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Abstract
Cytomegalovirus is common in adult recipients (prevalence of 40-90%). Children are typically seronegative but immunosuppression may prone to primary-infection or viral reactivation, with potentially severe consequences. CMV infection incidence in pediatric kidney transplant recipients has seldom been investigated.
The aim of our study was to evaluate the incidence and timing of CMV infection during the first year after renal transplantation. We assembled a retrospective cohort of 136 children who had received a kidney transplant between 2003 and 2014 with a year follow-up. The patients were classified regarding CMV infection as high risk (D+/R−), intermediate risk (R+) or low risk (D−/R−). CMV infection was defined by the viral replication remaining asymptomatic whereas CMV disease concerned viral replication with clinical and/or biological symptoms. Oral valganciclovir was used as prophylaxis for high-risk recipients.
A total of 38 patients (27.9%) developed CMV infection, 13 (40.6%) of the 32 D+/R-, 24 (45.3%) of the 53 R+ and 1 (2.0%) of the 51 D-/R-. Of these 38 infected patients, 10 developed tissue-invasive disease.
During the first year after kidney transplantation, 27.9% of recipients developed CMV infection. This study confirms the influence of donor and recipient CMV status on infection propensity and highlights the importance of adequate follow-up for intermediate risk patients.

Keywords: child; cytomegalovirus (CMV); ganciclovir; kidney transplantation; valganciclovir; viral prophylaxis

Introduction

Kidney transplantation (Tx) is considered the most effective treatment for children with end stage kidney disease. The drugs used to avoid transplant rejection suppress the immune system, predisposing the patient to opportunistic fungal, bacterial or viral infections [1], notably from cytomegalovirus (CMV), a ubiquitous virus from the *Herpesviridae* family [2,3]. Roughly 40 to 90% of humans are thought to be seropositive for CMV [2-4], with primary infection occurring mostly during childhood, predominantly through contact with secretions [5,6].

In contrast with adults, most children are seronegative before Tx [3] (59-67% versus 35-40% of adults [7]), making them at high risk of primary CMV infection. Transplantation can also reactivate latent infections [7]. CMV infection (primary or reactivated) is the most common infection in renal transplant patients [1], but tends to respond well to antiviral therapies [7]. Between 12.7% and 38.0% of children develop CMV infection in the first year after transplantation according to a small number of studies [8-11].

The main risk factors for CMV infection are the serostatuses of the donor (D) and recipient (R). Patients are classed as high risk if the donor is seropositive and the recipient seronegative (D+/R−), intermediate risk if the recipient is seropositive (R+, irrespective of the donor's serological status), and low risk if the donor and recipient are both seronegative (D−/R−) [3]. Other risk factors include acute rejection (because of the intensified immunosuppression required) [3,6], prolonged ischemia [3], elderly [3] or female recipients [12] and elderly or deceased donors [6]. CMV infection can itself lead to acute rejection [10,13-15], graft loss [13], reduced glomerular filtration rate (GFR) [9], tissue-invasive disease (e.g. gastrointestinal ulcers, hepatitis, pneumonia and retinopathy) [3,16], thrombotic micro-angiopathy (TMA) [2], chronic kidney disease [9,17], new-onset diabetes mellitus [18] and sometimes death [15].

Effective and safe anti-CMV prophylaxis is therefore crucial in pediatric kidney recipients. Two therapeutic strategies are used [3,6,7,16], whose effectiveness in pediatric patients has yet to be compared. The first one consists in a 3 to 6 months antiviral prophylaxis for all high-risk patients whereas the second closely monitors patients, initiating antiviral treatment as soon as viral replication is detected by polymerase chain reaction (PCR).

According to the 2013 international consensus guidelines on the management of CMV in solid-organ Tx [6], CMV disease in children younger than 12 years should be treated with intravenous ganciclovir, with doses adapted to their GFR. In cases of biological (symptom-free) CMV infection, patients under and over 5 years of age should be treated respectively with intravenous ganciclovir and oral valganciclovir. Children older than 12 years are expected to have an adult-like response to valganciclovir. Anti-CMV immunoglobulins are recommended for the treatment of CMV-related hypogammaglobulinemia, pneumonitis and enteritis.

Jongsma et al. [8] have documented CMV infection in a significant number of renal Tx children. The aim of this study was to evaluate the incidence and outcome of CMV infection during the first year after kidney Tx. Our hypothesis was that better outcome was obtained with prolonged prophylaxis adapted to CMV infection risk. Secondary objectives were to determine the risk factors and complications of CMV infection to help improve future clinical practices.

Patients and methods

Study design

The study was conducted on a single-center historical cohort. We retrospectively investigated potential risk factors for infection (primary or reactivated), namely, the donor-recipient serological matching risk (high: D+/R−; intermediate: R+; low: D−/R−), the occurrence of acute rejection before infection, the ischemia time (cold, warm, and total), the age and gender of the recipient, the number of organs transplanted, the age of the donor, and whether the donor was alive or deceased. From a prognostic perspective, we recorded the occurrence of the following complications of CMV infection: Acute rejection, decline in GFR, TMA and diabetes mellitus. Note that we considered complications, besides viral infection that arose within one year of Tx.

CMV IgG and IgM serology was assessed by a chemiluminescent microparticle immuno-assay (CMV IGG II, Liaison XL, DIASORIN and CMV IGM II, Liaison XL, DIASORIN).

As patient may be subject to consecutive Tx after graft loss, retransplanted patients were considered as independent recipients. All transplantations were analyzed and multiple Tx of a same patient were considered as independent.

