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TRANSCUTANEOUS NEAR INFRA-RED SPECTROSCOPY (NIRS) FOR MONITORING PAEDIATRIC RENAL ALLOGRAFT PERFUSION

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Abstract

Background. Kidney transplantation (KT) has become the treatment of choice for paediatric end-stage kidney disease. Nevertheless, paediatric KT might be affected by vascular complications, without specific clinical or biochemical signs. Transcutaneous near-infrared spectroscopy (NIRS) allows non-invasive, real-time, continuous monitoring of regional oxygenation of the haemoglobin (rSrO2). The primary aims of the project is to investigate risk factors for vascular complications and to validate NIRS monitoring of the kidney allograft perfusion in paediatric population.

Methods. A database included all the paediatric KTs from 2013 to 2021 in order to retrospectively investigate the influence of low body weight, vascular anomalies, and the operators' fatigue on the outcomes of KTs. As to NIRS, a systematic search gathered the current evidence to highlight the controversies. Then, a prospective observational study enrolled children that underwent KT at our department from March 2021. The kidney allograft was monitored by transcutaneous NIRS for five days. NIRS readings were compared to clinical, biochemical, and instrumental parameters, including a renal perfusion scintigraphy performed at day five. Finally, an experimental study involving an animal model of renal ischemia was designed to investigate the temporal relationship between NIRS modification and the outbreak of arterial or venous ischemia.

Results. The database included 130 paediatric KTs. Patients with a body weight inferior to 15 kg, allograft with vascular anomalies that required a complex bench surgery and transplantations performed during extraordinary working hours presented similar outcomes, in terms of early survival and rate of complications. The systematic review yielded only two studies dealing with the use of NIRS after paediatric KTs and included 53 patients. In our project, the transcutaneous NIRS monitoring was available for eight patients. Seven patients completed the monitoring, and the analysis showed a significant correlation with the modification of the serum creatinine. One patient experienced a graft venous thrombosis and the time-curve of NIRS readings was reported for the first time in the literature. A rat model of renal arterial and venous ischemia was designed, making transcutaneous NIRS monitoring feasible in a small animal model.

Conclusions. The standardization of the management of paediatric KTs is crucial to decrease the risk of complications and to improve the outcomes. Despite a limited sample size, this project reports positive preliminary results about the use of transcutaneous NIRS monitoring after paediatric KTs. The rat model should clarify the modifications of NIRS readings in case of vascular complications.

Background

Kidney transplantation (KT) has become the treatment of choice for paediatric end-stage renal disease [1]. Nevertheless, in comparison to adults, the small calibre of the vessels and the common size-mismatch between donors and recipients can predispose to vascular complications including allograft thrombosis. The latter may affect up to 10% of KTs and account for 35% of allograft losses in the first year [2]. Prompt recognition and treatment is clearly essential for the preservation of the allograft.

As to the prevention of vascular complications, several demographic, clinical, and surgical risk factors were investigated. A large series found that patients younger than six years old were more prone to develop vascular complications [3]. In these peculiar population, even the surgical approach might be controversial. Even though surgeons could prefer an intraperitoneal access to ease the nesting of the graft, this approach might increase the risk of adverse events. On the other hand, the extraperitoneal approach could be safer but requires higher surgical skills [4].

Another aspect that might influence on the occurrence of complications is the presence of vascular anomalies [5]. Multiple vessels could create a turbulence in blood flow that could end in a thrombosis. No evidence about the impact of microsurgical reconstruction during bench preparation of the graft was reported in the literature.

Moreover, human aspects, such as night-time or day-off surgery, have been investigated only recently [6]. Non-living donor KT is an emergent intervention that might be performed during extraordinary working hours. This might raise the risk of complications, due to professionals' fatigue [7]. On the other hand, the postponing of the intervention might prolong the cold ischemia time (CIT), increasing the risk of delayed graft function (DGF) [8].

As to the early diagnosis, it is relevant to report that vascular complications have no specific clinical or biochemical signs allowing for early diagnosis. Doppler-ultrasonography (DUS) and renal scintigraphy are reliable tools to assess allograft perfusion [9], but do not allow for continuous monitoring of the allograft and can miss early diagnosis, even if performed with a strict schedule in early follow-up of KT [10].

Transcutaneous near-infrared spectroscopy (NIRS) allows for non-invasive, real-time, continuous monitoring of regional oxygenation of the haemoglobin (rSrO2), which is an indirect measure of the blood flow and the metabolic state, of tissue placed deeper beyond the skin. Several clinical studies have tested the use of NIRS for monitoring cerebral and somatic perfusion in intensive care units and the viability of soft-tissue flaps [11,12]. Nevertheless, the main limit of this device concerns the tissue penetration, and the monitoring of solid organs may be altered by their deeper position. The region of interest of NIRS may reach up to 4 cm below the skin, according to the manufacturers, and its effectiveness in monitoring somatic perfusion was validated in infants weighing less than 10 kg and this body weight was sensibly lower than those of the patients undergone KT [13].

However, renal allograft is normally place in an extraperitoneal niche in the abdominal lower quadrant. This position is more superficial than the native kidneys, therefore NIRS has been proposed for the surveillance of allograft perfusion.

Experimental models compared the reliability between transcutaneous NIRS readings and the invasive monitoring of solid organ oxygenation. Kidney and liver NIRS monitoring was assessed porcine models, showing a good correlation with the invasive technique for both the organs [14,15].

In humans, preliminary studies in both paediatric and adult populations found encouraging results about NIRS monitoring of allograft perfusion during the first postoperative days [16,17,18].

Nevertheless, several points need further insight [19]. First, it was speculated that NIRS might anticipate the detection of severe complications, but no adverse events were reported in the series of paediatric KTs. The second concern about NIRS regarded the placement of the probes. The vascularization over the surgical wounds may be altered by the healing process, directly influencing NIRS readings. Currently, no study has investigated this aspect yet. Third, the normal range of renal rSrO2 values need to be established by investigating a larger number of patients. This process is crucial for the use of NIRS in the prompt identification of acute adverse events, which is the final aim of this non-invasive technique. Finally, further investigation should also relate rSrO2 to perfusion renal scintigraphy, which is the most objective modality to assess renal allograft vascularization and function.

As to the prompt treatment of allograft thrombosis and other vascular complications, no standard treatment has been proposed in the literature and the management of adverse events is tailored on the patients' characteristics due to their complexity.

Aim and objective

The scope of the project was about the prevention of postoperative complications after paediatric KT with the final aim of improving the perioperative management of this specific population. To reach this target, the project relied on different areas.

The first goal was to identify and to investigate controversial clinical factors that might be considered responsible for the occurrence of postoperative complications. These

risk factors were related not only to patients' characteristics, especially the body size and anatomical anomalies, but also to surgical aspects, such as bench microsurgery.

The second goal was to validate the use of a non-invasive real-time NIRS method of monitoring of the allograft perfusion. A device with those features could be extremely useful for a prompt recognition of ischemic events that could affect the allograft during the first postoperative days. This objective required several steps.

The first step regarded the identification of the aspects of NIRS monitoring that could need a further insight to improve the reliability of the method. Further, the clinical step consisted in testing the feasibility and effectiveness of NIRS monitoring in children during the first postoperative days after KT. Finally, the last phase consisted in the design and creation of a reliable experimental animal model of acute renal ischemia. This study aimed to investigate the modification of NIRS monitoring in the first moments after the outbreak of renal arterial or venous ischemia and to analyse a potential temporal relationship between the values of rSrO2 provided by NIRS and the markers of renal damage.

Material and Methods

Risk factors for postoperative complications

Study design and population

The clinical and surgical records were retrospectively reviewed to create a database about the patients that underwent KT at our Department from January 2013 to December 2021. The patients aged more than 18 years and patients with follow-up shorter than three months were excluded. Allografts from both non-living and living

donors were considered in the project. The dataset included demographic, clinical, biochemical, and surgical details, as shown in *Appendix A*.

Institutional bundle for paediatric KT

For the whole study period, our institutional protocol for induction therapy included: methylprednisolone (500 mg/m^2/die) and two doses of Basiliximab (10-20 mg/Kg), just before the transplantation and on day four after surgery. Within the first 24 hours 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/m^2/die aiming to a therapeutic trough level of 1.5-3.5 mg/l, and Methylprednisolone at an initial dose of 500 mg/m^2/die, to be progressively reduced in the following weeks.

The main surgeon and the assistant used personalized optical loupes with a 2.5–4 times magnification and microsurgical instruments appropriated for patient age and vascular structures dimension. All the grafts were implanted into the iliac fossa through an extraperitoneal access. The right side was the first choice. 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 patients weighting less than 15 kg or in case of significant mismatch between donor's and recipient's body size. 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 split urinary output of the transplanted kidney, especially in case of residual diuresis from the native kidneys.

The perioperative infusion of 5-10 units/kg/hours of unfractioned heparin was indicated in case of altered pre-operative coagulative screening, patients aging less than five years, weight less than 15 kg, considerable size mismatch (body weight ratio between donor and recipient higher than 1:4), donor kidney allograft with multiple vessels, apparent intimal lesion of the allograft renal artery, altered allograft perfusion immediately after implantation, such as venous congestion.

Variables and outcomes

A preliminary search of the current evidence identified three variables that should be considered as a risk factor and need a further investigation.

The first variable was recipient's body weight. Indeed, a body weight inferior to 15 kg might raise the complexity of the surgery and the risk of complications. The outcomes of the low-weight (LW) patients, that is to say inferior to 15 kg, were compared to the outcomes of the rest of the population, defined as normal-weight (NW) patients.

The second variable was the presence of vascular anomalies that required microsurgical reconstruction during bench surgery. The population was split into four groups according to the type of bench surgery. The first one included the allografts that required an elongation of the vein, the second the allografts with multiple veins and the third the allografts with multiple arteries. The outcomes of these groups were compared to the allografts with a normal vascular anatomy that did not require extraordinary bench surgery.

