Nailfold videocapillaroscopy in healthy children and adolescents: description of normal patterns

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ABSTRACT

Objective. *to describe normal patterns of nailfold videocapillaroscopy (NVC) in healthy children and adolescents; to quantify the relationship between age and capillary dimensions, intercapillary distance and number of capillaries/mm; to evaluate the inter and intraobserver concordance.*

Methods. *Cross-sectional study including 100 healthy participants aged 5 to 18 years. Capillary dimensions (capillary loop length, capillary width and intercapillary distance) and number of capillaries/mm were evaluated in 900 capillaries using stereomicroscope under 100x magnification. Intra and inter observer agreements were tested.*

Results. *The capillary dimensions (mean ± SD) were: capillary loop length 278.6±60.3 µm, intercapillary distance 124.1±28.1 µm, capillary width 15.0±2.6 µm. Teenagers between 15 and 18 years had longer and more enlarged capillaries than the other age groups ($p<0.001$ and $p=0.012$ respectively). We also found a significant increase in the number of capillaries/mm with age ($p<0.001$). There was a positive correlation between age and number of capillaries/mm, capillary length, and capillary width ($p<0.001$, $R=0.796$; $p<0.001$, $R=0.368$; $p=0.004$, $R=0.285$, respectively). There was a good intra and interobserver concordance. Enlarged capillary and avascular areas were present in 11% and 10% of capillaries respectively. A weak negative correlation was found between the intercapillary distance and the number of capillaries/mm ($p=0.05$; $R=-0.20$).*

Conclusion. *There is a wide variability in the capillary morphology among healthy individuals. There was a positive correlation between age and number of capillaries/mm, capillary length, and capillary width. In addition, NVC has been shown to be a reproducible method.*

Introduction

Nailfold capillaroscopy (NC) is a well-established method for the assessment of the microcirculation in individuals with Raynaud´s phenomenon (RP) and for the early diagnosis of systemic sclerosis in children and adults (1-6). It recently has been included into new classification of systemic sclerosis criteria (7). Especially in children, NC has been considered a safe, non-invasive, and cost-effective tool that may be useful in the detection of young individuals who are at risk for developing autoimmune rheumatic diseases (ARDs) (8-11).

Videocapillaroscopy (NVC) is an extension of the original widefield NC method that has been more recently used to assess the microvasculature in adults or children affected by ARDs (6-10). It uses higher magnification compared to widefield NC and provides a more objective analysis of the capillaries because of the inclusion of coupled software to perform quantitative assessments of the capillary loop dimensions (10-13). Another advantage is that the images acquired can be stored and analysed at a later time, which allows blind analysis of the data and the possibility of longitudinal follow-up in the same patient. Because of these factors, NVC has been considered more reliable than NC (12-14). Consequently, there has been a growing interest in the NVC method (15, 16).

The morphologic abnormalities in the microcirculation of individuals with ARDs have been widely investigated using NVC and has recently been standardised for healthy adults (4, 5, 15-20). The most important parameters studied include: capillary density (number of capillaries/mm), capillary width, intercapillary distance, and capillary length (5, 6, 21). Nevertheless,

there is a wide variability in the capillary morphology and pattern among the healthy population, and an accurate evaluation and interpretation of these findings are fundamental (9-11, 19, 20). In healthy children the normal nailfold capillary network appears to have some important differences in comparison to adults, including a lower number of loops per millimeter, a higher plexus visualisation score, and a higher frequency of atypical loops (8-11). A small amount of enlarged capillaries and avascular areas was observed in a study in healthy children and adolescents (8). Nonetheless, few studies have assessed the use of NVC in the paediatric population and evaluated the age-specific normal values (11, 22). In the study of Herrick *et al*. with 110 healthy children and adolescents in different ages capillary dimensions were calculated from one capillary, and capillary density from 3mm of nailbed (22). In 2005, Ingegnoli *et al*. analysed the microvasculature of 50 healthy children and 118 patients with ARDs and found an increase in the number of capillaries/mm with age, although the difference was not statistically significant (11). However, there is no data in the literature on the standardisation and reproducibility of NVC for healthy children and adolescents.

