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CHARACTERISATION OF REJECTION EPISODES IN AN INCIDENT POPULATION OF KIDNEY TRANSPLANT RECIPIENTS

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Introduction

Within the past decades, solid organ transplantation (SOT) has become the therapy of choice for many end-stage organ diseases. Much work has been done in the field of organ preservation, surgery, immunology and therapeutics resulting in improved short and long-term graft and patient survival. The one-year graft survival rate regarding deceased donor kidney transplantation is currently >85% (>95% for living donor) and these short-term results are comparable between Europe and the USA. However, long-term outcomes are considerably lower and differ between centers, as 10-year graft survival is significantly higher in Europe (70-90% depending on the type of donor) as compared to USA (30-50%) (1), possibly due to different medical follow-up and reimbursement systems.

Current data show that patients who undergo pre-emptive kidney transplantation benefit a better survival than the ones who first experienced dialysis, and that old vintage of dialysis is associated with worse life expectancy (2,3). For patients with end-stage renal diseases (ESRD), many studies have indeed confirmed that survival as well as quality of life is greater in transplanted patients as compared to patients that are maintained on chronic dialysis. Moreover, the type of donor graft, i.e. living vs. cadaveric, influences graft and patient outcomes. When it comes to a living donor, the graft is prepared and transplanted under favorable conditions, including careful selection of the donor and planning of the surgery as well as short cold ischemia time at the time of transplantation, which reduces noticeably the rate of complications. In addition, the donor's acute illness that has led to brain death, in the case of deceased donors, is associated with systemic inflammation with and activation of the innate immune response which can damage the graft even before organ retrieval (4).

1. Immune mechanisms of graft rejection

Adaptive immune responses to grafted tissues are a major obstacle to successful SOT. The main targets of the host's immune response to non-self tissues are the major histocompatibility complex (MHC) molecules, human leukocyte antigens (HLA) in humans, which are present on donor cells. T-cell recognition of genetically encoded polymorphisms between members of the same species (also referred to as allorecognition) is the main event that initiates graft rejection. Although most research efforts have focused on controlling the adaptive immune response to prevent rejection and graft loss, recent discoveries suggest that the innate immune system plays an important role as well. Thus, both innate and adaptive immune mediators participate in the host's immune response leading to the rejection of an allograft. Under current immunosuppressive protocols, the prevalence of rejection within the first year after kidney transplantation is 15-20% (5).

1.1. The innate immune response

Dendritic cells (DCs), macrophages and B cells are the main professional antigen presenting cells (APCs). To become fully immunogenic, these cells need to be activated by surface or intracellular receptors called pattern recognition receptors (PRRs) which are able to sense specific ligands such as pathogen-associated

molecular patterns (PAMPs) in the context of infectious pathogens or danger-associated molecular patterns (DAMPs) in the context of sterile tissue injury.

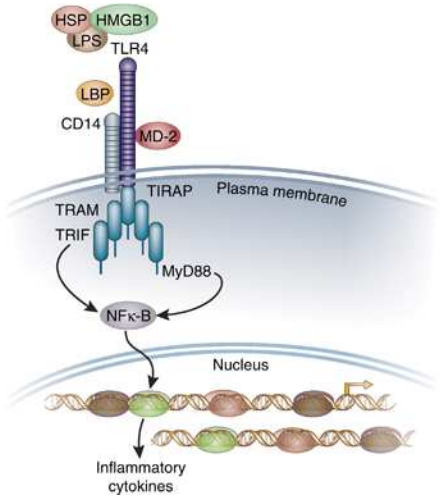

Figure 1. Activation of APCs

Under normal circumstances, the intracellular content is hidden from the immune system, but cellular stress and tissue injury will expose endogenous molecules, either released from necrotic cells or deriving from the degradation of the extracellular matrix (e.g. heat shock proteins, uric acid etc.) (6,7). This phenomenon could be of relevance in SOT. Indeed, the ischemia and reperfusion injury resulting from graft retrieval and implantation procedures at the time of transplantation induces some degree of cellular damage within the graft, generating DAMPs.

Toll-like receptors (TLRs) are a transmembranous group of PRR present either on plasma membranes or on endosomes, which DAMPs can bind. TLRs are expressed on various cell types of hematopoietic and non-hematopoietic origin (e.g. epithelial cells) and can be upregulated on APCs such as DCs and macrophages. After interacting with their ligands, TLRs will signal through adaptor molecules to activate the nuclear factor-kappa B (NF-κB), a transcription factor that induces immune mediators (**Figure 1**) (8-10). Thus, activated innate cells will produce inflammatory cytokines (IL-6, IL-12, TNF-α) and chemokines in order to recruit and stimulate other inflammatory cells. Activated APCs will also up-regulate MHC and co-stimulatory molecules, necessary for efficient presentation of antigens and full activation of T-lymphocytes (4).

Thus, in the context of SOT, the activation of innate cells through DAMPs (ischemia-reperfusion injury) or PAMPs (concomitant infections) will activate APCs and could contribute to acute rejection episodes.

1.2. The adaptive immune response

Adaptive immunity is defined by T and B lymphocytes activity. T cells are produced in the thymus as naive cells before being released in the peripheral blood and secondary lymphoid organs. While recirculating between secondary lymphoid organs, T cells encounter their specific antigens presented by APCs. To become effector cells, naive T cells need three activation signals: 1. Cognate antigen, 2. Costimulation, 3. Cytokines. Once activated, T cells differentiate into various subtypes of CD4+ helper and CD8+ cytotoxic T cells. Effector T cells produce cytokines to activate and differentiate other immune cells, including B cells, or are directly cytotoxic towards target cells. While most activated T cells are pro-inflammatory and induce rejection, there is a population of T cells with regulatory properties. Various subsets of regulatory T cells have been identified based on their ontogeny and phenotype, the best defined population being the thymus-derived naturally occurring CD4+Foxp3+ T cells (nTregs). These cells have the potential to control effector T cells and have been associated with immune tolerance in transplantation (11,12).

T cells play a central role in the immune response to an allograft. Depending on the APC subtype, the microenvironment and the cytokine milieu where they get activated, T cells can modulate the strength of the immune response (13). Moreover, CD4+ T cells help DCs to activate B cells in response to specific antigens. Allospecific B cells will differentiate into plasma cells that produce antibodies directed against the allograft (also referred to as donor-specific antibodies, DSA). A subset of activated B cells differentiates into memory B cells that can act as long-lasting APCs.

2. Clinical transplantation

2.1. MHC incompatibility

APCs present antigens to T cells in the context of MHC molecules as MHC:peptide complexes. In humans, HLA genes are encoded on chromosome 6 and are highly polymorphic, making each individual unique. MHC class I molecules (HLA A, B) are expressed on all nucleated cells of the organism and interact with the T-cell receptor (TCR) on CD8+ T cells, while MHC class II molecules (HLA DR, DQ, DP) are upregulated on activated professional APCs and interact with CD4+ T cells. As compared to other foreign peptide antigens such as derived from pathogens, donor HLA alloantigens are highly immunogenic, rendering the immune response to an allograft uniquely strong. Clinical studies in kidney transplantation have shown that donor-recipient HLA mismatches influence graft outcome and that poorly HLA-matched grafts are at greater risks of rejection (**Figure 2**) (5,14-16).

Figure 2. Graft outcome based on the number of HLA mismatches. (from reference 14)

2.2. Graft rejection

Allograft rejection is the consequence of a complex immune process initiated by the recognition of donor-derived antigens. As a result, the graft becomes infiltrated by immune effector cells resulting in organ dysfunction. In kidney transplantation, rejection will be clinically suspected by an increase of serum creatinine, in some instances associated with increasing proteinuria, hypertension and peripheral oedema. Graft biopsies are however required to confirm and classify rejection episodes and exclude other causes of graft dysfunction such as acute tubular necrosis, recurrence of initial nephropathy, drugs toxicity etc.(5,16). There are several types of rejection, defined by the underlying immune mechanisms and the timing after SOT. Two main categories can be distinguished: 1. Cellular rejection mediated mainly by T cells, 2. Humoral or antibody-mediated rejection.