The last variable regarded the timing of non-living donors' KTs. The outcomes of KTs performed in the night or in days-off were compared to KTs performed in the ordinary working days. The population was split into three groups according to the moment during which the intervention was performed. The time lapse between 8.00 AM and

6.30 PM during working-days was considered daytime according to the current policy of the University-Hospital. On the other hand, the interval between 6.30 PM and 8.00 AM was considered night-time. According to the Italian national calendar and the current policy of the University-Hospital, the third group included the KTs performed on Saturdays, Sundays, and public holidays.

For the different purposes of the project, the following endpoints were considered the outcomes to be compared between the groups: cold ischemia time (CIT), warm ischemia time (WIT), the length of surgery, the need for inotropic drugs in the early post-operative, the length of hospital stay, serum creatinine and estimated glomerular filtration rate (eGFR), calculated according to bedside Schwartz's formula [20] at discharge and after three months since the KT. The rate of surgical complications graded more than II, according to Clavien-Dindo Classification [21], such as bleedings, graft venous thrombosis, arterial stenosis, urinary obstructions, new onset of medical conditions, the rate of surgical re-interventions in the first 30 post-operative days, the occurrence of delayed graft function (DGF) [22] and primary graft non-function (PGNF) [23].

Update on transcutaneous NIRS monitoring of allograft perfusion

Study design

A review of the literature was performed to gather the current evidence about transcutaneous NIRS monitoring of allograft perfusion after solid organ transplantation and to identify controversial aspects that needed to be clarified.

Systematic review

The systematic search was performed according to the PRISMA guidelines [24]. PubMed, Scopus, and The Cochrane Library were searched in November 2020, using a combination of terms including “near-infrared spectroscopy”, “NIRS” and “transplantation”. The search was limited to English language and publications from 1991 to 2020. Papers dealing with both adult and paediatric population, liver or kidney allografts were included. Studies including animal models or invasive methods were excluded. A qualitative synthesis of the included studies was reported.

Clinical application of transcutaneous NIRS monitoring of the allograft perfusion after paediatric KT

Study design and population

The design of the study was prospective and monocentric. A transcutaneous NIRS monitoring was applied to all the patients undergone KT at our Department from March 2021, regardless of body weight or other clinical conditions. A further inclusion criterion was the age inferior to 18 years.

Protocol of NIRS monitoring

The transcutaneous NIRS monitoring was applied during the five first postoperative days. At the time of surgical intervention, the width of the abdominal was measured. The skin was marked in correspondence of the site of upper and lower kidney pole. As to the NIRS monitoring, a four-channel device called Root® and provided by Masimo (Irvine, CA, USA) was used. This device allowed to record rSO2 values every two seconds. The size of the probe O3® Regional Oximetry was chosen according to

the body weight. Overall, three probes were used for each patient, two for each kidney pole and one on the contralateral inferior arm, as a benchmark of peripheral perfusion. The monitoring was started after the KT at once until the fifth postoperative day. Blood pressure was recorded four times per day and the urinary output was reported for each single day. Serum creatinine was measured twice a day and serum cystatin C once a day. Urinary N-acetyl-beta-D-glucosidase (NAG) was measured in the first and fifth postoperative days. Doppler-ultrasonography (DUS) was performed immediately after the KT and once a day until the end of NIRS monitoring. The resistivity index (RI) was reported separately for each kidney pole. The monitoring ended with a Tc-99 MAG-3 renal scintigraphy. A qualitative assessment of the time-activity curve was performed according to Heaf and Iversen grading scale [25]. All the adverse events graded more than II according to Clavien-Dindo classification [21] were reported to investigate potential modifications of kidney rSrO2 provided by NIRS monitoring.

Design of an experimental animal model of renal ischemia for the application of transcutaneous NIRS monitoring

Choice of the animal species

The three-R principles were followed in the choice of the animal species. As to the replacement principle, the experiment could not be replicated in vivo for several reason. There is no paediatric disease that could cause acute renal ischemia and the occurrence of vascular complications after paediatric KTs is too low to gather a valid sample size. Moreover, the consequences of renal ischemia could be too complex to be replicated in cell cultures or organoids. Finally, the in vivo induction of acute renal ischemia is not ethical. As to the reduction principle, the lowest sample size was

estimated to produce valid results. The sample size could be adjusted in case of significant results. As to the refinement principle, all the surgical procedures were standardized and performed by experienced surgical team. For the experiment only well-appearing 8-week male animals were chosen, according to a body condition score of three and a body weight of around 250 gr [26]. Analgesia was administered to all the animals during the whole duration of the experiment. For these reasons, a wild-type Sprague Dawley rat presented the lowest level of neurological development and a body size to be fit for NIRS monitoring according to the devices that were currently produced.

Induction of renal ischemia and markers of renal damage

The literature was searched for standardized surgical models of renal ischemia in rats. A laparotomy was preferred for several reason. It allows a better access to the renal vascular pedicle and to the bladder to be punctured. Moreover, the NIRS probe should not covered the area of the surgical wound. The renal artery and vein could be clamped separately for the induction of ischemia. As to the specific markers of kidney damages, a rat model of renal ischemia found a significant with neutrophilgelatinase-associated lipocaine (NGAL) and Kim-1that were sampled from both urine and blood. The experiment will be replicated in a sham group, without clamping any renal vessel.

Determination of the sample size

The sample size was calculated a priori by using the open-source software G*Power (v. 3.1). The ANOVA test for repeated measures was chosen. The experiment by Soytas et al. provided the estimate of Cohen's effect size and its f coefficient of 0.344 [27]. A significance level of 0.05 and a statistical power of 0.8 were chosen for 3.600

measures in each of the three groups. The sample size was estimated in 69 rats, 23 rats for each group.

Statistics

The databases were created by using Microsoft® Excel. The statistical analysis was performed by using IBM® SPSS Inc. Version 26.0. The variables and the endpoints of the different groups were compared through a univariate analysis. Pearson's chi-squared tests were used for categorical variables and one-way ANOVA or Mann-Whitney U tests were used for continuous variables. The association between the continuous variable was estimated by Spearman's Rho correlation tests. Kaplan-Meier survival analysis was used for time-to-event analysis. The results of the survival analysis were compared by log rank tests among the different groups. P-value ≤0.05 was considered statistically significant.

Results

Risk factors for postoperative complications

Study population

One hundred and thirty KTs were performed in 128 patients from January 2013 to December 2021 at our Department. The characteristics were summarised in *Table 1*.

Table 1. Characteristics of the patients at the time of KT.

Gender *(n, %)*	Male 77 (59%)
	Female 53 (41%)
Age at KT *(median, IQR)*	11.3 (4.6-15.5) years
Cause of ESKD *(n, %)*	Urological malformation 43 (33%)
	Nephrological disease 87 (67%)
Kidney replacement therapy *(n, %)*	108 (83%)
Body weight at KT *(median, IQR)*	26 (13-43) kg
Living donor *(n, %)*	34 (26%)
Body weight ratio *(n, %)*	2.1 (1.1-3.5)

KTs in low-weight patients

One hundred and eight KTs, performed in 106 children, were considered for the purpose. LW 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). *Table 2* compared the perioperative characteristics of the two Groups. The only differences 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 frequently

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

Table 2. Perioperative characteristics of the population (*significant p-value ≤0.05).

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

Table 3 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). Serum creatinine at discharge was lower in the LW Group (p<0.001) and eGFR was higher in this Group (p=0.009). The urological diseases leading to ESKD did not influence operative time in the LW Group (260 ± 87 min versus 281 ± 84 min; p=0.30) and in the NW Group (253 ± 51 min versus 258 ± 45 min; p=0.27). Patients undergone peritoneal dialysis had a similar operative time in the LW and NW Groups (275 ± 75 min versus 245 ± 36 min; p=0.32). Patients in the LW Group who had suffered from nephrological diseases showed a longer hospital stay, when compared to those affected by urological diseases (22 ± 5.1 days versus 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 versus 18 ± 5.5 days; p=0.31). 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 reimplantation 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 versus 267 ± 54 min; p=0.16).

Table 3. Early outcomes of the KTs (*significant for p-value ≤0.05).

	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 *(umol/l; mean, SD)*	32 ± 10	80 ± 50	<0.001*
eGFR at discharge *(ml/min/1.73m^2; mean, SD)*	92 ± 35	78 ± 29	0.009*
Length of hospital stay *(days; mean, SD)*	19 ± 7.1	20 ± 8.6	0.83

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 4*.

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 hours 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, whilst 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

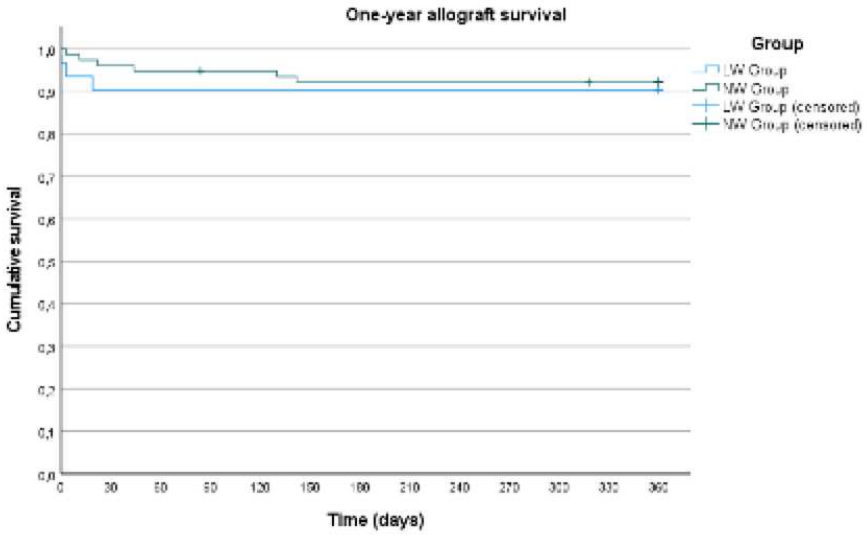
an altered coagulation screening before KT. In only one case, the thrombosis was related to the vascular human graft. The prompt administration of fibrinolytic agents succeeded in restoring the venous flow in only two patients. During the operation, no difficulty in wound closure or need of abdominal 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, due to 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).

