

# BMJ Open

## 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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3 **1 Ultrasonographic reference values for peripheral nerves and nerve**  
4 **2 roots in the normal population of children and adolescents: study**  
5 **3 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  $\geq 2$  and  $\leq 18$  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.

## 56 **Background**

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

## 80 **Methods/Design**

### 81 **Objectives and endpoints**

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

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3 85 diseases as it has been done in adults. The primary endpoint is to determine standard values of the  
4 86 cross-sectional area (CSA) of the C5 and C6 cervical roots, the vagus, median, ulnar, radial,  
5 87 superficial radial, peroneal, tibial, and the sural nerves in children and adolescents between  $\geq 2$  and  
6 88  $\leq 18$ . The secondary objectives are 1) to determine relations between CSA and epidemiological data  
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10 89 and 2) to assess inter- and intra-rater reliability of measurements.  
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## 14 91 **Study design**

15  
16 92 This is an observational-prospective, monocenter study with an estimated duration of 12 months. The  
17  
18 93 study was registered with ClinicalTrials.gov (Identifier: NCT02570802) and approved by the local  
19  
20 94 ethics committees (EKNZ 2015-210).  
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## 24 96 **Inclusion criteria**

- 25  
26 97 - Children and adolescents aged between  $\geq 2$  and  $\leq 18$  years  
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28 98 - Written informed consent of the caregivers and the children/adolescents between 10 and 18 years  
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30 99 - Oral assent by children under the age of 10 years  
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32

## 33 101 **Exclusion criteria**

- 34  
35 102 - Inability to meet study requirements  
36  
37 103 - Neuromuscular disease or symptoms/signs  
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## 40 104 41 105 **Methodology**

42  
43 106 Children/adolescents fulfilling the inclusion criteria and their caregivers will be informed about the  
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45 107 procedures and asked to participate either directly after a routine consultation in the outpatients'  
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47 108 department, during a hospitalisation at the University of Basel Children's Hospital (UKBB) or per  
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49 109 written letter addressed to school classes or sports clubs. Demographic and anthropometric data is  
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51 110 collected (height, weight, BMI, age, gender and handedness) and a neurologic examination is  
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53 111 performed. Inclusion and exclusion criteria are verified. Are the criteria met, the child will be enrolled  
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55 112 into the study (table 1).  
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114 **Table 1. Project flow chart.**

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| 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                    |
|  |                     |                      |

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

120

### 121 **Assessments of primary endpoint/outcome**

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

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3 129 well as posterior interosseous nerve at the supinator loge; peroneal nerve in the popliteal space and at  
4 130 the fibular head; tibial nerve in the popliteal space and at the medial malleolus and sural nerve  
5  
6 131 between lateral and medial gastrocnemius head in the calf. In addition the vagus nerve is analysed at  
7  
8 132 the lateral margins of the anterior cervical region beneath the sternocleidomastoid muscle and the  
9  
10 133 diameter of the 5th and 6th cervical nerve roots are measured in longitudinal scan below the  
11  
12 134 processus transversus. CSA is traced inside the hyperechoic rim of the nerve (Fig.1).

135

### 136 **Assessment of secondary endpoints**

137 Epidemiological data will be measured before performing the ultrasonographic examination. Height  
18  
19  
20 138 and weight are measured, BMI is calculated. The patient or the caregiver is asked about the age (Date  
21  
22 139 of birth), gender and handedness.

140

### 141 **Statistics**

142 The sample size was calculated in order to estimate the percentile curves with adequate accuracy.  
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28  
29 143 The accuracy of the estimation was quantified by the length of the bootstrapped 95% confidence  
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31 144 interval of the 50% percentile curve. The sample size estimation was based on the measurements of  
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33 145 medianus prox. forearm right side from the pilot data. 12 patients between age 3.75 and age 6.25  
34  
35 146 were used in this sample size estimation using a resampling method. Each sample size was evaluated  
36  
37 147 by estimating the 50% percentile curve together with its bootstrapped 95% confidence interval  $R = 100$   
38  
39 148 times. Each 95% confidence interval was estimated by simulating 99 times in individual patients, fitting  
40  
41 149 a "Generalized additive model for location, scale and shape" and estimating the 50 % percentile curve  
42  
43 150 from the fitted model. Then the 95% confidence interval of the 50% percentile curve was estimated  
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45 151 using a bootstrap approach using these 99 estimations. For each 50% percentile curve, it was  
46  
47 152 assessed whether the length of the 95% confidence interval was below the predefined margin of 1.2.  
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49 153 Assuming a drop-out rate of 2%, 205 patients should be recruited to ensure 200 evaluable patients.  
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51 154 This sample size allows in more than 80% of 100 hypothetical repetitions of the study (i.e., with a  
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53 155 power of 0.8) to estimate the 50% percentile curve with adequate accuracy (length of the 95%  
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55 156 confidence interval below the predefined margin of 1.2). Figure 2 shows how the sample size depends  
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57 157 on the pre-defined accuracy threshold of the estimate. Additional sample size estimation was

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3 158 performed in order to estimate the sample size needed to estimate the inter-rater and intra-rater  
4 159 reliability of the measurements. Reliability is expressed by the intraclass correlation coefficient (ICC).  
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6 160 Two scenarios have been calculated assuming two examinations in each child with different ICC for  
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8 161 intra- and inter-rater reliability. It is assumed that the ICC is 0.8 for intra-rater and 0.75 for inter-rater  
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10 162 reliability. The study should be able to estimate the ICC with a certain precision. This precision is  
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12 163 expressed as the width of a 95% confidence interval and is here defined to be 1/3. By applying the  
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14 164 sample size approximation of Bonett<sup>20</sup> and assuming a drop-out-rate of 5%, a sample size of 20  
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16 165 patients results (value rounded to the next higher integer) when assuming each child is examined  
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18 166 twice for intra-rater reliability (Fig. 3). A sample size of 30 results when assuming each child is  
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20 167 examined twice for inter-rater reliability (Fig. 4).  
21  
22 168 Figure 3 and 4 show how the sample size depends on the assumed ICC and the number of  
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24 169 examinations in each child.  
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26 170

### 171 **Primary Analysis**

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

### 177 **Secondary Analyses**

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

185 Inter-rater, intra-rater and inter-equipment reliability of the measurements will be investigated by  
186 estimating intraclass correlation coefficients (ICC) according to Streiner & Norman<sup>23</sup>.

187



## 188 **Data protection, archiving and destruction**

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

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

198

## 199 **Ethical considerations**

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

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

209

## 210 **Discussion**

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

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3 217 peripheral and spinal nerves at specific anatomic landmarks in children and adolescents under the  
4 218 age of 19 years. These normal values will guide clinicians in examining children and adolescents with  
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6 219 neuromuscular diseases by ultrasonography.  
7

8 **Strength:** The sample size estimation was based on the available measurements from pilot data.  
9  
10 221 Assuming a drop-out rate of 2%, 205 patients aged between 2 and 18 years should be recruited to  
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12 222 ensure 200 evaluable patients. This sample size allows in more than 80% of 100 hypothetical  
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14 223 repetitions of the study (i.e., with a power of 0.8) to estimate the 50% percentile curve for CSAs of the  
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16 224 most examined nerves at different clinically important locations with adequate accuracy.  
17

18 **Limitations:** Even though we plan to include a large cohort there still is the possibility of unrecognized  
19  
20 226 confounders. The trial is planned as a monocentric study.  
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## 23 228 **Trial status**

24  
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26 229 The trial started enrolment in November 2015 and is expected to be completed by the end of  
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28 230 December 2016.  
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## 31 232 **List of abbreviations**

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34 233 Cross sectional area (CSA)  
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36 234 Longitudinal anteroposterior diameter (LAPD)  
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38 235 University of Basel Children's Hospital (UKBB)  
39  
40 236 Intraclass correlation coefficient (ICC).  
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## 43 238 **Competing interests**

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46 239 The authors declare that they have no competing interests.  
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## 50 241 **Authors' contributions**

51  
52  
53 242 MR participated in the design of the study, acquired data and drafted the manuscript. BFD participated  
54  
55 243 in the design of the study and acquired data. SS participated in the design of the study, performed the  
56  
57 244 statistical analysis and calculated the sample size for the study. AG designed the study and acquired  
58  
59  
60

1  
2  
3 245 data. DF participated in the design of the study and revised the manuscript. PH designed and  
4 246 conducted the study, acquired data and revised the manuscript. All authors read and approved the  
5  
6 247 final manuscript.  
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10 248

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12  
13 250 We do confirm that there is no external funding of this project/study.  
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## 18 252 **Data sharing statement**

19  
20  
21 253 There are no additional unpublished data.  
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31 309 Figure 1: Standardized anatomical points for the measurement of CSA of the measured nerves and  
32 310 diameter of nerve roots

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34 312 Figure 2: Sensitivity of the sample size with respect to the predefined accuracy threshold of the  
35 313 estimate. The curves for a power of 0.7, 0.8 and 0.9 (i.e., 70 %, 80 % and 90 %) are shown. (The  
36 314 curves are smoothed and are shown for illustrative purposes only.)