2.2.1. Acute cellular rejection

Acute cellular rejection (ACR) usually occurs within the first months after transplantation, a time when immune activation is peaking following the first encounter between recipient's T cells and donor antigens. At the time of SOT, ischemia/reperfusion of the grafted organ triggers local innate immune activation and up-regulation of pro-inflammatory molecules (7). This will promote the maturation and migration of graft resident DCs towards recipient's secondary lymphoid organs where they to activate T cells. This pathway of antigen presentation (direct pathway) prevails in the early phase after SOT and induces very strong T cell responses. Recipient's DCs can also traffic to the graft, capture donor antigens, process and present them to recipient's T cells in secondary lymphoid organs (indirect pathway). Although this pathway of allorecognition results in weaker immune activation, it is ongoing throughout the lifespan of the graft (5). Once T cells have been specifically activated by their antigen, they differentiate and upregulate chemokine receptors and adhesion molecules that allow them to migrate to the allograft, adhere to the capillary endothelium and extravasate. Activated T cells can directly injure the graft as well as recruit other immune cells through the production of cytokines and chemokines, resulting in local inflammation, oedema and necrosis.

In kidney grafts, ACR starts in the tubulo-interstitial space with T lymphocytes infiltrating renal tubules and provoking tubulitis (**Figure 3**) (5). The inflammation cascade is there amplified by recruitment and activation of other cells. Tubular cells can regenerate after acute tubular necrosis, but if inflammation is severe or chronic, the cells can transform into mesenchymal myofibroblasts, inducing irreversible interstitial fibrosis and tubular atrophy (17,18). In the context of severe T-cell mediated ACR, the vascular compartment can also be injured. Inflamed endothelial cells up-regulate adhesion molecules (ICAM-1, VCAM) and chemokines, resulting in mononuclear invasion in the subendothelium and intima of arteries that can lead to thrombosis or interstitial haemorrhage, clinically manifesting as acute severe kidney dysfunction needing dialysis. At this later stage, graft injuries are rarely reversible.

2.2.2. Antibody-mediated rejection

In the context of blood group matched SOT, donor-reactive B cells differentiate into plasma cells that produce antibodies against donor HLA antigens (DSA) causing humoral or antibody-mediated rejection (AMR). Acute AMR refers to a process that occurs within days early after transplantation, chronic AMR implies a slow but continual deterioration of graft function with specific histological modifications (19,20).

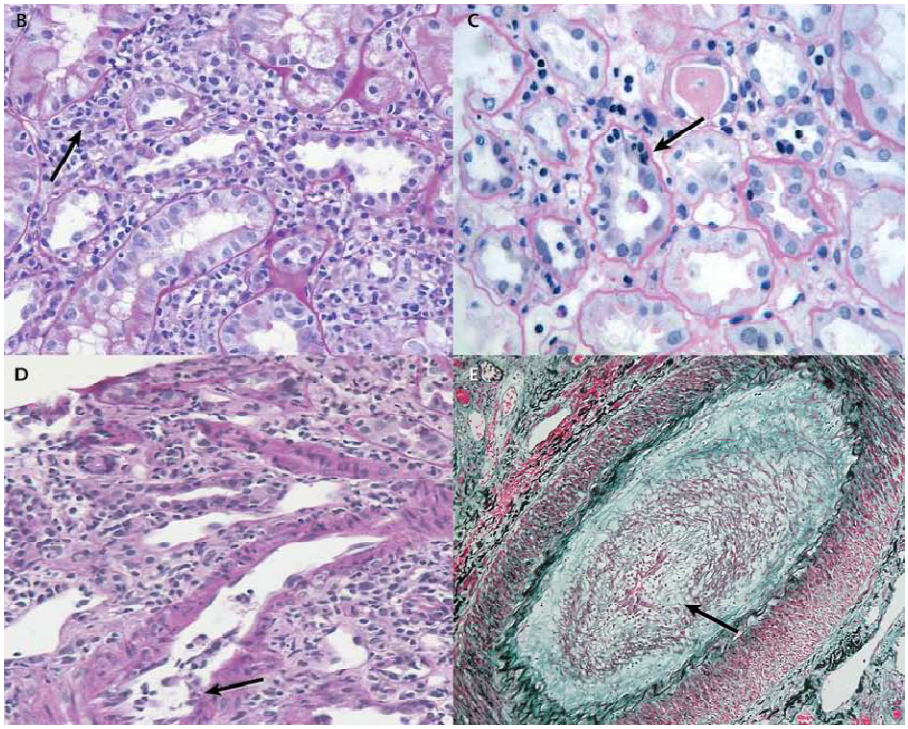

Figure 3. Acute cellular rejection (from reference 5)**. B.** Interstitial infiltration by inflammatory mononuclear cells; PAS. **C.** and **D.** Mononuclear cells, tubulitis; PAS. **E.** Vascular inflammation; HES. (from reference 5)

At the time of transplantation, DSA can be pre-existing in a subset of recipients that have been previously immunized, e.g. in the case of re-transplantation, transfusions or multiple pregnancies. DSA can also appear de novo after transplantation, in the case of insufficient immunosuppression to control the activation of B cells. Sensitized patients are more at risk of AMR and it has been stated that the occurrence of DSA correlates with an unfavorable outcome. HLA antigens are the most immunogenic molecules in SOT. Other non-HLA antigens have been described to contribute to host immunization but are not routinely screened in the clinic. In order to avoid AMR, anti-HLA antibodies are screened prior to transplantation and regularly thereafter. One method is the non-specific determination of panel-reactive antibodies (PRA). In brief, patient's serum is incubated with a mixture of cells carrying a broad spectrum of HLA antigens representative of potential donors. Anti-HLA antibodies, if present, will bind and lyse the cells carrying their target antigen in the presence of added complement factors. The result is given in percentage of destroyed panel cells (%PRA) (16). To determine the specificity of these anti-HLA antibodies, microbeads coated with single HLA antigens are incubated with the recipient's serum and a fluorescence-based method is used with results expressed as mean fluorescence intensity (MFI).

In kidney transplantation, circulating DSA typically first target donor MHC antigens expressed on the capillary endothelium in the peritubular interstitial compartment and or the glomerular tuft. Bound DSA then fix and activate locally the complement cascade causing endothelial injury. The damaged endothelium releases cytokines, chemokines and platelet aggregation factors (5,24). On biopsies, the signs of AMR

can be found as accumulation of neutrophils (PMN) and macrophages in dilated peritubular capillaries as well as acute glomerulitis (20). In severe cases, AMR can reach arteries and lead to vasculitis with thrombotic microangiopathy (TMA), hemorrhage and transmural necrosis (**Figure 4**) (5,20,21). C4d is an indicator of complement activation through the classical pathway and its positive staining in peritubular capillaries can be used as a marker for acute AMR.

The diagnosis of acute AMR relies on three main parameters: acute tissue injury particularly peritubular capillaritis, evidence of antibody activity, (usually C4d) and detection of DSA (**Table 1** in Material and Methods). The diagnosis of chronic AMR is based on chronic remodeling of glomeruli basement membranes and arteries and the presence of DSA leading to graft dysfunction and proteinuria; C4d positivity is not consistent (22). In some cases, often related to under-immunosuppression, it is possible to witness mixed rejections where AMR and ACR patterns co-exist.

