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Ultrasonographic reference values for peripheral nerves and nerve roots in the normal population of children and adolescents: study protocol for an observational-prospective trial

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Abstract

Background: High-resolution ultrasonography is a new and promising technique to evaluate peripheral and spinal nerves. Its validity as a diagnostic tool in neurological diseases has been demonstrated in adults. Up to now no reference values have been published in children and adolescents although this technique would be ideal in this population as it is fast and non-invasive.

Methods/Design: Our aim is to generate ultrasonographic reference values for several peripheral nerves (median, ulnar, radial, tibial, sural, peroneal and tibial nerve) as well as for the spinal nerves C5 and C6 and the vagus nerve in children and adolescents. In an observational-prospective study we will recruit 205 children and adolescents aged between ≥2 and ≤18 years without neuromuscular symptoms/signs and without a history of neuromuscular disease. After the collection of demographic and anthropometric data (height, weight, BMI, age, gender and handedness) and a neurologic examination, a high-resolution ultrasonography of peripheral and spinal nerves at several anatomic landmarks will be performed. These data will be used to estimate age-dependent percentile curves and to evaluate inter-rater, intra-rater and inter-equipment reliability of the measurements.

Discussion: This study will provide clinicians involved in the treatment of children and adolescents with neuromuscular diseases with useful reference values for the evaluation of peripheral and spinal nerves with high-resolution ultrasound.

Trial Registration: The study was registered with ClinicalTrials.gov (Identifier: NCT02570802) and approved by the local ethics committees (EKNZ 2015-210).

Strengths and limitations of this study:

- Sample size estimation is based on measurements from pilot data.
- The estimated sample size of 200 patients allows to estimate the 50% percentile curve for CSAs of the most examined nerves at different clinically important locations with adequate accuracy.
- Monocentric study.
- Unrecognized confounders could potentially alter our measurements.

Background

High-resolution ultrasonography is an emerging non-invasive technique for the investigation of peripheral nerves and is increasingly used worldwide in the diagnosis of peripheral nerve disorders. The value of peripheral nerve ultrasound for diagnosis of peripheral nerve damage in entrapment syndromes, nerve tumors and focal nerve lesions has been demonstrated clearly [1-6]. In adults it has become a useful supplementary tool for electrodiagnostic studies in these conditions. Characteristic nerve size changes in polyneuropathies have been reported as well [7-11] and are now further investigated. Nerve width (medial to lateral diameter), thickness (anterior to posterior diameter) and crosssectional area (CSA) measured on transverse scans, and anteroposterior diameter (LAPD) measured on longitudinal scans are the most frequently used quantitative parameters for the ultrasound investigation of peripheral nerves. Furthermore, ratios of CSA between different segments of the same nerve have also been used. Several reports have been published on reference values for the cross-sectional areas in nerves in adulthood, as well as normal values for cervical roots, radial nerve, lower limb nerves and pure sensory nerves [12-16]. In children the use of ultrasound was demonstrated in few studies of hereditary and immune-mediated neuropathies [17 18]. So far no work has been published on standard values for ultrasonography in children and adolescents. Especially in this population benefit of this fast and non-invasive technic is great because children can be examined much more stress-free. The aim of our study is to establish normal CSA values for C5 and C6 cervical roots, and several upper and lower limb nerves, including some pure sensory nerves, at pre-defined anatomical sites in children and adolescents, and to assess whether the CSAs correlates with height, age, gender and BMI. Furthermore, to test if such measurements are reliable in routine clinical practice, the intra- and inter-rater reliability of peripheral nerve ultrasound measurements will be assessed.

Methods/Design

Objectives and endpoints

The purpose of this study is to assess standard values of nerve ultrasonography in children and adolescents to use these values as a reference in clinical practice. This allows that nerve ultrasonography in children can be further evaluated and compared to standard values in different

diseases as it has been done in adults. The primary endpoint is to determine standard values of the cross-sectional area (CSA) of the C5 and C6 cervical roots, the vagus, median, ulnar, radial, superficial radial, peroneal, tibial, and the sural nerves in children and adolescents between ≥2 and ≤18. The secondary objectives are 1) to determine relations between CSA and epidemiological data and 2) to assess inter- and intra-rater reliability of measurements.

Study design

This is an observational-prospective, monocenter study with an estimated duration of 12 months. The study was registered with ClinicalTrials.gov (Identifier: NCT02570802) and approved by the local ethics committees (EKNZ 2015-210).

Inclusion criteria

- Children and adolescents aged between ≥2 and ≤18 years
- Written informed consent of the caregivers and the children/adolescents between 10 and 18 years
- Oral assent by children under the age of 10 years

Exclusion criteria

- Inability to meet study requirements
- Neuromuscular disease or symptoms/signs

Methodology

Children/adolescents fulfilling the inclusion criteria and their caregivers will be informed about the procedures and asked to participate either directly after a routine consultation in the outpatients' department, during a hospitalisation at the University of Basel Children's Hospital (UKBB) or per written letter addressed to school classes or sports clubs. Demographic and anthropometric data is collected (height, weight, BMI, age, gender and handedness) and a neurologic examination is performed. Inclusion and exclusion criteria are verified. Are the criteria met, the child will be enrolled into the study (table 1).

Table 1. Project flow chart.

Project Periods	Screening and Visit	Possible extra Visit
Visit	1	(2)
Time	60min	60min
Participant information and Informed Consent	x	
Demographics	x	
Anthropometric Measurements (Weight, Age)	x	
Medical History	x	
Clinical examination	x	
In- /Exclusion Criteria	x	x
Ultrasound (see list below)	x	x

Most of the children are examined once for about 60 minutes by one examiner. A number of 47 participants will be examined twice. Of these, 19 children will be examined again by the same examiner (intra-rater reliability), 28 children by another examiner (inter-rater reliability). The second examiner will be blinded to the results of the first examination.

Assessments of primary endpoint/outcome

Ultrasound measurements will be done in different nerves and at different locations. Ultrasound is performed using a high frequency probe real-time linear array scanner (Philipps Affiniti 50G and others). Ultrasound of different nerves at the upper and lower limbs and the neck are performed bilaterally. The nerves are scanned in axial planes, and the cross sectional area (CSA) of each nerve is measured at standardized anatomical points as described before [19]. In short: median nerve in the mid-upper arm, at the elbow, in the mid-forearm and at the carpal tunnel; ulnar nerve at mid-humerus, at the cubital tunnel and in the mid-forearm; radial nerve in the mid-upper arm and superficial radial as

well as posterior interosseous nerve at the supinator loge; peroneal nerve in the popliteal space and at the fibular head; tibial nerve in the popliteal space and at the medial malleolus and sural nerve between lateral and medial gastrocnemic head in the calf. In addition the vagus nerve is analysed at the lateral margins of the anterior cervical region beneath the sternocleidomastoid muscle and the diameter of the 5th and 6th cervical nerve roots are measured in longitudinal scan below the processus transversus. CSA is traced inside the hyperechoic rim of the nerve (Fig.1).

Assessment of secondary endpoints

Epidemiological data will be measured before performing the ultrasonographic examination. Height and weight are measured, BMI is calculated. The patient or the caregiver is asked about the age (Date of birth), gender and handedness.

Statistics

The sample size was calculated in order to estimate the percentile curves with adequate accuracy. The accuracy of the estimation was quantified by the length of the bootstrapped 95% confidence interval of the 50% percentile curve. The sample size estimation was based on the measurements of medianus prox. forearm right side from the pilot data. 12 patients between age 3.75 and age 6.25 were used in this sample size estimation using a resampling method. Each sample size was evaluated by estimating the 50% percentile curve together with its bootstrapped 95% confidence interval R = 100 times. Each 95% confidence interval was estimated by simulating 99 times in individual patients, fitting a “Generalized additive model for location, scale and shape” and estimating the 50 % percentile curve from the fitted model. Then the 95% confidence interval of the 50% percentile curve was estimated using a bootstrap approach using these 99 estimations. For each 50% percentile curve, it was assessed whether the length of the 95% confidence interval was below the predefined margin of 1.2. Assuming a drop-out rate of 2%, 205 patients should be recruited to ensure 200 evaluable patients. This sample size allows in more than 80% of 100 hypothetical repetitions of the study (i.e., with a power of 0.8) to estimate the 50% percentile curve with adequate accuracy (length of the 95% confidence interval below the predefined margin of 1.2). Figure 2 shows how the sample size depends on the pre-defined accuracy threshold of the estimate. Additional sample size estimation was

performed in order to estimate the sample size needed to estimate the inter-rater and intra-rater reliability of the measurements. Reliability is expressed by the intraclass correlaction coefficient (ICC). Two scenarios have been calculated assuming two examinations in each child with different ICC for intra- and inter-rater reliability. It is assumed that the ICC is 0.8 for intra-rater and 0.75 for inter-rater reliability. The study should be able to estimate the ICC with a certain precision. This precision is expressed as the width of a 95% confidence interval and is here defined to be 1/3. By applying the sample size approximation of Bonett [20] and assuming a drop-out-rate of 5%, a sample size of 20 patients results (value rounded to the next higher integer) when assuming each child is examined twice for intra-rater reliability (Fig. 3). A sample size of 30 results when assuming each child is examined twice for inter-rater reliability (Fig. 4).

