Nailfold capillaroscopy assessment in autoimmune disease: a clinical-morphological correlation

José Alves*, António Panarra**, Francisco Soromenho***, Manuel Vaz Riscado****

Abstract

Nailfold capillaroscopy is a non-invasive method for in vivo observation of micro-circulation. Several studies have been presented showing the relationship between capillary morphology and the presence of autoimmune diseases. Others addressed the relationship between specific characteristics found in essential hypertension, diabetes and the duration of the disease. Nevertheless, few quantitative controlled studies have been performed.

The authors present a case-control study in a population of women diagnosed with auto-immune diseases and a population of healthy individuals as control.

20 consecutive women from the Outpatients Clinic of the Auto-Immune Diseases Unit were considered (9 patients with systemic lupus erythematosus (SLE), seven with Systemic Sclerosis (SS) and 4 with other autoimmune diseases). Patients with hypertension, diabetes, hypercholesterolaemia or primary vascular diseases were excluded. A population of 20 healthy, age and gender matching controls were recruited among the hospital staff. Nailfold capillaroscopy was performed with a video stereo microscope and quantitative measurements were evaluated with the use of the metric scale included in the ocular system. The observations were performed in the same room, with a stabilized temperature. For each patient and control, measurements were made regarding capillary density and diameter, and the presence of minor or major structural changes. The Fagrell index was used to access the global microvascular damage.

Statistical significance was found with capillary density ($p<0.003$), diameter ($p<0.009$), and the presence of minor ($p < 0.001$) and major ($p<0.0002$) structural changes, between patients and controls. The Fagrell index was also significantly different ($p<0.01$). Lupus and systemic sclerosis patients were compared and differences were found regarding major structural changes ($p<0.02$) and the Fragell index ($p<0.01$), with higher values for SS patients.

The control group was compared with the SLE patients in the presence of minor structural changes was found more frequently in the lupus group ($p<0.001$). The other endpoints evaluated showed no significant difference. The SS patients showed differences regarding capillary density ($p<0.0001$) and diameter ($p<0.0001$) as well as the presence of major structural changes ($p<0.01$).

The results highlight the importance of nailfold capillaroscopy in the evaluation of patients with auto-immune diseases. The differences found are consistent with the microvascular involvement in these diseases. The difference pattern found in SS and SLE patients, can be explained by the different pathophysiology of these diseases. Fagrell classification can be an important index to evaluate the severity and evolution of pathological findings.

Keywords: naifold , capillaroscopy, systemic lupus erythematosus, systemic sclerosis, Fragrell index.

Introduction

Nailfold capillaroscopy (NFC) is a non-invasive method of observing the microcirculation, enabling a morphological and functional analysis of the capillary network. It is an old technique; the first microscopic observations of human capillaries were performed in 1879.[1] The adaptation of new techniques of direct optical observation, including video microscopy, and the use of fluorescent contrasts, have enabled a more complete analysis of haemorrheology phenomena.

Various studies have demonstrated alterations in the capillary network in autoimmune pathology, particularly in systemic sclerosis (SS),[2,3] systemic lupus erythematosus (SLE)[4] and rheumatoid arthritis (RA),[5] as well as in other diseases with impaired microcirculation such as diabetes mellitus,[6] atherosclerosis[7] and high blood pressure. [8]

In the absence of a clearly-defined nosological entity, capillaroscopy can play an important role in assessing possible morphological prognostic indicators[9] or therapeutic response.[10] In this context, it has been especially helpful in the differential diagnosis of patients with Raynaud's phenomenon.

Therefore, the possibility of assessing the degree of vascular impairment in each of the diseases consi-

*Resident to Internal Medicine Supplementary Internship

**Internal Medicine Interim Assistant

***Internal Medicine Senior Hospital Assistant

****Internal Medicine Head of Service

Medical Service 2 of the Hospital Curry Cabral, Lisbon

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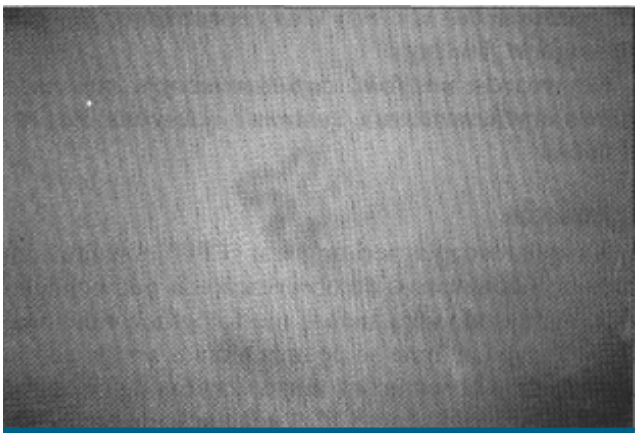

Image of S-shaped capillary.

