Study protocol

“HEALED”

Evaluation of acute and c**h**ronic n**e**phrotoxicity in **a**cute lymphatic **l**eukemia pati**e**nts using Ultrasoun**d** Localization Microscopy

Trial Short Title	HEALED
Trial Full Title	Evaluation of acute and c**h**ronic n**e**phrotoxicity in **a**cute lymphatic **l**eukemia pati**e**nts using Ultrasoun**d** Localization Microscopy
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STUDY TITLE, VERSION NUMBER, VERSION DATE

Study title

Evaluation of acute and chronic nephrotoxicity in acute lymphatic leukemia patients using Ultrasound Localization Microscopy (HEALED)

Version

1.3

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Overview

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23.04.2025	1.0	Draft	
23.05.2025	1.1	Draft	Inclusion of funding source
02.06.2025	1.2	Draft	Adaption of number of participants
06.07.2025	1.3	Draft	Addition of references to scientific background
02.09.2025	1.4	Draft	Addition of recommendations from the Ethics Committee

ZUSAMMENFASSUNG/PROJECT SUMMARY

With increasing survival rates in pediatric oncology, reports of long-term side effects persisting decades after treatment are also rising. Clinically evident nephropathies occur in about 5.5% of survivors more than five years after therapy. Chemotherapeutic agents such as ifosfamide, cisplatin, and carboplatin, as well as kidney-directed treatments like radiation, surgery, or stem cell transplantation, increase this risk. Acute kidney injury has also been described in association with cyclophosphamide and high-dose methotrexate, which are used in the treatment of acute lymphoblastic leukemia (ALL). Studies show a high prevalence of albuminuria (around 14.5% of childhood cancer survivors), an early marker of kidney damage, while standard parameters like creatinine often become abnormal only at later stages.
Leukemia survivors suffer from vascular late effects caused by persistent endothelial damage triggered by cancer therapies such as anthracyclines, cyclophosphamide, and asparaginase, which increase inflammation and thrombosis risk. These vascular changes may also contribute to kidney injury.
ULM is a high-resolution ultrasound technique that uses microbubbles to visualize the microvasculature and resolve dynamic blood flow changes with a resolution beyond the diffraction limit. ULM is independent of kidney or liver function, has been applied in various organs, and was recently used for the first time to visualize glomeruli—the smallest functional units of the kidney—in humans. This method enables early detection of glomerular injury as a consequence of vascular damage, even before albuminuria appears, potentially allowing earlier adaptation of follow-up and initiation of treatment.

This pilot project focuses on survivors of ALL, as they are the largest and best studied pediatric cancer patient group also regarding late effects and, therefore, a sufficient number of individuals can be expected for his monocentric approach. Vascular functional impairment of the kidney could be detected at an early stage and the follow-up structures and measures such as the early use of nephroprotective drugs could be adapted.

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Internal funds of the Children's Hospital Erlangen until application for external funds

SCIENTIFIC BACKGROUND

In parallel to increasing survival rates in pediatric oncology, a growing number of studies describe an increase in long-term side effects even decades after cancer treatment in childhood and adolescence [1].

Long-Term Nephrotoxicity in Childhood and Adolescent Cancer Survivors

Nephropathies occur in 5.5% of survivors more than 5 years after the end of therapy [2]. Chemotherapeutic agents with well documented early nephrotoxicity are ifosfamide, cisplatin, and carboplatin. A limited number of studies only evaluated long-term risk and risk factors for renal toxicity, and these studies were performed in selected groups of survivors with a maximum of 10 years' follow-up [3]. Other risk factors for nephrotoxicity include kidney radiotherapy (including whole body radiotherapy in the setting of stem cell transplantation), kidney surgery, or stem cell transplantation [4]. Acute nephrotoxicity has also been described with cyclophosphamide and high-dose methotrexate [3, 5, 6], both drugs used in the treatment of acute lymphoblastic leukaemia (ALL). There is a general lack of data or studies in other groups of cancer survivors.

Few studies describe a high prevalence and risk of reduced kidney function in the form of low GFR, albuminuria, or increased blood pressure in long-term survivors of childhood and adolescent cancer. For example, Knijnenburg et al. found albuminuria in 14.5% of 1442 childhood and adolescent cancer survivors analysed (0.7% in the normal population comparison group) [3]. Albuminuria is generally considered to be an early and sensitive marker in the presence of an often normal GFR [7]. Blood markers, such as creatinine or eGFR, are less sensitive because they are generally only detectable at late stages of kidney damage. However, given the lack of more sensitive alternatives as early indicators of chronic renal toxicity, markers such as creatinine or GFR are often used to monitor kidney function in regular (long-term) follow-up after childhood and adolescent cancer. Against this background, the establishment of sensitive methods facilitating and early detection of kidney damage appears to be of great value.

