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Time-dependent lymphocyte count after transplantation is associated with higher risk of graft failure and death.

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ABSTRACT

The transplantation field requires the identification of specific risk factors associated with the level of immunosuppression. Here, our aim was to analyze the association between the number of circulating lymphocytes, monitored routinely by complete blood cell counts during outpatient visits, and patient and graft survival. In total, 2,999 kidney or combined kidney-pancreas recipients transplanted between 2000 and 2016, from two University hospitals, were enrolled. We investigated the etiological relationship between time-dependent lymphocyte count beyond one year after transplantation and patient and graft survival, viral infection and cancer risk using time-dependent multivariate Cox models. Model 1 considered kidney function at one year and model 2 as time-dependent variable. At the time of inclusion (one year after transplantation), 584 patients (19.4%) had deep lymphopenia (under 750 /mm³) and 1,072 (35.7%) had a normal count (over 1,500 /mm³). A patient with deep lymphopenia at a given follow-up time had significantly higher risks of graft failure, death and viral infection than comparable patients with a normal lymphocyte count at the same time point. Thus, after the first year of transplantation, the occurrence of deep lymphopenia within a patient's follow-up is a risk factor for long-term graft failure, death and viral infection.

KEYWORDS : Post-transplant lymphopenia, graft failure, death risk, immune monitoring

INTRODUCTION

Kidney transplantation is still facing a high morbidity rate due to the use of immunosuppressive therapy ¹. Specifically, induction therapies may lead to lymphopenia ^{2,3} or blockade of lymphocyte activation, signaling and replication ⁴. In addition, the first year after transplantation is a high risk period for acute rejection, which might be treated using additional courses of immunosuppression. More specifically, after ATG induction, immune reconstitution is slow and can be delayed for up to more than two years ⁵. Long-term immunotherapies (CNIs, mycophenolate, steroids) can influence the re-expression of some T cell subsets such as regulatory T cells ⁶ and maintain various levels of immunosuppression in patients, leading to increased infectious and neoplastic complications and nephrotoxicity ^{7,8}. Conversely, a lack of immunosuppression can trigger graft-related immunological complications and shorten graft survival ^{9,10}.

Recently, studies have reported that the lymphocyte count could be a prognostic factor for invasive viral infections ^{11,12}. The frequency of some lymphocyte subsets could even correlate with the occurrence of de novo anti-HLA antibodies, including DSAs, and allograft rejection ^{13,14}. In HIV-positive kidney transplant recipients, low baseline CD4 counts were predictive of severe CD4 lymphopenia at one year after transplantation and were associated with higher probability of serious infection during the first six months after transplantation than high baseline CD4 counts ¹⁵.

Whereas long-term persistence of lymphopenia and its consequences have been well described, such as CD4 lymphopenia and occurrence of pneumocystis, skin cancer, atheroma and increased mortality ¹⁶⁻¹⁸, there is no large scale and longitudinal analysis of the whole lymphocyte count, and in particular of the effect of a long-term episode of lymphopenia on graft and patient outcomes. A recent study reported an association of long-term CD4 T cell lymphopenia, in 27 HIV-

negative kidney transplant recipients, with malignancy and accelerated decline in kidney allograft function ¹⁹.

We hypothesize that long-term lymphopenia could be associated with worse patient and graft outcomes. In this observational study, we investigated on 2,999 kidney transplant patients the etiological relationship between the time-dependent number of circulating lymphocytes (routinely counted during outpatient visits) and patient or death-censored graft survival. Our secondary endpoint was to establish an association between long-term lymphopenia and viral infections or cancer occurrence to assess the hypothesis that patients with the lowest lymphocyte count might be more exposed to infectious and neoplastic complications.

RESULTS

Demographic characteristics

Overall, 9,168 patients were screened and 4,395 met the inclusion criteria. After eliminating those with exclusion criteria, we enrolled 2,999 patients (Figure 1). The demographic characteristics are described in Table 1 and 2. After the first year of transplantation, 250 patients experienced a rejection episode (234 BPAR), 577 had a viral infection diagnosed, and 494 developed a cancer (details are provided in Table S1 and S2). At the time of inclusion (one year after transplantation), 584 patients (19.4%) had deep lymphopenia, 1,346 (44.8%) had mild lymphopenia and 1,072 (35.7%) had a normal lymphocyte count. Figure 2 represents the evolution of lymphocyte counts distribution at each year posttransplantation, Figure 3A shows the inpatient variability of lymphocyte count assessed by considering a representative 5% sample of randomly selected patients and Figure 3B shows the frequency of switching from one group to another all over the study follow-up. Within the follow-up period, 1,018 patients did not switch from one category to another, and 856 patients (28.5%) switched one time. The remaining 1,128 patients (37.6%) switched two times or more. In addition, as shown in Supplementary Figure 1 and 2, no sudden decrease of lymphocyte count occurred before final graft failure or death, respectively, and there was no relationship between the frequency of and the duration between visits and patient health state. The median number of biological collections was 5 (2 to 15) among patients alive with a functioning graft at the end of the follow-up versus 5 (2 to 16) among patients who died or lost their graft. The median duration between two visits was 367 (interquartile range from 350 to 392) days versus 367 (interquartile range from 347 to 401) in these groups of patients, respectively.

Time-dependent lymphopenia beyond 1 year after transplantation is associated with higher risk of graft failure

Among the 2,999 patients alive at 1-year posttransplantation, 423 lost their graft during follow-up. The death-censored graft survival values of the cohort at 2, 5, and 10 years

posttransplantation were 99.7%, 94.1%, and 78.5%, respectively (Supplementary Figure 3). At 10 years after transplantation, the graft survival values were 69.7%, 78.3% and 82.8%, respectively, for patients harboring deep lymphopenia, mild lymphopenia or a normal lymphocyte count at 1-year posttransplantation. Table 3 shows the results of the unadjusted analyses and the final multivariate Cox model for graft failure risk, using the number of circulating lymphocytes as a time-dependent covariate, unadjusted analyses and model 1 considered eGFR at one year and model 2 considered the renal function as time-dependent. From model 1, we observed a proportional relationship between the number of circulating lymphocytes beyond one year after transplantation and graft failure, with a 3-times higher risk of graft failure for patients with deep lymphopenia at a given follow-up time than for similar patients with a normal lymphocyte count at that follow-up time (HR 3.18, 95% CI 2.29-4.44, $p < 0.001$). The hazard ratio was 1.30 for patients with mild lymphopenia when compared to similar patients with a normal lymphocyte count (95% CI 0.99-1.71, $p = 0.057$). This association was independent of other confounding factors. In model 2, we observed that the effect size between the number of lymphocytes and the outcomes decreased but nevertheless, deep lymphopenia remained risk factor for graft loss (HR 2.21, 95% CI 1.58-3.09, $p < 0.001$).

Lymphopenia beyond one year after transplantation is associated with patient survival

During the first year after transplantation, 298 patients died with a functioning graft. Overall patient survival values (with graft failure censored), at 2 years, 5 years and 10 years were 99.7 %, 95.4 % and 84.9 %, respectively (Supplementary Figure 4). At 10 years, survival values were 87 %, 83.3% and 83.4%, respectively, for patients with a normal lymphocyte count, mild lymphopenia or deep lymphopenia at 1-year posttransplantation. The unadjusted analyses and the final multivariate Cox model using lymphocyte count as a time-dependent covariate are shown in Table 4. Patients with deep lymphopenia at a given time point were significantly more at risk of death than similar patients with a normal lymphocyte count at the same time point, independently of classical risk

factors for death when considering eGFR at one-year or as a time-dependent variable (HR 1.96, $p < 0.001$; and HR 1.91, $p < 0.001$, respectively) whereas patients with mild lymphopenia did not show this trend with risk of death. We performed other analyses to study the association between only the at one-year lymphocyte count and patient survival and graft survival (death censored) (Table S7 and S8 respectively) but we did not observe a significant relationship between lymphopenia and death risk or graft loss.