FIG. 1

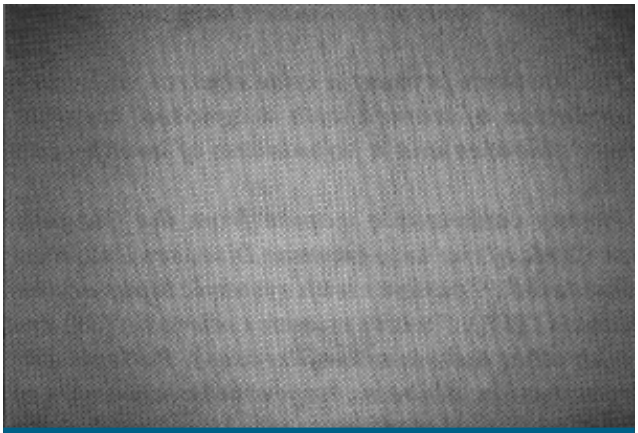

Image of "ball of wool" capillary.

FIG. 2

dered by a non-invasive method that is economic and easy to perform is, naturally, of particular importance. However, there have been no controlled studies focusing on the differential characterization of capillary morphology. On the other hand, the close relationship that exists between the capillary structures and the intervening factors in the mechanisms of haemostasis and fibrinolysis, as well as the identification of the endothelial origin of some coagulation activation markers,[11] raises the possibility of a relationship between coagulation parameters and alterations in microcirculation.

Our aim, therefore, is to perform a controlled study of a group of patients with autoimmune pathology (predominantly SLE and SS), analyzing the possible clinical-morphological relations of each of the pathologies and their relation to the control population.

Material and methods

Patients: female patients of between 18 and 65 years were considered for this study, all under regular observation in outpatient consultations at the Unit for Autoimmune Diseases at the Hospital de Curry Cabral. Exclusion criteria were defined as the presence of dyslipidaemias, diabetes mellitus, essential high blood pressure or primary vascular disease, pregnancy of more than 8 weeks, and the use of vasoactive drugs (particularly vasodilators). The study population included twenty Caucasian patients who met the requirements cited above and with a diagnosis of autoimmune disease. A control population was formed, consisting of twenty healthy women with similar epidemiological characteristics (gender, age and race) to those of the study population. All the participants gave their consent in writing.

Material: the morphological exam was carried out on a LEICA stereomicroscope, model Wild M3Z, with 10x ocular and 10x objective lenses, equipped with 6.5 to 40x zoom and a SONY video camera recorder, model DXC – 107A /107AP. The photographic material was prepared using a SONY Trinitron PVM – 1450MD video monitor for SONY video printer model UP-1200AEPM. Assessment of haemostasis: prothrombin time (PT), partial thromboplastin time (PTT), assay of factor VIII (FVIII) and fibrinogen (FNG) were carried out using an Automated Coagulation Laboratory – ACL 300 (Instrumentation Laboratory), with the following kit for each of the techniques: PT and FNG – IL Test PT-Fibrinogen HS Plus (Inst. Lab.), PTT – IL Test APTT Lyophilized silica (Inst. lab.), F VIII – IL Test Factor VIII abnormal control plasma (Inst. Lab.).

Methods: all the individuals (patients and control population) were assessed in the Office for Complementary Tests of the Autoimmune Disease Unit during the week following their selection in the abovementioned outpatient clinic. The tests were carried out by two of the authors, according to the previously outlined protocol. The room temperature was a constant 24ºC; the patients remained in the room for twenty minutes prior to the test. The observation was performed in the nailfold region of the 3rd and 4th fingers, after

TABLE I

Fagrell's morphological classification[12]

Stage	
A	0 - Period or comma shaped, with good "tonicity"
	1 - As in stage 0, but with less "tonicity"
	2 - Marked dilation, possible "microlakes"
B	3 - Non distinguishable capillaries (edema, sclerosis).
	4 - Capillary hemorrhages
C	5 - Only some capillaries visible in the whole field (<10)
	6 - No capillaries visible

local application of an oily substance to increase cutaneous transparency. Assessment of the quantitative parameters was carried out by direct measurement using a metric scale incorporated in the ocular system and confirmed by a 2nd investigator. Alterations in capillary morphology with a conserved base structure were considered to be "minor dysmorphism" (e.g. S-shaped capillary) (*Fig. 1*), while alterations resulting in a loss of this structure were considered to be "major dysmorphism" (e.g. "ball of wool" capillary) (*Fig. 2*). For each test, the morphological variations observed were quantified according to the Fagrell classification (*Table I*). Photographic records were obtained for each of the tests carried out. The records, together with the epidemiological data on each individual, were collected in a database developed for this purpose.

