Peripheral Microangiopathy in Pre-capillary Pulmonary Hypertension: A Nailfold Video Capillaroscopy Prospective Study

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Research article

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Abstract

Background: Although pulmonary vascular bed has been the main subject of research for many years in pulmonary hypertension (PH), interest has recently started to divert towards the possibility of a co-existing peripheral microangiopathy. The aim of this study was to investigate the presence of peripheral microangiopathy in patients with precapillary pulmonary hypertension by exploring nailfold video-capillaroscopic (NVC) structural changes among various subgroups with precapillary PH and to identify possible associations of NVC characteristics with markers of disease severity.

Methods: A cross-sectional study was performed in 46 consecutive patients with precapillary PH [14 with idiopathic pulmonary arterial hypertension (IPAH), 18 with PAH associated with connective tissue diseases (CTD-PAH) and 14 with chronic thromboembolic pulmonary hypertension (CTEPH)] and 30 healthy controls. NVC quantitative and qualitative parameters were evaluated.

Results: Patients with precapillary PH (71.7% were women, mean age 60.8 ± 13.4 years) presented reduced capillary density compared to healthy controls (7.5 ± 1.6 loops/mm vs. 9.7 ± 0.81 loops/mm, p<0.001) and increased capillary loop width (18.8 ± 6.7 μm vs. 11.5 ± 2.3 μm, p<0.001) detected mainly in PAH-CTD. Half of patients presented microhaemorrhages on capillary nailfold, while morphological capillary abnormalities where also detected compared to healthy controls. Log_{10} (NT-proBNP) was independently associated with capillary density in patients with precapillary PH [r= -0.68, B= -1.9, 95% CI (-3,3, -0.4) p=0.014].

Conclusion: Significant NVC microvascular changes were detected in patients with various types of precapillary PH, suggesting an impaired peripheral microcirculation associated with right ventricular remodeling parallel to pulmonary vasculopathy.

Introduction

Precapillary pulmonary hypertension (PH) represents a rare and heterogeneous group of pulmonary vasculopathies defined by elevated mean pulmonary arterial pressure (mPAP) ≥ 25 mmHg, normal pulmonary artery wedge pressure (PAWP) ≤ 15 mmHg and elevated pulmonary vascular resistance (PVR) ≥ 3 Wood Units at rest, that leads progressively to right heart failure and premature death, if not treated promptly [1]. Shear stress and hypoxia trigger pulmonary endothelial dysfunction and inflammation which play a vital role in the pathophysiology of pulmonary arterial hypertension (PAH), and lead to the remodeling of small and medium sized pulmonary arterioles, and thus to a progressive obstructive pulmonary vasculopathy [2]. Chronic thromboembolic pulmonary hypertension (CTEPH), on the other hand, develops from the occlusion of the pulmonary vascular bed with non-resolving thromboemboli, leading also to pulmonary vascular remodeling and increased PVR [3].

Although pulmonary vascular bed has been the main subject of research for many years [4, 5], interest has recently started to divert towards the possibility of a co-existing peripheral microangiopathy[6]. Nailfold video-capillaroscopy (NVC) is an established, validated, non-invasive imaging technique for the assessment of peripheral microcirculation, aiding in distinguishing different types of nailfold microvascular abnormalities [7]. It has been originally used in the assessment of Raynaud's phenomenon and the diagnosis of systemic sclerosis (SSc) [8], in which NVC changes may serve as an early prognostic marker that determines the risk of developing PAH [9–11]. Moreover, few NVC studies demonstrated evidence of peripheral microvascular dysfunction in patients with idiopathic PAH (IPAH), PAH associated with SSc and systemic lupus erythematosus (SLE), suggesting that changes in peripheral microcirculation may parallel pulmonary microangiopathy in patients with PAH [12–15]. However, no studies have assessed capillary rarefaction across the various types of precapillary PH including CTEPH and its potential correlations with indices of disease severity.

The aim of the present study was to: a) investigate the presence of peripheral microangiopathy in patients with precapillary PH, b) explore NVC structural differences among various subgroups with precapillary PH, c) identify possible associations of NVC characteristics with clinical, functional, biochemical, echocardiographic and hemodynamic parameters of disease severity in PH patients.

Methods

Study Design, Setting, Participants

This was a prospective case-control observational study which took place at a tertiary center for PH in collaboration with a tertiary center for rheumatic diseases with expertise in NVC in Northern Greece. Adult patients diagnosed with precapillary PH, and healthy controls were enrolled between February 2019 and April 2020. Written informed consent was obtained from all participants. The study received approval from the Aristotle University of Thessaloniki Ethics Committee and performed according to the Declaration of Helsinki.

Patients with pulmonary hypertension class 1 (Pulmonary Arterial Hypertension) and class 4 (Chronic Thromboembolic Pulmonary Hypertension) according to the classification of 2015 ESC/ERS guidelines [1], and healthy controls were enrolled. Patients with precapillary PH were either newly diagnosed and treatment naïve, as far as PAH specific therapy was concerned, or had established PH and have been receiving specific PAH medication. Diagnosis was based on the hemodynamic definition of 2015 ESC/ERS guidelines [1]. Patients with CTEPH additionally underwent a ventilation/perfusion lung scintigraphy or a computed tomographic pulmonary angiography to confirm diagnosis. In addition, patients with connective tissue disease associated PAH (CTD-PAH) underwent a high-resolution computed tomography scan to exclude significant lung fibrosis. Patients with PAH due to congenital heart disease were excluded from this study.

All the participants of the study underwent physical examination, 12-lead electrocardiogram, six-minute walking test (6MWT), laboratory evaluation including N- terminal pro-brain natriuretic peptide, TTE, spirometry with measurement of CO diffusing capacity and NVC at the same 1-week time interval, during their scheduled outpatient visits. Healthy controls underwent medical history, clinical examination and NVC.

