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Maintaining calcineurin inhibition after the diagnosis of post-transplant lymphoproliferative disorder improves renal graft survival

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Post-transplant lymphoproliferative disorder (PTLD) is an uncontrolled proliferation of transformed lymphocytes fostered by immunosuppression. In addition to chemotherapy, treatment of PTLD includes a reduction of maintenance immunosuppression. Patients with PTLD have an increased risk of graft loss, suggesting that reduced immunosuppression strategy needs to be optimized with regard to graft outcome. Here we retrospectively reviewed 101 cases involving PTLD to identify the risks associated with graft loss. During a median follow-up of 70 months, 39 patients died and 21 lost their graft. Multivariate analysis found that an eGFR under 30 ml/min per 1.73 m^2 at PTLD diagnosis, a biopsy-proven acute rejection episode following reduction of immunosuppression, and the absence of calcineurin inhibition in maintenance immunosuppression are independent risk factors for allograft loss. Neither the type of PTLD nor the chemotherapy regimen was predictive of allograft failure. Histological analysis of graft biopsies showed that maintaining calcineurin inhibition after the diagnosis of PTLD reduced the risk of developing *de novo* anti-HLA antibodies and humoral rejection. Remarkably, calcineurin inhibitor maintenance was neither associated with higher mortality nor with worse progression-free survival. Thus, maintaining calcineurin inhibition at a reduced dose after the diagnosis of PTLD seems safe and may improve renal graft outcome, possibly through better control of the recipient's humoral immune response.

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With a cumulative incidence of 2.1% at 10 years, post-transplant lymphoproliferative disorder (PTLD) is the second most frequent neoplasia after renal transplantation.[1,2] This life-threatening disease, first described in the early 1980s by Starzl *et al.*,[3] corresponds to an uncontrolled proliferation of lymphocytes that can be triggered by various factors. Mismatch in Epstein–Barr virus (EBV) serology (that is, recipient seronegative/donor seropositive) represents the most important independent risk factor for early PTLD.[4] EBV genes indeed encode for several functional homologs of B-cell proteins involved in cell cycle regulation, inhibition of apoptosis, and signal transduction that allow the virus to induce the transformation of these cells.[5] On the other hand, in late PTLD, lymphocyte transformation is thought to depend on chronic antigenic stimulation of recipient cells by donor antigens.

Histologically, PTLD encompasses a heterogeneous group of disorders ranging from EBV-driven polyclonal proliferation to aggressive monomorphic proliferations. The classification published in 2008 by the World Health Organization (WHO) recommends classifying PTLD into four categories: early lesions, polymorphic PTLD, monomorphic PTLD, and classic Hodgkin's lymphoma type.

In all cases, proliferation of transformed cells is facilitated in transplanted patients because immune surveillance mechanisms are impaired by immunosuppressive drugs.[6] Reduction of maintenance immunosuppression, which allows partial reconstitution of antitumor immunity, is therefore widely considered as the first therapeutic step for PTLD.[3,7–9]

Not surprisingly, reconstitution of a recipient's cellular immunity following reduction of immunosuppression is also associated with an increased incidence of graft rejection episodes[8,10] and a 5.5 times higher rate of death-censored graft loss.[1,11] These findings underline the urgent need for optimization of immunosuppression reduction strategies that

should aim not only at increasing the probability of PTLD remission but also preserving renal graft function. In this regard, it is interesting to note that almost all published studies comparing therapeutic options for PTLD have focused on treatment efficacy rather than graft outcome.

One difficulty in comparing the different immunosuppression reduction strategies is the lack of standardization. Reduction of immunosuppression is indeed largely physician or transplant center dependent. In particular, if most transplant physicians agree to continue treatment with steroids after a PTLD diagnosis, no consensus has been reached regarding what should be done with calcineurin inhibitors (CNIs). Immunosuppressive reduction algorithms[12–14] in most cases include the reduction of the CNI dose (25 to 50% of baseline). However, based on experimental results, which demonstrate the direct proneoplastic effects of these drugs (reviewed in Guba *et al.*[15] and Thaunat and Morelon[16]), some authors have proposed stopping CNIs.