Inclusion and exclusion criteria

The study cohort consisted of patients who underwent kidney Tx in the pediatric nephrology department of Lyon University Hospital between January 2003 and October 2014. Patients were excluded if they were older than 18 years at the time of Tx, if they died or lost their graft within the first week of Tx, or if they were lost to follow-up.

Pre- and post-transplantation data

Pre-Tx data were obtained from the national waiting list (www.agence-biomedecine.fr) and from the preoperative work-up. Follow-up data were collected from the hospital's digital information system and from the registry of annual work-ups.

CMV infection: Diagnosis

The biological diagnosis of CMV infection was made by real-time quantitative PCR. Viral DNA was extracted using an automatic nucleic acid platform (NucliSENS EasyMAG BioMérieux) from a whole blood sample (200 µL of blood eluted in 50 µL), as per the manufacturer's instructions. From January 2003 to May 2009, Hamar et al.'s technique was used [19]. In May 2009 the CMV R-Gene® approach (Argène, BioMérieux) became the local reference and was used until the end of the study period. The two techniques produced equivalent results, with the same units, and both involved measurements on an ABI PRISM 7500 real-time PCR system. The limit of detection and of quantification was 200 CMV DNA copies/mL and was considered as the cut-off DNAemia. The assay was linear between 500 and 7-log10 CMV.

To comply with recommendations [3,6] and ensure continuity in scientific publications, the following definitions are used herein:

• CMV infection: Evidence of CMV replication regardless of symptoms and method of detection (Nucleic acid testing, Antigen testing or culture);

• CMV disease: Evidence of CMV infection with clinical symptoms or biological signature. CMV disease can be further categorized as a viral syndrome with fever, malaise, leukopenia, and/or thrombocytopenia or as tissue-invasive disease;

• DNAemia: CMV infection defined by CMV DNA detection in blood or plasma.

Treatment

CMV monitoring by PCR was performed weekly for D+/R− and for R+ patients for the first

two months after Tx and in the month after valganciclovir was discontinued. CMV was then monitored monthly for all recipients. PCR was also used to confirm a suspected infection (from clinical and/or biological signs) and repeated weekly to monitor a proven infection.
CMV prophylaxis was prescribed according to published guidelines, which were updated during the study period [6,20-23]. Until May 2010, high-risk recipients received intravenous ganciclovir for 15 days, followed by two months of oral aciclovir or valaciclovir therapy. From 2010, high-risk recipients received intravenous ganciclovir until resumption of enteral feeding, followed by oral valganciclovir (900 mg/1,73 m²) until three months after Tx. After September 2012, prophylaxis was extended to six months for high-risk recipients.
Intermediate and low risk recipients did not receive prophylaxis.
In cases of CMV disease, intravenous preemptive ganciclovir was administered according to guidelines [3] with a dose adapted to the GFR (over 50 mL/min per 1.73 m^2 body surface area: 5 mg/kg in one hour, every 12 hours; between 25 and 50 mL/min per 1.73 m^2: 2.5 mg/kg in one hour, every 12 hours; between 10 and 25 mL/min per 1.73 m^2: 2.5 mg/kg in one hour every 24 hours; under 10 mL/min per 1.73 m^2: 1.25 mg/kg in one hour, every 24 hours) and after multidisciplinary discussion. Oral valganciclovir was used for non-severe infections (900 mg/1.73 m^2 twice a day or daily dose [in mg]: 7 * skin surface [m^2] * GFR, twice a day). Curative treatment was stopped if neutrophils fell below 0.5 g/L. CMV replication was monitored weekly during treatment. Antiviral treatment was given for at least three weeks and was extended if the PCR remained positive. No anti-CMV immunoglobulins were needed for CMV infection. Ganciclovir is renally excreted and renal function affects the dose which was adapted either in preventive or curative situation. Therapeutic drug monitoring was not included in the follow-up.
Acute rejection was always confirmed using the Banff classification, either from a protocol biopsy (3 and 12 months post-transplantation for all recipients) or upon noticing clinical or biological disturbances. GFR was estimated from the serum creatinine level (SCr, µmol/L) one month after Tx, then annually, according to the Schwartz-Lyon equation [24], namely k * height (in cm)/SCr, with k = 36.5 in males older than 13 years, k = 32.5 otherwise. TMA was diagnosed based on biological signs (thrombocytopenia, microangiopathic hemolytic anemia, with schistocytes, low haptoglobin levels, raised reticulocyte count and elevated lactate dehydrogenase [LDH]) or from a renal biopsy, while diabetes mellitus was diagnosed from routine biological assessments.
Immunosuppressive therapy was prescribed according to local protocol. Basilixumab is the main induction treatment. Anti-Thymocyte Globulin was used in case of iterative transplantation and/or immune recipient and daclizumab as a study. All patients received mycophenolate mofetil (MMF) plus ciclosporin (young recipient, EBV mismatch) or tacrolimus (adolescent, pre-adolescent, no EBV risk). Corticosteroid therapy (excluding situation of rejection) was limited (5 days) with tacrolimus and prolonged if ciclosporin was used.

Statistical methods

Analysis of primary judgment criteria
The probability of occurrence of CMV infection during the first year after renal transplantation was estimated by the proportion of transplants concerned by the infection. Time to infection delay was calculated from Tx date to infection date. Median and range of these variables were calculated in Tx concerned with infection.