Transthoracic Echocardiography

TTE was performed on all patients to assess right heart dysfunction using Vivid S70 (General Electric, Norway) based on the recommendations for the echocardiographic assessment of the right ventricle [16]. 2-D images of the right ventricle (RV)-focused apical four-chamber view were obtained. RV end-diastolic area (RV EDA) and RV end-systolic area (RV ESA) were measured. The RV fractional area change (FAC) was calculated as: (RV diastolic area – RV systolic area)/RV diastolic area X 100%. Tricuspid annular plane systolic excursion was acquired with M-mode placed on the lateral wall of the tricuspid annulus in the apical four-chamber view. Systolic displacement was measured from end-diastole to end-systole. In addition, tissue Doppler imaging (TDI) was applied on the lateral side of the tricuspid annulus. RV myocardial performance index (MPI) was calculated as follows: (isovolumic contraction time + isovolumic relaxation time)/ RV ejection time [17].

Nailfold videocapillaroscopy technique and image analysis

NVC was performed at room temperature (22–23 °C) with the subject seated and resting for 15 minutes. Subjects were asked to refrain from smoking and drinking alcohol or caffeinated drinks for at least 8 hours. Optilia Digital Capillaroscope (Optilia Instruments AB, Sollentuna, Sweden) was used for image acquisition (200x magnification). At least two adjacent fields of 1 millimeter in the middle of the nailfold were captured from all hands excluding thumbs. One drop of immersion oil was applied to the nailfold to maximize the transparency of the keratin layer. In total, 16 images from each patient were subsequently captured, coded, saved and manually analyzed using Optipix Lite software (Optilia Instruments AB) by a blinded trained examiner.

NVC images were quantitatively and qualitatively assessed. The following quantitative parameters were measured: capillary density (number of capillary loops per linear mm measured in the distal row following the 90° method) [18], avascular areas [19] (distinct areas in the nailfold where two or more capillaries are missing), capillary dimensions (total capillary width, arterial limb width, venous limb width, apical limb width and capillary length [19]; the mean value of each dimension of all capillaries per linear mm was eventually calculated), hemorrhages (defined as the presence of at least one hemorrhage in at least two different NVC images) and number of hemorrhages per linear mm, thromboses (number of thrombi per linear mm), edema (defined as the presence of edema in ≥ 50% of assessed fingers), capillary arrangement (capillary disorganization per linear mm defined as architectural disorientation) and capillary morphology (abnormal capillary shapes per linear mm including ramified, branched, bushy capillaries or other morphology that did not apply to normal shape). Tortuous or crossing capillaries were considered as non-specific variations of normal shapes.

As irregularly enlarged were characterized capillaries with apical limb width > 20 μm and < 50 μm and as giant, homogeneously enlarged capillaries with apical limb width ≥ 50 μm [20]. The mean of each capillaroscopic feature was calculated from the sum of consecutive images for each digit. Subsequently, the average values from eight fingers were added together and divided by the number of studied digits. The resulting value indicated the number of this capillaroscopic feature adjusted by each millimeter of the nailfold.

The “overall pattern recognition” was qualitatively assessed based on capillary density, the presence of irregularly enlarged or giant capillaries, hemorrhages and shape abnormalities using a fast track algorithm proposed by Smith et al. [7, 21]. Images were classified as “normal pattern”, “non-specific pattern” and “scleroderma pattern”. Furthermore, a semi-quantitative rating scale was adopted (score:0–3) based on these five capillaroscopic parameters in order to classify patients according to the severity of systemic microvascular disease. Capillary density was rated as follows: 0 for > 9 capillaries/mm, 1 for 7 to 9 capillaries/mm, 2 for 4 to 6 capillaries/mm and 3 for 1 to 3 capillaries per mm [22, 23]. Irregularly enlarged capillaries, giant capillaries, hemorrhages and shape abnormalities were scored accordingly: 0 equals to no changes, 1 to changes less than 33% of the total number of capillaries/mm, 2 to changes between 33% and 66% of the total number of capillaries/mm, and 3 to changes more than 66% of the total number of capillaries/mm [22, 23]. Total score was calculated by the sum of scores for each finger divided by the total number of fingers evaluated and was rounded to the next integer to define the risk group.

Statistical methods

Data are presented as mean ± standard deviation or as median (interquartile range) for continuous variables. Normal distribution was assessed using the Shapiro-Wilk test. Differences between patients with pre-capillary PH and healthy controls with respect to quantitative capillaroscopic characteristics were analysed using the Student’s t test for independent variables or the Mann Whitney test. For multiple comparisons among PH subgroups and controls, one-way ANOVA or the Kruskal-Wallis test were used. Bonferroni test was used for the correction of multiple comparisons. Categorical variables are presented as absolute count and percentage (%) and analysed using the chi-square test or Fisher’s exact test when appropriate.

Pearson or Spearman coefficient (r) was used to explore the correlation between capillaroscopic parameters and functional, laboratory, echocardiographic and hemodynamic parameters. N-terminal pro–brain natriuretic peptide did not follow the normal distribution, which was achieved using the common logarithm, $\log_{10}$(NT-proBNP). A multivariable linear regression analysis was conducted to examine parameters that were independently associated with capillary density in patients with precapillary PH. For normally distributed variables linear equation with 95% confidence intervals (CI) were also presented. A p-value < 0.05 was considered statistically significant. Data were analysed using IBM SPSS statistics (version 26.0) software.