After the test, 5c.c. of blood was collected from the vein for 2 tubes with sodium citrate at 30%. The haemostasis tests were carried out one hour after the blood collection.

Statistical Analysis: the statistical analysis of the data obtained was performed using the Statistical Package for the Social Sciences (SPSS). The Student t test and Fisher's test were performed, according to their respective indications.

Results

The classification of the study and control populations is represented in *Table II*. There are no significant differences in relation to the epidemiological data considered (race, age, gender), or values for prothrombin time (PT), partial thromboplastin time (PTT), factor VIII and fibrinogen (FNG), compared to the control population.

TABLE II

Classification of the study and control populations

	Patients	Control subjects	Significance
Number	20	20	—
Women	20	20	—
Caucasians	20	20	—
Age (years)	31.8 (22-52)	37.5 (20-58)	p=0.08 (NS)
PT	105.0 %	101.7 %	p=0.61 (NS)
PTT (ratio)	0.87	0.94	p=0.17 (NS)
Fibrinogen	396.4	350.8	p=0.12(NS)
Factor VIII	214.6	140.9	p=0.06 (NS)
SLE	9	—	—
SS	7	—	—
CREST	3	—	—
PSS	4	—	—
Others	4	—	—
Polymyositis	1	—	—
1st Raynaud	2	—	—
Acrocyanosis	1	—	—

Significant statistical differences were found between the population with autoimmune disease and the control population regarding the number of capillary loops/mm ($p < 0.003$), the difference between the diameters of the efferent and afferent branches ($p < 0.009$) and the existence of minor ($p < 0.001$) and major ($p < 0.002$) dysmorphism. The Fagrell index likewise revealed a relevant variation ($p < 0.001$). No difference was found between the two groups in relation to the presence of megacapillaries (*Table III*).

The aforementioned parameters were also considered in the comparison of the microvascular structure in the patients with SLE and SS, both between the groups and in relation

TABLE III

Capillaroscopy findings in the study and control populations

	Patients average (sd)	Control subjects average (sd)	Significance
No. loops	8.15 (3.31)	10.8 (1.32)	p < 0.003
A-V difference	1.25 (0.55)	0.39 (0.22)	p < 0.009
Minor D.	30.75 (22.43)	9.5 (12.34)	p < 0.001
Major D.	25.0 (31.3)	0.50 (2.23)	p < 0.002
Megacapillaries	4.25 (10.67)	0.00 (0.00)	p = 0.09 (NS)
Fagrell I.	2.65 (1.63)	1.00 (1.17)	p < 0.001

to the control group. There were no significant differences in age and haemostasis values of the three populations presently under consideration.

No statistically significant differences were found in regard to the number and diameter of capillary loops or the presence of minor dysmorphism and megacapillaries in patients with SLE and SS. However, relevant variations were observed (although they did not reach statistical significance) in the number of loops ¾ major in the SLE ¾ and the presence of megacapillaries ¾ predominant in the SS (p = 0.08 – NS, for both).

The number of major dysmorphism (p<0.02) and the Fagrell index (p<0.01) are significantly greater in the patients with SS in relation to the patients with SLE (*Table IV*).

In each of the nosological groups, individually, there was a greater number of minor dysmorphism in the patients with SLE in relation to healthy control subjects (p<0.0001), with no significant differences in relation to the other aspects previously considered (*Table V*). In the comparison between the patients with SS and the control group, significant variations were found regarding the number of loops/mm (p<0.0001), the difference in arterio-venous diameter (p<0.0001), the Fagrell index (p<0.0001) or the presence of major dysmorphism (p<0.01). No significant difference was identified in relation to the number of megacapillaries (*Table VI*).

The presence of the Raynaud's phenomenon was related to each of the aforementioned parameters of capillary classification, both together and individually, for the patients with SLE and SS.