Recognition of possible abnormal NVC patterns in childhood ARDs requires a previous definition of the normal variation of the capillaries as well as definition of the degree of variation of the capillary dimensions according to their percentiles in different age ranges. Therefore, the aims of the present study were to describe the normal patterns of NVC in healthy children and adolescents distributed between the different age groups and to assess the reproducibility of the method.

Methods

The present study was a cross-sectional observational study that analysed 900 capillaries from 100 healthy participants ranging from five to 18 years recruited from one primary and one secondary school.

Individuals who met the criteria for ARDs or other systemic diseases (diabetes mellitus, systemic arterial hypertension, thyroid disorders), who had a present or past history of RP, and who had first-degree relatives with ARDs were excluded from the study. Other exclusion criteria included: active smokers, use of any medication for acute or chronic disorders during the past thirty days before examination, or subjects who had any periungueal trauma or who had manipulated their cuticles in the past 30 days. The study was approved by the Institutional Ethics Committee. The participants and their guardians signed informed assent and consent forms, respectively.

NVC was performed using a stereomicroscope Trinocular coupled to a colour digital camera Qcolor 5 with 100x magnification. The software programme Image-Pro Plus v. 6.0 (Copyright Media Cybernetics) was used to perform the video-morphometric measurements.

To perform NVC, the participants first stayed in a climatised room of 24-25ºC over 20 minutes. Next, they sat with the hand stretched over the device, and a drop of immersion oil was applied on the nailfold of the assessed finger to improve the visualisation of the capillaries. Examination was performed on the fourth finger of the non-dominant hand, according to previous studies in the literature (11, 13, 22). Whenever that finger exhibited any trauma that made the visualisation of the capillaries impossible, the middle finger of the same hand was used. Under 10x magnification, the nailbed was divided in 3 equal regions. We localised the central one and under 100x magnification, this region was divided in three equal areas: (1) central region; (2): towards to radial side of the central region; and (3): towards to ulnar side of the central region. Images of three consecutive capillaries in each area were captured yielding a total of nine capillaries per participant. To assess capillary density we counted all distal capillaries of the central region from the captured images and measured in millimeters the length of this region in order to get the number of capillaries/mm. The following parameters were analysed in each image: number of capillaries/mm, capillary width at its apex (distance from one external margin of the capillary loop to another on the apical region), intercapillary distance (distance between two neighboring capillary loops, measured at the widest intercapillary space on the central capillary region), and capillary length (distance from the top of the capillary loop proximally to the point where that loop stopped being visible) (Fig. 1) (5, 6, 19, 21). The predominance of an open (more than 50%

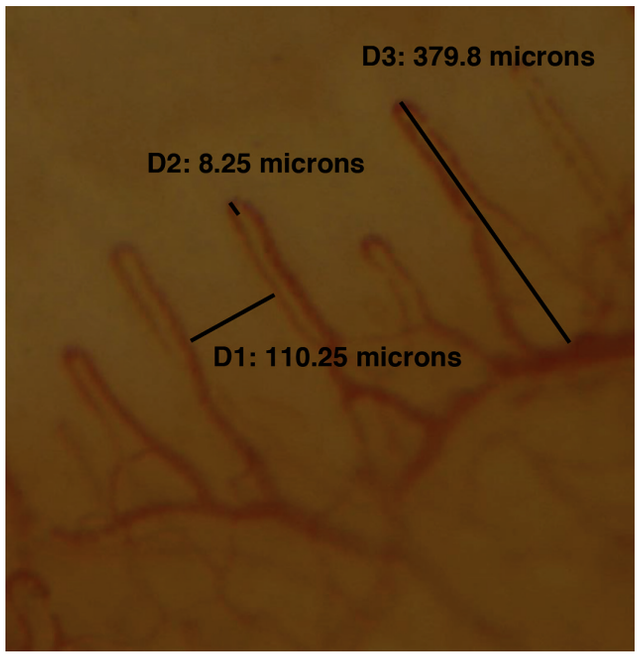

Fig. 1. Intercapillary distance (D1): the distance between two neighboring capillary loops measured at the widest intercapillary space on the central capillary region; capillary width (D2): the distance from one external margin of the capillary loop to another on the apical region; capillary length (D3): the distance from the top of the capillary loop proximally to the point where that loop stopped being visible. Magnification: 100x.