Lower lymphocyte level beyond one year since transplantation is associated with a higher risk of viral infection but not a higher risk of neoplasia

Starting at one year after transplantation to the end of follow-up, 577 patients developed a viral infection. Patients with deep lymphopenia were significantly more at risk of viral infections than patients with a normal lymphocyte count at the same time point (HR 2.18, CI 1.69-2.83 for Cox model 1 and HR 2.06, CI 1.59-2.67 for Cox model 2), regardless of the CMV serology mismatches between donor and recipient (data not shown). Regarding the treatment, neither the type of induction therapy nor the type of maintenance immunosuppressive therapy was associated with risk of viral infection in the multivariate model (Table 5). 494 patients were diagnosed with a neoplasia between the first anniversary of transplantation and the end of follow-up. The number of circulating lymphocytes (as a time-dependent variable) did not appear to be associated with cancer risk even for viro-induced skin cancer (Table 6 and S9). Induction or maintenance immunosuppressive therapies were not associated with cancer risk.

Finally, as we were not able to validate our observation in an external independent cohort, we modeled separately cohorts of Necker and Nantes centers to replicate our data internally at a lower scale and in two centers with different medical practices (Table 7). We confirmed our observations in both cohorts that deep lymphopenia is an independent risk factor of death-censored graft failure and late onset viral infection but not cancer. We only observed a discrepancy between the two centers for death without particular explanation.

DISCUSSION

We studied the potential etiological role of routinely monitored lymphocyte counts on patient and graft survival after kidney transplantation. Our results showed that half of the patients switched lymphocyte count category at least two times within their long-term follow-up beyond one year posttransplantation. When a patient's lymphocyte count was below 750 /mm³ at a given time of follow-up, the risk of graft failure and death was higher than that for a similar patient without lymphopenia at the same time point. However, if the patient with a low lymphocyte count subsequently returned to a normal lymphocyte count, he was no longer at risk. This observation suggests that lymphocyte count could be a risk factor for graft and patient outcomes. Finally, we showed that deep lymphopenia was not associated with occurrence of de novo neoplasia, but as expected, significantly increased the risk of viral infection, after adjusting for induction and maintenance immunosuppressive therapy. To inform a clinical point of view and for easier interpretation of our results in routine practice, we categorized lymphocyte level with thresholds based on empirical evaluation and the CTCAE ²⁰. We also performed the analyses by taking into account lymphocytes level as a continuous parameter (data not shown) and retained a proportional relationship between the number of circulating lymphocytes (time-dependent) and risks of graft failure, death and viral infection.

Our results are consistent with the hypothesis that a lower lymphocyte count is associated with a higher risk of graft failure and death. Nevertheless, to discard the possibility that lymphocyte count could be a marker of other dynamic processes such as renal function, we generated two models by taking into account eGFR either at one year (for the unadjusted analyses and for a first model) or as time-dependent (for a second model). Although we observed that the effect size between the number of lymphocytes and the outcomes decreased in model 2, possibly because we blocked the causal effect of the lymphocyte levels on the pathway related to time-dependent renal function, deep lymphopenia remained a risk factor for poor prognosis, suggesting that alternative

pathway were responsible. Lymphopenic patients are likely to be more exposed to severe complications such as viral infections (invasive CMV and BK virus), bacterial and opportunistic infections ²¹, and neoplasia than patients with normal lymphocyte counts, as suggested by the time-dependent association between the number of circulating lymphocytes and viral infection risk after one year of transplantation. These complications are linked to graft failure risk. They could negatively affect graft and patient outcomes, directly (acute kidney injury associated with the event or its treatment) ²² or indirectly when followed by a medical decision to alleviate immunosuppression therapy ²³⁻²⁵. In contrast, we found no significant association between the time-dependent number of circulating lymphocytes and cancer risk, despite a similar prevalence of both types of events. An association between lymphopenia and cancer risk was not established and remains a subject of discussion ^{26,27}. Ducloux et al. reported that CD4 lymphocytopenia was significantly inversely correlated with the occurrence of skin cancer but no correlation was found for other lymphocyte populations (CD8 and B cells), and this may explain why we did not find this correlation in our study ¹⁶. Furthermore, lymphopenia by itself has been proven to induce homeostatic proliferation of the remaining T cells, especially of activated memory cells that show resistance to tolerance and that are also the most efficient and aggressive immune cells targeting the graft ²⁸. In contrast, regulatory T cells proliferate poorly after ATG-induced lymphocytopenia ²⁹. Moreover, the majority of immunosuppressive drugs target T cells that may allow B cells to escape and exert their own deleterious effects. In 2016, Luque et al. ¹⁹ retrospectively studied patients with more than 10 years of graft function with severe CD4 lymphopenia. In agreement with our results, they found a significantly worse graft outcome in patients with severe lymphopenia. They had the opportunity to study histological lesions for seven patients and found significantly higher total inflammation and extensive fibrosis in lymphopenic patients than in normal individuals. Interestingly, the three groups of patients (according to their one year lymphocyte counts) also differed in terms of immunosuppressive therapies : patients with a lower lymphocyte count were

more often being treated with antiproliferative drugs and corticosteroids, and were more rarely being treated with mTOR inhibitors than patients with normal lymphocyte counts. One explanation could be that the treatment directly influences graft failure and that the lymphocyte count is only a consequence of the immunosuppressive therapy. However, this is not the case, since in our cohort, corticosteroids were associated with less graft failure risk and mTOR inhibitors were associated with higher graft failure risk; these results are exactly the opposite of what was observed with more patients who harbored lower lymphocyte counts being treated with antiproliferative drugs and corticosteroids and less patients with lower lymphocyte count being treated with mTOR inhibitors. Moreover, CNIs are the most cytotoxic maintenance drugs targeting lymphocytes ^{8,30}, and they are not significantly associated with graft failure risk. These data show that time-dependent lymphocyte count is associated with graft failure risk, independent of the treatment. Another explanation may be that transplant physicians, when faced with patients with a low lymphocyte count, often modify immunosuppressive therapy, either by pausing it or even sometimes by discontinuing such drugs, particularly mycophenolate, and switching toward less aggressive antiproliferative and corticosteroids therapies ³¹⁻³³. This could indirectly be the cause of the increased graft failure risk. This observation suggests that limited use of immunosuppressive therapy due to lymphopenia may amplify the phenomenon, because of the higher risk of graft failure, death and viral infection. It is of note that our model was only adjusted for maintenance therapies at 1-year posttransplantation and did not account for a potential switch in therapy. A randomized trial in lymphopenic patients comparing a steady dosage versus dose decrease could define what is more serious, the risk of infection or graft failure.