Results

Baseline characteristics

In total, 46 consecutive patients with precapillary PH (71.7% women, mean age 60.8 ± 13.4 years) and 30 healthy controls were included in the study. PAH-CTD subgroup consisted of 13 patients with PAH associated with SSc (SSc-PAH), 2 with SLE and rheumatoid arthritis, and 1 with mixed connective tissue disease (Table 1). About four out of ten patients (41.3%) were newly diagnosed and received no specific PAH treatment at the time of NVC examination, while one third received monotherapy (32.6%). Three patients had persistent CTEPH post pulmonary endarterectomy and one patient underwent multiple sessions of balloon pulmonary angioplasty. Median duration from diagnosis for prevalent PH population was 7 (41) months.

Table 1
Baseline characteristics of patients with precapillary PH and controls.

Variables	Precapillary PH	IPAH	CTD-PAH	CTEPH	Healthy Controls	P-value
N.	46	14	18	14	30	
Female, n (%)	33 (71.7)	10 (71.4)	14 (77.8)	9 (64.3)	21 (70.0)	0.14
Age, y	60.8 ± 13.4	53.1 ± 13.4	65.9 ± 10.2	60.9 ± 14.4	54.2 ± 13.6	0.12
BMI, kg/m2	28.6 ± 5.2	28.8 ± 6.8	27.2 ± 4.6	30.4 ± 3.8#	24.7 ± 3.1#	**0.02**
SpO2% rest	93.1 ± 4.1	93.7 ± 4.9	92.9 ± 4.2	92.6 ± 3.2		0.8
WHO FC						**< 0.001**
I	3 (6.8)	2 (14.2)	0	1 (7.1)		
II	22 (50.0)	6 (42.8)	10 (55.6)	7 (50.0)		
III	18 (41.0)	5 (35.7)	8 (44.4)	6 (42.9)		
IV	1 (2.3)	1 (7.1)	0	0		
6-MWD, (m)	417.2 ± 117.3	471.3 ± 133.1	405.7 ± 126.7	382.5 ± 103.8		0.24
Laboratory markers						
eGFR, ml/min/1.73 m^2	78.8 ± 25.6	80.5 ± 34.1	75.6 ± 27.1	81.1 ± 14.1		0.72
NT-proBNP (pg/ml)	384 (1178)	188 (2005)	933 (2508) *	335 (361) *		**0.016**
Right Heart Catheterization						
mPAP, mmHg	42.6 ± 11.8	44.4 ± 16.3	40.2 ± 9.1	44.1 ± 10.7		0.54
PAWP, mmHg	10.6 ± 3.1	10.6 ± 1.9	9.8 ± 3.3	11.7 ± 2.2		0.22
CI, ml/m^2	2.9 ± 0.9	3.2 ± 0.7	3.1 ± 1.2	2.7 ± 0.6		0.39
PVR, WU	6.6 ± 3.9	6.2 ± 4.1	6.6 ± 4.0	6.8 ± 3.9		0.91
Echocardiography						
RV FAC%	30.9 ± 9.6	26.8 ± 9.7	31.8 ± 10.5	33.1 ± 7.9		0.25
TAPSE, mm	18.8 ± 5.4	19.1 ± 7.5	18.8 ± 4.2	18.8 ± 4.9		0.98
RV MPI	0.39 (0.16)	0.42 ± 0.14	0.45 ± 0.16	0.40 ± 0.20		0.18
Pulmonary Function Tests						
FEV1/FVC %	80.3 ± 11.0	84.1 ± 9.7	77.4 ± 12.1	83.6 ± 7.7		0.36
DLCO %	60.6 ± 21.7	66.4 ± 25.2	56.6 ± 22.7	64.9 ± 6.4		0.64
PAH Treatment	27 (58.7)	11 (78.6)	10 (61.1)	6 (42.8)		**0.009**
Monotherapy	15 (32.6)	5 (41.6)	5 (27.8)	5 (35.7)		
Dual therapy	7 (15.2)	4 (28.5)	3 (16.7)	0		
Triple therapy	5 (10.8)	2 (14.3)	2 (11.1)	1 (7.1)		

Categorical variables are presented as frequency and percentage, n (%).

Continuous variables are presented as mean value ± standard deviation or median value with interquartile range.

Statistical significance among the three PAH subgroups: $p < 0.05$.

$p < 0.05$ between CTEPH vs. controls for continuous variables.

***** $p < 0.05$ between CTD-PAH vs. CTEPH for continuous variables.

BMI: body mass index; 6-MWD: 6-minute walk distance; bpm: beats per minute; CI: cardiac index; CTD-PAH: Pulmonary Arterial Hypertension associated with connective tissue disease, CTEPH: chronic thromboembolic pulmonary hypertension, DLCO: diffusing capacity for carbon monoxide; FAC: fractional area change; FC: functional class; FEV1: forced expiratory volume during the first second of expiration; FVC: forced vital capacity; GFR: glomerular filtration rate; IPAH: idiopathic pulmonary arterial hypertension; mPAP: mean pulmonary artery pressure; MPI: myocardial performance index, NT-proBNP: N-terminal pro-brain natriuretic peptide; PAH: pulmonary arterial hypertension; PAWP: pulmonary artery wedge pressure; PVR: pulmonary vascular resistance; RV: right ventricle; SpO2%: arterial oxygen saturation; TAPSE: tricuspid annular plane systolic excursion; WHO: World Health Organization; WU: Wood units.

Capillaroscopic alterations in precapillary PH

The majority of quantitative and qualitative capillaroscopic parameters were abnormal in patients with precapillary PH as compared to healthy controls (Table 2, Fig. 1). Patients with PH presented reduced capillary density and increased capillary dimensions, namely capillary width, loop width arterial and venous limb width compared to controls. Irregularly enlarged capillaries were present compared to controls, while capillary length was normal. Half of patients presented microhaemorrhages on capillary nailfold, while more than a quarter of them had significant edema (26.4%). In addition, PH patients had more abnormal, disorganized and thrombosed capillaries than healthy controls.

Table 2
Capillaroscopic abnormalities in patients with precapillary pulmonary hypertension and differences among subgroups.