Endothelial Injury and Long-Term Vascular Toxicity in Leukemia Survivors

Cancer therapies can lead to a broad spectrum of cardiovascular complications. Among these, cardiotoxicities remain of prime concern, but vascular toxicities have emerged as the second most common group [8]. Studies have shown that particularly leukemia survivors suffer from vascular late effects [9]. Growing evidence suggests that chemotherapeutics affect endothelial function, reduce nitric oxide (NO) bioavailability, and lead to endothelial cell activation.

Resulting endothelial dysfuntion is associated with tissue ischemia, hampered vascular barrier function, atherosclerosis, and altered hemodynamics [10]. Cancer therapies can induce and exacerbate endothelial injury through direct cytotoxic and cytostatic effects on endothelial cells, while also impairing repair mechanisms by reducing endothelial cell proliferation and the number of endothelial progenitor cells. Notably, endothelial dysfunction may persist long after treatment, as studies in long-term cancer survivors have shown signs of ongoing endothelial activation and elevated levels of circulating endothelial cells even decades after therapy.[8] Endothelial injury disrupts vascular homeostasis, leading to inflammation, impaired repair mechanisms, and a prothrombotic state, thereby contributing directly to the development of vascular damage.

Different drugs are used in the therapy of ALL that cause vascular damage. Anthracyclines cause endothelial damage [11], Cyclophosphamide metabolites are believed to cause oxidative stress and direct endothelial capillary damage [12], Asparaginase is is an essential drug for the treatment of ALL, may increase the risk of thrombosis or bleeding, the incidence of symptomatic asparaginase-associated thrombosis ranges from 2–7% in clinical trials [13].

As vascular late effects such as coronary artery disease caused by endothelial impairment are common late effects in childhood cancer survivors, it is reasonable that nephropathy in childhood cancer survivors may at least in part also be caused by vascular or endothelial effects in addition to a tubular damage [9, 14].

Visualizing Glomerular Injury: A New Frontier with Ultrasound Localization Microscopy

Ultrasound Localization Microscopy (ULM) is an **advanced and highly sensitive ultrasound imaging technique** that allows visualisation of the microvasculature and quantification of dynamic blood flow changes within the vascular architecture with unprecedented resolution. Tiny air bubbles (microbubbles) are administered intravenously as contrast agents, which can then be localised and tracked within each image of the ultrasound acquisition [15]. Accumulation of all these tracks on a 2D grid produces a microvascular map of the organ with a resolution beyond the diffraction limit of ultrasound waves. Unlike other types of contrast agents, the microbubbles are subsequently metabolised independently of kidney and liver function and will simply be washed out by respiration [16]. ULM has been used in several human organs [17-19]. Two studies have already been performed at the Department of Pediatrics and Adolescent Medicine (University Hospital Erlangen) using ULM to visualise the microvascular architecture in the neonatal brain and to quantify dynamic perfusion changes [18, 20]. Moreover, a study with ULM in kidney transplants has been successfully conducted, proving the feasibility of the method in patients with renal impairment. More recently, the glomerulus, the smallest functional unit of the kidney, has been visualised in the human body

with high resolution [21]. Glomerular injury is closely linked to vascular damage in the kidneys, as endothelial dysfunction and microvascular injury impair glomerular filtration and contribute to progressive renal dysfunction.
The aim of this study is to facilitate the early visualization of glomerular damage representing a vascular component of chemotherapy induced nephrotoxicity by ULM. This approach could contribute to the timely detection of functional kidney impairment - even earlier than potential albuminuria - and thus help to adapt follow-up structures and measures or even revise treatment protocols. In the frame of this pilot study, we focus on individuals with a history of ALL, as they represent by far the largest and most extensively studied subgroup of patients in pediatric oncology. Therefore, we expect a sufficient number of patients in this monocentric approach.

OBJECTIVES

Hypotheses

- ULM can be used to visualise and quantify the microvascular perfusion dynamics in the kidney and differs depending on the organ function
- Treatment of acute lymphoblastic leukaemia leads to (often subclinical) acute and chronic impairment of renal function also through vascular injury which can be visualized by ULM.