37 315

38 316 Figure 3: Sample size estimation for ICC – intra-rater reliability. The curve for a power of 0.8 is shown.

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40 318 Figure 4: Sample size estimation for ICC – inter-rater reliability. The curve for a power of 0.75 is  
41 319 shown.

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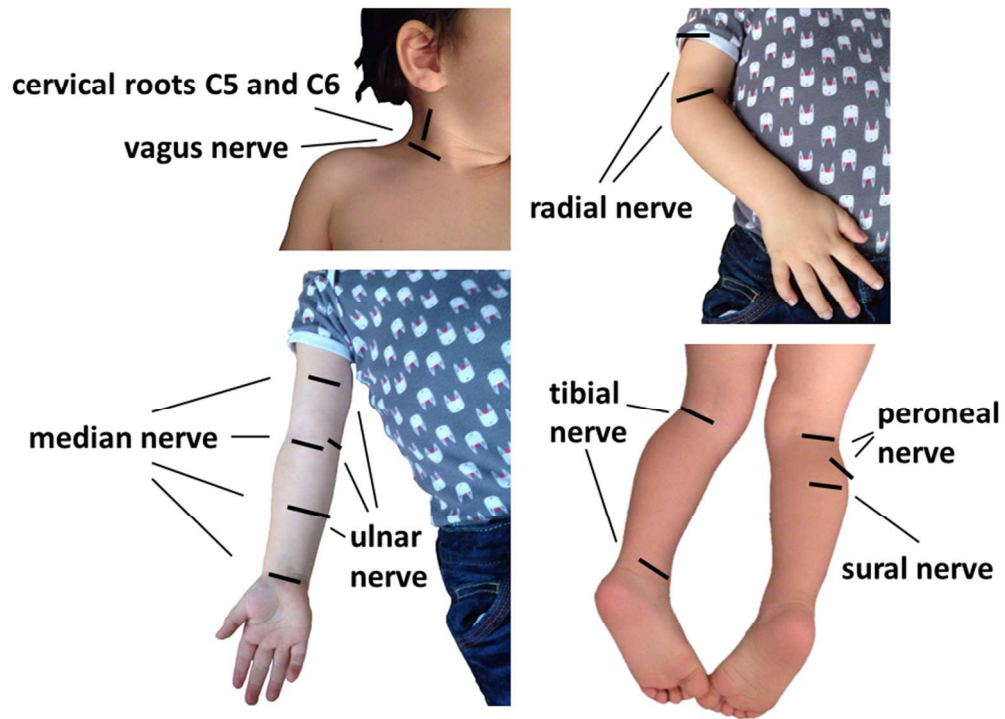


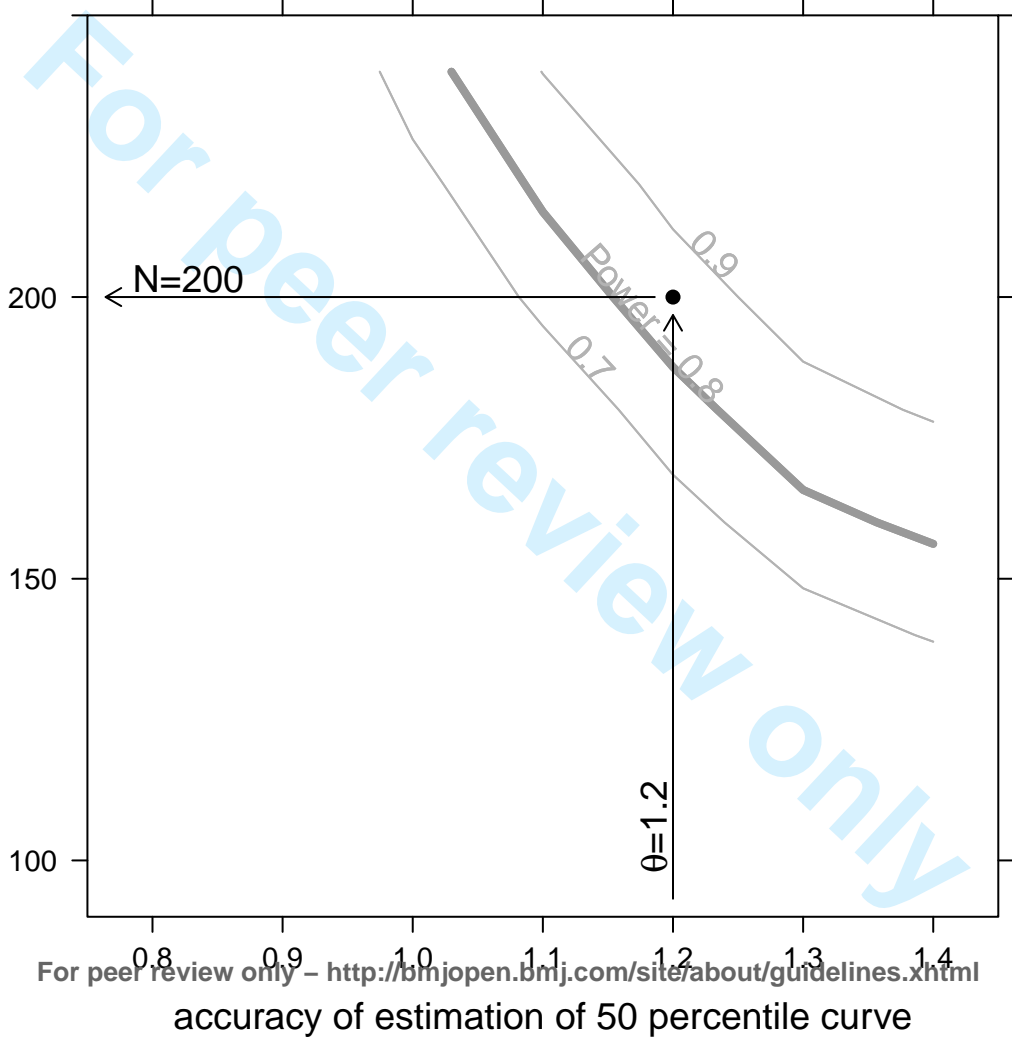
Figure 1: Standardized anatomical points for the measurement of CSA of the measured nerves and diameter of nerve roots

