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Implementation and evaluation of new training concepts for clinical teams to facilitate quality of sampling for sensitive pharmacokinetic and pharmacodynamic substances, applied within a multicentre paediatric study project

INAUGURAL-DISSERTATION

Zur Erlangung des Doktorgrades

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I. Erklärung zur Dissertation

Hiermit versichere ich an Eides statt, dass die vorgelegte Dissertation mit dem Titel:

> Implementation and evaluation of new training concepts for clinical teams to facilitate sampling of sensitive pharmacokinetic and pharmacodynamic substances applied within a multicentre paediatric study

von mir selbstständig und ohne unzulässige fremde Hilfe unter Beachtung der Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf erstellt worden ist. Die Dissertation wurde in der vorgelegten oder in ähnlicher Form noch bei keiner anderen Institution eingereicht. Ich habe bisher keinen erfolglosen Promotionsversuch unternommen.

Düsseldorf, den Agnes Maria Ciplea

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III. Zusammenfassung

Unzureichende Patientenrekrutierung und Schwierigkeiten bei der Studiendurchführung gehören zu den am häufigsten genannten Ursachen für den frühzeitigen Abbruch von pädiatrischen klinischen Studien. Um diese Risiken angemessen zu begegnen hat das EU-finanzierte LENA-Projekt (Labeling Enalapril from Neonates up to Adolescents), ein neuartiges, modulares Trainingskonzept entwickelt. Ziel des Projektes ist die Erforschung von Pharmakokinetik (PK) und Pharmakodynamik (PD) des ACE-Inhibitors Enalapril, der in den pädiatrischen Studien in Form von orodispersiblen Minitabletten für die Behandlung von herzinsuffizienten Kindern eingesetzt wird. Die erfolgreiche Bestimmung, der untersuchten, sensitiven Substanzen ist von anspruchsvollen prä-analytischen Abläufen abhängig, die über die klinische Routine hinausgehen.

Die vorliegende Arbeit beschreibt die Entwicklung, Implementierung und Evaluation zweier Module des individuell angepassten Trainingskonzeptes für beteiligte klinische Teams: Zum Ersten den auf die PK/PD-Probenentnahmen fokussierten Abschnitt des LENA-Simulationstrainings zur Probenentnahme für PK/PD-Untersuchungen und zur Kommunikation sowie dessen umfragebasierte Evaluation; zum Zweiten eine Machbarkeitsstudie, angelehnt an einer typischen LENA-Studienvisite, durchgeführt an den klinischen Zentren mit gesunden erwachsenen Freiwilligen. Durch diese wurden zeitgleich die Fähigkeiten der klinischen Teams, die erforderlichen Prozesse durchzuführen, sowie der Transport und die Bioanalyse der Proben bewertet. Weiter wurde eine Interimsanalyse der elektr. Patientenakten der pädiatrischen Patienten durchgeführt, um zu bestimmen, wie erfolgreich die Vorgaben zur

Probenentnahme und Probenaufarbeitung während der Studie umgesetzt wurden.

Dreiundzwanzig Teilnehmer aus fünf europäischen Ländern haben an dem Simulationstraining teilgenommen. Ihre Fähigkeit, die Probenabnahme und Probenaufarbeitungsprozesse für PK/PD-Untersuchungen durchzuführen, hat sich signifikant verbessert. Der Trainingseffekt wurde als langanhaltend und substanziell für die eigene Leistung während der Studie beitragend eingestuft. Die Machbarkeitsstudie wurde an fünf klinischen Zentren durchgeführt. Sie hat die Notwendigkeit für Nachschulungen aufgezeigt und zur Prozessoptimierung beigetragen, ohne pädiatrische Patienten zu gefährden. Die Interimsanalyse zur Studienprobenqualität basierend auf den elektr. Patientenakten hat gezeigt, dass die klinischen Teams die Vorgaben zur Probenentnahme und Probenaufarbeitung, welche die Aussagekraft der bioanalytischen Ergebnisse sicherstellen, erfolgreich umgesetzt haben.

Das individuell zugeschnittene Trainingskonzept hat es ermöglicht, die Lernkurve bezüglich studienspezifischer Abläufe erfolgreich vor den Einschluss des ersten Patienten zu verlagern und dadurch einen effizienten und qualitativ hochwertigen Studienablauf zu ermöglichen.

Diese Forschung wurde von der EU im Rahmen des 7. Forschungsrahmenprogramms (FP7/2007-2013) unter der Zuschussvereinbarungsnummer 602295 (LENA) finanziert.

IV. Summary

Insufficient recruitment and problems with trial conduct are among the most common reasons for early discontinuation of paediatric trials. To adequately address these identified challenges, a novel, modular training concept was developed for the EU-funded LENA project (Labeling Enalapril from Neonates up to Adolescents), which aims to investigate the pharmacokinetic (PK) and pharmacodynamic (PD) properties of the ACE inhibitor enalapril, administered as orodispersible minitablets to children with heart failure. Successful sampling for the sensitive substances investigated requires sophisticated pre-analytical procedures, exceeding clinical routine.

The present work describes the development, implementation, and evaluation of two modules of the tailored training concept for clinical teams. First, the PK/PD-focused section of the LENA simulation training on PK/PD sampling and communication, along with a survey-based evaluation. Second, an on-site feasibility study with healthy adult volunteers, mimicking the PK/PD sampling procedures of a regular LENA study visit. It simultaneously assessed the clinical teams' ability to adequately perform required procedures as well as transport and bioanalysis of the PK/PD samples. An interim analysis of the paediatric studies' eCRF records was performed to determine how successful clinical teams adhered to instructions for sampling and sample preparation during the trial.

Twenty-three participants from five European countries participated in the simulation-based training. The ability to successfully perform PK/PD sampling improved significantly. The training's impact was perceived as long lasting and substantially contributing to performance during the trials. The feasibility study

was conducted at five clinical sites. It revealed the need for retraining and helped optimise processes without endangering the vulnerable paediatric patients. The interim analysis of study sample quality, based on the study's eCRF records, showed that the clinical teams adhered very successfully to the challenging sampling and sample processing requirements, ensuring bioanalytical evaluability of study samples.

The tailored training concept enabled shifting the clinical teams' learning curves for critical study-specific procedures ahead of the start of recruitment and enabled efficient and high-quality conduct of the paediatric studies.

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n°602295 (LENA).

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VI. Abbreviations

ACE *angiotensin-converting enzyme*
ANG I *angiotensin I*

CI *Confidence interval*
CRF *case report form*

EMA *European Medicines Agency*

FDA *U.S. Food and Drug Administration*

GCP *Good Clinical Practice*

LENA *Labeling of Enalapril from Neonates up to Adolescents*

PD *pharmacodynamic(s)*
PK *pharmacokinetic(s)*
PRA *plasma renin activity*

RAA system *renin-angiotensin-aldosterone system*
RCT *randomised controlled trial*
REN *renin*

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1. Introduction

1.1. Current challenges for successful conduct of clinical trials

The complexity of clinical trial protocols has consistently increased across all phases of clinical research. The number and frequency of performed procedures—and in consequence the workload for clinical sites have considerably changed across all phases of clinical research (Getz, Campo, and Kaitin 2011). Driven by increasing regulatory demands regarding patient safety and efficacy, more and more procedures are included in study protocols (Getz 2014). The number of total study procedures performed during a typical phase II trial increased by 33.5% from 2000-2003 and 2004-2007 alone. For typical phase III trials, the increase was reported to be as high as 57.6% within the same period. This generated an increase in site work burden of up to 59.7% (Getz, Campo, and Kaitin 2011). These developments have several implications for trial conduct.

More narrowly defined eligibility criteria consequently limit the available patient pool for a study and therefore demand the most efficient patient recruitment and study conduct to achieve the required number of evaluable patients. However, it has been found that health professionals are less likely to refer, and patients less likely to participate, in more complex clinical studies (Ross et al. 1999). This finding is supported by the investigations of Madsen et al. (Madsen, Holm, and Riis 2001), who report that patients are significantly less willing to participate in studies with a demanding trial protocol. A 25% discontinuation rate for adult clinical trials has been reported, most frequently caused by insufficient patient recruitment (Kasenda et al. 2014; Carlisle et al. 2015). Rethinking current recruitment practices seems necessary to be able to improve recruitment success.

With rising trial complexity, there is an expected rise in deviations from trial protocol (Ghooi et al. 2016). Patients are not only more difficult to recruit into complex clinical trials, but ensuring their protocol conform participation required for collection of high-quality data requires increased effort. This suggests a need for new concepts to ensure familiarity of clinical trial staff with a trial's procedures ahead of the start of recruitment.

1.2. Additional challenges for paediatric trials

Clinical trials with children face additional hurdles compared to those performed with adults. The population is considerably smaller sized and more heterogeneous and is characterized by rapid individual (patho)physiological changes. Another factor is the limited availability of age-appropriate drug formulations, which can additionally impede research, especially for very young children. Furthermore, the recruitment process is more challenging compared to adults (Caldwell et al. 2004). Parents have been shown to be more reluctant to consent to their children's participating in a trial than if they themselves had been asked to participate (Caldwell, Butow, and Craig 2003). These factors are of special relevance as the validity of study results depends on the successful inclusion of the required number of suitable patients.

Furthermore, the extent to which invasive procedures common in adult trials can be incorporated into paediatric investigations is restricted due to ethical concerns (He 2008). Research is often limited to minimal risk procedures with a probability of harm or discomfort no greater than that ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests (He 2008). In line with these principles, trial-related blood withdrawal is strictly limited (though thresholds are not evidence-based). The amount of blood drawn should not exceed 3% of the total blood volume during a period of four weeks or 1% at any single time. (He 2008). This poses a challenge to analytical laboratories due to a lack of comprehensive availability of off-the-shelf low-volume analytical methods. A consequence may be a narrower scope of bioanalytical investigations in paediatric clinical trials. It has been found that barely 5% of interventional trials

performed in children below 12 years seek to collect PK and PD data simultaneously (Viergever, Rademaker, and Ghersi 2011).

All these factors play a role in the current underrepresentation of children in clinical trials and the high rate of early discontinuations of paediatric studies. Between 2005 and 2010, tenfold more non-paediatric trials than those involving children were listed on ClinicalTrials.gov (Pasquali et al. 2012), although about 26% of the world's population is below 14 years of age (The World Bank 2016). A substantial share of these clinical trials are discontinued prematurely. This is a reason of ethical concern and poses a waste of limited resources in clinical research. For randomised controlled trials (RCTs) performed in the paediatric population, a discontinuation rate of up to 40% has been reported (Pica and Bourgeois 2016). Insufficient recruitment of patients has been shown to be the main reason for discontinuation in 37% of cases, followed by conduct problems, including technical and logistical issues, which account for 12.5% of early terminations (Pica and Bourgeois 2016). It has to be noted, though, that these are not issues related to safety or efficacy, which would be ethical and absolutely acceptable reasons for an early termination.

1.3. The impact of variability in pre-analytical procedures on results of laboratory blood testing

The evaluation of clinical trial results is based in large part on the interpretation of obtained laboratory data, which are used to verify the assumed beneficial effects and safety of investigated drugs, devices, or medical procedures. The reliability of this decision basis is therefore of significant importance (Lippi et al. 2016; Yin, Lehmann, and Xu 2015).

The quality of pre-analytical procedures (e.g., sample preparation/storage/transport) can considerably affect the results of bioanalytical determinations. This has been reported for electrolytes (Baruah et al. 2014), metabolomics (Yin, Lehmann, and Xu 2015; Teahan et al. 2006; Yin et al. 2013), protein markers (Rai and Vitzthum 2006), and drugs (McGlasson et al. 2005; Chen and Hsieh 2005; Bush et al. 2001). It is an effect that cannot be compensated for by even the most sophisticated or precise method of determination applied later on (Yin, Lehmann, and Xu 2015). The utilization of central laboratories in multicentre trials may allow the decrease of analytical variability by applying consistent methods and instruments for specimen analysis, but the variability of the pre-analytical processes still remains a major source of bias if not standardised. This is of particular importance as the pre-analytical phase is the most error prone in the whole testing process. In routine clinical diagnostics, the pre-analytical phase is believed to account for 60%–80% of laboratory testing errors (Carraro, Zago, and Plebani 2012; Lippi et al. 2006; Szecsi and Ødum 2009).

Degradation processes occurring in a sample before analysis are often a result of chemical and/or enzymatic decomposition (Chen and Hsieh 2005). Depending on the substance of interest, factors such as test-tube type (with or without additives

such as antioxidants and inhibitors), storage temperature, time until separation of metabolically active cells from supernatant through centrifugation, spinning force during centrifugation, and pH value are of interest. Insufficient control of these pre-analytical procedures has been suspected as the cause for the unsuccessful reproduction and validation of promising biomarkers (Teahan et al. 2006) and the up to 95% of studies lost in the translational process from bench to bedside (Lippi et al. 2016). Thus, establishing standardised pre-analytical procedures should be adequately reflected during the trial-planning phase to ensure trial objectives can be met. This applies especially to trials in vulnerable populations so as to prevent unnecessary or repeated interventions.

The pre-analytical requirements of investigated substances are often overlooked in study design and are also underrepresented in existing guidance documents for quality testing, which is mainly focused on routine laboratory testing (Lippi et al. 2016). In order to be able to draw meaningful conclusions from laboratory testing, the preservation of substance concentration in study samples until analysis is paramount. Therefore, the standardisation of sampling and sample-processing procedures is essential. This can be a challenge, especially in multicentre clinical trials, including sites with varying levels of expertise and infrastructure (Dakappagari et al. 2017).

1.4. The LENA project

Background and objectives

The LENA project (Labeling of Enalapril from Neonates up to Adolescents) is a multinational academic research project. It aims to improve pharmaceutical care for children suffering from heart failure by developing a safe, effective, and age-appropriate formulation for the angiotensin-converting enzyme (ACE inhibitors enalapril in the form of an orodispersible minitablet. Comprehensive pharmacokinetic (PK) and pharmacodynamic (PD) investigations are performed to expand the knowledge of the maturating renin-angiotensin-aldosterone system (RAA system and its reaction to ACE-inhibitor exposure.

The RAA system is a hormone and enzyme system that plays an important role in regulating salt and fluid homeostasis, thereby directly affecting blood pressure regulation. Concentrations and ratios of its compounds are altered in the presence of different diseases of the cardiovascular system, such as hypertension or heart failure, which are associated with disease progression. Therefore, the RAA system is targeted by different drug classes used in the treatment of cardiovascular diseases such as heart failure, a disease known in children and adults. However, little is known about the maturation of the RAA system or its disease-related changes in paediatric patients as the etiology of heart failure in children differs significantly from the kind observed in adult patients. In the paediatric population, heart failure is mainly caused by congenital heart disease (characterised by structural abnormalities of the heart or its main blood vessels) or cardiomyopathy, whereas in adults, it is usually a consequence of hypertension, a myocardial infarction, or coronary artery disease.

Despite these differences, the pharmacotherapy for children with heart failure is extrapolated from adults and largely based on clinical experience due to the lack of PK and PD data for many drugs used in the paediatric population. ACE inhibitors are the most common unlicensed drugs prescribed in paediatrics (Langerova, Vrtal, and Urbanek 2014), which raises questions regarding the safety and efficacy of the current standard of pharmaceutical care for children suffering from heart failure.

The paediatric LENA project aims to address this knowledge gap by enabling paediatric investigations in children with heart failure due to either congenital heart disease or dilated cardiomyopathy. The PK properties of the ACE inhibitor enalapril, which has been widely used off-label in paediatrics for decades, are being investigated for the first time in a paediatric population, alongside four central parameters of the RAA system: aldosterone, renin (REN), plasma renin activity (PRA), and angiotensin I (ANG I). This is the first concise investigation of RAA system-related effects of ACE-inhibitor therapy in children below 12 years of age.

The complex sampling and sample preparation procedures are a consequence of the sensitive substances being investigated. For example, angiotensin I, a PD parameter of the RAA system, has an in-vitro half-life in non-anticoagulated whole blood of only 3 minutes (Oparil, Sanders, and Haber 1970), and renin’s precursor, prorenin, is cryoactivated when exposed to temperatures between −5°C and 4°C (Campbell et al. 2009). Therefore, adherence to specific temperature ranges and time limits until specimen storage at ultralow temperatures are a prerequisite for required sample quality.

The circumstances under which study samples are obtained during the paediatric LENA trials are of special interest as this information can impact the interpretation of analytical results. Deviations from procedures described in the *Sample collection manual for LENA paediatric studies* had to be conscientiously documented. This proposition impacted the study documentation practices for the paediatric trials. Forms collecting information on sampling circumstances have been designed and are reflected in the studies' eCRFs. An (electronic) case report form [(e)CRF] is a characteristic document of clinical research, individually designed for each trial, to systematically capture all relevant patient-related data generated during a patient's participation in a trial. It aids the collection of the specific information required to assess safety and answer the research question of the performed investigation.