In contrast with previous data ¹⁹, we also found that depleting therapy was associated with lower risk of death, but not with lower graft failure risk. After depleting induction therapy, complete immune reconstitution can take up to 2 years and depends on the baseline lymphocyte count ^{34,35}. Furthermore, even after normalization of the lymphocyte count, T cell function can still be

impaired, and this may prevent or minimize deleterious immunological events in the long term and even have a protective effect by tilting the balance in favor of a pro-tolerogenic environment as shown for several therapies^{36,37}. Nevertheless, with respect to our data, we may have expected that depleting induction therapy would have induced lymphopenia and thus higher risk of graft failure. The fact that we cannot make this direct link suggests a biological role independent of its basic lymphodepleting effect. Finally, induction therapy was neither associated with late onset viral infection (whereas lymphopenia was) nor cancer risk, reinforcing this hypothesis.

Our study has several limitations. For this large-scale analysis, we chose to perform a retrospective study, including the inherent biases. The missing data and patients lost to follow-up that we reported were not random leading to the exclusion from the multivariate models of patients who showed demographic differences from included patients. Additionally, we were only able to collect and analyze data that are routinely collected. As a consequence, only “for purpose” lymphocyte counts were available during the first year of transplantation, and thus were not analyzed to avoid bias in our study, even though they could be an interesting early marker of immunosuppression level. The occurrence of CMV viremia during the first year is also regarded as a marker of overimmunosuppression^{11,12}, even if which comes first between infection and lymphopenia is controversial. We observed no difference in the proportion of patients who developed a CMV infection within the first year posttransplantation regardless of lymphocyte count they belonged to, allowing us to exclude CMV infection from those factors that are affected by a lymphopenic state (at least at one year). Another consequence is that the complete blood count only included the total lymphocyte count with no phenotyping of lymphocyte subsets, as this analysis is not routinely performed, and we were limited by the number of samples for each patient. The elevated graft and patient survivals observed in this study can be explained by a selection bias, since all patients and grafts survived the first year of transplantation, and were regularly followed up in University Hospital. In addition, therapeutic interventions following immunological or infectious

events that could modify lymphocyte counts within the follow-up period could be possible confounders. Unfortunately, this information was not available for the whole cohort, and this is an additional limitation of our interpretations. Finally, an external validation of our results is of primary importance, as we were unable to find such a cohort for validation; however our study was bicentric which should limit the single-centre bias. A prospective study could improve on our study and could include a detailed analysis of whole blood cell subset phenotypes but this would obviously increase its cost and length.

In conclusion, the occurrence of deep lymphopenia in patient beyond one year of transplantation is a risk factor for long-term graft failure, death and viral infection. Such a finding should alert physicians to modify immunosuppressive therapies or to reinforce the monitoring of these at-risk patients.

PATIENTS AND METHODS

Population

Data were extracted from the prospective DIVAT cohort of kidney transplant recipients from Nantes and Necker university hospitals. In total, 2,999 adult recipients who received either a single kidney transplant or a simultaneous kidney-pancreas transplant between January 2000 and December 2016 and alive with a functioning graft at 1-year posttransplantation were enrolled. HIV-positive patients, patients with no lymphocyte count or a single lymphocyte count available after 1-year posttransplantation, and patients diagnosed with posttransplant lymphoproliferative disorders or leukemia were excluded.

Clinical and biological data

Classical risk factors likely to influence lymphocyte count changes and/or graft failure and/or patient risk of death were extracted from the database. Donor features included age, sex, living or deceased status, heart-beating vs non-heart-beating status, CMV serology, and extended criteria donor data ^{38,39}. The recipient characteristics were age, sex, history of comorbidities, CMV serology, initial nephropathy, pretransplant dialysis method, pretransplant anti-HLA immunization (defined as positive if at least one DSA was identified by Luminex® Single Antigen Bead technology within the 6 months pretransplantation, unless if at least one DSA was not identified but a later determination performed by Luminex® screening or other technology (ELISA or CDC) was positive in pretransplantation), induction therapy (depleting vs nondepleting) and initial maintenance therapy. The transplantation parameters were type of transplantation (kidney vs simultaneous pancreas-kidney), rank, cold ischemia time and number of HLA-A/B/DR mismatches. The following variables were collected within the first year posttransplantation: occurrence of delayed graft function, occurrence of BPAR, daily proteinuria and serum creatinine at one year posttransplantation. Any occurrence of viral infection, cancer (including nonsolid cancer and

squamous cell and basal cell skin cancers) or any type of BPAR during the follow-up was also noted. Finally, circulating lymphocyte counts at one year posttransplantation and all subsequent counts were noted. For an easier clinical interpretation, we defined “a priori” relevant thresholds instead of considering the lymphocyte level as a continuous variable. Thus, according to the laboratory boundaries, the lymphocyte count was classified as normal (between 1,500 and 4,000 /mm³, deep lymphopenia (lower than 750 /mm³ - half of 1,500 - according to empirical evaluation based on the Common Terminology Criteria for Adverse Events (CTCAE))²⁰ or mild lymphopenia (between 750 and 1,500 /mm³).

Statistics

Our main objective was to investigate the etiological relationship between time-dependent lymphocyte count and graft or patient survival. To avoid confounding results, all variables differentially distributed between lymphocyte count-based groups which were not consequences of lymphocyte count were included in multivariate models. Graft survival analysis was based on the time between one year posttransplantation and return to dialysis, and patient survival analysis was based on the time between one year posttransplantation and death with a functioning graft. Patients were observed only after one year of transplantation, when lymphocyte counts were assumed to have begun to steady, rather than within the first year, during which many factors impact the lymphocyte count, for instance induction therapy, antibiotic and anti-viral prophylaxis, or frequent acute rejection treatments. Our secondary objective was to assess the relationship between time-dependent lymphocyte counts and acute rejection, viral infection or cancer risks.

Analyses were performed in two steps. First, possible risk factors associated with the time-to-event of interest were selected by univariate analysis (log-rank test, $p \leq 0.20$). If the log-linearity assumption was unconfirmed, the variable was categorized according to thresholds traditionally used in the literature. The selected variables and confounding factors (induction and maintenance treatments) were further analyzed in a multivariate time-dependent Cox model⁴⁰. A cause-specific

hazards approach was used for survival data, as our etiological study aimed to describe the causal effect of the number of lymphocytes^{41,42}, and lymphocyte count was analyzed as a time-dependent variable since several counts per patient were performed during the follow-up. To account for the temporal relationship between lymphocyte count and outcomes, we employed a Cox model that considered time-dependent measures of lymphocyte count with multiple changes updated during the patient follow-up⁴³. Because lymphocyte count could be a marker for other dynamic processes and particularly renal function, we generated two models for each multivariate Cox analyses of the risk of graft failure (death censored), death (graft failure censored), cancer and viral infection (death and graft failure censored). Model 1 considered the renal function (eGFR) at one year and model 2 considered eGFR as time-dependent. Some interactions between explicative variables were tested according to relevant clinical interpretations. Nonsignificant variables were progressively removed respecting a step-by-step backward selection method ($p < 0.05$). Thereafter, clinically relevant interactions were tested. The proportional hazard assumption was evaluated by plotting log-minus-log survival curves and by analyzing the scaled Schoenfeld residuals⁴⁴. During the descending procedure, lymphocyte count and confounding factors were the only variables maintained in the model regardless of the corresponding p-values. For the secondary analysis, we also performed separate multivariate time-dependent Cox analyses to study the relationship between time-dependent lymphocyte count and the risk of 1) acute rejection, 2) viral infection or 3) cancer. All analyses were performed using R software V3.3.1.