	PH	IPAH	CTD-PAH	CTEPH	Controls	PH vs. controls	IPAH vs. Controls	CTD-PAH vs. Controls	CTEPH vs. Controls	IPAH vs. CTD-PAH	IPAH vs. CTEPH	CTD-PAH vs. CTEPH
N	46	14	18	14	30	P-Value*	P-Value*	P-Value*	P-Value*	P-Value*	P-Value*	P-Value*
Cap. Density (loops/mm)	7.5 ± 1.6	8.4 ± 1.2	6.5 ± 1.6	8.1 ± 1.2	9.7 ± 0.81	< 0.001	0.03	< 0.001	0.003	0.02	1.0	0.007
Avascular areas (n/mm)	0.82 ± 0.46	0.75 ± 0.26	0.99 ± 0.6	0.67 ± 0.42	0.15 ± 0.2	< 0.001	< 0.001	< 0.001	0.001	1.0	1.0	0.01
Avascular areas, N (%)	41 (89.1)	13 (92.8)	16 (88.8)	12 (85.7)	8 (26.6)	< 0.001	< 0.001	< 0.001	< 0.001	1.0	1.0	1.0
Cap. Width (µm)	46.1 ± 19.2	35.1 ± 5.1	58.5 ± 24.8	39.2 ± 4.6	29.5 ± 4.3	< 0.001	1.0	< 0.001	0.75	0.003	1.0	0.014
Loop Width (µm)	18.8 ± 6.7	15.7 ± 3.9	23.3 ± 10.1	15.8 ± 1.9	11.5 ± 2.3	< 0.001	0.74	< 0.001	0.54	0.03	1.0	0.025
Arterial limb (µm)	12.3 ± 5.2	10.2 ± 2.2	15.3 ± 6.7	10.3 ± 1.5	7.4 ± 1.3	< 0.001	0.71	< 0.001	0.58	0.03	1.0	0.021
Venous limb (µm)	15.1 ± 6.3	12.4 ± 2.4	18.8 ± 8.1	12.6 ± 2.4	8.8 ± 1.4	< 0.001	0.66	< 0.001	0.40	0.03	1.0	0.028
Irregularly enlarged (loops /mm)	1.5 ± 0.85	1.4 ± 1.0	1.9 ± 0.6	1.1 ± 0.7	0.4 ± 0.4	< 0.001	0.002	< 0.001	0.037	1.0	1.0	0.07
Giants (loops /mm)	0 (0.22)	0	0.26 ± 0.38	0	0	0.001	1.0	< 0.001	1.0	< 0.001	1.0	< 0.001
Giants, N%	7 (15.2)	0	7 (38.8)	0	0	0.02	1.0	< 0.001	1.0	< 0.001	1.0	< 0.001
Cap. Length (µm)	304.2 ± 97.8	323.8 ± 84.9	292.6 ± 95.2	315.2 ± 107.7	313.2 ± 83.9	0.53	1.0	1.0	1.0	1.0	1.0	1.0
Edema, N (%)	12 (26.1)	3 (21.4)	9 (50)	0	0	0.003	0.004	< 0.001	1.0	0.007	0.004	< 0.001
Microhemorrhages (n/mm)	0.2 (0.5)	0.25 (0.25)	0.25 (0.4)	0.12 (0.4)	0	< 0.001	0.16	0.026	0.92	1.0	1.0	1.0
Microhemorrhages, N (%)	23 (50)	8 (57.1)	10 (55.5)	5 (35.7)	0	< 0.001	< 0.001	< 0.001	0.002	0.7	0.08	0.09
Thrombosis (n/mm)	2.6 ± 1.2	2.2 ± 0.8	2.5 ± 1.2	3.2 ± 1.3	1.4 ± 0.8	< 0.001	0.41	0.006	< 0.001	1.0	0.06	0.35
Disorganized (loops/mm)	0.7 ± 0.6	0.5 ± 0.4	1.0 ± 0.7	0.4 ± 0.3	0.2 ± 0.4	< 0.001	1.0	< 0.001	1.0	0.057	1.0	0.013

Categorical variables are presented as frequency and percentage, n (%).

Continuous variables are presented as mean value ± standard deviation or median value and interquartile range.

*A p-value < 0.05 is considered statistically significant.

Cap.: capillary, PH: pulmonary hypertension; IPAH: idiopathic pulmonary arterial hypertension; CTD-PAH: Pulmonary Arterial Hypertension associated with connective tissue disease, CTEPH: chronic thromboembolic pulmonary hypertension

	PH	IPAH	CTD-PAH	CTEPH	Controls	PH vs. controls	IPAH vs. Controls	CTD-PAH vs. Controls	CTEPH vs. Controls	IPAH vs. CTD-PAH	IPAH vs. CTEPH	CTD-PAH vs. CTEPH
Shape Abnormalities (loops /mm)	2.4 ± 1.2	1.9 ± 1.0	2.2 ± 1.0	3.1 ± 1.1	1.1 ± 0.8	< 0.001	0.24	0.002	< 0.001	1.0	0.007	0.05
Ramified capillaries (loops /mm)	0.6 ± 0.5	0.5 ± 0.4	0.8 ± 0.7	0.7 ± 0.3	0.2 ± 0.2	< 0.001	0.69	< 0.001	0.004	0.63	1.0	1.0
Categorical variables are presented as frequency and percentage, n (%).												
Continuous variables are presented as mean value ± standard deviation or median value and interquartile range.												
*A p-value < 0.05 is considered statistically significant.												
Cap.: capillary, PH: pulmonary hypertension; IPAH: idiopathic pulmonary arterial hypertension; CTD-PAH: Pulmonary Arterial Hypertension associated with connective tissue disease, CTEPH: chronic thromboembolic pulmonary hypertension												

The majority of patients (69.5%) presented a non-specific NVC pattern, while one third of them had the scleroderma pattern. Moreover, according to the semi-quantitative classification, the majority of patients presented mild capillaroscopic changes (score = 1, 84.8%), (Fig. 2).