Table 4. Surgical complications of KTs (*significant for p-value ≤0.05).

	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

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 ± 5.3 %), while in the NW Group they were 95 ± 2.5 % and 92 ± 3.1 %, respectively. No difference was found between the two Groups (p=0.38; p=0.72). Kaplan-Meier curve is displayed in *Figure 1.* One-year eGFR was 80 ± 38 ml/min/1.73m^2 in the LW Group and 75 ± 26 ml/min/1.73m^2 in the NW Group (p=0.14).

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



KTs with complex bench surgery

Seventy-eight patients were considered for the purpose. Forty-six of them (58%) were male. The patients underwent 80 KTs. The median age was 11 (IQR 4.3-14) years, the median body weight was 24 (IQR 13-37) kg. The youngest patient was 2.2 years old, and the lowest body weight was 7.0 kg. The median body weight ratio (BWR) between donors and recipients was 2.1 (IQR 1.1-3.5), being the highest 6.3. Twenty-four children (30%) received a living-donor allograft. The median follow-up was 36 months (IQR 28-51 months).

Among 80 donor kidney allografts, 39 organs (49%) required a complex BS. In this case, the presence of vascular anatomical variants required microvascular anastomoses by using magnifying loops, microsurgical instruments and 7-0, 8-0 sutures, to create a single vein or artery. The following procedures were performed: elongation of the vein or artery (*Figure 2*), anastomosis between a polar artery and the main artery or anastomosis between multiple arteries or veins (*Figure 3*). For the elongation, we preferred to make an interrupted suture to avoid the risk of constructing a too long vessel, so the second part of the suture could be easily removed. All the grafts were irrigated by cold preservation solution during BS and the related procedures.

Figure 2. Elongation of the vein during bench surgery.

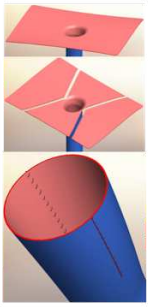
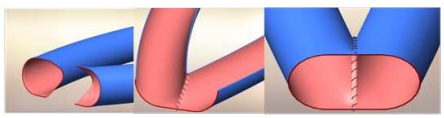
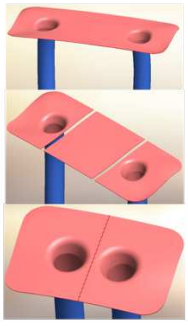


Figure 3. End-to-end anastomosis of multiple vessels and anastomosis of multiple vessels by using the vascular patch.





The complex BS was due to arterial anomalies in 16 (41%), venous anomalies in 23 (29%), 12 of which (31%) consisted in multiple veins and 11(28%) in a very short vein. In 32 out of 39 (82%) KTs undergone complex BS, a single venous anastomosis with single arterial anastomosis was possible. In one case, the elongated vein had to be intraoperatively shortened because of a kinking. No differences in the perioperative factors were found among the four groups, as shown in *Table 5.*

Table 5. Perioperative characteristics of KTs (*significant for p-value ≤0.05).

	Arterial anomalies *(n=16)*	**Venous anomalies** *(n=12)*	**Elongation of the vein** *(n=11)*	**Standard bench surgery** *(n=41)*	***p-value***
Age *(years;median,IQR)*	13 (7.2-14)	14 (6.7-14)	4.9 (3.9-12)	9.9 (4.2-16)	0.75
Body weight *(kg;median IQR)*	28 (19-37)	27 (16-41)	15 (11-32)	24 (13-37)	0.94
Male gender *(n,%)*	7 (44)	5 (42)	7 (64)	27 (66)	0.28
BWR *(median,IQR)*	2.2 (1.1-2.7)	1.7 (1.1-2.3)	3.3 (1.2-4.6)	2.1 (1.1-3.6)	0.58
Living donors *(n,%)*	4 (25)	3 (25)	0 (0)	17 (42)	0.17
Cold ischemia time *(h;median, IQR)*	9.6 (5.8-14)	11 (4.0-13)	12 (10-13)	9.5 (1.5-12)	0.12
Heparin *(n,%)*	13 (81)	8 (67)	7 (64)	24 (59)	0.45
Inotropic drugs *(n,%)*	9 (56)	4 (33)	7 (64)	19 (46)	0.46
Vasoactive drugs *(n,%)*	7 (44)	6 (50)	6 (55)	11 (27)	0.22

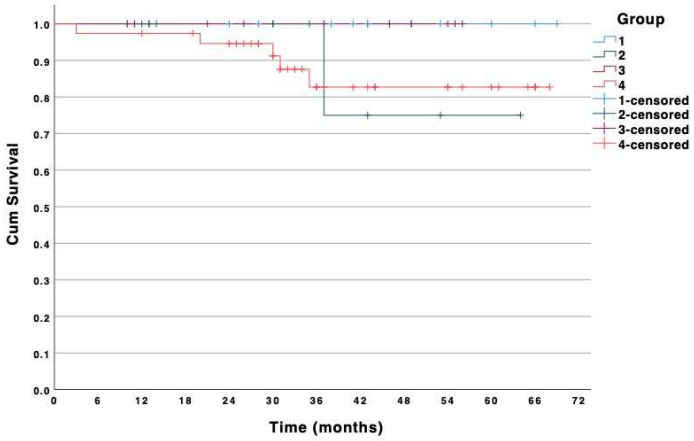
Table 6 shows the outcomes of KTs. First RI, serum creatinine at discharge and length of hospital stay were not different among the groups (p=0.92, p=0.48, p=0.49 respectively). The occurrence of DGF was similar (p=0.72). Only one case of PGNF was reported and it affected an allograft undergone standard BS (p=0.97).

Table 6. Outcomes of KT (*significant for p-value ≤0.05).

	Arterial anomalies *(n=16)*	**Venous anomalies** *(n=12)*	**Elongation of the vein** *(n=11)*	**Standard bench surgery** *(n=41)*	***p-value***
Resistive index *(median,IQR)*	0.67 (0.60-0.71)	0.63 (0.60-0.65)	0.70 (0.64-0.75)	0.65 (0.60-0.71)	0.92
Primary graft non-function *(n,%)*	0 (0)	0 (0)	0 (0)	1 (2.4)	0.97
Delayed graft function *(n,%)*	3 (19)	1 (8.3)	0 (0)	5 (12)	0.72
Serum creatinine at discharge *(umol/l,median,IQR)*	64 (51-84)	65 (34-78)	36 (34-63)	55 (35-89)	0.48
Length of hospital stay *(day,median,IQR)*	19 (15-23)	16 (12-22)	19 (16-20)	20 (15-25)	0.49
Follow-up *(months,median,IQR)*	43 (35-50)	30 (14-40)	41 (22-53)	34 (29-54)	0.43
Overall survival *(%,standard error)*	100	75 (22)	100	83 (7.3)	0.27

The allografts with venous anomalies presented the lowest survival rate (75%) in a median follow-up of 30 months (IQR 14-40 months). However, this rate was not different among the groups (p=0.27). The curve showing the estimated survival is displayed on *Figure 4*.

Figure 4. Survival curve of the allografts grouped according to the complexity of BS (1: arterial anomalies; 2: venous anomalies; 3: elongation of the vein; 4: standard bench surgery).



The donor kidneys undergone complex BS presented a median warm ischemia time of 65 min, longer than the standard BS (p=0.01). These data are reported in *Table 7*. Twelve vascular complications (15%) occurred in the whole population. Bleeding was observed in 6 cases (7.7%). Only two patients were treated conservatively; the others needed a surgical exploration to control the bleeding and to avoid the compression of the urinary tract, which required the placement of a double-J stent in two patients. Nevertheless, no graft was lost because of the bleedings.

Graft venous thrombosis was observed in five cases (6.3%), within the first 72 hours after transplantation. The graft could be saved only in two patients. In one of them it was removed and after having performed the thrombectomy in a bench procedure, the kidney was irrigated with fibrinolytic drugs and successfully re-implanted. Only one case (1.3%) of kinking of the renal artery was reported, in a graft without any vascular anomaly. This event did not require any surgical treatment. No graft arterial thrombosis occurred during the study period, and no complications were found in those KTs that needed multiple vascular anastomosis. The rates of overall vascular complications and bleedings were compared between the group undergone standard BS and those

undergone vascular procedures during BS. These rates were similar (respectively p=0.51, p=0.59), as shown in *Table 7*. Two cases of graft venous thrombosis were encountered in the groups undergone complex BS. The first case presented a donor kidney with two renal veins and the thrombosis led to the loss of the organ. The second case, where the elongation of the renal vein had been performed, was treated with anticoagulation therapy. However, no difference in the occurrence of graft venous thrombosis was identified among the four groups (p=0.78).

Table 7. Outcomes of surgery (*significant for p-value ≤0.05).

	Arterial anomalies *(n=16)*	**Venous anomalies** *(n=12)*	**Elongation of the vein** *(n=11)*	**Standard bench surgery** *(n=41)*	***p-value***
Warm ischemia time *(min,median,IQR)*	65 (60-71)	65 (60-66)	60 (58-70)	60 (55-61)	0.01*
Vascular complications *(n,%)*	1 (6.3)	2 (17)	3 (27)	6 (15)	0.51
Bleeding *(n,%)*	1 (6.3)	1 (8.3)	2 (18)	2 (4.9)	0.59
Venous graft thrombosis *(n,%)*	0 (0)	1 (8.3)	1 (9.1)	3 (7.3)	0.78
Vascular stenosis or kinking *(n,%)*	0 (0)	0 (0)	0 (0)	1 (2.4)	0.97

KTs performed during night-time and day-off

One hundred and thirty KTs were performed during the nine-year study period. Among those, 96 (74%) were non-living donors urgent KTs. The median age and body weight

at surgery were 11 (IQR 4.3-14) years and 26 (13-50) kg, respectively. Forty-five patients (47%) were females and fifty-one (53%) were males.