In this study, we retrospectively reviewed 101 cases of PTLD diagnosed in two French transplantation centers to identify the risk factors associated with renal graft loss. A particular effort was made to analyze the impact of the type of PTLD, the type of chemotherapy, and the maintenance immunosuppressive regimen on kidney graft survival and the outcome of PTLD.

RESULTS

Description of the study population

Since its first description,[3] 101 cases of PTLD were diagnosed in the two French university hospitals involved in the study.

The characteristics of the patients and PTLD are presented in Table 1.

Briefly, the period of transplantation extended from 1967 to 2008, the median age at PTLD diagnosis was 55 years (range: 14.5–72), and the median time from transplantation to PTLD was 9 years (range: 0.3–32.5).

Most of the PTLDs (82%) were monomorphic B-cell lymphomas, 50% of which were EBV related.

First-line therapy consisted of immunosuppression reduction for 93 patients (92.1%); immunosuppression reduction was not mentioned in the files of the remaining 8 patients. PTLD treatment consisted of immunosuppression reduction alone for 3 patients (2.97%), rituximab alone for 16 patients (15.84%), chemotherapy alone for 32 patients (31.68%), and rituximab + chemotherapy for 41 patients (40.59%). Surgery was performed in 14 patients (13.86%) and radiotherapy in 11 (10.89%).

Over the follow-up period (median 70 months), 39 patients died (Figure 1a). Overall survival of PTLD patients was 76% at 1 year and 65.4% at 5 years after PTLD diagnosis. In all, 70 patients obtained complete remission.

Renal graft survival was 95.3% at 1 year and 76.4% at 5 years after PTLD diagnosis (Figure 1b). After the diagnosis of PTLD, 21 patients lost their graft.

Identification of risk factors for renal graft failure in PTLD patients

To evaluate the impact of maintenance immunosuppressive regimen on renal graft outcome following the diagnosis of PTLD, we split the 101 patients into three groups. The first group included the patients for whom a CNI was maintained at a reduced dose ($n = 31$; CNI group). The mean percentages of dose and trough-level reduction for CNIs were, respectively, $35 \pm 24\%$ (Figure 1c) and $30 \pm 40\%$ (Figure 1d). The second group included patients who were treated with corticosteroids alone ($n = 54$; CS-only group). Patients who received a combination of immunosuppressive (IS) drugs without CNIs were gathered in the third group ($n = 16$; Other IS group). The characteristics of the patients of the different groups were not different at transplantation in terms of age at transplantation, age of the donor, type of donor, and human leukocyte antigen (HLA) mismatches (Table 1). The estimated glomerular filtration rate (eGFR) at PTLD diagnosis and the type and severity of PTLD were similar in the three groups, as was the immunological status of the patients, that is, HLA sensitization, previous episode of rejection, or cytomegalovirus infections (Table 1).

We observed that the maintenance immunosuppressive regimen after PTLD diagnosis had an influence on graft outcome: the risk of graft loss was increased for patients treated with corticosteroids alone as compared with patients for whom a CNI was maintained at a reduced dose (Figure 1e).

To confirm this univariate analysis, a multivariate analysis was conducted (Table 2). The eGFR of <30 ml/min per $1.73\,m^2$ at the time of PTLD diagnosis was identified as an independent risk factor for graft failure (hazard ratio (HR): 20.02 (2.92–137.15)). This expected result validates the notion that the 'quality' of the renal graft at the time of PTLD is highly predictive of transplantation outcome. Unfortunately, this nonmodifiable parameter cannot be targeted for therapy.

More interestingly, multivariate analysis also found that the occurrence of an acute graft rejection episode after the diagnosis of PTLD was an independent risk factor for graft failure (HR: 45.36 (7.94–258.89)), as was the nature of the maintenance immunosuppressive regimen. The risk for graft loss was indeed more than 20 times higher for patients on corticosteroids alone as compared with patients on CNIs. Despite a similar trend for patients maintained on a combination of immunosuppressive drugs without CNIs, the difference did not reach statistical significance owing to the heterogeneity and the small number of patients in this group ($n = 16$).

These results identify the intensity of recipients' alloimmune response as a major force driving renal graft destruction after a PTLD.

Histological analysis of graft rejection episodes

Among the 101 patients of the cohort, 13 presented an episode of biopsy-proven acute rejection after the diagnosis