SOPHOCLES

StOPping Hypertension and imprOving Children's Lives after KidnEy TranSplantation

Studie zur Verbesserung der Herz-Kreislauf-Gesundheit nach Nierentransplantation im Kindesalter durch intensivierte Therapie des Blutdrucks

Study Protocol

Version 1.1, 5.6.2024

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1. Summary

Cardiovascular (CV) disease is a major morbidity in children after kidney transplantation (KTx), limiting life expectancy and impairing graft function. Arterial hypertension (AH) is the dominant CV risk factor, and highly abundant in this patient group. AH can cause left ventricular hypertrophy (LVH), which is predictive of CV death. LVH can be non-invasively assessed by measuring left ventricular mass index (LVMI). Analyses of observational data showed that blood pressure (BP) levels <75th percentile (pct) were associated with a significant reduction of LVMI. Guidelines give BP goals for children with chronic kidney disease (CKD). No guidelines, however, exist on the treatment of AH in pediatric KTx patients. In the proposed multicenter, randomized, parallel group trial with blinded endpoint evaluation we aim to assess n=500 pediatric patients >12 months after KTx at several KTx centers. Patients will be randomly assigned 1:1 to an intensified BP management group (BP target ≤60th pct) and a standard BP management group (BP target <90th pct). The primary endpoint is LVMI after 24 months. Secondary endpoints are estimated glomerular filtration rate (eGFR), pulse wave velocity (PWV) and intima media thickness (IMT) after 24 months. BP control will be guaranteed for both groups through BP telemonitoring, which will be transmitted in real time to the treating physician and the trial's centralized BP office. By defining the adequate BP goal, the results of the proposed study will have direct implications for the care of children after KTx. The results will define an important element of post-KTx care and help to lower CV morbidity and subsequently CV mortality of pediatric KTx patients.

2. Responsibilities

Principal investigator: Anette Melk, MD PhD
Dept. of Pediatric Kidney, Liver, and Metabolic Diseases
Hannover Medical School (MHH)
Carl-Neuberg-Str. 1, 30625 Hannover
Email: melk.anette@mh-hannover.de

Statistician: Anika Großhennig, PhD
Inst. of Biostatistics, MHH
Carl-Neuberg-Str. 1, 30625 Hannover

Central laboratory: Synlab Heidelberg
Wasserturmstraße 71, 69214 Eppelheim

Biobanking: Hannover Unified Biobank (HUB)
Feodor-Lynen-Str.15, 30625 Hannover

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3. Scientific Background

Life expectancy in children with chronic kidney disease (CKD) after KTx has improved steadily[1]. Therefore, factors affecting long-term health, especially cardiovascular (CV) disease, carry increased relevance. CV morbidity is caused by a variety of risk factors. Of those, arterial hypertension (AH) is of particular importance, as it is strongly correlated with CV organ damage[2, 3]. CV events are among the most common causes of death in pediatric KTx

recipients[4-6]. Registry studies demonstrated a high prevalence of AH in pediatric KTx recipients[7, 8] with more than 70% requiring antihypertensive medication[9] and more than one third showing actual BP levels higher than the 95th percentile (pct)[8]. Our longitudinal analysis from the Cooperative European Pediatric Renal Transplant Initiative (CERTAIN) Registry of 336 pediatric KTx recipients showed a prevalence of AH of 84% at discharge after KTx that declined only slightly to 77% after three years of follow-up[8]. We extended this finding, demonstrating sharply increased odds for AH for patients after pediatric KTx when compared to matched healthy children in a case-control study[10]. Long-term data report AH in more than 60% of pediatric KTx recipients after 10 years[11]. Several studies demonstrated the negative impact of AH on CV health and transplant survival in children after KTx[12, 13].