Table I. Capillary length and width, intercapillary distance, and number of capillaries/mm per age range (n=100).

	Age range in years, n					
	Group 1 5-7 n=17	Group 2 8-10 n=24	Group 3 11-14 n=30	Group 4 15-18 n=29	Total (n=100)	p^*
Capillary length, µm	263.1 ± 69.0	256.1 ± 72.6	266.6 ± 66.9	318.7 ± 64.4	278.6 ± 60.3	<0.001**
Intercapillary distance, µm	132.3 ± 42.3	126.6 ± 43.5	127.8 ± 44.5	113.4 ± 43.6	124.1 ± 28.1	0.088
Capillary width, µm	13.7 ± 3.4	14.4 ± 4.2	14.9 ± 3.5	16.2 ± 3.3	15.0 ± 2.6	0.012**
Number of capillaries/mm	6.1 ± 0.2	7.0 ± 0.9	8.0 ± 1.3	9.3 ± 1.1	7.8 ± 1.5	<0.001**

Mean ± standard deviation; *ANOVA with two fixed factors **p<0.05.
Tukey's test (p<0.05):
Capillary length: groups 1 *vs.* 2, p=0.979; 1 *vs.* 3, p=0.997; 1 *vs.* 4, p=0.008; 2 *vs.* 3, p=0.902; 2 *vs.* 4, p=0.001; 3 *vs.* 4 p=0.003.
Intercapillary distance: groups 1 *vs.* 2, p=0.908; 1 *vs.* 3, p=0.944; 1 *vs.* 4 p=0.106; 2 *vs.* 3, p=0.999; 2 *vs.* 4, p=0.294; 3 *vs.* 4 p=0.179.
Capillary width: groups 1 *vs.* 2, p=0.853; 1 *vs.* 3, p=0.433; 1 *vs.* 4 p=0.014; 2 *vs.* 3, p=0.876; 2 *vs.* 4, p=0.067; 3 *vs.* 4 p=0.255.
Number of capillaries/mm: groups 1 *vs.* 2, p=0.027; 1 *vs.* 3, p<0.001; 1 *vs.* 4, p<0.001; 2 *vs.* 3, p=0.003; 2 *vs.* 4, p<0.001; 3 *vs.* 4 p<0.001.

of hairpin capillary loop) or tortuous pattern (more than 50% of tortuosity of capillaries) was also evaluated. Enlarged capillary was defined as a capillary width above the 97.5th percentile for each age range. Avascular areas were considered as areas in which the number of capillaries/mm was below the 2.5th percentile for age and/or the intercapillary distance was larger than the 97.5th percentile for age. In addition, presence of micro-haemorrhages, capillaries with one crossing or with two intersections (meandering capillary), and bizarre (morphology different from the usual) capillaries were also analysed (2).

Statistical analysis

The descriptive analysis included the following measures: means, 2.5th, 50th and 97.5th percentiles, minimum and maximum values, standard deviations (SDs), and absolute and relative (percentage) frequencies. Analysis of variance (ANOVA) was performed in blocks to compare the capillary length and width and the intercapillary distance among capillaries and areas. ANOVA with two fixed factors was used to compare the capillary length and width and intercapillary distance based on gender and age range followed by Tukey's multiple comparison test as needed. The Pearson linear correlation coefficient was calculated to quantify the linear relationship between the age and the capillary dimensions and also to quantify the intercapillary distance and the number of capillaries/mm. In an attempt to establish the normal patterns, the quantile curves corresponding to the 2.5th, 50th and 97.5th percentiles of the nine capillaries assessed in each participant were calculated by means of parametric quantile regression (23).

Intra- and interobserver reliability was evaluated by performing both examinations in 25 individuals on 2 different days and by 2 long-term experienced observers (JSK/DGPP). The intraclass coefficient correlation (ICC) was used to compare the capillary dimensions. The software programmes SPSS (Statistical Package for the Social Sciences) v. 19.0, Statistical v. 12.0, and R project v. 2.15.2 for the quantile curves were used. The significance level was set as p<0.05.