All the patients with SS presented Raynaud's phenomenon (100%), while only three of the nine patients with SLE presented this type of manifestation (33.3%). In regard to the study patient population, statistically significant differences were observed when considered individually with and without Raynaud's phenomenon. These differences translate into higher values of the Fagrell index (p<0.007) and the existence of a greater number of major dysmorphism (p<0.02) in the patients who clinically express Raynaud's disease. A relationship was also found between the existence of Raynaud's phenomenon and prothrombin time (p<0.03). The FNG and PTT values were not significantly different in the two groups considered.

TABLE IV

Comparison of populations with SLE and PSS

	SLE average (sd)	PSS average (sd)	Significance
Number	9	7	—
Age (age)	35.2 (13.8)	41.7 (8.4)	p = 0.29 (NS)
PT	98.8 % (14.9)	123.5 % (9.2)	p = 0.07 (NS)
PTT (ratio)	0.85 (0.12)	0.93 (0.29)	p = 0.13 (NS)
Fibrinogen	386.8 (68.6)	425.0 (25.4)	p = 0.48 (NS)
Factor VIII	162.7 (47.3)	422.0 (0.0)	p < 0.01
No. loops	8.8 (3.1)	6.1 (2.3)	p = 0.08 (NS)
A-V difference	4.4 (0.46)	0.2 (0.5)	p = 0.32 (NS)
Minor D.	38.9 (17.6)	23.6 (30.2)	p = 0.22 (NS)
Major D.	10.0 (12.2)	47.1 (34.5)	p < 0.02
Megacapillaries	0 (0)	12.1 (15.7)	p = 0.08 (NS)
Fagrell I.	1.7 (1.0)	4.0 (1.3)	p < 0.01

Discussion

The results of this study show the morphological alterations that exist in the presence of autoimmune pathology. The patient population presents very significant variations in capillary morphology, although the majority are not very specific. The analysis of these results was prejudiced by the number of elements in each group. However, the level of significance observed in the comparisons carried out enabled some coherence

TABLE V

Comparison between the patients with SLE and the control population

	SLE average (sd)	Control subjects average (sd)	Significance
Number	9	20	—
Age (age)	35.2 (13.8)	41.7 (8.4)	p = 0.51 (NS)
PT	98.8 % (14.9)	101.0 % (13.1)	p = 0.65 (NS)
PTT (ratio)	0.85 (0.12)	0.94 (0.008)	p = 0.07 (NS)
Fibrinogen	386.8 (68.6)	350.8 (89.8)	p = 0.38 (NS)
Factor VIII	162.7 (47.3)	140.9 (50.5)	p = 0.44 (NS)
No. loops	8.8 (3.1)	10.8 (1.3)	p = 0.08 (NS)
A-V difference	4.4 (0.46)	0.4 (0.2)	p = 0.06 (NS)
Minor D.	38.9 (17.6)	9.5 (12.3)	p < 0.0001
Major D.	10.0 (12.2)	0.5 (2.2)	p < 0.04
Megacapillaries	0 (0)	0.0 (0.0)	—
Fagrell I.	1.7 (1.0)	1.0 (1.2)	p < 0.15

TABLE VI

Comparison between the patients with SS and the control population

	PSS average (sd)	Control subjects average (sd)	Significance
Number	7	20	-
Age (age)	41.7 (8.4)	31.8 (7.9)	p < 0.01
PT	123.5 % (9.2)	101.7 % (13.1)	p < 0.03
PTT (ratio)	0.93 (0.29)	0.94 (0.008)	p = 0.96 (NS)
Fibrinogen	425.0 (25.4)	350.8 (89.8)	p = 0.27 (NS)
Factor VIII	422.0 (0.0)	140.9 (50.5)	-
No. loops	6.1 (2.3)	10.8 (1.3)	p < 0.0001
A-V difference	0.2 (0.5)	0.4 (0.2)	p < 0.0001
Minor D.	23.6 (30.2)	9.5 (12.3)	p = 0.09 (NS)
Major D.	47.1 (34.5)	0.5 (2.2)	p < 0.01
Megacapillaries	12.1 (15.7)	0.0 (0.0)	p = 0.08 (NS)
Fagrell I.	4.0 (1.3)	1.0 (1.2)	p < 0.0001