ABBREVIATIONS

ATG, anti-thymoglobulin

BKV, BK virus

BPAR, Biopsy proven acute rejection

CD4 (CD8), cluster of differentiation 4 (8)

CMV, cytomegalovirus

CNI, calcineurin inhibitor

DIVAT, données informatisées et validées en transplantation (computerized and validated data in transplantation)

DSA, donor specific antibody

HIV, human immunodeficiency virus

HSV, Herpes simplex virus

HLA, human leukocyte antigen

mTOR, mechanistic target of rapamycin

PRA, panel-reactive antibody

VZV, Varicella zoster virus

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DISCLOSURE

The authors declare conflict of interest with Roche Pharma, Novartis and Sanofi and Astellas laboratories for supporting the DIVAT cohort.

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AUTHORS' CONTRIBUTIONS

Amaury Dujardin: Conceptualization, Methodology, Formal analysis, Writing - original draft, review and editing. Magali Giral: Conceptualization, Methodology, Writing - review and editing. Sophie Brouard: Conceptualization, Methodology, Writing - review and editing. Marine Lorent and Yohann Foucher : Biostatistic analysis, Writing - review and editing. Christophe Legendre : Data acquisition, Writing - review and editing. Clarisse Kerleau: data management.

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FIGURES AND TABLES

Figure 1. Flow-chart of the study.

Figure 2. Boxplot representing the evolution of lymphocyte counts distribution at each year post-transplantation for the whole cohort.

Figure 3A. Representation of intra-patient variability of lymphocyte counts, assessed by considering a representative 5% sample of randomly selected patients. Each colored line represents a separate patient.

Figure 3B. Frequency of switching from one lymphocyte group to another all over the study follow-up.

Table 1. Description of recipient, donor, and transplantation characteristics of the global study population (qualitative variables).

Table 2. Description of recipient, donor, and transplantation characteristics of the global study population (quantitative variables).

Table 3. Cox unadjusted analyses and Cox multivariate analysis of graft failure risk (with death-censored) with lymphocyte count as time-dependent variable (738 patients were removed from the analysis because of missing data as detailed in Table S3 and S4).

Table 4. Cox unadjusted analyses and Cox multivariate analysis of patient death risk (with a functioning graft) with lymphocyte count as time-dependent variable (374 patients were removed from the analysis because of missing data as detailed in Table S5 et S6).

Table 5. Cox multivariate analysis of viral infection risk (with death and return to dialysis censored) with lymphocyte count as time-dependent variable (344 patients were removed from the analysis because of missing data).

Table 6. Cox multivariate analysis of cancer risk (with death and return to dialysis censored) with lymphocyte count as time-dependent variable.

Table 7. Cox multivariate analyses per center (Nantes: n= 1632, Paris Necker: n=1367).

SUPPLEMENTARY MATERIAL

Figure S1. Kinetics of lymphocytes count prior to “graft failure” event. Each colored line represents a patient that presented a graft failure during follow-up.

Figure S2. Kinetics of lymphocytes count prior to “death” event. Each colored line represents a patient that died during follow-up.

Figure S3. Death-censored graft survival probability in function of the time since the transplantation from Kaplan–Meier estimator (solid line), and their corresponding 95% confidence intervals (dashed lines).

Figure S4. Patient survival probability in function of the time since the transplantation from Kaplan–Meier estimator (solid line), and their corresponding 95% confidence intervals (dashed lines).

Table S1. Details of the type of acute rejection episodes after one year of transplantation.

Table S2. Details of the type of cancers and of principal viral infections after one year of transplantation.

Table S3. Difference between excluded and included patients from Cox multivariate analysis of graft failure risk (quantitative variables). Results are presented as mean \pm SD.

Table S4. Difference between excluded and included patients from Cox multivariate analysis of graft failure risk (qualitative variables). Results are presented as effective numbers (with percentages).

Table S5. Difference between excluded and included patients from Cox multivariate analysis of death risk (quantitative variables). Results are presented as mean \pm SD.

Table S6. Difference between excluded and included patients from Cox multivariate analysis of death risk (qualitative variables). Results are presented as effective numbers (with percentages).

Table S7. Cox multivariate analysis of patient death risk (with a functioning graft) with lymphocyte count at 1-year post-transplantation.

Table S8. Cox multivariate analysis of graft failure risk (with death-censored) with lymphocyte count at 1-year post-transplantation. (734 patients were removed from the analysis because of missing data).

Table S9. Cox multivariate analysis of viro-induced skin cancer (with death and return to dialysis censored) with or without the lymphocyte count as time-dependent variable.

Supplementary information is available at [Kidney International’s website](#).

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DIVAT

N = 9,168 kidney transplant recipients registered in March 2018 in Nantes and Necker