Left ventricular hypertrophy (LVH) is the most prominent manifestation of CV organ damage as a result of AH[14]. LVH is associated with death due to major CV events[15]. It affects about 40% of children one year after KTx[16, 17]. We have previously shown that the left-ventricular mass index (LVMI), an echocardiographic parameter to quantify LVH, increases in the early course after pre-emptive KTx and is mainly determined by BP and not by the mode of kidney replacement therapy[18]. LVH is a strong predictor of CV morbidity and mortality[19-22], therefore measurement of left ventricular mass is a cornerstone of CV risk assessment[14, 23].

In children with CKD not yet requiring kidney replacement therapy, we have shown in the randomized controlled ESCAPE trial that intensified BP control using angiotensin-converting-enzyme (ACE) inhibition reduces CKD progression[24]. This pivotal study has led to the implementation of BP goals for patients prior to kidney replacement therapy. However, currently **no specific recommendations or guidelines exist on BP management in pediatric patients after KTx** that are based on prospective data. To discern the effect of different BP levels and thereby the importance of BP control on LVH in KTx patients we analyzed long-term follow up data from the 4C-T study[25], a sub-study of the 4C study (www.4c-study.eu)[26], which prospectively assessed CV morbidity in children prior to and after KTx. We analyzed LVMI depending on blood pressure (BP) control in 94 pediatric KTx recipients. The cumulative systolic/diastolic BP exposure was calculated as time-averaged area under the curve and categorized according to pct ranges (≤50th, >50th-≤75th, >75th-≤90th, >90th pct). We show that a cumulative exposure to systolic BP values ≤75th pct is associated with lower LVMI: Compared to patients with a cumulative systolic BP exposure >90th pct, a significant LVMI reduction of -5.24g/m$^{2.16}$ is seen in patients exposed to systolic BP between 50th to ≤75th pct (p=0.007). A similar tendency is observed in those exposed to ≤50th pct (ß=-3.70 g/m$^{2.16}$; p=0.067), while no LVMI reduction is found in patients with cumulative systolic BP exposure between 75th to ≤90th pct. The cumulative exposure to lower diastolic BP levels was also associated with lower LVMI with no large differences in the magnitude of the effect on LVMI across the three strata. Patients exposed to systolic BP ≤50th or between 50th to ≤75th pct had a significant risk reduction of 79% or 83% lower odds for the development of LVH, respectively. A similar tendency was seen for diastolic BP with patients exposed ≤50th pct showing 82% lower odds for LVH[25].

These findings substantiate that continuous exposure to high BP levels leads to LVH progression in pediatric KTx recipients, but also clearly point out that the conservative BP goal <90th pct is most likely insufficient to lower LVMI, especially for systolic BP. This further supports the hypothesis that a lower BP goal will be beneficial with regard to LVH, leading to

a reduction in CV disease and improved long-term patient survival. However, as these **observations are inferred from observational data only** a randomized controlled trial (RCT) prospectively evaluating the effect of different BP goals on LVMI (primary endpoint) is warranted. In fact, the KDIGO (Kidney Disease: Improving Global Outcomes) guideline on BP in patients with CKD recommends performing an RCT on the adequate BP goal in children, ideally using home- or office-based BP measurements[27].

Measurements for subclinical vascular damage as well as for early kidney injury will comprise our secondary endpoints. The surrogate markers for vascular damage will be aortic pulse-wave velocity (PWV) and carotid intima-media thickness. Both have been shown to be predictive of CV mortality in adults[28, 29] and are recommended parameters to be used as CV endpoints in studies by the American Heart organization [30]. We have demonstrated in a variety of both single-center and multi-center observational studies that both PWV and IMT can be non-invasively and reprodicubly assessed in a variety of pediatric cohorts [17, 31-34]. We will assess albuminuria and the estimated glomerular filtration rate (eGFR) as measures of kidney injury, as, again both can be easily measured.