According to the inclusion criteria, only 27 KTs (28%) were performed during ordinary days. Most of them was performed during after-hours. Twenty-eight KTs (29%) were performed during days-off and the remaining 41 (43%) were during night-time. The demographic and perioperative characteristics of the groups were compared, as reported in *Table 8*.

Table 8. Demographic and perioperative characteristics of the study population (*significant for $p \leq 0.05$).

	Ordinary-day KT *(n=27)*	**Day-off KT** *(n=28)*	**Night-time KT** *(n=41)*	*p-value*
Age at KT *(median, IQR)*	10 (3.7-13) years	13 (5.6-16) years	8.7 (4.3-13) years	0.698
Female gender *(n,%)*	12 (44)	13 (46)	19 (46)	0.985
Body weight *(n, %)*	24 (12-36) kg	32 (14-49) kg	22 (14-36)	0.414
Body weight ratio *(median, IQR)*	1.9 (1.0-3.0)	1.7 (1.0-2.3)	2.1 (1.0-3.5)	0.597
Low-weight (< 15kg) patients *(n, %)*	9 (33)	8 (29)	11 (27)	0.844
Vascular anatomic variants *(n, %)*	18 (67)	17 (28)	19 (46)	0.217
Anticoagulant therapy *(n, %)*	17 (63)	14 (50)	32 (78)	0.052

The clinical endpoints were compared among the groups, as reported in *Table 9*. The length of surgery was longer for the KTs performed in the days-off (p=0.011). As to

the serum creatinine and the eGFR, no difference was found at discharge (p=0.432, p=0.521) and at 3-month follow-up (p=0.371, p=0.093).

Table 9. Clinical endpoints compared among the groups (*significant for p≤ 0.05).

	Ordinary-day KT *(n=27)*	**Day-off KT** *(n=28)*	**Night-time KT** *(n=41)*	*p-value*
Cold ischemia time *(median, IQR)*	12 (10-13) hours	10 (8.5-12) hours	12 (10-15) hours	0.769
Warm ischemia time *(median, IQR)*	60 (59-65) minutes	65 (60-71) minutes	62 (55-69) minutes	0.526
Operative time *(median, IQR)*	255 (232-283) minutes	285 (251-330) minutes	235 (203-293) minutes	0.011
Inotropic drugs *(n, %)*	8 (30)	15 (54)	15 (37)	0.168
Length of hospital stay *(median, IQR)*	19 (15-22) days	17 (15-24)	19 (14-24) days	0.571
Serum creatinine at discharge *(median, IQR)*	55 (36-82) umol/l	81 (44-106) umol/l	57 (37-91) umol/l	0.432
eGFR at discharge *(median, IQR)*	78 (66-96) ml/min/1.73 m^2	67 (54-94) ml/min/1.73 m^2	77 (60-109) ml/min/1.73 m^2	0.521
Serum creatunine at 3-month follow-up *(median, IQR)*	55 (43-79) umol/l	73 (54-112) umol/l	54 (36-69) umol/l	0.371
eGFR at 3-month follow-up *(median, IQR)*	76 (68-85) ml/min/1.73 m^2	67 (50-82) ml/min/1.73 m^2	81 (62-100) ml/min/1.73 m^2	0.093
3-month graft loss *(n, %)*	0 (0)	2 (7.1)	4 (9.8)	0.469

A total of 32 adverse events occurred in 31 patients. *Figure 5* displayed the distribution of the cutting time throughout the day for the KTs affected by adverse events. The overall rate of complications was similar among the groups (p=0.669), as reported in *Table 10*. However, the risk for post-operative bleeding was significant higher for KTs performed during days-off (0.003).

Figure 5. Distribution throughout the day of the cutting time for the KTs affected by adverse events.

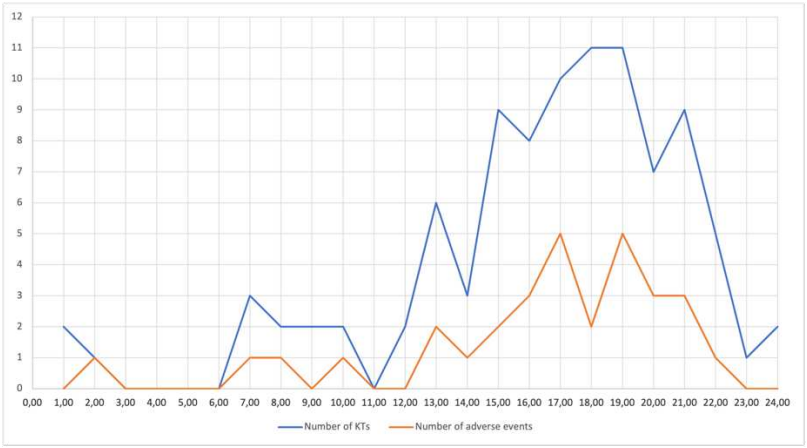


Table 10. Rate of complications and adverse events compared among the groups (*significant for p≤ 0.05).

	Ordinary-day KT *(n=27)*	**Day-off KT** *(n=28)*	**Night-time KT** *(n=41)*	*p-value*
Overall complications *(n,%)*	6 (22)	9 (32)	10 (24)	0.669
Bleeding *(n,%)*	0 (0)	6 (21)	0 (0)	0.003*
Graft venous thrombosis *(n,%)*	0 (0)	0 (0)	3 (7.3)	0.456
Arterial stenosis *(n,%)*	1 (3.7)	0 (0)	1 (2.4)	0.412
Urinary obstruction *(n,%)*	2 (7.4)	2 (7.1)	4 (9.8)	0.909
Medical conditions *(n,%)*	3 (11)	1 (3.6)	2 (4.9)	0.458
Delayed graft function *(n,%)*	0 (0)	3 (11)	3 (7.3)	0.383
Primary graft non-function *(n,%)*	0 (0)	0 (0)	1 (2.4)	0.949

During the first 30 post-operative days, only one patient (3.7%) required a further endourological procedure. Five patients (18%) in the day-off group required seven re-interventions, six laparotomies and one endourological procedure. Finally, seven patients (17%) belonging to night-time group reported a further intervention for each of them. Four endourological procedures and three laparotomies. Even though, the rate of re-intervention was higher in the after-hours KTs, no significant difference was found (0.211). The details about the re-interventions were described in *Table 11*.

Table 11. Re-interventions for adverse events during the first 30 post-operative days.

	Ordinary-day KT *(n=27)*	**Day-off KT** *(n=28)*	**Night-time KT** *(n=41)*
Re-interventions	1 ureteral stenting	2 laparotomies for bleeding 1 laparotomy for bleeding followed by graft removal for graft venous thrombosis 1 graft removal for PGNF 1 nephrostomy followed by re-do of the ureterovesical anastomoses	2 nephrostomies 2 ureteral stentings 2 laparotomies for graft removal (1 PNGF, 1 graft venous thrombosis 1 laparotomy for re-do of the arterial anastomoses

The occurrence of DGF and PGNG was similar (p=0.383; p=0.949). During the three-month follow-up, no graft was lost among the KTs performed during working time. Two of the day-off group grafts (7.1%) failed because of venous graft thrombosis. In the night-time group, four graft failures (9.8%) were recorded: two cases due to venous graft thrombosis in two cases, one case of primary graft non-function and one case of relapse of the underlying nephropathy. No difference was found (p=0.469).

<u>Update on NIRS monitoring of allograft perfusion</u>

Results of the systematic review

According to the inclusion criteria, 1313 papers were screened. The search yielded five pertinent articles as reported in *Appendix B*. Three of them investigated kidney graft perfusion, accounting for a total of 53 pediatric patients and 50 adult patients [16, 17, 28]. The remaining two papers dealt with 50 adults and two children undergone liver transplantation (LT) [18; 29]. The quality assessment, according to the Newcastle-

Ottawa Scale, showed a high risk of bias for all studies in *Appendix C* [30]. This might be due to the small size of the cohorts selected for the studies. Main findings of the selected papers are summarized in the *Table 12*. NIRS monitoring was used maximum for the first 72 hours after transplant in a cohort of children undergone KT. The reference values of rSrO2 were reported in four of the studies and these were quite variable. Adverse events were generally rare, but most studies find a correlation between abnormal NIRS values and complications with decrease in organ perfusion for both KT and LT.

Table 12. Clinical studies assessing the feasibility of real-time NIRS monitoring of allograft perfusion.

Study	Country	Design	Population	NIRS monitoring	Values of allograft rSrO2	Adverse events	Main findings
Vidal *et al*., 2014 [16]	Italy	Prospective	24 pediatric kidney transplants	72 h after transplant	At the beginning: 68.8 % (IQR 59.3–76.2) At the end: 83.6% (IQR 79.2–90.4)	4 delayed graft functions	rSrO2 correlated with serum creatinine, eGFR and u-NGAL.
Malakasioti *et al*., 2018 [28]	UK	Prospective	29 pediatric kidney transplants	2 min (during annual or biannual routine follow-up)	Upper pole: 78.8% ± 7.0% Lower pole: 79.3% ± 10.7%	None reported	rSrO2 correlated with resistive index by DUS and systolic blood pressure.
Shiba *et al*., 2018 [29]	Japan	Case Report	2 pediatric liver transplants	After the transplant until the discharge from Intensive Care Unit	Case 1: decline of 23% Case 2: decline of 26%	2 acute cellular rejections	Decrease in rSrO2 anticipated vascular complications.
Pérez Civantos *et al*., 2019 [17]	Spain	Prospective	61 adult kidney transplants	24 h after transplant	At the beginning: 81% ± 6% At the end: not statistically different	3 bleedings 1 arterial thrombosis 1 venous thrombosis	Decrease in rSrO2 anticipated vascular complications. rSrO2 correlated with serum lactate and initial diuresis.
Pérez Civantos *et al*., 2019 [18]	Spain	Prospective	50 adult liver transplants	24 h after transplant	At the beginning: 74% ± 5.7% At the end: 76% ± 4.1%	12 bleedings 2 low cardiac outputs 1 septic shock 1 bronchospasm	Decrease in rSrO2 anticipated vascular complications.