409 patients with transplantation other than kidney or kidney-pancreas

3,123 patients transplanted before 01/01/2000 or after 31/12/2016

149 patients under 18 years of age

1,092 patients without a functioning graft at one year post transplantation

N = 4,395 kidney transplant recipients met the inclusion criteria

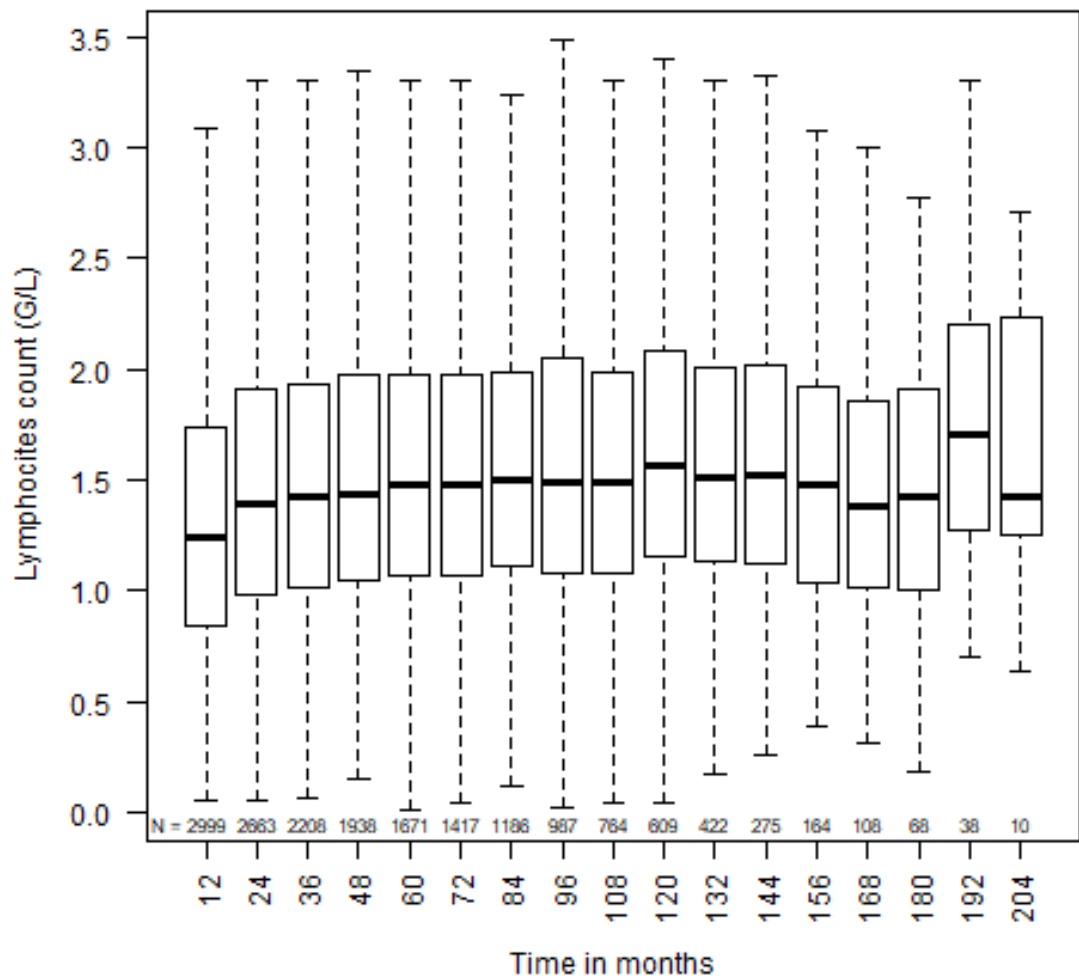
Exclusion criteria

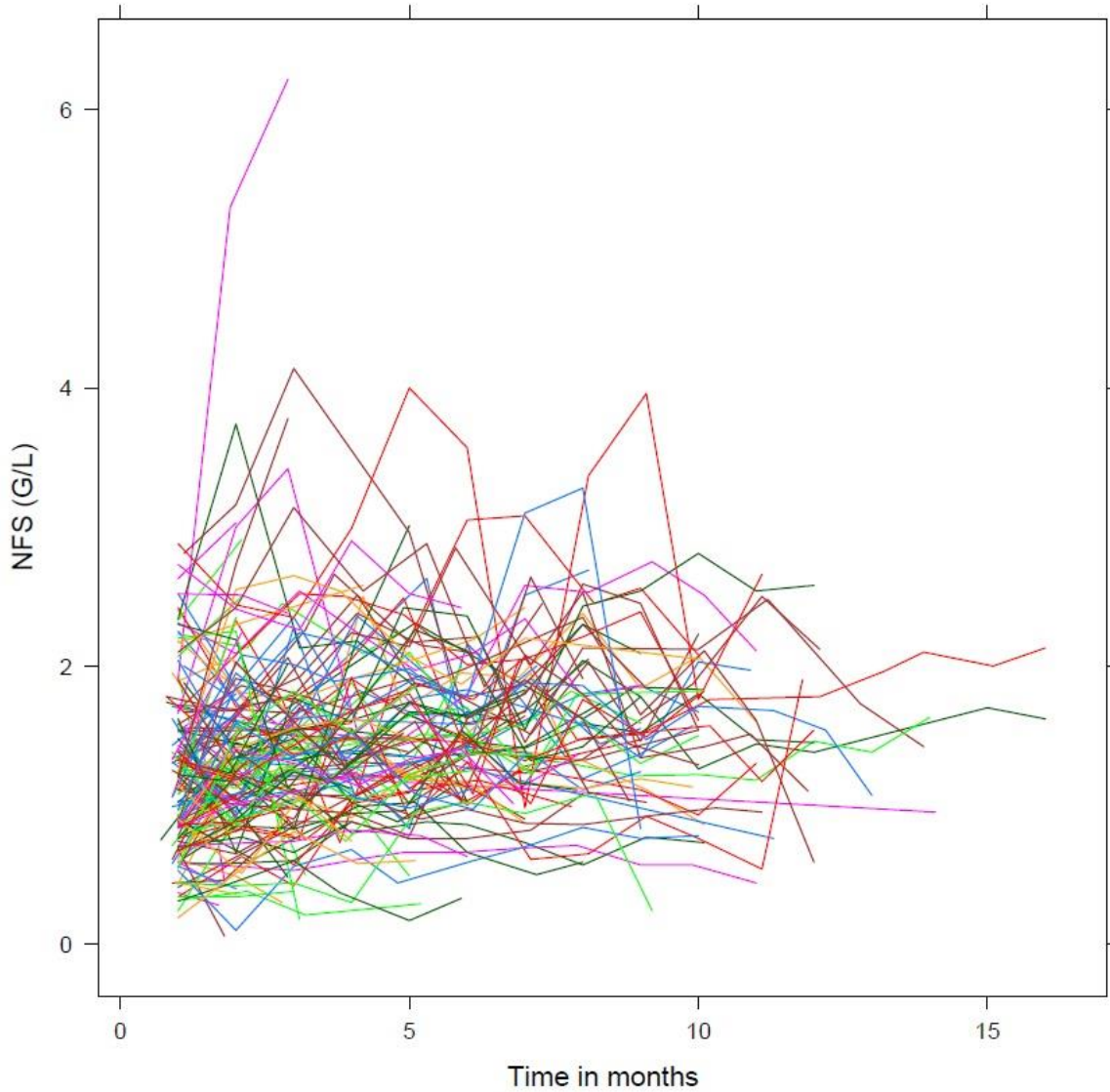
71 patients with positive HIV serology

1,276 patients with no lymphocyte count or a single lymphocyte count after 1-year post-transplantation

49 patients diagnosed with post-transplant lymphoproliferative disorder

N = 2,999 kidney transplant recipients included in the study





Switching sequences Between groups	Frequence (n)	Switching sequences Between groups	Frequence (n)
1*	545	32121	13
2**	344	32123	2
3***	129	32132	1
12	169	32312	2
13	17	32321	4
21	378	32323	12
23	78	121212	17
31	29	121231	1
32	185	121312	2
121	152	123121	1
123	7	123212	2
131	7	131212	1
132	4	132121	1
212	147	132323	1
213	13	212121	23
231	8	212123	1
232	78	212321	1
312	8	212323	4
313	1	213121	2
321	44	213212	2
323	55	213232	1
1212	60	231212	2
1213	5	232323	5
1231	1	312121	1
1232	13	321212	3
1321	4	321232	3
2121	99	323212	2
2123	10	323232	8
2131	6	1212121	9
2132	7	1212123	2
2312	5	1212312	1
2321	13	1212321	1
2323	21	1232121	1
3121	7	1312132	1
3132	1	2121212	4
3212	21	2121232	4
3213	2	2121323	1
3232	30	2123123	1
12121	41	2123231	1
12123	5	2323212	1
12131	1	3121212	1
12132	1	3212121	1
12312	2	3212123	1
12321	1	3232121	1
13232	2	3232323	2
21212	37	12121212	4
21213	4	12121232	1
21232	7	12323232	1
21312	1	21212121	4
21323	1	23212121	1
23121	3	32121212	1
23123	1	32323232	1
23132	1	232121232	1
23212	6	323212121	1
23213	1	1321212121	1
23231	1		
23232	11		
31212	2		

Table 1. Description of recipient, donor, and transplantation characteristics of the global study population (qualitative variables).

