplant recipients.

	Multivariate analysis		
	OR	95% CI	*P*-value
HCV+	6.9	1.9–23	0.003
Serum creatinine > 1.5 mg/dl*	4.8	1.1–21	0.03

HCV, hepatitis C virus.
*At the time TB was diagnosed (in cases) and at the same time after transplantation (in controls).

described as an important risk factor for TB [4]. HCV infection is the main cause of chronic liver disease after renal transplantation, and chronic liver disease is a well known risk factor for TB [10]. Otherwise renal disease is a well known complication of HCV infection after renal transplantation [16], and it could have contributed to chronic deterioration of renal function in some of our cases. However, in our opinion, HCV infection by itself might play an outstanding role contributing to increase the immunosuppression in KRT. In a previous study, we demonstrated that HCV infection has a direct role influencing cellular immune response in KRT and favoring a lower rejection rate and an increased rate of opportunistic infections in this population [5]. In the same way, the deteriorated cellular immune response on account of HCV infection would facilitate the development of intracellular infections, including TB. In fact, there were no patients with symptoms, signs or laboratory findings suggestive of severe chronic liver disease among our HCV-infected KRT and liver dysfunction was not retained in the final multivariate model so that the contribution of HCV infection to the development of TB should be explained mostly by the immunomodulative effect of the virus. In a previous descriptive report, HCV-infected patients showed a four times greater tendency to develop TB after renal transplantation [17], but to our knowledge this is the first case–control study that by multivariate logistic regression analysis demonstrates an increased risk of post-transplant TB in KTR infected with HCV (Table 3).

Prevention of TB in organ transplantation is a complex issue and the description of HCV infection as a new risk factor for TB in KTR makes our decision even more complex as to the use of prophylaxis with INH in this population. This is a controversial issue on account of the high risk of hepatotoxicity in these patients. KTR are often anergic before transplantation on account of the prolonged time in dialysis, thereby making preoperative screening with PPD skin tests frequently unrevealing. This may explain why pretransplantation PPD skin tests were not generally used in this series. Indeed, PPD skin testing was performed in only 18.7% of our patients.

Our study had some limitations, the most important of them is the small number of outcomes in this case–control study because of the relative uncommon nature of TB after renal transplantation in our country [7]. Given the retrospective nature of this study, it was susceptible to bias, however many of the variables analyzed were objective in nature, so this effect was limited. Taking into account that the majority of the patients had mild HCV hepatitis, HCV viral load was only performed in a minority of the patients and, therefore, such information was not included in this study. Finally discordant findings may be attributable to geographic factors and to the different immunosuppressive drugs and protocols used along the period of time analyzed**.**

In summary, this study confirms that chronic graft dysfunction and HCV infection are two important risk factors for TB in KTR. The effect of HCV infection does not appear to be related with the development of chronic liver disease, which suggested the existence of complex pathogenic immunomodulator mechanisms in HCV infection which favored the development of TB in these patients.

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Authorship

TJ: Performed research and wrote the paper. JMA: Designed research and wrote the paper. RSJ: Analyzed data and wrote the paper. AA: Designed research. PS: Collected data. FL-M: Collected data. JMM: Designed research.

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