Interpretation of the results

A preliminary study by Vidal *et al.* reported transcutaneous NIRS measurements in 24 children after KT. Kidney rSrO2 measured by NIRS significantly increased over time during the first three days after surgery and a significant correlation was found with serum creatinine, eGFR and with the decrease of post-operative urinary NGAL, which is a marker of tubular injury reflecting the ischemic and reperfusion damage of the kidney. In this series, DUS did not identify any abnormality in kidney vascularization and no vascular complications were reported. To date, four patients experienced a DGF without any peculiar modifications of rSrO2. However, the Authors suggested that NIRS might help in discriminating the causes of oligo-anuria in the early post-operative time after KT, helping in managing the postoperative fluid balance. Malakasioti *et al*. identified a significant correlation between kidney rSrO2 measured by NIRS and resistive index derived from DUS in a series of 29 paediatric patients. Once again, no complications were reported in this cohort. At the moment, NIRS contributed to the early diagnosis of vascular complications in two children undergone liver transplantation, as reported by Shiba *et al*.

Pérez Civantos *et al.* assessed renal rSrO2 in 61 adults undergone KT, using probes with a maximum depth of 2.5 cm. NIRS readings were significantly correlated to the decreasing values of serum lactate at eight hours and 24 hours, which is another marker of ischemic injury. Furthermore, kidney rSrO2 was related to initial diuresis at three hours and to mixed central venous oxygen saturation. On the other hand, no correlation with DUS was found. In addition, in this series arterial thrombosis and bleedings were encountered, showing a maintained decrease in rSrO2 which might anticipate the vascular events. It is relevant to point out that the same study was

replicated in liver transplantations. Even in this scenario, NIRS decreased early during bleeding or in case of thrombosis.

Clinical application of transcutaneous NIRS monitoring of the allograft perfusion after paediatric KT

Study population

During the study period, eight patients were enrolled for NIRS monitoring after KT. The monitoring was completed in seven of them. One patient (#4) suffered from a graft venous thrombosis that resulted in the loss of the allograft during the first postoperative day. The Table *13* summarized the characteristics of the eight patients included in the study.

Table 13. Characteristics of the patients included in the NIRS monitoring project.

	Age and Gender	Cause of ESKD	Body weight	eGFR	Living donor	Donor's body weight and BWR	Vascular anomalies of the allograft	Cold ischemia time	Warm ischemia time	Length of surgery	Heparin during PO	Inotropes during PO	Length of stay in pICU
Patient #1	4.7 yrs; M	Posterior urethral valves	17 kg	12 ml/min/1.73m^2	no	85 kg; 1:5	3 arteries and 2 veins	16 hrs	90 min	330 min	yes	dopamine	10 hours
Patient #2	11.1 yrs; M	Joubert's syndrome	27 kg	4.5 ml/min/1.73m^2	yes	75 kg; 1:2.8	no	1 hrs	70 min	300 min	yes	-	24 hours
Patient #3	11.3 yrs; F	Congenital kidney hypodysplasia	40 kg	5.2 ml/min/1.73m^2	no	53 kg; 1:1.3	no	9 hrs	85 min	290 min	no	-	15 hours
Patient #4	0.9 yrs; M	Posterior urethral valves	7.6 kg	8.2 ml/min/1.73m^2	no	23 kg; 1:3	2 arteries and 2 veins	10 hrs	120 min	330 min	-	-	-
Patient #5	1.9 yrs; M	Vesical-ureteral reflux	9.8 kg	15 ml/min/1.73m^2	no	33 kg; 1:3.4	no	13 hrs	55 min	260 min	yes	dopamine	16 hours
Patient #6	15.5 yrs; M	Congenital kidney hypodysplasia	47 kg	11 ml/min/1.73m^2	no	45 kg; 1:1	no	10 hrs	65 min	235 min	no	-	16 hours
Patient #7	9.7 yrs; M	Vesical-ureteral reflux	27 kg	5.5 ml/min/1.73m^2	no	56 kg; 1:2	2 arteries and lengthening of the vein	10 hrs	70 min	220 min	no	dopamine	12 hours
Patient #8	12.5 yrs; F	Nephronophthisis	36 kg	13 ml/min/1.73m^2	no	28 kg; 1:0.8	no	16 hrs	55 min	205 min	yes	-	11 hours

Results of NIRS monitoring

The data about NIRS monitoring were available for seven patients (#1, #2, #3, #5, #6, #7, #8). None of the patients experienced major adverse events during the five postoperative days.

The clinical and biochemical parameters that were registered during the monitoring are displayed for each postoperative day in the following *Tables (14,15,16,17,18)*.

Table 14. Clinical and biochemical parameters during the first postoperative day.

	Median systolic blood pressure	**Urinary output from ureteral stent**	**Serum creatinine**	**Serum Cystatin C**	**Urinary NAG**
Patient #1	115 mmHg	2430 ml	196 umol/l	1.84 mg/l	1 U/l
Patient #2	125 mmHg	-	135 umol/l	-	1 U/l
Patient #3	149 mmHg	1797 ml	170 umol/l	2.5 mg/l	1 U/l
Patient #5	118 mmHg	500 ml	167 umol/l	1.22 mg/l	1 U/l
Patient #6	136 mmHg	5000 ml	218 umol/l	0.97 mg/l	-
Patient #7	101 mmHg	465 ml	645 umol/l	2.11 mg/l	1 U/l
Patient #8	105 mmHg	3700 ml	707 umol/l	-	-

Table 15. Clinical and biochemical parameters during the second postoperative day.

	Median systolic blood pressure	**Urinary output from ureteral stent**	**Serum creatinine**	**Serum Cystatin C**
Patient #1	120 mmHg	1805 ml	127 umol/l	1.52 mg/l
Patient #2	119 mmHg	525 ml	36 umol/l	0.86 mg/l
Patient #3	149 mmHg	335 ml	80 umol/l	-
Patient #5	104 mmHg	499 ml	43 umol/l	0.81 mg/l
Patient #6	134 mmHg	4190 ml	95 umol/l	1.16 mg/l
Patient #7	118 mmHg	505 ml	323 umol/l	1.82 mg/l
Patient #8	100 mmHg	1910 ml	197 umol/l	1.22 mg/l

Table 16. Clinical and biochemical parameters during the third postoperative day.

	Median systolic blood pressure	**Urinary output from ureteral stent**	**Serum creatinine**	**Serum Cystatin C**
Patient #1	117 mmHg	1555 ml	106 umol/l	-
Patient #2	105 mmHg	165 ml	36 umol/l	0.85 mg/l
Patient #3	142 mmHg	1705 ml	78 umol/l	1.59 mg/l
Patient #5	110 mmHg	311 ml	38 umol/l	0.98 mg/l
Patient #6	124 mmHg	4990 ml	90 umol/l	1.18 mg/l
Patient #7	118 mmHg	35 ml	198 umol/l	-
Patient #8	101 mmHg	1720 ml	114 umol/l	1.70 mg/l

Table 17. Clinical and biochemical parameters during the fourth postoperative day.

	Median systolic blood pressure	**Urinary output from ureteral stent**	**Serum creatinine**	**Serum Cystatin C**
Patient #1	138 mmHg	1845 ml	85 umol/l	1.69 mg/l
Patient #2	105 mmHg	675 ml	42 umol/l	-
Patient #3	153 mmHg	1045 ml	101 umol/l	2.11 mg/l
Patient #5	116 mmHg	312 ml	37 umol/l	0.99 mg/l
Patient #6	124 mmHg	6620 ml	95 umol/l	1.20 mg/l
Patient #7	126 mmHg	20 ml	143 umol/l	-
Patient #8	110 mmHg	1660 ml	99 umol/l	1.74 mg/l

Table 18. Clinical and biochemical parameters during the fifth postoperative day.

	Median systolic blood pressure	**Urinary output from ureteral stent**	**Serum creatinine**	**Serum Cystatin C**	**Urinary NAG**
Patient #1	124 mmHg	960 ml	70 umol/l	1.49 mg/l	1.43 U/l
Patient #2	99 mmHg	262 ml	43 umol/l	1.21 mg/l	8.48 U/l
Patient #3	151 mmHg	-	110 umol/l	2.18 mg/l	18.18 U/l
Patient #5	-	-	-	-	1,13 U/l
Patient #6	-	-	-	-	-
Patient #7	121 mmHg	885 ml	93 umol/l	1.42 mg/l	1.20 U/l
Patient #8	-	-	-	-	-

Table 19 and *Table 20* show the values of rSrO2 together the values of RI assessed by DUS for the upper and lower kidney pole. *Table 21* reports the values of rSrO2 of the contralateral lower limb, that was chosen as a benchmark of peripheral perfusion.

Table 19. The values of rSrO2 and RI for the upper kidney pole.

Patient	**Width of the abdominal wall**	**First day**		**Second day**		**Third day**		**Fourth day**		**Fifth day**	
		Median rSrO2	*RI*	*Median rSrO2*	*RI*	*Median rSrO2*	*RI*	*Median rSrO2*	*RI*	*Median rSrO2*	*RI*
#1	2.0 cm	84%	0.81	86%	0.71	88%	0.62	86%	0.66	83%	0.66
#2	3.0 cm	85%	0.76	83%	0.84	79%	0.80	78%	0.69	79%	0.71
#3	4.5 cm	86%	0.53	87%	0.55	87%	0.55	85%	0.43	78%	0.76
#5	2.0 cm	85%	0.66	88%	0.62	89%	0.65	83%	0.69	-	-
#6	2.7 cm	85%	0.56	85%	0.54	84%	0.54	82%	0.50	-	-
#7	2.5 cm	79%	1.00	82%	0.50	81%	0.50	80%	0.54	80%	0.65
#8	4.0 cm	84%	0.75	85%	0.72	85%	0.72	84%	0.71	-	-

Table 20. The values of rSrO2 and RI for the lower kidney pole.

