




ORIGINAL ARTICLE

Extraperitoneal kidney transplantation: a comparison between children weighting ≤ 15 kg and > 15 kg. Experience of a single institution

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SUMMARY

Extraperitoneal approach is sometimes recommended for kidney transplantation (KT) in children weighting < 15 kg. We hypothesized that this approach might be as successful as in patients with normal weight. Data of all consecutive KTs performed between 2013 and 2019 were retrospectively reviewed. Early outcomes and surgical complications were compared between children weighting ≤ 15 kg (low-weight (LW) group) and those weighting > 15 kg (Normal-weight (NW) group). All the 108 KTs were performed through an extraperitoneal approach. The LW group included 31 patients (mean age 3.5 ± 1.4 years), whose mean weight was 11.1 ± 2.0 kg. In the LW group,—a primary graft nonfunction (PNGF) occurred in one patient (3.2%), surgical complications occurred in nine (29%), with four venous thrombosis. In the NW group, PNGF occurred in one case (1.3%), delayed graft function (DGF) in eight (10%), surgical complications in 11 (14%) with only one case of venous thrombosis. In both groups, no need for patch during wound closure and no wound dehiscence were reported. The extraperitoneal approach can be effectively used in LW children. No differences were observed in the overall complication rate ($P = 0.10$), except for the occurrence of venous thrombosis ($P = 0.02$). This might be related to patients' characteristics of the LW group.

Transplant International 2021; 34: 2394–2402

Key words

complications, extraperitoneal approach, kidney transplantation, low-weight children

Received: 15 March 2021; Revision requested: 5 August 2021; Accepted: 15 August 2021;

Published online: 22 September 2021

Introduction

Kidney transplantation (KT) is indicated as the preferred approach in the treatment of end-stage kidney disease (ESKD) in children [1]. Early KTs positively influences both patients' survival and the long-term survival of the allografts [2]. In addition, an early KT spares a long period of renal replacement therapy along with its side effects [3]. Thus, nephrologists, pediatric surgeons, and urologists made KT accessible also to low-weight (LW) children, despite the reduced caliber of the major vessels and the potential disparity in dimension of the organs. All these aspects may contribute to the increased rate of complications in this peculiar population [4].

Traditionally, most surgeons have recommended the intraperitoneal technique for the smallest patients, weighting <15 kg. These recommendations aim to ease the access to the aorta and to the vena cava, which are the preferred vessels for the anastomoses in LW patients, and consent more space for the allograft, in order to avoid complications during the closure of the abdominal wall [5]. This approach was successfully used in LW patients receiving organs from adult living donor [6].

Despite these putative advantages, however, severe episodes of bowel obstruction from intestinal adhesions may occur, as well as iatrogenic lesions of the bowel or twisting of the renal graft because of the hypermobility of the allograft vascular pedicle in the abdominal cavity [7,8]. All these abdominal complications might put at the risk the vitality of the allograft.

The aim of the study was to compare the surgical outcomes of extraperitoneal KT between children weighting ≤ 15 kg and patients weighting > 15 kg, treated in our Institution. This might allow to establish if the extraperitoneal approach does not increase the risk of adverse event in small children and, for this reason, represent a good choice in this peculiar population.

Materials and methods

Population

All the KTs, consecutively performed from January 2013 to December 2019 in the Department of Women's and Children's Health of the University-Hospital of Padua, were included. All clinical data were obtained from the medical records, and all patients' legal guardians gave their written consent for the collection of these data.