Analysis of secondary judgment criteria
Risk factors of CMV infection were estimated fitting univariate unconditional logistic regression models. Two-tailed likelihood ratio tests were performed. Estimated odds-ratio and corresponding 95% confidence interval were provided. Mean cold, warm and total

ischemia times were compared in CMV infected and non-infected TX using Student T test. In complication analysis, changes in GFR rate were analysed using Student T tests. The type-1 error rate was fixed at $\alpha = 0.05$ in all analysis.

Results

Cohort characteristics

From January 1st 2003 to October 31st 2014, 145 pediatric kidney Txs were performed in our unit. All patients were younger than 18 years at the time of Tx. The baseline characteristics of the patients are reported in *Table 1*. Patients were excluded if the graft was lost (n = 2), if they died after surgery (n = 1), or if less than one year of follow-up data were available (n = 6, these patients were followed up in other treatment centers). The cohort consisted of 130 children, of which six were re-transplanted during follow-up (i.e. 136 transplantations in total). The high (D+/R−), intermediate (R+) and low (D−/R−) risk groups comprised 32 (23.5%), 53 (39.0%) and 51 (37.5%) patients, respectively. Eighty-three of the recipients (61.0%) were seronegative at the time of Tx (32 high risk and 51 low risk patients).

Primary outcomes

Incidence of infection (primary and reactivated) during the first year

In the first year after Tx, 38 patients (27.9% of the cohort) had positive PCR results for CMV. The 38 patients did not have repeated episodes after a second transplantation. These included 15 primary-infections and 23 reactivations and involved 13 (40.6%) of the 32 high risk Tx, 24 (45.3%) of the 53 intermediate risk Tx, and one (2.0%) of the 51 low risk Tx (*Figure 1*). 28 infections occurred before September 2012 (26.9% out of 104 recipients) versus 31.2% (10 infections out of 32 recipients) after new recommendations.

Time to infection

The median time to infection was 77 days (108 and 63 days respectively for the high and intermediate risk recipients). Specifically, the median time to DNAemia for the high risk patients was 108 days after Tx (8-334 days), or 31 days after viral prophylaxis was halted. Two of the 32 high-risk recipients (6.5%) experienced infection during prophylaxis. Most infections occurred within the first four months after Tx (*Figures 2 and 3*).

Severity of the infection

CMV disease occurred in 10 of the 38 CMV infection (26.3%), namely 6 of the 32 high risk recipients (18.7%), 3 of the 53 in intermediate risk patients (5.9%), and 1 of the 51 low-risk recipients. The clinical presentation was variable (*Table 2*), being in five instances unspecific with isolated fever while the five other patients showed an impaired general condition. Respiratory symptoms were reported in two cases: One case of respiratory distress syndrome and one of pleural effusion associated with ascites. Four cases had digestive involvement: One duodenal bleeding, two colitis and one severe ascites (with CMV identified in the ascitic fluid) that required a transfer to intensive care. Mild hepatic cytolysis was observed in four cases, all of which improved rapidly. Significant neutropenia (viz. of 210, 810, 1,050 and 1,260 copies/mm^3) was reported in four invasive infections but none was complicated by a bacterial or fungal infection. Nephritis was noted in two cases but without any neurological symptoms or retinitis.

Secondary end points

The results of our investigation for the major risk factors of CMV infection in pediatric renal

Tx are summarized in *Table 3*. The relative risk of infection was defined with respect to the low risk patients, of which only one (2.0%) was infected. The risk of infection was significantly higher for the D+/R− patients (Odds Ratio [OR] 34.21; Confidence Interval [CI] 4.18-279.76; *P*<0.001) despite antiviral prophylaxis. Intermediate risk (R+) recipients, who did not receive antiviral prophylaxis, were also more at risk of CMV infection (OR 41.38; CI 6.32-322.08; *P*<0.001).

Among the intermediate risk recipients, there was no statistically significant difference between the 2 subgroups even if D+/R+ tended to be more at risk than D-/R+ (OR 1.93; CI 0.66-5,94; *P*=0.27).

During the first year after Tx, confirmed acute graft rejection occurred in 23 cases (16.9%), only two of which (5.3%) were followed by CMV infection (both T-Cell Mediated Rejection). This does not represent a statically significant risk (OR 0.25; CI 0.05-1.12) (*Figure 4*). Prolonged ischemia was not identified as a risk factor for CMV infection, neither was cold or warm ischemia time (*Table 3*).

The characteristics of the recipient were not identified as risk factors. The age at Tx was of 10.00 ± 5.01 years (median ± SD) and infections was not associated with age (OR 0.99 per additional year; CI 0.92-2.06; *P*=0.706). CMV infection occurred in as many boys as girls (n=19), and among the 15 patients receiving multiple organs, 5 were infected (OR 1.33; CI 0.42-4.19; *P*=0.621). The characteristics of the donor had no significant effect on the risk of CMV infection. The donor was 17.0 ± 11.2 years old on average, and recipients of older and younger donors were equally likely to be infected (OR 1.01 per additional year; CI 0.97-1.04; *P*=0.741). Eighteen (13.2%) of the Txs were performed from a living donor and eight of the corresponding recipients were infected during the first year. In comparison, 30 (25.4 %) of the 118 recipients from deceased donors were infected. This 2.35 times higher risk of CMV infection for recipients from living donors is nonetheless not statistically significant (OR 2.35; CI 0.85-6.49; *P*=0.107).