Results

NVC was performed in 123 healthy children and adolescents, but 23 participants were excluded due to poor technical quality and/or poor sharpness of the images. Therefore, the final sample was comprised of 100 children and adolescents (55% female) who were distributed among four age ranges: five to seven (17), eight to 10 (24), 11 to 14 (30), and 15 to 18 (29). In only five cases (5%), NVC had to be performed on the middle finger of the non-dominant hand due to the presence of local trauma on the fourth finger that made assessment impossible. A total of 300 images and 900 capillaries were analysed.

Initially analysis between the three nailfold areas in each individual was performed. The three nailfold areas were homogeneous, *i.e.* they did not differ significantly in capillary length (p=0.083), capillary width (p=0.735), or intercapillary distance (p=0.116) measurements. Comparison between the nine capillaries assessed in each participant showed significant differences in the intercapillary distance (p=0.033) and capillary length (p=0.042), but not in the capillary width (p=0.379). Thus, we decided to calculate the average values for the three capillaries per area and to use the average value of the three areas in the statistical analyses.

The capillary measurements were obtained among the 100 subjects and no difference was observed between genders with exception of intercapillary distance that was significantly higher in girls. (p=0.011).

The comparison between the four age ranges showed significantly longer capillaries in the participants aged 15 to 18 years as compared to the remaining groups (p<0.001), as well as significantly higher capillary width in the participants aged 15 to 18 years as compared with the participants aged five to seven years (p=0.012). The capillary density exhibited a significant increase with age (p<0.001); this parameter differed among all four groups (p<0.05). The intercapillary distance remained constant over time (p=0.088) (Table I).

Additionally, there was a positive correlation between age and the number of capillaries/mm, capillary length, and capillary width (p<0.001, R=0.796; p<0.001, R=0.368; p=0.004, R=0.285, respectively). There was no significant correlation between age and intercapillary distance (p=0.069, R=0.182).

In an attempt to define standard normal values, the 2.5th, 50th, and 97.5th percentiles of the nine capillaries assessed in each participant were calculated. The distribution of the measurements of capillary length and width, intercapillary distance, and number of capillaries/mm per age range are described in Table II.

The representation of the quantile curves of the normal percentiles for capillary length and width, intercapillary distance, and number of capillaries/mm per age range is provided in Figure 2.

The intra-class correlation coefficient analysis showed an excellent intra- and inter-observer ICC for all parameters evaluated (Table III).

With respect to the descriptive findings on NVC, 98% had a predominantly open capillary pattern. Micro haemorrhages were found in 4% of the participants, 18% presented bizarre capillaries, 8% had capillaries with one crossing, and none had meandering capillaries. Enlarged capillary was found in 11 out of 100 participants (width above the 97.5th percentile) and was most frequent among participants aged eight to 10 years (five participants). No capillaries presented a width larger than 50 microns. Approximately 10% of the sample exhibited potentially avascular areas (intercapillary distance above the 97.5th percentile for age). Nevertheless, based on the number of capillaries/mm (bellow the 2.5th percentile for age), no avascular areas were identified. A weak negative correlation was found between the intercapillary distance and the number of capillaries/mm (p=0.05; R=-0.20).

Table II. Distribution of the measurements of capillary length and width, intercapillary distance, and number of capillaries/mm per age range.