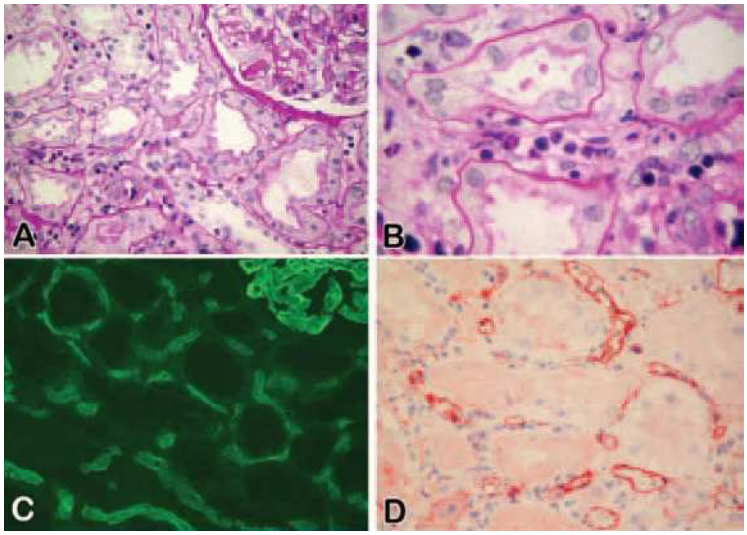

Figure 4. **Acute humoral rejection. A.** peritubular and glomerular capillaries with PMN, mononuclear cells and fibrin thrombus; PAS. **B.** PMN in peritubular capillary; PAS. **C.** C4d in peritubular capillaries; immunofluorescence. **D.** C4d, IHC (peritubular capillaries). (from reference 5)

2.3. Chronic allograft dysfunction

Besides acute rejection episodes, progressive deteriorating processes occur in the allograft, leading to chronic allograft dysfunction (CAD), proteinuria and arterial hypertension (23). Immune and non-immune factors are involved in CAD. On biopsies, non-specific chronic alterations can be observed including glomerular and vascular remodeling leading to glomerulosclerosis, interstitial fibrosis and tubular atrophy (22).

Immune factors are mainly related to inadequate immunosuppression with resulting chronic activation of CD4+ T cells leading to memory T- and B-cell responses as well as the production of DSA. Non-immune factors consist of various graft injuries. Donor age is naturally relevant in this context as aging causes cellular exhaustion, epithelial

and endothelial dysfunction and atrophy. In the same way, the quality of the organ at the time of transplantation influences future CAD development. Living donor transplantation is associated with improved graft survival. This can be explained by pre-existing or early organ injuries at the time of retrieval of cadaveric organs (brain death, ischemia/reperfusion injury, donor conditions such as hypertension or diabetes mellitus). Recipient's factors may also influence graft function, in particular cardiovascular diseases and viral infections (for example BK or CMV). Another important factor in CAD is drugs toxicity and in particular calcineurin inhibitors (CNI) nephrotoxicity. CNI enhance the release of vasoconstrictors and reduce vasodilators producing a chronic ischemic state. Moreover, they increase TGB-β release, which induces fibrosis. Chronic CNI administration thus creates histological changes like arteriolar vacuolization and necrosis as well as hyalinosis, and glomerulosclerosis (24,25). Finally, recurrence of the recipient's nephropathy can also alter graft outcome and contribute to CAD.

2.4 Infection and transplantation

Infectious events are an important concern after transplantation as chronic immunosuppressive treatments in the recipient favors their occurrence and severity. A pre-transplantation evaluation is necessary to assess whether the recipient harbors latent organisms that can be reactivated by immunosuppression, or whether the donor can transmit infectious agents through the graft. Infections are strongly linked to rejection as through the induced inflammatory state, they can activate innate and subsequent adaptive immunity. On the other hand the treatment of rejection episodes implies the increase of immunosuppressive treatments which will in turn promote infections. Thus, the balance between infections and rejection episodes is dependant on the amount of given immunosuppression. CMV and EBV are two viruses that are routinely screened as acute infections can alter patient's and graft's outcome. CMV has been shown to promote other infections and increase the risk of acute rejection. EBV has a promoting role in post-transplant lymphoproliferative disease (PTLD) (16). The situation that is considered most at risk is a primary infection in the context of a sero-postive donor (D+) while the recipient is not (R-).

In the field of kidney transplantation, specific attention is also given to the BK virus. It is a polyomavirus, normally dormant in the urinary tract of adults that can be reactivated by immunosuppression. This viral infection is asymptomatic but can lead to tubule-interstitial nephritis (BK associated nephropathy or PVAN) with progressive fibrosis and graft loss. BK nephropathy is related to the overall state of immunosuppression and currently, the only treatment is to decrease immunosuppression. The level of BK viremia has been shown to correlate with the severity of nephritis, and viremia is routinely screened following kidney transplantation during the 1st year and thereafter in the case of graft dysfunction.

3. Immunosuppressive therapy

Immunosuppression (IS) is a fundamental element in SOT outcome because it is the only available way to prevent and treat rejection episodes nowadays. It comprises two parts: the induction therapy, which is given at the time of transplantation in order

to inhibit the immediate strong immune response by depleting T lymphocytes or modulating their function; and the maintenance therapy, which is a chronic treatment aiming at constantly down-regulating the immune system.

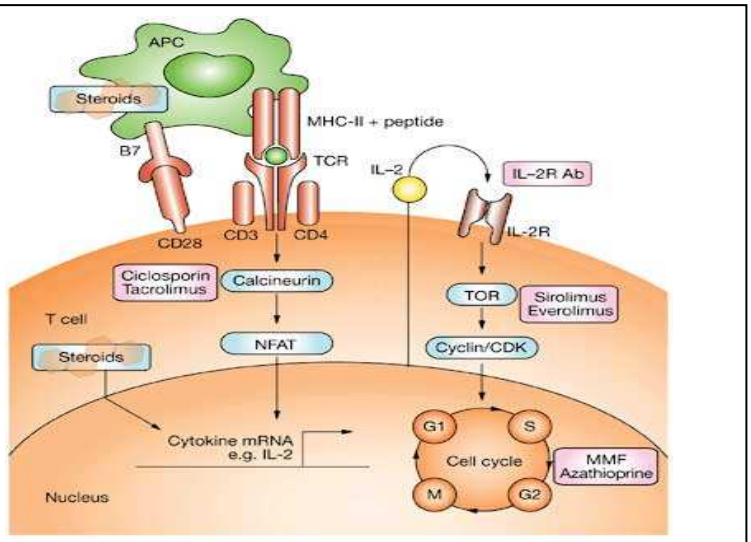

Figure 5. Targets of immunosuppressive drugs

Basiliximab is a monoclonal anti-IL-2 receptor antibody used in induction therapy. Since the IL-2 receptor alpha-chain is up-regulated after T-cell activation, it will only block activated T lymphocytes without depleting them. Basiliximab can be replaced or combined with Thymoglobulin (rabbit polyclonal anti-thymocyte globulin) in patients at high immunological risk of rejection (immunized recipients) or in the case of delayed graft function (DGF). Unlike Basiliximab, Thymoglobulin is a T-cell depleting immunosuppressive drug. In the presence of DSA, intravenous Ig (IvIg) can be administered together with Thymoglobulin. In these cases, plasmapheresis can also be performed.

Maintenance therapy usually involves a combination of 2-3 drugs: CNI (tacrolimus or cyclosporine A), anti-metabolites (azathioprine or mycophenolic acid derivatives) and prednisone. CNI act on signal 1 by blocking the TCR early downstream signaling after interaction with the MHC:peptide complex, while anti-metabolites impede cell cycle and proliferation non-specifically (signal 3). Sirolimus and everolimus are other anti-proliferative drugs that are used in SOT; they inhibit the mTOR pathway. More recently, the drug Belatacept was introduced in the clinic. This is a costimulation-blocker (signal 2) that can be used in induction and maintenance therapy instead of CNI, to avoid nephrotoxicity or TMA.

Current IS mainly target T cells and prevent ACR episodes, but to some extent, they also modulate B-cell activation. More specific B-cell agents also exist, such as Rituximab, an anti-CD20 monoclonal antibody depleting circulating B cells. In the presence of DSA and acute AMR, current treatment consists of plasmapheresis and/or Rituximab and IvIg.