Induction treatment was mainly basilixumab (124 recipient), 10 receiving anti-thymocyte globulin and 2 daclizumab. There was therefore no association between the induction treatment and CMV infection (*P=0.51*). 28.2% of basilixumab group presented an infection against 30.0% of anti-thymocyte globulin (*Table 4*). The long term immunosuppressive therapy was neither identified as risk factor (*P=0.48*) as 65 received ciclosporin and 71 tacrolimus (*Table 4*).

Complications

Among the 23 instances of acute rejection documented in the first year after Tx, three occurred after CMV infection whereas 20 occurred without previous CMV infection, of which only two were followed by CMV infection (both T-Cell Mediated Rejection). There was therefore no association between CMV infection and acute rejection (*P*=0.81). A mild decrease in GFR was observed in CMV infected patients during the first year after Tx, but these rates were not significantly lower than those of non-infected patients (*Table 5*). Two cases of TMA were recorded but neither patient experienced CMV infection in the first year after Tx. Three cases of diabetes mellitus requiring insulin were reported, none in CMV-infected patients.

Treatment

CMV infection was not systematically treated with antiviral drugs if DNAemia was low without evidence of CMV disease and under close monitoring. The viral load was reduced in 12 (31.6%) of the 38 infections simply by reducing immunosuppression. The mean viral load in these cases was 1596 ± 1606 copies/mL. Intravenous ganciclovir was immediately given to 11 (28.9%) of the 38 infected patients because they developed CMV disease or because their viral load increased rapidly. Oral valganciclovir was used to relay ganciclovir or as a first line of treatment for cases of symptom-free CMV infection. No major side ef-

fects were observed. There was no evidence of anti-viral resistance (progression in CMV viremia or clinical disease during prolonged antiviral therapy) and CMV infections that occurred during oral prophylaxis were successfully treated with intravenous ganciclovir. Antiviral levels were not biologically monitored.

Two of the 9 recipients receiving the 6 months prophylaxis used since September 2012 had an infection (*Table 6*) against four out of 8 receiving the 3 months prophylaxis (OR 3.50; CI 0.43-28.46; *P*=0.335) and 7 out of fifteen receiving the 2 months prophylaxis (OR 3.06; CI 0.47-19.89; *P*=0.389).

Discussion

The incidence of CMV infection within one year of Tx in this study group (27.9%) is similar to those reported in the literature (between 12.7 and 38.0%) [8-11]. Eighty three (61.0%) of the recipients were seronegative at the time of Tx. This proportion falls within the range reported by Matas et al. [7] describing between 58.7 and 67.2% of seronegative pediatric recipients at the time of Tx.

In this cohort of 136 kidney transplant recipients, most CMV infections occurred within four months of Tx. Of the high risk patients who developed CMV infection, most received antiviral prophylaxis for just three months. The increased rate of infection observed here after three months prophylaxis supports the current recommendation to prescribe oral valganciclovir for up to six months after Tx, aiming to prolong time before seroconversion post-transplantation and avoid early CMV disease. Arthurs et al. [25] have shown that continued viral surveillance and close clinical follow-up is warranted after prophylaxis is complete due to the risk of late-onset CMV infection, even if Kotton et al. [6] specified that a hybrid strategy is not recommended in any group due to limitations of the available data.

Positive DNAemia tests were returned for four patients at the work-up one year after Tx. The short interval between infection (in the fourth trimester after Tx for all four) and data recovery makes these results difficult to interpret because of the lack of information on the outcome of the infection.

The occurrence of CMV disease in this cohort (26.3% of infected patients) is slightly lower than reported elsewhere. Höcker et al. [26] reported 10 cases of tissue-invasive disease among 35 pediatric CMV infections (28.6%), Jongsma et al. [8] 20 among 61 children (32.8%) and Witzke et al. [27] 19 among 58 adult recipients (32.8%). Apart from one patient who was treated briefly in intensive care for severe ascites, none of our patients developed a life-threating disease. While tissue biopsies could have been used to detect CMV DNA and document tissue invasion more precisely, these were only taken when the benefits were expected to outweigh the risk of side effects for the patient. No analysis was done to identify risk factors of CMV disease. Immunosuppressive regimen (neither the induction therapy nor the long-term therapy) was not identified as a risk factor modifying the infection occurrence.

Notwithstanding its description by Humar et al., CMV-associated retinitis seems rare and none of our patient suffered retinal damage [16]. We do note however that contrary to 2013 international consensus guidelines [6], ophthalmologic examinations were not routinely performed for all CMV-infected patients in this study. These exams are important because retinitis can occur despite very low to undetectable blood viral loads [3].

Our study confirms that D+/R− recipients are at significant risk of CMV infection (OR 34.21; *P*<0.001) despite prophylaxis. However, we found that this rate of reactivation is even higher (OR 41.38; *P*<0.001) for R+ recipients (classed as intermediate risk not receiving prophylaxis). This result confirms that careful subclinical and biological surveillance is recommended for intermediate risk recipients, whose CMV infection can be reactivated, whether they received a prophylaxis or they are monitored for pre-emptive therapy.

Although it is not statistically significant, the protective effect of acute rejection against CMV infection suggested in this cohort (OR 0.25; CI 0.05-1.1; *P*=0.035) differs from

previous reports in literature. The management of acute rejection requires a higher level of immunosuppression, leading to a higher sensitivity to infections. Razonable et al. reported that allograft rejection was associated with the occurrence of late-onset CMV disease [28]. A possible explanation for our result is therefore that the follow-up period is too short. Indeed, Razonable et al. found that the delay between allograft rejection and CMV infection was 4.5 months on average, which highlights the importance of prolonged monitoring. Longer-term monitoring would also have allowed us to describe the impact of CMV infection on the decline in GFR and the appearance of chronic allograft dysfunction.