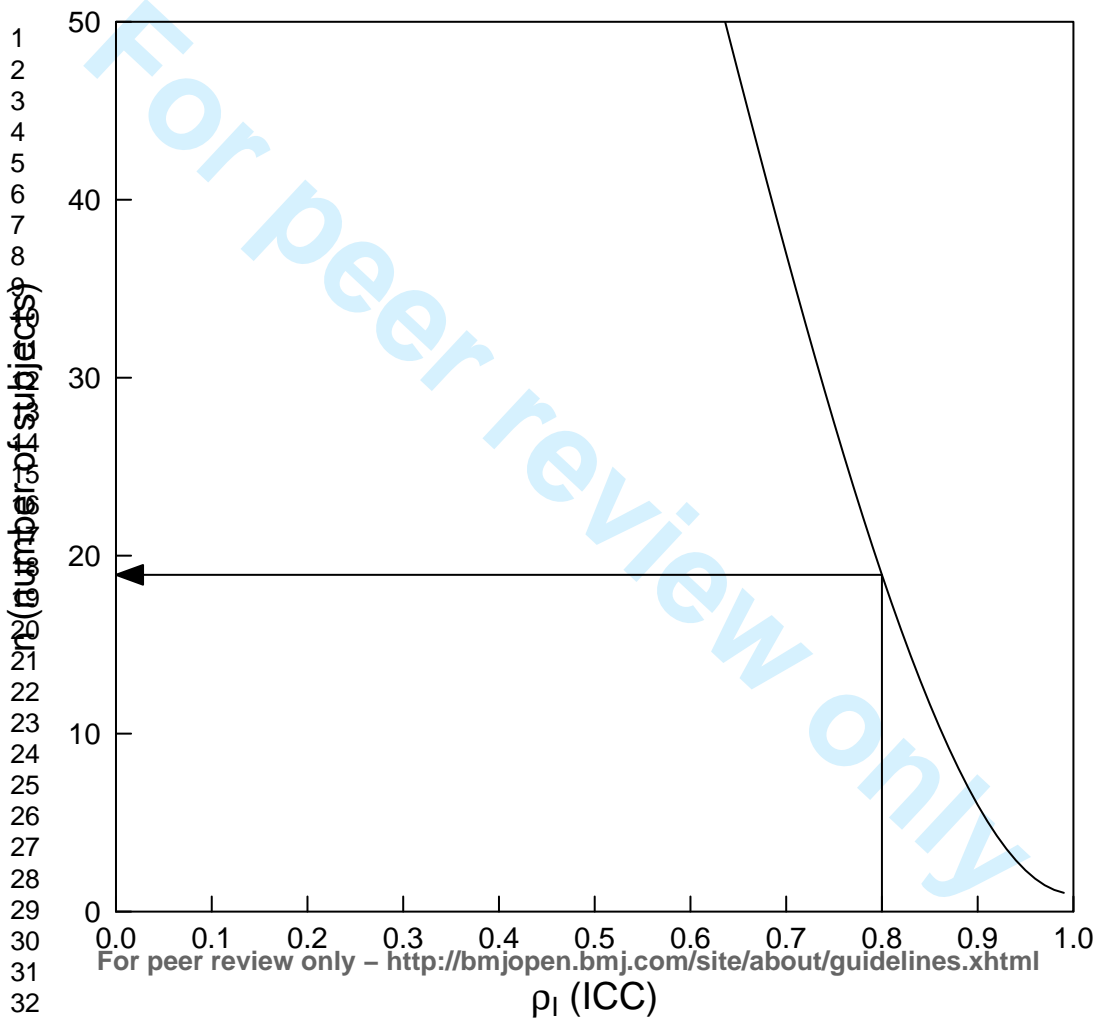
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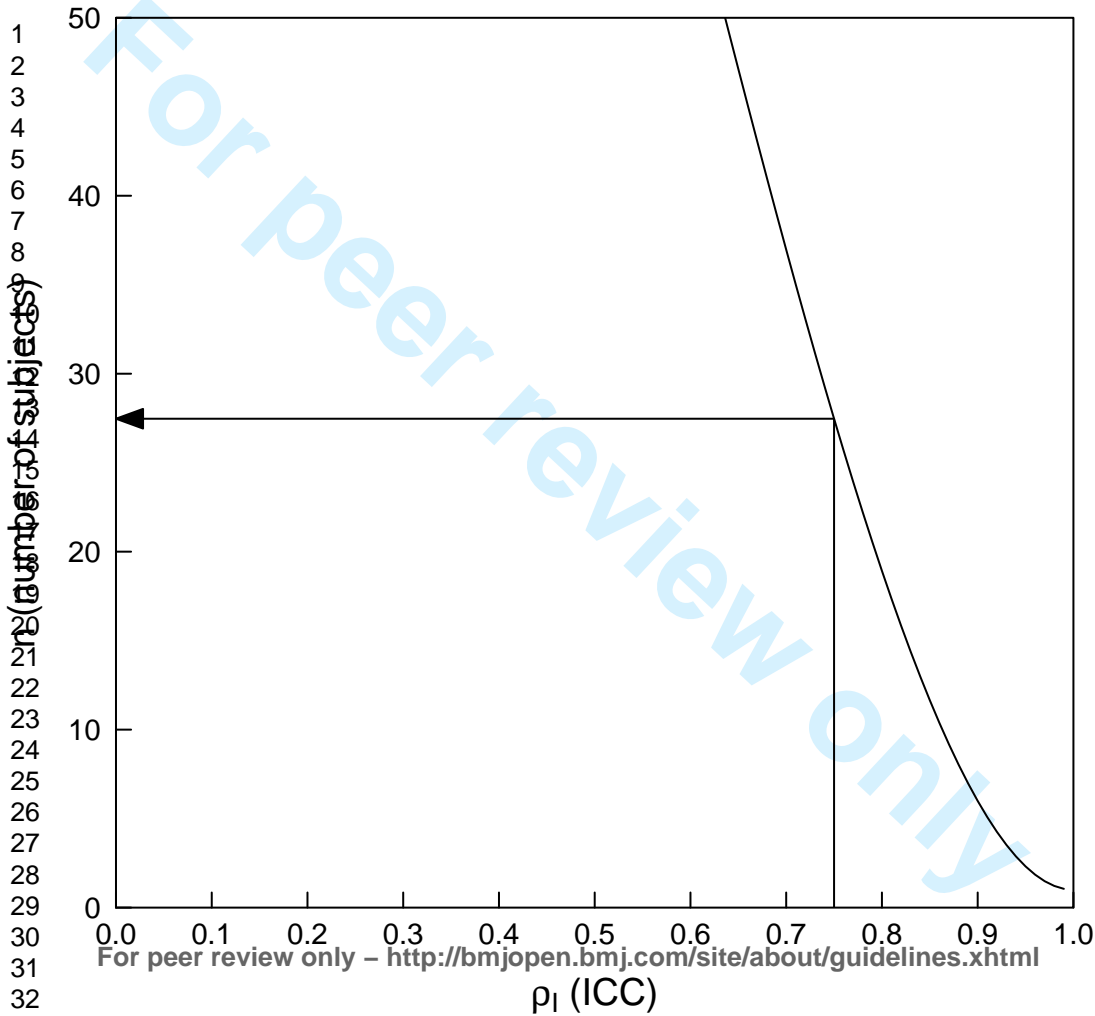
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# BMJ Open

## 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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3 **1 Ultrasonographic reference values for peripheral nerves and nerve**  
4 **2 roots in the normal population of children and adolescents: study**  
5 **3 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  $\geq 2$  and  $\leq 18$  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.

## 56 Background

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

## 80 Methods/Design

### 81 Objectives and endpoints

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

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3 85 diseases as it has been done in adults. The primary endpoint is to determine standard values of the  
4 86 cross-sectional area (CSA) of the C5 and C6 cervical roots, the vagus, median, ulnar, radial,  
5 87 superficial radial, peroneal, tibial, and the sural nerves in children and adolescents between  $\geq 2$  and  
6 88  $\leq 18$ . The secondary objectives are 1) to determine relations between CSA and epidemiological data  
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10 89 and 2) to assess inter- and intra-rater reliability of measurements.  
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## 14 91 **Study design**

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16 92 This is an observational-prospective, monocenter study with an estimated duration of 12 months. The  
17  
18 93 study was registered with ClinicalTrials.gov (Identifier: NCT02570802) and approved by the local  
19  
20 94 ethics committees (EKNZ 2015-210).  
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## 23 96 **Inclusion criteria**

- 24  
25 97 - Children and adolescents aged between  $\geq 2$  and  $\leq 18$  years  
26  
27 98 - Written informed consent of the caregivers and the children/adolescents between 10 and 18 years  
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29 99 - Oral assent by children under the age of 10 years  
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31

## 32 100 **Exclusion criteria**

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35 102 - Inability to meet study requirements  
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37 103 - Neuromuscular disease or symptoms/signs  
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39

## 40 104 **Methodology**

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43 106 Children/adolescents fulfilling the inclusion criteria and their caregivers will be informed about the  
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45 107 procedures and asked to participate either directly after a routine consultation in the outpatients'  
46  
47 108 department, during a hospitalisation at the University of Basel Children's Hospital (UKBB), per written  
48  
49 109 letter addressed to school classes or sports clubs and from the authors' our circle of acquaintances  
50  
51 110 and colleagues. Demographic and anthropometric data is collected (height, weight, BMI, age, gender  
52  
53 111 and handedness) and a neurologic examination is performed. Inclusion and exclusion criteria are  
54  
55 112 verified. Are the criteria met, the child will be enrolled into the study (table 1).  
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114 **Table 1. Project flow chart.**

115

| 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                    |
|  |                     |                      |

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

120

### 121 **Assessments of primary endpoint/outcome**

122 Ultrasound measurements will be done in different nerves and at different locations. Ultrasound is  
 123 performed using a high frequency probe real-time linear array scanner ( Philipps Affiniti 50G and  
 124 others). Ultrasound of different nerves at the upper and lower limbs and the neck are performed  
 125 bilaterally. The nerves are scanned in axial planes, and the cross sectional area (CSA) of each nerve  
 126 is measured at standardized anatomical points as described before <sup>25</sup>. In short: median nerve in the  
 127 mid-upper arm, at the elbow, in the mid-forearm and at the carpal tunnel; ulnar nerve at mid-humerus,  
 128 at the cubital tunnel and in the mid-forearm; radial nerve in the mid-upper arm and superficial radial as

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3 129 well as posterior interosseous nerve at the supinator loge; peroneal nerve in the popliteal space and at  
4 130 the fibular head; tibial nerve in the popliteal space and at the medial malleolus and sural nerve  
5  
6 131 between lateral and medial gastrocnemius head in the calf. In addition the vagus nerve is analysed at  
7  
8 132 the lateral margins of the anterior cervical region beneath the sternocleidomastoid muscle and the  
9  
10 133 diameter and CSA of the 5th and 6th cervical nerve roots are measured in longitudinal scan below the  
11  
12 134 processus transversus. CSA is traced inside the hyperechoic rim of the nerve (Fig.1).