Figure 3 and 4 show how the sample size depends on the assumed ICC and the number of examinations in each child.

Primary Analysis

The age-dependent percentile curves will be estimated using a “Generalized additive model for location, scale and shape” as suggested by the WHO Multicentre Growth Reference Study Group [21] using the R-package Rigby & Stasinopoulos [22].The analysis will be performed on the full analysis data set.

Secondary Analyses

The same percentile curves as described in the main analysis will be estimated depending on size and weight. The models will be compared to the main model in order to investigate whether a growth curve in dependence of size or weight is more applicable than a growth curve depending on age. The association between gender and handedness and the thickness of the nerves will be investigated by including these variables as covariables in the main model in an exploratory manner. If gender has a relevant influence on thickness of the nerves, separate growth curves for each gender will be considered.

Inter-rater, intra-rater and inter-equipment reliability of the measurements will be investigated by estimating intraclass correlation coefficients (ICC) according to Streiner & Norman [23].

Data protection, archiving and destruction

In this study personal patient data will be captured. This data will be encoded and is only accessible to experts. The appropriate experts of the sponsor (or their designees) can survey the conduct of the study with monitoring or audits. In case of inspections these experts and also members of the appropriate authorities can get access to the original data. Also the responsible Ethics Committee can get access to the original data. The confidentiality of the data will be strictly protected during the whole study and when performing the mentioned controls. The name of the patient will not be published in no way in reports or publications arisen from the study.

The paper documents will be stored in a lockable room during 10 years in the archive of the UKBB in a dedicated shelf.

Ethical considerations

To generate ultrasonographic reference values in children and adolescents it is inevitable to include subjects requiring particular protection (children under the age of 18 years) into this trial. The participation in this study is voluntary. The parents and the patient can withdraw their consent at every time point without giving any reason. In case of withdrawal the data collected until this time point will be used.

As the ultrasonography of peripheral nerves is a non-invasive and painless examination the benefit of generating normal values and therefore providing a tool to complement and minimase more invasive electrophysiological examinations legitimates the recruitment and examination of subjects requiring particular protection.

Discussion

Standard values for nerve ultrasonography in children and adolescents have not been published so far. This fast and non-invasive technique may provide great benefit especially in children because they can be examined much more stress-free. Therefore, the main aim of our study is to establish normal CSA values for C5 and C6 cervical roots, and several upper and lower limb nerves, including some pure sensory nerves, at pre-defined anatomical sites in children and adolescents. This study will provide these urgently needed reference values for the ultrasonographic evaluation of several

peripheral and spinal nerves at specific anatomic landmarks in children and adolescents under the age of 19 years. These normal values will guide clinicians in examining children and adolescents with neuromuscular diseases by ultrasonography.

Strength: The sample size estimation was based on the available measurements from pilot data. Assuming a drop-out rate of 2%, 205 patients aged between 2 and 18 years should be recruited to ensure 200 evaluable patients. This sample size allows in more than 80% of 100 hypothetical repetitions of the study (i.e., with a power of 0.8) to estimate the 50% percentile curve for CSAs of the most examined nerves at different clinically important locations with adequate accuracy.

Limitations: Even though we plan to include a large cohort there still is the possibility of unrecognized confounders. The trial is planned as a monocentric study.

Trial status

The trial started enrolment in November 2015 and is expected to be completed by the end of December 2016.

List of abbreviations

Cross sectional area (CSA)

Longitudinal anteroposterior diameter (LAPD)

University of Basel Children's Hospital (UKBB)

Intraclass correlation coefficient (ICC).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MR participated in the design of the study, acquired data and drafted the manuscript. BFD participated in the design of the study and acquired data. SS participated in the design of the study, performed the statistical analysis and calculated the sample size for the study. AG designed the study and acquired

data. DF participated in the design of the study and revised the manuscript. PH designed and conducted the study, acquired data and revised the manuscript. All authors read and approved the final manuscript.

Funding

We do confirm that there is no external funding of this project/study.

Data sharing statement

There are no additional unpublished data.

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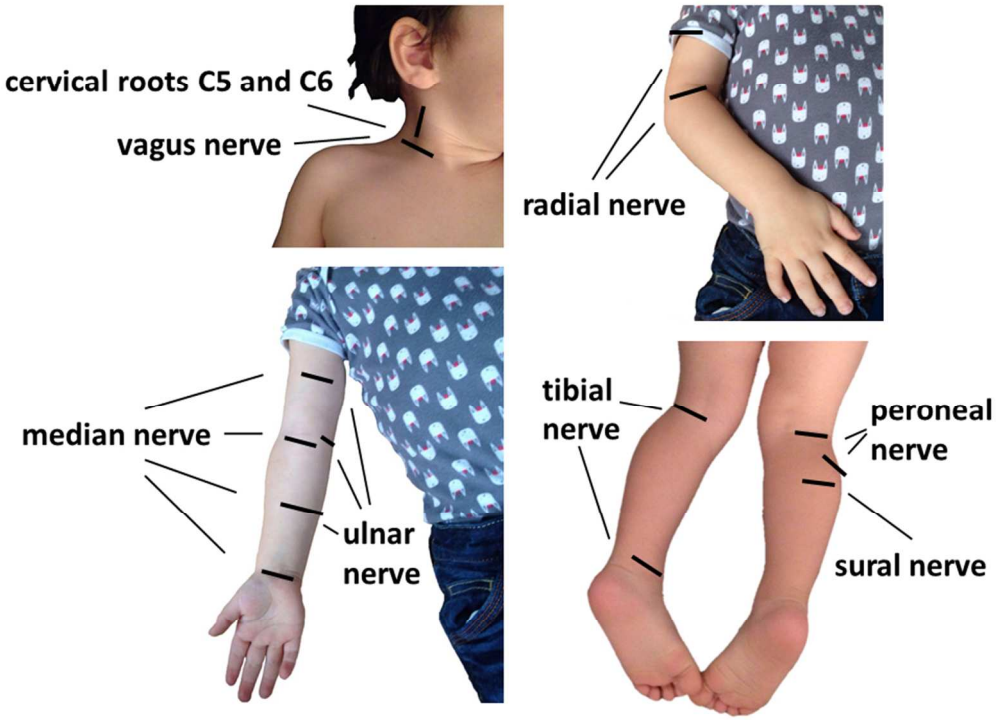
Figure 1: Standardized anatomical points for the measurement of CSA of the measured nerves and diameter of nerve roots

Figure 2: Sensitivity of the sample size with respect to the predefined accuracy threshold of the estimate. The curves for a power of 0.7, 0.8 and 0.9 (i.e., 70 %, 80 % and 90 %) are shown. (The curves are smoothed and are shown for illustrative purposes only.)

Figure 3: Sample size estimation for ICC – intra-rater reliability. The curve for a power of 0.8 is shown.

Figure 4: Sample size estimation for ICC – inter-rater reliability. The curve for a power of 0.75 is shown.