Challenges and strategies for project success

Following a risk-based approach, challenges to successful trial completion have been ascertained to enable a timely adoption of precautionary measures. During this process, two main challenges were identified. One challenge was the recruitment of the required number of patients. To achieve this, the clinical staff has to be able to adequately meet the concerns of patients and parents regarding general and trial-specific aspects of participation. The second big challenge identified concerns the complex procedures required for scheduled PK/PD investigations. The pre-analytical requirements of the substances of interest surpass clinical routine and have to be performed directly on the ward. Only by assuring a comparable sample quality across all clinical sites can reliable study data be obtained and the objectives of the project met.

With these challenges in mind, a comprehensive training concept for clinical staff has been developed to promote successful study conduct, patient recruitment, and the generation of high-quality data.

1.5. Aim of thesis

The aims of this thesis were to develop and implement tailored training modules as part of the LENA training concept, targeting the projects' complex sampling and the sample preparation process, and to assess the impact of the developed training concept on participants' performance and study conduct.

1.6. Comment on the structure of the thesis

The further work presented within this thesis is divided into three main chapters, each dealing with a distinctive component of the LENA training or the evaluation of conduct quality within the subsequent paediatric LENA trials. Each of these chapters is structured according to the IMRaD format (Introduction, Methods, Results, and Discussion), allowing individual focus on the specifics of each part of the topic. This structure is complemented by a conclusive discussion.

2. The LENA simulation training

2.1. Introduction

The EU Directive on Good Clinical Practice (GCP) states that “each individual involved in conducting a trial shall be qualified by education, training, and experience to perform his tasks.” However, it does not specify how trial-specific trainings are to be performed. Usually, site personnel are trained on protocol and study procedures during the investigator meeting or the site-initiation visit. The challenges identified for the LENA studies were found to require a more sophisticated approach to prepare clinical staff for the most efficient study conduct.

Simulation in preparation of clinical trials is a novel approach to prepare clinical teams for trial-specific challenges. Simulation-based medical education has successfully been applied in various medical disciplines such as anaesthesia (Schwid et al. 2002; Chopra et al. 1994), nursing educational programs (Boling et al. 2016; Conner-Warren, Hillman, and Murphy 2014), surgery (Sturm et al. 2008), and emergency medicine (Okuda et al. 2008). It allows for knowledge, skills, and attitudes to be acquired in a safe and efficient manner, without putting patients at risk (Aggarwal et al. 2010). The subsequent learning curve within the clinical environment was found to be both shorter and flatter for simulation-based pre-trained individuals (Aggarwal et al. 2007). Though simulations are particularly suitable to train technical and procedural skills (Aggarwal et al. 2010; Lateef 2010) as frequent repetition leads to faster automaticity and proficiency (Barry Issenberg et al. 2005), authors also underlined the potential for simulation training focusing on communication in health care settings (Aggarwal et al. 2010). Thus, simulation-

based training was a promising new approach to train abilities required within the context of clinical trials.

As the concept of a simulation training in preparation for clinical trials is a novel application of simulation-based training within the field of medical teaching, its initiators aimed to evaluate the impact and suitability of this approach. A survey-based evaluation for trainers and participants was given to assess its short- and long-term effects on participants' trial-related knowledge and abilities and to determine the acceptability of this new concept to clinical staff. The survey's results will help to inform the impact of the new training approach in preparation for clinical trials and its possibilities for further application.

2.2. Methods

Ethical approval

Applications for ethical approval were filed at the responsible committees at Salzburg, where the simulation training was performed, and in Dusseldorf, where the survey data was analysed. The ethical committee of Salzburg confirmed that no approval was required (No. 415-E/1909/2-2015), and the ethical committee from the medical faculty in Dusseldorf approved the survey-based evaluation (study no. 5138).

Training site

The training was conducted at the Clinical Research Centre (CRCS) in Salzburg, Austria. The setting consisted of three rooms: the simulation room itself, a control room, and an observation/debriefing room. The simulation room was equipped like a typical hospital room, with a baby with artificial blood and all study consumables, laboratory equipment, and documentation materials necessary for the trial. The simulation room's audio and video system, operated from the control room, was connected to the observation room to enable observation by other team members. Trainers were able to contact the simulation participants via a microphone from the control room. Training sessions were videotaped so that records could be used during the debriefing.

Training on PK/PD

The frequent small blood volume sampling (up to six PK samples and two times all four PD samples per LENA study day) all required different preparation procedures, with variances in temperature ranges and the maximally allowed time frame (≤ 15 to ≤ 30 min depending on the parameter of interest) until specimen freezing was required to obtain reliable values; the PK/PD schedule thus classified as complex. The encompassing training was designed to mimic the PK/PD sampling procedures of a typical study visit. The schedule for this PK/PD training followed a step-by-step approach to teach the clinical teams the required skills in sampling and sampling preparation outlined in the *Sample collection manual for LENA paediatric studies.* An introductory presentation gave an overview on the sampling of PK/PD parameters investigated in the paediatric LENA trials. The presentation included background, schedules, and detailed information for each parameter of interest as well as associated official trial documentation. The presentation was followed by a practical demonstration in the simulation room. The participants then performed one to two sampling and sample preparation rounds on their own. Adherence to the correct sequence of procedures, to the timelines, and to several quality criteria were monitored by the trainers and documented in a checklist. These observations, supported by the study documentation generated by the participants and the video recording, formed the foundation for the debriefing following each training round.

Training on communication

Six peer-reviewed scenarios were prepared by the trainer team, addressing expected communicative challenging situations during trial recruitment and conduct. Small groups of up to three people actively took part in the role-plays while being observed by the remaining participants from the observation room. An extensive debriefing session with participants, trainers, and observers concluded each session.

Trainers

The training on PK/PD sampling and sample preparation was conducted by two clinical pharmacists with expertise in bioanalysis, one of whom is the author of this thesis.

The training session on communication was conducted by a multilingual clinical pharmacist with an additional educational/coaching background and expertise in communication in complex healthcare settings.

Survey-based evaluation

Surveys for training participants

A survey-based evaluation for participants was performed that focused on short- and long-term effects as well as the perceived usefulness of the simulation training. The survey was conducted at four different time points: before and after the simulation training, before study start, and during recruitment at the cut-off date of June 1, 2017. At this point, an almost 70% project-wide inclusion rate was reached. The survey contained questions that can be categorized as related to demographics, communication abilities/performance during communication scenarios, PK/PD sampling abilities/performance during sampling scenarios, the usefulness of trained topics/suitability of approach, and long-term effects and benefit. Overall, the four surveys contained 11 recurring questions and 13 one-time questions. Apart from demographics, all questions featured 5-point Likert scales.

All clinical staff who attended the simulation training were eligible to participate in the survey-based evaluation (total number: 23). Informed consent had to be provided before receiving the first survey. Participants who left the LENA project during the course of events were excluded from further participation. The last survey was distributed only among eligible clinical staff from study sites that had enrolled at least one patient and had faced the challenges of trial conduct on-site.

Survey for trainers

The initiators and trainers of the simulation training compiled a survey to assess the performance of each participant after completion of the training. It focused on the individual performance of participants and the improvement of abilities throughout the consecutive training sessions.

The rating of abilities related to PK/PD sampling and sample preparation was based on adherence to correct sequence of procedures, timelines, and to several quality criteria monitored by the PK/PD trainers and documented in a checklist. This was complemented by the documentation completed by the participants during the performed sampling and sample preparation procedures.

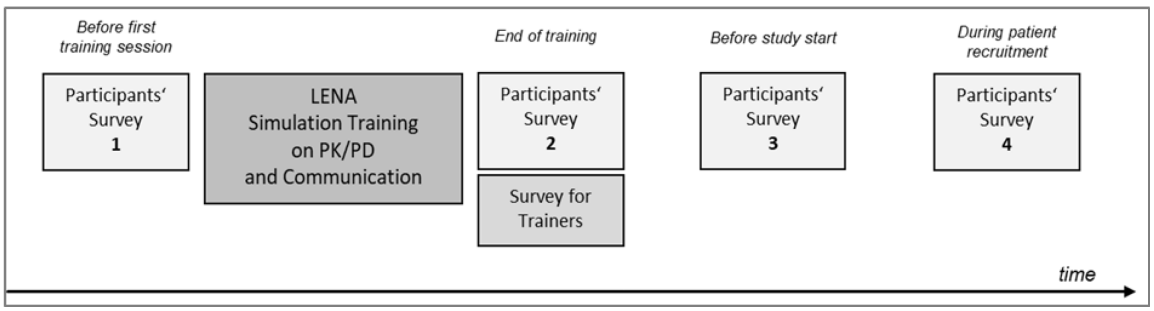

Figure 1 Timeline of survey distribution

Statistical analysis

Data extraction from returned surveys was performed with the four-eye principle. The 5-point Likert scale items were transformed into numerical items from 1 to 5 for statistical analysis. The Wilcoxon-test, appropriate for non-normal distributed data, was chosen for the calculation of statistically significant differences and confidence intervals (CI). The statistical level of significance was defined at $p \leq 0.05$. Calculations were performed in R Studio. Data extraction from returned questionnaires was reviewed by a second person. Inconclusive answers were

excluded from analysis. Results are depicted as a Hodges-Lehman Estimator (pseudomedian) with corresponding CI or as a percental distribution of answers. Calculations were performed in R Studio.

2.3. Results

The simulation training was hosted four different times between July and October 2015 in accordance with the availability of the clinical teams. Twenty-three participants from six different clinical sites in Europe took part: Austria (1), Hungary (1), the Netherlands (2), the Republic of Serbia, (1) and the United Kingdom (1). In total, 13 (56.5%) participants were physicians and 10 (43.5%) (study) nurses.

Training on PK/PD

The PK/PD training was conducted by 19 active participants. Where possible, the training groups were put together per hospital to reflect the study teams that will work together during the clinical trials. Based on the experiences of the participant's ability to conduct the sampling, the difficulty was increased to a realistic setting within two to three rounds. After following the presentation and following the demonstration by the trainer, participants were able to prepare 62.5% of all samples in time but with partial incorrect handling (first hands-on session). This was increased in the second round. Even in stressful situations with unforeseeable events, the strict timelines for PD parameters of ≤ 15 minutes were kept more than 91% of the time. Bearing the sensitivity with reported half-lives of 1–2 minutes in mind, the improvement was crucial for reliable data generation. The correctness of trial documentation was increased to 100% from the first to the last round per team. The correctness of comprehensive sample preparation increased to 97.4%.

Training on communication

Thirteen participants impersonated the challenged physician/study nurse during the role-plays. The role-plays were conducted in either English or the participants' native language. The most requested scenario was the informed consent process.

Survey-based evaluation

Distribution and response rate

Surveys were distributed before and after completion of the simulation training in 2015, shortly before the anticipated start of recruitment (depending on individual study start-up procedures of the clinical sites) and at the cut-off date of June 1, 2017, at which almost 70% of the project-wide anticipated number of patients had been recruited. Exact dates are outlined in **Table 1**. Total follow-up time ranged from approximately 19 to 23 months.

Surveys for participants of the simulation training	**Survey 1**	**Survey 2**	**Survey 3**	**Survey 4**
Time of assessment	Before training	After training	Before study start	During recruitment
Date of distribution				
Simulation Training 1	01.07.15	02.07.15	04.12.15	01.06.17
Simulation Training 2	06.07.15	07.07.15	11/17.11.15	01.06.17
Simulation Training 3	04.10.15	05.10.15	23.05.16	01.06.17
Simulation Training 4	05.10.15	06.10.15	19.01.16	01.06.17

Table 1 Overview of survey distribution dates for participants of the simulation training

All 23 participants of the simulation training gave informed consent to take part in its evaluation. For the first three surveys, all participants in the training remained eligible to complete the surveys, but for survey four, three participants had to be excluded as their site had not enrolled any patients before the cut-off date of June 1, 2017. Response rates are shown in **Table 2**.

Surveys for participants of the simulation training	**Survey 1**	**Survey 2**	**Survey 3**	**Survey 4**
Time of assessment	Before training	After training	Before study start	During recruitment
eligible participants	23	23	23	20
response rate	23 (100%)	23 (100%)	23 (100%)	19 (95%)

Table 2 Overview of survey response rates

Trainers completed a survey, assessing each participant after the simulation training.

Survey results for training on PK/PD

Results for perceived improvement of trial-related PK/PD sampling and sample preparation abilities improved significantly as shown in **Figure 2** [2 (1.5-2.5) to 4.5 (4.0-5.0) [HLE (95%)], $p \leq 0.05$]. Overall, participants rated their own performances during the scenarios as “good”.

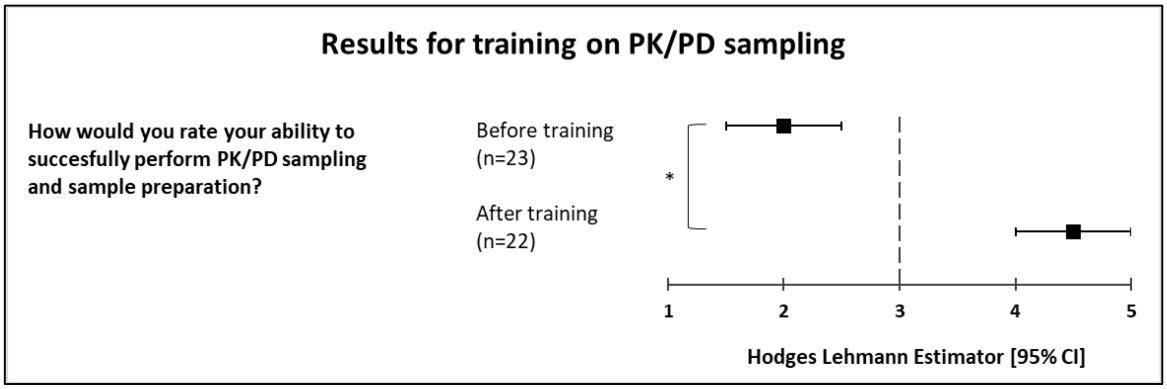

Figure 2 Results for training on PK/PD sampling
Participants’ self-assessment using a 5-point Likert scale (1 = very poor, 5 = very good, $*p \leq 0.05$)

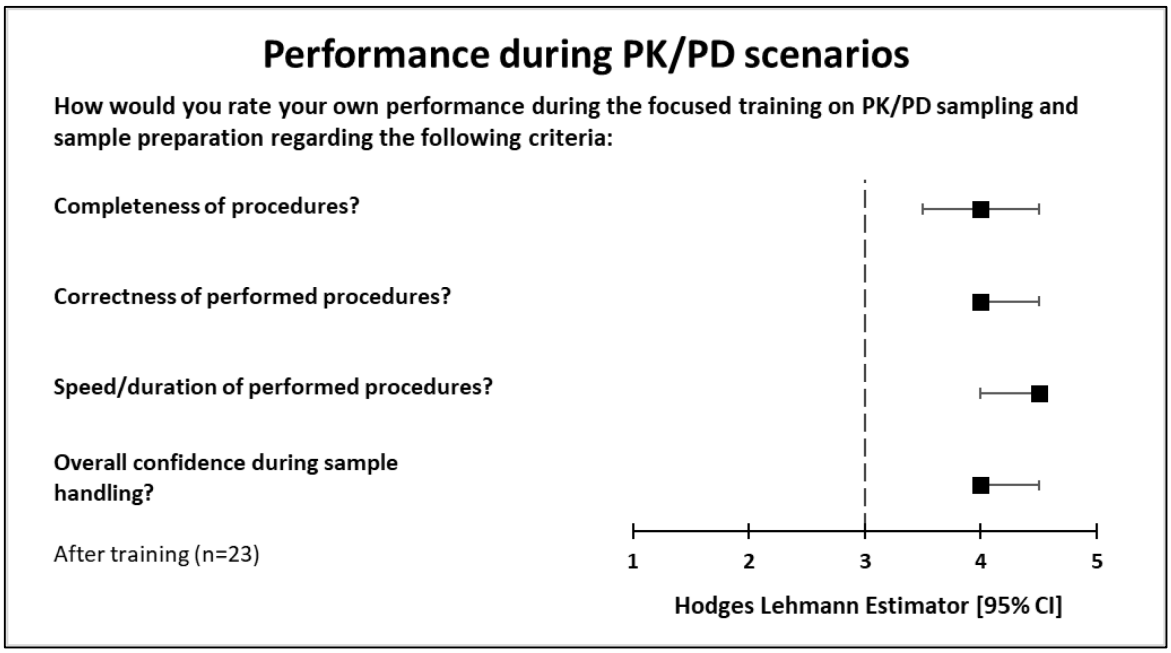

Figure 3 Self-evaluation of performance in training scenarios on PK/PD
Participants’ self-assessment using a 5-point Likert scale (1 = very poor, 5 = very good, $*p \leq 0.05$)

Trainers observed a significant improvement in the abilities of participants between the first round of simulation training and the last as shown in **Figure 4**. The self-assessment of participants regarding their abilities after completion of the training seems accurate as the results match the trainers' assessment as shown in **Figure 5**.