Characteristics	Missing data		N=2999	
	n	%	Effective	%
RECIPIENT				
Male gender	-	-	1,814	60.49
Rank of the graft \geq 2	-	-	620	20.65
Initial nephropathy: Glomerulonephritis	-	-	818	27.28
Pyelonephritis			1197	39.91
Vascular nephropathy			177	5.90
Diabetic nephropathy			409	13.64
Other disease and unknown			398	13.27
Replacement therapy: Preemptive transplantation	-	-	513	17.11
Peritoneal dialysis			201	6.70
Hemodialysis			2,285	76.19
Delayed Graft Function	24	0.80	888	29.85
Past history of diabetes	-	-	588	19.61
Past history of neoplasia	-	-	317	10.57
Positive CMV serology	17	0.57	1,718	57.61
DONOR				
Positive CMV serology	13	0.43	1,518	50.84
Male gender	1	0.03	1,694	56.50
Type of donor: Living	1	0.03	533	17.78
Heart beating			2,410	80.39
Non heart-beating			55	1.83
ECD	555	18.51	951	38.91
TREATMENTS				
Maintenance immunosuppressive by CNI	-	-	2,909	96.97
Maintenance immunosuppressive by mTOR inhibitor	-	-	146	4.87
Maintenance immunosuppressive by MMF/MPA/AZA	-	-	2,831	94.39
Maintenance immunosuppressive by Corticosteroids	-	-	2,600	86.70
Induction: No induction	-	-	113	3.77
Non depletant therapy			1,630	54.35
Depletant therapy			1,256	41.88
IMMUNOLOGY				
Number of HLA-A/B/DR mismatches $>$ 4	5	0.17	515	17.20
Positive anti-class I HLA antigens	339	11.29	973	36.57
Positive anti-class II HLA antigens	419	13.96	964	37.36
Pretransplantation DSA	863	28.75	332	15.54
Rejection in the 1st year post-transplantation	-	-	552	18.40

Table 2. Description of recipient, donor, and transplantation characteristics of the global study population (quantitative variables).

Characteristics	Missing data		N=2999			
	n	%	Mean	SD	Min	Max
Recipient age (years)	-	-	48.31	13.69	18	84
Cold ischemia time (min)	2	0.07	1021.82	593.66	30	2915
Donor age (years)	-	-	50.41	15.68	3	88
1-year daily proteinuria (g.L ⁻¹)	441	14.69	0.36	0.63	0	10.16
1-yr serum creatinine (μmol.L ⁻¹)	6	0.20	135.32	49.05	36	555
1-year number of circulating lymphocytes (Giga.L ⁻¹)	-	-	1.38	0.78	0.06	7.86
2-year number of circulating lymphocytes (Giga.L ⁻¹)	336	11.19	1.54	0.83	0.06	8.3
3-year number of circulating lymphocytes (Giga.L ⁻¹)	794	26.45	1.57	0.82	0.07	9.88
4-year number of circulating lymphocytes (Giga.L ⁻¹)	1064	35.44	1.60	0.82	0.15	12.11
5-year number of circulating lymphocytes (Giga.L ⁻¹)	1331	44.34	1.61	0.79	0.01	7.48
6-year number of circulating lymphocytes (Giga.L ⁻¹)	2393	79.71	1.69	0.84	0.04	10.18

Table 3. Cox unadjusted analyses and Cox multivariate analysis of graft failure risk (with death-censored) with lymphocyte count as time-dependent variable (738 patients were removed from the analysis because of missing data as detailed in Table S3 and S4).

	Unadjusted Cox models			Cox model 1			Cox model 2		
	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value
Number of lymphocytes (ref >1.500 G/L) (normal)									
≤ 0.750 (severe lymphopenia)	3.54	2.73-4.60	<0.001	3.18	2.29-4.44	<0.001	2.21	1.58-3.09	<0.001
]0.750-1.500] (mild lymphopenia)	1.45	1.16-1.80	<0.001	1.30	0.99-1.71	0.057	1.20	0.92-1.58	0.184
TRANSPLANTATION									
Kidney-pancreas transplantation	0.85	0.60-1.20	0.343	-	-	-	-	-	-
Graft rank (>1 versus 1)	1.53	1.22-1.91	<0.001	1.36	0.99-1.88	0.061	1.25	0.89-1.75	0.193
Cold ischemia time (hours)	1.02	1.01-1.03	<0.001	-	-	-	-	-	-
Number of dialysis post-transplantation > 1	1.76	1.45-2.13	<0.001	-	-	-	-	-	-
DONOR									
Donor age (ref ≤50 years)									
51-60 years	1.17	0.93-1.51		-	-	-	-	-	-
> 60 years	1.76	1.40-2.21		-	-	-	-	-	-
Donor gender (men vs women)	0.99	0.81-1.20	0.901	-	-	-	-	-	-
Deceased donor	1.73	1.25-2.40	0.001	-	-	-	-	-	-
Type of donor (ref living donor)									
Heart-beating	1.73	1.25-2.40		-	-	-	-	-	-
Non-heart-beating	2.32	0.92-5.90		-	-	-	-	-	-
Positive CMV serology	1.12	0.93-1.36	0.19	-	-	-	-	-	-
RECIPIENT									
Recipient age (> 55 vs ≤55 years)	1.30	1.05-1.60	0.014	1.07	0.83-1.37	0.604	1.25	0.68-1.14	0.327
Recipient gender (men vs women)	0.91	0.75-1.11	0.369	-	-	-	-	-	-
Initial nephropathy (ref Glomerulonephritis)									
Diabetic nephropathy	1.04	0.77-1.41		-	-	-	-	-	-
Vascular nephropathy	1.02	0.65-1.61		-	-	-	-	-	-
Pyelonephritis / interstitial nephritis	0.96	0.76-1.21		-	-	-	-	-	-
Other disease and unknown causes	0.90	0.65-1.24		-	-	-	-	-	-
Dialysis technique (ref pre-emptive)									
Peritoneal	1.10	0.65-1.87		-	-	-	-	-	-
Hemodialysis	1.40	1.04-1.89		-	-	-	-	-	-
History of diabetes (yes vs no)	1.33	1.06-1.67	0.013	1.30	0.98-1.73	0.067	1.18	0.90-1.57	0.231
History of neoplasia (yes vs no)	1.05	0.74-1.50	0.777	-	-	-	-	-	-
Positive CMV serology	0.87	0.72-1.05	0.149	-	-	-	-	-	-
IMMUNOLOGY									
HLA-ABDR mismatches >4	1.28	1.01-1.63	0.038	1.29	0.96-1.74	0.093	1.27	0.93-1.73	0.129
Positive Panel-Reactive Antibody anti-class I	1.72	1.37-2.17	<0.001	1.63	1.23-2.15	0.001	1.32	0.99-1.75	0.054
Positive Panel-Reactive Antibody anti-class II	1.33	1.05-1.69	0.017	-	-	-	-	-	-
INITIAL TREATMENT									
Induction therapy (depleting vs non-depleting)	1.32	1.09-1.60	0.004	0.88	0.67-1.15	0.343	0.96	0.73-1.26	0.771
Maintenance therapy									
Calcineurin inhibitor	1.72	0.99-2.99	0.055	1.71	0.80-3.69	0.166	1.06	0.50-2.25	0.872
mTOR inhibitor	1.26	0.81-1.95	0.309	2.15	1.03-4.49	0.040	1.61	0.72-3.59	0.245
Antiproliferative drug	0.81	0.55-1.19	0.288	1.03	0.57-1.89	0.913	0.98	0.49-1.95	0.956
Prednisone	0.86	0.67-1.11	0.253	0.76	0.56-1.02	0.073	0.74	0.54-1.02	0.067
TIME-DEPENDENT PARAMETERS									
eGFR* (per 10 ml/min)	0.63	0.59-0.68	<0.001	0.67	0.62-0.72	<0.001	0.19	0.16-0.22	<0.001
1-year daily proteinuria (g.L ⁻¹)	1.39	1.31-1.48	<0.001	1.32	1.22-1.43	<0.001	1.08	0.98-1.19	0.105
Rejection in the 1st year post-transplantation	1.71	1.38-2.12	<0.001	1.28	0.98-1.67	0.007	1.18	0.91-1.56	0.210

* The eGFR was considered at 1-year for the unadjusted analyses and the model 1 and as time-dependent marker for the model 2.