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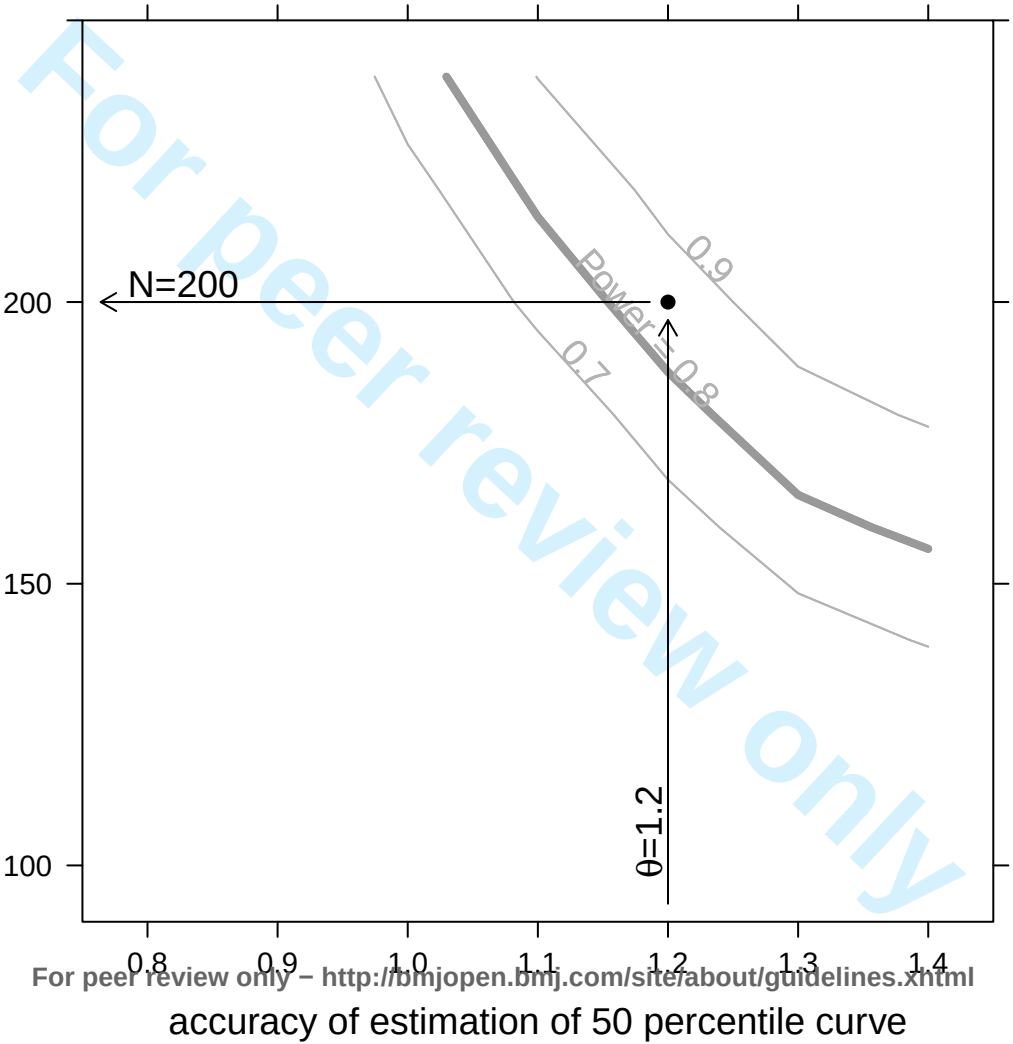

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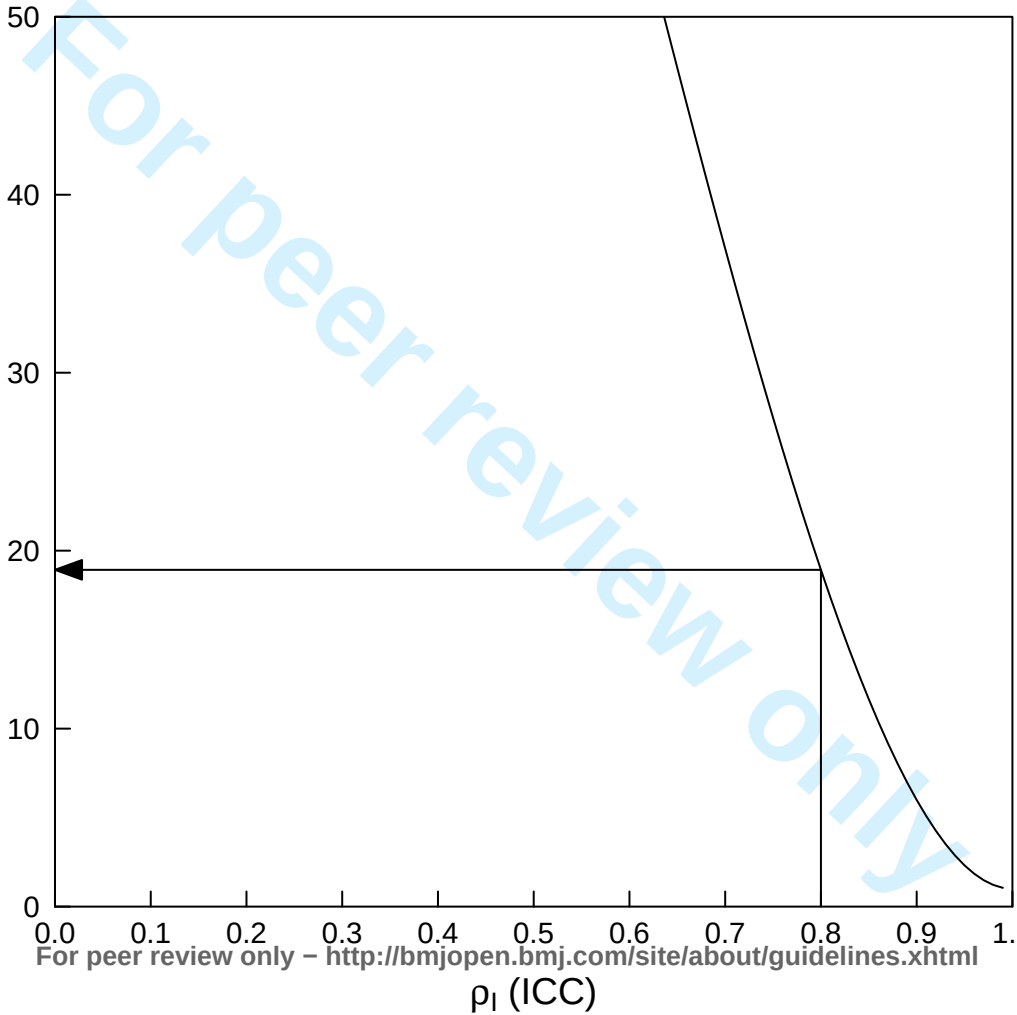

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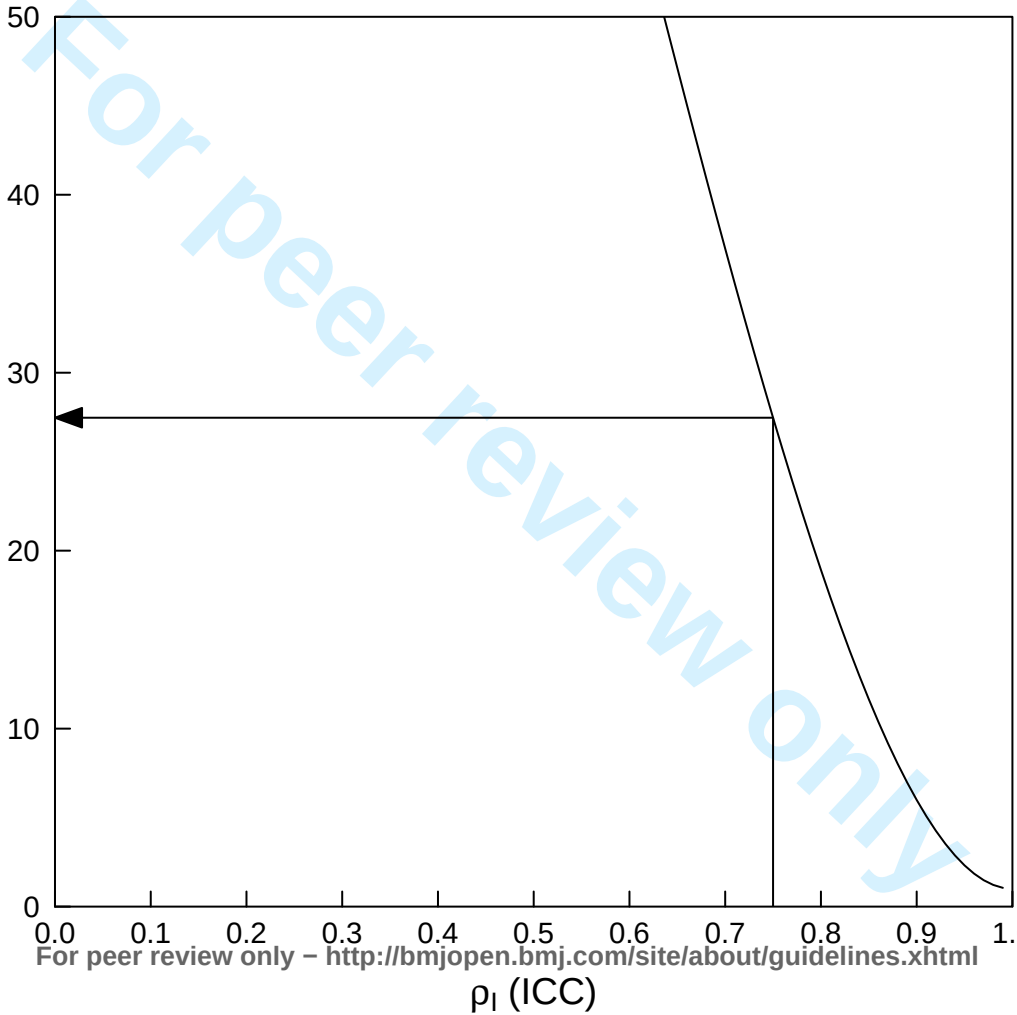