Capillaroscopic alterations among PH subgroups

All PH subgroups had a significantly reduced capillary density compared to healthy controls with areas of avascularity (Table 2). As expected, patients with IPAH and CTEPH presented less reduced capillary density in comparison to patients with CTD-PAH. In addition, patients with CTD-PAH had significantly increased capillary dimensions (capillary width, loop width, arterial limb width and venous limb width) as compared to patients with IPAH, CTEPH and healthy controls. On the other hand, patients with IPAH and CTEPH had similar capillary dimensions to healthy controls, although an increased number of irregularly enlarged capillaries have been identified in these subgroups, too.

Although microhemorrhages were present in the nailfold of all PH subgroups, a significantly increased number was spotted only in CTD-PAH group compared to healthy controls. Similarly, capillary disorganization was present in CTD-PAH at a greater extent compared to CTEPH and healthy controls, while no difference was found compared to IPAH. Furthermore, patients with CTEPH demonstrated a significant number of capillary thrombi compared to healthy controls and more abnormal capillaries compared to IPAH and controls.

All patients with IPAH and CTEPH presented a non-specific capillaroscopic pattern, while the vast majority of patients with CTD-PAH (77.8%) had the scleroderma pattern (3 patients presented early pattern, 7 patients active and 4 patients late scleroderma pattern). According to the semi-quantitative classification, the majority of PH subgroups presented mild capillaroscopic changes, while 16.7% of patients with CTD-PAH had more severe changes (score: 2) (Fig. 2).

Correlations between capillaroscopic characteristics and markers of renal and cardiac function

A significant negative linear correlation was identified between capillary density and $\log_{10}$(NT-proBNP) [r = − 0.37, B= -1.0, 95% CI (-1.8, -0.2), p = 0.016] in patients with precapillary PH (Fig. 3), which was enhanced in multivariable analysis [r= -0.68, B= -1.9, 95% CI (-3,3, -0.4) p = 0.014] (Table 3). No other biochemical, functional, echocardiographic or hemodynamic variables were correlated with capillary density or other capillary features in the total PH population .

Table 3
Correlations of demographic, functional and hemodynamic characteristics with capillary density in patients with precapillary pulmonary hypertension.

	Univariable analysis		Multivariable analysis	
Variable	**R coefficient**	**P-Value**	**R coefficient**	**P-Value**
Age, years	-0.06	0.69	-0.09	0.73
BMI, kg/m^2	-0.07	0.63	-0.3	0.21
SpO2%	-0.2	0.23	-0.38	0.16
6-MWD, m	0.04	0.81	-0.07	0.79
Log_{10} (NT-proBNP)	-0.37	**0.016**	-0.68	**0.014**
GFR, ml/min	0.19	0.2	0.43	0.67
mPAP, mmHg	0.07	0.65	0.01	0.97
CI, L/min/m^2	0.08	0.95	-0.05	0.85
PVR, WU	0.07	0.66	0.17	0.07

Statistical significance $p < 0.05$, BMI: body mass index; 6-MWD: 6-minute walk distance; bpm: beats per minute; GFR: glomerular filtration rate; NT-proBNP: N-terminal pro-brain natriuretic peptide; SpO2% arterial oxygen saturation %, mPAP: mean pulmonary arterial pressure, CI: cardiac index, PVR: pulmonary vascular resistance

Among PH subgroups, eGFR was negatively correlated with capillary density in CTD-PAH patients [r=-0.63, B = 0.04, 95% CI (0.01, 0.06), p = 0.007] (Fig. 3), while TAPSE was positively associated with loop width (r = 0.55, p = 0.03). In CTEPH, a negative linear correlation between SpO2% and capillary density was detected [r = − 0.58, B=- 0.2, 95% CI (-0.4, -0.02) p = 0.037]. In addition, RV MPI was negatively associated both with capillary density (r = -0.69, p = 0.02) and with abnormal capillaries (r= -0.68, p = 0.02) (Supplementary Material).

Discussion

The main finding of our study is the demonstration of peripheral microvascular impairment assessed by NVC among all the different precapillary PH subgroups included in the study. In particular, PH individuals presented both reduced capillary density and increased capillary dimensions compared to controls, whilst microhaemorrhages, thrombi, shape abnormalities and capillary disorganization were also observed in all PH subgroups. To the best of our knowledge this is the first study to report such microvascular alterations in a wide spectrum of precapillary PH, indicating a more generalized microangiopathy in this population.

The results of the study suggest that different types of precapillary PH share common pathogenetic mechanisms that could explain the presence of systemic vasculopathy. For example, the imbalance between vasodilation and vasoconstrictor mediators, culminating in excessive vasoconstriction, endothelial and smooth muscle proliferation may account for pulmonary vascular remodeling as well as peripheral vascular changes in PH [4]. Vascular endothelial growth factor (VEGF) and proinflammatory cytokines are likely mediators of chronic hypoxia-driven pulmonary and peripheral vascular remodeling in pre-capillary PH, by mobilizing endothelial progenitor cells (EPCs) [24–27]. A severe reduction in circulating EPCs despite VEGF stimulus in late stages of SSc and its positive correlation with the reduction in capillary density may explain peripheral microvascular alterations [26], observed among various entities of precapillary PH in our study. To lend more support the strong inverse correlation between NT-proBNP - a significant prognostic biomarker in PH[1]- and reduced capillary density, indicates neurohormonal activation due to compromised RV function as another common pathway leading to systemic microvascular dysfunction in patients with precapillary PH.