Dimensions	Age range (years)	Minimum	2.5th perc.	Median perc.	97.5th	Maximum
Capillary width (μm)	5-7	6.9	8.0	13.5	20.3	21.2
n=900	8-10	6.8	7.3	14.3	23.9	30.9
	11-14	7.3	8.7	14.8	23.5	28.7
	15-18	8.0	9.4	16.3	22.7	26.2
	Total	6.9	8.3	15.0	23.1	30.9
Intercapillary distance (μm)	5-7	54.6	62.9	128.0	232.4	246.3
n=900	8-10	31.6	48.9	121.7	208.9	242.6
	11-14	32.2	47.0	123.6	236.0	284.7
	15-18	36.2	43.5	109.7	223.9	243.9
	Total	31.6	46.8	121.1	224.1	284.7
Capillary length (μm)	5-7	99.4	146.5	256.9	396.9	407.2
n=900	8-10	87.9	131.6	247.2	393.0	496.8
	11-14	105.0	140.5	256.8	395.1	494.0
	15-18	145.3	170.1	323.5	416.3	445.3
	Total	87.9	145.3	278.2	405.5	496.8
Number of capillaries/mm	5-7	6.0	6.0	6.0	7.0	7.0
n=100	8-10	6.0	6.0	7.0	9.0	9.0
	11-14	6.0	6.0	7.0	12.0	12.0
	15-18	7.0	7.0	9.0	12.0	12.0
	Total	6.0	6.0	7.0	12.0	12.0

Discussion

There is a wide variability in the capillary morphology between healthy individuals (2, 8-11, 19, 22). In children, the capillaroscopic parameters and the threshold between normal and pathological states are less defined. The present study was the first to describe the normal patterns of NVC in healthy children and adolescents based on different age ranges.

Our results indicate that NVC measurements vary with age throughout childhood. There was a positive correlation between age and the number of capillaries/mm, capillary length, and capillary width. Our study also showed a significant difference in the capillary length and width between the older age group and younger participants. There were no significant differences in the capillaroscopic parameters between male and female children. In addition, NVC has been showed to be a reproducible method. We evaluated the most important and frequent parameters evaluated previously by NVC in a large group of healthy children (22). To evaluate a possible influence of different age ranges on the capillary parameters, the subjects were divided into four different age groups.

The apical capillary width showed progressive increase with age. The apical region is the most common area evaluated in other studies. We found a five-fold variation of the normal width of the apical capillaries in the investigated population. In a previous NVC study conducted with children, Herrick *et al*. assessed the apical width of the venous and arterial branches of one single capillary in 110 healthy children and adolescents using 200x and 600x magnifications (22). They did not show a significant difference in this parameter according to age (22). Nonetheless, the values found by Herrick *et al*. were higher compared with our results for all age ranges (22). However, their results were similar to our results in the 97.5th percentile and thus within the range of normality. Increased capillary width is associated with the characterisation of enlarged and giant capillaries; in adults, according to Cutolo *et al*., they are defined as a width greater than 20 μm and 50 μm, respectively (5). Because we found a progressive increase in the apical width with age, we decided not to use that definition, which was formulated for adults. However, no capillaries presented a width larger than 50 microns. Despite diverse definition of capillary diameter, giant capillaries were consistently not found in healthy children (10). In our study, 11% of the sample exhibited capillary ectasia. The presence of capillary ectasia in healthy children and adolescents is in agreement with a previous study, which detected 10% of ectasia in healthy children submitted to NC (8).

The intercapillary distance is an indirect parameter to assess the presence of avascular areas (11, 19). There was no significant correlation between age and intercapillary distance. In the study by Ingegnoli *et al*., the intercapillary distance found in adults was wider than