Primary objectives

- ULM: Visualisation of microvascular architecture and Quantification of microvascular perfusion dynamics in the kidney before, during, and after treatment of acute lymphoblastic leukaemia

Secondary objectives

- ULM: Correlation of the vascular architecture of the kidney **visualised** by ULM before, during and after treatment of acute lymphoblastic leukaemia (e.g. number of segmented glomeruli) with laboratory chemical parameters (including renal function parameters, inflammation parameters, immunological parameters)
- ULM: Correlation of vascular architecture of the kidney **visualised** using ULM before, during and after treatment of acute lymphoblastic leukaemia (e.g. number of segmented glomeruli) with sonographic parameters (e.g. resistance index (RI), flow velocity)
- ULM: Correlation of parameters of **quantified** microvascular perfusion dynamics of the kidney before, during and after treatment of acute lymphoblastic leukaemia with

laboratory chemical parameters (e.g. renal function parameters, inflammation parameters)

- ULM: Correlation of parameters of the **quantified** microvascular perfusion dynamics of the kidney before, during and after treatment of acute lymphoblastic leukaemia with sonographic parameters (including resistance index (RI), flow velocity)

Study type

Prospective, monocentric, diagnostic study

OUTCOME MEASURES

All ULM measurements will be performed on the kidney, which is better visualized in sonography.

Primary outcome measures collected by ULM

ULM: Visualisation of the microvascular architecture of the kidney

- ULM: Quantification of perfusion dynamics in one kidney (including measurement of peak enhancement (PE), wash-in area under the curve (WIAUC), wash-in perfusion index (WiPI), wash-in rate (WIR), wash-out area under the curve (WoAUC), wash-in and wash-out area under the curve (WiWoAUC), wash-out rate (WoR), time to peak (TTP), rise time (RT), mean transit time (local) (mTTI) and fall time (FT)).

Secondary targets determined by laboratory chemistry

- Laboratory chemistry parameters (including BB, Diff, Glucose, Na, K, Cl, Ca, LDH, Crea, Cystatin C, Uric Acid, CRP, Albumin, Ferritin, Total Protein)
- Urinalysis: including urinary status, proteinuria/haematuria

Secondary outcome measures to be determined sonographically

- Sonographic parameters (including resistance index (RI), Doppler signal, flow velocity, and others)

Routine clinical practice measures (not related to the study)

- Collection of clinical data from medical records (these outcome measures are available from medical records or will also be collected at initial presentation)

STUDY DESIGN

Monocentric / multicentric

This is a monocentric study

Study arms: interventional/control

Patients fulfilling the inclusion criteria will receive ULM of one kidney. Patients with recently diagnosed ALL will receive ULM at two timepoints whereas participants in the oncological follow up after treatment for ALL will be examined at a single visit.