Limited amount of data point towards the presence of a widespread vascular injury as determined by forearm blood flow dilation after brachial artery occlusion in precapillary PH [28, 29]. The majority of existing NVC studies until today have focused on systemic microvascular changes in SSc-PAH individuals by demonstrating significant reduction in capillary density in parallel with increase in capillary loop width, both of which have been correlated with hemodynamic severity such as mPAP [12, 15, 30]. In the context of IPAH, Hofstee et al. reported a significant decrease in capillary density between 20 patients with IPAH and 21 healthy controls [15], and these observations were confirmed by Corrado et al., who also displayed increased loop width in IPAH patients [12].

The present study supports and further expands previous observations by establishing reduced capillary density as a common NVC feature across different subgroups of precapillary PH. In line with previous studies [15], it appears that widening of capillaries is attributable to vascular phenotype associated with SSc rather an indicator of PH. However, other morphological markers of microvascular dysregulation, namely irregularly enlarged capillaries were also detected among IPAH and CTEPH patients at a greater extent compared to controls, whereas capillary edema and disorganized capillaries, have been identified for the first time in IPAH.

Besides an obstructive macrovascular disorder, CTEPH also encapsulates a “secondary arteriopathy” component of small sized pulmonary arterioles in which inflammation, oxidative stress and endothelial dysfunction play an important role [27]. In the current study, patients with CTEPH presented not

only with mild capillaroscopic alterations similar to NVC changes observed in IPAH, but also with a higher degree of abnormal capillaries and capillary thrombi compared to IPAH patients. Taking into account the negative linear correlation between rest arterial oxygen saturation and capillary density, enhanced NVC abnormalities in CTEPH group could be explained on the basis of hypoxia-induced peripheral vasculogenesis. On the other hand, the negative correlation of RV MPI with capillary density and abnormal capillary shapes may indicate a possible association of impaired right ventricular function and diminished vascularity. Taking altogether, these novel findings suggest the presence of impaired peripheral microcirculation in CTEPH and may shed new light on the pathophysiology of the disease.

The main limitation of our study is the small sample size, which could explain the lack of a significant association between capillaroscopic and functional or hemodynamic markers of cardiac dysfunction. Another limitation is that the majority of patients have been receiving PAH specific therapy which could be a confounding factor for NVC changes. The novelty of our study compared to previous ones is the recruitment of patients across the whole spectrum of precapillary PH. NVC was conducted in all fingers except thumbs and the acquisition of two adjacent images from each finger according to the updated EULAR recommendations [7]. Several studies acquired images only from the fourth finger of the non-dominant hand, which limited the generalizability of their results [15, 31]. In addition, qualitative and semi-quantitative assessment was performed based on a validated algorithm proposed by Smith et al, which facilitates parameters that can be reliably measured by a trained examiner [7, 21, 32]. The fact that the image analysis was conducted by one examiner could be a limitation, however it was conducted in a blinded manner. This is the first NVC study that comprehensively analyzed in a systemic manner all capillaroscopic features in a PH population representative of the spectrum of PH patients attending outpatient clinics in referral centers in Europe.

Conclusion

This study demonstrated significant NVC microvascular changes in patients with precapillary PH suggestive of an impaired peripheral microcirculation associated with right ventricular remodeling. Further prospective multi-center studies are warranted to confirm our results and reveal potential NVC markers as predictors of clinical outcomes in PH cohorts.

Declarations

Ethics approval and consent to participate: The study received approval from the Aristotle University of Thessaloniki Ethics Committee (protocol number 264) and was performed according to the Declaration of Helsinki.

Consent for publication: Acquired from the subjects to publish their nailfold videocapillaroscopic images.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: AA is the recipient of the International Training and Research Fellowship EMAH Stiftung Karla Voellm, Krefeld, Germany. The rest of the authors declare that they have no competing interests

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Authors' contributions: AA performed the NVC examination, stored and coded the images. AA collected, statistically analyzed and interpreted all data and drafted the manuscript. ET analyzed the NVC images in a blinded manner. CF and GG performed the right heart catheterization and the transthoracic echocardiography. AA, GG and TD conceived the idea, designed the study and critically revised the manuscript. AG and HK critically revised the manuscript for important intellectual content.

