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CLINICAL INVESTIGATION

Blood volumes in pediatric clinical trials: a review of current regulations and guidance for research studies

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Significant progress is being made in advancing the development of clinical research in pediatrics. In continuing this trend, we must safeguard the interests and welfare of children participating in increasingly expansive and complex clinical trials designed to maximize clinical and scientific outputs. The potential impact of taking multiple blood samples and sometimes substantial blood volumes from children is at present unclear. Available guidelines define safe limits of 1–5% total blood volume, but these guidelines vary and are not evidence-based. The current review looks at the basis for these guidelines, summarizes previously published studies directly addressing this important issue and discusses factors that should be considered in the design and implementation of clinical research involving blood sampling in children.

Keywords: biological samples • blood volumes • children • clinical trial design • ethics • minimal risk • pediatrics

Over the past two decades, significant progress has been made to advance the development of clinical trials and research in pediatrics. Of particular note, regulatory and legislative changes in Europe and the USA, in the form of the EU Pediatric Regulation and the Best Pharmaceuticals for Children Act, respectively, have encouraged the development of new treatments for children and positively impacted the number of clinical studies conducted in a pediatric setting [1,2]. It is hoped that this trend will continue, with greater numbers of new drugs being trialed in children leading to improved pediatric drug labeling and an increased number of new therapeutics being more widely available for use in children across all disease types. Facilitating the development and availability of medicines for children, however, also brings with it significant challenges. While ensuring that clinical trials are carried out in pediatric patient populations, to allow the authorization and safe use of new drugs in children of all ages, we must also safeguard the interests and welfare of all children who take part in clinical research, both in terms of clinical trials of investigational medicinal products and more widely. The current report is predominantly designed to review current guidelines relating to the collection of blood samples in children of all ages. While this issue is particularly pertinent in the case of neonates and infants it is not our intention to provide a specific focus in this age group.

Clinical research in the modern era

Modern day clinical trials have advanced considerably in terms of level of complexity and the number of additional 'substudies' that are frequently built into the trial design to learn as much as possible about the impact of new treatments on the target patient population. In addition to the routine collection of multiple blood samples as part of the clinical care of the patient, clinical samples are increasingly sought for a wide range of testing in areas such as biomarker and pharmacodynamic studies, pharmacogenomics, pharmacokinetics, cytogenetics and biobanking to facilitate future research projects. Many if not all of these clinical trial substudies will require the collection of specific biological samples,

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of which blood is the most commonly sought. Indeed, many of these additional studies will frequently request the collection of multiple samples over a defined time period in order to answer important scientific questions and maximize the clinical benefit of the new treatment, either for the patient on study or for those who will receive similar treatment in the future. As an example of the potential complexity of running a clinical trial to answer important scientific questions in the setting of childhood cancer, Table 1 summarizes the requirement for clinical samples for children with childhood acute lymphoblastic leukemia (ALL) being treated on an ongoing European trial (UKALL 2011; ISRCTN identifier: 64515327). While patients will not provide clinical samples for all of the optional substudies shown in Table 1, the study provides a good example of how complex many trials can be. Childhood ALL provides an arguably unparalleled example of how advances in drug development and clinical research can positively impact on the successful treatment of children with cancer, with long-term survival rates having increased from 10 to 90% over the past 50 years [3]. These advances have been achieved on the back of well planned and intuitive trials such as UKALL 2011, leading to the inclusion of increased numbers of effective drugs and drug combinations which have positively impacted on patient outcome.

With increasing numbers of complex and challenging clinical trials being carried out in pediatric patient populations, in addition to clinical research taking place outside of clinical trials of investigational medicinal products, a fundamental question that may be raised relates to the potential impact of taking multiple blood samples and sometimes substantial blood volumes from children. While we should embrace the running of intuitive clinical research projects and drug development trials in pediatrics, it is clearly important that clinicians and researchers understand the importance of ensuring the safety and welfare of the patients involved, particularly in the case of younger children. In some instances it may be possible to utilize data obtained from studies in adults to design trials in such a way as to reduce the number of children that need to be studied and/or minimize the requirements for collection of blood samples and volumes. Guidance on the extrapolation of data is available from the EMA [4] and an excellent review paper on the subject has been published by the US FDA [5]. Similarly, it may be feasible to design trials to predominantly involve the recruitment of older children as opposed to younger patients, where repeated blood sampling and/or the collection of significant blood volumes is less likely to be a concern. However, there will always be clinical situations where it is necessary to include younger children, infants and neonates. For example, in the field of oncology there are numerous tumor types, including neuroblastoma and retinoblastoma, which are very rare in patient populations other than infants and young children [6,7]. For clinical trials involving these very young patients in particular, it is critical that guidance is available to clinicians and researchers as to what are deemed to be safe blood sample volume limits. Although in some areas the collection of less invasive biological fluids, including urine, saliva and sweat, may provide potential alternatives to blood sampling, the collection of whole blood remains a requirement of the majority of clinical research studies.