Randomization

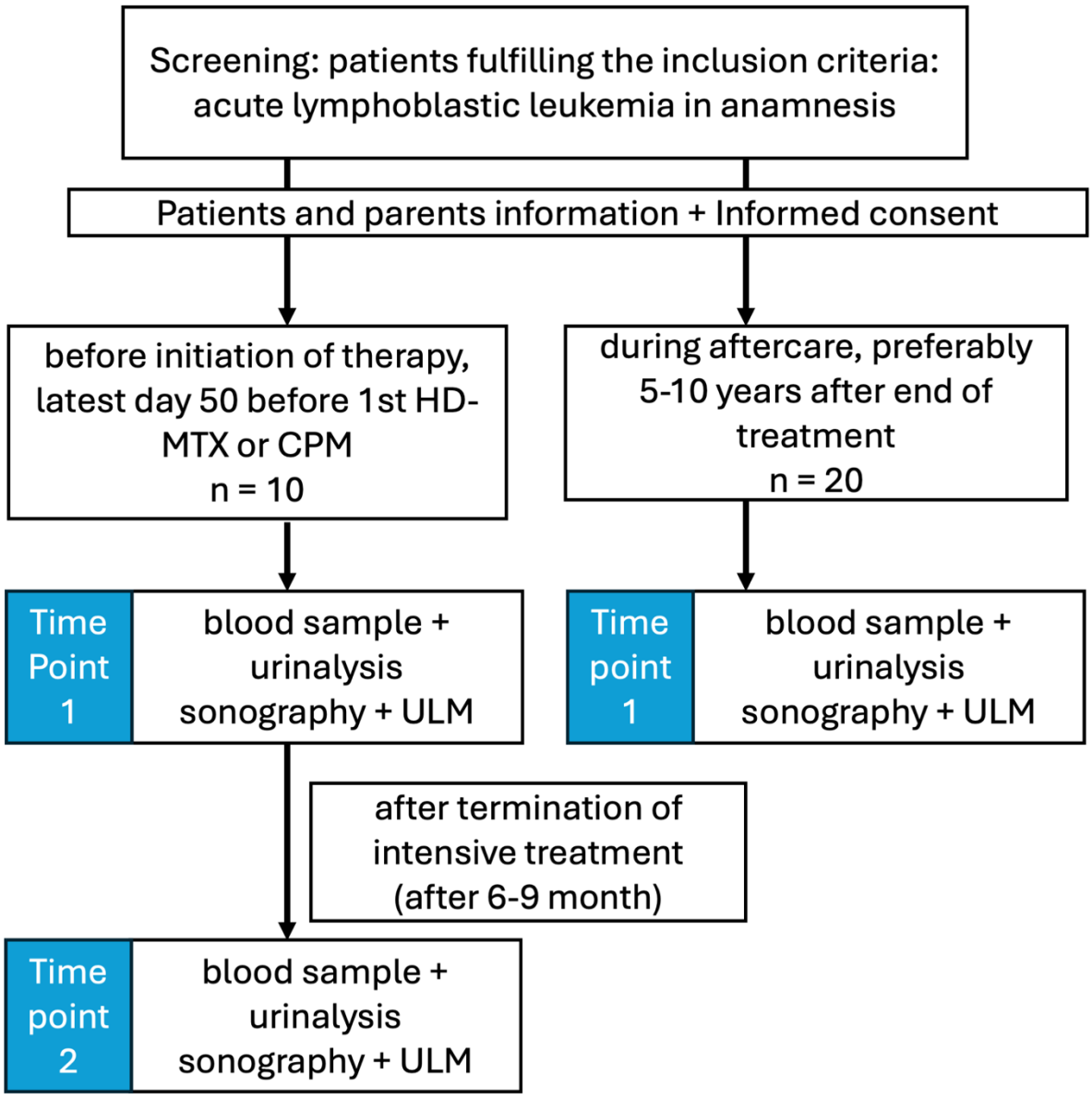
Randomization is not planned

Blinding

Blinding to the study is not possible. Blinding of patients/subjects is not necessary

Graphical design of study flow

Figure 1: Course of Study



STUDY POPULATION / IN- AND EXCLUSION CRITERIA

Early therapeutic effects	Late therapeutic effects
Planned number of patients	
N = 10 ALL (recently diagnosed ALL)	N = 20 ALL (completed oncological treatment)
Planned number of examinations for each participant	
2 (before first CPM according to therapy protocol and after completion of intensive therapy)	1
Inclusion criteria	
- Diagnosed acute lymphatic leukemia - Treatment day < 50 according to therapy protocol / no administration of CPM before first examination - From 3 years to < 18 years	- Diagnosed acute lymphatic leukemia - Completed oncological treatment - From 3 years to < 18 years
Exclusion criteria	
- Known allergic disposition to SonoVue / other contrast agents - Tattoo in the area of the examination field - Pregnancy - Breastfeeding mothers - Contraindication for the use of Sonovue - Critical condition	- Known clinically evident renal impairment - Known allergic disposition to SonoVue / other contrast agents - Tattoo in the area of the examination field - Pregnancy - Breastfeeding mothers - Contraindication for the use of Sonovue - Critical condition

Number of patients

As this is a pilot study, it is not possible to calculate the exact number of patients. It is planned to include 30 patients (in total 40 examinations). The planned sample size of 30 participants (10 during therapy and 20 in follow-up) was determined based on the annual incidence of ALL at our center, which is approximately 15 cases per year. Recruitment within the intended timeframe of 12–18 months is therefore considered feasible. In addition, the study has a pilot character, aiming to provide the first data set for estimating effect sizes and to serve as a foundation for the design of larger subsequent studies. 10 patients who will undergo an ULM examination of the kidney before the start of chemotherapy, latest before therapy day 50 before the first application of high-dose methotrexate or cyclophosphamide (Timepoint 1). After completion of intensive therapy (after administration of cyclophosphamide and high-dose methotrexate), they will receive a follow-up ULM

examination (Timepoint 2) approx. 6-9 months after the first ULM examination. In addition, 20 different patients who have completed treatment for ALL and are seen during aftercare will be included in the study.