4. Aim of the study

The proposed trial will confirmatory test whether intensified treatment of AH (i.e BP control ≤60th pct, compared to standard BP control <90th pct) will lead to a lower LVMI in KTx recipients. Reducing LVMI will reduce long-term morbidity, improving quality of life and life expectancy in this population particularly vulnerable to CV disease. SOPHOCLES targets a central clinical problem that is highly relevant for the studied patient population, and addresses a major gap of knowledge. Its results have great potential to change future clinical practice.

5. Outcome parameter

Primary outcome parameter

SOPHOCLES' primary endpoint is **LVMI at 24 months** after randomization.

In children and young adults after KTx, the incidence of CV events (e.g. myocardial infarction or stroke), despite being 100-fold higher compared to the normal population, is still too low to provide adequate power for an RCT with reasonable duration. LVH has not only been shown to predict the incidence of CV events and CV mortality [19], but more importantly, a decrease in left ventricular mass is associated with a decrease of CV risk [35-38]. This makes LVMI a unique endpoint that ideally reflects CV risk [14]. Furthermore, LVH assessment is also recommended in the European guidelines for pediatric hypertension [23]. Therefore, LVMI is the most suitable endpoint for our trial.

Secondary outcome parameter

Secondary endpoints will be **eGFR** as a measure of kidney function, **IMT** as measure of atherosclerosis and **PWV** as measure of arteriosclerosis. eGFR will be calculated using the Schwartz bedside formula [39]. IMT will be assessed from the recordings of the common carotid arteries, following the Mannheim consensus [40]. PWV will be provided from Vicorder readings following established standardized operating procedures [3].

6. Design of the Trial

The trial is a multicenter, randomized, controlled, parallel group trial with blinded endpoint evaluation comparing the effect of intensified vs. standard BP control on left ventricular mass. The flow chart (**Fig. 1**) illustrates the trial design and depicts the patient numbers from enrolment to analysis.

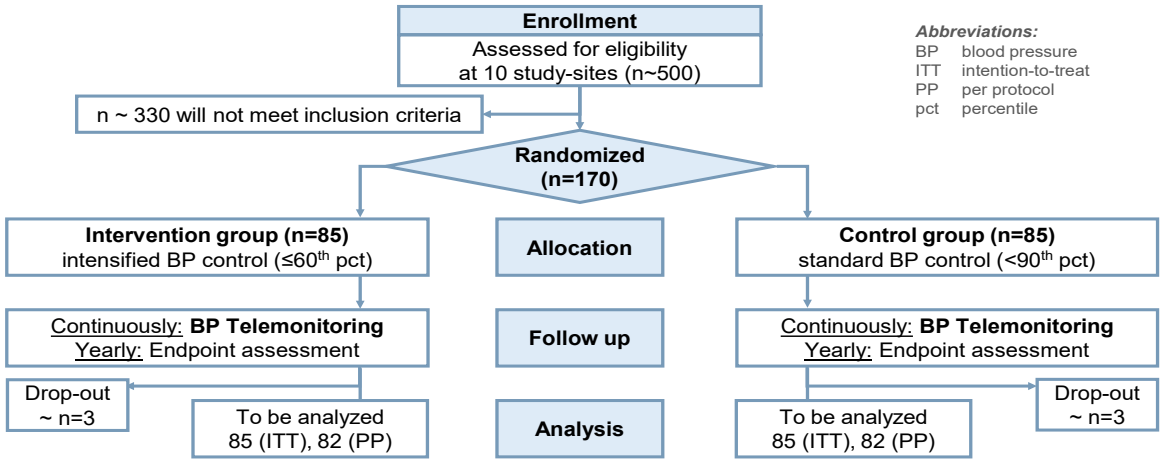

Fig. 1 Patient flow chart

7. Study Population

7.1. Inclusion and exclusion criteria

Inclusion criteria:

Age between 6 and 18 years

Kidney transplantation >12 months ago

AH, i.e. systolic and/or diastolic BP >95th percentile (pct) or on antihypertensive treatment

Exclusion criteria:

Cardiac malformation

Treatment for a rejection episode within three months prior to inclusion

7.2. Patient numbers

The planned patient numbers are depicted in **Figure 1**.