In recent years, a lot of efforts have been invested in finding the best combination of treatment to avoid cellular and humoral rejection, with as little as possible toxicity for the graft and the patient.

Aims

The aims of this study were

- to update the database of the Lausanne/CHUV kidney transplant recipients cohort in order to characterize this population
- to identify the determinants associated with graft rejection and graft outcome at 1-year post transplantation

Material and methods

Patients. This is a retrospective study based on clinical, biological and histological data available on patients who underwent kidney transplantation and were followed at the CTO (*centre de transplantation d'organes*) of the CHUV (University Hospital of Lausanne). We included all patients transplanted from November 2003 to December 2011, except patients under 20 years of age who were followed by pediatricians. Part of the data used in this study was previously gathered by Jeremy Jankovic as part of his Master project.

Data collection and database. Data collection was based on medical paper files and follow-up charts available at CTO and was completed by electronic centralized files of the CHUV: documents archived on *Arichimède* (biopsy reports, biological results), information from *SOARIAN* as well as from the *Transplantation coordination* database (donor and transplantation data). The following parameters were registered in our database: data related to the **donor** (age, living vs. cadaveric), the **transplantation procedure** (cold ischemia time); and to the **recipient** on the day of transplantation (induction therapy), the early **post-operative period** (DGF) and at **1-year follow-up** (maintenance IS, graft function, patient survival). General **recipient**'s clinical data were recorded, such as age, gender, weight, waiting list time, time and type of dialysis, number and type of transplantation, diagnostic of nephropathy. In addition we recorded immunological data: number of donor-recipient HLA mismatches, anti-HLA antibodies and DSA at transplantation and up to 1 year after; infectious events focusing on BK virus.

Immunosuppression protocols after kidney transplantation. Recipients were divided in low or high immunological risk patients based on their immunological and previous transplantation history. Low risk patients were patients receiving a 1st graft, from a living or cadaveric donor, with no prior immunization. High risk patients comprised all other cases (≥ 2 grafts, prior immunization) or if DGF occurred. DGF is defined as the need to dialysis during the first week or if there is no significant reduction in serum creatinine within 4-7 days after transplantation.

Induction therapy was prescribed according to the immunological risk. Low risk patients received Basiliximab (2 doses, day 0 and 4), whereas high risk patients received Thymoglobulin (1-1.5mg/kg/day for CD3 depletion<20 cells/mm^3) ± IvIg. In the 1st year, the maintenance treatment was usually composed of a combination of

CNI, anti-metabolite and prednisone. The CNI dose was adjusted according to therapeutic drug monitoring, while anti-metabolites were administered according to digestive and hematological tolerability and prednisone following a tapering protocol (20mg/day at day 15, tapering down to 5mg/day at 1 year).

Viral infections prophylaxis. All patients received systematic CMV prophylaxis based on D/R sero-status. The dose of the medication was adjusted to the glomerular filtration rate (GFR).

CMV Status		Medication
D	R	
+	-	Valgancyclovir during 3-6 months
+ or -	+	Valgancyclovir during 3 months
-	-	Valacyclovir during 3 months

BK viremia was screened at 3-6-12 months after transplantation or in the case of graft dysfunction.

Immunological analysis. Class I and II anti-HLA antibodies were routinely screened before transplantation, at day 7, at months 3 and/or 6 and yearly thereafter. Whenever anti-HLA antibodies were detected, DSA were screened by the luminex technique.

Rejection episodes and biopsies. Biopsies were performed in the case of graft dysfunction (>20% rise in serum creatinine, proteinuria, DGF) or in the presence of DSA. Rejection events were classified either as clinically-suspected or confirmed by biopsies. Biopsies were assessed by light microscopy, electron microscopy and immunofluorescence for C4d-deposition and classified by the pathologist according to Banff criteria (see **Table 1**). For this study, we reviewed all biopsies performed in our cohort, whatever the indication and the final diagnosis. Of note, some patients may have had >1 biopsy, and/or >1 histological diagnostic. We classified the biopsies as follows:

1) Acute cellular rejection
2) Acute AMR
3) Chronic AMR (a. C4d positive, b. glomerular and/or peritubular capillary remodelling, c. vasculopathy)
4) Renal disease recurrence
5) CNI toxicity
6) BK nephropathy
7) TMA (a. HUS, b. drug-mediated)
8) ATN
9) Other
10) Borderline rejection
11) Mixed cellular and humoral rejection

Table 1. Banff 97 diagnostic categories for renal allograft biopsies-Banff '09 update (from reference 22)

1. Normal
2. Antibody-mediated rejection C4d+, presence of circulating DSA, no signs of rejection. Cases with simultaneous borderline changes are considered as indeterminate. **Acute antibody-mediated rejection** C4d+, presence of circulating DSA, morphologic evidence of acute tissue injury, such as **I.** ATN-like minimal inflammation **II.** Capillary and/or glomerular inflammation and/or thromboses **III.** Arterial **Chronic active antibody-mediated rejection** C4d+, presence of circulating DSA, morphologic evidence of chronic tissue injury, such as glomerular double contours and/or peritubular capillary basement membrane multilayering and/or interstitial fibrosis/tubular atrophy and/or fibrous intimal thickening in arteries
3. Borderline changes Suspicious for acute T-cell mediated rejection. This category is used when no intimal arteritis is present, but there are foci of tubulitis with minor interstitial infiltration or interstitial infiltration with mild tubulitis.
4. T-cell mediated rejection **Acute T-cell mediated rejection (Type/Grade)** IA. Cases with significant interstitial infiltration and foci of moderate tubulitis IB. Cases with significant interstitial infiltration and foci of severe tubulitis IIA. Cases with mild to moderate intimal arteritis IIB. Cases with severe intimal arteritis comprising >25% of the luminal area III. Cases with transmural arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocytic inflammation **Chronic active T-cell mediated rejection** chronic allograft arteriopathy (arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neo-intima).
5. Interstitial fibrosis and tubular atrophy, no evidence of any specific etiology Interstitial fibrosis and tubular atrophy
6. Other: Changes not considered to be due to rejection - acute and/or chronic

Graft and patient outcome. We assessed renal function at 1-year follow-up using serum creatinine measurements in two formulae to calculate GFR: Cockroft-Gault and CKD-EPI. Of note, there is currently no consensus on how to calculate GFR in kidney transplant recipients. The Cockcroft-Gault formula is imprecise in obese and elderly patients. The more recently introduced CKD-EPI method is more accurate than the MDRD method, especially for high creatinine clearances (27,28).

Cockroft-Gault: $Clcr=\frac{(140-A)\times M\times k}{[Cr]}$

CKD-EPI: $GFR= 141 \times \min\left(\frac{[Cr]}{\kappa}, 1\right)^{\alpha} \times \max\left(\frac{[Cr]}{\kappa}, 1\right)^{-1.209} \times 0.993^{Age} \times 1.018[if\ ♀] \times 1.159[if\ black]$

Clcr: Creatinine clearance, GFR: Glomerular filtration rate, A: Age [y], M: Mass [kg], [Cr]: Creatininemia [µmol/l], k: 1.25 for male and 1.05 for female, κ: 0.9 for male and 0.7 for female, α: -0.411 for male and -0.329 for female.

According to the GFR, renal failure is usually separated in 5 stages.

CKD stage	Disease description	GFR
I	Kidney damage with normal or ↑ GFR	≥ 90
II	Kidney damage with mild ↓ GFR	60–89
III	Moderate ↓ GFR	30–59
IV	Severe ↓ GFR	15–29
V	Kidney failure	<15

As most kidney transplant patients have some degree of organ dysfunction (single kidney) with a GFR which is rarely ≥90ml/min, CKD stage I and II patients were grouped for statistical analysis. The great majority of the recipients maintain a working allograft during the 1st year. Severe graft dysfunction requiring renal replacement therapy or a 2nd transplantation was recorded as graft loss, as well as in rare occasions the need for graft explantation. As some patients can die with a functioning graft, their data were excluded in graft survival analysis (death-censored graft survival).