Patient	Width of the abdominal wall	First day		Second day		Third day		Fourth day		Fifth day	
		Median rSrO2	*RI*	*Median rSrO2*	*RI*	*Median rSrO2*	*RI*	*Median rSrO2*	*RI*	*Median rSrO2*	*RI*
#1	1.8 cm	86%	0.70	89%	0.69	87%	0.63	83%	0.65	72%	0.65
#2	2.3 cm	86%	0.69	81%	0.80	81%	0.70	78%	0.53	77%	0.68
#3	4.5 cm	87%	0.58	86%	0.54	85%	0.54	83%	0.47	80%	-
#5	2.0 cm	86%	0.65	88%	0.65	84%	0.70	79%	0.64	-	-
#6	2.7 cm	86%	0.50	82%	0.49	82%	0.51	80%	0.60	-	-
#7	2.5 cm	84%	0.91	83%	0.50	82%	0.38	83%	0.61	79%	0.74
#8	4.0 cm	86%	0.73	83%	0.70	81%	0.60	81%	0.70	-	-

Table 21. The values of rSrO2 of the contralateral lower limb.

Patient	First day	Second day	Third day	Fourth day	Fifth day
	Median rSrO2	*Median rSrO2*	*Median rSrO2*	*Median rSrO2*	*Median rSrO2*
#1	85%	81%	84%	82%	73%
#2	82%	80%	77%	76%	75%
#3	85%	82%	82%	77%	-
#5	83%	81%	79%	79%	-
#6	85%	80%	81%	80%	-
#7	82%	79%	77%	78%	74%
#8	84%	81%	76%	78%	-

At the end of the NIRS monitoring, a Tc-99 MAG-3 renal scintigraphy was performed. In all of the patients, the qualitative assessment of the time-activity curve showed a grade 1 pattern according to Heaf and Iversen grading scale.

Association with rSrO2 values provided by NIRS monitoring

Table 22 reported the results of Spearman's Rho correlation tests between clinical, biochemical and ultrasonographic parameters and rSrO2 values. The correlation between serum creatinine values and rSrO2 values of the lower pole was significant (p=0.0081). However, even though the correlation was not significant, an association with the urinary output (p=0.0985) and RI values (p=0.1329) could be found for the upper pole.

Table 22. Association between clinical, biochemical and ultrasonographic parameters and rSrO2 values (*significant for p≤ 0.05).

		p-value
Systolic blood pressure	*Upper pole*	0.2472
	Lower pole	0.2759
Urinary output	*Upper pole*	0.0985
	Lower pole	0.2626
Serum creatinine	*Upper pole*	0.9456
	Lower pole	0.0081*
Serum cystatin C	*Upper pole*	0.6635
	Lower pole	0.3917
RI values	*Upper pole*	0.1329
	Lower pole	0.9692

NIRS monitoring of an episode of graft venous thrombosis

An episode of graft venous thrombosis was reported during NIRS monitoring. Patient #4 suffered from ESKD due to the consequences of severe posterior urethral valves. The characteristics of the patient and of the allograft were displayed in *Table 13*. It is relevant to report that the allograft presented two veins with a patch of inferior vena cava (IVC) and two arteries with a small aortic patch. This required a microsurgical reconstruction during bench surgery. The kidney was implanted in the right iliac fossa through an extraperitoneal approach. An end-to-side anastomosis was fashioned between the venous patch and the IVC and another single end-to-side anastomosis between the arteries and the aorta, according to our institutional protocol. After removing the vascular clamps, the allograft was bluish and venous congestion was suspected. The venous anastomosis was inspected, finding a stenosis due to the proximity of the wall suture to the anastomotic suture. The allograft was washed with heparin solution and the venous anastomosis was re-fashioned. After removing the vascular clamps for the second time, the aspect of the allograft seemed to be vital, but

the lower pole was still darker. Administration of intravenous heparin (20 UI/kg/h) was started during the abdominal wall closure to help in restoring the perfusion in the congested lower pole. The thickness of the abdominal wall was directly estimated at the end of the intervention, resulting in a measure of less than 2 cm at both the poles. The first probe was placed on the skin above the lower pole, the second on the skin above the upper pole and the third on the contralateral leg, as a benchmark of patient's oxygenation. The recording of rSO2 values was started after patient's arrival in Paediatric Intensive Care Unit.

During the monitoring period, the patient required mechanical ventilation and O2 saturation levels were steady, ranging from 98% to 100%. At the end of KT and in the early post-operative, the patient presented with severe hypotension that required the administration of multiple inotropic drugs (dopamine, noradrenaline, adrenaline). Drain output was high (7-10 ml/kg/h) and, after the detection of decreasing haemoglobin levels from 9.8 gr/dl to 6.6 gr/dl, two blood transfusions were administrated. Despite the resuscitation, clinical conditions worsened to hypovolemic shock and haemoglobin levels further decreased to 4.0 gr/dl. Urinary output from ureterostomy was low and the patient was oliguric during all the early post-operative. A DUS evaluation was performed by a senior paediatric nephrologist six hours after the KT, and this found no signs of perfusion with arterial reverse flow that suggested the occurrence of a venous graft thrombosis (*Figure 6*). Then, the allograft was explanted, and the histopathological findings confirmed the presence of venous thrombosis involving the whole allograft.

Figure 6. Doppler-ultrasonographic picture taken at the renal hilum showing diastolic reverse flow highly suspicious for graft venous thrombosis.

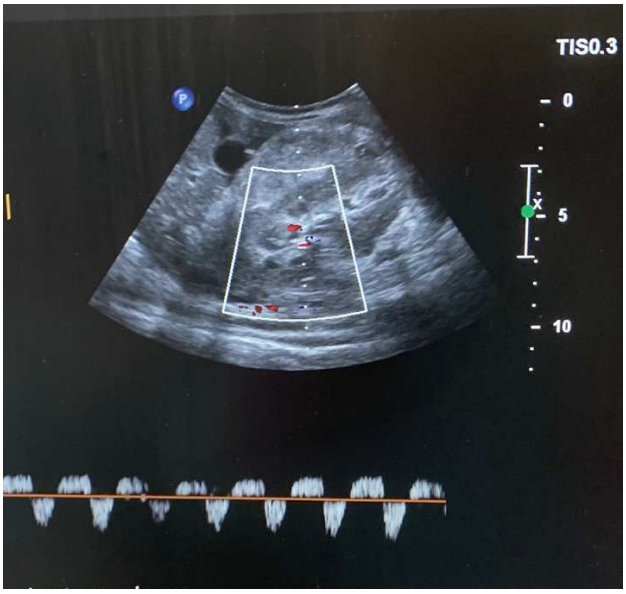


Figure 7 shows the trend of readings of the three transcutaneous NIRS probes during the post-operative. The recording started from patient's arrival in the paediatric intensive care unit until the removal of the probes to ease the execution of the DUS. This lasted for 348 minutes. *Table 23* reported median, interquartile range and the minimum rSrO2 values recorded by the three probes. The lower pole presented the lowest rSrO2 values ($p<0.0001$).

Figure 7. Trend of the rSrO2 values during the period of monitoring.

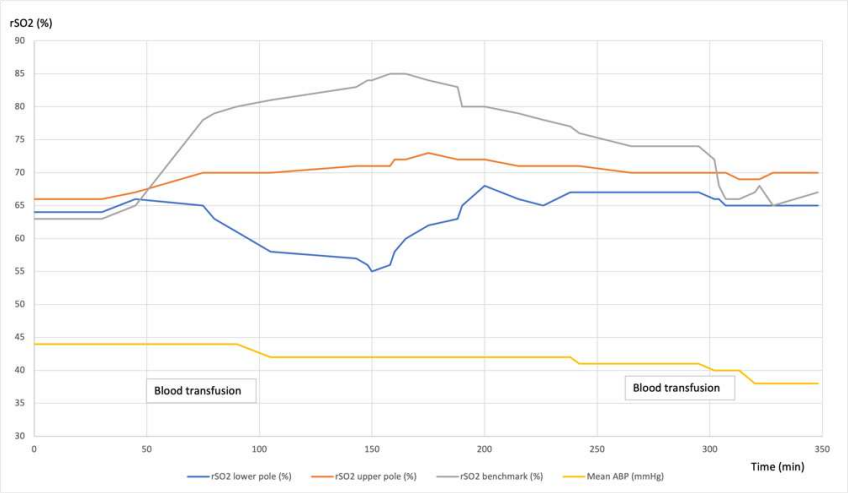


Table 23. rSrO2 values during NIRS monitoring.

	Lower pole rSrO2	Upper pole rSrO2	Benchmark rSrO2
Median	65%	70%	78%
IQR	62-66%	70-71%	68-82%
Minimum	55%	66%	63%

Design of the rat model for the NIRS monitoring of kidney perfusion

Study population

Only Sprague-Dawley wild-type male rats are used. The body condition score, the age and the body weight should be 3, eight weeks and 250 gr, respectively. According to the a priori calculation of the sample size, the population of 69 rats is split into three groups. Each group consists of 23 rats. The first group is composed by sham animals, and it is the control group. Arterial ischemia is induced by the ligation of the renal artery in the rats belonging to the group A and, venous ischemia is induced by the ligation of the renal vein in the rats belonging to the group V. *Table 24* displays the characteristics of the three groups.

Table 24. Characteristics of the three groups.

Gruppo	Number of rats	Description	Surgical procedure
Sham	23	Control group	Identification of the left renal vascular pedicle
Group A	23	Arterial ischemia	Identification of the left renal vascular pedicle and ligation of the renal artery
Group V	23	Venous ischemia	Identification of the left renal vascular pedicle and ligation of the renal vein

Surgical procedure

The entire experiment will be conducted under general anaesthesia. A 3-3.5% mix of Sevoflurane will be administered by inhalation. Asepsis will be provided by disinfection with a 10% solution of iodopovidone. The left renal vascular pedicle will be isolated, and the procedure will end according to the group.