Surgical technique

Our institutional protocol for KT requires the use of optical loupes with a 2.5–4 times magnification and microsurgical instruments. The extraperitoneal approach was chosen for all patients. A "hockey stick" incision was used to have an optimal visualization of the retroperitoneum and bladder, even in those patients who had a previous surgery. All grafts were implanted into the iliac fossa through an extraperitoneal access, dissecting the peritoneum, that needed to be intact, from the lateral abdominal wall, and visualizing the ileo-psoas muscle and the iliac vessels. The right side was the first choice. In case of severe previous urinary tract infections, leading to inflammatory adhesions, the implantation in the left iliac fossa was considered. One Collin's and two Doyen's abdominal retractors were used in order to medialize the peritoneum and reach an optimal view of the vessels. This setting of the instruments allowed a good retraction of the liver even in case of hepatomegaly. The renal vein and artery were sutured in an end-to-side fashion, to the iliac vessels or to the vena cava and aorta, in case of LW patients. The ureteral-vesical anastomoses were performed through an extra-vesical approach according to the Lich-Gregoire technique. A trans-anastomotic external stent was inserted up to the renal pelvis in all the patients, to preserve the patency of the anastomosis and to monitor the urinary output of the transplanted kidney, especially in case of the presence of diuresis originating from the native kidneys.

The perioperative infusion of 5–10 units/kg/h of unfractionated heparin was indicated in case of altered preoperative coagulative screening, patients aging < 5 years, weight < 15 kg, considerable size mismatch (body weight ratio between donor and recipient more than 1:4), donor kidney allograft with multiple vessels, intimal lesion of the allograft renal artery, altered allograft perfusion immediately after implantation, such as venous congestion.

Immunosuppression therapy

The induction therapy included methylprednisolone (500 mg/m²/die) and two doses of basiliximab (10–20 mg/Kg), one at KT and one after four days. Within the first 24 h after KT, the maintenance therapy was initiated and included tacrolimus, at an initial dose of 0.3 mg/kg aiming to a therapeutic trough level of 10–12 ng/ml, mycophenolate mofetil, at an initial dose of 600 mg/m²/die aiming to a therapeutic trough level of

1.5–3.5 mg/l, and methylprednisolone at an initial dose of 500 mg/m²/die, to be reduced in the following weeks.

Study design

This study is retrospective and single-centered.

For the purpose of the study, patients were grouped into two samples, according to the body weight at the time of KT: LW group—children weighing <15 kg; normal-weight (NW) group—children weighing more than 15 kg.

The following perioperative variables were collected: patients' age and weight, body weight ratio between donor and recipient (BWR), cause of ESKD, replacement therapy, previous surgical interventions, ischemia times, vascular anomalies of the donor allograft kidney (multiple veins or arteries), need of intra- and postoperative administration of heparin, inotropic drugs (dopamine, norepinephrine), and vasodilating drugs (fenoldopam, esmolol).

The following early outcomes, related to the transplantation, were investigated: the value of the Resistive Index (RI), assessed by doppler ultrasonography (DUS) at first postoperative day, occurrence of delayed graft function (DGF), defined as an acute kidney injury requiring dialysis during the first week after KT [9], or primary graft nonfunction (PGNF), defined as a permanent graft failure without a detectable immunological or vascular cause [10], serum creatinine at discharge, estimated glomerular filtration rate (eGFR) at discharge, calculated by creatinine-based “Bedside Schwartz” equation [11], and the length of the hospital stay.

Concerning the outcomes related to the extraperitoneal approach, the following surgical complications were considered and compared between the two groups: intra- and postoperative bleedings, need of abdominal patch or difficult closure of the abdominal wall, compartmental syndrome, graft venous thrombosis, wound dehiscence, episodes of ileus, lymphocele, and urinary tract complications. Only the events classified as Clavien-Dindo grade IIIa or more [12] were reported in the study.

Besides the routine clinical and biochemical follow-up, a conclusive US-guided percutaneous biopsy of the allograft was performed at 1-year follow-up. The results were reported according to Banff Classification [13].

Three-month and one-year estimated allograft survival, defined as the time to allograft failure, were assessed. Three-month and one-year patients' survival were also estimated. Finally, one-year eGFR and the

episodes of acute allograft rejection during the first year after KT were reported.

Statistics

Categorical variables were reported as number (%), while continuous variables were reported as their mean value and standard deviation (SD). For the comparison between the two groups, Fisher's exact tests and Mann–Whitney *U* tests were used.