7.3. Patient recruitment

The participating centers provide care to a large number of pediatric KTx recipients allowing to screen more than 500 patients for eligibility. As highlighted in the trial's flow chart (**Fig. 1**), we expect patients to be excluded because of age or not suffering from AH. Past experience taught us that pediatric KTx patients have a close relationship to their treating physicians, and show a high willingness to participate in clinical studies. We expect to screen no more than 250 patients assuring the recruitment of the necessary number of patients within 24 months. Less than 20% of patients will come from study sites outside of Germany. No additional measures are planned to recruit patients.

8. Study Procedures

8.1. Informed consent

Each family will receive detailed information on the purpose and procedures of the trial. In addition to thorough verbal information, a patient information form (provided in German, English, Turkish, Kurdish, Farsi, Ukrainian, and Russian) adapted to the respective age of the child will be handed out (see appendix), and any questions arising will be answered. Signature will be given by the legal guardians ("Sorgeberechtigte") and if possible by the child itself. In the case a teenager reaches the age of 18 during the course of the study, he or she will give informed consent at the following study visit, again. No study specific tasks will be performed before reaching informed consent. The signed informed consent forms will be filed by the local investigators.

8.2. Study procedures

This trial will compare two different BP treatment goals. Pediatric KTx recipients will be randomly assigned to the **intensified BP group** or the **standard BP group**. Treatment goal in the intensified group will be lowering BP ≤60th pct. Data justifying this treatment goal are discussed in the introduction. The treatment goal for the standard group is to achieve BP levels <90th pct in accordance with pediatric guidelines for AH[23, 41]. The chosen antihypertensive drug regimen is in the discretion of the treating physician.

All patients will measure BP at home using a telemonitoring device (Tel-O-Graph, IEM GmbH, Aachen, Germany) providing current BP data and allowing for the patients' accustomed mode of documentation. The device enables telemonitoring through real-time transmission of BP data, which will be accessible to the MHH trial physician at the trial's centralized BP office and to the treating physician on-site allowing for timely interventions and as an important safety feature.

Physicians caring for the patients will have no restrictions in treating medical conditions (e.g. intercurrent infections or rejections) or changing any medication as part of the regular clinical care. Changes in medication and any adverse events (AE) or serious adverse events (SAE) will be recorded in the electronic case report form (eCRF).

8.3. Investigational plan, assessment of endpoints

The primary endpoint is **LVMI at 24 months after randomization**.

Secondary endpoints will be eGFR as a measure of kidney function, IMT as measure of atherosclerosis and PWV as measure of arteriosclerosis. **Table 1** provides the timepoints at which the endpoints will be assessed.

Table 1. Frequency and scope of patients' trial visits

	Screening	Inclusion	Month							
			1	3	6	9	12	15	18	24
Informed consent	X									
Inclusion/exclusion criteria	X	X								
Past medical history	X									
Anthropometrics*	X	X	X	X	X	X	X	X	X	X
Documentation of medication	X	X	X	X	X	X	X	X	X	X
Local routine lab documentation	X	X	X	X	X	X	X	X	X	X
Office BP measurement	X	X	X	X	X	X	X	X	X	X
AE assessment			X	X	X	X	X	X	X	X
Echocardiography		X					X			X

PWV		X					X			X
IMT		X					X			X
ABPM		X					X			X
Centralized lab**		X					X			X
Biosampling (blood, urine)		X					X			X
*height, weight; **creatinine, cystatin C, urea, electrolytes, blood count, lipids, bone metabolism (PTH, AP, 25-OH-D3), urine analysis (protein/creatinine ratio). Abbreviations: ABPM, ambulatory blood pressure monitoring; AE, adverse event; BP, blood pressure; IMT, intima media thickness; PWV, pulse wave velocity										