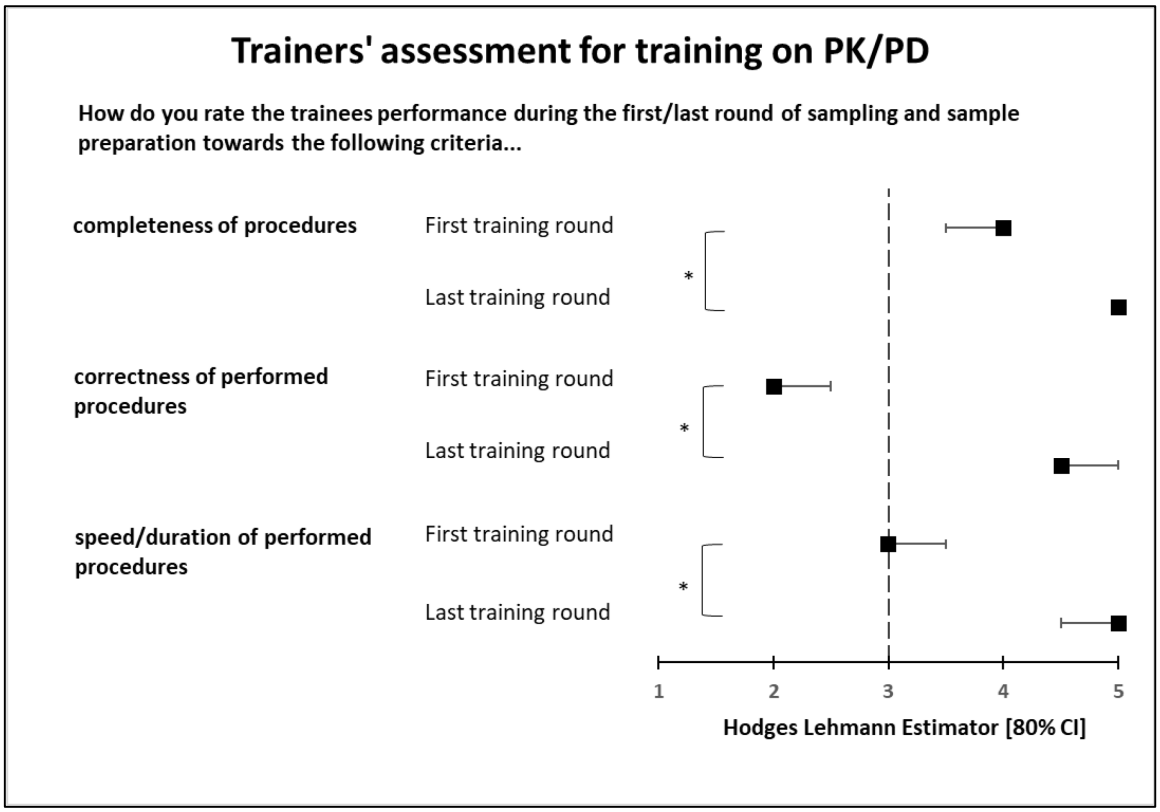

Figure 4 Trainers' assessment for training on PK/PD
Trainers assessment using a 5-point Likert scale (1 = very poor, 5 = very good, $*p \leq 0.05$)

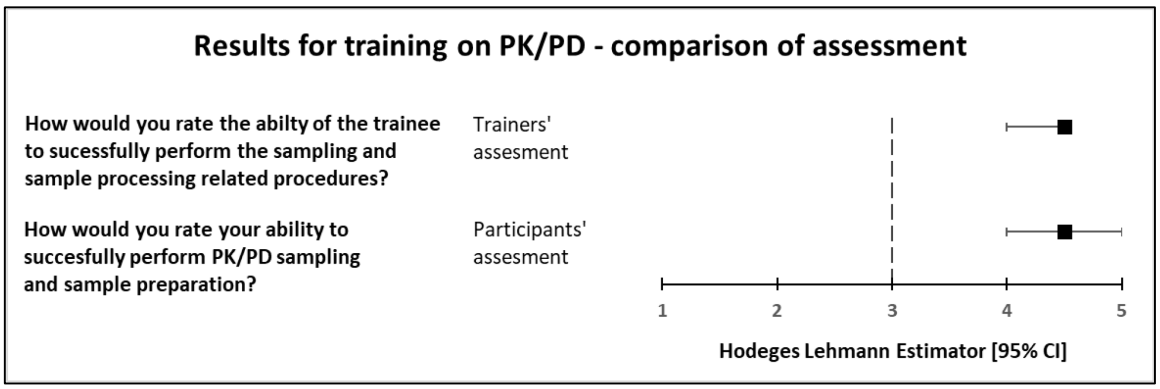

Figure 5 Comparison between trainers' and participants' evaluation of PK/PD sampling and sample preparation abilities
5-point Likert scale (1 = very poor, 5 = very good, $*p \leq 0.05$)

Survey results for training on communication

All results regarding perceived improvement in communication-related abilities were significant. The ability to communicate the core elements of the clinical trials to different target audiences (patients/parents/colleagues) increased significantly after the training. The same applies to the preparation for dealing with trial-related communicative challenging situations (**Figure 6**). Overall, participants rated their own performance during the communication training as “good” (**Figure 7**).

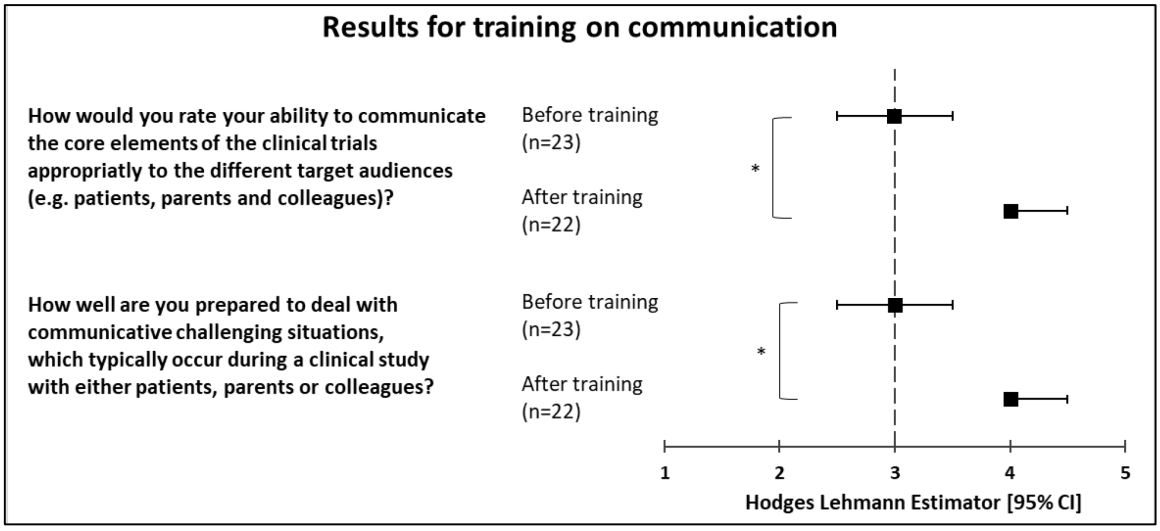

Figure 6 Results for training on communication
Participants’ self-assessment using a 5-point Likert scale (1 = very poor, 5 = very good, $*p \leq 0.05$)

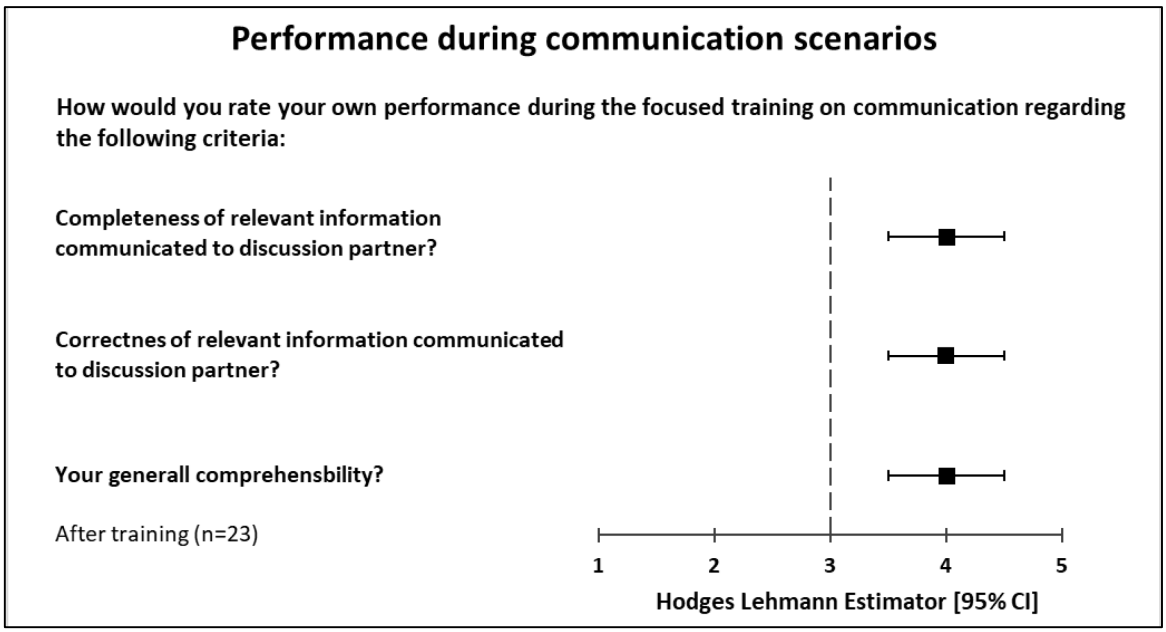

Figure 7 Self-evaluation of performance in training scenarios on communication Participants' self-assessment using a 5-point Likert scale (1 = very poor, 5 = very good, $*p \leq 0.05$)

Long-term effects and overall concept

The positive perception of the simulation training's impact has shown to be long-lasting, without significant differences throughout the surveyed time period. Results on long-term effects on knowledge, attitudes, and modes of practice are shown in **Figure 8**. The approach of a simulation-based training in preparation for clinical trials was unanimously perceived as suitable (**Figure 9**). The simulation training had been tailored to the specific needs of the paediatric trials of the LENA project. It aimed to select topics relevant to trial conduct and to successful trial completion. The participants of the training agreed to the choice of training topics and rated them as useful throughout the complete survey series (**Figure 10**). Participation was perceived as beneficial to one's own performance during the paediatric trials (**Table 3**). Participants unanimously recommended simulation-based training in preparation for clinical trials (**Table 3**).

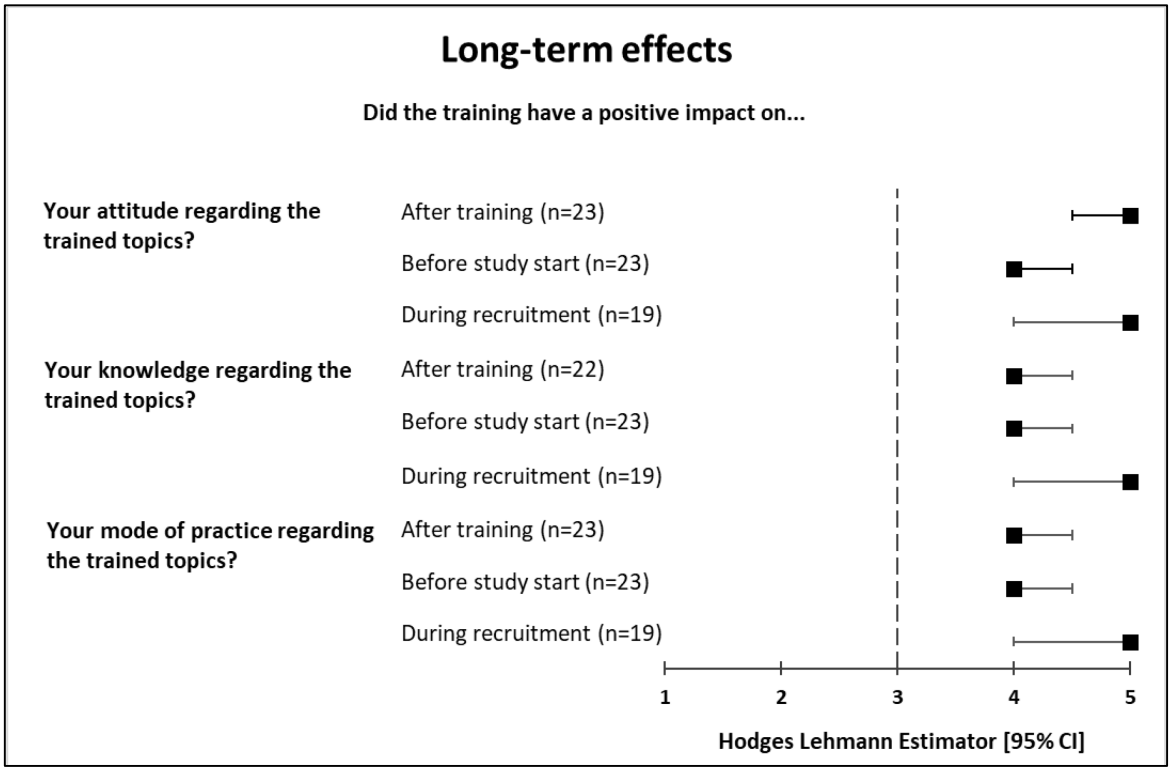

Figure 8 Perceived long-term effects on attitude, knowledge, and mode of practice Using 5-point Likert scale (1 = strongly disagree, 5 = strongly agree, $*p \leq 0.05$)

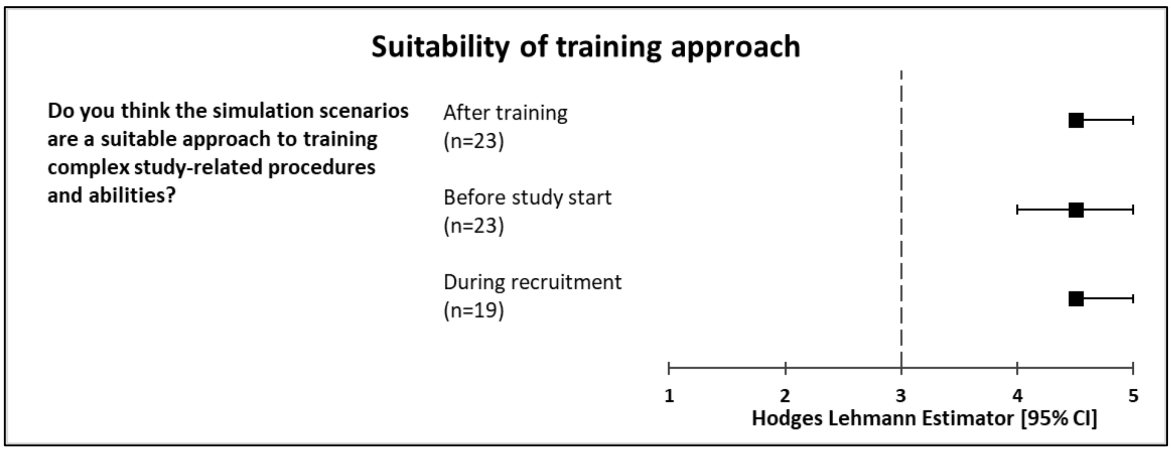

Figure 9 Suitability of simulation scenarios as training approach
Using 5-point Likert scale (1 = strongly disagree, 5 = strongly agree, $*p \leq 0.05$)

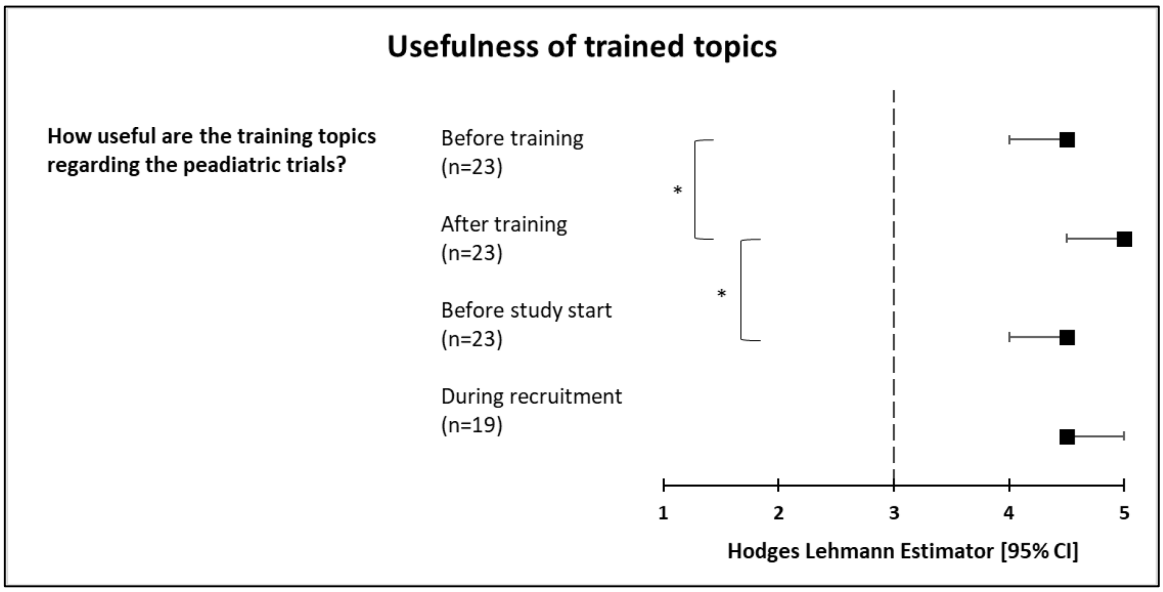

Figure 10 Participants' evaluation of usefulness of trained topics
Using 5-point Likert scale (1 = not relevant, 5 = very relevant, HLE [95% CI], $*p \leq 0.05$)

	Time of assessment	**1**	**2**	**3**	**4**	**5**
Did the experiences from the simulation training have an impact on your preparation procedures?	before study start	1 (4%)	-	-	10 (44%)	12 (52%)
Would you recommend a simulation-based training on the most challenging aspects of a clinical study as part of the preparation procedures?	during recruitment	-	-	1 (5%)	3 (16%)	15 (79%)
Do you think your performance during the study benefited from the training?	during recruitment	-	-	1 (5%)	5 (21%)	13 (68%)

Table 3 Evaluations of training concept
(1 = strongly disagree, 5 = strongly agree; *n* = number of votes (%))

2.4. Discussion

Twenty-three participants from six clinical sites participated in the tailored 2-day LENA simulation training focusing on PK/PD sampling and communication. The simulation-based training significantly improved the participants' performance. PK/PD sampling and trial-related communicational abilities benefited considerably. The training was perceived as contributing substantially to performance during the paediatric trials. The setup as a simulation-based training was rated as a suitable approach to preparing clinical teams for the upcoming trials. The participants unambiguously recommended such a simulation-based training focusing on a trial's most challenging aspects.