Table 5. Cox multivariate analysis of viral infection risk (with death and return to dialysis censored) with lymphocyte count as time-dependent variable (344 patients were removed from the analysis because of missing data).

	Cox model 1			Cox model 2		
	HR	95% CI	P-value	HR	95% CI	P-value
Number of lymphocytes (ref>1.500 G/L)						
≤ 0.750	2.18	1.69-2.83	<0.001	2.06	1.59-2.67	<0.001
]0.750-1.500[1.24	1.01-1.52	0.041	1.21	0.98-1.48	0.073
Donor age (ref ≤50 years)						
51-60 years	1.26	1.01-1.58	0.038	1.14	0.91-1.43	0.240
> 60 years	1.41	1.14-1.75	0.002	1.17	0.94-1.48	0.155
Recipient gender (men vs women)	0.81	0.68-0.97	0.023	0.84	0.70-1.01	0.060
History of neoplasia (yes vs no)	1.35	1.03-1.77	0.028	1.33	1.02-1.75	0.037
HLA-ABDR mismatches >4	1.36	1.09-1.70	0.007	1.38	1.11-1.73	0.004
Positive Panel-Reactive Antibody anti-class I	1.84	1.51-2.23	<0.001	1.85	1.53-2.24	<0.001
Rejection in the 1st year post-transplantation	1.28	1.04-1.60	0.018	1.20	0.97-1.47	0.456
eGFR* (per 10 ml/min)	-	-	-	0.89	0.84-0.93	<0.001
INITIAL TREATMENT						
Induction therapy (depleting vs non-depleting)	0.96	0.78-1.16	0.661	0.93	0.76-1.13	0.456
Maintenance therapy						
Calcineurin inhibitor	0.98	0.59-1.63	0.953	0.94	0.57-1.57	0.826
mTOR inhibitor	1.32	0.77-2.25	0.316	1.28	0.75-2.20	0.365
Antiproliferative drug	1.04	0.65-1.67	0.867	1.06	0.67-1.71	0.790
Prednisone	0.95	0.73-1.24	0.714	0.97	0.75-1.27	0.846

* The eGFR was considered as time-dependent marker for the model 2.

Table 6. Cox multivariate analysis of cancer risk (with death and return to dialysis censored) with lymphocyte count as time-dependent variable.

	Cox model 1			Cox model 2		
	HR	95% CI	P-value	HR	95% CI	P-value
Number of lymphocytes (ref>1.500 G/L)						
≤ 0.750	0.93	0.69-1.26	0.648	0.92	0.68-1.26	0.615
]0.750-1.500[1.08	0.89-1.31	0.442	1.08	0.88-1.31	0.468
Graft rank (>1 versus 1)	1.58	1.25-2.01	0.001	1.59	1.25-2.02	0.001
Donor age (ref ≤50 years)						
51-60 years	1.24	0.97-1.58	0.083	1.21	0.95-1.56	0.116
> 60 years	1.56	1.22-2.01	<0.001	1.51	1.17-1.97	0.002
Recipient age (> 55 vs ≤55 years)	2.51	2.02-3.14	<0.001	2.52	2.02-3.13	<0.001
History of neoplasia (yes vs no)	1.69	1.32-2.15	<0.001	1.68	1.32-2.15	<0.001
eGFR* (per 10 ml/min)	-	-	-	0.98	0.93-1.03	0.422
INITIAL TREATMENT						
Induction therapy (depleting vs non-depleting)	0.96	0.77-1.18	0.677	0.95	0.77-1.18	0.652
Maintenance therapy						
Calcineurin inhibitor	0.96	0.56-1.65	0.889	0.95	0.56-1.63	0.861
mTOR inhibitor	0.85	0.47-1.51	0.573	0.84	0.47-1.50	0.554
Antiproliferative drug	0.77	0.49-1.21	0.257	0.77	0.49-1.22	0.266
Prednisone	0.89	0.70-1.14	0.368	0.89	0.70-1.14	0.369

* The eGFR was considered as time-dependent marker for the model 2.

Table 7. Cox multivariate analyses per center (Nantes: n= 1632, Paris Necker: n=1367).

	Overall cohort		Nantes		Necker	
	HR	95% CI	HR	95% CI	HR	95% CI
Cox MODEL 1 *						
Graft failure (ref>1.500 G/L)						
≤ 0.750 (severe lymphopenia)	3.18	2.29-4.44	3.57	2.37-5.38	2.61	1.44-4.72
]0.750-1.500] (mild lymphopenia)	1.30	0.99-1.17	1.58	1.12-2.22	0.87	0.53-1.41
Patient death (ref>1.500 G/L)						
≤ 0.750 (severe lymphopenia)	1.96	1.35-2.86	2.37	1.41-3.99	1.46	0.81-2.60
]0.750-1.500] (mild lymphopenia)	1.07	0.81-1.41	0.64	0.41-1.02	1.49	1.02-2.17
Viral infection (ref>1.500 G/L)						
≤ 0.750 (severe lymphopenia)	2.18	1.69-2.83	2.41	1.67-3.52	1.97	1.37-2.83
]0.750-1.500] (mild lymphopenia)	1.24	1.14-1.75	1.25	0.92-1.70	1.27	0.96-1.67
Cancer (ref>1.500 G/L)						
≤ 0.750 (severe lymphopenia)	0.93	0.69-1.26	0.88	0.60-1.30	1.08	0.65-1.81
]0.750-1.500] (mild lymphopenia)	1.08	0.89-1.31	1.03	0.81-1.30	1.17	0.83-1.64
Cox MODEL 2**						
Graft failure (ref>1.500 G/L)						
≤ 0.750 (severe lymphopenia)	2.21	1.58-3.09	2.11	1.39-3.19	2.22	1.20-4.13
]0.750-1.500] (mild lymphopenia)	1.20	0.92-1.58	1.51	1.07-2.12	0.71	0.43-1.17
Patient death (ref>1.500 G/L)						
≤ 0.750 (severe lymphopenia)	1.91	1.31-2.78	2.37	1.41-3.98	1.40	0.79-2.50
]0.750-1.500] (mild lymphopenia)	1.06	0.90-1.41	0.66	0.42-1.03	1.45	0.99-2.12
Viral infection (ref>1.500 G/L)						
≤ 0.750 (severe lymphopenia)	2.06	1.59-2.67	2.32	1.60-3.39	1.80	1.29-2.59
]0.750-1.500] (mild lymphopenia)	1.21	0.98-1.48	1.24	0.91-1.68	1.20	0.90-1.58
Cancer (ref>1.500 G/L)						
≤ 0.750 (severe lymphopenia)	0.92	0.68-1.26	0.89	0.61-1.31	1.06	0.63-1.77
]0.750-1.500] (mild lymphopenia)	1.08	0.88-1.31	1.03	0.81-1.31	1.14	0.81-1.61

* eGFR was considered at 1-year for the Cox model 1 analysis

***: eGFR was considered as time-dependent marker for the model 2 analysis

Table 4. Cox unadjusted analyses and Cox multivariate analysis of patient death risk (with a functioning graft) with lymphocyte count as time-dependent variable (374 patients were removed from the analysis because of missing data as detailed in Table S5 and S6).