Recruitment

Patients (and parents) will be informed about the possibility to participate in the study when visiting the pediatric clinic for hematology and oncology (including its outpatient clinics) or attending the follow-up consultation (Department for internal Medicine / Med 5 University Hospital Erlangen). If patients and their parents are interested to participate, they will be fully informed about the aims and methods (especially about the scientific/explorative nature of the study), the benefits and risks, and the revocability of participation in the study before giving their consent prior to study initiation. Patients in childhood and adolescence are additionally informed and educated about the study and its procedure in an age-appropriate manner.

STUDY PROCEDURES

Procedure for informing and obtaining consent

Patients or subjects can only be enrolled in the study after written informed consent has been obtained. The written informed consent requires an oral and written explanation to the patients/subjects, as well as their parents or legal guardians, about the aims and methods (incl. scientific-explorative character of the study), benefits and risks as well as the revocability of the study participation. Children and adolescents are informed by means of age-appropriate, comprehensible patient information sheets. By giving written informed consent, the patients/participants as well as their parents/guardians declare that they agree with the collection and storage of study-relevant data and their review by monitoring or authorities. It must be clearly conveyed to the study participant that withdrawal of consent is possible at any time and without any disadvantage. Furthermore, all study participants/subjects and parents/guardians are informed that this study is a purely scientific study without any current diagnostic or therapeutic benefit. In case of incidental findings, the study participants/test persons and parents/guardians will be informed and further clarification will be initiated if indicated.

The original consent form will be kept in the study folder at the study site. The patient/proband and parent/guardian will be given a copy of the patient information and consent form. The patient information and informed consent form can be found in the appendix of this study protocol.

Measures and recording the target values

Together with the patient and the patient's family, a date for the study is set. After the patient has been informed about the study by phone or in person at least one day in advance, a routine blood sample is taken through a venous access during the chemotherapy or follow-up visit. Patients currently undergoing treatment will already have a venous access used for chemotherapy (CVC or Hickman catheter or port catheter). This can then be used for the study-related contrast agent, so no additional intervention is required. The region to be examined in all patients is one kidney and its immediate surroundings. Patients can lie relaxed during the scan.

Timing and duration of the study

Patients will be examined using ULM during an outpatient or inpatient stay for chemotherapy or aftercare. A total of 30 patients will be included in the study.

The expected duration of the study for individual patients is 30 minutes to 1 hour.

Total duration of the study

The expected total duration of the study is 18 months.

RISK-BENEFIT-ANALSYSIS

Study-related risks with ULM

Insertion of a peripheral venous catheter (pVK)

For the examination using ULM in the 20 individuals who have completed treatment for ALL and are seen during aftercare it is necessary to insert a peripheral venous catheter (pVK) (e.g. in the hand, crook of the elbow). The insertion of a peripheral venous catheter is generally feasible without limitations even in children after completion of chemotherapy. For this study, catheter placement will be combined with routine blood sampling to avoid additional burden. As with any blood sampling, this can result in injury to the skin at the entry point. There is also a risk of infection at the entry site, but this is very low given the short time of insertion of approx. 2 hours.

In the 10 individuals who will be assessed before therapy day 50 (Visit 1) and after completion of intensive chemotherapy (Visit 2), the routinely implanted central venous catheter will be used for the administration of the contrast agent. In these cases, no additional pVK is required. Laboratory chemistry parameters (including BB, Diff, Glucose, Na, K, Cl, Ca, LDH, Crea, Cystatin C, Uric Acid, CRP, Albumin, Ferritin, Total Protein) and urinalysis including urinary status, proteinuria/haematuria as well as conventional ultrasound assessment are routinely performed during therapy and aftercare. So, in addition to ULM, no extra procedure will be undertaken in the frame of this study.

Contrast agent application

A contrast medium (SonoVue®) is administered venously, a potential intolerance to the contrast medium (allergic reaction) is a possible however very low risk of this study. It is an already established contrast agent the tolerability of which has been investigated in several studies: In a large study , 23,188 patients examined showed a rate of 0.0086% ‘adverse events’ (18). In a separate investigation, 30,222 patients were examined, with a rate of 0.02% (19). The adverse events included self-limiting symptoms such as redness, epistaxis, vomiting, nausea, back pain, numbness and symptoms of incipient anaphylaxis (18, 19).

In our working group, children have already been examined several times using ULM and SonoVue contrast enhancement.