The concept of a simulation-based training has been successfully applied to other areas of medicine and shows promising potential for an application in the preparation for clinical trials. It has been shown, as also indicated by our survey results, that skills acquired during such a training indeed translate into improved on-site performance (Sturm et al. 2008; McGaghie et al. 2011). Furthermore, simulation training has been shown to improve teamwork within clinical teams (Shapiro et al. 2004), a prerequisite to successfully performing interlocking and time-critical procedures as required for the paediatric studies of the LENA project. As shown on other occasions (Shapiro et al. 2004; Howard et al. 1992), our survey confirmed that this education tool is well-accepted by participants.

It can be estimated that over 10 million Euros of funding may be lost every year in clinical trials in the European Union due to pre-analytical and analytical problems (Lippi et al. 2016). The vast majority of specimens (over 90%) rejected by laboratories are found unsuitable due to incorrect procedures for collection and/or

transportation (Lippi et al. 2016). The simulation training provided the possibility of familiarization with study procedures ahead of recruitment to enable the most efficient collection of study samples possible.

Investigators often do not receive formal training in seeking informed consent for research but mostly learn by observing more experienced colleagues (Mason and Allmark 2000). This practice is perceived as insufficient by many professionals involved in recruitment of study participants. They suggest that every professional who is going to seek consent from study participants should undergo hands-on training as inadequate education and training contribute to deficiencies in the informed consent process (Nusbaum et al. 2017). Our finding suggests that simulation-based training as performed in the LENA training could be a suitable tool to be included in such a training.

Further investigations are required to determine the effectiveness of simulation trainings regarding trial outcome objectives. Currently, different approaches are applied to assess the effectiveness of simulation-based training in medicine. Among these are knowledge and confidence scores (Boling et al. 2016; Rhodes et al. 2016; Young and Burke 2010); self-assessment of performance (DeVita et al. 2005; Weller, Wilson, and Robinson 2003); application of established, structured assessment scores (Price et al. 2011; Fann et al. 2008); and reduction of medical complications (Cohen et al. 2010). To complement the findings of the performed survey, the participants of which credit the simulation training with a long-lasting impact on their performance during the trial, objective criteria related to study conduct should be consulted.

Regarding the high rate of early trial terminations, with all the ethical and financial implications, new approaches have to be adopted to counteract. The surveys'

results show a potential of simulation-based training in clinical trial preparation that deserves further exploration. Results regarding acceptance of trained topics indicate that immanent relevance to the trial conduct of the study is a key issue. This requires an individual tailoring according to a study's characteristic challenges and processes, which should be considered for communicative and practical skills-oriented training sessions.

3. LENA feasibility study focused on PK/PD sampling and sample preparation

3.1. Introduction

Small-scale studies such as pilot or feasibility studies conducted prior to a main trial have received increasing interest from the research community (Lancaster 2015; Williams 2016; Eldridge et al. 2016) as part of the preparation process for larger trials. Size and scope of these preliminary studies can vary significantly. They can range from questionnaire-based inquiries regarding the size of the available patient pool and site infrastructure during the site-selection process (Mehta and Bhatt 2013) to the performance of the complete study on a smaller scale (Karwalajtys et al. 2009). These preliminary studies help to inform the planning for the main trial and are performed to increase chances of successful completion (Thabane et al. 2010). The aspects examined belong to either processes, resources, management, and/or scientific facets of the planned main study (Thabane et al. 2010). Pilot and feasibility studies informed sample size calculations, recruitment rate, and patient retention (Corder et al. 2016) and helped to prevent unfeasible trials (Olsen et al. 2015). They also helped to streamline assessment processes, train staff, and install recruitment procedures ahead of the main trial (O'Malley et al. 2005). Ideally, they should target identified risk factors for successful completion of the main trial (Craig et al. 2008).

Clinical trials that are to be performed in such a vulnerable population as paediatric patients should especially be verified for feasibility to prevent or help improve planned investigations unsuitable to meet their objectives. Researchers even

claimed that it seems unethical to conduct a study when its feasibility has not been verified (Thabane et al. 2010). Thousands of children have been enrolled and undergone medical investigations in ultimately discontinued trials (Pica and Bourgeois 2016). Only a minority, 12.5%, of those trials were discontinued due to informative reasons (e.g., related to safety or efficacy). This raises ethical concerns regarding the patients from the vast majority of terminated trials, the efforts of which do not translate into results contributing to medical knowledge or the improvement of patient care.

The investigators of the LENA project decided to perform a preparational study to verify the feasibility of the most critical planned procedures. The identified biggest challenges of trial conduct were the complex procedures related to sampling and sample preparation of pharmacokinetic (PK) and pharmacodynamic (PD) parameters. They enable correct determination of temperature-sensitive substances with short half-lives. The subsequently designed feasibility study assessed the capability of involved clinical teams to perform the challenging sampling-related procedures and to obtain study samples in a manner of adequate quality. Furthermore, it evaluated planned procedures for logistics and bioanalysis simultaneously.

3.2. Methods

Ethical approvals

Clinical sites obtained ethical approval (or confirmation that an approval for this specific kind of study was not required) before participating in the feasibility study (EK 1690/2015 at the Medical University of Vienna, WT/aj/247957 at the Erasmus Medical Center in Rotterdam, WAG/mb/15/037193 at the University Medical Center Utrecht, and REC no. 16/SC/0124 at the Great Ormond Street Hospital). In addition, the University Clinics' ethics committee in Dusseldorf, where the project's central laboratory is located, agreed on the protocol (Protocol No. 5118). The data protection concept of the investigation complied with the regulations of North Rhine Westphalia (Germany) being regarded as one of the strictest in Europe.

Inclusion/exclusion criteria for participation in the feasibility study

Healthy adults without cardiovascular diseases and without any current medication (self-reported), aged between 18 and 50 years, were eligible for participation. Volunteers whose (self-reported) health condition raised medical concerns against blood withdrawal (e.g., anaemia) were excluded. All participants provided informed consent prior to the start of the study.

Study design and conduct

The feasibility study was designed to mimic a regular LENA study visit within the paediatric studies. Details on performed procedures are provided in Figure 1. The exact date of conduct was chosen individually for each clinical site, according to the anticipated start of recruitment. Areas assessed at the clinical sites included sampling, on-site sample preparation, documentation and dispatch of samples to the central laboratory of the LENA project for subsequent analysis as well as evaluation of results. Each clinical site performed corresponding procedures on three adult volunteers. Staff members were encouraged to rotate tasks, e.g., blood collection, documentation, and sample preparation. This ensured that every member of staff ran through all procedures that would come up during the trials. In this feasibility study, clinical teams of five clinical sites from four countries (Great Britain, The Netherlands, Austria, and Hungary) were involved.

Sampling requirements encompassed blood withdrawal for the compounds of interest in the paediatric trials of the LENA project. For the PK-related primary outcomes, the compounds are enalapril and its metabolite enalaprilat. The humoral parameters aldosterone, renin, plasma renin activity and angiotensin I are of interest as secondary outcome measures for exploratory PD investigations (ClinicalTrials.gov ; ClinicalTrials.gov ; ClinicalTrials.gov). All samples were collected and labeled in accordance with the approved pseudonymisation process. Due to the sensitivity and poor stability of investigated peptides and hormones, all samples required immediate on-site sample preparation. Procedural instructions were applied as outlined in the corresponding LENA manual on sampling and sample preparation. This included a fixed sampling and sample preparation

sequence, a strict time limit and specific temperature conditions to be applied during sampling, clotting time as well as centrifugation, depending on the type of the sample matrix. Blood samples for temperature sensitive humoral parameters were drawn with the blood collection tube enveloped in an ice pack and transported on ice to further preparation. All samples were centrifuged for 10 minutes at 2000g, at either room temperature or 4 °C depending on temperature sensitivity. The supernatant was subsequently transferred to cryo tubes and snap frozen in a methanol/dry ice bath. Depending on the sensitivity of the substance of interest, applicable time limits to proceed from sampling to snap freezing of the supernatant were defined as 15 or 30 minutes. The consumables used and all on-site laboratory equipment utilised were identical to equipment intended for the paediatric trials, including the small-scale sampling material for paediatric use, the layout for labels and all form sheets required.

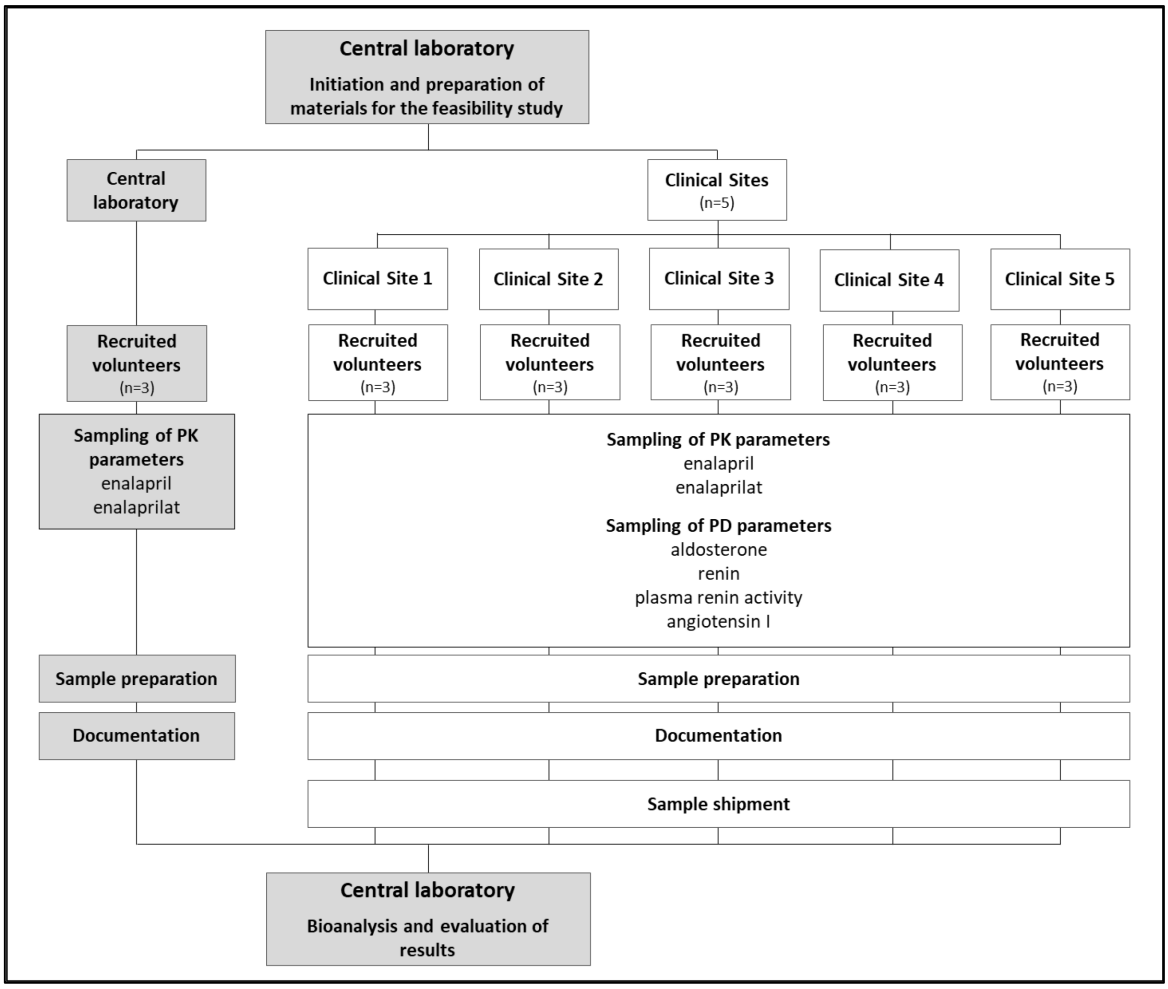

Figure 11 Flow chart of the feasibility study procedures

By-pass of drug administration

Bearing in mind ethical constraints on unnecessary drug administration to healthy volunteers, a special concept to by-pass drug application was developed to avoid administration of a drug during the feasibility study. This avoidance was achieved by pre-spiking the blood collection tubes with a stock solution of the drugs. The spiking took place in batches at the Institute of Clinical Pharmacy and Pharmacotherapy (Dusseldorf, Germany). The spiking procedure is illustrated in **Figure 12**. The volume and concentration of the spiked drug solution is based on calculations regarding the final concentrations per blood-filled tube without substantial dilution of blood collected. Targeted concentrations in whole blood were 50 ng/mL enalapril with 25 ng/mL enalaprilat and 12.5 ng/mL enalapril with 6.25 ng/mL enalaprilat. These concentration levels reflect expected Cmax of both compounds in a neonate/toddler as well as in an adolescent and were reached during the withdrawal of blood as drug solution and freshly collected blood mixed up. To avoid deviations in filling volume, thus affecting determined concentrations, discard tubes were provided to remove the air from the connective tubing before the first sample was drawn. Therefore, it was assumed that a comparable maximal filling volume could be obtained in all samples if sampling was performed correctly.

Evaluation of bioanalytical results for PK samples (enalapril/enalaprilat)

Acceptable range of results

To consider characteristics of the applied by-pass procedure for drug administration for the evaluation of results, setting specific factors had to be considered to establish an acceptable range of results. The range was based on following variables: First, based on applicable guidelines on bioanalytical method validation by the European Medicines Agency (EMA) (Committee for Medicinal Products for Human Use 2011) and the U.S. Food and Drug Administration (FDA) (Food and Drug Administration Center for Drug Evaluation and Research 2013), acceptable accuracy is defined as a maximum deviation to the nominal/reference value of ±15%. Second, the pipetting error during pre-spiking and variability in the filling volume were also considered. The total pipetting error was compounded by a systematic and random error provided by the pipette manufacturer (Eppendorf, Hamburg, Germany) as 1.6% (model: Multipette® M4). Pre-examinations had indicated that the filling volume differed by 1.1% (using the vacuum approach) from that of the blood collection tubes used (Sarstedt, Nuembrecht, Germany). Third, a factor that had to be considered, due to its elementary influence within the concept of pre-spiked blood collection tubes, was the haematocrit. As both enalapril and enalaprilat have a blood to plasma ratio <1, their individual haematocrit affects the distribution volume of the drug and metabolite by defining the available serum fraction.

As no individual determination of haematocrit was performed for, the lower reference value for females (35.8%) and the upper reference value for males (51.0%) were used to define the expected haematocrit range for the healthy volunteer population. Thereby, it is ensured that calculations account for

volunteers with haematocrit values up to the margins of reference values for healthy adults. As no international standardised reference values are established, the reference values used were calculated as the mean of reference values applied in the participating clinical sites' routine laboratories. The 15.2 percentage points (pp) difference between 51.0% and 35.8% constitute a maximal variability of 29.8% between volunteers.

Based on these identified variables, the acceptance range was defined as 58% to 153% of the mean reference concentration of enalapril and its active metabolite enalaprilat represented by **Equations 2** and **3.**

*Limit (%) = 100 * acceptable maximal variation according to the EMA/FDA guidelines (%) * maximum haematocrit difference in healthy adults (%) * variability in the filling volume of blood collection tubes (%) * pipetting error (%)* **(1)**

Equation 1 Formula for the calculation of acceptable ranges of accuracy for drug/metabolite results from pre-spiked blood sampling tubes compared to reference values

*Lower limit: 100% * 0.85 * 0.702 * 0.989 * 0.984 = 58%* **(2)**

*Upper limit: 100% * 1.15 * 1.298 * 1.011 *1.016 = 153%* **(3)**

Equations 2 and **3** Calculated acceptance range of accuracy for the feasibility study, for drug/metabolite results from the pre-spiked blood sampling tubes compared to reference values.