Statistical analysis. Data was gathered in a Microsoft Office Excel database, before being simplified and exported to STATA for statistical analysis. In a first phase, descriptive statistics were performed using all the patients who were included in the study to have general characteristics of our population and of the transplantations. After excluding graft losses and deceased patients, we proceeded to general descriptions of the population at 1 year after transplantation.

We then analyzed and compared patients based on their rejection status (at least 1 episode vs. none), no matter the type of rejection. Secondly, we compared patients that had ACR vs. patients with acute AMR. The assumption of normality was controlled for each continuous variable by means of the kurtosis test for normality. Normal distributed variables were assessed by mean-comparison tests (t-test). Group comparisons using Wilcoxon rank-sum test was performed for skewed variables (P Kurtosis<0.05). Comparisons between discrete variables were undertaken using the $\chi 2$ test. P value allowed asserting whether the differences in the compared groups were significant (*P value≤0.05*). Finally, multivariate analysis was applied to determine the factors associated with 1-year graft function (linear regression) and with rejection episodes (logistic regression of several variables).

Glossary

ACR	acute cellular rejection	**HLA**	human leucocyte antigen
AMR	antibody-mediated rejection	**HUS**	haemolytic uremic syndrome
Anti-HLAdn	anti-HLA de novo	**IHC**	immuno histochemistry
Anti-HLAp	pre-existing anti-HLA	**ISH**	in situ hybridisation
APC	antigen presenting cell	**IvIg**	intravenous immunoglobulins
AR	acute rejection	**MHC**	major histocompatibility complex
ATN	acute tubular necrosis	**MMF**	mycophenolate mofetil
AZA	azathioprine	**MPA** **EC-MPS**	mycophenolic acid enteric-coated mycophenolate sodium
B	Basilixmab	**P**	prednisone
CAD	chronic allograft dysfunction	**PAS**	periodic acid-Schiff staining
CNI	calcineurin inhibitor	**PMN**	polymorphonuclear leukocytes
CSA	cyclosporine A	**PRR**	pattern recognition receptor
DC	dendritic cell	**PTLD**	post-tranplant lymphoproliferative disease
DSA	donor specific antibody	**STCS**	Swiss transplant cohort study
DSAdn	DSA de novo	**T**	Thymoglobulin
DSAp	pre-existing DSA	**TCR**	T-cell receptor
FK	tacrolimus	**TLR**	Toll-like receptor
GFR	glomerular filtration rate	**TMA**	thrombotic microangiopathy
HES	hematoxylin eosin staining	**SOT**	solid organ transplantation

Results and discussion

The statistical analysis of the clinical, immunological and histological data that were gathered for this study is described under the following 3 headings in the results section:

1. Characteristics of the population at the time of transplantation
2. Patient and graft outcomes 1 year after kidney transplantation
3. Predictive factors of rejection and graft outcome at 1 year

1. Characteristics of the population at the time of transplantation

1.1. Patient characteristics

Table 2
Recipients characteristics (n, %)

Sex	Male	194 (68.5%)
Number of Tx	First Tx	216 (78.3%)
Dialysis	Pre-emptive	48 (17.6%)
	Hemodialysis	180 (65.9%)
	Peritoneal dialysis	25 (9.2%)
	HD+PD	20 (7.3%)
Pre-Tx disease	Unknown origin	18 (6.5%)
	Diabetes	30 (10.9%)
	Hypertension / nephroangiosclerosis / renovascular	27 (9.8%)
	Glomerulonephritis / vasculitis	64 (23.2%)
	ADPKD	32 (11.6%)
	Other genetic familial diseases	10 (3.6%)
	Interstitial nephritis	11 (4.0%)
	HIV	1 (0.4%)
	Obstructive/reflux nephritis	14 (5.1%)
	Previous graft insufficiency	46 (16.7%)
	Congenital diseases/malformation	8 (2.9%)
	CNI toxicity	10 (3.6%)
	Other	5 (1.8%)
Mean age in years		56 [21-79]
Mean waiting time on list in days		665 [2-3552]
Mean weight in kg		72.6 [46-108]

Table 2 summarizes the basic characteristics of all the patients that received a kidney graft from November 2003 until December 2011 in CHUV, with the exception of pediatric patients. A total of 280 patients were transplanted in our study period, with an impressive 17.6% grafts performed pre-emptively (before starting dialysis), mainly thanks to the possibility of living donation. Sixty-eight percent of recipients were men, reflecting a previously described higher prevalence of ESRD in this population. Surprisingly, in a period when cardiovascular diseases such as diabetes and hypertension are reaching epidemic levels, glomerulonephritis remains the main cause of ESRD in our population (23.2%), followed by autosomal dominant polycystic kidney disease (ADPKD), perhaps reflecting the lack of efficient treatment for these nephropathies that can prevent the evolution towards ESRD. Interestingly, previous

graft failure constitutes 16.7% of the causes, testifying needed improvement in current medical and pharmacological strategies to improve long-term survival of grafts after kidney transplantation.

We also examined overall kidney transplantation activity in the CHUV during our study period (**Figure 6**). The number of transplantations has gradually increased, with a progressive increase in the proportion of living, related (RLD) or unrelated (ULD), vs. cadaveric donors (CD). This could partly be explained by the fact that transplantation has become a public issue, with more information and encouragement to donate a kidney. Moreover, because of the aging of the population and of the increasing prevalence of ESRD, the waiting list is increasing with insufficient cadaveric donors. This tendency is reflected by the time on waiting list (mean 665 days in our population, range 2-3552 days).

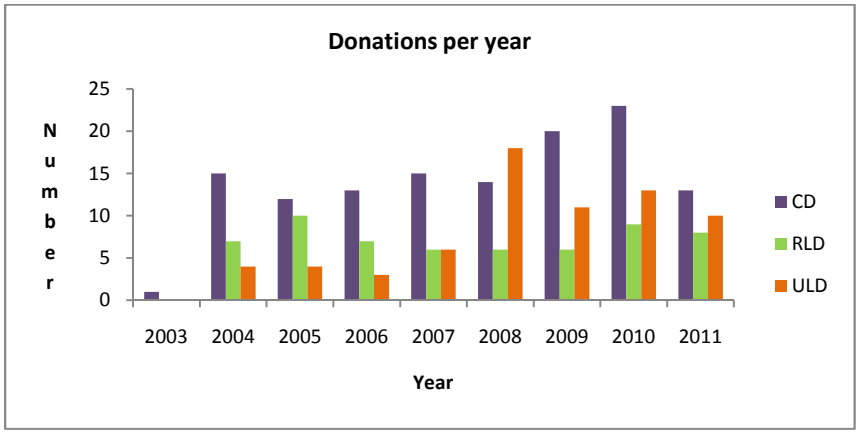

Figure 6. Kidney transplantation activity at CHUV

	Year of renal transplantation							
Donor type	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**
CD	15	12	13	15	14	20	23	13
RLD	7	10	7	6	6	6	9	8
ULD	4	4	3	6	18	11	13	10

1.2. Graft and immunological characteristics

Table 3
Transplantation characteristics (n, %)

		Total n (%)	2003-2008	2008-2011
Donor type	Cadaveric donor	145 (51.8%)	66 (56.4%)	79 (48.5%)
	Total living donors	135 (48.2%)	51 (43.6%)	84 (51.5%)
	Related	61 (21.8%)	32 (27.3%)	29 (17.8%)
	Unrelated	74 (26.4%)	19 (16.3%)	55 (33.7%)
Mean cold ischemia time in hours		11 [3-24]		
HLA mismatches	0	13 (4.6%)		
	1	7 (2.5%)		
	2	28 (10.0%)		
	3	58 (20.7%)		
	4	61 (21.8%)		
	5	63 (22.5%)		
	6	50 (17.9%)		
Presence of antibodies	Anti-HLA	54 (19.9%)		
	DSA	16 (6.0%)		
Mean last PRA		3.6% [0-88]		