Current guidelines

For a clinical trial to take place it will generally need to pass rigorous ethical and regulatory review to ensure

Table 1. Clinical sample collection for children with childhood acute lymphoblastic leukemia being treated on an ongoing European trial (UKALL 2011; ISRCTN identifier: 64515327).

Test	Clinical sample	Optional
Morphology†	Blood/bone marrow	No
MRD†	Bone marrow/blood	No
Flow cytometry	Blood/bone marrow	No
Cytogenetics	Bone marrow	No
Asparaginase study†	Blood/bone marrow aspirate	Yes
TPMT genotyping	Blood	No
Thiopurine study†	Blood	Yes
Dexamethasone PK study†	Blood/saliva	Yes
Vincristine PK study†	Blood/saliva	Yes

†Samples collected at multiple time points and/or stages of treatment (diagnosis, induction, consolidation and maintenance therapy). Blood volumes for individual tests range from 2 to 30 ml.
MRD: Minimal residual disease; PK: Pharmacokinetic; TPMT: Thiopurine methyltransferase.

that provisions are in place to protect the well-being and safety of the study participants. In this respect there are clear differences in carrying out research in children as compared with adults, one of which relates to differences in what is deemed to be an acceptable level of risk for children participating in clinical trials, with international guidelines advocating 'minimal risk' standards which should be adhered to [8–10]. With respect to the question of what are safe limits of blood that can be drawn in the pediatric setting, the ethical and regulatory bodies themselves will need clear guidelines by which they can review clinical trial submissions. One could argue that it is impossible to define 'risk' without knowing what defines safe limits of blood volume loss. But what guidelines or policies are available for these bodies to base their decisions and to what extent are the guidelines available evidence-based? The availability of international guidelines on safe blood sample volume limits for pediatric clinical research has recently been reviewed by Howie, who identified nine relevant policies through a literature search-based approach [11]. These policies were obtained from various different children's hospitals, universities and healthcare partners, predominantly from North American institutions, and in general identified blood volume limits of 1–5% total blood volume either on a single blood draw or over a 24-h period and a maximum of ≤10% over an 8-week period. In Europe, requirements for the conduct of EU clinical trials are covered in Directive 2001/20/EC, with a guidelines document produced in 2008 concerning ethical considerations for clinical trials on medicinal products conducted within the pediatric population [12]. This document states that trial-related blood loss should not exceed 3% total blood volume during a period of 4 weeks and should not exceed 1% at any single time. With total blood volume generally accepted as being most appropriately estimated at 80–90 ml/kg body weight, a total of 3% total blood volume equates to approximately 2.4 ml blood per kg body weight [12]. However, these European guidelines also state that the limits recommended are not evidence-based and that any deviation from these guidelines should be justified by the investigator responsible for the trial. Clearly this gray area relating to deviations from the guidelines and the lack of available evidence-based knowledge can make the task of approving clinical trial applications somewhat challenging. But is there a way that definitive data could be generated to scientifically support the somewhat vague guidelines currently available?

Studies investigating the impact of blood sampling in children

To date, very few studies have been published that have attempted to directly address the potential impact of frequent blood sampling in children [13–16]. These include a retrospective study in 11 cancer patients aged 1–19 years [13] and a prospective study in girls with central precocious puberty aged 1.6–9.2 years [14]. Both of these studies assessed hemoglobin levels alongside additional hematological clinical parameters in patients participating in clinical trials at appropriate intervals following withdrawal of serial blood volumes. The study by Cole *et al.* [13] in a childhood cancer setting was limited by the small number of patients involved and was confounded by variation in clinical practice regarding indications for blood transfusions, but benefited from including patients with a wide range of blood volumes drawn for research purposes, from 1 to 10% of total blood volume. Data indicated a trend toward removal of blood samples for research purposes, in addition to those taken for routine clinical care, and a fall in hemoglobin levels in children. However, no relationship was observed between the actual decreases in hemoglobin observed and the percentage of total blood volume taken from individual patients. In addition, the age of the patient did not seem to be a factor influencing the clinical impact of serial blood sampling in this small patient cohort.