The survival was assessed through Kaplan–Meier analysis. The results of the two groups were compared by using Mantel–Cox's test.

IBM[®] SPSS Inc. Version 26.0 provided the results of the statistical analysis.

P-value ≤ 0.05 was considered statistically significant.

Results

Characteristics of the population

During this period, at our institution, 108 KTs were performed in 106 children. Sixty patients were male (56%). At the time of KT, the mean age was 10 ± 5.6 years and the mean body weight was 29 ± 17 kg. In 77 recipients (71%), body weight was more than 15 Kg, in 31 less than 15 kg (29%). The mean body weight ratio between donor and recipient (BWR) was 2.5 ± 1.6. Thirty-one children (29%) received a living-donor organ. All the procedures were performed through an extraperitoneal approach.

Composition of the two groups

Low-weight group included 31 patients (29%), 17 males (55%), with a mean age of 3.5 ± 1.4 years (range 1.5–9.2 years). The mean body weight was 11 ± 2.0 kg (range 6.7–15 kg). NW group included 77 patients, 43 males (56%), with a mean age of 13 ± 4.2 years (range: 4.4–21 years). The mean body weight was 36 ± 16 kg (range: 15–88 kg). There were no differences in the causes of ESKD: urological diseases, including vesicoureteral reflux and posterior ureteral valves, accounted for 52% in LW group and 39% in NW group (*P* = 0.23).

Table 1 compared the perioperative characteristics of the two groups. The only difference regarded the BWR, which was higher in the LW group (*P* = 0.03), the type of kidney replacement therapy before KT (*P* < 0.0001), the rate of native kidney nephrectomy (*P* = 0.007), the rate of administration of inotropic drugs, more

Table 1. Perioperative characteristics of the population.

	LW Group (<i>n</i> = 31)	NW Group (<i>n</i> = 77)	<i>P</i> -value
Male sex (<i>n</i> , %)	17 (55)	43 (56)	1.0
Urological diseases as cause of ESKD (<i>n</i> , %)	16 (52)	30 (39)	0.23
Kidney replacement therapy (<i>n</i> , %)	None 2 (6.5)	None 15 (19)	<0.0001*
	Peritoneal 25 (81)	Peritoneal 23 (30)	
	Hemofiltration 2 (6.5)	Hemofiltration 21 (27)	
	Both 2 (6.5)	Both 18 (23)	
BWR (mean, SD)	3.7 ± 1.7	1.9 ± 1.2	0.03*
Living donors (<i>n</i> , %)	8.0 (26)	23 (30)	0.82
Nephrectomy of the native kidneys (<i>n</i> , %)	Monolateral 4 (13)	Monolateral 2 (2.6)	0.007*
	Bilateral 5 (16)	Bilateral 3 (3.9)	
Cold ischemia time-nonliving donors (h; mean, SD)	14 ± 4.9	11 ± 4.3	0.04
Cold ischemia time-living donors (h; mean, SD)	1.4 ± 0.2	1.7 ± 0.3	0.37
Warm ischemia time (min; mean, SD)	58 ± 2.2	60 ± 11	0.94
Anatomic variants (<i>n</i> , %)	9.0 (29)	32 (42)	0.28
Operative time (min; mean, SD)	272 ± 74	256 ± 47	0.57
Heparin (<i>n</i> , %)	24 (77)	44 (57)	0.08
Inotropic drugs (<i>n</i> , %)	22 (71)	24 (31)	<0.001*
Vasodilating drugs (<i>n</i> , %)	7.0 (23)	32 (42)	0.08

*Significant *P*-value ≤ 0.05.

frequently administered in LW patients ($P < 0.001$) and cold ischemia time, which was longer in LW patients ($P = 0.04$).

Early outcomes of KT

Table 2 reports and compares the early outcomes of KT between the two groups. No differences in terms of DGF or PGNF were reported ($P = 0.10$; $P = 0.49$). Among the eight patients who presented DGF, seven had hemofiltration as transient replacement therapy and one peritoneal dialysis.