Table 3 shows the transplantation characteristics. During the whole period of the study, slightly more cadaveric transplantations were performed as compared to living donors (51.8 vs. 48.2%). However, in the more recent 2008-2011 period, the tendency has reversed in the favor of living donation, and in particular the proportion of living unrelated donors has increased. Around 20% of the patients in our cohort were sensitized (presence of anti-HLA) at the time of transplantation, with 6% having pre-existing DSA. These DSA were probably present at low levels, not affecting the complement-dependent-cytotoxicity (CDC) crossmatch. Indeed, the serum of the recipient is tested against donor cells prior to transplantation, to detect the presence of anti-HLA antibodies that can bind and lyse donor cells (CDC crossmatch). Transplantation is performed only if the test is negative. Pre-sensitization can occur due to previous transplantations, transfusions or pregnancies. Of note, the proportion of patients with pre-exisiting DSA may have been underestimated as DSA monitoring and, in particular, class II DQ anti-HLA antibodies were not routinely tested until in recent years.

1.3. Immunosuppression

Table 4
Immunosuppression at the time of Tx (n, %)

Induction	B	184 (67.6%)
	B+T	34 (12.5%)
	T (up to 4days)	36 (13.2%)
	T (>4days)	5 (1.8%)
	T+IvIg	13 (4.8%)
Maintenance	FK+MMF+P	241 (89.9%)
	CSA+MMF+P	11 (4.1%)
	CSA+P	1 (0.4%)
	FK+P	2 (0.7%)
	FK+AZA+P	13 (4.8%)

At the time of transplantation, the standard IS treatment consisted of Basiliximab induction, followed by FK+MMF+P maintenance therapy (**Table 4**). In high immunological risk patients, Thymoglobulin induction (3-4 days) was instead administered (13,2%). In the case of DGF, Basiliximab induction therapy was combined with Thymoglobulin (B+T), or prolonged Thymoglobulin (>4 days) was administered in order to delay the introduction of potentially nephrotoxic CNI, until recovery of graft function. A little number of patients switched MMF to AZA even before initial hospital discharge, mainly due to gastro-intestinal side-effects. CSA was preferred to FK in some patients, mainly due to the risk of post-transplant diabetes, chronic HCV or depending on the underlying nephropathy (e.g. focal segmental glomerulosclerosis). No patient was started on mTOR-inhibitor-based maintenance IS.

2. Patient and graft outcomes one year after kidney transplantation

During the 1st year after transplantation, there were 19 graft losses (6.7%) and 9 patients (3.2%) died, leaving 252 patients (out of total 280 transplanted) with functioning grafts for further analysis (**Table 5**). Graft losses were mainly due to primary non-function (due to renal vessels thrombosis) and early explantation. In one case, non-function was due to severe TMA. Because of the small number of deaths, we did not systematically look at the causes but this should be done in future work.

2.1. Outcomes at 1 year

Table 5
Status one year after Tx (n, %)

Antibodies	Anti-HLA	62 (25.1%)
	Anti-HLAp	43 (71.7%)
	Anti-HLAdn	17 (28.3%)
	DSA	15 (6.5%)
	DSAp	10 (76.9%)
	DSAdn	3 (23.1%)
Renal function (ml/min)	Cockroft-Gault	64.4 [14.9-134.2]
	CKD-EPI	54.8 [11.9-104.1]
CKD stage (by CKD-EPI)	≥60	93 (36.8%)
	30-59	145 (57.3%)
	15-29	13 (5.1%)
	0-14	2 (0.8%)
BK virus infection		40 (18.6%)
Graft loss		19 (6.7%)
Death		9 (3.2%)

One year after transplantation, anti-HLA antibodies were present in 25.1% of recipients, of which 71.7% pre-existing and 28.3% de novo (including 23.1% DSA). For 2 patients in our cohort, some immunological data were missing and we could not determine whether the anti-HLA antibodies present at 1 year were pre-existing or de

novo. The proportion of patients with pre-exisiting anti-HLA antibodies reflects the pre-sensitized patients, while de novo anti-HLA antibodies, and in particular de novo DSA, are associated with under- or inadequate IS.

The mean GFR at 1 year was 64.4 ml/min calculated by the Cockroft-Gault method and 54.8 ml/min using the CKD-EPI formula (which should be more accurate as Cockroft-Gault tends to overestimate renal function). The majority of patients (57.3%) were in CKD stage III, but 36.8% had even better creatinine clearances $\geq$60 ml/min. This is important as kidney function at 1 year after transplantation has been shown to be an important factor influencing graft and patient outcome (29). Only, a small proportion (5.1%) of the recipients had severe CKD and 2 patients (0.8%) were in ESRD. Graft dysfunction at 1 year can have several causes, such as the quality of the graft at implantation (donor's age and comorbidities, injuries related to the surgical procedure), rejection episodes, BK nephropathy, CNI toxicity and in some cases recurrence of nephropathy.

BK virus infection, defined here as either high level BK viremia (viremia>10'000 copies) or based on histological diagnosis of BK nephropathy, occurred in 18.6% of the patients. Not all patients in whom BK viremia was detected underwent biopsy, in some instances because of comorbidities and potential risks of the procedure but also because viremia has been shown to correlate with kidney injury.

2.2. Maintenance immunosuppression at one year

Table 6
Immunosuppression at one year (n, %)

CNI	FK	234 (92.5%)
	CSA	14 (5.5%)
	Sirolimus/everolimus	4 (1.6%)
Anti-proliferative	MMF/EC-MPS	217 (86.1%)
	AZA	19 (7.5%)
Prednisone		237 (94.0%)

Careful study of all patients' charts revealed that maintenance IS was relatively unchanged during the 1st year after transplantation (**Table 6**). At one year, the majority of the patients had been maintained on triple IS, based on tacrolimus together with mycophenolic acid derivatives and prednisone (FK+MMF+P). Some (5.5%) were switched to cyclosporine or mTOR inhibitors (1.6%). The switch from FK to CSA was mainly due to post-transplant diabetes or the occurrence of BK nephropathy. In this later group, MMF dosage was usually decreased or even stopped. A little number of patients (7.5%) received AZA instead of MMF/EC-MPS, mainly due to gastro-intestinal side-effects. The switch to mTOR inhibitors was motivated by CNI-induced TMA or the occurrence of neoplasia. Ninety-four percent of patients were still receiving prednisone (usually 5mg/day) and were not yet weaned at 1 year.

2.3. Kidney biopsies

We reviewed the reports of all the biopsies that were performed during the first year after transplantation in our study population. All the biopsies with the diagnostic of rejection were scored by our pathologist according to Banff criteria (**Table 1**). For further analysis, we classified these biopsies according to the underlying immune mechanism, as described in Material and Methods. If there were 2 or more diagnostics for the same biopsy, we always considered the most prominent one (for example “rejection” rather than “mild signs of CNI toxicity”) or classified them in mixed groups if all diagnostics were clinically relevant.
Protocol biopsies were not performed in CHUV but only in the case of graft dysfunction (>20% rise in serum creatinine, proteinuria, DGF) or in the presence of DSA. Eighty-seven biopsies were performed in our cohort during the study period. The main histological diagnosis was ACR (16.1% of biopsies) (**Table 7**). The 14 episodes of ACR combined with 5 of borderline rejection, when reported to the total number of 252 patients in our cohort, represented a relatively low incidence (7.5%) of T-cell mediated rejection in the first year after kidney transplantation when compared to published data. Acute AMR, represented 11.5% of all biopsies. In 4 cases, histological criteria for both acute cellular and humoral rejection were present, reflecting insufficient IS and sensitization of the recipient. There were 6 cases of BK nephropathy, but interestingly in 6 other cases, criteria for both BK nephropathy and rejection (either cellular or humoral) were present, illustrating the delicate balance between over- and under-immunosuppression and current therapeutic challenges.