This study shows that CMV infection does not lead to major morbidity. CMV disease with flu-like symptoms requires appropriate management but no significant differences were found between infected and non-infected patients in terms of the acute rejection rate, decline of GFR, occurrence of TMA or diabetes mellitus. Our results nonetheless indicate that regarding potential complications, all recipients should benefit from a close monitoring of CMV infection. Indeed, R+ recipients-considered at intermediate risk, were followed closely (pre-emptive treatment opposed to prophylaxis) and experienced higher rates if CMV infection, but overall did not have larger rates of CMV disease. We cannot directly compared R+ recipients and D+/R- recipients as they did not experience the same prevention. Intermediate-risk patients may benefit from CMV prophylaxis.

There are therapeutic consequences for recipients identified as being at high risk of infection. Six months prophylaxis with oral valganciclovir has been shown to reduce the rates of CMV disease and viremia in high risk recipients but had no effect on the acute rejection rate [29]. Furthermore, prolonging oral valganciclovir therapy does not seem to increase the number of adverse events [29]. The most suitable duration of CMV replication prevention is not defined even if our study shows a trend to retain the 6 months prophylaxis even if we did not highlight a statistically significant difference. The different prevention protocols could be compared on retrospective studies with larger numbers of recipients.

The one year follow-up period in this study was defined from Tx rather than CMV infection to facilitate its incorporation in and comparison with long-term survival studies. Indeed, Smedbraten et al. found that in their cohort with a median observation period of 13.7 years, early CMV infection was predictive of increased mortality [13].

Conclusion

Our study indicates that CMV infection occurs in roughly one third of pediatric kidney recipients and that the serological matching of the donor and the recipient is the most crucial factor involved. High risk recipients (D+/R−) must receive prophylaxis. Intermediate risk recipients (R+) showed a high CMV infection incidence, similar to high risk recipients receiving prophylaxis. Pre-emptive therapy seems appropriated since we did not observe more complication in intermediate risk recipients. Long term complications might be argument for universal prophylaxis and needs be studied in prospective studies.

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Compliance with ethical standards. All procedures performed in this study were approved by the institutional research committee (PPC Lyon Sud Est II, IRN # 00009118) and were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, formal patient consent is not required.

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Figure 1. CMV infection flow chart after enrollment and according to donor and recipient serostatus.
Tx: transplantation
Figure 2. Infection-free survival rate in the first year after pediatric kidney transplantation.
Figure 3. Infection-free survival rate in the first year after pediatric kidney transplantation according to CMV infection risk.
Figure 4. Chronology of acute rejection and CMV infection in the first year after pediatric kidney transplantation.
Tx: transplantation