135

### 136 **Assessment of secondary endpoints**

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

140

### 141 **Statistics**

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

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3 158 performed in order to estimate the sample size needed to estimate the inter-rater and intra-rater  
4 159 reliability of the measurements. Reliability is expressed by the intraclass correlation coefficient (ICC).  
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6 160 Two scenarios have been calculated assuming two examinations in each child with different ICC for  
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8 161 intra- and inter-rater reliability. It is assumed that the ICC is 0.8 for intra-rater and 0.75 for inter-rater  
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10 162 reliability. The study should be able to estimate the ICC with a certain precision. This precision is  
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12 163 expressed as the width of a 95% confidence interval and is here defined to be 1/3. By applying the  
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14 164 sample size approximation of Bonett <sup>26</sup> and assuming a drop-out-rate of 5%, a sample size of 20  
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16 165 patients results (value rounded to the next higher integer) when assuming each child is examined  
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18 166 twice for intra-rater reliability (Fig. 3). A sample size of 30 results when assuming each child is  
19  
20 167 examined twice for inter-rater reliability (Fig. 4).

21 168 Figure 3 and 4 show how the sample size depends on the assumed ICC and the number of  
22  
23 169 examinations in each child.  
24  
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### 26 27 171 **Primary Analysis**

28  
29 172 The age-dependent percentile curves will be estimated using a “Generalized additive model for  
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31 173 location, scale and shape” as suggested by the WHO Multicentre Growth Reference Study Group <sup>27</sup>  
32  
33 174 using the R-package Rigby & Stasinopoulos <sup>28</sup>. The analysis will be performed on the full analysis data  
34  
35 175 set.  
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### 38 39 177 **Secondary Analyses**

40  
41 178 The same percentile curves as described in the main analysis will be estimated depending on size and  
42  
43 179 weight. The models will be compared to the main model in order to investigate whether a growth curve  
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45 180 in dependence of size or weight is more applicable than a growth curve depending on age. The  
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47 181 association between gender and handedness and the thickness of the nerves will be investigated by  
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49 182 including these variables as covariables in the main model in an exploratory manner. If gender has a  
50  
51 183 relevant influence on thickness of the nerves, separate growth curves for each gender will be  
52  
53 184 considered.

54 185 Inter-rater, intra-rater and inter-equipment reliability of the measurements will be investigated by  
55  
56 186 estimating intraclass correlation coefficients (ICC) according to Streiner & Norman <sup>29</sup>.  
57  
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## 188 **Data protection, archiving and destruction**

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

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

198

## 199 **Ethical considerations**

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

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

209

## 210 **Discussion**

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

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

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

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

## 230 Trial status

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

## 234 List of abbreviations

235 Cross sectional area (CSA)  
236 Longitudinal anteroposterior diameter (LAPD)  
237 University of Basel Children's Hospital (UKBB)  
238 Intraclass correlation coefficient (ICC).

## 240 Competing interests

241 The authors declare that they have no competing interests.

## 243 Authors' contributions

1  
2  
3 244 MR participated in the design of the study, acquired data and drafted the manuscript. BFD participated  
4  
5 245 in the design of the study and acquired data. SS participated in the design of the study, performed the  
6  
7 246 statistical analysis and calculated the sample size for the study. AG designed the study and acquired  
8  
9 247 data. DF participated in the design of the study and revised the manuscript. PH designed and  
10  
11 248 conducted the study, acquired data and revised the manuscript. All authors read and approved the  
12  
13 249 final manuscript.  
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252 We do confirm that there is no external funding of this project/study.  
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## 254 Data sharing statement

255 There are no additional unpublished data.  
256

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

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3 328 Figure 2: Sensitivity of the sample size with respect to the predefined accuracy threshold of the  
4 329 estimate. The curves for a power of 0.7, 0.8 and 0.9 (i.e., 70 %, 80 % and 90 %) are shown. (The  
5 330 curves are smoothed and are shown for illustrative purposes only.)  
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8 331  
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10 332 Figure 3: Sample size estimation for ICC – intra-rater reliability. The curve for a power of 0.8 is shown.  
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14 334 Figure 4: Sample size estimation for ICC – inter-rater reliability. The curve for a power of 0.75 is  
15 335 shown.  
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For peer review only

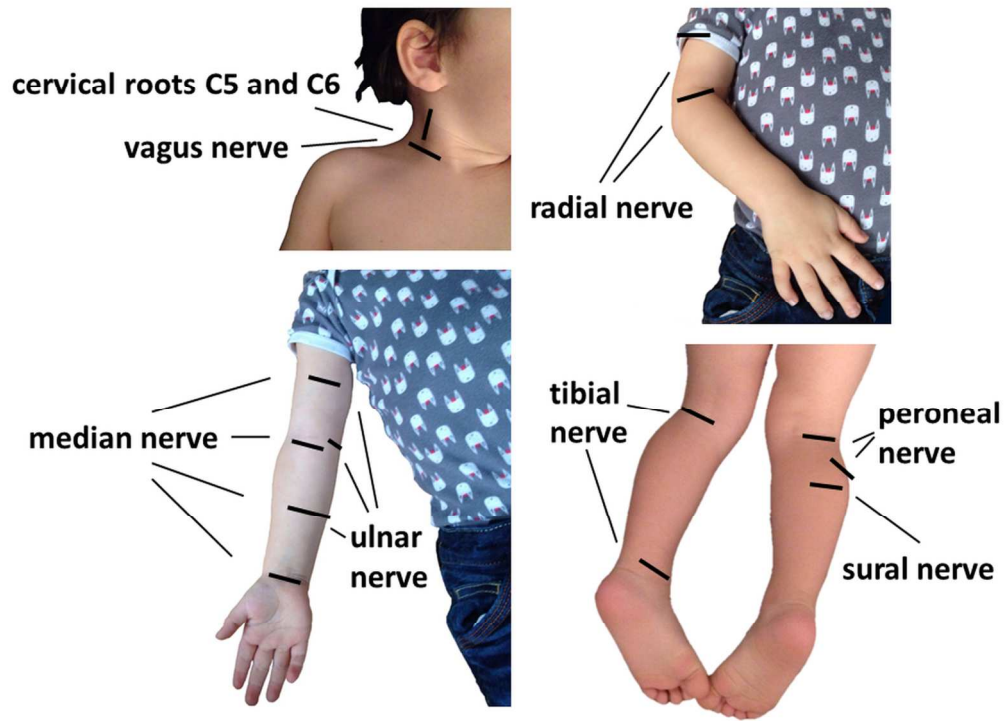


Figure 1: Standardized anatomical points for the measurement of CSA of the measured nerves and diameter of nerve roots

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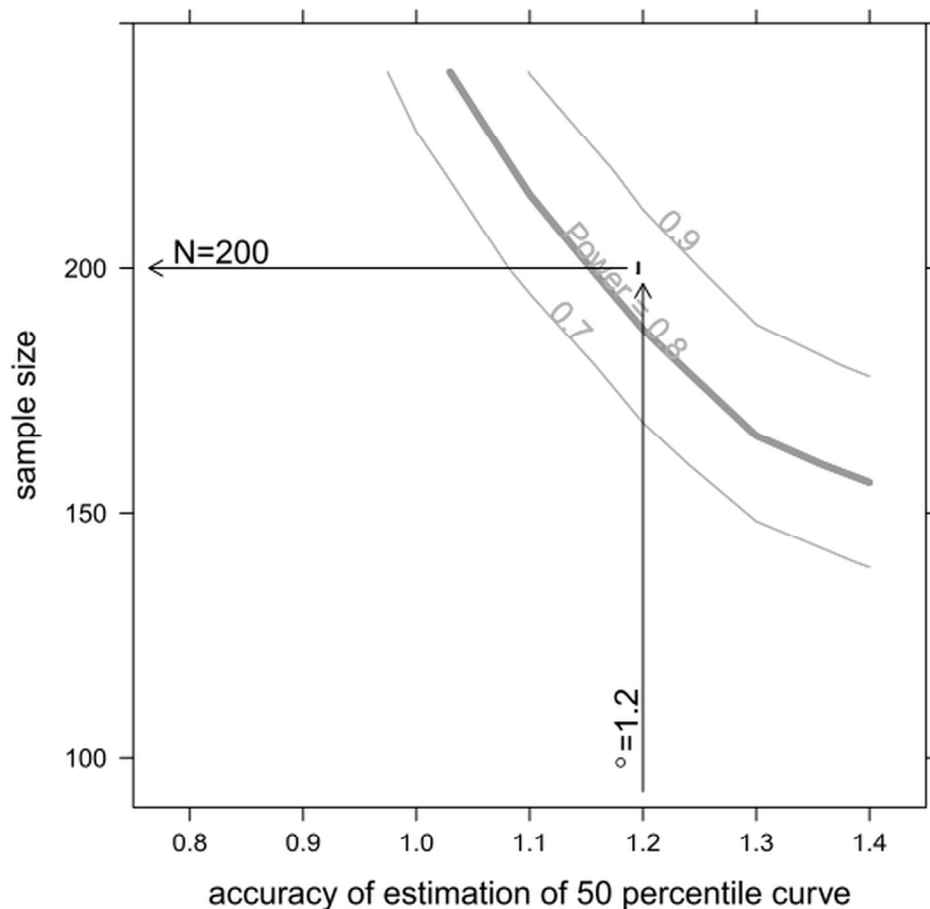