Table 7

Biopsies (n=87)	
ACR	14 (16.1% of all biopsies)
Acute AMR	10 (11.5%)
Borderline rejection	5 (5.7%)
Mixed cellular and humoral rejection	4 (4.6%)
BK nephropathy	6 (6.9%)
BK and rejection	6 (6.9%)
Recurrence of nephropathy	2 (2.3%)
CNI toxicity	10 (11.5%)
TMA	4 (4.6%)
ATN	9 (10.3%)
Other	17 (19.5%)

3. Predictive factors of rejection and graft outcome at 1 year

3.1. Rejection

We first analyzed the incidence and type of rejection occurring during the 1st year after transplantation (**Table 8**).

Table 8

Acute rejection episodes

Patients with rejection	40 (15.9%)
Mean in all patients	0.2 [0-3 episodes]
Type of rejection	
Clinical	1 (2.5%)
Acute cellular	19 (47.5%)
Acute AMR	10 (25%)
Mixed cellular and humoral	4 (10%)
BK and rejection	6 (15%)

At 1 year, 15.9% of the patients had suffered acute rejection, of whom 47.5% cellular and 25% humoral. Most patients had only 1 episode of rejection during the 1st year, but some patients suffered from up to 3 episodes. No diagnostic of chronic AMR was ascertained during the first year follow-up.

Starting with univariate analysis, we compared 2 populations based on their rejection status: patients that had no rejection vs. patients that had suffered from at least 1 episode of acute rejection, independent of the type of rejection. In order to simplify statistical analysis, we decided to consider only 1 rejection episode, the strongest (with the worst histology), per person. However, when there was, an episode of cellular rejection and another of humoral rejection for the same patient, we chose to describe one sole event as mixed cellular and humoral rejection. Indeed, when looking carefully in the patients files, we realized that in many cases, repeated biopsies in the same patient during the 1st year reflected a continuum of the same rejection episode that had not resolved or was insufficiently treated.

Table 9
Clinical characteristics and acute rejection

	No rejection	Rejection	P value
Continuous data	*Mean±SD*	*Mean±SD*	
Age	51.3±12.9	49.9±13.5	0.643
Creatinine at 1yr	125.9±37.8	160.4±64.2	*0.001*
GFR (CKD-EPI) at 1yr	56.6±15.9	44.7±16.6	*0.001*
PRA last	2.5±9.5	7.3±17.7	*0.030*
Categorical data	*n (%)*	*n (%)*	
Male sex	147 (69.0)	26 (66.7)	0.771
Prior dialysis	174 (81.7)	34 (87.2)	0.406
Cadaveric donor	103 (48.4)	21 (53.9)	0.528
Prior Tx	41 (19.2)	13 (33.3)	*0.049*
BK virus infection	31 (17.5)	9 (25)	0.294

P values that are significant are in italics.

We first analyzed in our population, patient's or graft's related factors that could predict rejection (**Table 9).** There was no correlation between recipients' age, sex, prior dialysis status or the type of donor. No association was found between rejection and BK virus infection. However, this could be due to insufficient number of patients in this group. But it is to be noted that in 6 biopsies we found both BK nephropathy and signs of rejection, either ACR or AMR. Pre-sensitized patients, such as patients who underwent a prior transplantation or had positive last PRA values, were more at risk of acute rejection, despite having received IS and in particular induction treatment (usually Thymoglobulin) adapted to their known high immunological risk. As expected, these risk factors were associated with acute AMR (**Table 10**). Interestingly, all 10 episodes of acute AMR occurred in patients with prior dialysis, perhaps reflecting the difficulties of performing pre-emptive transplantation in pre-sensitized patients due to lack of suitable living donors, de-sensitization protocols and long waiting time on list.

Significantly decreased kidney function at 1 year was associated with acute rejection, here of course rather as a consequence of immune-mediated graft injury.

We then analyzed more closely the correlation between the immunological status of the recipient and the occurrence and type of acute rejection (**Table 11, Table 12**). In our population, pre-existing anti-HLA antibodies and pre-existing DSA were the main predictors of acute rejection, mainly AMR, in the first year. There was a trend towards more ACR with increasing number of donor-recipient HLA mismatches. Post-transplant de novo DSA were not associated with acute rejection in this time-frame.

Table 10 Clinical characteristics and type of acute rejection

Continuous data	**ACR** *Mean±SD*	**Acute AMR** *Mean±SD*	**P value**
Age	50.6±15.1	51.1±12.7	0.945
PRA last	0±0	13±15.1	*<0.001*
Categorical data	*n (%)*	*n (%)*	
Prior dialysis	15 (78.9)	10 (100)	0.118
Prior Tx	2 (10.5)	8 (80.0)	*0.001*
BK virus infection	3 (18.7)	2 (20.0)	0.937

In this analysis, borderline or mixed cellular and humoral rejection episodes were not included.

Table 11
Immunological data and acute rejection

HLA mismatches	**No rejection** *n (%)*	**Rejection** *n (%)*	**P value** 0.130
0	13 (6.2)	0 (0)	
1	6 (2.9)	1 (2.6)	
2	17 (8.1)	8 (20.5)	
3	41 (19.5)	7 (17.9)	
4	50 (23.8)	5 (12.8)	
5	46 (21.9)	9 (23.1)	
6	37 (17.6)	9 (23.1)	
Antibody status			
Pre-existing anti-HLA	34 (16.5)	14 (36.8)	*0.004*
Pre-existing DSA	6 (3.0)	7 (20.6)	*0.001*
Anti-HLA dn	13 (30.9)	3 (17.6)	0.298
DSA dn	3 (42.9)	0 (0.0)	0.067

In this analysis, all rejection episodes were included, except 1 clinical episode.

Table 12 Immunological data and type of acute rejection

HLA mismatches	**ACR** *n (%)*	**Acute AMR** *n (%)*	**P value** 0.146
0	0	0	
1	0	0	
2	2 (10.5)	5 (50.0)	
3	4 (21.0)	2 (20.0)	
4	4 (21.0)	0	
5	6 (31.6)	2 (20.0)	
6	3 (15.8)	1 (10.0)	
Antibody status			
Pre-existing anti-HLA	3 (15.8)	7 (77.8)	*0.001*
Pre-existing DSA	1 (5.6)	5 (71.4)	*0.001*

In this analysis, all rejection episodes were included, except 1 clinical and mixed episodes.

We next examined the influence of immunosuppressive treatments on graft rejection (**Table 13**). Having received Basiliximab induction therapy was associated with the absence of rejection during the 1st year, reflecting probably more the low immunological risk profile of these patients at the time of transplantation than a protective effect of the treatment. Regarding the use of CNI, FK-based IS tended to protect against acute rejection, but data did not reach statistical significance.

Table 13 Immunosuppressive treatment and acute rejection

Ât Tx	**No rejection**	**Rejection**	**P value**
Induction	*n (%)*	*n (%)*	
B	154 (72.3)	21 (53.8)	*0.001*
B+T	22 (10.3)	5 (12.8)	
T (3-4d)	30 (14.1)	2 (5.1)	
T (>4d)	2 (0.9)	3 (7.7)	
T+Ivlg	5 (2.4)	8 (20.5)	
Maintenance			
FK+MMF+P	195 (91.5)	32 (82.0)	0.055
CSA+MMF+P	7 (3.3)	3 (7.7)	
CSA+P	0 (0.0)	1 (2.6)	
FK+P	1 (0.5)	1 (2.6)	
FK+AZA+P	10 (4.7)	2 (5.1)	
At 1 yr			
FK	197 (92.9)	35 (89.7)	0.087
CSA	11 (5.2)	3 (7.7)	
Sirolimus	4 (1.9)	0 (0.0)	
MMF/MPA	181 (85.8)	34 (87.2)	
AZA	17 (8.1)	2 (5.1)	0.779
Prednisone	196 (92.9)	39 (100)	0.086

For cadaveric donors transplantation (**Table 14**), longer waiting times before transplantation tended to favor acute rejection episodes in the 1st year; however this was not statistically significant. There was no effect of cold ischemia time on rejection, probably mainly because it is anyway relatively short in Switzerland due to short distances between centers and the good organization of the teams involved in SOT.

