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Posttransplant lymphoproliferative disorders in renal allograft recipients: report of 53 cases of a French multicenter study

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Abstract New immunosuppressive therapies are currently being developed in renal transplantation and their relative risk in terms of posttransplant lymphoproliferative disorders (PTLD) must be carefully evaluated. For this purpose, a French registry of PTLD occurring after renal transplantation was set up. Among 10000 patients presently followed up in 30 French renal transplantation centers, we prospectively identified 53 new PTLD (0.5%) since January 1998. Patients (34 male, 19 female) ranged from 3 to 72 years (mean age: 46 years), and the median time between grafting and diagnosis of PTLD was 63 months (2 months to 14 years). Ninety percent of recipients were Epstein-Barr virus (EBV) positive before transplantation. Most patients received a quadruple sequential therapy with polyclonal anti-lymphocyte globulin. Sites involved in PTLD were isolated lymph nodes in 13 cases, stomach or bowel in 10 cases, allograft in 14 cases, central nervous system in 6 cases, oropharynx in 4 cases, and skin or mucosa in 4 cases. Only three PTLD expressed markers of T lineage. Out of 40 studied tumors, 31 (78%) were EBV positive. Tumors were classified as polymorph in 26 cases and monomorph in 23 cases. Genotype studies in 18 PTLD showed a monoclonal pattern in 13 cases. In most patients, treatment consisted of reduction of immune suppression, 21 patients were given additional anti-viral therapy, 13 patients had anti-CD20, 23 patients underwent chemotherapy, and 4 patients were given cerebral radiotherapy. Five patients underwent transplantectomy. Sixteen patients (30%) died within the 1st year and 7 patients returned to dialysis (13%). The outcome of patients with PTLD remains poor, and the optimal approach to therapy is largely unknown. This ongoing registry is not only a national observatory but also a task force designed to improve the treatment strategy of PTLD.

Key words Posttransplant lymphoproliferative disorders · Renal transplantation · Chemotherapy

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Introduction

It is well established that renal transplant recipients under immunosuppressive therapy have an increased risk of developing de novo malignancies. The most common malignancies observed are skin cancers and posttransplant lymphoproliferative disorders (PTLD). The risk for a renal allograft recipient to develop a lymphoproliferative disorder is estimated to be about 30–40 times greater than that of general population [11, 13].

In kidney transplant recipients, PTLD occurred in about 1% of patients [5]. PTLD comprise a group of disorders ranging from a benign self-limiting form of lymphoproliferation to aggressive and widely disseminated disease. It is usually associated with B cell proliferation, although T cell and null cell lesions have been described. Histologically, these tumors include both polyclonal B cell hyperplasia without cytogenetic abnormalities and more typical monoclonal lymphomas with clonal chromosomal abnormalities [7]. Intermediate forms with variable clonality, invasiveness, and cellular atypia are common.

It has been hypothesized that this complication arises from impaired immune surveillance, chronic antigenic stimulation from the allograft, oncogenic effects of immunosuppressive therapy, oncogenic role of Epstein-Barr virus (EBV) or a combination of these factors [3, 4, 6]. PTLD appear to differ in pathogenesis, clinical presentation, histopathological findings, response to treatment, and evolution from malignant lymphoma occurring in immunocompetent patients. Given these differences, and in order to better understand the lymphomagenesis and to propose an adapted and uniform therapeutic strategy, we carried out a prospective multicenter study to evaluate the clinical, virological, and histopathological characteristics, the course, and the therapeutic outcome of PTLD occurring in kidney allograft recipients of French transplantation centers within 1 year.

Patients and methods

A registry was initiated by a French scientific committee to obtain a consensus follow-up chart. All the French transplantation centers were asked to declare all their new cases of PTLD and to fill out a form sheet for clinical and biological data.

Patients

Between 15 January 1998 and 15 January 1999, 51 kidney allograft recipients were newly diagnosed with PTLD, two more patients, whose lymphomas were diagnosed shortly after this period were also included for clinical description. Patients were evaluated prospectively at one of the 30 French kidney transplant institutions for age at PTLD diagnosis, sex, time elapsed between transplantation and PTLD diagnosis, viral status, number of rejection episodes, and immunosuppressive regimens.