NIRS monitoring

The rSrO2 will be recorded by using a four-channel Root® with O3® Regional Oximetry, provided by Masimo® (Irvine, CA, USA). This device allowed to record rSO2 values every two seconds, for a total of 3600 readings. A neonatal probe will be placed over the shaved skin in correspondence of the left kidney, immediately after the closure of the abdominal wall. The monitoring will continue for 120 minutes under general anaesthesia by inhalation of a 2-2.5% mix of Sevoflurane. The rat will be supported by the administration of 0.9% saline solution at a dose of 10 ml/kg.

A blood and urine sample will be taken by the puncture of the lateral tail vein and of the bladder after 30, 60 and 120 minutes. Each sample will contain 0.5 ml of urine and 0.1 ml of blood respectively. The concentration of NGAL and Kim-1 will be measured in the serum and in the urine through ELISA method. The serum concentration of creatinine will be assessed at the same intervals. The experiment will end after 120 minutes, and the rat will be sacrificed according to the current institutional and national policy. *Table 25* summarizes the procedure of the experiment.

Table 25. Protocol of the experiment.

0 minutes		**30 minutes**		**60 minutes**		**120 minutes**	**End**
End of the surgical procedure and placement of the NIRS probe		Blood and urine sample		Blood and urine sample		Blood and urine sample	Sacrifice of the rat
Real-time continuous NIRS monitoring							

Analysis of the results

The results will be collected in a MS Excel® database. A statistical analysis will compare the modification of rSrO2 values between the three groups and will test a potential correlation between the modification of rSrO2 values and the marker of renal damages in case of acute ischemia.

Discussion

Interpretation of the results

Risk factor for surgical complications after paediatric KT

Three risk factors for postoperative complications were analysed in the project. First, the impact of a body weight inferior to 15 kg was investigated. The LW patients were not expose to an overall higher rate of surgical complications and the survival rate was similar to the rest of the population. Nevertheless, 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. Second, the presence of vascular variants that required a complex bench surgery was investigated. The microsurgical procedures consisted in

elongations of the vein, or end-to side anastomosis between anomalous vessels. Even though the warm ischemia time was longer in the group that underwent complex bench surgery, the early outcomes of KT and the rate of complications were similar. Moreover, none of the living-donor allografts affected by vascular anomalies experienced an adverse event. Finally, the project investigated surgeons' fatigue that was represented by operative time during night-time and days-off. Indeed, in our series less than 30% of non-living donor paediatric KTs were performed during the ordinary working hours. No difference in terms of short-term outcomes and overall rate of complications were found in comparison to KTs performed during ordinary working hours. Nevertheless, KTs performed during days-off presented a longer length of surgery and a higher risk of post-operative bleedings.

Clinical application of transcutaneous NIRS monitoring of the allograft perfusion after paediatric KT

Transcutaneous NIRS monitoring was well tolerated by the patients. rSrO2 values were superior to 80% for most of the time. Despite the limited sample size, a correlation was found with the values of serum creatinine. The urinary output and the values of RI assessed by DUS showed a mild correlation with rSrO2 values, even though the statistical analysis showed no significance. A patient from the series experienced a graft venous thrombosis during the transcutaneous NIRS monitoring. rSrO2 values were inferior to those of the other patients. The time-curve showed a decrease of the rSrO2 values in correspondence of worsening clinical conditions.

Design of an experimental animal model of renal ischemia for the application of transcutaneous NIRS monitoring

The rat model of renal ischemia should test the modification of rSrO2 values provided by transcutaneous NIRS monitoring and to investigate the correlation with the specific marker of renal damage, such as NGAL and Kim-1, to prove the efficacy of NIRS monitoring in the prediction of vascular complications. Careful attention will be placed on the temporal relationship between the different exposure of arterial or venous ischemia and the modifications o rSrO2 values. Indeed, the aim of transcutaneous NIRS monitoring consists in the prompt recognition of vascular complications that may affect KTs.

Clinical implications

Patients' body weight is one of the main aspects to consider during the planification of paediatric KT. A body weight inferior to 15 kg, because of the age or the growth retardation due to the underlying disease [31], could make a matching organ hard to retrieve. 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 [32]. The BWR was chosen to assess the disparity between donor and recipient and this parameter resulted sensibly higher in LW patients [33]. Moreover, even though the extraperitoneal approach is the first choice for pediatric KTs, due to the preservation of the peritoneal cavity, this approach may generate 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 [34]. A comparison between the

intraperitoneal and extraperitoneal techniques reported similar long-term outcomes in graft function and survival [35]. Furthermore, it is relevant to highlight that the extraperitoneal approach may avoid episodes of intestinal obstruction or twisting of the allograft on its vascular pedicle [36,37]. Finally, 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 [38]. For these reasons, the extraperitoneal approach should be recommended for LW patients. Another aspect to consider is the presence of vascular anomalies of the donor kidney. These anomalies are present in more than 30% of organ, but no difference in terms of outcomes was found between the grafts with standard anatomy and those with vascular arterial or venous variants and did not represent a contraindication for transplantation [5]. However, vascular anomalies might the anastomosis during KT more difficult, especially in case of multiple vessels. For this reason, a microsurgical reconstruction during bench surgery should be indicated in order to improve the outcome of the KT. To achieve this goal, of this work aimed to standardize the most common procedures performed during bench surgery. The early outcomes and the rate of complications was not different after complex microsurgical reconstruction. Furthermore, it is relevant to report that in our series seven living donor allograft underwent complex BS and none of them experienced vascular complications, confirming that vascular anomalies might not be a contraindication even in case of a living donor KT [5]. On the other hand, the donor kidneys with arterial or venous anomalies presented a longer warm ischemia time than those undergone standard bench surgery. The extended warm ischemia time might be due to the caution and precision that were required during vascular anastomoses and particular attention

should be paid in the choice of the correct position of the graft to avoid kinking or other misplacement that might influence the blood flow, reflecting the importance of surgeons' experience in performing meticulous, microsurgical techniques [39]. Finally, the presence of vascular anomalies or complex BS is an indication for the administration of unfractioned heparin in our protocol. In literature its use was questioned, due to the lack of strong evidence and to the increased risk of post-operative bleedings [40,41]. This protocol has not been changed since the positive outcomes of our series.

Surgeons' fatigue could play a role in the risk for adverse events after pediatric KTs. Even though, a systematic review and meta-analysis did not find an increased hazard for the KTs performed after-hours [42] and, more recently, two pilot studies found that night-time KT did not present an increased risk of complications in the adult population [6,7], pediatric KTs might be more challenging for the surgeons, due to the low body weight or the presence of complex somatic and vascular malformations [43]. Our rate of after-hours KTs was considerably high and might be explained by several aspects such as logistic. The KT was frequently delayed after the ongoing elective surgery because no operating rooms were available, and KT was started immediately after the availability of the results for the compatibility to reduce the length of the CIT [8]. For this reason, more than two thirds of the KTs in our series were started between 5.00 and 9.00 PM. In this span of time, the surgeons might have just finished their ordinary activity without resting before entering on-call shift for KTs. This can certainly lead to sleep deprivation and fatigue. Consequently, surgeon's performance might be diminished, raising the risk of complications. It has been proved that sleep deprivation, fatigue, and stress due to the workload increase the risk of human error and prolong the surgical times for procedures that require concentration and caution [44].

Surprisingly, night-time KTs presented a reduced operative time, since the main operator could be more fatigued, willing to end earlier the intervention. On the other hand, KTs performed during days-off presented a longer length of surgery probably due to the fatigue accumulated during the previous ordinary working days. Furthermore, most of the documented complications were concentrated in the span of time between 5.00 and 9.00 PM or at the end of the night-time. Once again, sleep deprivation might have influenced this outcome. The analysis of our data found no difference in terms of overall complications and short-terms outcomes among the groups. These results were consistent with the findings in the adult's population [6,7]. It is relevant to report that the occurrence of post-operative bleedings was higher in KTs performed during days-off. This might be a warning sign of fatigue. For this reason, the promising results of the hypothermic machine perfusion of the allografts in the adult population might help the planification of paediatric KTs and, consequently, to reduce the risk of adverse events due to surgeons' fatigue [45].

Graft venous thrombosis occurs in 0.1- 4.2% of KTs. This is a fearful event because it is one of the major causes for the loss of the allograft, due to vascular complication [46]. Recipients' and donors' age represented the main risk factor for vascular complications [3]. Our study confirmed this trend and graft venous thrombosis occurred more often in LW patients in our series. It is difficult to speculate about the risk factors for graft venous thrombosis in this peculiar population and the event could be generated by a multifactorial predisposition. In our work all of the patients showed several risk factors for thromboembolic events, such as pro-coagulative status or vascular anomalies. None of them presented consistent BWR between donor and recipient and none encountered episodes of abdominal compartment syndrome.

Finally, a prompt treatment of graft venous thrombosis in a LW patient was described for the first time in literature. This treatment consisted in explantation of the allograft, followed by bench irrigation with fibrinolytic agents and successive autotransplantation of the allograft. This treatment combined the benefits of a high dose of fibrinolytic agents, without the risk of bleeding, together with the mechanic washing of the blood clots. Finally, the auto-transplantation was a safe procedure, already performed for renovascular, ureteral and malignant pathologies [47]. This staged procedure allowed a complete thrombectomy and a complete recovery of the allograft.

For these reasons, transcutaneous NIRS monitoring could be extremely helpful for the postoperative follow-up after paediatric KTs. Especially LW patients may benefit from this innovative device because they are more prone to vascular complications in the immediate postoperative. These peculiar patients present a limited width of the abdominal wall that may increase the accuracy for the measurement of rSrO2 provided by transcutaneous NIRS.