Echocardiography will follow a standardized operating procedure established during the 4C study in accordance with the American Society of Echocardiography guidelines[42]. LVMI will be calculated by dividing left-ventricular mass in grams (according to Devereux[43]) by height in meters to the 2.16th with a correction factor of 0.09[44]. LVH is defined as an LVMI ≥45 g/m$^{2.16}$ independent of sex or age. eGFR will be calculated using the Schwartz bedside formula[39]. IMT will be assessed using ultrasound with a high-resolution linear probe, by averaging five measurements from the far wall of the common carotid artery 1-2 cm proximal of the carotid bulb, following the Mannheim consensus[40]. PWV will be provided from oscillometric measurements using the Vicorder device (Skidmore Medical Ltd, Bristol, United Kingdom) following established standardized operating procedures[3]. Appropriate normative data allow for PWV and IMT to be normalized by age and sex and to be expressed as z-scores. Blood and urine samples will be analyzed. eGFR will be calculated using the modified pediatric Schwartz formula Schwartz 2012], albuminuria will be measured in spot urine and given a albumin-to-creatinine ratio.

8.4. Study timeline, study duration

Trial duration will be 24 months for each patient (**Fig. 2**). This time span is sufficient to reach the BP goal and to induce changes in LVMI. The stringent BP monitoring allows for a fast adaption of antihypertensive treatment with the aim to reach the target within three months. A shorter trial duration is not advisable as LVMI changes need time[45]. We expect the first visible changes to occur no sooner than after 6 months in the intensified BP group. As the magnitude of these changes increases over time, a longer trial duration allows for the recruitment of a smaller number of patients. Our analysis of 4C-T data[25] demonstrated a robust difference in LVMI dependent of BP control after a median follow-up of 26 months. Hence, we based the trial duration on these data.

Pediatric patients >12 month after KTx are routinely seen every 4-6 weeks in the outpatient clinic. The proposed quarterly trial visits (**Tab. 1**) can therefore be easily implemented without additional strain on patients. The annual evaluation will take approximately 45 minutes per patient. The non-invasive measurements include evaluation of LVMI by echocardiography, ambulatory blood pressure monitoring (ABPM) and assessment of secondary endpoints that will be executed by the same investigator team. Moreover, collected biomaterial (plasma, serum, urine) will be analyzed centrally to guarantee standardization of laboratory values.

The duration of the whole study will be five years.

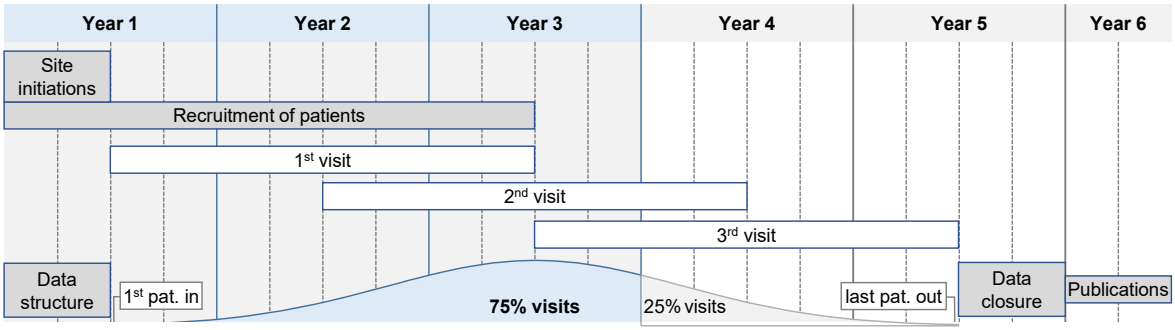

Fig. 2 Study timeline

8.5. Adherence / Rate of Loss to Follow-Up

From our experience from previous studies including RCTs, we expect an excellent follow-up adherence resulting in a low number of drop-outs[24, 46]. This is facilitated as all pediatric KTx recipients are under close, continuous surveillance at the participating centers; generally seen every 4-6 weeks. Thereby, the additional burden through the study is very low. The number of visits to the outpatient clinic essentially remains unchanged; only during the annual visits patients and their families will have to take extra time for the CV examinations. Echocardiography and the other CV examinations are non-invasive and known to be well-tolerated. Blood samples will be collected in context of a routine blood draw. The trial's design using telemonitoring approach will also facilitate superior protocol adherence.

