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Elliptical broken line method for calculating capillary density in nailfold capillaroscopy: Proposal and evaluation

Abdolamir Karbalaie [a,*], Farhad Abtahi [b,e], Alimohammad Fatemi [c], Mahnaz Etehadtavakol [d], Zahra Emrani [d], Björn-Erik Erlandsson [a]

[a] School of Technology and Health, Royal Institute of Technology (KTH), SE-100 44, Stockholm, Sweden
[b] Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden
[c] Department of Rheumatology, Alzahra Hospital, Isfahan University of Medical Sciences, Isfahan, Iran
[d] Medical Image and Signal Processing Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[e] Department of Clinical Physiology, Karolinska University Hospital, Stockholm, Sweden

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ABSTRACT

Nailfold capillaroscopy is a practical method for identifying and obtaining morphological changes in capillaries which might reveal relevant information about diseases and health. Capillaroscopy is harmless, and seems simple and repeatable. However, there is lack of established guidelines and instructions for acquisition as well as the interpretation of the obtained images; which might lead to various ambiguities. In addition, assessment and interpretation of the acquired images are very subjective. In an attempt to overcome some of these problems, in this study a new modified technique for assessment of nailfold capillary density is introduced. The new method is named elliptic broken line (EBL) which is an extension of the two previously known methods by defining clear criteria for finding the apex of capillaries in different scenarios by using a fitted elliptic. A graphical user interface (GUI) is developed for pre-processing, manual assessment of capillary apexes and automatic correction of selected apexes based on 90° rule. Intra- and inter-observer reliability of EBL and corrected EBL is evaluated in this study. Four independent observers familiar with capillaroscopy performed the assessment for 200 nailfold videocapillaroscopy images, form healthy subject and systemic lupus erythematosus patients, in two different sessions. The results show elevation from moderate (ICC = 0.691) and good (ICC = 0.753) agreements to good (ICC = 0.750) and good (ICC = 0.801) for intra- and inter-observer reliability after automatic correction of EBL. This clearly shows the potential of this method to improve the reliability and repeatability of assessment which motivates us for further development of automatic tool for EBL method.

1. Introduction

Capillaries are playing a key role in exchange of nutrients, waste products and gases between the tissues and circulation (Shore, 2000). The pathogenic role of endothelial dysfunction in systemic autoimmune diseases has been studied before. Previous investigations have been demonstrated endothelial injury and microvascular impairment in rheumatic diseases such as systemic sclerosis, rheumatoid arthritis, SLE, Sjogren's syndrome and spondyloarthropathies and they might be associated with induction and propagation of atherosclerosis (Murdaca et al., 2012) These observations can explain the increased incidence of cardiovascular morality in the patients with rheumatic diseases. (Castaneda et al., 2016) In addition, the association of microcirculation impairment with other medical condition such as sepsis (Den Uil et al., 2008), renal failure (Den Uil et al., 2008) and metabolic syndrome (Wiernsperger et al., 2007) has been addressed previously. Interestingly, Francischetti et al. showed the negative correlation between components of metabolic syndrome such as central and global obesity and cutaneous capillary density (Francischetti et al., 2011). Another study showed that number of tortuous loops in ring finger increased in the obese group compare with healthy group (Chin et al., 1999). The abovementioned statements reflect an important role for the microcirculation in health and diseases. Furthermore, they reveal the need of non-invasive methods to assess microcirculation in clinical practice.

Investigating the capillaries function is very interesting and might reveal information about health and disease, however, non-invasive

Abbreviations: SLE, systemic lupus erythematosus; NFC, nailfold capillaroscopy; NVC, nailfold videocapillaroscopy; EBL, elliptical broken line; GUI, graphical user interface; ICC, intra-class correlation coefficient; DO, direct observation.

* Corresponding author at: School of Technology and Health, Royal Institute of Technology (KTH), SE-100 44, Stockholm, Sweden.
E-mail addresses: abdolamir.karbalaie@sth.kth.se (A. Karbalaie), farhad.abtahi@ki.se (F. Abtahi), a_fatemi@med.mui.ac.ir (A. Fatemi), mahetehad@gmail.com (M. Etehadtavakol), Zahra_emrani@mail.mui.ac.ir (Z. Emrani), bjorn-erik.erlandsson@sth.kth.se (B.-E. Erlandsson).

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access to the vascular bed is limited. Advances in medical device development in the microcirculation area *i.e., capillaroscopy* allow better assessment of the skin capillaries. Nailfold capillaroscopy (NFC) is one of the potential affordable and non-invasive imaging techniques to investigate skin microcirculation. Among various nailfold imaging instruments, nailfold videocapillaroscopy (NVC) is a great tool to assess the morphology and density of the nailfold area (Lambova, 2016). The NVC is reported to have higher usability for physicians, however, there is no evidence of difference between diagnostic performance of NVC compare to other capillary imaging techniques *e.g., stereomicroscope* (Emrani et al., 2017; Sekiyama et al., 2013). Typically, the NVC device is a mobile hand-held with optical magnification of 20–200 times. The general capillaries architecture can be studied by using lower magnification *e.g., 50×* while assessing morphological details of a single capillary need higher magnification *e.g., 200×*.