Reference samples

Determined concentrations of each adult were compared to the mean concentration of three reference samples collected under ideal conditions at the central laboratory (Institute of Clinical Pharmacy and Pharmacotherapy, Heinrich-Heine University, Dusseldorf, Germany). This approach addresses possible distribution processes between plasma and blood cells as well as plasma protein binding and inevitable degradation processes during sample preparation. Blood collection tubes used to obtain these reference samples originated from the same batch of pre-spiked collection tubes used at the clinical site. The spiking procedure is illustrated in **Figure 12**.

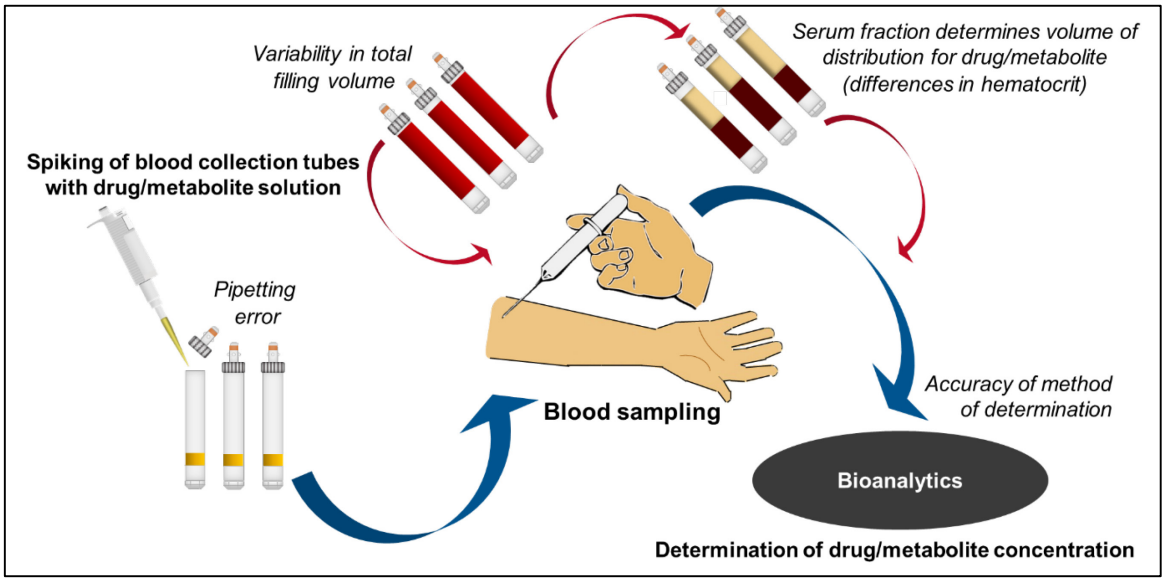

Figure 12 Circumvention of drug administration in the feasibility study**.**
Procedures from spiking of blood collection tubes to determine drug concentration are connected by bold/blue arrows. Additional variability in results induced by this approach is indicated by thin/red arrows

Evaluation of bioanalytical results for PD-related humoral parameters (aldosterone, renin, plasma renin activity, and angiotensin I)

Criteria to assess the acceptability of results obtained for concentration/activity of the RAA system-related substances investigated were derived from established reference ranges for adults. Deviations from recommended sample preparation procedures can cause major degradation. Due to their sensitivity and poor stability, prompt, complete and correct on-site sample preparation was mandatory for high-quality and reliable sample results. Therefore, sample preparation had to take place immediately and be conducted by the clinical staff on the ward. Since these procedures exceed daily routine by far, it is prone to handling errors affecting the sample quality. Correct sampling and detectable values of concentration/activity in collected samples were defined as criteria for successful conduct. Detected values were expected to be above the lower limit of reference values in adults, as far as established. As sampling was performed in a sitting position without obligatory resting time, reference values for standing position were used for evaluation in case-distinct reference values according to posture (supine vs. standing) were given. According to available reference values in adults, the concentration level had to exceed the following limits: 22.1 pg/mL for aldosterone (MVZ Laboratory Dr. Limbach, Heidelberg, Germany), 1.68 pg/mL for renin (Laboratory Dr. Spranger, Ingolstadt, Germany) and 0.06 ng/(mL*h) for plasma renin activity (Laboratory Dr. Spranger, Ingolstadt, Germany). For angiotensin I, no lower reference value was provided by the analysing laboratory.

Evaluation of logistics

Since the courier services by DHL were contracted for the paediatric studies through the company's recently introduced clinical trial-specific service called "Medical Express", the services and their reliability were assessed in the feasibility study. Due to limited access to dry ice at the clinical sites and with regard to the generally high workload on the wards, a care-free shipment approach was established by the central laboratory. Parcel pick-up was initiated remotely, and clinical sites were provided with parcels pre-filled with dry ice and required labels/documentation for sample transport. For both shipments together (outward and return), a total turnaround time of three days was scheduled. The timeliness, tracking function and reliability of the transport as well as the condition of the parcels (damaged/undamaged), functionality of provided temperature data loggers and general quality of the service were assessed.

Evaluation of laboratory procedures

Obtained samples were shipped on dry ice to the central laboratory (Bioanalytical Laboratory of the Institute for Clinical Pharmacy and Pharmacotherapy, Heinrich-Heine University, Germany). Determinations for enalapril, enalaprilat, aldosterone, renin, plasma renin activity and angiotensin I were performed at the institute or by an external partner (Dr Spranger Laboratory, Ingolstadt, Germany). Drug concentrations of enalapril and its metabolite (enalaprilat) were determined using a low-volume LC/MS-MS method (Burckhardt and Laeer 2015), whereas humoral parameters (aldosterone, renin, plasma renin activity and angiotensin I) were determined using enzyme-linked immunosorbent assays (ELISA) (Schaefer et al. 2017b, 2017a) or radioimmunoassays (RIA). The bioanalysis at the laboratories

was performed blinded. The interface management of sample dispatch, sample registration, and storage as well as bioanalysis were assessed in the feasibility study.

3.3. Results

Between November 2015 and March 2016, five clinical centers involved in the LENA project (83% of all recruiting sites at this stage) performed the on-site feasibility study. The clinical sites were located in the Netherlands (2 sites), Austria (1 site), Hungary (1 site) and the United Kingdom (1 site). The sixth site in Serbia was not able to participate, due to national regulatory restrictions.

Study population

In total, 18 apparently healthy volunteers were recruited at the five clinical sites, whereof three were enrolled per site. Since one site repeated the feasibility study, three additional volunteers were recruited, thus resulting in a total of 18 participants.

To obtain reference samples, additional nine volunteers were recruited at the central laboratory. Main characteristics of the volunteer population are presented in **Table 4**.

	Volunteers' Clinical Sites (N = 18)	Volunteers' Reference (N = 9)
Age* in years (± SD)	33 (± 7)	29 (± 3)
No. of females (%)	10 (55 %)	5 (55 %)

*Calculated, as volunteers provided only the year of birth. Presumed birthday is December 31 of a given year.

Table 4 Demographics of volunteers

Results for the PK samples (enalapril and enalaprilat)

Using the pre-spiked blood collection tubes for evaluation of PK sampling performance by each site enabled determination of eligible concentration levels at all five clinical sites. The pre-spiking approach appeared to be suitable to evaluate the clinical team's ability to perform the PK sampling according to the intended trial procedures.

In total, 29 of 30 planned PK samples were collected during the first attempt of the feasibility study. Due to a technical handling error of the sampling material at clinical site 1, one PK sample with low concentration levels was lost. At four out of the five sites, results of both concentration levels and compounds were determined between 73% and 120% when compared to the reference values, hence within the defined acceptable accuracy range of 58% to 153%. Obtained results for the two differently concentrated pre-spiked samples, displayed as accuracy (%) relative to the results of reference samples, are illustrated in **Figure 13**.

In its initial performance, clinical site 4 exceeded the allowed deviation for one sample, where relative concentration values were determined as 168% for enalapril and 177% for enalaprilat. This result indicates too little blood was drawn within the respective tube to allow for appropriate dilution of the drug solution inside. In addition, this site had the highest variability of results, and its obtained accuracy levels varied between 96% and 177%. This was considered to be an unsatisfactory outcome, and it contributed to the decision to repeat the feasibility study at this site, which was completed successfully in its second attempt.

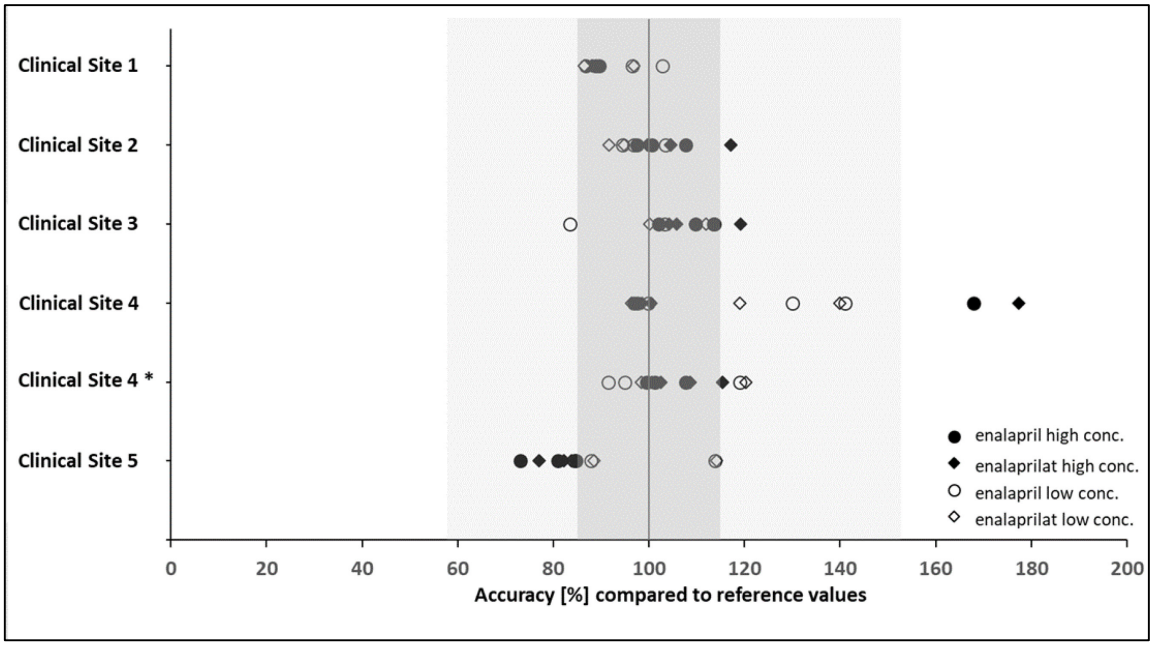

Figure 13 Accuracy of enalapril/enalaprilat samples, obtained with pre-spiked blood collection tubes, in relation to reference values.
The dark grey area indicates the acceptable accuracy of the bioanalytical method according to EMA/FDA Guidelines (± 15%). The light grey area indicates the extended tolerance range based on additional influencing factors of the artificial setting of the feasibility study (58% to 153%). * denotes results from the re-run for the site that did not pass the feasibility study in its first attempt.

Results for the PD-related humoral parameters aldosterone, renin, plasma renin activity and angiotensin I

The developed on-site sample preparation appeared appropriate and effective in collecting reliable results of humoral parameters at all sites. Unless no handling error during sample collection and on-site preparation occurred, detectable levels of all humoral parameters investigated were measured. Analytical results of samples eligible for analysis are illustrated in **Figure 14 (A–D)**. Determined aldosterone levels were within the applied reference range of 22.1–353 pg/mL (standing position) for aldosterone (MVZ Laboratory Dr. Limbach, Heidelberg, Germany). Levels of plasma renin activity were found to be higher than the reference range of 0.06–4.96 ng/(mL*h) (Dr Spranger Laboratory, Ingolstadt, Germany) in six different volunteers from four different clinical sites, with a maximal measured value of 8.2 ng/(mL*h). The same applies to renin, of which three samples from three different sites had values above the reference range of 1.68–27.66 pg/mL (Dr Spranger Laboratory, Ingolstadt, Germany). The highest renin concentration determined was 30.42 pg/mL. Angiotensin I could be detected in all samples analysed. However, not all samples could be collected per volunteer. From a total of 60 pharmacodynamic samples that had to be drawn originally (four per volunteer), four samples had to be excluded from analysis. This was required because of either insufficient volume or handling errors resulting during transfer of blood not suitable for bioanalysis. Three of these samples originated from clinical site 4. Analogue to the results for PK samples at clinical site 4, the accumulation of ineligible samples at this site contributed to the decision to re-train staff in PK plus PD sampling and perform a re-run of the study. In the re-run, one pharmacodynamic sample was missed due to a pipetting error.

The overall findings correspond to the study's expectation of results above the lower limit of reference values for adults, as apparently healthy volunteers were recruited.

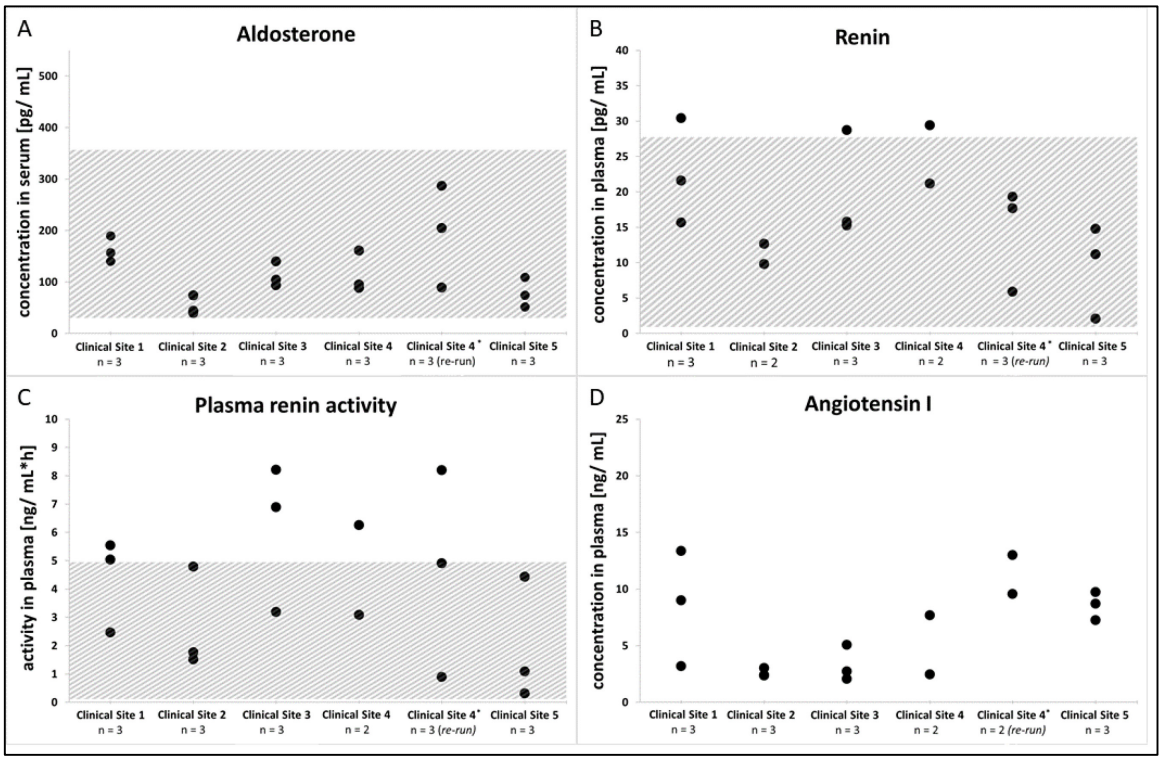

Figure 14 (A–D) Results for humoral parameters aldosterone, renin, plasma renin activity and angiotensin I.
Each black circle represents an analytical result for an evaluable sample. Hatched areas indicate applied reference ranges for healthy adults, as far as established or given by the laboratory. * denotes analytical results from the re-run for sites that did not pass the feasibility study in their first attempt.

Results for logistics

All parcels shipped by DHL “Medical Express” reached their destination intact, the day after dispatch, although not all were delivered within the announced time window. Information provided by the online tracking was accurate and available with an acceptably short lag time. Unfortunately, no automatic notifications were sent in case of delays in transport. Insulation and storage capacity proved to be sufficient to maintain dry ice for the duration of a three-day sample shipment cycle. Provided temperature data loggers were found to be unsuitable for use on dry ice. The batteries drained too fast when exposed to these extremely low temperatures to enable any relevant data recording. Therefore, the purchase of suitable devices (testo® 184 t4) was initiated, to be used for shipments during the paediatric trials. Overall, services provided by DHL were assessed as appropriate to be used for the paediatric trials after calculation of cost-risk ratio.

Results for laboratory procedures

Recently implemented sample registration and storage documentation procedures, tailored to high sample throughput, allowed for fast and traceable sample handling at the laboratory. Established workflows in accordance with Good Clinical Laboratory Practice proved the bioanalytical teams’ preparedness for timely sample preparation and measurements using ELISA and LC-MS/MS. Scheduled assay runs could be performed in time without protocol deviations, properly documented and reported.