Clinical evaluation

Baseline evaluation was completed at diagnosis. All patients in whom PTLD was diagnosed before death underwent a thorough work up to detect lymphoproliferative sites, including CT scans of the chest, abdomen, pelvis, and brain, bone marrow biopsy, and cerebrospinal fluid analysis or gastrointestinal endoscopy according to clinical belongings. Sites of disease are classified as previously proposed [8]: (1) exclusive lymphoreticular disease, with disease limited to lymph nodes, spleen, liver, and bone marrow, and (2) extralymphoreticular disease, with all locations other than the aforesaid areas, and disease in both sites. A subclass of PTLD involving kidney allograft was considered apart given their particular clinical course.

Histopathology and immune phenotype

Diagnosis of PTLD was based on the histology of the material obtained by core needle biopsy, surgical biopsy, or analysis of a resected specimen. The following two types were distinguished: (1) polymorph PTLD with a spectrum of lymphoid cells and (2) monomorph PTLD, mostly consisting of immunoblasts. Immune phenotype studies were done using Ig staining. Clonality of B cell and T cell was assessed by rearrangement studies of the Ig gene and of the gene for T cell receptor β chain on tumor specimens whenever technically feasible.

EBV studies

EBV serological markers were studied and immunohistological studies with antibodies against EBNA2 and LMP1 were performed to detect EBV antigens in tumor specimens. In some samples, presence of EBV RNA was also determined using a more sensitive in situ hybridization technique with EBER1 probe.

Treatment

Treatment modalities of PTLD varied according to the different transplant centers and were documented, ranging from the simple dose reduction in immunosuppressants combined with antiviral therapy to aggressive chemotherapy, surgery, and radiotherapy. Standard response criteria were used to define complete remission, partial remission, absence of response, and progressive disease.

Results

Incidence

Of about 10000 kidney transplant recipients followed up in 30 transplantation centers, 53 cases of PTLD were diagnosed and declared by 24 centers yielding an annual incidence of about 5‰. PTLD occurred in 49 first allograft recipients, 3 recipients of a second allograft, and 1 third allograft recipient. Two patients had received a living allograft from a related donor, the remaining 51 patients had a cadaver allograft.

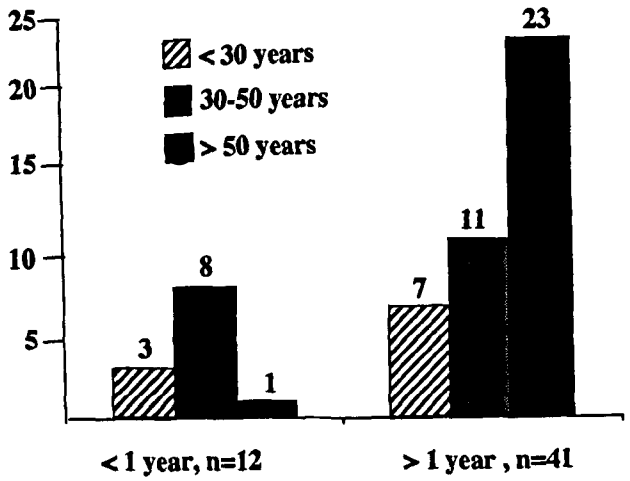

Fig. 1 Age and delay after transplantation at time of diagnosis

Clinical characteristics

At the time of PTLD diagnosis, patients' age ranged from 3 to 72 years. Ten patients were younger than 30 years, 19 patients were aged between 30 and 50 years, and 24 patients were older than 50. The male to female sex ratio was 1.9:1 (34/19). PTLD was diagnosed between 2 months and 14 years (median 63 months) after transplantation. In 12 patients this interval was less than 1 year (Fig. 1). Recipient EBV serology before transplantation was negative in 5 cases (10%).