Number of capillaries is one of the important quantitative parameters which might be related to autoantibodies, digital ulcer, pulmonary arterial hypertension as well as classification of the capillaroscopic patterns in systemic sclerosis (SSc) patients (Emrani et al., 2017). Studying these parameters is possible even with lower magnification instruments (20–50×) *e.g.*, dermatoscope and stereo-zoom microscope. The reliability of capillary density *i.e., number of capillaries in one millimeter square span of the distal row in each finger or toe* with high and low magnifications is previously reported (Hofstee et al., 2011b; Karbalaie et al., 2016), however, it is just valid when the same area is being counted for both intra and inter-observer (Cheng et al., 2015; Murray et al., 2012). Despite potential clinical applications of this technology, there is a little standardization for quantifying capillary density. To the author's knowledge there is to date just one publication describes a computer aided tool to standardize the capillary quantification process. Cheng et al. reported a semi-automated method to calculate the number of capillaries in 1 mm^2. However, capillary density in the most of studies is defined as the number of capillaries in a one millimeter span of the distal row in each finger or toe. Counting number of capillaries in 1 mm^2 might be useful to count the number of capillary in skin area (Cheng et al., 2015).

In a basic technique used in most studies, *sometimes called direct observation* (DO), each capillary loop is counted by manually marking the loops in the distal row (Lambova et al., 2011; Schiavon et al., 1999; Selva-O'Callaghan et al., 2010). In a study for determining the capillary density, an alternative method to the direct observation has been proposed (Hofstee et al., 2011a). The alternative method, *called 90° methods*, is counting a capillary loop if the angle between the apex of a capillary and the apex of its adjacent capillaries is >90°. In absence of standardized approach, most authors use the direct observation or the 90° method. However, both approaches are underestimating the effect of different capillary loop shapes by just considering a classical physiological pattern of a nailfold capillary loop as a "reverse U-shaped" or "hairpin-like". The ramified capillary, *occupies more than one dermal papilla*, can be seen in most of active and late Scleroderma patterns. However, the direct observation or the 90° methods are not clear how to count such ramified capillaries. Determining which capillaries are in the distal row is another problem that may arise from measuring the number of capillaries. It is important to find one method to count number of capillary in both class of capillaries, when the capillary density is high, or when the nailfold aria include different shape of capillary. Ability to develop an automatic or semi-automatic counting method can be an advantage to reduce counting time for observer. However, this need better definition of different type of capillaries and specific rules for counting which are covered by proposed method *i.e.*, EBL method. The aim of this study was to describe and evaluate a reproducible method for calculating the nailfold capillary density and assess the intra- and inter-observer reliability. In this study we use different type of image from healthy and systemic lupus erythematosus (SLE) patients for evaluation of proposed assessment method. However, this study was not design to evaluate screening or diagnostic value of nailfold capillaroscopy for SLE diseases.

2. Materials

2.1. Subjects

34 (13 males, 21 females) patients who fulfilled the American college of rheumatology (Hochberg, 1997) criteria for SLE and 11 healthy (3 males, 8 females) were enrolled the study. The age range was 24–63 years and 24–63 years for SLE patients and healthy subjects, respectively. A total of 280 nailfold images were captured. Among these image collection, 140 from SLE patients and 60 from healthy were chosen for the analysis. Unsuitable images resulted from darkly pigmented skin, extensively manicured nailfold, thickened and dry nailfold skin were excluded from the study. SLE patients typically have more variation of capillary shapes and hence are chosen in addition to healthy subjects for evaluation of proposed assessment method.

Patients were recruited from rheumatology outpatient clinics on the basis of clinical diagnoses established by an experienced rheumatologist (AF). The local ethics committee approved the study (ID: 293075 and 293147). Participants were properly informed about this study and their rights consequently a written informed consent was obtained for each patient.

2.2. Nailfold capillary microscopy

Patients and controls were asked to refrain from caffeine-containing drinks and smoking for 5 h prior to the examination (however, the smoking status as current or ex-smoker was not considered). Each subject was at rest in room temperature, *22–25 °C*, for a minimum of 15–20 min before the examination, depends on the outside temperature. During acclimatization time, subject's hands positioned at their heart level as previously described (Dolezalova et al., 2003; Etehad Tavakol et al., 2015; Ingegnoli et al., 2013; Sebastiani et al., 2009). Immersion oil was placed on each nailfold to improve visibility. Since the fourth and fifth fingers of both hands have a higher skin transparency, these two fingers were examined.

Six consecutive images (1500 × 2000 pixels) were taken from the middle of a nailfold by using Optilia Digital Capillaroscopy System, *Optilia Mediscope, Optilia Instruments AB, Sweden* set at 200 times magnification, see Fig. 1. NVC images were recorded and processed by an attached computer using the respective software, *OptiPix Optilia Mediscope, Optilia Instruments AB, Sweden*. According to anatomical variation nailfolds vary in size and hence each nailfold image is of different size, representing a different nail width and including a variable number of capillaries. To be able to study reliability and repeatability of proposed method, it was necessary to study the same length of capillary nail bed on each occasion. Therefore, images were cropped to the same size with different position, representing 3 mm of the nail bed. The total examination time of one patient, including capturing images from all 4 nailfolds, was around 8–12 min. All procedures were performed by one investigator (AK), who was fully trained in videocapillaroscopy and blinded to the patients' clinical conditions and laboratory data.

2.3. Selection of images

Ideally, selecting capillary images for calculating the capillary density should be performed while observing the movement of red blood cells and plasma gaps through the capillaries during video replay. This imaging condition may clarify whether a given red dot is a capillary or a knot of vessels indicating one or two capillaries. However, this is not always possible (Shore, 2000) due to several limitations *e.g., increasing stress, tension and the measurement time*. In addition, in most cases operators who perform the NVC are not the same observers who are assessing the image for calculating the capillary density. In this study, an image for counting is selected among six images corresponds to different circulatory cycle, see Fig. 1.