Transpl Int (2002) 15: 393–396
DOI 10.1007/s00147-002-0410-4

ORIGINAL ARTICLE

Lymphocyte subsets and assessment of cancer risk in renal transplant recipients

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Received: 6 February 2001
Revised: 7 September 2001
Accepted: 26 February 2002
Published online: 21 August 2002

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Abstract Renal transplant recipients have a well-recognized increased risk of de novo neoplasia. In this study, we investigated whether lymphocyte subset count could predict the risk of developing noncutaneous neoplasia (NCSC) in renal transplant recipients (RTR). Between January 1995 and December 1995, lymphocyte subsets (CD4, CD8, CD19) were measured in 281 RTR. This population was studied until November 1999 for the development of NCSC. The mean follow-up was 42 ± 9 months. Neoplasm was diagnosed in 22 patients (7.9%). Patients who developed a cancer were significantly older (53.8 ± 6 years vs 38 ± 16 years, $P < 0.0001$), had lower CD4 (234 ± 126/mm^3 vs 543 ± 214/mm^3, $P < 0.005$) and CD19 (19 ± 9/mm^3 vs 51 ± 22/mm^3, $P < 0.0001$) levels, and more frequently had past histories of skin cancer (24% vs 4%, $P < 0.01$). Cox regression revealed that high CD4 levels (RR 0.73, 95% CI 0.62–0.89 for each 100/mm^3 increase in CD4 cell count) were associated with decreased risk of NCSC, whereas age (RR 2.49, 95% CI 1.12–5.92 for each 10-year increase in age) was predictive of the subsequent development of NCSC. To conclude, CD4 cell depletion is associated with the development of solid cancers and lymphoma in RTR.

Keywords Renal transplantation · Cancer · Immunosuppression · Lymphocyte subsets

Introduction

Renal transplant recipients (RTR) have a well-recognized increased risk of de novo neoplasia [10, 13]. The main factors affecting the risk of de novo cancer in these patients are age, gender, and length of exposure to immunosuppressant drugs after transplantation [2, 3]. Immunosuppression is supposed to be a major factor in the increased rate of neoplasia in the setting of transplantation, especially for virus-induced malignant disorders. Diminished immune surveillance of virus-transformed cells, increase in the frequency of viral infection, and in-viral load in transplant recipients might explain this result [11]. Nevertheless, the risk of non-virus-linked cancer is also increased in transplant patients [10].

Moreover, an increasing incidence of cancer with the exposure to multiple immunosuppressive drugs has been reported with the introduction of new drugs during the last years (calcineurin inhibitors, mycophenolate mofetil, monoclonal antibodies) which have significantly improved patient and allograft survival after renal transplantation. Two explanations may account for this increased incidence of cancer. First, as long-term survival is improving, the length of exposure to immunosuppression is also increasing. Second, the immunosuppressive properties and the dosage of the drugs may have important and early consequences. Thus, recent reports have suggested that the link between cancers and immunosuppression might be dose-related, even in the early post-transplant period [3, 7]. As a consequence, immunosuppressive therapy is believed to be the most

important and the only modifiable risk factor for the development of cancers in RTR [3]. Nevertheless, it remains difficult to establish the actual influence of overimmunosuppression and to quantify the individual risk of complications linked to immunosuppressive therapy.

We previously reported that CD4 lymphocytopenia was associated with the occurrence of skin cancers in long-term RTR [5]. We also showed that CD4 cell depletion was associated with late opportunistic infection [4] and monoclonal gammopathies [6], suggesting that CD4 cell count may be a marker of overimmunosuppression in this population.

In this study, we investigated whether the lymphocyte subset count could predict the risk of developing noncutaneous solid cancer (NCSC) in RTR.

Subjects and methods

Between January 1995 and December 1995, 281 RTR had lymphocyte subset determination in our center. Because there are great variations in lymphocyte subset counts in the first year posttransplant, we only studied patients transplanted more than 1 year before inclusion.

This population was studied until November 1999 for the development of neoplasia. Only histologically confirmed neoplasias (NCSC and lymphoma) were included in the study.

Clinical parameters (age, gender, primary nephropathy, dialysis duration before transplantation, transplant duration, immunosuppressive regimen, past history of acute rejection, past history of cutaneous cancer) and biological parameters (serum creatinine concentration, lymphocyte subset) were obtained through medical records.

The majority of the patients (92%) had received induction by polyclonal antithymocytes. Patients were classified according to both the use of induction and the type of immunosuppressive association used. Both ATG Fresenius (total dose 21 mg/kg) and thymoglobulin Merieux (total dose 8 mg/kg) were used. Three maintenance immunosuppressive therapies were used: cyclosporin A (CsA), (AZA), and prednisone (88%); CsA and prednisone (5%); and AZA and prednisone (7%). Mean CsA through levels were 101 ± 26 ng/ml. Mean azathioprine dosage was 64 ± 29 mg/day. Mean steroid dosage was 5 ± 1 mg/day.