Serum creatinine at discharge was lower in the LW group ($P < 0.001$) and eGFR was higher in this group ($P = 0.009$), although no differences were found in RI values ($P = 0.76$), and length of hospital stay ($P = 0.83$).

The urological diseases leading to ESKD did not influence operative time in the LW group (260 ± 87 min vs. 281 ± 84 min; $p = 0.30$) and in the NW group (253 ± 51 min vs. 258 ± 45 min; $p = 0.27$). Patients undergone peritoneal dialysis had a similar operative time in the LW and NW groups (275 ± 75 min vs. 245 ± 36 min; $P = 0.32$).

Patients in the LW group who had suffered from nephrological diseases showed a longer hospital stay, when compared with those affected by urological diseases (22 ± 5.1 days vs. 16 ± 7.5 days; $P = 0.003$). In the NW group, the causes leading to ESKD did not

influence the length of hospital stay (21 ± 9.9 days vs. 18 ± 5.5 days; $P = 0.31$). Patients undergone peritoneal dialysis had a similar length of hospital stay (19 ± 6.9 days vs. 23 ± 11 days; $P = 0.10$).

Five LW patients (16%) underwent major bladder procedures before KT: closure of a urinary fistula in three patients affected by anorectal malformation, bilateral ureterostomy in one patient, and a previous KT in another one. Twenty NW patients (26%) underwent major bladder procedures before KT: previous KTs in 14 patients, closure of a urinary fistula in three patients affected by anorectal malformation, ureteral reimplantations in three children, a vesicostomy in one patient, and Mitrofanoff appendicovesicostomy in another one. There was no difference between the two groups ($P = 0.27$). Only two LW patients and three NW patients required a ureterostomy after KT ($P = 0.21$). Previous major bladder surgeries did not influence operative time in LW group (276 ± 73 min vs. 253 ± 76 min; $P = 0.61$) and NW group (253 ± 44 min vs. 267 ± 54 min; $P = 0.16$).

Surgical complications

Twenty surgical complications (19%) were observed overall. Nine occurred in the LW group (29%) and 11 in the NW group (14%). However, this rate was not different between the two samples ($P = 0.10$), as reported in Table 3.

Table 2. Early outcomes of the KTs.

	LW Group (n = 31)	NW Group (n = 77)	P-value
RI value (mean, SD)	0.68 ± 0.17	0.66 ± 0.13	0.76
DGF (n, %)	0 (0)	8.0 (10)	0.10
PGNF (n, %)	1.0 (3.2)	1.0 (1.3)	0.49
Serum Creatinine at discharge (μmol/l; mean, SD)	32 ± 10	80 ± 50	<0.001*
eGFR at discharge (ml/min/1.73 m ² ; mean, SD)	92 ± 35	78 ± 29	0.009*
Length of hospital stay (days; mean, SD)	19 ± 7.1	20 ± 8.6	0.83

*Significant for P -value ≤ 0.05 .

Table 3. Surgical complications of KTs.

	LW Group (n = 31)	NW Group (n = 77)	P-value
Overall surgical complications (n, %)	9 (29)	11 (14)	0.10
Bleeding (n, %)	2 (6.5)	5 (6.5)	1.00
Graft venous thrombosis (n, %)	4 (13)	1 (1.3)	0.02*
Urinary tract complications (n, %)	2 (6.5)	4 (5.2)	1.00
Other surgical complications (n, %)	1 (1.3)	1 (3.2)	0.49

*Significant for P -value ≤ 0.05 .

The most frequent complication was postoperative bleeding, occurring in two children (6.5%) in the LW group and in five patients (6.5%) in the NW group ($P = 1.0$). Both patients from the LW group underwent a surgical exploration: in one, only a moderate but continuous oozing was found without a precise source of bleeding. In the other, who developed hypovolemic shock after 72 h from KT, a laceration of the renal capsule was identified. Both had a good recovery after the operation, even after suspension of heparin. In the NW group, only one patient required a further surgical intervention to control the bleeding, while the others were conservatively treated.