Figure 2: Sensitivity of the sample size with respect to the predefined accuracy threshold of the estimate. The curves for a power of 0.7, 0.8 and 0.9 (i.e., 70 %, 80 % and 90 %) are shown. (The curves are smoothed and are shown for illustrative purposes only.)

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**number of examinations in one child: 2**

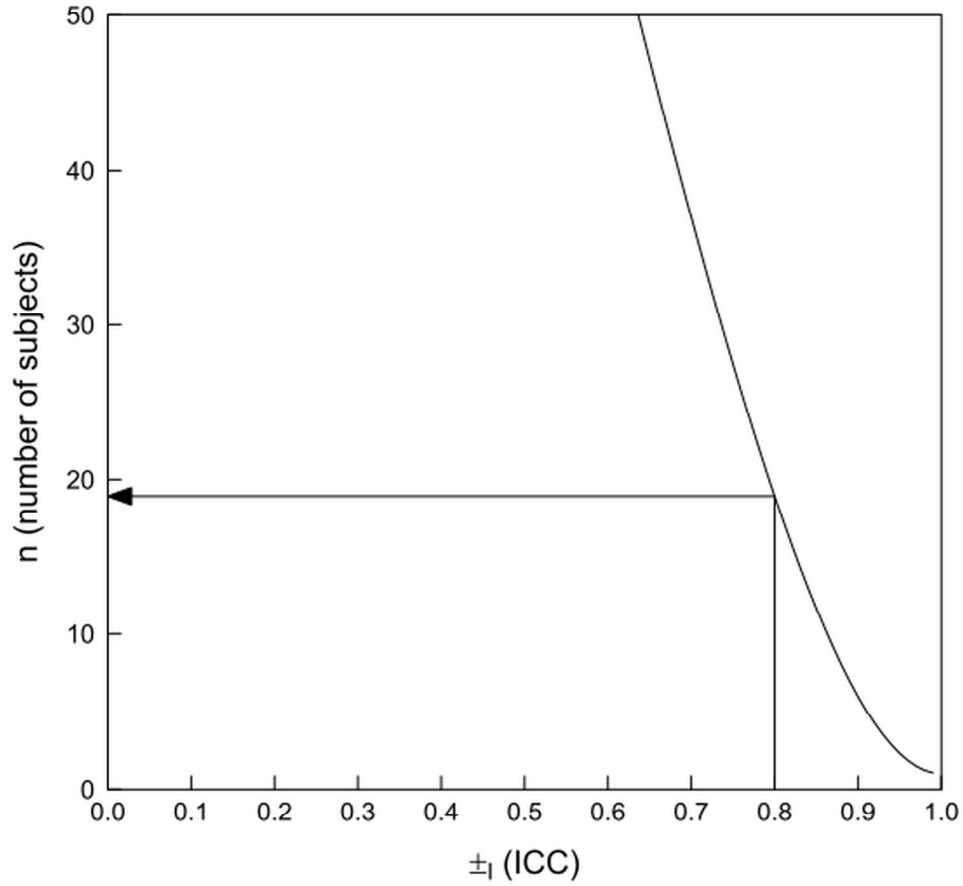


Figure 3: Sample size estimation for ICC – intra-rater reliability. The curve for a power of 0.8 is shown.

152x152mm (300 x 300 DPI)





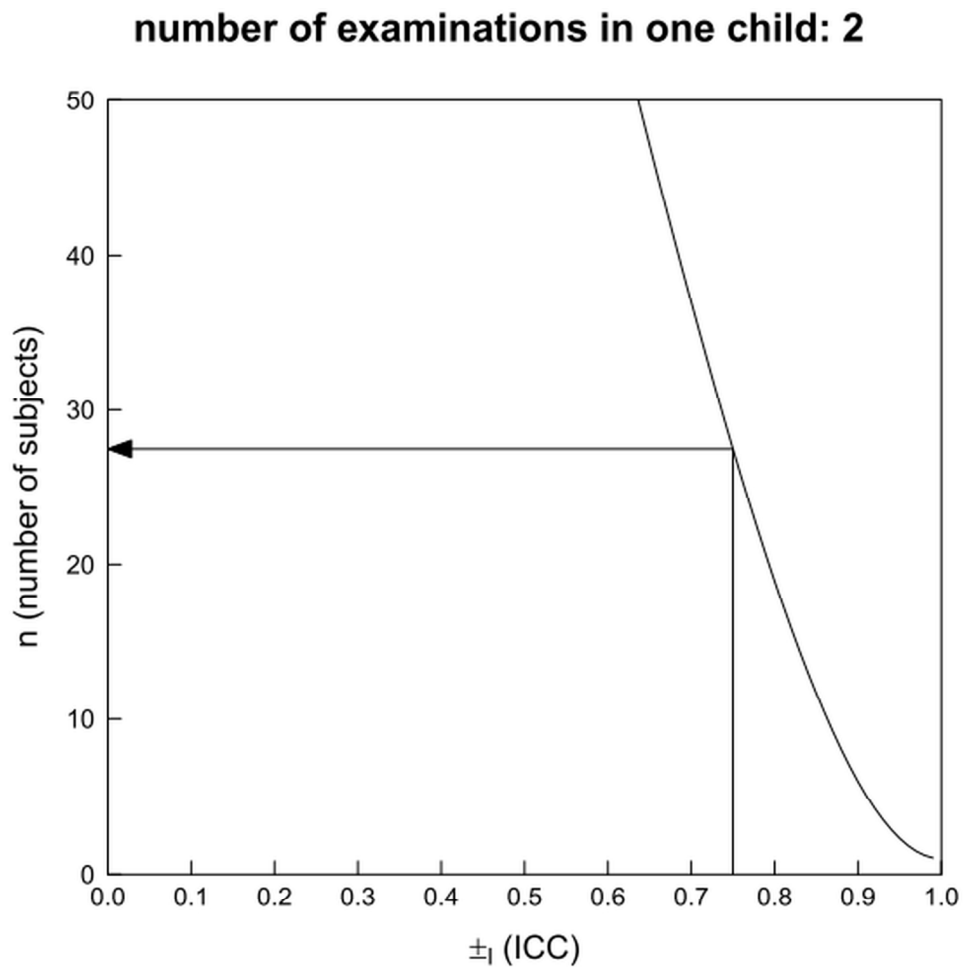


Figure 4: Sample size estimation for ICC – inter-rater reliability. The curve for a power of 0.75 is shown.