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well as posterior interosseous nerve at the supinator loge; peroneal nerve in the popliteal space and at the fibular head; tibial nerve in the popliteal space and at the medial malleolus and sural nerve between lateral and medial gastrocnemic head in the calf. In addition the vagus nerve is analysed at the lateral margins of the anterior cervical region beneath the sternocleidomastoid muscle and the diameter and CSA of the 5th and 6th cervical nerve roots are measured in longitudinal scan below the processus transversus. CSA is traced inside the hyperechoic rim of the nerve (Fig.1).

Assessment of secondary endpoints

Epidemiological data will be measured before performing the ultrasonographic examination. Height and weight are measured, BMI is calculated. The patient or the caregiver is asked about the age (Date of birth), gender and handedness.

Statistics

The sample size was calculated in order to estimate the percentile curves with adequate accuracy. The accuracy of the estimation was quantified by the length of the bootstrapped 95% confidence interval of the 50% percentile curve. The sample size estimation was based on the measurements of medianus prox. forearm right side from the pilot data. 12 patients between age 3.75 and age 6.25 were used in this sample size estimation using a resampling method. Each sample size was evaluated by estimating the 50% percentile curve together with its bootstrapped 95% confidence interval R = 100 times. Each 95% confidence interval was estimated by simulating 99 times in individual patients, fitting a “Generalized additive model for location, scale and shape” and estimating the 50 % percentile curve from the fitted model. Then the 95% confidence interval of the 50% percentile curve was estimated using a bootstrap approach using these 99 estimations. For each 50% percentile curve, it was assessed whether the length of the 95% confidence interval was below the predefined margin of 1.2. Assuming a drop-out rate of 2%, 205 patients should be recruited to ensure 200 evaluable patients. This sample size allows in more than 80% of 100 hypothetical repetitions of the study (i.e., with a power of 0.8) to estimate the 50% percentile curve with adequate accuracy (length of the 95% confidence interval below the predefined margin of 1.2). Figure 2 shows how the sample size depends on the pre-defined accuracy threshold of the estimate. Additional sample size estimation was

performed in order to estimate the sample size needed to estimate the inter-rater and intra-rater reliability of the measurements. Reliability is expressed by the intraclass correlaction coefficient (ICC). Two scenarios have been calculated assuming two examinations in each child with different ICC for intra- and inter-rater reliability. It is assumed that the ICC is 0.8 for intra-rater and 0.75 for inter-rater reliability. The study should be able to estimate the ICC with a certain precision. This precision is expressed as the width of a 95% confidence interval and is here defined to be 1/3. By applying the sample size approximation of Bonett [26] and assuming a drop-out-rate of 5%, a sample size of 20 patients results (value rounded to the next higher integer) when assuming each child is examined twice for intra-rater reliability (Fig. 3). A sample size of 30 results when assuming each child is examined twice for inter-rater reliability (Fig. 4).

Figure 3 and 4 show how the sample size depends on the assumed ICC and the number of examinations in each child.

Primary Analysis

The age-dependent percentile curves will be estimated using a “Generalized additive model for location, scale and shape” as suggested by the WHO Multicentre Growth Reference Study Group [27] using the R-package Rigby & Stasinopoulos [28].The analysis will be performed on the full analysis data set.

Secondary Analyses

The same percentile curves as described in the main analysis will be estimated depending on size and weight. The models will be compared to the main model in order to investigate whether a growth curve in dependence of size or weight is more applicable than a growth curve depending on age. The association between gender and handedness and the thickness of the nerves will be investigated by including these variables as covariables in the main model in an exploratory manner. If gender has a relevant influence on thickness of the nerves, separate growth curves for each gender will be considered.

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The paper documents will be stored in a lockable room during 10 years in the archive of the UKBB in a dedicated shelf.

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To generate ultrasonographic reference values in children and adolescents it is inevitable to include subjects requiring particular protection (children under the age of 18 years) into this trial. The participation in this study is voluntary. The parents and the patient can withdraw their consent at every time point without giving any reason. In case of withdrawal the data collected until this time point will be used.

As the ultrasonography of peripheral nerves is a non-invasive and painless examination the benefit of generating normal values and therefore providing a tool to complement and minimase more invasive electrophysiological examinations legitimates the recruitment and examination of subjects requiring particular protection.

Discussion

Standard values for nerve ultrasonography in children and adolescents have not been published so far. This fast and non-invasive technique may provide great benefit especially in children because they can be examined much more stress-free. Therefore, the main aim of our study is to establish normal CSA values for C5 and C6 cervical roots, and several upper and lower limb nerves, including some pure sensory nerves, at pre-defined anatomical sites in children and adolescents. This study will provide these urgently needed reference values for the ultrasonographic evaluation of several

peripheral and spinal nerves at specific anatomic landmarks in children and adolescents under the age of 19 years. These normal values will guide clinicians in examining children and adolescents with neuromuscular diseases by ultrasonography.

Strength: The sample size estimation was based on the available measurements from pilot data. Assuming a drop-out rate of 2%, 205 patients aged between 2 and 18 years should be recruited to ensure 200 evaluable patients. This sample size allows in more than 80% of 100 hypothetical repetitions of the study (i.e., with a power of 0.8) to estimate the 50% percentile curve for CSAs of the most examined nerves at different clinically important locations with adequate accuracy.

Limitations: Even though we plan to include a large cohort there still is the possibility of unrecognized confounders. The trial is planned as a monocentric study. By not only including patients from our hospital but also from schools, sports classes and the authors' circle of acquaintances we will try to reduce selection bias.

Trial status

The trial started enrolment in November 2015 and is expected to be completed by the end of December 2016.

List of abbreviations

Cross sectional area (CSA)

Longitudinal anteroposterior diameter (LAPD)

University of Basel Children's Hospital (UKBB)

Intraclass correlation coefficient (ICC).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MR participated in the design of the study, acquired data and drafted the manuscript. BFD participated in the design of the study and acquired data. SS participated in the design of the study, performed the statistical analysis and calculated the sample size for the study. AG designed the study and acquired data. DF participated in the design of the study and revised the manuscript. PH designed and conducted the study, acquired data and revised the manuscript. All authors read and approved the final manuscript.

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Data sharing statement

There are no additional unpublished data.

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Figure 1: Standardized anatomical points for the measurement of CSA of the measured nerves and diameter of nerve roots

Figure 2: Sensitivity of the sample size with respect to the predefined accuracy threshold of the estimate. The curves for a power of 0.7, 0.8 and 0.9 (i.e., 70 %, 80 % and 90 %) are shown. (The curves are smoothed and are shown for illustrative purposes only.)

Figure 3: Sample size estimation for ICC – intra-rater reliability. The curve for a power of 0.8 is shown.

Figure 4: Sample size estimation for ICC – inter-rater reliability. The curve for a power of 0.75 is shown.