In our series transcutaneous NIRS monitoring was used in eight patients. The range of rSrO2 values was similar to those reported in the other two studies in the paediatric population, proving the feasibility of this procedure [16,28]. Our work failed to confirm a strong correlation between clinical, biochemical and ultrasonographic parameters and rSrO2 values, despite the presence of a significant association with the values of serum creatinine and a mild but not significant association with the urinary output and the values of RI. These results might be due to the limited sample size that undermined the statistical power rather than to an issue related to transcutaneous NIRS monitoring. Moreover, these results could not be undermined by the position of the probes as postulated by Skowno et al. [19]. First, the probes were not placed over the surgical wound. Second, the width of the abdominal wall was inferior to 4 cm, except

for a probe of the upper pole in one case. This measure was the limit for the accuracy of the readings reported by the manufacturer of the device.

Another controversial aspect about transcutaneous NIRS monitoring was investigated. Our study design encompassed a perfusion renal scintigraphy at the end of the monitoring. This exam was performed in the seven patients that positively ended the monitoring. The assessment of the time-curves showed good results and, even though the evaluation was only qualitative, a correlation with the rSrO2 values might be postulated. It is relevant to point out this aspect because, even if perfusion renal scintigraphy is a point-evaluation, this is the most objective modality to assess both kidney vascularization and function, also adding information about the early prognosis of the KT [48].

The modification of SrO2 values assessed by transcutaneous NIRS due to a venous allograft thrombosis after pediatric KT belonging to our series was documented for the first time. Larger pediatric series did not encounter any vascular complication [16,28] and, the paper by Shiba et al. reported the use of transcutaneous NIRS monitoring in two children facing with vascular complications after LT. In these cases, the modification of rSrO2 values successfully predicted the occurrence of vascular events [29]. Two series including only adult patients found that a decrease of more than 10% in allograft rSrO2 was associated with vascular complications after solid organ transplantation and suggested this value as a cut-off [17,18].

In this episode, the lower pole showed the lowest rSO2 when compared to those of the upper pole or to the other patients [16,28]. It is relevant to outline that this aspect reflected the findings during the intervention in which the surgeons outlined a congested and darker lower pole.

A further consideration, concerning the trend of rSO2 values, should be taken into account. The arterial blood flow might be preserved after an acute venous occlusion until venous pressure overcame arterial pressure. For this reason, rSO2 values might be maintained in the early phases after graft venous thrombosis, as occurred in this episode [49].

Since the rSrO2 values provided by transcutaneous NIRS devices during vascular complications might be hard to collect due to the epidemiology and ethical concerns, the lack of evidence should be filled by experimental studies on animal models that might help the interpretation of NIRS readings and improve the detection of the early onset of vascular adverse events. For this reason, a part of this project dealt with the design of animal model of renal ischemia due to arterial or venous occlusion that could be feasible and reliable to test the NIRS monitoring. The current literature yielded a rat model that provided an optimal compromise between the complexity of the experiment and its feasibility.

Future perspectives

The preliminary results of this project showed the potential benefits of NIRS. However, a wide use of transcutaneous NIRS device after paediatric KT might produce further evidence about its advantages in the postoperative management and which patients might benefit more from the monitoring. Our series showed that paediatric KT in LW patients and the presence of vascular anomalies presented a challenge not only during surgery but also during the early postoperative follow-up. Therefore, these categories might take a huge advantage from a real-time continuous monitoring of the allograft perfusion. Another concern about NIRS is the duration of the monitoring. Most of the severe complications occurred within the first 72 hours, as reported in our series. For this reason, the monitoring could be reduced to three days in order to reduce the

burden for the patients [16]. Since NIRS devices are not wireless, the mobilization might be compromised by the monitoring and the mobilization could negatively impact on NIRS readings. A larger series could clarify this relevant aspect.

Finally, the execution of the animal experimental study could clarify the temporal relationship between the outbreak of renal ischemia and the modifications of rSrO2 values. This aspect is crucial to highlight the effectiveness of the innovative technique and to confirm its advantages.

Limitations

The main limitations of the clinical works included in the project reside in the retrospective design that could lead to the lack of standardization in reporting the outcomes of paediatric KT [50]. The limited sample size, especially for the series about transcutaneous NIRS monitoring, has influenced the statistical significance and the generalization of the results. Moreover, it is relevant to outline that the complexity of postoperative management after KT could have influenced the collection and report of clinical parameters. Finally, the results of an experimental model involving larger animals could be easier to translate to a clinical context, but the feasibility was not cost-effective, and a rat model was judged sufficiently reliable.

Conclusions

A standardized protocol is crucial for the management of paediatric KTs especially when dealing with peculiar risk factors such as a low body weight and vascular anomalies. Since it could be difficult to reduce the outbreak of vascular complications, an early diagnosis is crucial for the preservation of the allograft. A transcutaneous NIRS device, that is continuous, real-time, and non-invasive, could fulfil this role. The preliminary results of this series showed a good correlation of NIRS readings with the

other clinical, biochemical, and instrumental parameters that are currently used for the postoperative follow-up. However, these results should be implemented with a larger sample size. The rat model should clarify the modifications of NIRS readings in case of vascular complications.

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Appendix

Appendix A. List of demographics, clinical, biochemical, and surgical details collected in the main database.

- Gender
- Age at KT
- Leading cause to ESKD
- Need for kidney replacement therapy before KT
- Recipient's and donor's body weight
- Body weight ratio
- Living-donor allograft
- Vascular anomalies and bench reconstruction of the allograft
- Cold and warm ischemia time
- Length of surgery
- Number and site of vascular anastomosis
- Administration of heparin, inotropes, or other drugs
- Length of hospital stay after KT
- eGFR at discharge
- post-operative complications

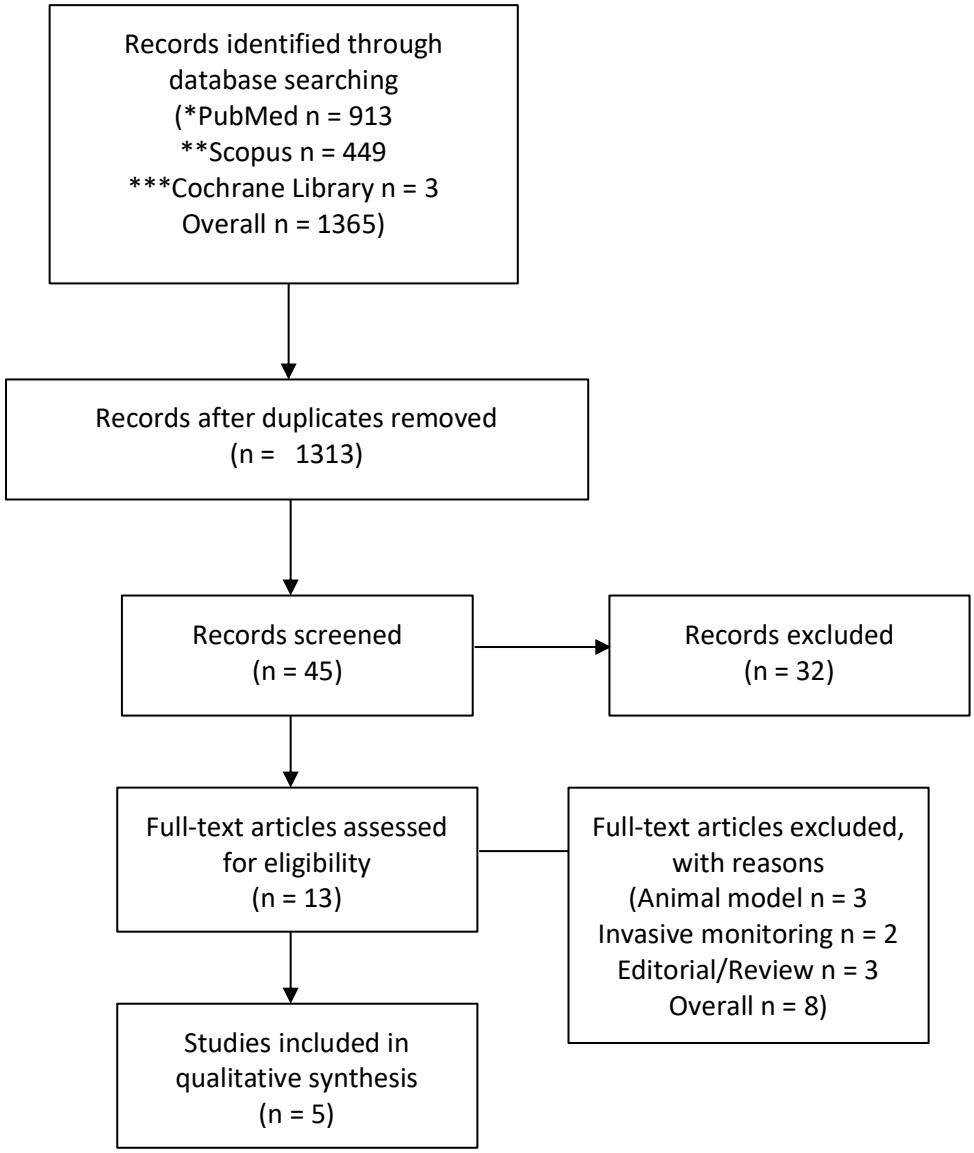
Appendix B. Systematic Review according to the PRISMA guidelines.

Search queries, limited to articles published from January1991 to November 2020

*Pubmed: *(((near-infrared AND spectroscopy) OR NIRS OR oxymetry) AND (transplant OR transplantation OR graft)) NOT (cerebral OR brain)*

**Scopus: *ALL ("NIRS" AND transplantation) AND (LIMIT-TO (SUBJAREA , "MEDI"))*

***The Cochrane Library: *NIRS AND transplantation*



Appendix C. Quality assessment of the selected articles according to Newcastle-Ottawa Scale.

Study	Study design	Selection				Comparability		Exposure/Outcome		
		1	2	3	4	a	b	1	2	3
Vidal *et al*., 2014 [16]	Cohort			*	*	*		*		*
Malakasioti *et al*., 2018 [28]	Cohort			*	*	*		*		*
Shiba *et al*., 2018 [29]	Cohort			*		*		*	*	*
Pérez Civantos *et al*., 2019 [17]	Cohort			*	*	*	*	*		*
Pérez Civantos *et al*., 2019 [18]	Cohort			*	*	*	*	*		*