9. Methods

9.1. Measurement of Cardiovascular Endpoints

Echocardiography will follow a standardized operating procedure established during the 4C study in accordance with the American Society of Echocardiography guidelines[42]. LVMI will be calculated by dividing left-ventricular mass in grams (according to Devereux[43]) by height in meters to the 2.16th with a correction factor of 0.09[44]. LVH is defined as an LVMI ≥45 g/m$^{2.16}$ independent of sex or age. In a sub-cohort perfusion of the kidney transplant will be assessed by abdominal doppler ultrasound. IMT will be assessed using ultrasound with a high-resolution linear probe, by averaging five measurements from the far wall of the common carotid artery 1-2 cm proximal of the carotid bulb, following the Mannheim consensus[40]. PWV will be provided from oscillometric measurements using the Vicorder device (Skidmore Medical Ltd, Bristol, United Kingdom) following established standardized operating procedures[3]. Appropriate normative data allow for PWV and IMT to be normalized by age and sex and to be expressed as z-scores. Blood and urine samples will be analyzed. eGFR will be calculated using the Schwartz bedside formula[39], albuminuria will be measured in spot urine and given a albumin-to-creatinine ratio.

9.2. Blood samples

The amount of blood needed at the annual visits is 10-15 ml, the amount of urine will be 10-30 ml. DNA analysis will require only 5 ml at one timepoint. The analyses of biochemical parameters will be performed in a centralized laboratory in Germany to guarantee comparability. Blood and urine samples will be stored in the Hannover Unified Biobank for future analysis with novel analytical methods (e.g. new biomarkers, proteomics).

10. Statistical analysis

10.1. Sample size calculation

Our data from the 4C-T study[25] is the best fundament for sample size calculation. To simulate SOPHOCLES, we performed an additional analysis on a sub-group of 37 patients very closely resembling the inclusion and exclusion criteria for our planned trial. At baseline, these patients were 6-16 years old, were transplanted ≥9 months ago and presented with AH. We observed the lowest LVMI corrected mean value in patients who were exposed to cumulative systolic BP of ≤75th pct (47.4±12.0 g/m$^{2.16}$) when compared to patients exposed to values >90th pct (52.3±11.5 g/m$^{2.16}$) or patients between >75th and ≤90th pct (52.7±11.4 g/m$^{2.16}$). We consider the observed difference of 5.3 g/m$^{2.16}$ in LVMI achieved between the ≤75th pct and the >75th-≤90th pct as clinically relevant. A closer look at patients below ≤75th pct showed a skewed distribution with a median systolic BP exposure of 57.5th pct (IQR: 51st-66th pct, maximum: 71st pct) supporting our proposed BP target of ≤60th pct.

A sample size calculation based on the corrected means of the sub-group analysis (47.4 vs. 52.7 g/m$^{2.16}$), a common standard deviation of 12 g/m$^{2.16}$, an unpaired t-test with a two-sided type-one error of 5% and a power of 80% resulted in a sample size of at least 82 patients for each group (nQuery® 9.2.1.0.). We expect only few drop-outs and therefore only added 3 patients per group to account for withdrawal of consent. This results in an overall sample size of 170 patients. Importantly, it is assumed that the planned mixed model approach in the primary analysis will increase power compared to the t-test used for sample size calculation because of stratification, highlighting our conservative approach in setting up this trial.