Immunosuppressive regimens varied because of the different protocols used in the centers. Eighty-five percent of patients (45/53) received a sequential quadruple therapy consisting of anti-thymocyte globulin (ATG) or OKT3 (5 patients), steroids, azathioprine ($n = 38$) or mycophenolate mofetil (MMF; $n = 6$), and cyclosporine ($n = 43$) or FK506 ($n = 2$). Six were switched to MMF and 4 to MMF and FK506. Eight patients were on triple therapy using ATG/azathioprine/cyclosporine ($n = 1$), ATG/steroid/cyclosporine ($n = 2$), ATG/cyclosporine/MMF ($n = 1$), or steroid/azathioprine/cyclosporine ($n = 4$). One patient was switched to FK 506 and 1 to MMF. Finally, 44 patients received ATG and 5 OKT3 for induction, 17 patients were treated by MMF, and 7 by tacrolimus. One patient had received anti-LFA1 at the time of transplantation.

Rejection episodes

Nineteen patients (35%) sustained one (13 cases) or more (6 cases) episodes of allograft rejection prior to diagnosis of PTLD. All but 4 patients were treated by steroid bolus. Only 4 patients received anti lymphocyte antibodies as either ATG (2 patients), OKT3 (1 patient), or ATG and OKT3 (1 patient) for rescue therapy.

Table 1 Sites of posttransplant lymphoproliferative disorders

Lymphoreticular	13	Extralymphoreticular	40
Associated with:		Hilar graft	10
Spleen or liver	6	Parenchymal graft	4
Bone marrow	3	Esophagus (1), stomach (3)	4
		Small or large bowel	7
		Pancreas	2
		Brain	6
		Pharynx	4
		Skin or mucosa	4
		Lung	2
		Pleural effusion and ascites	2

Sites of PTLD involvement

The locations are summarized in Table 1. Four patients presented with a malignant mononucleosis-like syndrome. Extralymphoreticular disease was most common ($n = 30$) involving esophagus ($n = 1$), stomach ($n = 3$), small or large bowel ($n = 7$), pancreas ($n = 2$), brain ($n = 6$), pharynx ($n = 4$), skin or mucosa ($n = 4$), and lung ($n = 2$). Disease confined to the lymphoreticular system was less frequent ($n = 13$) with spleen or liver involvement in 6 cases and bone marrow involvement in 3 cases. PTLD was located at the hilar graft exclusively in 10 cases, and in 4 other cases the renal graft was involved along with other extrarenal sites. Two patients had pleural effusion and ascites. Eleven patients presented signs of disease in two or more sites.

Renal function

Thirty-two patients (60%) had renal insufficiency (serum creatinine > 120 µmol/l) at the time of diagnosis presenting in some cases with the features of a rejection episode.

Histological findings

The immune phenotype of the lymphoid cells was studied in 52 of the 53 cases. Forty-nine (94%) expressed immunological markers of B cell lineage whereas three PTLD were classified as T cell lymphoma. Morphological histopathological data were available for 49 studied specimens. Twenty-six lymphomas were classified as polymorph lympho-proliferations and were composed of small lymphocytes, small and large uncleaved cells, and a large proportion of immunoblasts, often showing plasmocytic differentiation. The other 23 cases were classified as monomorph proliferation with uniform large, uncleaved lymphocytes and immunoblasts with plasmocytic differentiation.

Clonality was assessed by detection of surface immunoglobulins kappa or lambda and genotype studies. Twenty-two cases were defined as monotypes, 5 as polytypes and 1 oligotype; 25 cases were undetermined. Genotype studies were performed in 18 cases, where monoclonality of the tumor was confirmed in 13 cases.

EBV studies

Only 5 patients were EBV seronegative before transplantation. The presence of EBV in the tumor was assessed in 40 PTLD. Thirty-one (78%) expressed EBV markers by immunochemistry or by in situ hybridization. Nine tumors were considered negative. In 13 cases EBV could not be determined because of the absence of a specimen or lack of sensitivity of the technique.

Therapeutic approaches and response

In all but 3 patients, immunosuppression was reduced or stopped, and steroids maintained as the only treatment. Cyclosporine or FK506 were reduced in about 50% of the patients and withdrawn in the other 50%. Azathioprine was stopped in 72% of the patients and reduced in 9%. Likewise MMF was stopped in most patients (91%) and only reduced in 1 patient. Steroid therapy was maintained in most patients (90%).