The most dangerous complication was the graft venous thrombosis, which occurred in four children (13%) from the LW group and in only one child (1.3%) from the NW group ($P = 0.02$). In the LW group, none of them had prolonged ischemia time, however, one patient had multiple renal allograft veins, and the others (75%) showed an altered coagulation screening before KT. In only one case, the thrombosis was related to the vascular human graft. The prompt administration of fibrinolytic agents succeeded in restoring the venous flow in only two patients.

Moreover, five patients (19%), without graft thrombosis, presented a prothrombotic status ($P = 0.04$). Nevertheless, all the patients with preoperative

coagulative screening received unfractionated heparin after KT.

During the operation, no difficulty in wound closure or need of abdominal patch were reported. In the postoperative period, wound dehiscence, clinical signs of compartmental syndrome, or ileus were not documented. Only one child in the LW group developed a lymphocele, which was conservatively treated.

Obstruction of the urinary tract occurred in two LW (6.5%) and four NW children (5.2%) ($P = 1.00$): LW patients were treated by ureteral stenting, whereas a patient in the NW underwent a redo reimplantation of the transplant ureter, because of the development of ischemic stenosis.

Finally, the urological diseases leading to ESKD did not influence the rate of complications in the LW group ($P = 0.13$) and in the NW group ($P = 0.74$). Moreover, the occurrence of adverse events was not influenced by major bladder surgery ($P = 0.32$) and peritoneal dialysis ($P = 0.97$).

Survival and acute allograft rejection

At one year from KT, all the patients were alive. The estimated allograft survivals at three months and one year was the same in the LW group ($90 \pm 5.3\%$), while in the NW group they were $95 \pm 2.5\%$ and $92 \pm 3.1\%$,

respectively. No difference was found between the two Groups ($P = 0.38$; $P = 0.72$). Kaplan-Meier curve is displayed in Fig. 1.

One-year eGFR was 80 ± 38 ml/min/1.73 m² in the LW group and 75 ± 26 ml/min/1.73 m² in the NW group ($P = 0.14$).

The occurrence of allograft acute rejection during the first year was similar ($P = 0.44$), with nine cases (29%) occurred in the LW group and 17 (22%) in the NW group.

The results of biopsies performed at one year from KT were available for 26 patients (84%) in the LW group and 64 patients (83%) in the NW group: LW patients presented a higher number of Banff II biopsies ($P < 0.0001$) (Table 4).

Discussion

The benefits and advantages of extraperitoneal KT are well recognized even for LW patients and, in the last years, some reports have documented good outcomes also in those who received a living donor KT [14,15]. It is undeniable, however, that nephrologists, pediatric surgeons, urologists, and anesthesiologists need to face some challenging issues when planning the transplant.

Patients' age and weight or size are the first aspects to consider: for children weighting <15 kg, because of their age or because serious growth retardation because of their disease [16], it is hard to retrieve an organ that could match, and, even more so, a living-donor KT is less likely. For the general population, the age of 20–25 years was defined by the North American Pediatric Renal Trials and Collaborative Studies as the optimal

donor age but even a kidney from young donors may represent a serious problem in this group of children [17]. And in fact, the BWR, chosen to assess the disparity between donor and recipient, resulted sensibly higher in our patients, as already demonstrated [18]. However, it is relevant to point out that, in our country, the organs from underage nonliving donors are assigned to pediatric patients with priority [19].

In our series, the nephrectomy of native kidneys was more common in the LW group. This might be because of the higher prevalence of polycystic kidney disease in this sample, that required monolateral or bilateral nephrectomies in order to improve their respiratory dynamic and the management of arterial hypertension. It is relevant to underline that our policy is to perform nephrectomies before KT, to decrease the risk of complications [20].