Acute rejection episodes were initially treated with intravenous methylprednisolone (5 mg/kg for 3 days with progressive tapered doses). Corticosteroid-resistant acute rejection was treated by OKT3 for 10 days.

Lymphocyte subset determination

Lymphocyte subsets were measured by flow cytometry. Total blood samples were incubated with anti-CD4, anti-CD8, and anti-CD19 monoclonal antibodies labeled with fluorescein isothiocyanate (FITC) (Diaclone, France). After red cell lysis, the samples were analyzed on a facs calibur flow cytometer (Becton-Dickinson). A repeated measurement was performed 3 months after the first one. CD4 cell count was the mean of the two values. CD4 lymphocytopenia was defined by a CD4 cell count persistently less than $300/mm^3$.

Statistics

Student's t-test was used for comparing differences between groups, and the Spearman test was used for estimating relationships between variables. The ordinal data were analyzed using a chi-squared test.

We analyzed the incidence of cancers in two groups of patients having CD4 cell count of either $> 300/mm^3$ or $< 300/mm^3$. Results are expressed as mean $\pm$ SD.

Univariate Cox proportional hazards analysis was carried out to select among some parameters that were linked with the development of NCSC. We first performed a univariate Cox analysis, keeping covariates with P values of < 0.2 to include in the model. The selected covariates ($P < 0.2$) were then included in the Cox model, and a backward stepwise selection process was performed. Because the time from kidney transplant to entry in the study varied among patients, transplant duration was forced into the Cox model. This way, we took this variation into account.

Results

The mean follow-up was 42 ± 9 months. At the end of the study, 236 patients (84%) were still alive with a functioning graft, 22 (7.8%) were on dialysis, and 23 (8.2%) were dead.

A neoplasia had been diagnosed in 22 patients (7.8%) during the study period (1.9 cancer/1000 patient-months). Four patients (18%) had lymphoma and 18 (82%) NCSC (colon 5, lung 4, kidney 3, uterus 2, breast 2, ovary 1, tongue 1). Ten patients suffering from neoplasia (45%) died during the study period. The mean interval between CD4 count and the diagnosis of cancer was 23 ± 15 months.

Table 1 depicts clinical and biological characteristics of the patients with and without cancers.

Patients who developed cancers were significantly older (53.8 ± 6 vs 38 ± 16, $P < 0.0001$), had lower CD4 levels (234 ± 126 vs $543 \pm 214/mm^3$, $P < 0.005$) and CD19 levels (19 ± 9 vs $51 \pm 22/mm^3$, $P < 0.0001$), and more frequently had past histories of skin cancer (24% vs 4%, $P < 0.01$).

The immunosuppressive regimen did not differ between patients who developed cancers and those who did not. The cumulative dose of polyclonal antibodies was similar in patients with and without neoplasia. There was no difference in primary nephropathy. Especially, the prevalence of glomerulonephritis was nearly the same in the two groups (18% vs 15%, $P = 0.42$).

Eleven cancers (20%) occurred in 54 patients with CD4 cell counts of $< 300/mm^3$ and eleven (4.9%) in 227 with CD4 cell counts of $> 300/mm^3$ ($P < 0.01$). The positive and negative predictive values of CD4 cell

Table 1. Characteristics of the patients with and without cancer

	Patients with cancer	Patients without cancer	*P* value
Age (years)	53.8 ± 6	38 ± 16	< 0.0001
Gender (M/F)	15/7	173/86	0.15
Transplant duration (months)	72 ± 46	78 ± 50	0.45
Hemodialysis duration (months)	24 ± 12	21 ± 15	0.21
Past history of acute rejection	21%	18.5%	0.28
Serum creatinine concentration (çmol/l)	127 ± 32	156 ± 47	0.09
CD4 (/mm^3)	234 ± 126	543 ± 214	< 0.0005
CD8 (/mm^3)	328 ± 265	640 ± 302	0.1
CD19 (/mm^3)	19 ± 9	51 ± 22	< 0.0001
Past history of skin cancer	24%	4%	< 0.01

counts of < 300/mm^3 for the subsequent development of a neoplasia were 20% and 95%, respectively.

Cox regression revealed that high CD4 levels (RR 0.73, 95% CI 0.62–0.89 for each 100/mm^3 increase in CD4 cell count) were associated with decreased risk of NCSC, whereas age (RR 2.49, 95% CI 1.12–5.92 for each 10-year increase in age) was predictive of the subsequent development of NCSC. CD19 levels' effect on the development of NCSC was near significance (RR 0.87, 95% CI 0.59–1.02 for each 10/mm^3 increase in CD4 cell count, $P = 0.07$) (Table 2). When excluding lymphoma from analysis, results did not change for CD4 cell count (RR 0.71, 95% CI 0.61–0.85 for each 100/mm^3 increase in CD4 cell count) and age (RR 2.89, 95% CI 1.27–6.37 for each 10-year increase in age). By contrast, CD19 cell count was not more associated with the occurrence of cancer (RR 0.94, 95% CI 0.68–1.34 for each 10/mm^3 increase in CD4 cell count, $P = 0.17$).