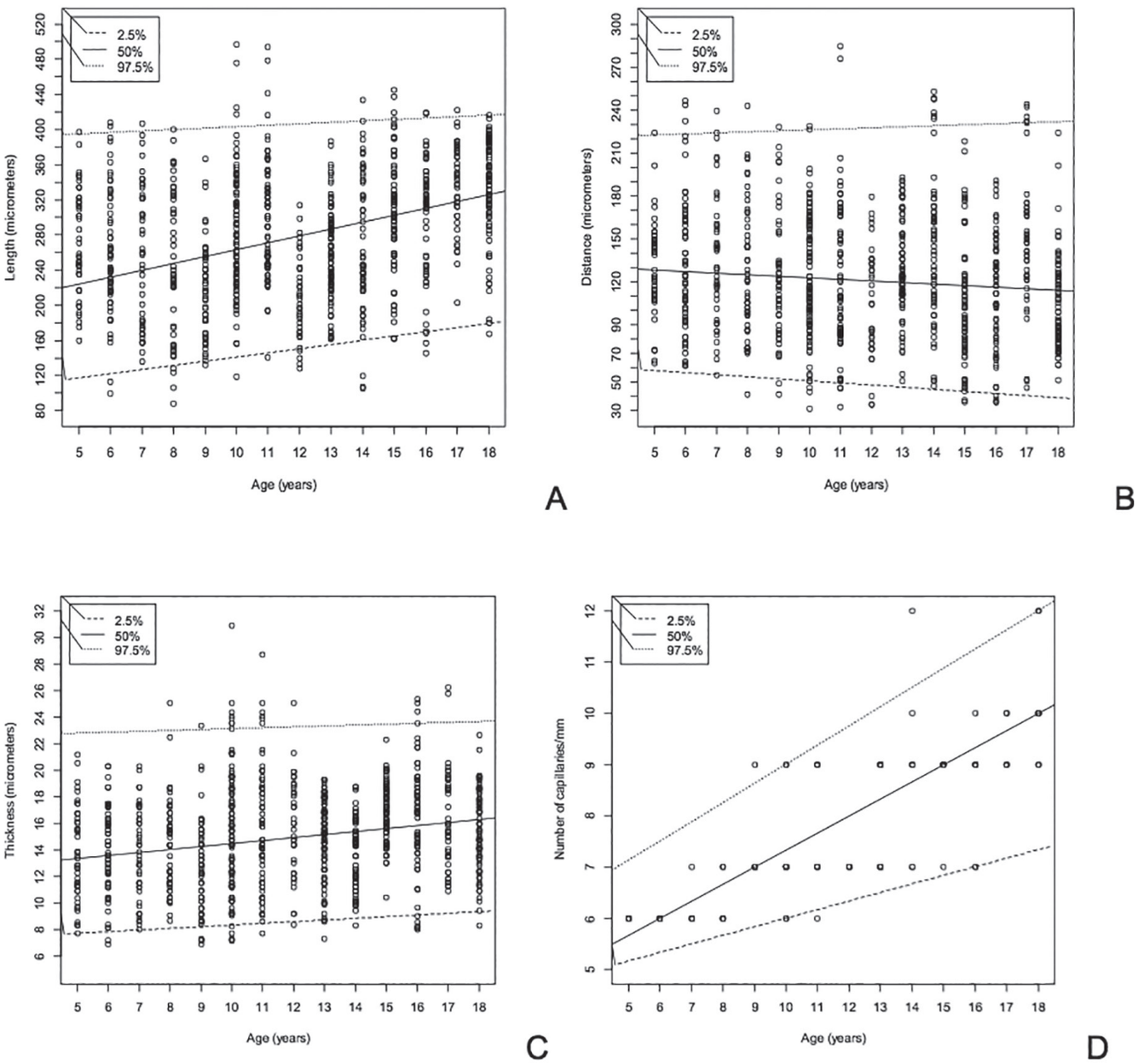

Fig. 2. Curves of normal percentiles: (**A**) capillary length (N=900); (**B**) intercapillary distance (n=900 capillaries); (**C**) capillary width (n=900); (**D**) number of capillaries/mm (n=100 subjects).

the distance we found in any of the investigated age ranges (19). We believe that such a discrepancy is due to the age of the investigated populations as well as to methodological differences because we defined the intercapillary distance as the widest distance between two neighboring capillary loops on the central area of capillaries, while Ingegnoli et al measured the distance between apexes (19). We found that the intercapillary distance was the only measurement that did not change in parallel with age. However, the intercapillary distance was significantly greater in girls compared with boys. Avascular areas were detected in 10% of the sample based on the intercapillary distance. In a study of NC a low grade of avascular areas was also detected in 2% of a population of healthy children based on intercapillary distance (8). We found a nine-fold variation of the values within the normal range, which is wider compared with the other assessed parameters (capillary length and width). In addition, another study found that the variation in the intercapillary distance was quite wide (19). These findings lead us to question whether intercapillary distance might represent the best parameter to define the presence of avascular areas because clinical practice shows that the distribution of capillaries is irregular in patients who exhibit the scleroderma pattern, which does not allow for measuring the intercapillary distance in a standardised manner.