10.2. Primary analysis

According to the intention-to-treat (ITT) principle, analysis of the primary endpoint will include all randomized patients. A linear mixed model for repeated measures (MMRM) will be used to analyze the contrast in LVMI (least squares means at month 24 after randomization) between groups. Treatment group, LVMI at baseline, visit, the stratification factors (center, sex, age group) and treatment-by-visit interaction will be included as fixed effects. Patient will be included as random effect, an unstructured covariance pattern is assumed to model the within-patient errors. Restricted maximum likelihood (REML) in combination with the Newton-Raphson algorithm will be used to obtain parameter estimates. The Kenward-Rogers method will be used for estimation of the denominator degrees of freedom. Superiority of intensified treatment will be concluded, if the upper boundary of the 95% confidence interval for the estimated treatment effect (contrast of intensified minus control group at month 24) is below 0. In the unlikely situation that the model does not converge, a simplified model (i.e. simplified covariance pattern, such as compound symmetry, or a fixed effects model) will be used for the primary analysis. Missing values will be implicitly imputed by the MMRM.

To assess the robustness of the model, various sensitivity analyses will be performed. Specifically, the impact of missing values (e.g. replacement by last-observation-carried-forward in an ANCOVA model) will be examined. Nature and extent of missing values will be compared between treatment groups and a per-protocol analysis, which consists of all patients who complete the study in accordance to the study protocol, will be conducted. Furthermore, subgroup analyses will be performed for age group, sex and center.

10.3. Secondary and exploratory analysis

Analysis of the three secondary endpoints (eGFR, IMT, PWV at month 24 after randomization) will be in line with the primary analysis.

AEs and SAEs will be documented and recorded. We will calculate relative and absolute frequencies of AEs per intervention arm at the event level and at the patient level. A two-sided chi-squared test will be used to compare the intervention and control group. Expected AE related to the study intervention can occur due to lowering of BP per se (dizziness, nausea, weakness, but also acute worsening of kidney function) and due to side effects of the antihypertensive medications (e.g. edema from calcium antagonists, cough from ACE inhibitors, electrolyte disorders from diuretics).

10.4. Measures against Bias

Permuted block randomization with variable block length will be used to allocate patients 1:1 to both groups. The following strata will be used: study-center, sex (male or female), age group (<11 or ≥11 years) and left ventricular hypertrophy (<45 $g/m^{2.16}$ vs ≥45 $g/m^{2.16}$). The randomization list is generated centrally by the Institute of Biostatistics, allocation is performed via the eCRF system. During the trial only the statistician and the unblinded data manager will have access to the randomization list.

All echocardiographic and secondary endpoint readings will be performed by the same investigators who will be uniformly trained according to the standards established as part of the 4C study[26]. The analyses of these readings will be performed centrally by investigators blinded with regard to the patient's intervention group and time point of the measurement.

As a result of an increased awareness for BP control by treating physicians on site, we expect an improvement in BP levels also in the standard group during the trial. The telemonitoring approach, however, will assure clear differentiation between both groups. The transmission of BP data and the close contact between MHH trial physician and treating physicians will assure strict target control and immediate corrections, if targets are not met. For generalizability, we aim to recruit a representative patient population. In consequence, the only exclusion criterion are cardiac abnormalities that very rarely occur, but would impede measurement of the primary endpoint.

11. Stopping Rules

On the **individual level**, the patients receive no specific treatment but are assigned to different treatment goals. In this setting, we believe that any stopping rules carry the risk of inducing bias into our study. Patients' safety is guaranteed by telemonitoring of blood pressure values allowing for timely reaction if blood pressure levels are too low. This situation then will prompt adaption of treatment but not exclusion from the study. The only reason for early stopping of the study is withdrawal of patients' consent, unforeseeable new ethical or medical aspects. A **single center** might wish to stop the study due to logistic reasons.

In context of the **whole study** the primary endpoint is left ventricular mass measured after two years. The recruitment period also spans two years (see Fig. 2 Study timeline). Hence a meaningful preliminary endpoint assessment of e.g. half of the patients would only be possible very late in the trial. Therefore, stopping the trial at this time point e.g. due to an overwhelming positive result would not be reasonable.