Discussion

Our study demonstrates that lymphocytopenia and especially CD4 lymphocytopenia are associated with the occurrence of noncutaneous malignancy in long-term RTR. This result is concordant with our previous reports on the relationships between CD4 cell count and different kinds of complications of immunosuppression in the transplant population [4, 5, 6]. Thus, CD4 cell count could represent an objective marker of overimmunosuppression in RTR.

A dose-effect relationship between cancer and immunosuppression has been previously suggested. Indeed, Dantal and colleagues [3] reported the development of cancer in two groups of patients who had been allocated, at 1 year after transplantation, to either a low- or a high-target therapeutic range for CsA. They showed that the high-target-range group had more cancers. Even though a specific role of CsA was recently suggested [15], the possibility that this reported effect was restricted to the use of CsA seems highly unlikely. The global quantity of immunosuppression, that is the combination of the individual genetic background and the sum of immunosuppressant drugs, is more likely to account for the increased rate of cancer in the high-target-range group. CD4 cell count may at least partly reflect the global immunosuppression of RTR.

The reasons for the persistence of low CD4 cell count in some RTR remain unclear. Muller et al. [12] demonstrated that polyclonal antilymphocyte globulins may induce persistent changes in lymphocyte subsets characterized by low CD4 cell count with CD8 expansion. Because most of our patients had received induction with polyclonal antithymocyte globulins, it remains difficult in our study to assess the specific role of this treatment in CD4 lymphocytopenia and in the occurrence of NCSC. Nevertheless, other causes of CD4 lymphocytopenia are unlikely. Suppression of cell-mediated immunity by infectious agents other than human immunodeficiency virus has long been recognized as a cause of CD4 lymphocytopenia. Indeed, a variety of acute and chronic infections may be associated with the depletion of CD4 cells, which is usually transient and accompanied by CD8 lymphocytosis [12]. Finally, both corticosteroids and antimetabolites (azathioprine, mycophenolate mofetil) may induce CD4 lymphocytopenia.

An unresolved question is whether CD4 lymphocytopenia is just a marker of overimmunosuppression or whether it directly participates in the increased risk of cancer in RTR. Indeed, it is conceivable that the diminution of T cell mediated immune surveillance might allow neoplasic cells to get out of control. In lymphoma, suppression of the control systems on B cell proliferation for a prolonged period might also play a major role in the development of the disease. In this hypothesis, an increase in CD4 cell count could be associated with a decrease in the risk of developing complications linked to immunosuppression. Even when we could not

Table 2. Parameters associated with the occurrence of NCSC (Cox regression analysis)

Parameters	RR	CI
CD4 levels (for each 100/mm^3 increase in CD4 cell count)	0.73	0.62 to 0.89
Age (for each 10-year increase in age)	2.49	1.12 to 5.92
CD19 for each 10/mm^3 increase in CD4 cell count)	0.87	0.59 to 1.02

demonstrate a relationship between the number and dosage of immunosuppressive drugs and CD4 cell count, there is some evidence that immunosuppressant therapy, and not only past use of polyclonal antithymocyte globulins, interferes with lymphocyte subsets. We recently observed a significant decrease in CD4 cell count in RTR who underwent conversion from azathioprine to mycophenolate mofetil because of chronic allograft dysfunction (unpublished data). Moreover, there is a trend towards an increase in CD4 cell count after azathioprine withdrawal in patients with profound CD4 lymphocytopenia under triple conventional immunosuppression (unpublished data). As a consequence, in view of the consequences of CD4 lymphocytopenia, physicians should probably consider the possibility of decreasing immunosuppressive therapy in transplant patients with low CD4 levels.

Even when not significant in multivariate analysis, we observed a trend towards a lower CD19 cell count in patients who subsequently developed NCSC during the follow-up period. This effect was almost entirely due to the association between low CD19 cell count and the occurrence of lymphoma. Another important result is that patients with past histories of skin cancer seem more prone to developing other malignancies. This result is in accordance with our previous results on the association between CD4 lymphocytopenia and skin cancers in RTR [5]. Some forms of cancers have been also reported with a higher incidence in patients with a past history of skin cancers [1, 8, 14]. Even when genetic factors may influence the successive development of these tumors, viral infections and overimmunosuppression are likely to be the two main factors responsible for this association.

CD4 cell depletion is associated with the development of solid cancers and lymphoma in RTR. Lymphocyte subsets should be assessed in long-term RTR to estimate the risk of overimmunosuppression and to guide decreases in immunosuppressive therapy. Other studies are required to appreciate the effect of these strategies on both CD4 cell count and CD4 lymphocytopenia-related complications.

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