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Figures

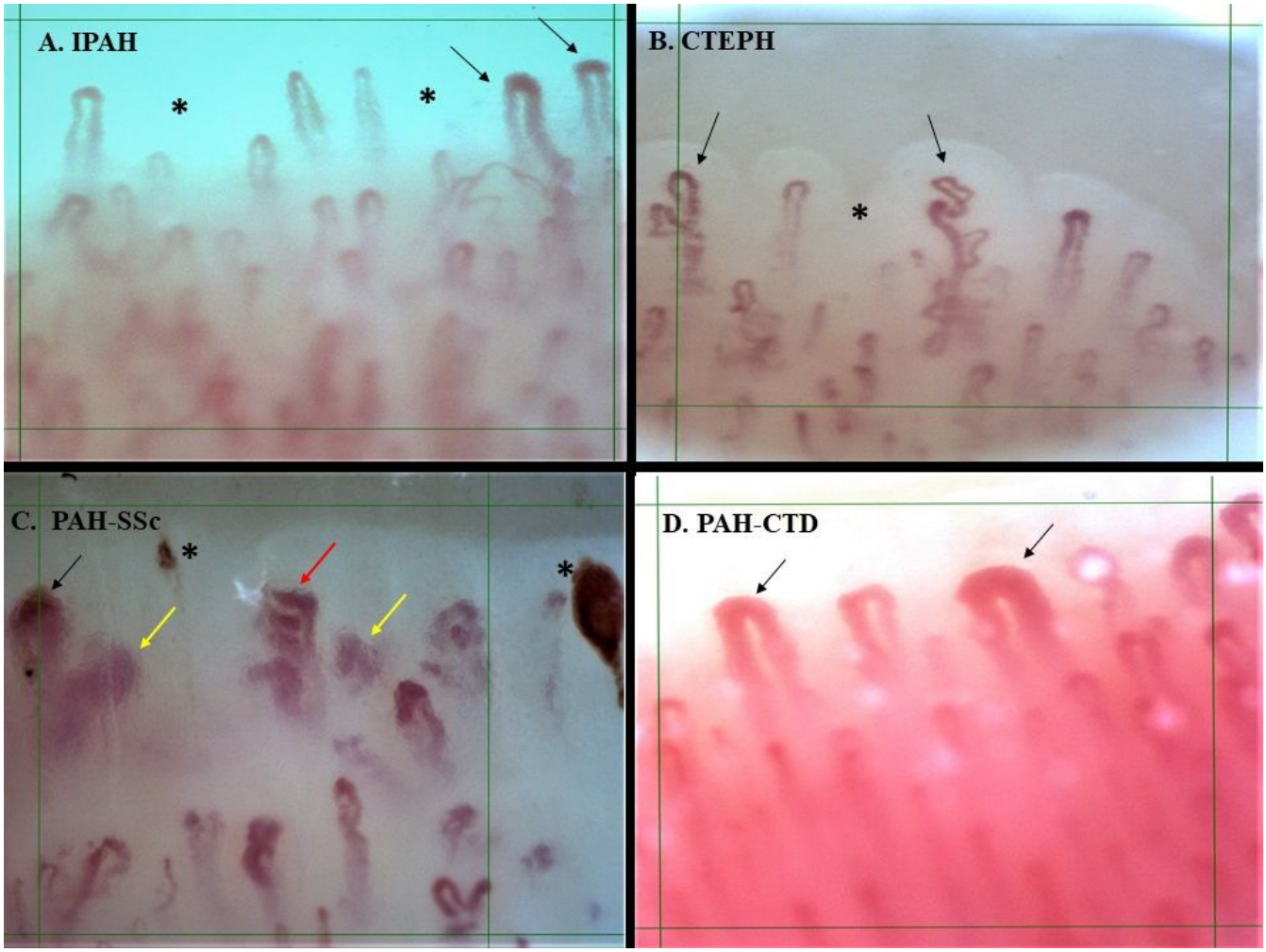

Figure 1

Nailfold video-capillaroscopic changes in patients with precapillary pulmonary hypertension. A. Idiopathic pulmonary arterial hypertension (IPAH). Reduced capillary density (5 capillary loops/mm), avascular areas (asterisk) and irregularly enlarged capillaries (<50µm, arrows). B. Persistent chronic thromboembolic pulmonary hypertension (CTEPH) post pulmonary endarterectomy. Mildly reduced capillary density (7 capillary loops/mm), avascular areas (asterisk) and abnormal capillary shapes (arrows). C. PAH associated with systemic sclerosis (SSc-PAH). Active scleroderma pattern with reduced capillary density (5 capillary loops/mm), giant capillaries (black arrow), disorganized capillaries (yellow arrows), abnormal capillary shapes (red arrow) and microhemmorhages (asterisk). D. PAH associated with mixed connective tissue disease (CTD-PAH). Early scleroderma pattern with mildly reduced capillary density (6 capillary loops/mm) and giant capillaries (black arrows). Grid pattern represents area of 1mm2. Magnification 200×.