12. Risk-benefit assessment

The **principle study aim is ethically justified because of the scientific uncertainty** which of the two interventions (standard vs. intensified BP management) will result in a more favorable risk-benefit profile. While observational data suggest that stricter BP control will translate into a reduction of left ventricular mass and better patient survival, the intensified protocol might have disadvantages as well. These may be due to worsening of overall treatment adherence because of an increased drug burden, but also due to potential side effects of lowering BP (such as dizziness or even acute worsening of kidney function), or side effects coming from antihypertensive medication itself (e.g. edema from calcium antagonists, cough from ACE inhibitors, electrolyte disorders from diuretics). In conclusion, we believe that **clinical equipoise is assured**.

Both groups will benefit from improved monitoring of BP between outpatient visits based on telemonitoring and timely response to BP levels outside the target range. Thereby, the standard treatment group will profit as well due to greater awareness of their AH, as in every day practice AH is not treated sufficiently, with one third of KTx patients displaying BP values >95th pct[8, 17].

Before and during the clinical trial, regular risk management will be performed according to ICH-GCP. Every subject participating in the trial will be insured against any trial-related illness/injuries. An independent data safety monitoring board (DSMB) will be established, consisting of three independent experts, that oversees patient safety and assure the risk/benefit assessment while the trial is ongoing. Throughout the trial, the DSMB will monitor safety data according to a pre-specified plan (DSMB charter). DSMB members are independent from the PIs, the trial's investigators and the medical institutions involved. Together, the DSMB members form an independent multidisciplinary group of clinicians and biostatisticians that, collectively, have experience in the management of pediatric KTx recipients and the conduct, monitoring and statistical analysis of RCTs. The DSMB will give advice to PIs and the trial steering committee (TSC) whether to continue, modify, or stop the trial. Importantly, this will include the assessment whether the separation of BP values between the intensified and the standard group is sufficient. If not, the DSMB will discuss the issue with the trial steering committee. This will allow to take additional measures to reinforce BP goals early enough during the course of the trial. Detailed rules on the flow of data and communication between the parties involved in the trial and the DSMB will be set forth in a separate DSMB charter. The TSC also involves independent external experts that will review the trial's recruitment and progress

13. Data Security

An eCRF will be used by employing an electronic data capture system. The system has been fully validated according to the applicable criteria and regulations (e.g. GCP). Personal data of all participating patients will be handled in accordance with the General Data Protection Regulation (GDPR) of the European Union, all respective patient rights will be granted (Art. 12-23- DSGVO). Pseudonymized data will be entered only by authorized and trained staff. A clinical trial data base allows data entries remotely via the eCRF. Data quality will be verified by centralized and on-site monitoring as well as via range, validity and consistency checks programmed in the system. All changes of data entered in the eCRF will be documented by an

audit trail. A quality control will be performed and documented before the database is closed. The complete data set will be archived in the central electronic MHH archive in compliance with the corresponding regulations. After data transfer, data analyses will be performed by the responsible biostatistician.

Responsible for data management will be the principal investigator, Prof. Melk. The data safety manager at MHH is Joachim Barke.

14. Biobanking

Collected biomaterials will be stored at Hannover Unified Biobank (HUB), which follows highest quality standards and is certified according to DIN EN ISO 9001:2015.

Samples from this study in this pediatric cohort with long-term follow-up are unique as they will never be retrieved again in a similar set-up. Because these samples are extremely precious, the patients and parents will be asked to allow us to store them without time restriction.

15. Insurance

The subjects participating in the trial will be insured against any trial-related illness/injuries (“Jahresvertrag zur Probanden- und Wege-Unfall-Versicherung” Nr. 79 153194 03016/03192, Insurance company: HDI Global SE, Customer number: 2330 99 0302 / 1502, application number 03022024301).

16. Publication rules

We intend to publish the results of the trial. The publication rights are with the principal investigator. The principal investigator will assure that contributions of participating individuals and centers are acknowledged.

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