The more recently published study by Broder-Fingert *et al.* [14] involved the collection of hematological data from patients undergoing frequent venous sampling at 3–6-month intervals over a 3–4-year period, with a blood volume limit of 9–10 ml/kg defined (~12% of total blood volume). Data obtained from this study suggested similar falls in hemoglobin levels following blood sampling to the study by Cole *et al.*, but indicated a recovery in levels at 3-month follow-up and no significant differences in levels of hemoglobin or additional hematological parameters over the duration of the study. While the authors concluded that no significant adverse effects attributable to serial blood sampling were observed in this trial, it should be noted that patients did receive an equivalent volume of saline to replace the blood collected on each draw and were also treated with iron supplementation for 6 weeks after each hospital visit. While no definitive conclusions could be drawn from either of these published studies therefore, it is interesting to note that neither study showed a clear detrimental effect on the study participants, despite the fact that the blood volumes taken were markedly in excess of those recommended in the various guidelines available. These findings, although from a very limited number of studies involving relatively few patients, can provide us with some confidence that the guidelines currently available are appropriate, arguably erring on the side of caution as opposed to being overly liberal. However, although challenging in their design, well-planned prospective

studies involving larger patient numbers are required to more appropriately assess and define safe blood volume limits in children of all ages.

Considerations in carrying out clinical research in children

With a certain lack of clarity concerning what reflects acceptable clinical practice in relation to blood sample volumes taken for research studies, it is imperative that researchers take every opportunity to minimize the likely impact on the study participants. Box 1 highlights a number of factors that should be considered when designing and conducting clinical research in a pediatric setting. While some of these factors may be specific to particular types of clinical trials, they act to highlight ways by which the potential clinical impact of carrying out clinical studies in children can be minimized as much as possible. This subject has previously been discussed in some detail by Hawcutt *et al.* [17].

One consideration that is not always appreciated in the design of clinical research projects and trials relating to the issue of safe limits for blood volumes is that while a study protocol will identify samples that need to be taken as part of the study itself, there are additional sampling demands on the patient. For children taking part in clinical research in a hospital setting there are likely to be blood draws that need to be made as part of the routine clinical care of the patient that will be made outside of the research protocol stipulations. In addition it is important to consider the difference between taking a single large blood volume and the collection of multiple smaller blood volumes from a central line or intravenous catheter. From experience in the setting of pediatric oncology in the UK, significant differences in practice exist between centers as to whether deadspace samples (taken immediately prior to the collection of a blood sample for clinical or research purposes) are returned to the patient or discarded and as to the deadspace volume that is taken. This issue was investigated in a study by Cole *et al.*, involving 70 children with a range of different types of central venous catheter inserted as part of their standard clinical treatment [18]. The study showed that the withdrawal of a 3 ml deadspace or discard volume was sufficient to ensure that the subsequent blood sample was not diluted or contaminated by residual intraluminal fluid. For hospitals routinely withdrawing 5 ml deadspace or discard volumes therefore, an additional 2 ml of blood is in effect being removed from the patient unnecessarily with each sample collection. This is a particularly relevant consideration for the conduct of pharmacokinetic studies in children where multiple blood draws are often required over a defined time frame.

Additional considerations that are likely to be applicable to the design and implementation of the vast majority of clinical research projects and trials involving blood sampling in children, include the collection of samples for research studies alongside those being taken for routine patient care wherever possible. This is applicable to the issue of deadspace volume highlighted above, but is also important in terms of minimizing the number of times a central line or intravenous catheter

Box 1. Factors to consider in the design and implementation of clinical research involving blood sampling in children.

- Clear justification for collection of all samples and clear definition of which samples are optional samples being taken for research purposes as opposed to patient care within the trial setting.
- Careful consideration of blood volumes being taken in any single 24-h period and across other time periods (as appropriate to the guidelines being adhered to).
- Consideration of the impact of deadspace/discard blood volumes that are drawn prior to clinical samples being taken on the cumulative blood volume collected (minimal deadspace volumes should be taken).
- Collection of samples for research studies alongside those being taken for routine clinical care of the patient to avoid multiple blood draws and reduce deadspace/discard blood volume waste.
- Potential for using single blood sample for multiple tests as opposed to drawing multiple samples (may be possible to separate different blood constituents if appropriate).
- Utilization of procedures to minimize any pain and distress associated with blood sampling (use of intravenous catheters, collection from central catheters if in place, use of local anesthesia for needle sticks, etc.).
- Inclusion of reduced or limited sampling approaches for infants and smaller children based on previously published studies in children and adults where appropriate.
- Development of assays that allow for reduced blood volumes and/or sparse sampling approaches (may allow for finger prick blood collection/use of dried blood spots).
- Consideration of less-invasive alternatives to blood collection for research studies, for example, saliva for extraction of genomic DNA.
- Use of population pharmacokinetic studies involving larger numbers of children but a reduced number of blood samples collected from individual patients.

needs to be accessed and particularly in situations involving venepuncture for blood collection. It may also be possible in certain situations to utilize a sample collected from a single blood draw for several tests by separating out the different constituents (plasma, white blood cells, etc.), providing the integrity of the individual constituents is maintained prior to sample analysis. However, this may not be applicable to all studies and individual scenarios need to be assessed appropriately. Researchers need to be able to articulate why blood collection and analysis is required and how risk is minimized for study participants. Where applicable, it may be possible to consider less invasive techniques, such as the collection of genomic DNA from saliva samples as opposed to whole blood [19]. Finally, in clinical pharmacology studies the development of sensitive assays to allow the effective analysis of drug levels in minimal sample volumes, the use of limited sampling approaches based on available pharmacokinetic data obtained from studies in both adults and children, and the utility of population pharmacokinetic approaches should all be considered where appropriate, to minimize the need for frequent collection of blood samples within a narrow time frame [20].