	Unadjusted Cox models			Cox model 1			Cox model 2		
	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value
Number of lymphocytes (ref>>1.500 G/L) (normal)									
≤ 0.750 (severe lymphopenia)	2.66	1.92-3.68	<0.001	1.96	1.35-2.86	0.004	1.91	1.31-2.78	0.001
]0.750-1.500] (mild lymphopenia)	1.39	1.08-1.79	0.011	1.07	0.81-1.41	0.378	1.06	0.80-1.41	0.669
TRANSPLANTATION									
Kidney-pancreas transplantation	0.50	0.30-0.85	0.010	-	-	-	-	-	-
Graft rank (>1 versus 1)	1.51	1.16-1.97	0.002	1.62	1.11-2.35	0.012	1.59	1.09-2.31	0.015
Cold ischemia time (> 12 vs ≤ 12 hours)	1.78	1.28-2.47	<0.001	-	-	-	-	-	-
Number of dialysis post-transplantation > 1	1.85	1.47-2.33	<0.001	1.55	1.11-2.35	0.001	1.52	1.17-1.98	0.002
DONOR									
Donor age (ref ≤50 years)									
51-60 years	1.69	1.25-2.29		-	-	-	-	-	-
> 60 years	3.39	2.60-4.43		-	-	-	-	-	-
Donor gender (men vs women)	1.06	0.84-1.34	0.628	-	-	-	-	-	-
Deceased donor	2.84	1.76-4.58	0.001	-	-	-	-	-	-
Type of donor (ref living donor)									
Heart-beating	2.84	1.76-4.58		-	-	-	-	-	-
Non-heart-beating	2.86	0.84-9.74		-	-	-	-	-	-
Positive CMV serology	1.11	0.88-1.39	0.384	-	-	-	-	-	-
RECIPIENT									
Recipient age (> 55 vs ≤55 years)	3.89	3.09-4.90	<0.001	2.93	2.22-3.86	<0.001	2.90	2.21-3.81	<0.001
Recipient gender (men vs women)	1.10	0.87-1.39	0.439	-	-	-	-	-	-
Initial nephropathy (ref Glomerulonephritis)									
Diabetic nephropathy	1.52	1.06-2.18		1.49	0.86-2.62	0.156	1.48	0.85-2.60	0.161
Vascular nephropathy	2.50	1.63-3.83		2.63	1.62-4.27	<0.001	2.53	1.56-4.11	0.002
Pyelonephritis	1.19	0.88-1.61		1.48	1.05-2.08	0.027	1.47	1.03-2.07	0.029
Other disease and unknown causes	1.23	0.83-1.83		0.98	0.62-1.56	0.952	0.98	0.62-1.57	0.961
Dialysis technique (ref pre-emptive)									
Peritoneal	1.30	0.59-2.85		1.60	0.67-3.83	0.287	1.62	0.67-3.87	0.277
Hemodialysis	2.63	1.67-4.15		2.15	1.25-3.69	0.006	2.09	1.21-3.59	0.008
History of diabetes (yes vs no)	1.64	1.27-2.12	<0.001	1.81	1.18-2.76	0.006	1.78	1.17-2.73	0.007
History of neoplasia (yes vs no)	2.40	1.77-3.25	<0.001	1.83	1.29-2.59	0.007	1.86	1.32-2.64	0.004
Positive CMV serology	1.71	1.33-2.19	<0.001	1.54	1.15-2.05	0.003	1.53	1.15-2.04	0.003
IMMUNOLOGY									
HLA-ABDR mismatches >4	0.75	0.54-1.05	0.096	-	-	-	-	-	-
Positive Panel-Reactive Antibody anti-class I	1.49	1.13-1.96	0.006	1.39	1.03-1.88	0.032	1.37	1.02-1.86	0.037
Positive Panel-Reactive Antibody anti-class II	1.18	0.90-1.57	0.242	-	-	-	-	-	-
INITIAL TREATMENT									
Induction therapy (depleting vs non-depleting)	0.88	0.70-1.12	0.293	0.62	0.46-0.84	0.002	0.63	0.46-0.85	0.002
Maintenance therapy									
Calcineurin inhibitor	1.70	0.87-3.30	0.160	0.86	0.41-1.77	0.677	0.80	0.39-1.66	0.552
mTOR inhibitor	0.76	0.39-1.49	0.427	0.69	0.25-1.88	0.464	0.63	0.23-1.75	0.378
Antiproliferative drug	1.35	0.76-2.41	0.267	1.08	0.51-2.29	0.844	1.09	0.51-2.32	0.812
Prednisone	1.00	0.73-1.36	0.994	1.15	0.79-1.66	0.457	1.17	0.81-1.70	0.475
1-YEAR PARAMETERS									
eGFR* (per 10 ml/min)	0.80	0.74-0.86	<0.001	0.91	0.83-0.98	0.011	0.86	0.80-0.92	<0.001
1-year daily proteinuria (g.L ⁻¹)	1.14	0.97-1.33	0.107	-	-	-	-	-	-
Rejection in the 1st year post-transplantation	1.03	0.77-1.38	0.833	-	-	-	-	-	-

* The eGFR was considered at 1-year for the unadjusted analyses and the model 1 and as time-dependent marker for the model 2.

Time-dependent lymphocyte count after transplantation is associated with higher risk of graft failure and death.



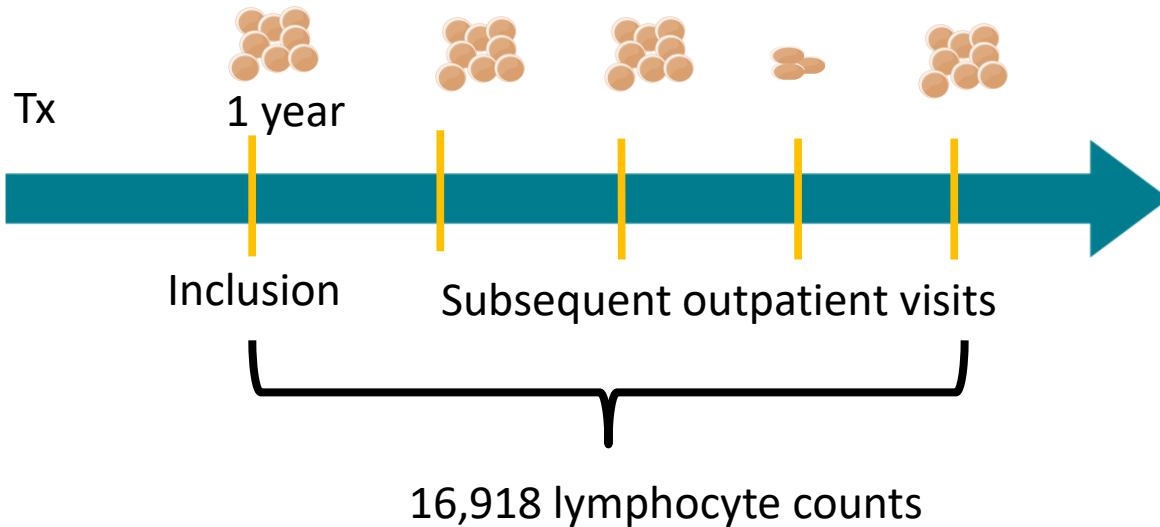
2,999 kidney transplant recipients



Normal Lymphocyte count [1,500-4,000/mm³]



Deep lymphopenia [< 750 /mm³]



Deep lymphopenia at a given time of the follow-up increases the risk of

Graft failure

HR 3.18

IC 2.29-4.44

Mortality

HR 1.96

IC 1.35-2.86

Viral infections

HR 2.18

IC 1.69-2.83

CONCLUSION:

After the first year of transplantation, the occurrence of a deep lymphopenia at a given time of the patient's follow-up is a risk factor of long term graft failure, death and viral infection