In one patient, diagnosis was made just before death, and in two others, treatment consisted of antibiotic therapy against *Helicobacter pylori* for MALT lymphomas. Antiviral treatment consisting of parenteral gancyclovir and oral valacyclovir was given in 21 patients. Thirteen patients had surgical excision when the tumor was located (5 transplant removals). Cerebral radiotherapy was used in 4 patients with isolated central nervous system lesions. Monoclonal anti-CD20 antibodies were given in 13 cases. This approach was considered effective in 7 cases, and ineffective in 3, whereas severe and even lethal effects were observed in 3 others. Finally, chemotherapy was chosen for 23 patients. Protocols varied according to the center including basically two associations: cyclophosphamide-doxorubicin-vincristin-prednisone (CHOP) and adriamycin-cyclophosphamide-vincristin-bleomycin-prednisone (ACVBP).

Remission duration and survival

Sixteen patients (30%) died. Deaths were related to multiorgan failure ($n = 6$), pancreatitis ($n = 1$), acute respiratory distress syndrome ($n = 1$), agranulocytosis after chemotherapy ($n = 2$), intracerebral bleeding ($n = 2$), small bowel perforation in 2 patients (after anti-CD20 administration in one case of small bowel PTLD), and unknown causes ($n = 2$). In these patients, time to death ranged from a few days after diagnosis to 12 months. Of note, 14 patients who died belonged to the group of the 41 patients with late PTLD diagnosed more than 1 year after transplantation (34%). Conversely, only 2 of the 12 patients with early PTLD (< 1 year) died rapidly after diagnosis.

In this short duration survey, 24 treated patients (45%) were considered to have either partial ($n = 5$) or complete remission ($n = 19$) with a functioning graft in 17 (32%) after 6 months. One patient experienced PTLD rapid reoccurrence after initial remission. Seven patients returned to dialysis after graft removal and/or interruption of immunosuppression. In 13 patients (25%), the followup was judged to be insufficient (< 6 months) to evaluate the response to treatment. Only 1 patient experienced acute allograft rejection after PTLD diagnosis.

Patients with allograft PTLD

Fourteen patients (26%) developed isolated allograft disease. In 12 cases allograft PTLD occurred early, i.e., within eighteen months after transplantation. Only 2 patients had a late occurrence of PTLD (99 and 104 months). In 4 cases the lymphoma involved the renal parenchyma, whereas in 10 the location was restricted to the graft hilum. In all patients, a decline of graft function was observed at the time of diagnosis and often revealed the disease. Nephrectomy was performed in 5 cases, mainly to allow withdrawal of immunosuppressive therapy. Of note, the extension check-up yielded one unique location in all which was the hilar graft PTLD.

A previous rejection episode was noted in only 4 patients. Patients with PTLD allograft involvement were treated with either cyclosporine (11/14) or FK506 (3/14) associated with MMF in 7 patients. All but one, PTLD were of the B phenotype. Histological analysis disclosed a polymorph aspect in six and monomorph in five (not reported in three), and by immunohistochemistry or clonal analysis seven were monoclonal. In nine out of ten samples the presence of EBV was shown. Treatment of allograft PTLD consisted in a reduction of the immunosuppressive drugs in all combined with an antiviral therapy in three. The graft was immediately removed in 2 cases to allow withdrawal of immunosuppressive therapy, and in 2 more patients after failure of a treatment by anti-CD20 monoclonal antibodies or chemotherapy. Four patients were treated with the anti-CD20 monoclonal antibody and 5 with aggressive chemotherapy. Finally, among the 11 patients with allograft PTLD with a sufficient followup, 2 died (1 on dialysis) and 3 returned to dialysis.