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# BMJ Open

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

|                                    |   |
|------------------------------------|---|
| Journal:                           | <i>BMJ Open</i>   |
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| Complete List of Authors:          | Rasenack, Maria; Basel University Hospital, Department of Neurology<br>Décard, Bernhard; Basel University Hospital, Department of Neurology<br>Schaedelin, Sabine; Basel University Hospital, Clinical Tria Unit<br>Grimm, Alexander; Tübingen University Hospital<br>Fischer, Dirk; University of Basel Children's Hospital, Division of<br>Neuropaediatrics; Kantonsspital Baselland, Division of Neurology, Medical<br>University Clinic<br>Hafner, Patricia; University of Basel Children's Hospital, Division of<br>Neuropaediatrics; Kantonsspital Baselland, Division of Neurology, Medical<br>University Clinic |
| <b>Primary Subject<br>Heading</b>: | Neurology   |
| Secondary Subject Heading:         | Diagnostics, Paediatrics, Radiology and imaging   |
| Keywords:                          | High resolution nerve ultrasound, normal values, peripheral nerve system  |
|                                    |   |

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Manuscripts

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3 **1 Ultrasonographic reference values for peripheral nerves and nerve**  
4 **2 roots in the normal population of children and adolescents: study**  
5 **3 protocol for an observational-prospective trial**  
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44 **Keywords**  
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46 High resolution nerve ultrasound, normal values, peripheral nerve system  
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51 Word count: 1982  
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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  $\geq 2$  and  $\leq 18$  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

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3 56 High-resolution ultrasonography is an emerging non-invasive technique for the investigation of  
4 57 peripheral nerves and is increasingly used worldwide in the diagnosis of peripheral nerve disorders.  
5 58 The value of peripheral nerve ultrasound for diagnosis of peripheral nerve damage in entrapment  
6 59 syndromes, nerve tumors and focal nerve lesions has been demonstrated clearly <sup>1-9</sup>. In adults it has  
7 60 become a useful supplementary tool for electrodiagnostic studies in these conditions. Characteristic  
8 61 nerve size changes in polyneuropathies have been reported as well <sup>10-16</sup> and are now further  
9 62 investigated. Nerve width (medial to lateral diameter), thickness (anterior to posterior diameter) and  
10 63 cross-sectional area (CSA) measured on transverse scans, and anteroposterior diameter (LAPD)  
11 64 measured on longitudinal scans are the most frequently used quantitative parameters for the  
12 65 ultrasound investigation of peripheral nerves. Furthermore, ratios of CSA between different segments  
13 66 of the same nerve have also been used. Several reports have been published on reference values for  
14 67 the cross-sectional areas in nerves in adulthood, as well as normal values for cervical roots, radial  
15 68 nerve, lower limb nerves and pure sensory nerves <sup>17-22</sup>. In children the use of ultrasound was  
16 69 demonstrated in few studies of hereditary and immune-mediated neuropathies <sup>23 24</sup>. So far no work  
17 70 has been published on standard values for ultrasonography in children and adolescents. Especially in  
18 71 this population benefit of this fast and non-invasive technic is great because children can be examined  
19 72 much more stress-free. The aim of our study is to establish normal CSA values for C5 and C6 cervical  
20 73 roots, and several upper and lower limb nerves, including some pure sensory nerves, at pre-defined  
21 74 anatomical sites in children and adolescents, and to assess whether the CSAs correlates with height,  
22 75 age, gender and BMI. Furthermore, to test if such measurements are reliable in routine clinical  
23 76 practice, the intra- and inter-rater reliability of peripheral nerve ultrasound measurements will be  
24 77 assessed.  
25 78

## 29 **Methods/Design**

### 30 **Objectives and endpoints**

31 81 The purpose of this study is to assess standard values of nerve ultrasonography in children and  
32 82 adolescents to use these values as a reference in clinical practice. This allows that nerve  
33 83 ultrasonography in children can be further evaluated and compared to standard values in different  
34 84 diseases as it has been done in adults. The primary endpoint is to determine standard values of the  
35 85 cross-sectional area (CSA) of the C5 and C6 cervical roots, the vagus, median, ulnar, radial,

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3 86 superficial radial, peroneal, tibial, and the sural nerves in children and adolescents between  $\geq 2$  and  
4 87  $\leq 18$ . The secondary objectives are 1) to determine relations between CSA and epidemiological data  
5  
6 88 and 2) to assess inter- and intra-rater reliability of measurements.  
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## 10 **Study design**

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12 91 This is an observational-prospective, monocenter study with an estimated duration of 12 months. The  
13  
14 92 study was registered with ClinicalTrials.gov (Identifier: NCT02570802) and approved by the local  
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16 93 ethics committees (EKNZ 2015-210).  
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## 20 **Inclusion criteria**

- 21  
22 96 - Children and adolescents aged between  $\geq 2$  and  $\leq 18$  years  
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24 97 - Written informed consent of the caregivers and the children/adolescents between 10 and 18 years  
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26 98 - Oral assent by children under the age of 10 years  
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## 30 **Exclusion criteria**

- 31  
32 101 - Inability to meet study requirements  
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34 102 - Neuromuscular disease or symptoms/signs  
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## 38 **Methodology**

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40 105 Children/adolescents fulfilling the inclusion criteria and their caregivers will be informed about the  
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42 106 procedures and asked to participate either directly after a routine consultation in the outpatients'  
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44 107 department, during a hospitalisation at the University of Basel Children's Hospital (UKBB), per written  
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46 108 letter addressed to school classes or sports clubs and from the authors' our circle of acquaintances  
47  
48 109 and colleagues. Demographic and anthropometric data is collected (height, weight, BMI, age, gender  
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50 110 and handedness) and a neurologic examination is performed. Inclusion and exclusion criteria are  
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52 111 verified. Are the criteria met, the child will be enrolled into the study (table 1).  
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## 56 **Table 1. Project flow chart.**

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| 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                    |
|  |                     |                      |

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

119

### 120 **Assessments of primary endpoint/outcome**

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

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3 130 between lateral and medial gastrocnemius head in the calf. In addition the vagus nerve is analysed at  
4 131 the lateral margins of the anterior cervical region beneath the sternocleidomastoid muscle and the  
5 132 diameter and CSA of the 5th and 6th cervical nerve roots are measured in longitudinal scan below the  
6 133 processus transversus. CSA is traced inside the hyperechoic rim of the nerve (Fig.1).  
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### 11 135 **Assessment of secondary endpoints**

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14 136 Epidemiological data will be measured before performing the ultrasonographic examination. Height  
15 137 and weight are measured, BMI is calculated. The patient or the caregiver is asked about the age (Date  
16 138 of birth), gender and handedness.  
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### 21 140 **Statistics**

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24 141 The sample size was calculated in order to estimate the percentile curves with adequate accuracy.  
25 142 The accuracy of the estimation was quantified by the length of the bootstrapped 95% confidence  
26 143 interval of the 50% percentile curve. The sample size estimation was based on the measurements of  
27 144 medianus prox. forearm right side from the pilot data. 12 patients between age 3.75 and age 6.25  
28 145 were used in this sample size estimation using a resampling method. Each sample size was evaluated  
29 146 by estimating the 50% percentile curve together with its bootstrapped 95% confidence interval R = 100  
30 147 times. Each 95% confidence interval was estimated by simulating 99 times in individual patients, fitting  
31 148 a “Generalized additive model for location, scale and shape” and estimating the 50 % percentile curve  
32 149 from the fitted model. Then the 95% confidence interval of the 50% percentile curve was estimated  
33 150 using a bootstrap approach using these 99 estimations. For each 50% percentile curve, it was  
34 151 assessed whether the length of the 95% confidence interval was below the predefined margin of 1.2.  
35 152 Assuming a drop-out rate of 2%, 205 patients should be recruited to ensure 200 evaluable patients.  
36 153 This sample size allows in more than 80% of 100 hypothetical repetitions of the study (i.e., with a  
37 154 power of 0.8) to estimate the 50% percentile curve with adequate accuracy (length of the 95%  
38 155 confidence interval below the predefined margin of 1.2). Figure 2 shows how the sample size depends  
39 156 on the pre-defined accuracy threshold of the estimate. Additional sample size estimation was  
40 157 performed in order to estimate the sample size needed to estimate the inter-rater and intra-rater  
41 158 reliability of the measurements. Reliability is expressed by the intraclass correlation coefficient (ICC).  
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3 159 Two scenarios have been calculated assuming two examinations in each child with different ICC for  
4 160 intra- and inter-rater reliability. It is assumed that the ICC is 0.8 for intra-rater and 0.75 for inter-rater  
5 161 reliability. The study should be able to estimate the ICC with a certain precision. This precision is  
6 162 expressed as the width of a 95% confidence interval and is here defined to be 1/3. By applying the  
7  
8 163 sample size approximation of Bonett <sup>26</sup> and assuming a drop-out-rate of 5%, a sample size of 20  
9 164 patients results (value rounded to the next higher integer) when assuming each child is examined  
10 165 twice for intra-rater reliability (Fig. 3). A sample size of 30 results when assuming each child is  
11 166 examined twice for inter-rater reliability (Fig. 4).

12 167 Figure 3 and 4 show how the sample size depends on the assumed ICC and the number of  
13 168 examinations in each child.

14 169

### 15 170 **Primary Analysis**

16 171 The age-dependent percentile curves will be estimated using a “Generalized additive model for  
17 172 location, scale and shape” as suggested by the WHO Multicentre Growth Reference Study Group <sup>27</sup>  
18 173 using the R-package Rigby & Stasinopoulos <sup>28</sup>. The analysis will be performed on the full analysis data  
19 174 set.