3.4. Discussion

This concept of a feasibility study that assesses on-site PK/PD sampling, sample preparation, logistics and bioanalysis simultaneously has proved to be a promising tool for trial preparation and is likely to have substantially contributed to the good quality of obtained samples during the main trials. Planned procedures and adequate training of clinical teams could be assessed by performing study-specific sampling for all PK/PD parameters of interest on healthy adult volunteers before investigations in the more vulnerable population of diseased children were initiated. Substances of interest were determined at expected levels, and planned procedures were found to be suitable for being applied within the paediatric trials.

Performing the current study to verify the feasibility of planned procedures for the main trials, as recommended by researchers (Thabane et al. 2010), had a substantial impact on the preparatory procedures. It became apparent that one site required re-training concerning study-related sampling and sample preparation procedures. It succeeded to obtain blood samples according to the pre-defined requirements only in its second attempt. The feasibility study appeared to be a capable tool for detecting sampling errors as too low volume and transfer of the whole blood instead of serum/plasma for analysis. It enabled retraining of clinical staff before study procedures were performed in children.

As recommended by researchers (Whitehead, Sully, and Campbell 2014), the feasibility study focused on the complex PK/PD sampling procedures identified as a main risk for study failure for the planned trials. This complexity is a consequence of the sensitive substances investigated, e.g., angiotensin I; a PD parameter of the RAA system has an in-vitro half-life in non-anticoagulated whole blood of 3 minutes

(Oparil, Sanders, and Haber 1970), and renin´s precursor pro-renin is cryo-activated when exposed to temperatures between -5°C to 4°C (Campbell et al. 2009). Other investigators had focused in a similar manner but on other parameters of a planned study, such as feasibility and acceptability of randomisation (Crawley et al. 2013); cost-effectiveness, retention and suitability of outcome measures (Hu et al. 2016); as well as feasibility of a planned intervention (Boogerd et al. 2014; Crawley et al. 2013). Thus, the current results demonstrate that highly sophisticated pre-analytical procedures should be an objective of feasibility studies performed prior to a main trial.

The validity of the results of the current feasibility study is not limited by the study's conduct in healthy adults instead of in children, the population of the main trial. This was an ethically motivated decision, as it seemed inappropriate to perform invasive investigations in paediatric patients without benefit but only for testing purposes (He 2008). Furthermore, the investigated peptides/hormones aldosterone, renin, plasma renin activity and angiotensin I are inherent components of the RAA system. They are physiologically present in all human beings. The absolute levels may vary between healthy individuals and paediatric patients, but they are not decisive for the verification process, as investigating the impact of heart disease and/or age on RAA system-related substances was no an objective of the feasibility study. Therefore, the inclusion of healthy adults was adequate to meet the goals of the study.

The decision to circumvent drug application was motivated by ethical and practical considerations. By pre-spiking, the blood collection tubes with the enalapril/enalaprilat solution, considerations regarding inter-individual differences in enalapril absorption and metabolism could be disregarded. This approach

additionally allowed for a less time-consuming conduct of the feasibility study on-site. As no additional time was required to allow for drug absorption and metabolism after an oral application, sampling procedures could start right away. The substantial influence of the haematocrit on the established range of acceptable accuracy is because no simultaneous determination of the haematocrit was performed. Without the possibility of individual adjustment of analytical results, the whole range of normal haematocrits for participants of both genders had to be considered. However, this approach highly simplified processes to obtain ethical approvals, as it reduced the risk to which volunteers were exposed without limiting the objectives of the feasibility study.

Corresponding to the expectations, the pharmacodynamic humoral parameters investigated were found to be above given physiological lower reference values and within normal ranges for most of the volunteers. Some samples exhibited elevated values of renin and plasma renin activity. RAA system-related substances are known to be influenced by, for example, circadian rhythm, physical activity and posture during sampling. The elevated results were attributed to the study design, which did not dictate a specific time of day for sampling nor a preceding resting period.

Applicability of the here presented concept to other studies depends on the respective substances of interest. With regard to tested peptides/hormones, their presence in healthy adults would determine whether this concept for a feasibility study is applicable. If these criteria are met, such a feasibility study can aid a main trials' start-up process with regard to the training of clinical staff and help evaluate as well as optimize planned procedures for studies with complex and demanding sampling procedures.

4. LENA paediatric trial performance—quality of PK/PD sampling and sample preparation

4.1. Introduction

Optimistic projections based on sample quality for routine laboratory testing assume that over 100,000 blood samples collected during the European clinical trials ongoing in 2014 had to be rejected by the analysing laboratory. These samples could not have been processed in a way that ensured the quality of data is preserved (Lippi et al. 2016). Although there have been increasing efforts to investigate and improve sample quality for routine laboratory testing in hospitals (Ashakiran, Sumati, and Murthy 2011; Bonini et al. 2002; Tapper et al. 2017), few data are available on the quality of clinical study samples. The evaluation of adherence to study SOPs in a multicentre epidemiologic study revealed that 9.3% of all blood samples were classified as haemolysed, 4% had insufficient volume, and one clinical site delayed processing 11% of samples (Peplies et al. 2011).

The circumstances under which study samples during trials are of special interest as this information can impact the interpretation of analytical results. Therefore, deviations from procedures described in the *Sample collection manual for LENA paediatric studies* had to be conscientiously documented during the paediatric LENA trials. To enable an efficient collection of this relevant information, the corresponding eCRF sections had been designed for all PK and PD samples to be drawn during the trials.

Promoting the quality of PK/PD samples obtained during the paediatric trials was one of the main scopes of the intense training efforts. To properly assess the

success of these efforts, the evaluation of the clinical teams' performance in these highly study outcome related areas is required. Therefore, the eCRF sections documenting the quality of the sampling and sampling preparation procedure have been analysed within an interim analysis. The share of paediatric study samples reportedly affected by deviations have been determined, serving as an indicator of the clinical teams' capability to perform related study procedures according to the predefined requirements.

4.2. Methods

To evaluate the adherence of study teams to sampling procedures outlined in the *Sample collection manual for LENA paediatric studies*, an interim analysis of relevant data captured in the eCRF has been performed. Therefore, the share of study samples affected by deviations from the established sampling and sample preparation procedures could be determined.

Reviewed trial period

The analysis was performed for samples originating from study visits performed until (and including) the date of June 1, 2017, and which had been entered to the eCRF until the time of excerpt extraction. This was 19 months since the Site Initiation Visit for the first clinical site to start the active trial phase and 11 months for the last site to start.

Information extracted from the eCRF

The eCRF provides a standardized form to document deviations from the procedures described in the *Sample collection manual for LENA paediatric studies.* This includes but is not limited to deviations from time limits until specimen freezing to be observed (30 min. and 15 min. respectively), temperature ranges to be applied during sampling/centrifugation, modes of centrifugation, and snap freezing.

The following section provides an overview on the information collected within these forms.

eCRF questions accessing sample quality for PK samples

1) *Sample done according to manual instructions?*

- *Yes/*
- *No/*
- *not done*

If option “No” is selected:

Specify sampling deviation:

- *Time interval from blood sampling to snap freezing out of allowed time frame/*
- *Other*

If option ”Time interval…” is selected:

Specify exact time of snap freezing: hh:mm

If option “Other” is selected:

Specify “Other”: (free-text field)

2) *Sample frozen according to manual instructions?*

- *Yes/*
- *No/*
- *not done*

If option “No” is selected:

Specify Deviation:

- *Transport not performed on ice/*
- *Other*

If option “Other” is selected:

Specify “Other”: (free-text field)

A screenshot of the respective eCRF form is provided in **Figure 15**.

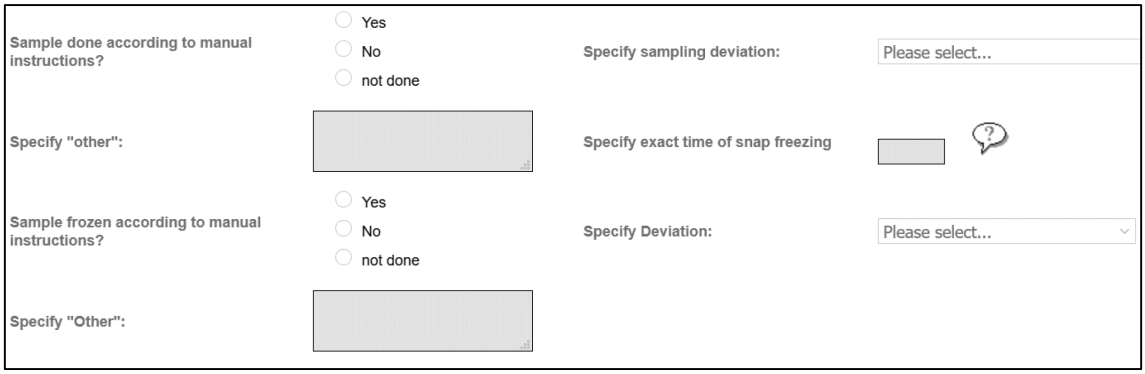

Figure 15 Screenshot of eCRF form assessing adherence to manual instructions for PK samples

eCRF questions accessing sample quality for PD samples

1) *Any sample(s) not done according to manual instructions?*

   - *Yes/*
   - *No/*
   - *not done*

   If option “Yes” is chosen, the following options become available for each PD sample (renin, plasma renin activity, and/or angiotensin I) chosen with a tick box:

   Specify sampling deviation:

   - *Time interval from blood sampling to snap freezing out of allowed time frame/*
   - *Other*

   If option “Time interval…” is selected:

   Specify exact time of snap freezing: hh:mm

   If option “Other” is selected:

   Specify “Other”: (free-text field)

2) *Any sample(s) not frozen according to manual instructions?*

- *Yes/*
- *No/*
- *not done*

If option “No” is selected:

Specify Deviation:

- *Transport not performed on ice/*
- *Other*

If option “Other” is selected:

Specify “Other”: (free-text field)

A screenshot of the respective eCRF form is provided in **Figure 16**.

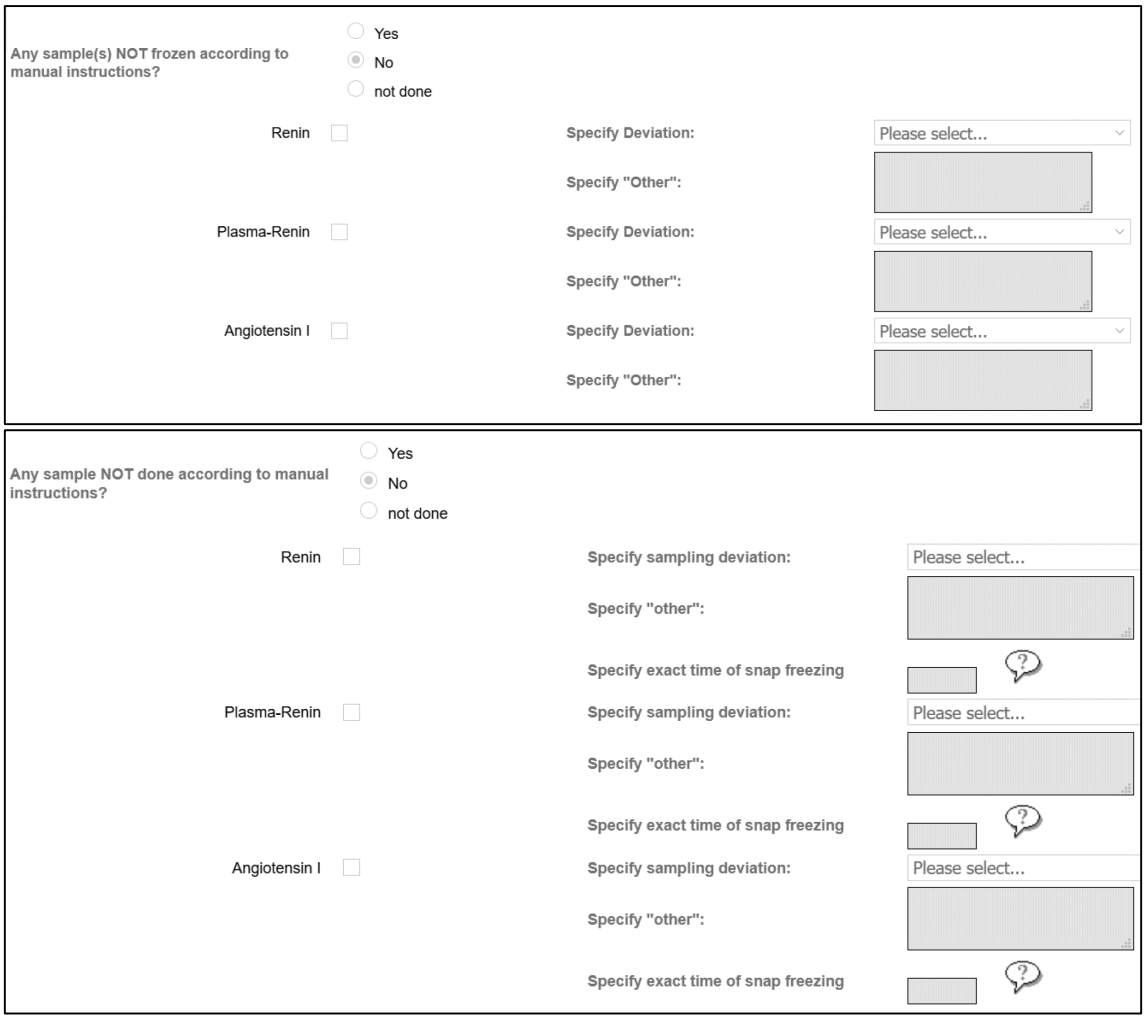

Figure 16 Screenshot of eCRF form assessing adherence to manual instructions for PD samples

Data analysis

Data extraction and review

This interim analysis was based on an excerpt of the eCRF database for the paediatric LENA trials. With regards to data privacy and confidentiality, the excerpt was carefully limited to the eCRF fields required for this evaluation.

Evaluation of eCRF entries

Both areas—sampling (including sample preparation) and freezing (addressed via questions 1 and 2 as in the respective eCRF form) have to be confirmed as being performed without deviations to qualify a sample as obtained according to manual instructions. Only in this case, adherence to manual instructions is given for the respective sampling procedure.

Subgroup analysis

In addition to the overall adherence to the applicable instructions for sampling and sample preparation for all samples obtained, the following subgroups of samples have been analysed:

1) Samples originating from the same clinical site
2) Samples with the same parameter of interest:
    - PK
    - Aldosterone
    - Renin
    - Plasma renin activity
    - Angiotensin I

Review of eCRF deviation entries

All deviations reported by the clinical site staff have been reviewed. In this case, the described incidents did not qualify as a deviation according to the applicable LENA manual, and the respective samples were reclassified. The tables and figures in the results section will show numbers as calculated *after* review of deviation comments and recalculation unless stated otherwise.

Consideration of combined sampling

For the PK substances enalapril/enalaprilat and the humoral parameter aldosterone, combined sampling is performed in case both parameters are to be sampled at the respective time point. Both analytic determinations are then performed from the same serum sample, with PK analysis being the first priority. Different scenarios are possible; either PK parameters and aldosterone or only one of them are to be determined from such a combined sample. This depends on the sampling time point as the designated sampling time points for aldosterone are consistent with those for the other PD substances.

Independent of how many analytes have been determined from such a combined sample, it has been considered only once for determination of the total amount of samples drawn as it represents only one single sampling procedure. Therefore, the total number of PK/PD samples per site is comprised of PK (and aldosterone) samples, aldosterone-only samples, renin, plasma, renin activity, and angiotensin I samples obtained. The subgroup analysis per parameter is not affected by this consideration.

Descriptive statistical calculations

The share of samples that had been reportedly sampled and frozen according to the manual has been determined and given as a percent of total samples obtained as well as in absolute numbers. Data capture and calculations have been performed in Excel.

Inconsistent data

ECRF data is subject to intense scrutiny during the paediatric LENA trials. Inconsistent or missing data are routinely queried by clinical monitors and responsible staff at the central laboratory. However, data inconsistencies detected during analysis that have not been already been subject of a query have been addressed with the clinical sites.

4.3. Results

The eCRF excerpt for this interim analysis was retrieved on September 18, 2017, from the LENA eCRF database. Personnel involved in this interim analysis had signed applicable nondisclosure agreements prior to gaining access to confidential eCRF information. Results have been independently verified by a second person. At the cut-off date chosen for the interim analysis (June 1, 2017), five clinical sites had completed at least one study visit including sampling for PK/PD.

Review of eCRF entries

Incidents reported by the clinical site staff have been checked to see if they qualify as deviations according to the laboratory manual. If they do not, the respective samples have been reclassified. An overview of incidents confirmed and rejected as deviations are provided in **Table 5** and **Table 6**.