Table 14

Cadaveric donors data

	No rejection	**Rejection**	**P value**
	Mean±SD	*Mean±SD*	
Waiting time (days)	632±558	954±777	0.074
Cold ischaemia time (hours)	11±4	10±3	0.641

Finally, multivariate analysis using logistic regression tests were applied to determine the factors associated with rejection episodes within the first year after transplantation. When all the potential risk factors were analyzed together, none was associated with rejection with significance (**Table 15**). This may be due to low total numbers of acute rejection episodes within the 1st year to reach statistical significance.

Table 15
Predictors of acute rejection

Model	Odds ratio	P value
Constant	39.37±80.05	0.071
Recipient age	0.96±0.02	0.055
Female sex	0.59±0.33	0.345
Prior dialysis	1.79±1.31	0.423
Prior Tx	1.31±1.17	0.766
Donor type	0.81±0.44	0.696
BK virus infection	1.27±0.71	0.672
Induction treatment		
B+T	0.92±0.70	0.936
T (3-4d)	0.40±0.48	0.444
T (>4d)	0.20±0.36	0.373
T+IvIg	3.81±4.44	0.252
Maintenance treatment		
CSA+MMF+P	1.72±1.14	0.870
CSA+P	1	
FK+P	1	
FK+AZA+P	1.56±1.58	0.658
PRA last	1.04±0.27	0.106
Pre-existing anti-HLA	0.41±0.48	0.446
Pre-existing DSA	2.32±3.06	0.522

Using the same model, we completed our analysis by comparing separately immunological factors that may promote acute rejection in the 1st year after transplantation (**Table 16, Table 17**). Regarding induction therapy, the prolonged use of Thymoglobulin or its combination with IvIg was associated with acute rejection. The maintenance IS prescribed at hospital discharge early after transplantation or that the patient was taking at 1 year did not significantly influence rejection (data not shown). Pre-existing DSA were again found to be predictors of acute rejection.

Table 16
Induction therapy and acute rejection

Model	Odds ratio	P value
Constant	0.13±0.03	*0.001*
B+T	1.67±0.91	0.351
T (3-4d)	0.49±0.37	0.350
T (>4d)	11.00±10.36	*0.011*
T+IvIg	11.73±7.22	*0.001*

Table 17 Rejection and immunological status		
Model	**Odds ratio**	**P value**
Constant	0.14±0.03	*0.001*
PRA last	1.02±0.02	0.274
Pre-existing Anti-HLA	0.70±0.54	0.648
Pre-existing DSA	8.62±7.39	*0.012*

3.2. Renal function

Using multivariate analysis and linear regression tests, we analyzed the predictive factors of graft outcome at 1 year, as defined by kidney function calculated with the CKD-EPI formula. The results are summarized in **Table 18**.

Table 18 Renal function at 1 year		
Model	**Coefficient±SD**	**P value**
Constant	78.59±5.04 ml/min	
Recipient age	-0.38±0.08 ml/min/yr	*0.001*
Female sex	-2.75±2.24	0.221
Prior dialysis	-2.39±2.89	0.410
Prior Tx	3.26±2.54	0.201
Cadaveric donor	-1.16±2.21	0.599
Cold ischemia time	0.48±0.45	0.288
BK virus infection	-0.09±2.60	0.971
Induction treatment		
Compared to B		
B+T	-4.06±4.26	0.344
T (3-4d)	-0.51±5.34	0.924
T (>4d)	-31.30±10.42	*0.004*
T+IvIg	3.91±12.72	0.760
Maintenance treatment		
Compared to FK+MMF+P		
CSA+MMF+P	-25.10±10.32	*0.018*
CSA+P	0	
FK+P	0	
FK+AZA+P	-7.12±6.25	0.259
PRA last	0.00±0.14	0.989
Pre-existing anti-HLA	10.65±5.31	*0.049*
Pre-existing DSA	-26.39±11.96	*0.031*
Rejection	-14.54±2.74	*0.001*

The constant signifies that a man, with age of 0, without any of the listed factors would have, in accordance with this model, a creatinine clearance of 78.6 ml/min at 1 yr, after a first pre-emptive living donor Tx without any complication.

In our cohort, donor and recipient demographic data, prior dialysis, the type of donor or the cold ischemia time did not significantly influence graft function at 1 year after transplantation. Surprisingly, BK virus infection did not affect the creatinine clearance at 1 year but this should be further evaluated as the number of events was low.

Prolonged Thymoglobulin induction treatment was associated with an important decrease in GFR. It could reflect DGF and early post-transplantation complications and/or early acute rejection. Regarding maintenance IS, CSA-based triple therapy was associated with significant decreased renal function as compared to FK+MMF+P. Rejection episodes were also deleterious to graft function at 1 year. Because of the small number of events, we didn't further detail the analysis according to the type of rejection. Pre-existing DSA were associated with poorer graft function, but unexpectedly, this was not the case for pre-existing anti-HLA. These later data need confirmation.

Conclusion and perspectives

The aim of this study was to identify the determinants associated with graft rejection and outcome at 1-year post transplantation, analyzing the CHUV kidney transplantation cohort established from November 2003 up to December 2011.

The main diagnosis found on the renal biopsies performed during the 1st year was acute rejection with ACR being the most frequent diagnosis (16.1% of all biopsies), followed by acute AMR (11.5% of all biopsies). CNI toxicity and acute tubular necrosis accounted for around 11% of the biopsies, BK nephropathy for around 7% of the cases.

At 1 year, 15.9% of the patients had suffered acute rejection, of whom 47.5% ACR and 25% AMR. In our analysis, we mainly found predictors of acute AMR and in particular pre-sensitization (prior transplantation, positive last PRA values and pre-existing DSA). AMR occurred even if these patients were considered as high immunological risk recipients based on their immunization history and received Thymoglobulin-based induction therapy. This illustrates the paucity of current treatment in targeting B-cell and antibody responses. Our study period was too short to see any effect of de novo DSA, in particular on chronic humoral rejection. With the exception of a trend for the number of donor-recipient MHC mismatches, we could not identify clear determinants of ACR, maybe because of lack of statistical power in our analysis. One could also argue that the relatively low numbers of ACR episodes reflects a good control of T-cell responses by current induction and maintenance IS. Indeed, although the P value did not reach significance standard FK+MMF+P maintenance was less associated to rejection as compared to other treatments.

Acute rejection was associated with significantly decreased renal function (by around 14 ml/min of GFR in the first year). In the same way, pre-existing DSA was associated with decreasing GFR. BK virus infection did not significantly impact renal function but these results have to be taken with caution because of the low number of events. CSA-based IS also significantly affected renal function at 1 year (loss of mean 25 ml/min GFR).

Overall, the 1-year patients and grafts outcomes of the CHUV kidney transplant recipients correspond to published reports under current standard of care (5,14,16). A limitation in our study that aimed to find causal factors of rejection episodes was the lack of statistical power. Bigger multicenter prospective cohorts with long-term follow-up are thus needed to unravel predictors of outcome after kidney transplantation. This will help improve future therapeutic strategies. Moreover, detailed cellular and molecular studies based on patients' biological samples are essential in understanding underlying mechanisms of graft rejection. The ongoing Swiss Transplant Cohort Study follows these aims (30).

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