20 175

### 21 176 **Secondary Analyses**

22 177 The same percentile curves as described in the main analysis will be estimated depending on size and  
23 178 weight. The models will be compared to the main model in order to investigate whether a growth curve  
24 179 in dependence of size or weight is more applicable than a growth curve depending on age. The  
25 180 association between gender and handedness and the thickness of the nerves will be investigated by  
26 181 including these variables as covariables in the main model in an exploratory manner. If gender has a  
27 182 relevant influence on thickness of the nerves, separate growth curves for each gender will be  
28 183 considered.

29 184 Inter-rater, intra-rater and inter-equipment reliability of the measurements will be investigated by  
30 185 estimating intraclass correlation coefficients (ICC) according to Streiner & Norman <sup>29</sup>.

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### 32 187 **Data protection, archiving and destruction**

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3 188 In this study personal patient data will be captured. This data will be encoded and is only accessible to  
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5 189 experts. The appropriate experts of the sponsor (or their designees) can survey the conduct of the  
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7 190 study with monitoring or audits. In case of inspections these experts and also members of the  
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9 191 appropriate authorities can get access to the original data. Also the responsible Ethics Committee can  
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11 192 get access to the original data. The confidentiality of the data will be strictly protected during the whole  
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13 193 study and when performing the mentioned controls. The name of the patient will not be published in no  
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15 194 way in reports or publications arisen from the study.

16 195 The paper documents will be stored in a lockable room during 10 years in the archive of the UKBB in a  
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18 196 dedicated shelf.

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## 21 198 **Ethical considerations**

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23 199 To generate ultrasonographic reference values in children and adolescents it is inevitable to include  
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25 200 subjects requiring particular protection (children under the age of 18 years) into this trial. The  
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27 201 participation in this study is voluntary. The parents and the patient can withdraw their consent at every  
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29 202 time point without giving any reason. In case of withdrawal the data collected until this time point will  
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31 203 be used.

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33 204 As the ultrasonography of peripheral nerves is a non-invasive and painless examination the benefit of  
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35 205 generating normal values and therefore providing a tool to complement and minimise more invasive  
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37 206 electrophysiological examinations legitimates the recruitment and examination of subjects requiring  
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39 207 particular protection.

40 208 Local Ethics Committee approval was obtained (EKNZ 2015-210) and the study is registered online  
41  
42 209 with [clinicaltrials.gov](http://clinicaltrials.gov) (NCT02570802).

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## 45 46 47 211 **Dissemination**

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49 212 The results of this study will be published in peer-reviewed journals and presented at national and  
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51 213 international scientific meetings and congresses to ensure the applicability of its findings into clinical  
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53 214 practice.

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## 56 57 58 216 **Discussion**

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3 217 Standard values for nerve ultrasonography in children and adolescents have not been published so  
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5 218 far. This fast and non-invasive technique may provide great benefit especially in children because they  
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7 219 can be examined much more stress-free. Therefore, the main aim of our study is to establish normal  
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9 220 CSA values for C5 and C6 cervical roots, and several upper and lower limb nerves, including some  
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11 221 pure sensory nerves, at pre-defined anatomical sites in children and adolescents. This study will  
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13 222 provide these urgently needed reference values for the ultrasonographic evaluation of several  
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15 223 peripheral and spinal nerves at specific anatomic landmarks in children and adolescents under the  
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17 224 age of 19 years. These normal values will guide clinicians in examining children and adolescents with  
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19 225 neuromuscular diseases by ultrasonography.

20 226 **Strength:** The sample size estimation was based on the available measurements from pilot data.  
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22 227 Assuming a drop-out rate of 2%, 205 patients aged between 2 and 18 years should be recruited to  
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24 228 ensure 200 evaluable patients. This sample size allows in more than 80% of 100 hypothetical  
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26 229 repetitions of the study (i.e., with a power of 0.8) to estimate the 50% percentile curve for CSAs of the  
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28 230 most examined nerves at different clinically important locations with adequate accuracy.

29 231 **Limitations:** Even though we plan to include a large cohort there still is the possibility of unrecognized  
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31 232 confounders. The trial is planned as a monocentric study. By not only including patients from our  
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33 233 hospital but also from schools, sports classes and the authors' circle of acquaintances we will try to  
34  
35 234 reduce selection bias.

36 235

## 37 38 236 **Trial status**

39  
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41 237 The trial started enrolment in November 2015 and is expected to be completed by the end of  
42  
43 238 December 2017.

44  
45 239

## 46 47 240 **List of abbreviations**

48  
49 241 Cross sectional area (CSA)

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51 242 Longitudinal anteroposterior diameter (LAPD)

52  
53 243 University of Basel Children's Hospital (UKBB)

54  
55 244 Intraclass correlation coefficient (ICC).

56  
57 245

## 246 **Competing interests**

247 The authors declare that they have no competing interests.

248

## 249 **Authors' contributions**

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

256

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

259

## 260 **Data sharing statement**

261 There are no additional unpublished data.

262

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7 331 Figure 1: Standardized anatomical points for the measurement of CSA of the measured nerves and  
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12 334 Figure 2: Sensitivity of the sample size with respect to the predefined accuracy threshold of the  
13 335 estimate. The curves for a power of 0.7, 0.8 and 0.9 (i.e., 70 %, 80 % and 90 %) are shown. (The  
14 336 curves are smoothed and are shown for illustrative purposes only.)  
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20 338 Figure 3: Sample size estimation for ICC – intra-rater reliability. The curve for a power of 0.8 is shown.  
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24 340 Figure 4: Sample size estimation for ICC – inter-rater reliability. The curve for a power of 0.75 is  
25 341 shown.  
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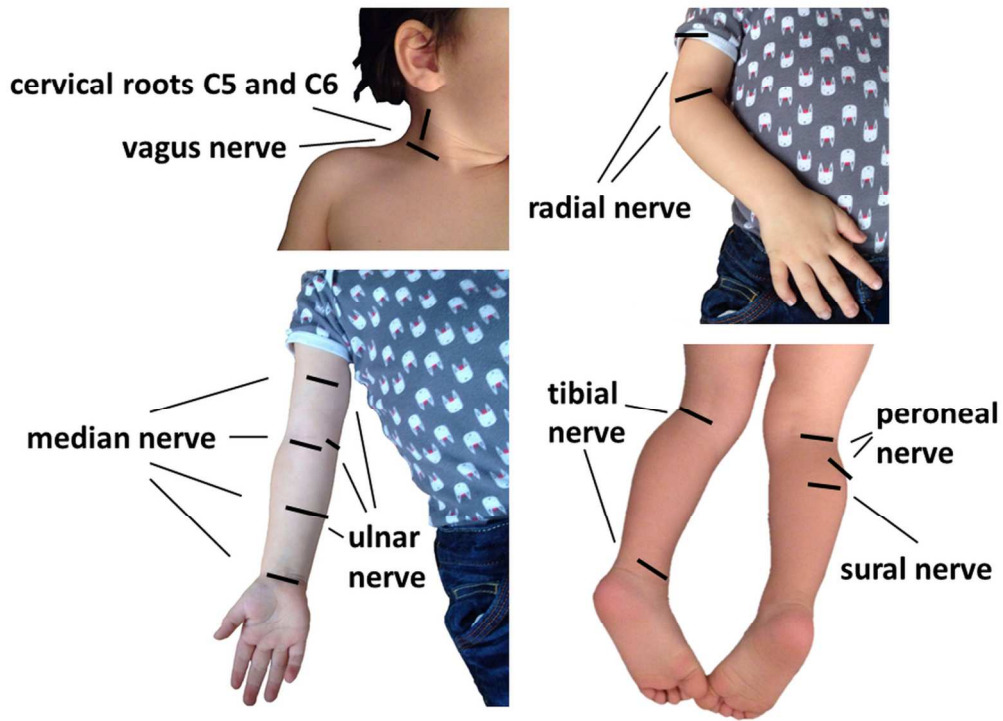


Figure 1: Standardized anatomical points for the measurement of CSA of the measured nerves and diameter of nerve roots

170x127mm (300 x 300 DPI)