Deviation	Site	Parameter of interest	(*n*) samples affected
Capillary blood instead of venous blood	1	PK + Aldosterone	7
	1	Renin	3
	1	Plasma renin activity	2
	1	Angiotensin i	2
Double centrifugation due to insufficient supernatant	1	PK + Aldosterone	1
	4	PK + Aldosterone	1
Overstepping of time limit from sampling to snap freezing	1	Renin	5
	1	Plasma renin activity	2
	1	Angiotensin I	1
	5	Renin	4
	5	Angiotensin 1	1
Haemolysed blood	1	Renin	1
	1	Plasma renin activity	1
Use of syringe (for transfer into Monovette)	1	Plasma renin activity	2
	1	Angiotensin I	1
Mix up of colour-coded cryostorage tubes (colour indicates parameter of interest)	5	Plasma renin activity	1
	5	Angiotensin I	1
Deviation of sampling sequence	2	Angiotensin I	1

Table 5 Incidents confirmed as deviations according to manual instructions

The reported collection of capillary blood has been the most extensive deviation reported as it is not only a deviation from the laboratory manual but from clinical protocol. The most commonly reported deviation has been the overstepping of time limits from sampling to freezing, outlined in the manual. Its clinical relevance may only become apparent after data analysis, if affected samples are identified as unexpected outliers. The same applies to samples characterised as haemolysed.

The central laboratory detected the reported mix-up of colour-coded storage tubes (the colours of which indicate the type of sample and the required analysis) in time to adapt the analytical processes.

The most common incident that was rejected as a deviation during the analysis was the specification of sampling material. Site 5 primarily classified the used sampling material (microtube or Monovette) by using the eCRF function to report deviations (**Table 6**). The eCRF design allows classification of the sampling material only once for all samples obtained at a designated time point. The clinical teams aimed to include the information regarding their switch of device during the sampling procedures by using the deviation reporting function, which, however, was contrary to its original scope.

Incident	**Site**	**Parameter of interest**	**(*n*) samples affected**
Stating of overstepping the time limit in cases were provided sampling/freezing times did not confirm this classification	5	Renin	1
	5	Plasma renin activity	2
	5	Angiotensin I	5
Switching of sampling device during attempt of sampling	5	Aldosterone	1
	5	Plasma renin activity	1
Missing (optional) B sample	2	PK + Aldosterone	2
Specification of sampling material (microtube for dripping vs. Monovette®)	4	Angiotensin I	1
	5	Renin	17
	5	Plasma renin activity	9
	5	Angiotensin I	23

Table 6 Incidents rejected as deviations according to manual instructions

The results shown in the following chapters depict the calculated share of samples obtained according to manual instructions after verification (and if necessary, reclassification) of reported deviations in the eCRF.

Inconsistent data

The eCRF data is subject to intense scrutiny. However, one case of inconsistent data was identified during the interim analysis. The same set of study-visit information was entered into the eCRF for two different patients of a clinical site. This included the date of the visit and unique sample identifiers. One of these double data sets has been excluded from analysis. The respective eCRF section was subject of a query to the respective clinical site.

Results for all obtained PK/PD samples

In total, 1977 PK/PD samples had been collected over the course of the paediatric trials until the cut-off date for the interim analysis (**Table 7**). Samples obtained per site vary, depending on the number of patients recruited and individual on-site periods of study activity. The majority, 1001 (50.6%) samples, originated from Site 5, the site scheduled to recruit the most patients. Sites 1 to 4 each obtained between 172 (8.7%) and 304 (15.4%) of all PK/PD samples.

According to the performed interim analysis, a very satisfying 98.1% of all samples obtained were sampled and prepared according to the applicable instructions from the *Sample collection manual for LENA paediatric studies* (**Figure 17**). The most common deviation reported was the overstepping of time limits stated in the manual, applicable for the time frame between sampling to snap freezing of supernatant, which affected 13 samples in total (**Table 5**). Remarkably, Site 3 did not report one single deviation for the whole evaluated time period. Except for Site 1, all other clinical sites achieved an adherence to instructions of above 99%. Site 1 achieved noticeably less (87.7%), mainly due to the collection of capillary blood instead of venous blood (14 samples affected). Aside from nonadherence to the sample collection manual, this represents a deviation from the approved clinical study protocol. These deviations had been discovered only after already 14 samples had been affected. The clinical team was retrained accordingly. This interim analysis found that after the retraining, no further capillary blood samples were obtained by the respective team. Apart from the deviations concerning capillary blood, the results do not suggest a significant difference in adherence to sampling instructions among the clinical sites. Detailed results for subgroup

analysis according to parameter of interest are presented in the subsequent sections.

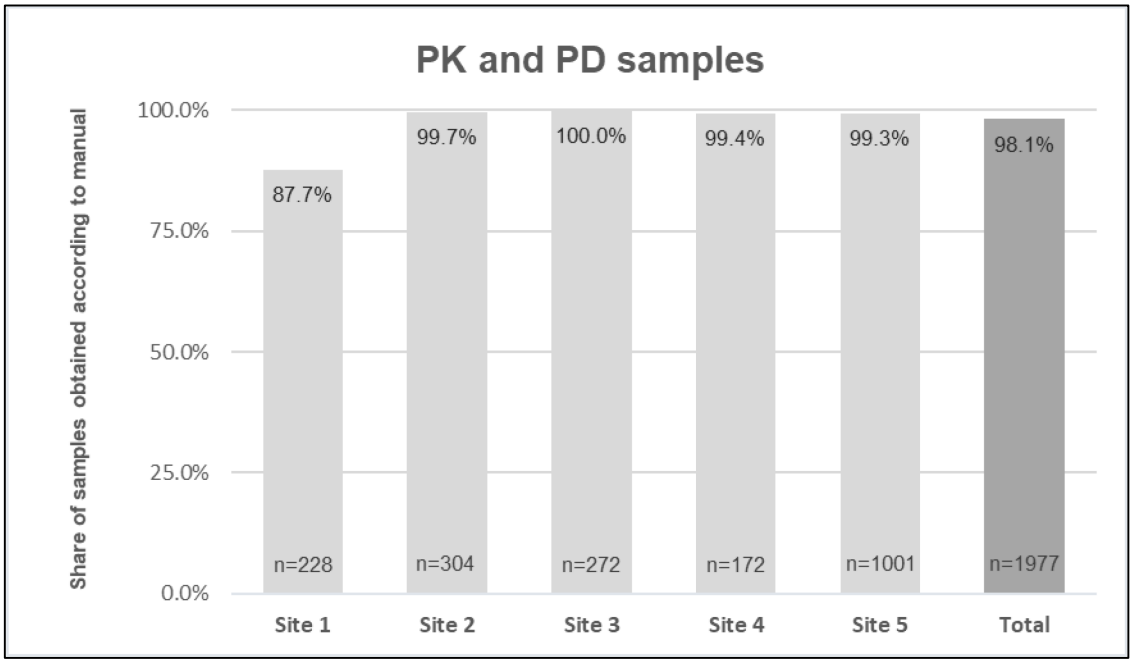

Figure 17 Evaluation of sample quality for all PK/PD samples obtained

All PK/PD samples	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Total**
Number of samples obtained	228	304	272	172	1001	1977
Sampling deviations*	28 (12.3%)	1 (0.3%)	0 (0%)	1 (0.6%)	5 (0.5%)	35 (1.8%)
Freezing deviations*	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (0.2%)	2 (0.1%)
Number of samples obtained according to manual	200 (87.7%)	303 (99.7%)	272 (100%)	171 (98.4%)	994 (99.3%)	1940 (98.1%)

* *No cases of simultaneous sampling and freezing deviation reported*

Table 7 Evaluation of sample quality for all PK/PD samples obtained

Results for all obtained PK samples

PK investigations are the primary objective of the paediatric LENA trials. Consequently, PK (and aldosterone) samples are the most frequently obtained sample type. They made up 30.4% of all samples collected within the evaluated trial period, 601 in total (**Table 8**). Overall adherence to procedures described in the applicable manual for obtained PK samples is 98.5%, with individual results for clinical sites varying between 89.3% and 100% (**Figure 18, Table 8**). In line with the overall results (**Figure 17**), lowest adherence has been reported at Site 1. Sites 2, 3, and 5 achieved 100% adherence to manual instructions.

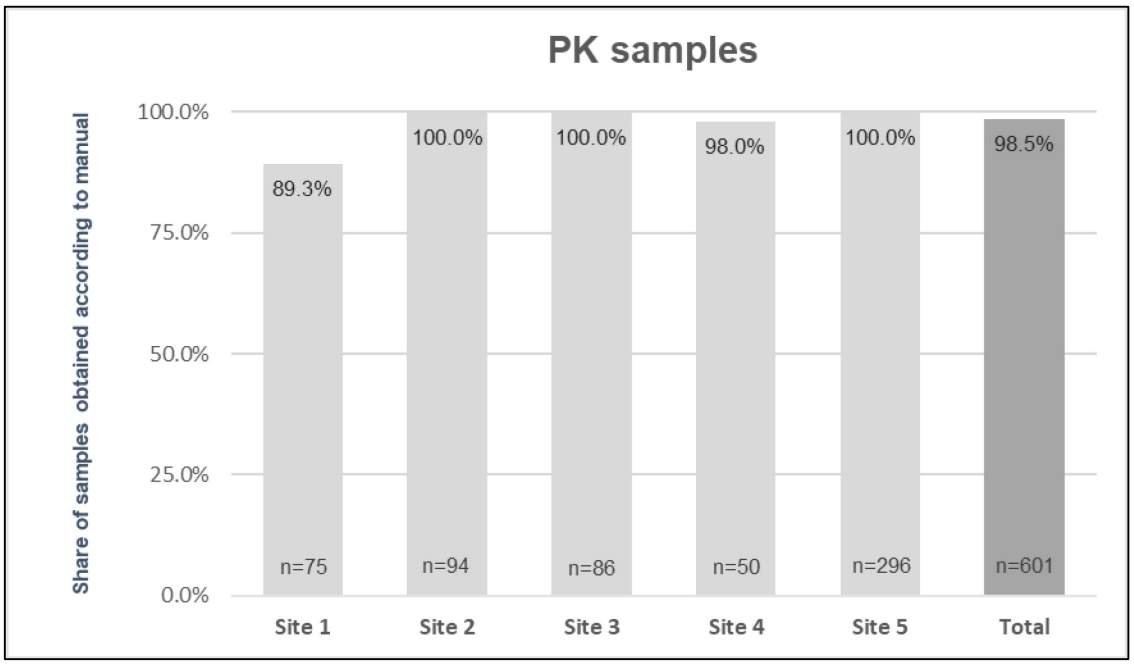

Figure 18 Evaluation of sample quality for all PK samples obtained

PK samples	Site 1	Site 2	Site 3	Site 4	Site 5	Total
Number of samples obtained	75	94	86	50	296	601
Sampling deviations*	8 (10.7%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	9 (1.5%)
Freezing deviations*	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Number of samples obtained according to manual	67 (89.3%)	94 (100%)	86 (100%)	49 (98%)	296 (100%)	592 (98.5%)

* *No cases of simultaneous sampling and freezing deviation reported*

Table 8 Evaluation of sample quality for all PK samples obtained

Results for all obtained aldosterone samples

The obtaining of blood for aldosterone determinations is regularly included in the PK (and aldosterone) samples for the designated time points where sampling of PD parameters are scheduled. So, the obtained amount of samples is very similar for the four PD parameters investigated. The total number of aldosterone samples is 458. This includes 23 “aldosterone only” samples, obtained at occasions where PK investigations were no longer performed due to discontinuation of study medication. One originated from Site 1 and 22 from Site 5. No deviations were reported for any of these. Overall adherence to manual instructions for all aldosterone samples collected ranges from 86.2% to 100% and is 98.0 % overall (**Figure 19**, **Table 9**).

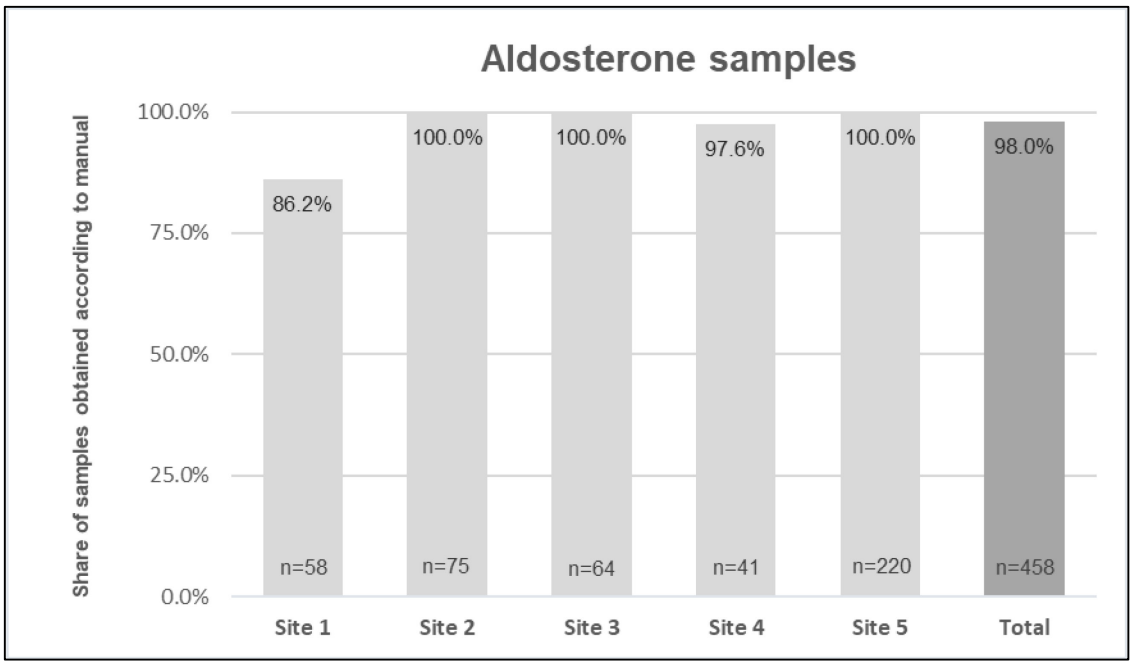

Figure 19 Evaluation of sample quality for all aldosterone samples obtained

Aldosterone samples	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Total**
Number of samples obtained	58	75	64	41	220	458
Sampling deviations*	8 (13.8%)	0 (0%)	0 (0%)	1 (2.4%)	0 (0%)	9 (2.0%)
Freezing deviations*	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Number of samples obtained according to manual	50 (86.2%)	75 (100%)	64 (100%)	40 (97.6%)	220 (100%)	449 (98.0%)

* *No cases of simultaneous sampling and freezing deviation reported*

Table 9 Evaluation of sample quality for all aldosterone samples obtained

Results for all obtained renin samples

From 456 renin samples collected during the evaluated trial period, 97.1% were reported to be obtained according to manual instructions (**Figure 20**, **Table 10**). Sampling for this substance was least successful overall, with a reported adherence of > 98%. As for the other types of samples, three sites (2, 3 and 4) reported 100% adherence. Site 1 reported its highest share of deviations for a given type of sample, 17%.

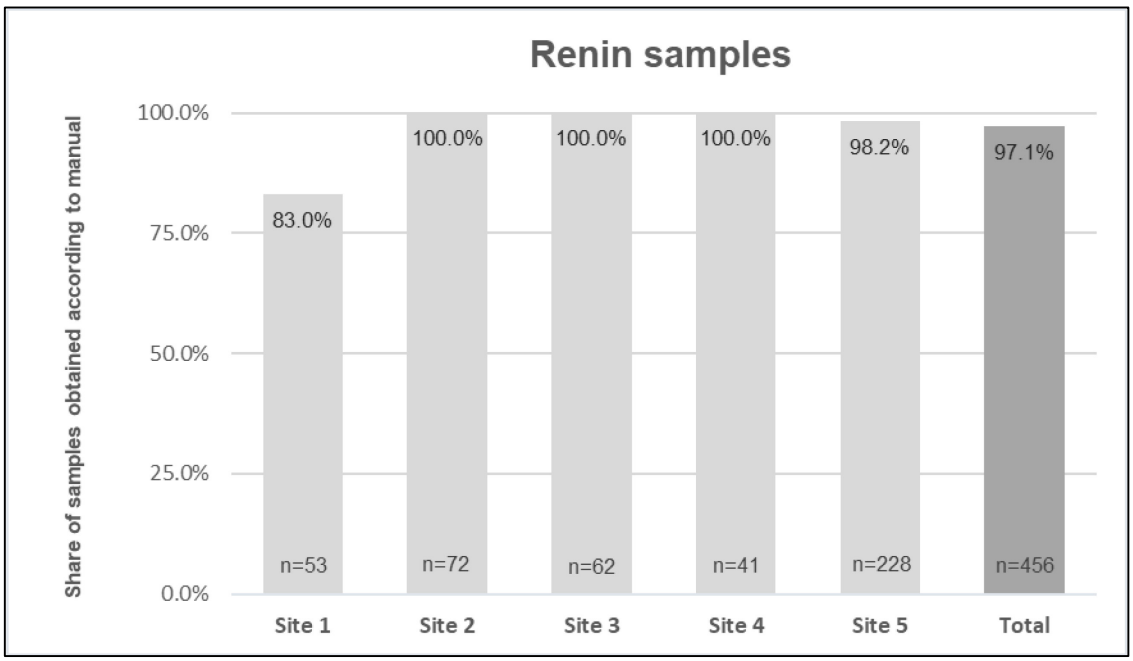

Figure 20 Evaluation of sample quality for all renin samples obtained

Renin samples	Site 1	Site 2	Site 3	Site 4	Site 5	Total
Number of samples obtained	53	72	62	41	228	456
Sampling deviations*	9 (17%)	0 (0%)	0 (0%)	0 (0%)	2 (0.9%)	11 (2.4%)
Freezing deviations*	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (0.9%)	2 (0.5%)
Number of samples obtained according to manual	44 (83%)	72 (100%)	62 (100%)	41 (100%)	224 (98.2%)	443 (97.1%)

* *No cases of simultaneous sampling and freezing deviation reported*

Table 10 Evaluation of sample quality for all renin samples obtained

Results for all obtained plasma renin activity samples

From 450 plasma renin activity samples obtained during the evaluated period of time, 98.2% were reported to be obtained and prepared according to manual instruction (**Figure 21**, **Table 11**). Sites 2, 3, and 4 reported 100% adherence without a single deviation. The highest share of deviations was reported by Site 1 with 14.3%.