Discussion

In this study we have prospectively collected the data concerning new cases of PTLD in French transplant centers over 1 year. Twenty-four centers declared at least one PTLD yielding an estimated overall annual incidence of 0.5%, comparable to data published in numerous large studies [5, 11, 13]. Previous reports have pointed out the increased risk of developing PTLD in the organ transplant population with modern immunosuppressive regimens including an induction therapy with poly- or monoclonal antibodies, associated with an anti-calcineurin drug and/or MMF [12, 14]. Eighty-five percent of the patients of this series had been treated with this immunosuppressive approach. Nonetheless, the answer to what extent these immunosuppressive treatments favor the development of PTLD in renal transplant patients remains to be determined. Of note, we also did not find any evidence for a direct correlation with overimmunosuppression due to treatment rejection, since only a few patients received ATG or OKT3 for rejection therapy.

Diverse histopathological classifications have been proposed to describe non-Hodgkin lymphomas [2], but are in fact inadequate for PTLD lesions. Nalesnik [9] distinguished, as we did, morphological aspects of PTLD in polymorph or monomorph forms. More recently, Knowles proposed a classification based on molecular genetic characteristics, implicating the clonality, the type of EBV replication inside the tumor, and the presence or absence of tumor suppressor gene alteration [7].

Previous reports have outlined the high prevalence of PTLD located at the allograft site especially in heart, lung, and kidney transplant recipents indicating a possible role for the local immune response against the allograft [10, 11]. In our series, we found a high incidence of 26% of PTLD at the site of allograft (hilar or parenchymal; 14/53). Of note, most of the patients (12/14) developed their PTLD early after transplantation and diagnosis was almost always made in the context of graft failure. Particular characteristics of renal graft PTLD can be stressed. In most cases they are limited to the graft hilum, EBV-associated, of B phenotype, and monotypic. Remission was observed in some cases after simple reduction of immunosuppressants [16]. The place of a more aggressive therapy associated or not with graft removal is still to be defined.

PTLD is the result of a complex interaction between several factors including loss of surveillance mechanisms for B cell proliferation, chronic antigenic stimulation by the allograft, viral, i.e., mainly EBV oncogenesis, and accumulative action of immunosuppressants [3]. Most PTLD tumor cells in our series present an activated B cell phenotype and an unrestricted pattern of latent EBV gene products. Of note, only a few patients presented serum conversion for EBV leading to a primary infection.

There is no consensus on the treatment of PTLD other than reducing or discontinuing immunosuppression, which in some cases may lead to tumor regression [16]. Antiviral therapy was recommended by certain centers, especially in polyclonal EBV-associated PTLD [15], but with inconstant results in our series. In half the patients, immunosuppression was reduced and combined with an antiviral therapy, and a complementary treatment was initiated in most patients. Infusion of monoclonal anti-B cell antibodies was reported to be successful in extracerebral oligoclonal or monoclonal B cell PTLD [1]. In our series the efficacy of this immunotherapeutic approach was efficient in only half of the cases. Finally, opting for more effective treatment of PTLD, the place of the association of chemotherapy [17] and B cell monoclonal antibody treatment is yet to be defined.

There are only a few data on prognosis and survival of renal transplant patients with PTLD [8]. From the initial short followup of our study, it appears that the response to decreased immunosuppression associated with either recent chemotherapy regimen or monoclonal antibody administration is not as good as previously reported with a death rate of 30% in the first months after diagnosis similar to previous registries.

In conclusion, these preliminary data obtained from the French PTLD registry gave us the opportunity to report 53 cases arising within about 1 year in 30 French centers. The poor prognosis of these tumors remained a matter of concern for renal transplant physicians. The relatively homogeneous description of PTLD of the graft allows us to differentiate this type of lymphoma, both on clinical and histopathological grounds. A certain number of studies are actually underway to analyze the pathoanatomical findings. The principal goals of the working group are:

1. To identify the risk factors for the development of PTLD and clearly define the clinicoanatomical correlations.
2. To evaluate the impact of the reconstitution of an efficient immune response, especially anti-EBV, on the viral load in order to restrict the aggressive therapeutic indications to those cases where a mere reduction of immunosuppression fails.
3. To institute a multicenter trial aimed to assess the potential benefit of anti-monoclonal antibody therapy and chemotherapy, respectively, for a homogeneous therapeutic approach of PTLD.

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