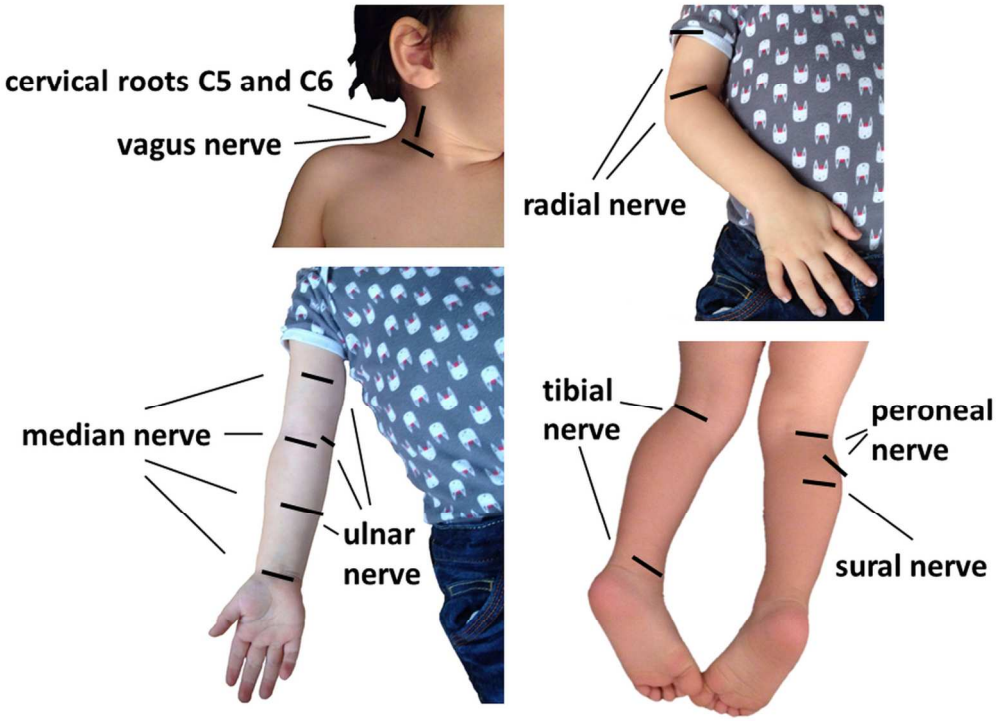

Figure 1: Standardized anatomical points for the measurement of CSA of the measured nerves and diameter of nerve roots

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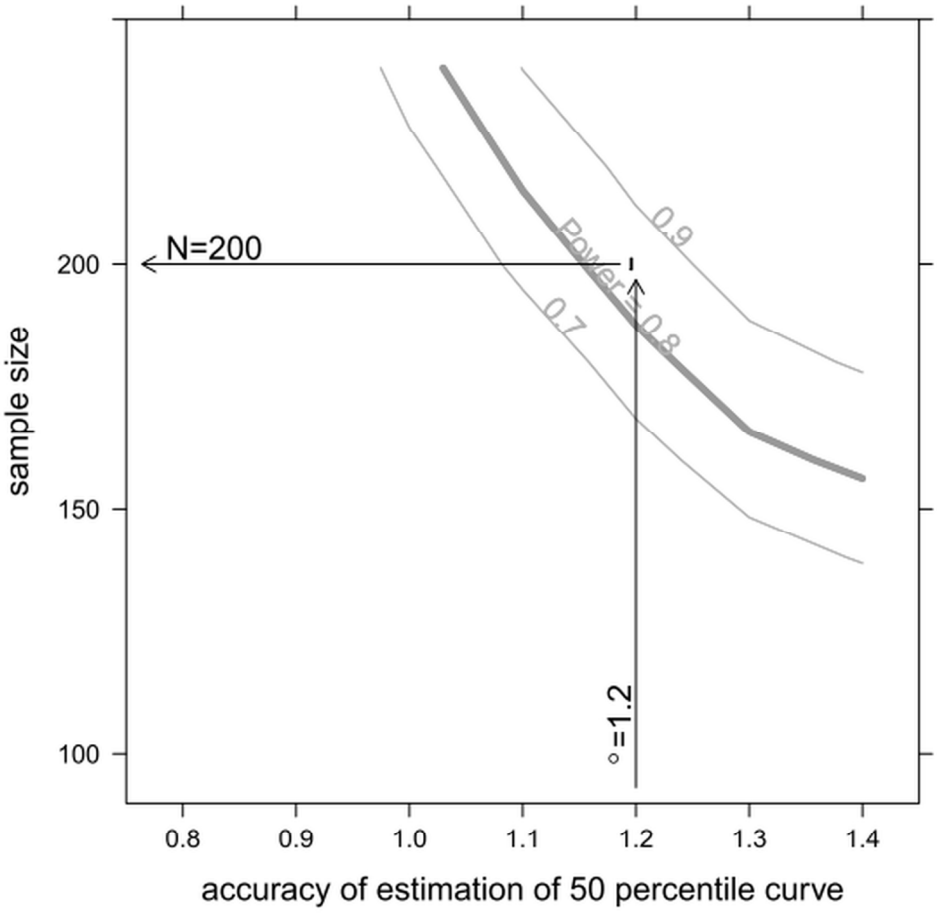

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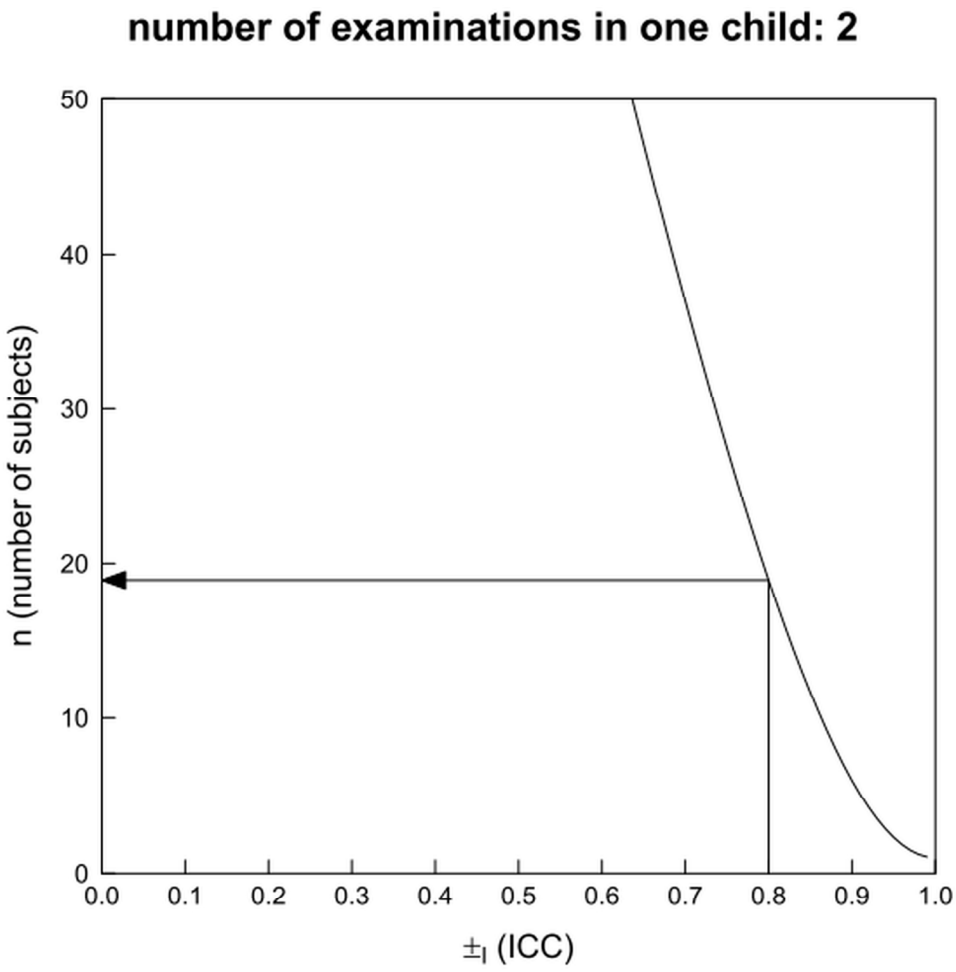