Another aspect is related to the pharmacologic treatment of these patients. First, the dimensional mismatch between donor and recipient, one of the main risk factors for renal graft venous thrombosis, may require the administration of heparin, that might increase the chance of postoperative bleeding. Second, patients with fewer nutritional reserves at the time of KT have been demonstrated to necessitate the frequent administration of inotropic drugs, during the postoperative intensive care [16], with possible damage of the vascular anastomoses, or the kidney itself. This aspect emphasizes the importance of hemodynamic stability and fluid balance. During and after transplant, recognizing the importance of vasoactive drugs to preserve graft perfusion and maintain blood pressure within normal ranges, also avoiding pulmonary edema in case of arterial hypertension [21].

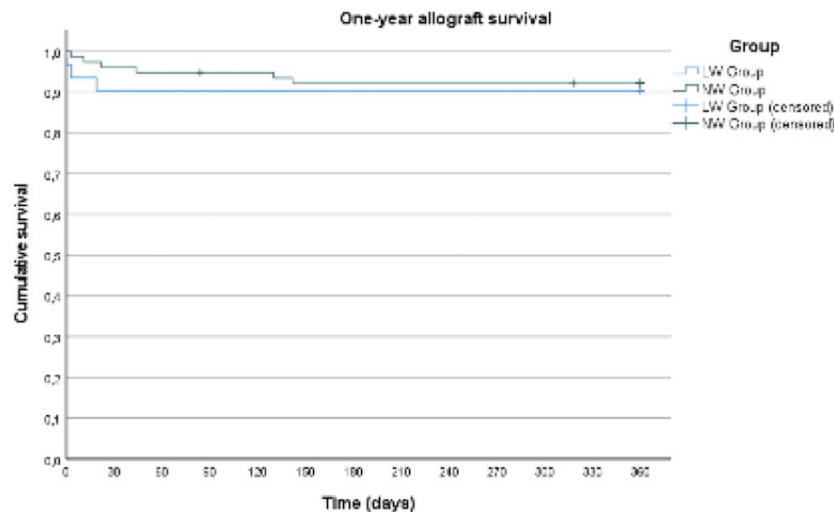


Figure 1 Kaplan-Meier curve estimating one-year allograft survival.

Table 4. Results of one-year allograft biopsies.

	LW Group (n = 26)	NW Group (n = 64)	P-value
Banff I Normal biopsy or nonspecific changes (n, %)	17 (65)	47 (73)	0.44
Banff II Antibody mediated changes (n, %)	7 (27)	0 (0)	<0.0001*
Banff III Suspicious (borderline) for acute T-cell mediated rejection (n, %)	1 (3.8)	9 (14)	0.16
Banff IV T-cell mediated rejection (n, %)	1 (3.8)	8 (13)	0.21

*Significant P-value ≤ 0.05 .

Another factor to consider is the presence of multiple veins or arteries in the allograft, requiring delicate surgical maneuvers during the bench preparation and the anastomosis, and might create an alteration of blood flow, increasing the risk of thrombosis. In our series, one third of the donor kidneys presented a number of anatomic variants of renal vessels, which was similar in the two groups: the different anatomy may have increased the duration of vascular anastomosis, and therefore the length of warm ischemia time. However, this aspect did not increase the risk of thrombosis, as a previous investigation had reported [22].

Concerning the site of the transplant, the extraperitoneal approach did not determine any particular problem in both groups and it remained the procedure of choice, preserving the integrity of the peritoneum with regard to the abdominal cavity [23]. It is true that the extraperitoneal approach may create some difficulties in LW patients for several reasons: limited space to nest the allograft, limited access to the vena cava and aorta, which are the preferable sites of anastomosis in LW patients, and the possible dissection of the lymphatics running close to the great vessels, that may determine a lymphocele [24].

Another questionable aspect, regarding the surgical technique, was the use of prophylactic ureteric stent. Although the catheter may be a source of infective or mechanical complications [25], we preferred to place a splint ureteric stent in all our patients, to monitor the urinary output of the transplanted kidney, and preserve the urinary anastomosis. A lower rate of complications was observed, when compared it with the double-J stent [26,27].