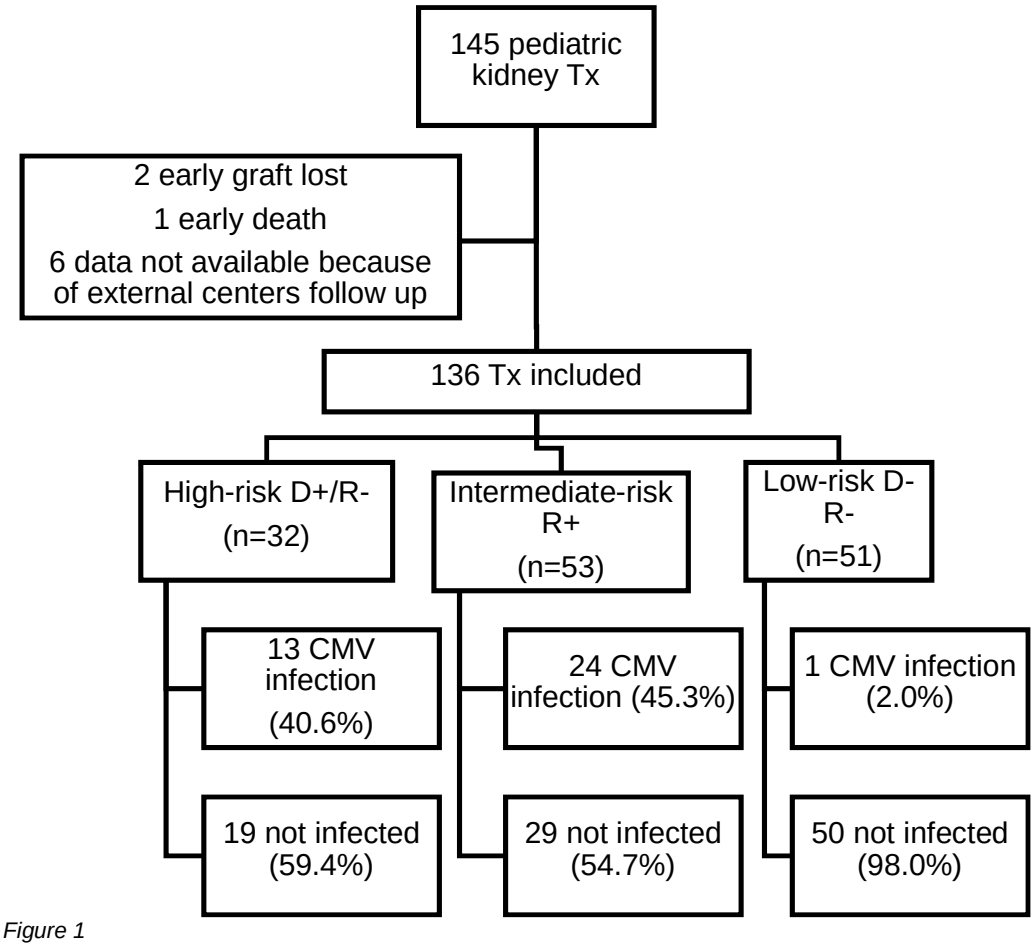

Figure 1

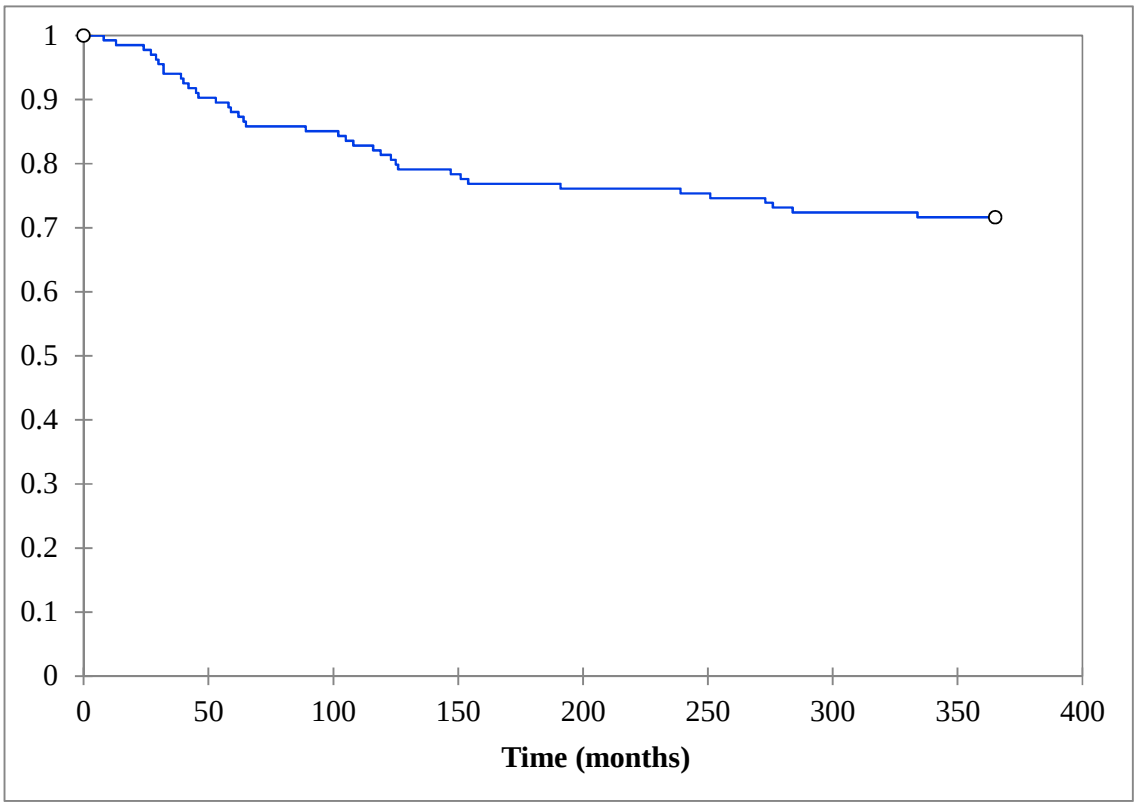

Figure 2

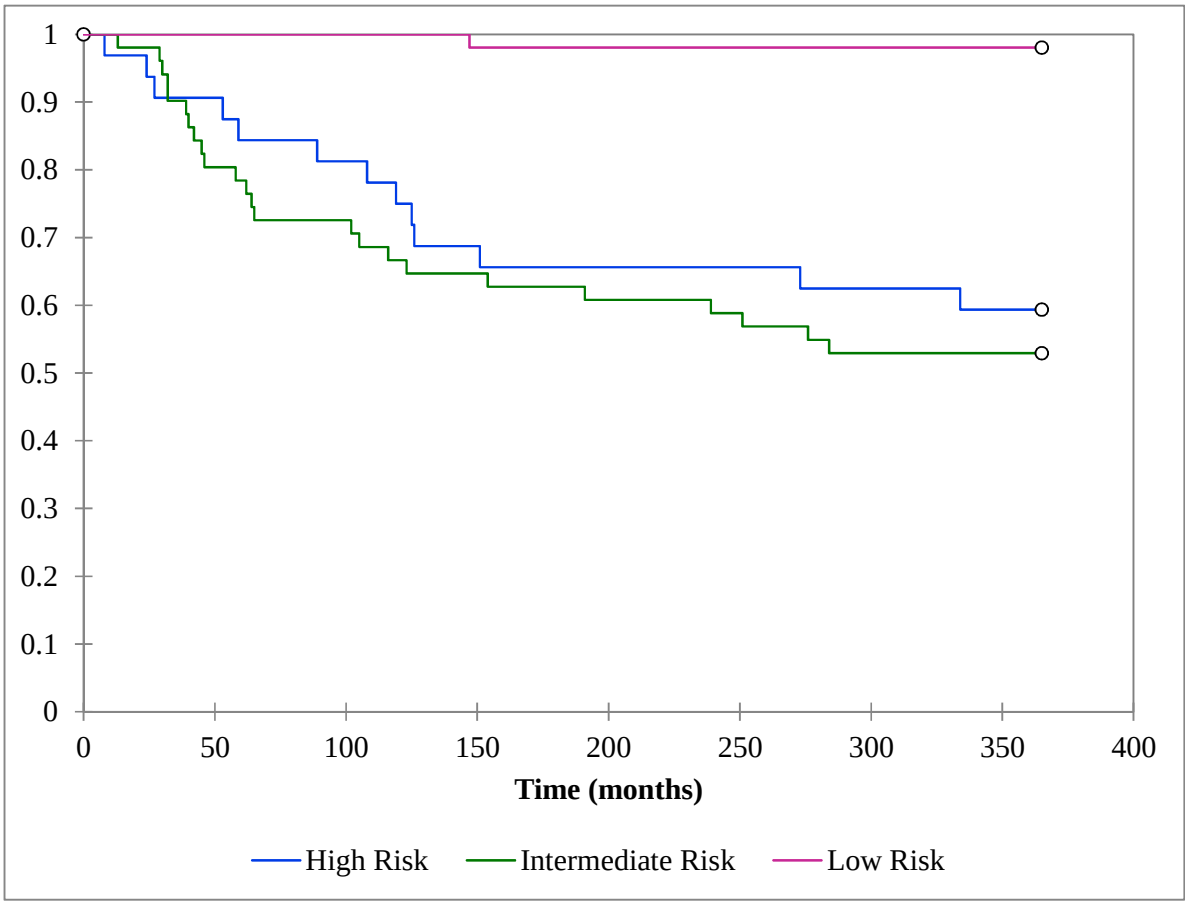
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
50
100
150
200
250
300
350
400
Time (months)
High Risk
Intermediate Risk
Low Risk