The information for healthcare professionals on SonoVue reports very rare cases of reactions with fatal outcome that were temporally related to the use of SonoVue®. Notably, the patients concerned were already at high risk of cardiac complications.

Repeated applications during an examination are permitted, overdoses have not been described. SonoVue® has no drug approval for this examination or intravenous use in children and will be used off-label. SonoVue® is not nephrotoxic, remains intravascular, and is

eliminated via the lungs and phospholipid metabolism. It can be safely administered even in patients with impaired renal function.
The ULM measurements are performed using a CE certified clinical ultrasound system.

Study related benefits

The data obtained in this study may provide important insights into the acute and chronic nephrotoxicity of ALL therapy. The potentially quantifiable differences in ULM could be used in the future as markers to predict nephropathy. Follow-up methods and measures, schedules and treatment plans could be adapted.

Withdrawal criteria

Withdrawal criteria are an allergic reaction to the ultrasound contrast agent and reddening or warming of the skin during optoacoustic imaging.
The entire study will be stopped if a reassessment of the expected and unexpected side effects of the drugs or devices used indicates an increased risk to the subjects.

Statement on medical justifiability

Based on experience to date, the risk of adverse events is considered to be extremely low.
With ULM, the rate of side effects is very low and if they do occur, they are rapidly reversible and easily treatable.
Given the current lack of diagnostic tools and data on post-chemotherapy nephrotoxicity, we assume this method will provide new diagnostic tools that will significantly improve long-term follow up care in the future.

Calculation of case numbers

As this is a pilot study and no information is yet available on the expected differences between the different groups, no calculation of the number of cases has been made. The number of cases given is an estimate.

Statistics

The data is given as a mean value with standard deviation. Correlations are indicated with the non-parametric Spearman correlation coefficient (Rs). Differences in the mean values are analysed statically using a non-parametric T-test (Mann-Whitney test) or ANOVA. In all analyses, an error level of <0.05 is considered statically significant.

DATA MANAGEMENT AND DATA PROTECTION

Data collection and data storage

The participation of the individual subjects in the study is documented and the principal investigator keeps a separate list to identify the participating subjects. This list contains the name and date of birth as well as the study date and pseudonymisation code of the subjects. The principal investigator is responsible for the quality of data collection and data storage. The data is stored exclusively on computers or specially designated network drives at the University Hospital Erlangen.

Pseudonymisation

Prior to a scientific analysis of the data from this study, all information is pseudonymised in accordance with the guidelines of the Federal Data Protection Act.

Revocation, data deletion

If consent is withdrawn, data collected up to this point may be considered and retained. However, test persons have the right to demand that the data be destroyed, provided that there are no legal provisions to the contrary.

Participant insurance

All participants in the study are insured via the CCS Erlangen group contract. This will be pointed out separately in the patient information.

STATEMENT ON THE INCLUSION OF MINORS IN ACCORDANCE WITH ARTICLE 20 OF THE DECLARATION OF HELSINKI (2024)

The inclusion of children and adolescents (aged 3–<18 years) is indispensable, as the research question specifically and exclusively pertains to this patient population. Data derived from adults cannot be extrapolated, given the substantial differences in disease characteristics, treatment protocols, and late effects.

Scientific Rationale

Ultrasound Localization Microscopy (ULM) allows for the early and non-invasive detection of glomerular damage. This approach provides novel opportunities for preventive measures and for the adaptation of long-term follow-up care in survivors of acute lymphoblastic leukemia (ALL).

Risk–Benefit Assessment

ULM is a non-invasive procedure and will be performed via pre-existing central venous access or, in the follow-up setting, in combination with routine blood sampling where applicable.
The contrast agent (SonoVue®) has an excellent safety profile and is associated with only a very low risk of adverse reactions.
The examination itself is of short duration (approximately 30–60 minutes).
Accordingly, the associated risks are minimal and clearly outweighed by the anticipated scientific and clinical benefit.

Information and Consent

Parents or legal guardians will receive comprehensive information and provide written informed consent prior to participation.
Children and adolescents will be informed in an age-appropriate manner and their assent will be sought.
Withdrawal from participation will be possible at any time without any disadvantages.

Conclusion

The inclusion of minors is scientifically necessary and ethically justifiable. It will be conducted with the utmost consideration for their specific need for protection and in strict adherence to ethical and regulatory standards.

SIGNATURES

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Dr. med. A. Dierl

PD Dr. med. Axel Karow

Dr. med. H. Mandelbaum 

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