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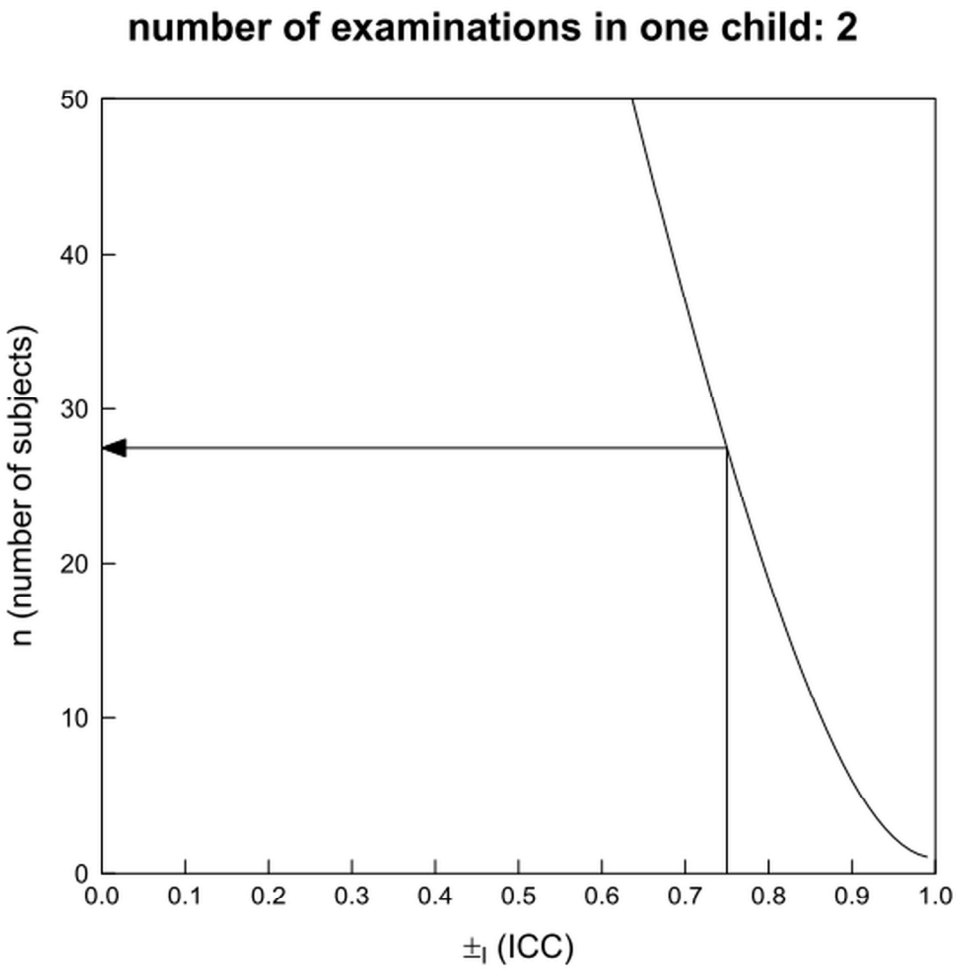

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Ultrasonographic reference values for peripheral nerves and nerve roots in the normal population of children and adolescents: study protocol for an observational-prospective trial

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Keywords:	High resolution nerve ultrasound, normal values, peripheral nerve system

Ultrasonographic reference values for peripheral nerves and nerve roots in the normal population of children and adolescents: study protocol for an observational-prospective trial

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Keywords

High resolution nerve ultrasound, normal values, peripheral nerve system

Word count: 1982

Abstract

Background: High-resolution ultrasonography is a new and promising technique to evaluate peripheral and spinal nerves. Its validity as a diagnostic tool in neurological diseases has been demonstrated in adults. Up to now no reference values have been published in children and adolescents although this technique would be ideal in this population as it is fast and non-invasive.

Methods/Design: Our aim is to generate ultrasonographic reference values for several peripheral nerves (median, ulnar, radial, tibial, sural, peroneal and tibial nerve) as well as for the spinal nerves C5 and C6 and the vagus nerve in children and adolescents. In an observational-prospective study we will recruit 205 children and adolescents aged between ≥2 and ≤18 years without neuromuscular symptoms/signs and without a history of neuromuscular disease. After the collection of demographic and anthropometric data (height, weight, BMI, age, gender and handedness) and a neurologic examination, a high-resolution ultrasonography of peripheral and spinal nerves at several anatomic landmarks will be performed. These data will be used to estimate age-dependent percentile curves and to evaluate inter-rater, intra-rater and inter-equipment reliability of the measurements.

Ethics and Dissemination: This study was approved by the local ethics committee (EKNZ 2015-210). The findings from this study will be disseminated through peer-reviewed publications and conference presentations.

Trial Registration: The study was registered with ClinicalTrials.gov (Identifier: NCT02570802).

Strengths and limitations of this study:

- Sample size estimation is based on measurements from pilot data.
- The estimated sample size of 200 patients allows to estimate the 50% percentile curve for CSAs of the most examined nerves at different clinically important locations with adequate accuracy.
- Monocentric study.
- Unrecognized confounders could potentially alter our measurements.

Background

High-resolution ultrasonography is an emerging non-invasive technique for the investigation of peripheral nerves and is increasingly used worldwide in the diagnosis of peripheral nerve disorders. The value of peripheral nerve ultrasound for diagnosis of peripheral nerve damage in entrapment syndromes, nerve tumors and focal nerve lesions has been demonstrated clearly [1-9]. In adults it has become a useful supplementary tool for electrodiagnostic studies in these conditions. Characteristic nerve size changes in polyneuropathies have been reported as well [10-16] and are now further investigated. Nerve width (medial to lateral diameter), thickness (anterior to posterior diameter) and crosssectional area (CSA) measured on transverse scans, and anteroposterior diameter (LAPD) measured on longitudinal scans are the most frequently used quantitative parameters for the ultrasound investigation of peripheral nerves. Furthermore, ratios of CSA between different segments of the same nerve have also been used. Several reports have been published on reference values for the cross-sectional areas in nerves in adulthood, as well as normal values for cervical roots, radial nerve, lower limb nerves and pure sensory nerves [17-22]. In children the use of ultrasound was demonstrated in few studies of hereditary and immune-mediated neuropathies [23 24]. So far no work has been published on standard values for ultrasonography in children and adolescents. Especially in this population benefit of this fast and non-invasive technic is great because children can be examined much more stress-free. The aim of our study is to establish normal CSA values for C5 and C6 cervical roots, and several upper and lower limb nerves, including some pure sensory nerves, at pre-defined anatomical sites in children and adolescents, and to assess whether the CSAs correlates with height, age, gender and BMI. Furthermore, to test if such measurements are reliable in routine clinical practice, the intra- and inter-rater reliability of peripheral nerve ultrasound measurements will be assessed.

Methods/Design

Objectives and endpoints

The purpose of this study is to assess standard values of nerve ultrasonography in children and adolescents to use these values as a reference in clinical practice. This allows that nerve ultrasonography in children can be further evaluated and compared to standard values in different diseases as it has been done in adults. The primary endpoint is to determine standard values of the cross-sectional area (CSA) of the C5 and C6 cervical roots, the vagus, median, ulnar, radial,

superficial radial, peroneal, tibial, and the sural nerves in children and adolescents between ≥2 and ≤18. The secondary objectives are 1) to determine relations between CSA and epidemiological data and 2) to assess inter- and intra-rater reliability of measurements.

Study design

This is an observational-prospective, monocenter study with an estimated duration of 12 months. The study was registered with ClinicalTrials.gov (Identifier: NCT02570802) and approved by the local ethics committees (EKNZ 2015-210).

Inclusion criteria

- Children and adolescents aged between ≥2 and ≤18 years
- Written informed consent of the caregivers and the children/adolescents between 10 and 18 years
- Oral assent by children under the age of 10 years

Exclusion criteria

- Inability to meet study requirements
- Neuromuscular disease or symptoms/signs

Methodology

Children/adolescents fulfilling the inclusion criteria and their caregivers will be informed about the procedures and asked to participate either directly after a routine consultation in the outpatients' department, during a hospitalisation at the University of Basel Children's Hospital (UKBB), per written letter addressed to school classes or sports clubs and from the authors' our circle of acquaintances and colleagues. Demographic and anthropometric data is collected (height, weight, BMI, age, gender and handedness) and a neurologic examination is performed. Inclusion and exclusion criteria are verified. Are the criteria met, the child will be enrolled into the study (table 1).

Table 1. Project flow chart.

Project Periods	Screening and Visit	Possible extra Visit
Visit	1	(2)
Time	60min	60min
Participant information and Informed Consent	x	
Demographics	x	
Anthropometric Measurements (Weight, Age)	x	
Medical History	x	
Clinical examination	x	
In- /Exclusion Criteria	x	x
Ultrasound (see list below)	x	x

Most of the children are examined once for about 60 minutes by one examiner. A number of 47 participants will be examined twice. Of these, 19 children will be examined again by the same examiner (intra-rater reliability), 28 children by another examiner (inter-rater reliability). The second examiner will be blinded to the results of the first examination.