The capillary density (number of capillaries/mm) is considered the most accurate parameter used for the definition

of the degree of normality of avascular areas (19, 21). Additionally, a decrease in the capillary density is characteristic of the scleroderma pattern both on NVC and NC (5, 11, 13, 18, 19). Although avascular areas have been seen in healthy children, patients with ARD have more prominent alterations than the formers (5, 11, 18). In our study, capillary density ranged from 6 to 12 capillaries/mm, with a mean of 7.8 capillaries/mm. Although the differences in the devices and lens magnification used in the literature this finding is in agreement with previous studies (2, 8-11, 19-22). There was a significantly increase in the number of capillaries/mm according to age, suggesting that this is part of the childhood maturation process (9). Therefore, this finding should be taken into account in order to define a pathologic pattern when evaluating children with suspected ARDs. The number of capillaries/mm is more reliable than the intercapillary distance as a parameter to define the presence of avascular areas (10, 19). A reduction in the number of capillaries/mm is characteristic of the scleroderma pattern both on NVC and NC (5, 13, 18, 21). The presence of fewer than six capillaries/mm has 92% specificity to predict the occurrence of Raynaud's secondary to ARDs in adults (24). In our study there were no avascular areas based on the number of capillaries/mm. Although the intercapillary distance is not the most adequate parameter to define the presence of avascular areas, we found a negative correlation with the number of capillaries/mm, which shows that these are complementary measurements.

In agreement with a previous study, there was a positive correlation between age and capillary length (11). In our study, the capillary length was higher in participants aged 15 to 18 years. The capillary length presented also a wide variability in the measurement among the different age ranges assessed. The variation within the range of normal was as high as almost five-fold. Previous studies have reported two- to ten-fold variation in capillary length measurements; such variability among normal capillaries might hinder the definition of the limits of normal (11, 19, 20). The reason for such wide variation might be the difficulty in establishing where a capillary loop ends due to the loss of focus at the loop base close to the venous sub-papillary plexus (19). Additionally, Ingegnoli *et al*. mentioned difficulty in the assessment of the capillary length due to the obliquity of the angle of some loops with consequent distortion of their real length (19).

Table III. Intraclass correlation coefficient (ICC) relative to capillary length and width, intercapillary distance, and number of capillaries/mm.

	Intra-examiner ICC (%)	Inter-examiner ICC (%)
Capillary length, μm	98.5	98.6
Intercapillary distance, μm	97.2	94.7
Capillary width, μm	98.7	82.8
Number of capillaries/mm	98.6	94.7

NVC was shown to be reproducible and sensitive in our study. Nevertheless, poor technical quality of images and poor visibility of the capillaries, which made measurements impossible, limited the performance of NVC in some of the participants in the present study. Additionally, patients should be able to sit quietly during the procedure and in children, the longer time needed to perform the test limits the use in this age range. We expect that such limitations might be overcome in the future through the elaboration of more sophisticated software for image acquisition and analysis. The main limitation of the study was the low number of patients per age range, being partially offset by using multiple images per finger. This is the first study to suggest percentiles for normal capillary dimensions and numbers of capillaries by NVC for children and adolescents per age range and also the second largest evaluating nailfold capillary parameters in children and adolescents using this method. As differential features, we call attention to the adequate selection of the sample, including various age ranges, even preschoolers, in addition to having shown the excellent intra- and inter-observer reproducibility of NVC. The present results and parameters might be used as the range of normality in healthy children and might be helpful in analysing children with suspected ARDs in further studies. We believe that NVC might provide a more precise and objective method for the assessment of vascular abnormalities detected on previous screening using NC and that it might contribute to the diagnosis of ARDs through the detection of the scleroderma pattern on objective measurements of capillary dimensions. Finally, we stress that the variables assessed on NVC should be interpreted cautiously due to the wide variability in the corresponding measurements, which reflects the broad normal range in the healthy paediatric population. Future studies comparing NVC data collected in the paediatric population with data from children with ARDs might confirm the reproducibility and sensitivity of this method.

Key messages

- There is a wide variability in the capillary morphology among healthy paediatric population.
- There was a positive correlation between age and number of capillaries/mm, length and width of capillary.
- Videocapillaroscopy has been shown to be a reproducible method.

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