Figure 3

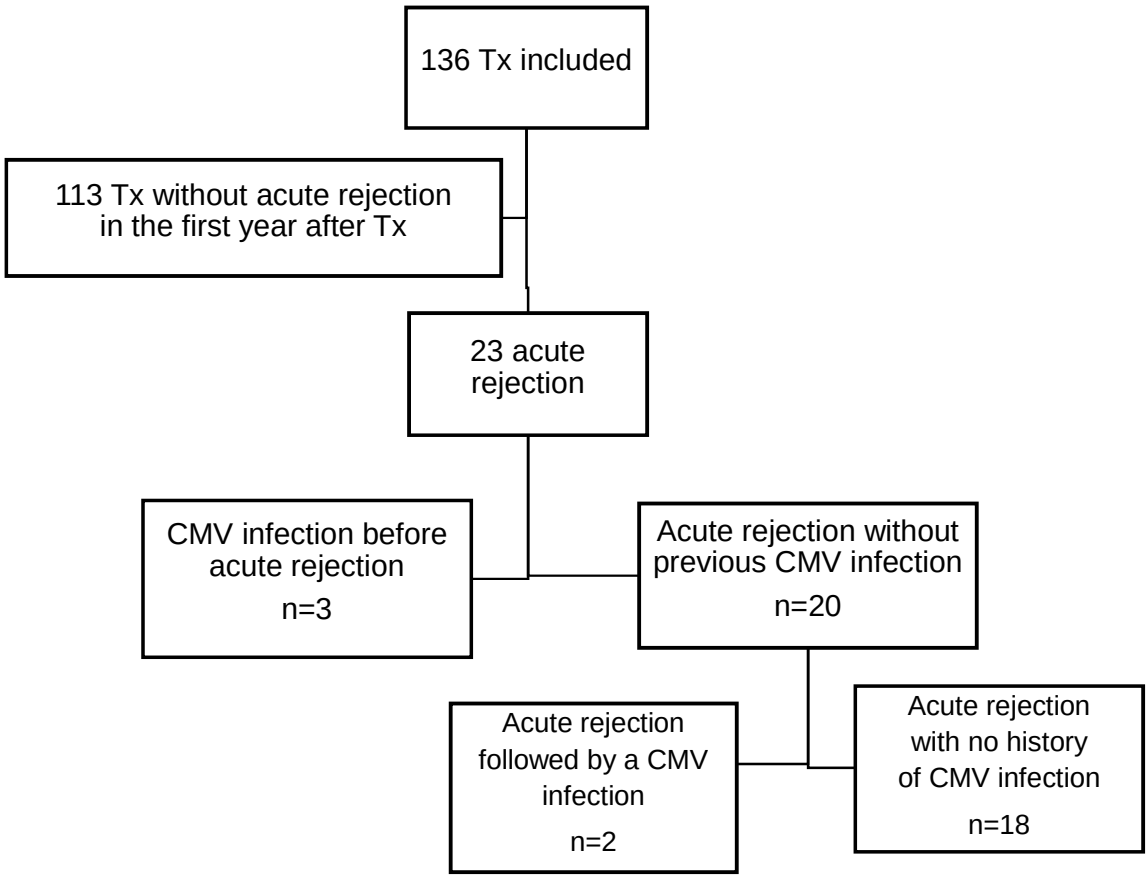
136 Tx included
113 Tx without acute rejection in the first year after Tx
23 acute rejection
CMV infection before acute rejection
n=3
Acute rejection without previous CMV infection
n=20
Acute rejection followed by a CMV infection
n=2
Acute rejection with no history of CMV infection
n=18