A comparison between the intraperitoneal and extraperitoneal techniques reported similar long-term outcomes in graft function and survival [28]. It is relevant to highlight, however, that the extraperitoneal approach may avoid episodes of intestinal obstruction

or twisting of the allograft on its vascular pedicle, as reported in other series, in which the intraperitoneal implantation was the preferred choice [7,8]. During the extraperitoneal procedure, a careful dissection of the peritoneum up to the diaphragm creates a sufficient space for the kidney, and the use of optical loupes and microsurgical instruments with nonabsorbable monofilament sutures may reduce possible adverse events, without increasing morbidity [29].

Considering the early outcomes, the rate of DGF and PGNF in the LW group were comparable to other series [14,15]. In the LW group serum creatinine was lower in relation to the different body composition [30]. Furthermore, patients belonging to the LW group presented a higher eGFR at discharge. Nevertheless, one-year eGFR was similar between the two groups. The reason of this early improvement of allograft function was unclear, as reported by Heap *et al.* [14]. LW patients suffering from nephrological diseases presented a longer hospitalization, related to metabolic instability and presence of comorbidities.

Overall, the rate of surgical complications was comparable between the two groups. The rate of adverse events in LW patients was steady during the period of the study and similar to other series dealing with KT recipients weighting <15 kg [31]. The only difference concerned the graft venous thrombosis, which occurred more often in LW patients. However, the overall rate of thrombosis and allograft loss is similar to a larger North American series [32]. All of them showed further risk factors for thromboembolic event, such as pro-coagulative status or vascular anomalies. None of them, however, presented consistent BWR between donor and recipient and none encountered episodes of abdominal compartment syndrome.

Finally, a higher rate of antibody-mediated changes was detected by scheduled biopsies in the LW group.

This might be because of a larger variability of the immunosuppressant trough levels in this group, since this is considered one of the main risk factors for the development of antibody-mediated rejection [33].

The limitations of this study reside mainly in the sample size. Despite the lack of standardization in reporting the outcomes of pediatric KT [34], in our center, KT protocols are well established, and our data included most of the clinical variables, except the routine monitoring of intra-abdominal pressure that may represent an important index in LW children with limited space available for the graft.

Despite the surgical challenges, extraperitoneal KT is accepted also in LW patients and does not expose to a higher rate of surgical complications, when compared with NW patients. However, graft venous thrombosis is more frequent in the LW group. The findings of this study suggest that this event might be related to dimensional mismatch, pro-coagulative status and longer cold ischemia time rather than to the extraperitoneal approach and surgical technique of transplantation. Nonetheless, both groups presented a similar three-month and one-year cumulative survival. Of course, a careful follow-up is recommended to identify a possible thrombosis and start a proper treatment for the survival of the graft.

Authorship

FG and PD participated in the design of the study. FG, AR, SM, AG, and EB performed the collection of the data. FG, AR, and PD performed the analysis of the

data and wrote the first draft of the manuscript. FDC, FFL, EV, MP, NZ, MG, LA, CT, GM, MC, EB, and PG reviewed and commented the manuscript. All Authors read and approved the final manuscript.

Funding

Open Access Funding provided by Universita degli Studi di Padova within the CRUI-CARE Agreement. WOA Institution: Universita degli Studi di Padova. Blended DEAL: CARE.

Conflicts of interest

The authors declare no conflicts of interest.

Compliance with ethical standards

This is an observational study performed in accordance with the ethical standards laid down in an appropriate version of the 2000 Declaration of Helsinki as well as the Declaration of Istanbul 2008. No ethical approval is required. However, a notification regarding this study was sent to the local Ethics Committee.

Written informed consent was obtained from legal guardians of all individual participants included in the study.

Data availability statement

The data underlying this article will be shared on reasonable request to the Corresponding Author.

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