Assessments of primary endpoint/outcome

Ultrasound measurements will be done in different nerves and at different locations. Ultrasound is performed using a high frequency probe real-time linear array scanner (Philipps Affiniti 50G and others). Ultrasound of different nerves at the upper and lower limbs and the neck are performed bilaterally. The nerves are scanned in axial planes, and the cross sectional area (CSA) of each nerve is measured at standardized anatomical points as described before [25]. In short: median nerve in the mid-upper arm, at the elbow, in the mid-forearm and at the carpal tunnel; ulnar nerve at mid-humerus, at the cubital tunnel and in the mid-forearm; radial nerve in the mid-upper arm and superficial radial as well as posterior interosseous nerve at the supinator loge; peroneal nerve in the popliteal space and at the fibular head; tibial nerve in the popliteal space and at the medial malleolus and sural nerve

between lateral and medial gastrocnemic head in the calf. In addition the vagus nerve is analysed at the lateral margins of the anterior cervical region beneath the sternocleidomastoid muscle and the diameter and CSA of the 5th and 6th cervical nerve roots are measured in longitudinal scan below the processus transversus. CSA is traced inside the hyperechoic rim of the nerve (Fig.1).

Assessment of secondary endpoints

Epidemiological data will be measured before performing the ultrasonographic examination. Height and weight are measured, BMI is calculated. The patient or the caregiver is asked about the age (Date of birth), gender and handedness.

Statistics

The sample size was calculated in order to estimate the percentile curves with adequate accuracy. The accuracy of the estimation was quantified by the length of the bootstrapped 95% confidence interval of the 50% percentile curve. The sample size estimation was based on the measurements of medianus prox. forearm right side from the pilot data. 12 patients between age 3.75 and age 6.25 were used in this sample size estimation using a resampling method. Each sample size was evaluated by estimating the 50% percentile curve together with its bootstrapped 95% confidence interval R = 100 times. Each 95% confidence interval was estimated by simulating 99 times in individual patients, fitting a “Generalized additive model for location, scale and shape” and estimating the 50 % percentile curve from the fitted model. Then the 95% confidence interval of the 50% percentile curve was estimated using a bootstrap approach using these 99 estimations. For each 50% percentile curve, it was assessed whether the length of the 95% confidence interval was below the predefined margin of 1.2. Assuming a drop-out rate of 2%, 205 patients should be recruited to ensure 200 evaluable patients. This sample size allows in more than 80% of 100 hypothetical repetitions of the study (i.e., with a power of 0.8) to estimate the 50% percentile curve with adequate accuracy (length of the 95% confidence interval below the predefined margin of 1.2). Figure 2 shows how the sample size depends on the pre-defined accuracy threshold of the estimate. Additional sample size estimation was performed in order to estimate the sample size needed to estimate the inter-rater and intra-rater reliability of the measurements. Reliability is expressed by the intraclass correlaction coefficient (ICC).

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The paper documents will be stored in a lockable room during 10 years in the archive of the UKBB in a dedicated shelf.

Ethical considerations

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As the ultrasonography of peripheral nerves is a non-invasive and painless examination the benefit of generating normal values and therefore providing a tool to complement and minimase more invasive electrophysiological examinations legitimates the recruitment and examination of subjects requiring particular protection.

Local Ethics Committee approval was obtained (EKNZ 2015-210) and the study is registered online with clinicaltrials.gov (NCT02570802).

Dissemination

The results of this study will be published in peer-reviewed journals and presented at national and international scientific meetings and congresses to ensure the applicability of its findings into clinical practice.

Discussion

Standard values for nerve ultrasonography in children and adolescents have not been published so far. This fast and non-invasive technique may provide great benefit especially in children because they can be examined much more stress-free. Therefore, the main aim of our study is to establish normal CSA values for C5 and C6 cervical roots, and several upper and lower limb nerves, including some pure sensory nerves, at pre-defined anatomical sites in children and adolescents. This study will provide these urgently needed reference values for the ultrasonographic evaluation of several peripheral and spinal nerves at specific anatomic landmarks in children and adolescents under the age of 19 years. These normal values will guide clinicians in examining children and adolescents with neuromuscular diseases by ultrasonography.

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Limitations: Even though we plan to include a large cohort there still is the possibility of unrecognized confounders. The trial is planned as a monocentric study. By not only including patients from our hospital but also from schools, sports classes and the authors' circle of acquaintances we will try to reduce selection bias.

Trial status

The trial started enrolment in November 2015 and is expected to be completed by the end of December 2017.

List of abbreviations

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Longitudinal anteroposterior diameter (LAPD)

University of Basel Children's Hospital (UKBB)

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MR participated in the design of the study, acquired data and drafted the manuscript. BFD participated in the design of the study and acquired data. SS participated in the design of the study, performed the statistical analysis and calculated the sample size for the study. AG designed the study and acquired data. DF participated in the design of the study and revised the manuscript. PH designed and conducted the study, acquired data and revised the manuscript. All authors read and approved the final manuscript.

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Data sharing statement

There are no additional unpublished data.

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Figure 1: Standardized anatomical points for the measurement of CSA of the measured nerves and diameter of nerve roots

Figure 2: Sensitivity of the sample size with respect to the predefined accuracy threshold of the estimate. The curves for a power of 0.7, 0.8 and 0.9 (i.e., 70 %, 80 % and 90 %) are shown. (The curves are smoothed and are shown for illustrative purposes only.)

Figure 3: Sample size estimation for ICC – intra-rater reliability. The curve for a power of 0.8 is shown.

Figure 4: Sample size estimation for ICC – inter-rater reliability. The curve for a power of 0.75 is shown.

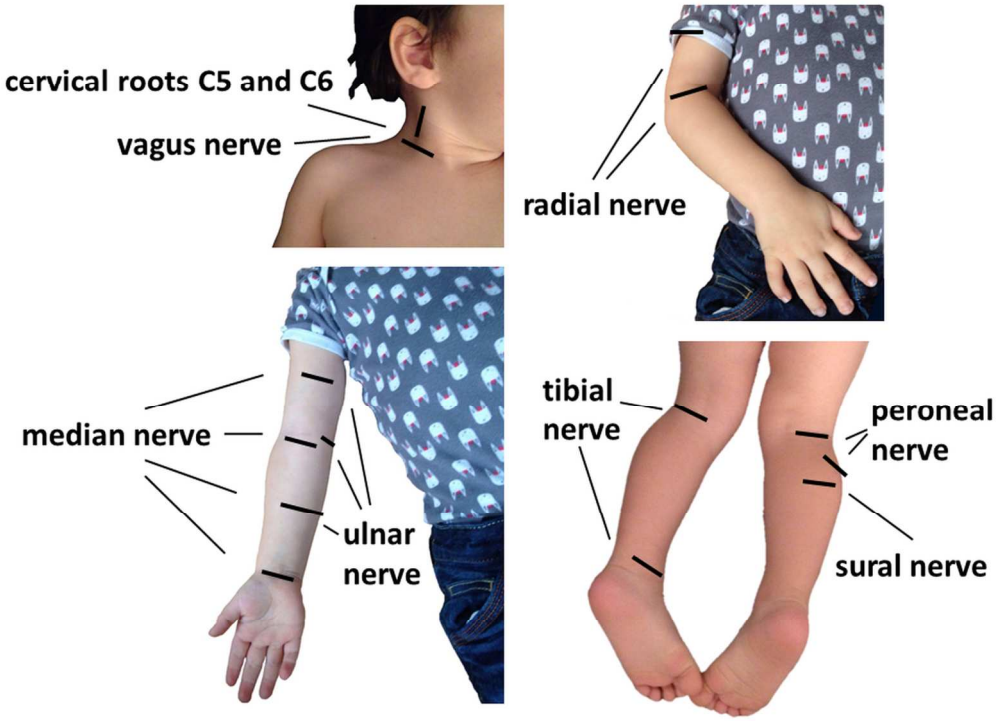

Figure 1: Standardized anatomical points for the measurement of CSA of the measured nerves and diameter of nerve roots