Figure 4

Table 1
Baseline characteristics of the recipients

Variable		Included
Recipients		(n=136)
Average age in year (SD)		10.0 (5.1)
Sex		
	Male (%)	68 (50.0)
	Female (%)	68 (50.0)
Ischemia (average)		
	Cold (SD)	12h59min (6h13min)
	Warm (SD)	30min (10min)
	Total (SD)	12h23min (6h10min)
Infectious risk		
	High D+/R- (%)	32 (23.5)
	Intermediate R+ (%)	53 (39.0)
	Low D-/R- (%)	51 (37.5)
Donors		
	Alive (%)	18 (13.2)
	Cadaveric (%)	118 (86.8)
	Average age in year (SD)	17.0 (11.2)
CMV infection		38
	Primary-infection (%)	15 (39.5)
	Reactivation (%)	23 (60.5)
	CMV disease (%)	10 (26.3)
	Asymptomatic CMV infection (%)	28 (73.7)
Rejection (%)		23 (16.9)
Multi organ transplant (%)		15 (11.0)

SD: Standard deviation; h: hour; min: minute; D: donor CMV serostatus; R: recipient CMV serostatus

Table 2
Distribution of CMV disease symptom after pediatric kidney transplantation

CMV disease	n=10	D+/R- (n=6)	R+ (n=3)	D-/R- (n=1)
Fever	5	4	0	1
Impaired general condition	5	3	1	1
Respiratory symptoms	2	1	1	0
Digestive symptoms	4	2	1	1
Hepatic cytolysis	4	2	2	0
Nephrology symptoms	2	2	0	0
Neutropenia	4	4	0	0
Neurological symptoms	0	0	0	0
Retinitis	0	0	0	0

D: donor CMV serostatus; R: recipient CMV serostatus

Table 3
CMV infection risk factors after pediatric kidney transplantation

CMV Infection risk factors		CMV infected	CMV non-infected	Odd-ratio	95% Confidence Interval	*P* (likelihood ratio*)
Basis risk						
Low risk D-/R-		1/38 (2.6%)	50/98 (51.0%)	1.00		<0.001
Intermediate risk R+		24/38 (63.2%)	29/98 (29.6%)	41.38	6.32-322.08	
High Risk D+/R-		13/38 (34.2%)	19/98 (19.4%)	34.21	4.18-279.76	
Acute rejection without previous infection	No	36/38 (94.7%)	80/98 (81.6%)	1.00		0.035
	Yes	2/38 (5.3%)	18/98 (18.4%)	0.25	0.05-1.12	
Ischemia time (hours)						
Cold ischemia time [(mean(SD))]		0.52 (0.25)	0.55 (0.24)			0.577
Warm ischemia time [(mean(SD))]		0.02 (0.01)	0.02 (0.01)			0.206
Total ischemia time [(mean(SD))]		0.56 (0.29)	0.57 (0.24)			0.777
Recipient characteristics						
Recipient age				0.99 †	0.92-2.06	0.706
Recipient gender	Male	19/38 (50.0%)	49/98 (50.0%)	1.00		
	Female	19/38 (50.0%)	49/98 (50.0%)	1.00		
Multi-organ transplantation	No	33/38 (86.8%)	88/98 (89.8%)	1.00		
	Yes	5/38 (13.2%)	10/98 (10.2%)	1.33	0.42-4.19	0.621
Donor characteristics						
Donor age				1.01 †	0.97-1.04	0.741

Cadaveric donor	30/38 (78.9%)	88/98 (89.8%)	1.00		0.107
Alive donor	8/38 (21.1%)	10/98 (10.2%)	2.35	0.85-6.49	

CMV: cytomegalovirus ; SD: standard deviation ; R: recipient CMV serostatus; *: likelihood ratio test for heterogeneity; †: per additional year (of age); D: donor CMV serostatus

Table 4
CMV infection risk factor compared to induction and long term immunosuppressive therapy in pediatric kidney transplantation

CMV infection risk factors	CMV infected	CMV non-infected	Odd-ratio	95% Confidence interval	*P* (likelihood ration*)
Induction therapy					
Simulect	35/38 (92.1%)	89/98 (90.8%)	1.00		0.513
Anti-thymocyt globulin	3/38 (7.9%)	7/98 (7.2%)	1.09	0.27-4.45	
Daclizumab	0/38 (0.0%)	2/98 (2.0%)	0.00	-	
Long term immunosuppression					
Tacrolimus	20/38 (52.6%)	45/98 (45.9%)	1.00		0.482
Ciclosporin	18/38 (47.4%)	53/98 (54.1%)	1.31	0.62-2.77	

CMV: cytomegalovirus; *: likelihood ratio test for heterogeneity

Table 5
Variation in glomerular filtration rate according to CMV infection between immediate post-transplantation baseline and first year annual monitoring

Decline of glomerular filtration		*P**
Difference of creatinine at one year	Mean (µmol/L)	
CMV infected	+38.32	0.414
CMV non-infected	+25.52	
Difference of clearance at one year	Mean (mL/min/1.73 m^2)	
CMV infected	-32.32	0.642
CMV non-infected	-28.81	

*: *P* for average comparison

Table 6
Comparison of successive protocols for prevention of CMV infection in high-risk recipients

Successive prevention protocols	CMV infected	CMV non-infected	Odd-ratio	95% Confidence interval	*P* (likelihood ratio*)
2 months prophylaxis (until May 2010)	7/15 (46.7%)	8/15 (53.3%)	3.06	0.47-18.89	0.389
3 months prophylaxis (May 2010 to Sept 2012)	4/8 (50.0%)	4/8 (50.0%)	3.50	0.43-28.46	0.335
6 months prophylaxis (Sept 2012 to Dec 2014)	2/9 (22.2%)	7/9 (77.8%)	1.00		

CMV: cytomegalovirus *: likelihood ratio test for heterogeneity Sept: September Dec: December