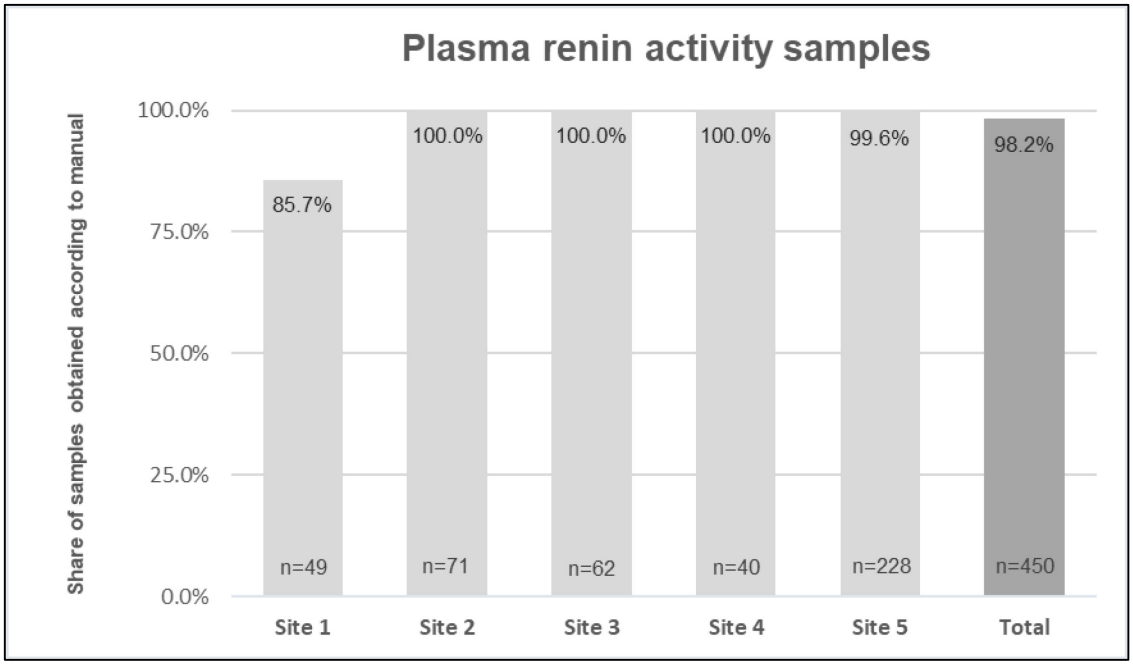

Figure 21 Evaluation of sample quality for all plasma renin activity samples obtained

Plasma renin activity samples	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Total**
Number of samples obtained	49	71	62	40	228	450
Sampling deviations*	7 (14.3%)	0 (0%)	0 (0%)	0 (0%)	1 (0.4%)	8 (1.8%)
Freezing deviations*	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Number of samples obtained according to manual	42 (85.7%)	71 (100%)	62 (100%)	40 (100%)	227 (99.6%)	442 (98.2%)

* *No cases of simultaneous sampling and freezing deviation reported*

Table 11 Evaluation of sample quality for all plasma renin activity samples obtained

Results for all obtained angiotensin I samples

From 447 angiotensin I samples obtained in total, 98.4% have been found to be obtained according to manual instructions. Results from the individual sites vary between 92.0% and 98.4% (**Figure 22**, **Table 12**). Angiotensin I is the parameter sampled by far most successfully at Site 1, with 92.0% adherence to instructions.

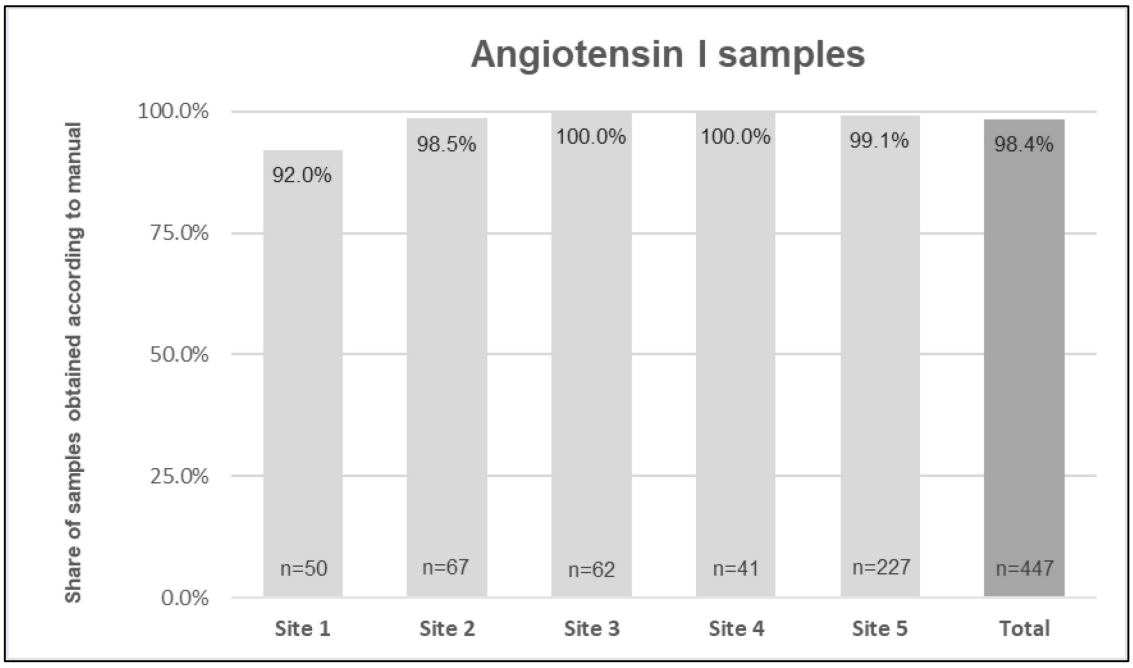

Figure 22 Evaluation of sample quality for all angiotensin I samples obtained

Angiotensin I samples	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Total**
Number of samples obtained	50	67	62	41	227	447
Sampling deviations*	4 (8%)	1 (1.5%)	0 (0%)	0 (0%)	2 (0.9%)	7 (1.6%)
Freezing deviations*	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Number of samples obtained according to manual	46 (92%)	66 (98.5%)	62 (100%)	41 (100%)	225 (99.1%)	440 (98.4%)

* *No cases of simultaneous sampling and freezing deviation reported*

Table 12 Evaluation of sample quality for all angiotensin I samples obtained

4.4. Discussion

The overall self-reported adherence to studies sampling manual was determined as excellent 98.1% for all PK/PD samples obtained. Shares of samples successfully obtained according to manual instructions have been determined as 98.5% for PK, 98.0% for aldosterone, 97.1% for renin, 98.2% for plasma renin activity, and 98.4% for angiotensin I. These results do not suggest significant differences in sample quality for the different parameters investigated but are in line with the premise that renin samples are the most challenging to prepare within the given time frame.

The share of samples successfully prepared varies between clinical sites. A considerable share of deviations that occurred at Site 1 are related to the collection of capillary blood instead of venous blood when difficult venous conditions did not allow for venipuncture. As the comparability of concentration levels between venous and capillary blood have not been investigated for either of the substances of interest, the study protocol specified venous blood as the single eligible sample type for study samples. Although the clinical team was alerted after the deviation has been discovered, 14 samples were already affected. This represents a substantial share (6.1%) of all 228 samples originating from this site. In consequence of this protocol deviation, the affected samples had to be excluded from analysis. However, this exclusion of data points did not affect the evaluability of the respective patients for primary and secondary study objectives.

Aside from these samples, there is no conspicuous accumulation of deviations in one site, though the 100% adherence reported in Site 3 is a notable exception. But as data from clinical trials is the object of intense scrutiny (e.g., verified by clinical monitors), it is to be assumed accurate. At the cut-off date for this interim analysis,

almost 70% of envisaged patients had been recruited project-wide, and the majority of samples envisaged had been obtained. Therefore, these results can be presumed to be a reliable estimate of the overall sample quality achieved during the paediatric trials.

The comparison of results with other clinical studies is hardly feasible due to a trial's individual characteristics. Pre-analytical variables to be considered and the difficulty of adequately assure them during trial conduct vary depending on the parameters of interest and objectives of the investigation. Therefore, the challenge to comply with relevant study manuals and SOP may differ significantly between trials. A publication generally advising on standardisation of pre-analytical sample preparation states a time frame of two to four hours until freezing the specimens as a realistic target (Yin, Lehmann, and Xu 2015), which highlights the extraordinary requirements accomplished during the paediatric LENA trials. In any case, trial-specific evaluation and standardisation of relevant pre-analytical variables ahead of trial conduct is strongly recommended (Lippi et al. 2016; Banfi and Lippi 2016; Dakappagari et al. 2017). Though retrospective analysis regarding the frequency and potential effect of sample collection and sample processing on biomarker results in a clinical study has been performed occasionally (Peplies et al. 2011), the impact of pre-analytical variability in clinical trials is still underestimated and often not addressed appropriately (Banfi and Lippi 2016).

The results of this interim analysis are very positive, suggesting that procedures for sampling and sample preparation were adequately trained. The teams from all five sites for which the quality of samples was evaluated participated in the LENA simulation training, and four of them also completed the feasibility study. Although

the results are very promising, some limitations have to be kept in mind. For example, the analysis does not account for planned samples that could not be obtained (e.g., due to clotting of the line) or were missed for other reasons. Furthermore, it does not include the perspective of the central laboratory—for example, the number of samples classified as haemolysed by laboratory staff or whether the samples had enough volume to obtain a valid analytical result. Nonetheless, it provides valuable insight into the overall quality of trial conduct and the successful implementation of standardised procedures for study-related sampling.

5. Discussion

The training concept for the paediatric LENA trials enabled the collection of high-quality data right from the beginning of the paediatric trials. The impact of the simulation training was perceived as long-lasting and substantially contributing to the trial performance by the participants themselves but was additionally verified by an on-site feasibility study and objective site performance criteria captured in the eCRF during trial conduct.

With regards to sampling and sample preparation, the learning curve typically passed through by clinical teams at the beginning of a clinical trial has been successfully shifted ahead of the recruitment period for the paediatric LENA trials. It has been found that due to this learning curve for clinical staff, queries and protocol deviations are significantly more common in the first patients included at a clinical site (Taekman et al. 2010; Macias et al. 2004). Apart from further investigations regarding this effect, the authors recommend its consideration during the trial planning process as it may have an extensive impact on patient safety, ethics, and research quality as well as economic implications for trial conduct (Macias et al. 2004). The tailored LENA training concept accounted for the expected learning curve and aimed to shift it ahead of active trial conduct. With regards to the special vulnerability of the paediatric population and the sensitivity of substances investigated, it ensured clinical staff were appropriately experienced in the most critical trial procedures before including the first patient.

To complement the findings of the performed survey-based evaluation, further investigations regarding the impact of the communication-based training on trial

outcome objectives are recommended. For example, ratios of screened or approached to recruited patients could be of interest to further evaluate the impact of the training. Though recommended to be regularly included into publications of clinical trials, a great share of trials have been found to not provide this information, even though it can provide valuable insights into recruitment efforts (Gross et al. 2002). With clinical trials typically requiring almost double the planned timelines (Getz 2015) to complete recruitment and up to 29% not finally reaching envisaged patient numbers (Carlisle et al. 2015), information on recruitment periods could also be of interest to evaluate the effect of training efforts.

The LENA training elements have been embedded into the study start-up phase for the paediatric clinical trials. This added a further burden to this already resource-intensive and time-critical stage of trial conduct (Krafcik, Doros, and Malikova 2017). But by scheduling training appointments in consideration with the individual site timeline for trial start-up, the training concept could be seamlessly integrated into trial preparations and not delay the start of recruitment. Though further research is required, obtained results suggest that additional investment of time and resources into trial-specific training results in more effective trial conduct later on.

6. Conclusion

The tailored training concept developed for the paediatric LENA trials has been shown to have a substantial impact on the clinical teams' performance and study conduct. The modular concept enabled the efficient acquisition of skills required and the upfront investigation of on-site abilities to appropriately conduct complex sampling and sample preparation procedures as well as the verification of sample logistics and bioanalytical processes. It facilitated the determination of bottlenecks and optimised processes without endangering vulnerable paediatric patients and also helped avoiding low-quality data in the pivotal studies.

With increasing complexity of clinical trials, and given the rising financial risks attached (Collier 2009), new preparation and training concepts are required to increase the share of successfully completed studies. Tailored training modules in the form of simulation training and feasibility studies for the most critical components of a trial prove to be a promising tool to facilitate successful and high-quality conduct for studies with complex procedures, exceeding clinical routine.

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9. Appendix

Parts of this thesis have already been published in international journals or were previously presented at conferences:

Publications:

Ciplea AM, Laeer S, Burckhardt BB, A feasibility study prior to an international multicentre paediatric study to assess pharmacokinetic/pharmacodynamic sampling and sample preparation procedures, logistics and bioanalysis (manuscript submitted to Contemporary Clinical Trials Communications, April 2018)

The first author Agnes M. Ciplea substantially contributed to the development of the concept and the study protocol. She was responsible for the conduct of the investigation, data interpretation and the drafting of the manuscript.

Poster and oral presentations:

15th Congress of the European Society for Developmental, Perinatal and Paediatric Pharmacology (ESDPPP), 23-26 June 2015 (Belgrade, Serbia)
“Development and establishment of a quality-framework for the LENA project” - by A. M. Ciplea, K. Kleine, B. B. Burckhardt, S. Läer, J. Breitkreutz, L. Špatenková, I. Klingmann – [Poster]

50th Annual Meeting of the Association for European Paediatric and Congenital Cardiology (AEPC); 01-04 June 2016, (Rome, Italy)
“A novel simulation-based approach to train study teams for clinical trials in neonates, infants and children with heart failure“ - by Burckhardt BB, Ciplea AM, Laven A, Klingmann I, Laeer S, Lagler F. - [Poster]

Annual meeting of the *Deutsche Pharmazeutische Gesellschaft* (DPhG); 05-07 October 2016, (Munich, Germany)

“The PILOT-Study – part of the LENA project, a dry-run as training-concept for paediatric clinical studies with complex sampling requirements” – by A.M. Ciplea, B. Burckhardt, L. Ablonczy, J. Breur, M. Burch, M. Dalinghaus, S. deWildt, K. Kleine, I. Klingmann, V. Swoboda, A. Szatmari, S. Läer – [Poster]

16th Congress of the European Society for Developmental, Perinatal and Paediatric Pharmacology (ESDPPP), 20-23 June 2017 (Leuven, Netherlands)

“Novel tailored training concept to facilitate successful study conduct and optimize recruitment in paediatric clinical trials” – by Burckhardt BB, Ciplea AM, Kleine K, Ablonczy L, Breur JMPJ, Dalinghaus M, van der Meulen M, Klingmann I, Spatenkova L, Swoboda V, Bajcetic M, Keatley-Clark A, Laeer S, Lagler F - [oral presentation]

Annual Meeting of the American Heart Association (AHA), 11-15 November 2017 (Anaheim, CA, USA)

“Innovative training concept to overcome main reasons for failed pediatric clinical trials and to facilitate successful study conduct and recruitment” – by Burckhardt, B.B.; Ciplea A.M.; Laven, A.; Kleine, K.; Ablonczy, L.; Breur, JMPJ; Dalinghaus, M.; van der Meulen, M.; Klingmann, I.; Spatenkova L.; Swoboda, V.; Bajcetic, M.; Keatley-Clark, A.; Läer, S.; Lagler, F. B. - [Poster]

Annual Meeting of the American Society for Pharmacology and Therapeutics (ASCPT), 21-28 March 2018 (Orlando, F, USA)

“Tailored simulation training as an innovative training concept to facilitate successful study conduct and recruitment encompassing its assessment” – by Burckhardt, B.B.; Ciplea A.M.; Laven, A.; Kleine, K.; Ablonczy, L.; Breur, JMPJ; Dalinghaus, M.; van der Meulen, M.; Klingmann, I.; Spatenkova L.; Swoboda, V.; Bajcetic, M.; Keatley-Clark, A.; Läer, S.; Lagler, F. B. - [Poster]