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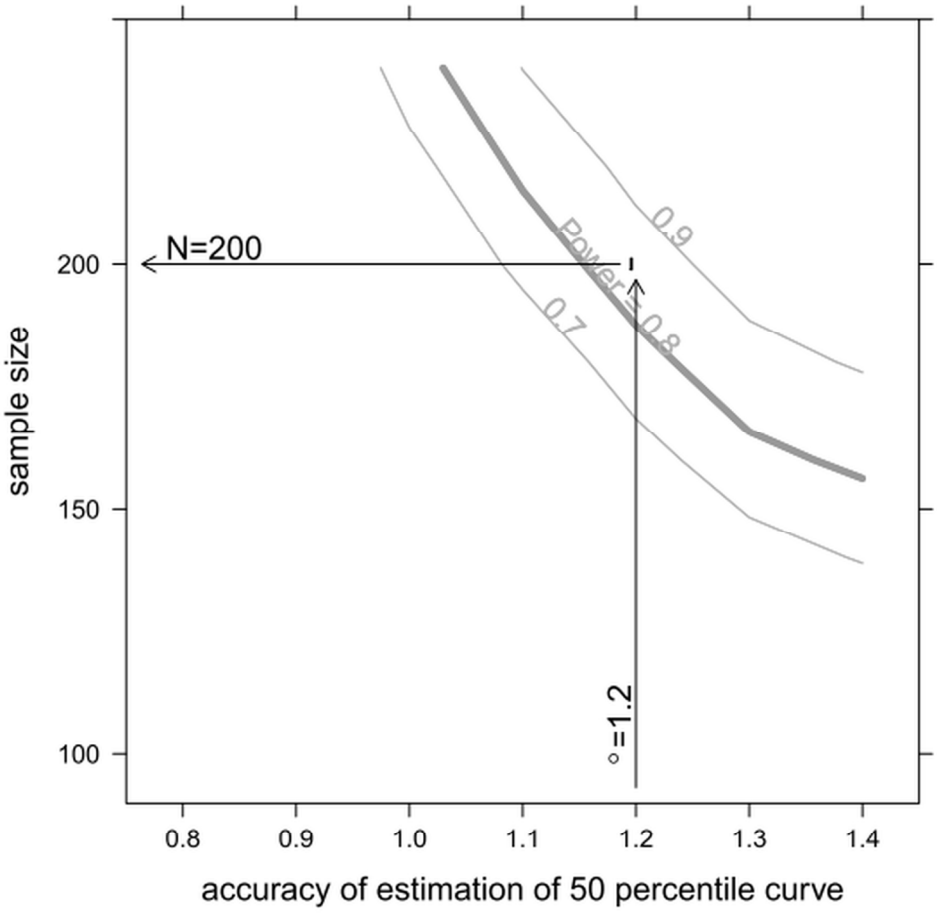

Figure 2: Sensitivity of the sample size with respect to the predefined accuracy threshold of the estimate. The curves for a power of 0.7, 0.8 and 0.9 (i.e., 70 %, 80 % and 90 %) are shown. (The curves are smoothed and are shown for illustrative purposes only.)

152x152mm (300 x 300 DPI)

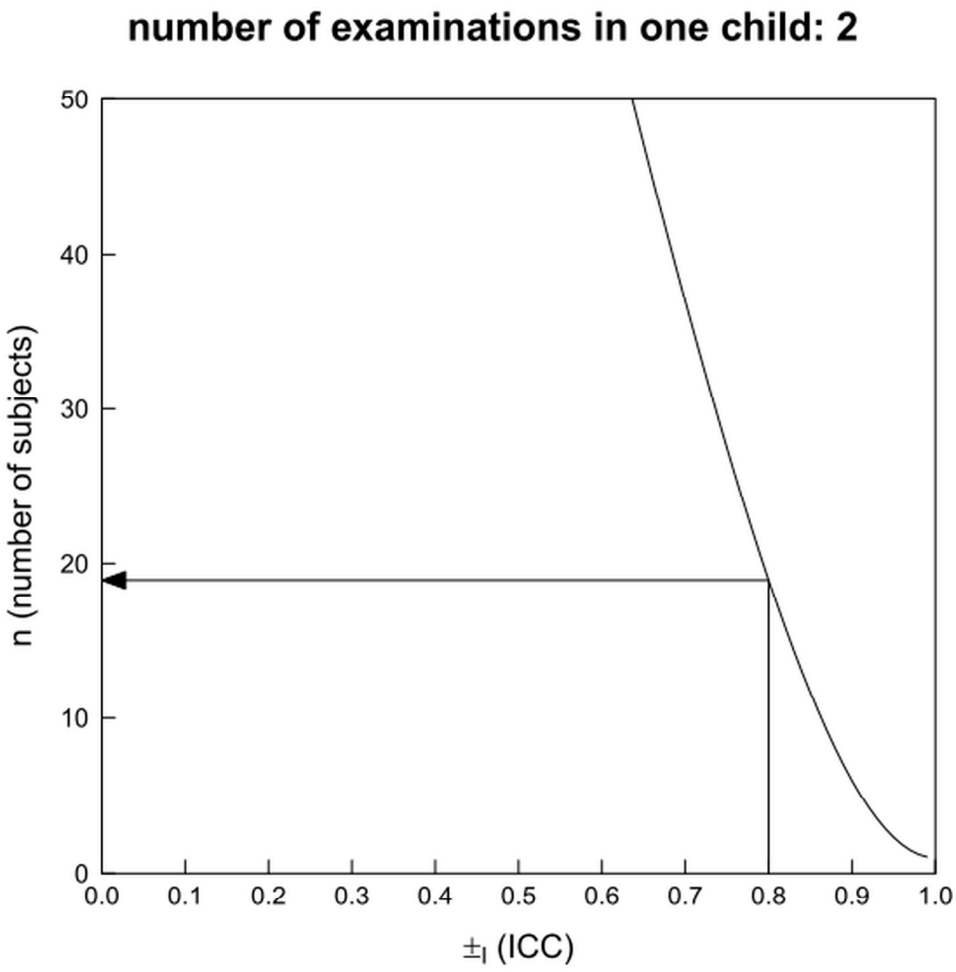

Figure 3: Sample size estimation for ICC – intra-rater reliability. The curve for a power of 0.8 is shown.

152x152mm (300 x 300 DPI)

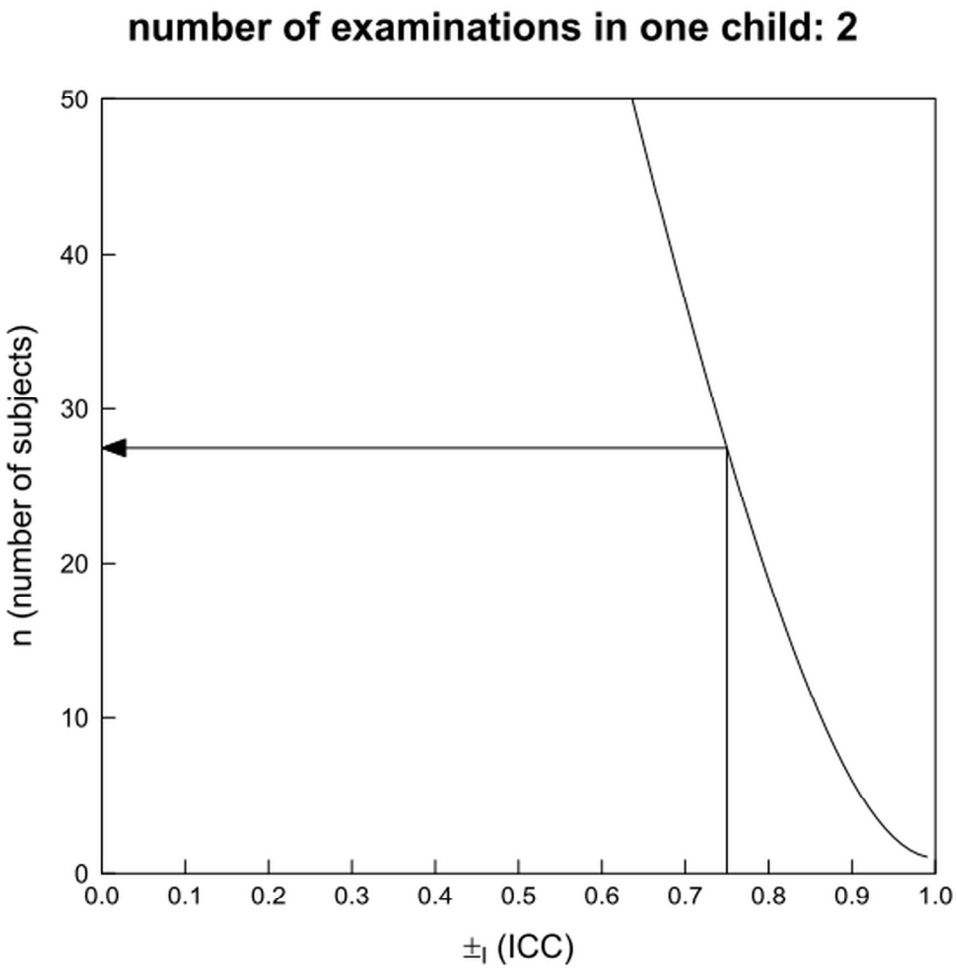

Figure 4: Sample size estimation for ICC – inter-rater reliability. The curve for a power of 0.75 is shown.

152x152mm (300 x 300 DPI)