in the conclusions obtained. The differences recorded are established not only from a quantitative point of view (number of capillaries – p<0.003), but also from a qualitative perspective, with important divergences in regard to the diameter of the efferent branch (p<0.009) and morphological variations (minor p<0.001 / major p<0.002). The Fagrell index is presented as a comprehensive assessment scale, as it considers all these variables in its design, proving to be especially useful in the monitoring of patients. The presence of these differences reinforces the notion of impaired microcirculation in these nosological entities,[13] often preceding clinical manifestations of vascular involvement.[14] The absence of significance in regard to the number of megacapillaries is highlighted (contradicting the results reported in the literature),[15] justified by the small number of patients in the two study groups, and possibly, the fact that some of them are at a more advanced phase of systemic sclerosis, therefore with a predominance of capillary rarefaction and fibrosis, with no characteristic capillary structures being found in this stage. Basic assessment of the parameters for haemostasis did not show significant results in the analysis of the two groups.

In the comparative analysis of SLE and SS, the differences mentioned take on different aspects when considering the aspects considered within this group of patients. The present study also found no significant variations in relation to the basic concepts for the classification of the populations, as age and haemostasis values. No differences were found between the group of patients with SLE and with SS in terms of the number and diameter of capillary loops, and the presence of minor dysmorphism and megacapillaries. Aside from the small number in the sample, the similarity of these values appears to be based on a common involvement of the two entities under analysis. However, the existence of different physiopathological mechanisms is emphasized, through without evident reflection on this type of test. Even so, the number of capillaries was substantially different (average number in the SLE group: 8.77 / average number in the SS group: 6.14 – p=0.08), and in this aspect, the size of the sample may be relevant. The numerical

factor is certainly the reason for the non-existence of significant differences regarding the presence of megacapillaries, as it is one of the more specific alterations in the microcirculation for a specific (SS) pathology.[2] The smaller number of capillaries in SS appears to be in accordance with the predominant chronic ischemia in this entity in relation to SLE.[16] The existence of inflammatory activity, which could sometimes be interpreted only by endothelial activation, may explain the alterations in capillary morphology in the patients with SLE in relation to the control population. This difference is very significant when "minor dysmorphism" ($p<0.0001$) are considered. A possible explanation for the absence of significance in the comparison of the other parameters (major dysmorphisms, number and diameter of capillaries) may relate to the fact that SLE does not alter the extracellular space as frequently or dramatically, for which reason the conformational alterations are predominantly attributed to the lesion/inflammation of the inner layers of the vessel, particularly the endothelium. Studies focused not only vascular structure but also on tissue and interstitial components of the skin could confirm or rule out the hypothesis presented.

In the comparison of patients with SS and the control group, more evident and global differences have already been observed, with significant variations in capillary number and diameter and the presence of major dysmorphism. This greater disparity may result in greater interstitial damage due to ischemia and fibrosis, besides the actual vascular involvement. The Fagrell index, because it considers the cumulative aspects of quantity and quality, presents a very significant difference, which is much higher in patients with SS than in the controls ($p<0.0001$). Also, in this case, there was no significant difference in relation to the presence of megacapillaries, a fact that may be due to the size of the sample and the type of patients.

The presence of the Raynaud's phenomenon, frequently considered to be an independent factor,[17] differed in frequency between the groups of patients with SLE (33.3%) and those with SS (100.0%), according to the literature.[18] Considered as a classification factor, a relationship was found between its clinical manifestation and a higher value of the Fagrell index ($p<0.007$), or a greater number of major dysmorphism. Curiously, it was also possible to establish a relationship between the existence of Raynaud's and a lower prothrombin time (average with Raynaud's: 116.7% / average without Raynaud's: 100.0%). These differences between patients with and without Raynaud's may be due to the fact that this phenomenon is more frequent in the patients with SS, in whom these observations had already been made, compared with the control population.

Final considerations

Nailfold capillaroscopy is a non-invasive, easy-to--use technique that requires observer experience and enables the identification of alterations in microcirculation, before the appearance of clinical evidence. Although not particularly specific, it presents very good sensitivity to pathology that involves some form of capillary circulation. Within this, autoimmune diseases are naturally an important group. Enabling differences to be established between sick individuals and the healthy population, its greatest use in this context, in our opinion, is the possibility of anticipating the clinical onset of disease in this nosological group, or confirming more probable evolutions in cases of "connectivites of incomplete expression" or in "non-specific autoimmune disturbances". Although not addressed in this study, capillaroscopy, particularly with the increases brought by the adaptation of new technologies (video, computer analysis and the introduction of contrasts) can play an important role in the study of therapeutic efficiency or in monitoring the evolution of the disease. ■

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