Conclusion

While progress continues to be made in the development of clinical trials and research in pediatrics, it is important that we safeguard the interests and welfare of the children who take part in these studies. This is particularly relevant to many expansive and complex clinical trial designs that are increasingly

Executive summary

Background

- Significant progress has been made in advancing the development of clinical trials and research in pediatrics. While ensuring that this positive trend continues, we must also safeguard the interests and welfare of the children who take part in research studies. One particular concern relates to the requirement for multiple blood samples and sometimes substantial blood volumes as part of pediatric clinical trials.

Clinical research in the modern era

- In addition to the routine collection of multiple blood samples as part of the standard clinical care of the patient, clinical samples are increasingly sought for a wide range of testing in areas such as biomarker and pharmacodynamic studies, pharmacogenomics, pharmacokinetics, cytogenetics and biobanking to facilitate future research projects. This frequently involves the collection of multiple blood samples over a defined period of time to answer important scientific questions and maximize clinical benefit.

Current guidelines

- While guidelines exist and policies are available in Europe and the USA through government bodies, children's hospitals, universities and healthcare partners, it is accepted that these guidelines are largely not evidence-based.

Studies investigating the impact of blood sampling in children

- Only a limited number of studies have been published, which have attempted to directly address the potential impact of frequent blood sampling in children. While no definitive conclusions can be drawn from these studies, all of which have several limitations, the data available would suggest that current guidelines are generally appropriate.

Considerations in carrying out clinical research in children

- It is clearly imperative that researchers and clinicians take every opportunity to minimize the likely impact of blood sampling on children taking part in clinical trials and research studies. In designing studies and carrying out research, factors such as sample collection alongside those being taken for routine clinical care, use of single samples for multiple tests, use of alternative less invasive biological fluids and the development of assays that allow for reduced blood volumes or sparse sampling approaches should all be considered as appropriate.

Conclusion

- Guidelines that currently exist relating to blood sample volume limits are designed to ensure 'minimal risk' for participants and should be used appropriately to design pediatric clinical trials, with blood volume requirements clearly detailed in trial protocols and patient information sheets. However, it is accepted that these guidelines are largely not evidence-based and this issue should ideally be addressed in future studies.

Future perspective

- Although challenging in their design, well-planned prospective studies are required to more clearly define safe limits of blood loss over appropriate periods of time relevant to different clinical trial settings. In the meantime a continued focus on developing more sensitive assays and increasingly insightful research design strategies, to allow studies to be carried out with a diminished requirement for blood samples in terms of number and volume, should be encouraged.

being developed in order to maximize the clinical and scientific outputs from studies. The guidelines that currently exist relating to blood sample volume limits are designed to ensure 'minimal risk' for participants and should be used appropriately to design pediatric clinical trials, with blood volume requirements clearly detailed in trial protocols and patient information sheets. However, it is accepted that these guidelines are largely not evidence-based and this issue should ideally be addressed in future prospective randomized studies, designed to more clearly define safe limits of blood loss over appropriate periods of time relevant to different clinical trial settings. Until such studies are carried out it is important that pediatric clinical trials are carried out in such a way as to minimize the impact on the children involved, with requirements for blood samples limited as much as possible while maintaining the scientific integrity of the studies. The approaches being taken should be clearly explained to research study participants and their families, alongside the potential risks and benefits of participation.

Future perspective

Based on the limited evidence available, current guidelines available to researchers and clinicians involved in pediatric research would appear to offer rational advice relating to safe limits of blood volume loss in children participating in clinical studies. Over the next 5–10 years it is highly likely that clinical trial protocols will continue to become even more complex, with increased requirements for access to biological samples including blood, to facilitate important scientific advances. While more carefully planned prospective studies may allow for the generation of more conclusive data relating to safe blood volume limits in children, it is difficult to foresee who will embark on the coordination of such studies. This is particularly the case when we consider that from an ethical point of view, researchers should be confident that any study currently being carried out or being planned will not impact negatively on the research participants involved. In the meantime the most sensible approach would seem to be a continued focus on developing more sensitive assays and increasingly insightful research design strategies, to allow studies to be carried out with a diminished requirement for clinical samples in terms of number and volume. Similarly, further research to provide confidence in utilizing less invasive biological fluids, thus reducing requirements for analysis of blood samples, should be strongly encouraged.

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