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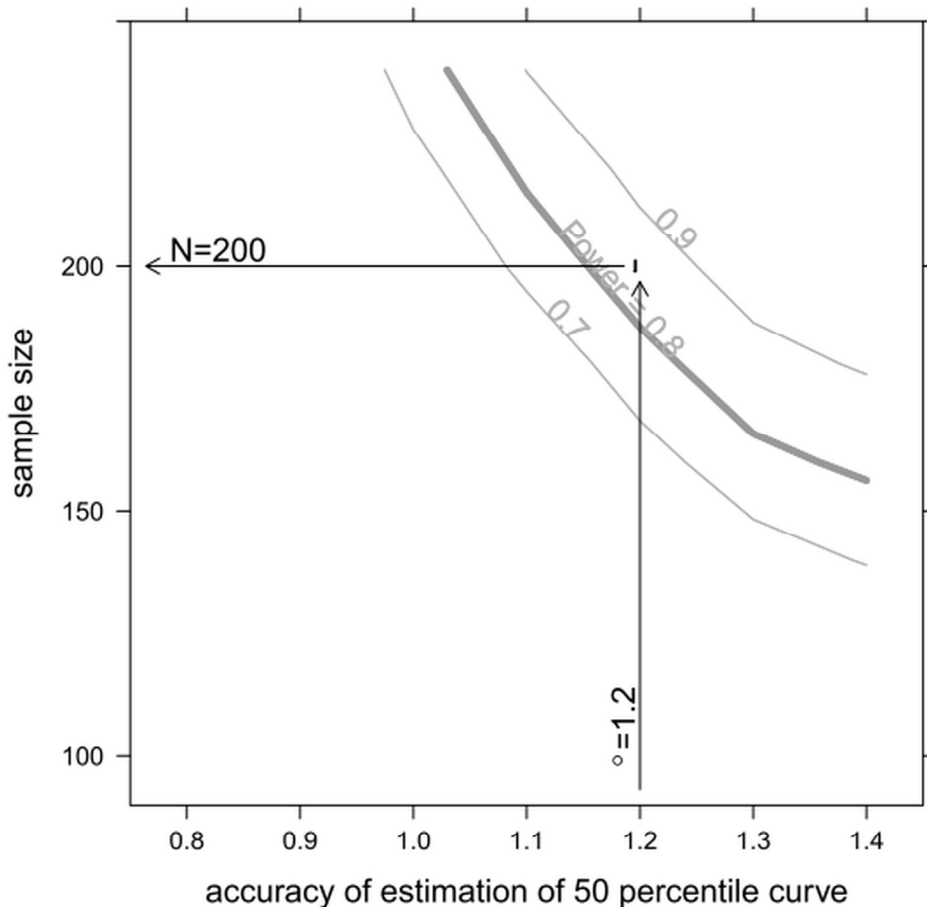


Figure 2: Sensitivity of the sample size with respect to the predefined accuracy threshold of the estimate. The curves for a power of 0.7, 0.8 and 0.9 (i.e., 70 %, 80 % and 90 %) are shown. (The curves are smoothed and are shown for illustrative purposes only.)

152x152mm (300 x 300 DPI)



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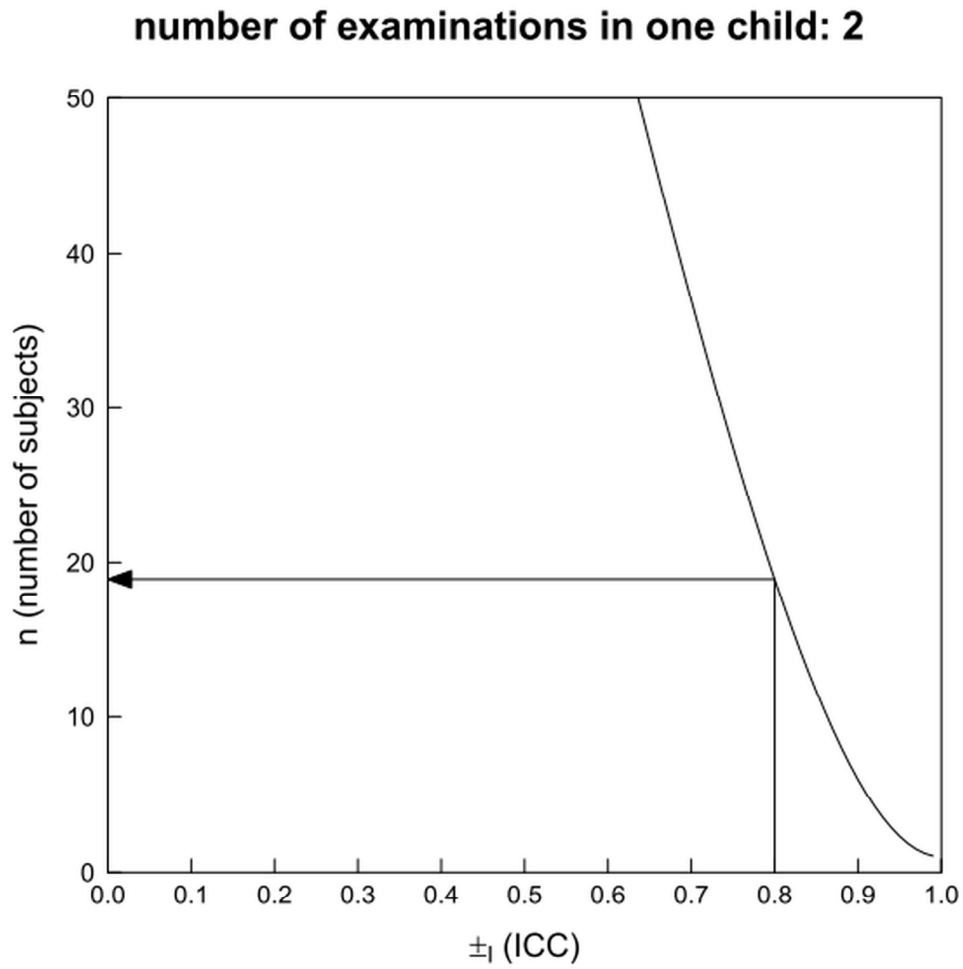


Figure 3: Sample size estimation for ICC – intra-rater reliability. The curve for a power of 0.8 is shown.

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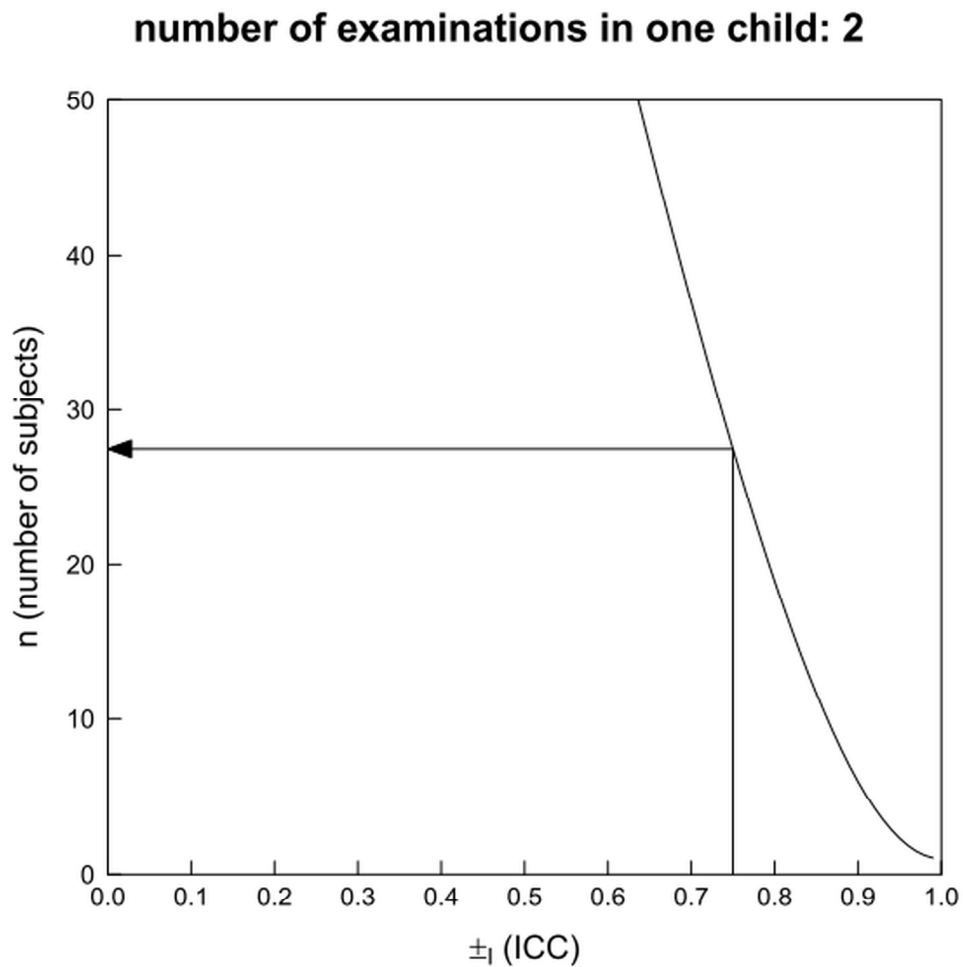


Figure 4: Sample size estimation for ICC – inter-rater reliability. The curve for a power of 0.75 is shown.

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