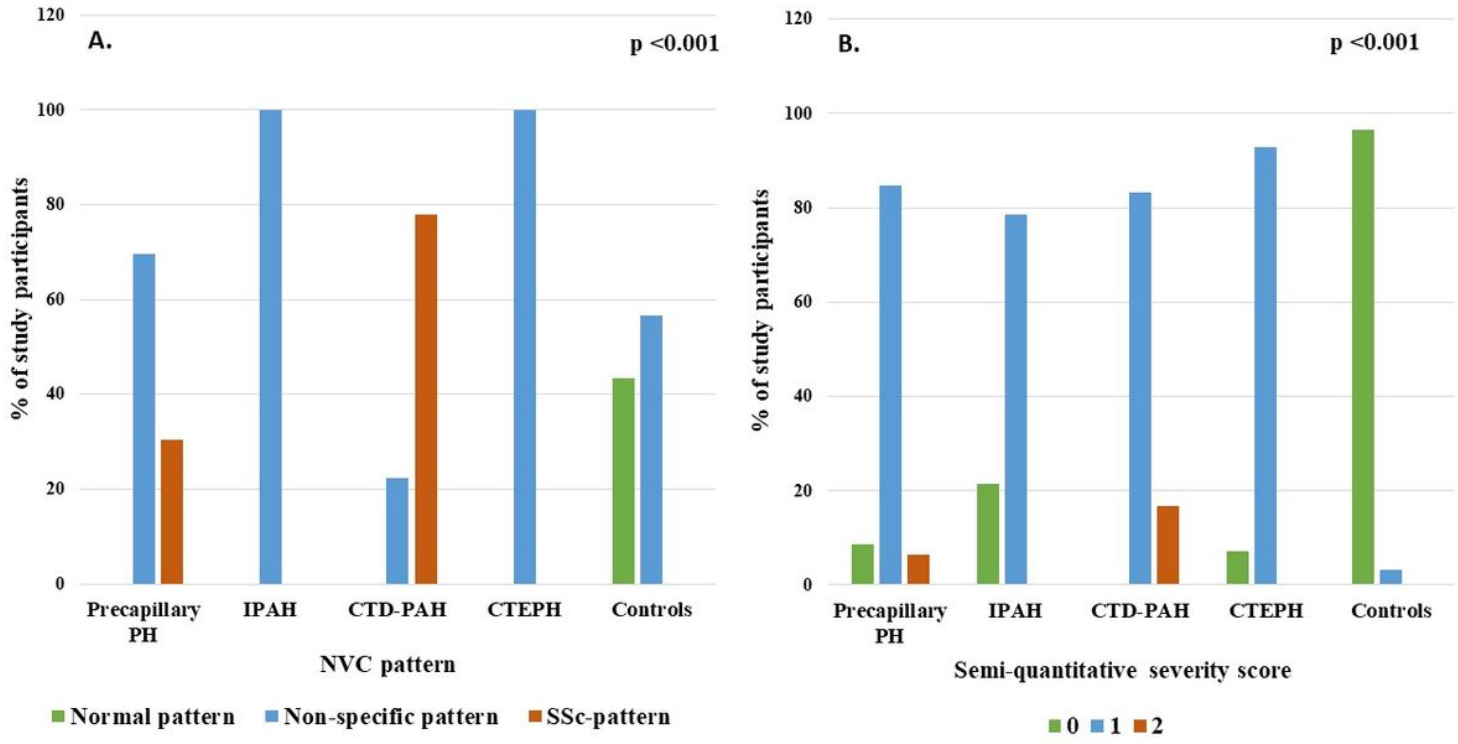

Figure 2

Qualitative and semi-quantitative nailfold video-capillaroscopic (NVC) evaluation of patients with precapillary PH. A. Differences in NVC pattern among various subgroups of precapillary pulmonary hypertension and healthy controls. NVC was qualitatively assessed based on capillary density, the presence of irregularly enlarged or giant capillaries, hemorrhages and shape abnormalities. Images were classified as “normal pattern”, “non-specific pattern” and “scleroderma pattern”; B. NVC semi-quantitative score representing the severity of microangiopathy among 46 patients with precapillary pulmonary hypertension (PH) and 30 healthy controls. A scoring system was adopted using capillary density, the presence of irregularly enlarged or giant capillaries, hemorrhages and shape abnormalities. Total score was calculated by the sum of scores for each finger divided by the total number of fingers evaluated and was rounded to the next integer to define the risk group. A p-value <0.05 is considered statistically significant. PH, pulmonary hypertension; IPAH, idiopathic pulmonary hypertension; CTD-PAH, pulmonary arterial hypertension associated with connective tissue disease; CTEPH, chronic thromboembolic pulmonary hypertension; SSc, systemic sclerosis

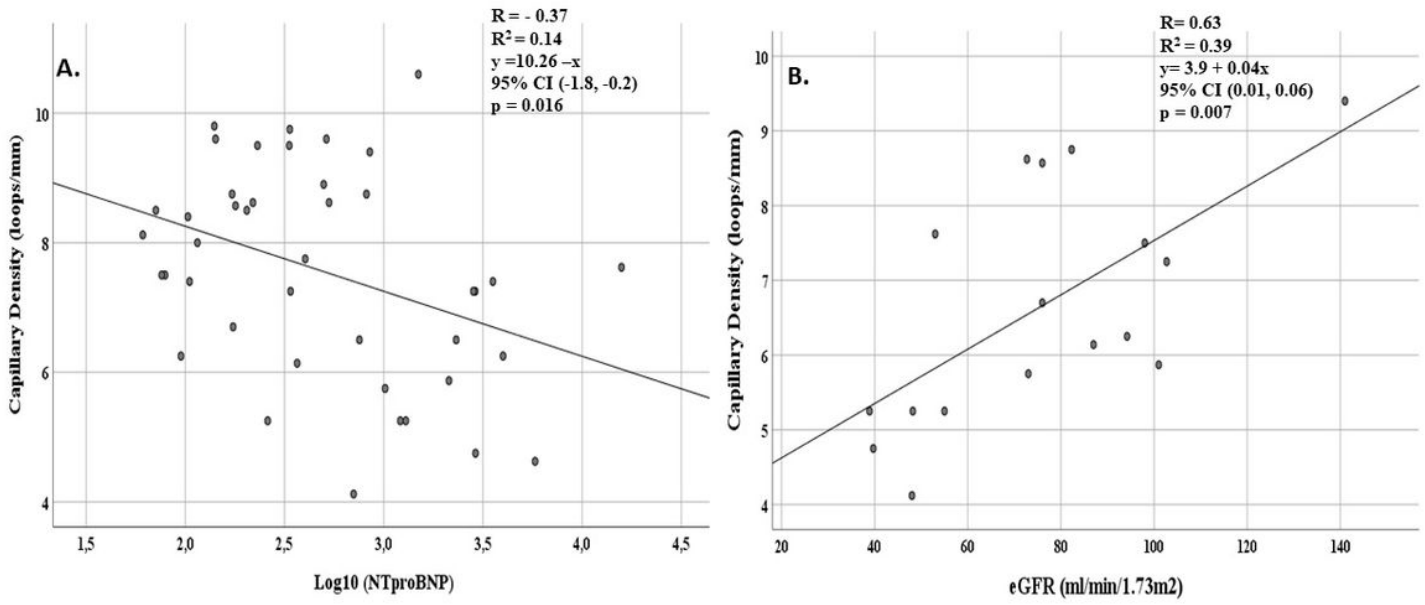

Figure 3

Scatter plots depicting linear correlations between capillary density and markers of cardiac and renal function. A) Negative linear correlation between capillary density (loops / mm) and Log10 (NT-proBNP) in patients with precapillary pulmonary hypertension; B) Positive linear correlation between capillary density (loops / mm) and eGFR (ml / min /1.73 m2) in patients with pulmonary arterial hypertension associated with connective tissue disease. Univariate linear regression analysis was used. R-coefficient and linear equations with 95% Confidence intervals (CI) are depicted. A p-value <0.05 is considered statistically significant. NT-proBNP: N-terminal pro-brain natriuretic peptide; GFR: glomerular filtration rate

Supplementary Files

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